ion torrent

Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics

USER GUIDE

Publication Number MAN0018810

Revision A.0

IVD

For In Vitro Diagnostic Use.

ThermoFisher SCIENTIFIC

Life Technologies Holdings Pte Ltd |
Block 33 |
Marsiling Industrial Estate Road 3 |
#07-06, Singapore 739256

Products manufactured in Singapore:

Ion PGM™ Dx Instrument System
Ion PGM™ Dx Sequencer
Ion OneTouch™ Dx Instrument
Ion OneTouch™ ES Dx Instrument
Ion PGM™ Dx Chip Minifuge (120V)
Ion PGM™ Wireless Scanner
Ion Torrent™ Server
Torrent Suite™ Dx Software
Veriti™ Dx 96-well Thermal Cycler, 0.2 mL

Life Technologies Corporation |
7335 Executive Way |
Frederick, MD 21704 | USA

Products manufactured in Frederick:

Oncomine™ Dx Target Test Kit
Ion Torrent Dx FFPE Sample Preparation Kit
Ion PGM™ Dx Library Kit
Ion OneTouch™ Dx Template Kit
Ion PGM™ Dx Sequencing Kit
Ion 318™ Dx Chip
Ion OneTouch™ Rack Kit
DynaMag™ Dx 96-Well Plate Magnet
DynaMag™ Dx 16 2-mL Magnet

The information in this guide is subject to change without notice.

DISCLAIMER

TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0018810

Revision	Date	Description
A.0	3 September 2020	Oncomine™ Dx Target Test user guide for FDA submission—updated for Torrent Suite™ Dx Software 5.12.5

Contents

About this guide

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Purpose of this guide

This user guide describes the intended use, theory of operation, and performance characteristics of the Oncomine™ Dx Target Test when used on the Ion PGM™ Dx System. This guide also includes a list of DNA variants and fusion isoforms targeted by the test for companion diagnostic (cDx) claims, as well as variants that show evidence of clinical significance.

Oncomine™ Dx Target Test Kit user guides

This user guide is part of a five-guide set.

- *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide*
- *Oncomine™ Dx Target Test Part II: Sample and Library Preparation User Guide*
- *Oncomine™ Dx Target Test Part III: Template Preparation User Guide*
- *Oncomine™ Dx Target Test Part IV: Sequencing and Results Reports User Guide*
- *Oncomine™ Dx Target Test Part V: Torrent Suite™ Dx Software 5.12.5 Reference User Guide*

All five guides are required to complete the entire Oncomine™ Dx Target Test workflow.

Note: The procedures in these guides supersede the instructions in the *Ion PGM™ Dx System User Guide* when using the Ion PGM™ Dx System with the Oncomine™ Dx Target Test.

1 Product information

Oncomine™ Dx Target Test

The Oncomine™ Dx Target Test is an *in vitro* diagnostic next-generation sequencing test to detect somatic changes in human DNA and RNA isolated from non-small cell lung cancer (NSCLC) tumor specimens in formalin-fixed, paraffin-embedded (FFPE) tissue samples. Detection of these variants is performed using the Ion PGM™ Dx System.

The Oncomine™ Dx Target Test (Cat. No. A49755) provides a set of primers in two panels that target key regions of 23 genes related to NSCLC.

Intended use

The Oncomine™ Dx Target Test is a qualitative *in vitro* diagnostic test that uses targeted high-throughput, parallel-sequencing technology to detect single nucleotide variants (SNVs) and deletions in 23 genes from DNA and fusions in ROS1 and RET from RNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor tissue samples from patients with non-small cell lung cancer (NSCLC) using the Ion PGM™ Dx System.

The test is indicated to aid in selecting NSCLC patients for treatment with the targeted therapy listed in Table 1 in accordance with the approved therapeutic product labeling.

Table 1 List of variants for therapeutic use

Gene	Variant	Tissue type	Targeted therapy
BRAF	BRAF V600E mutation	NSCLC	TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib)
EGFR	EGFR L858R mutation, EGFR Exon 19 deletions	NSCLC	IRESSA® (gefitinib)
RET	RET fusions	NSCLC	GAVRETO™ (pralsetinib)
ROS1	ROS1 fusions	NSCLC	XALKORI® (crizotinib)

Safe and effective use has not been established for selecting therapies using this device for the variants other than those listed in Table 1.

Results other than those listed in Table 1 are indicated for use only in patients who have already been considered for all appropriate therapies (including those listed in Table 1). Analytical performance using NSCLC specimens has been established for the variants listed in Table 2.

Table 2 List of variants with established analytical performance only

Gene	Variant ID/type	Amino acid change	Nucleotide change
KRAS	COSM512	p.Gly12Phe	c.34_35delGGinsTT
KRAS	COSM516	p.Gly12Cys	c.34G>T
MET	COSM707	p.Thr1010Ile	c.3029C>T
PIK3CA	COSM754	p.Asn345Lys	c.1035T>A

The test is not indicated to be used for standalone diagnostic purposes, screening, monitoring, risk assessment, or prognosis.

Theory of operation

Overview

DNA and RNA are isolated from tumor tissue samples prepared as FFPE sections on slides. The amounts of DNA and RNA in a sample are quantified, and if they meet the minimum required amounts for the test, cDNA is prepared from the RNA. The DNA and cDNA are made into amplicon libraries using the Ion PGM™ Dx Library Kit and the Oncomine™ Dx Target Test DNA and RNA Panel, which target the variants and gene fusions of interest for the test. No-template libraries and control libraries specific to the test are also prepared.

Each amplicon library is templated onto Ion PGM™ Dx Ion Sphere™ Particles (ISPs), loaded onto an Ion 318™ Dx Chip, and sequenced using the Ion PGM™ Dx Sequencer.

The signal generated by the sequencing reaction is translated into base calls and then reads, which are mapped to a reference sequence. Using parameters in the specific Assay Definition File designed for particular set of targets and therapies, Torrent Suite™ Dx Software generates reports containing a summary of the samples, test results, and any recommended therapies associated with the detected variants and gene fusions.

Sample and library preparation

The system has been validated with DNA and RNA isolated from FFPE tissue samples using the Ion Torrent Dx FFPE Sample Preparation Kit. Samples are prepared as slide-mounted 5-micron FFPE sections, which are deparaffinized before use. The samples must be macrodissected and enriched for tumor content if the tumor content is less than 20% and the tumor content in the region of interest is greater than or equal to 10%, or if the tissue is highly necrotic. The samples are digested, then the DNA and RNA are isolated and quantified. The minimum concentration and R^2 values that are required for library preparation are shown in Table 3.

Table 3 Required sample concentrations and R^2 values from the linear regression of the standards

Sample type	Required concentration	Required R^2 value
DNA	≥0.83 ng/µL	≥0.99
RNA	≥1.43 ng/µL	≥0.98

The RNA is transcribed into cDNA using the Ion Torrent Dx cDNA Synthesis Kit, and sample and control amplicon libraries are prepared from the cDNA and DNA using primers and reagents in the Oncomine™ Dx Target Test, Controls, and Diluent Kit and Ion PGM™ Dx Library Kit. Libraries created using these kits have a distinguishing nucleic acid sequence barcode that is incorporated into each amplicon. Information about each sample and its resulting libraries are entered into Torrent Suite™ Dx Software, which tracks the progress of the sample from library preparation through analysis. The specific Assay Definition File for particular set of targets and therapies defines the sample and library information required and tracked by the software.

Template preparation and sequencing

Using the Ion OneTouch™ Dx Instrument and the process of emulsion PCR, the library molecules are bound to Ion PGM™ Dx ISPs and each nucleic acid sequence is clonally amplified over the ISP surface. The templated ISPs are enriched and collected using the Ion OneTouch™ ES Dx Instrument. Sequencing primer is annealed to the single-stranded template, sequencing enzyme is added, and the ISPs are loaded onto the Ion 318™ Dx Chip. Chip loading occurs through use of the Ion PGM™ Dx Chip Minifuge. The chip is then placed onto the Ion PGM™ Dx Sequencer, where the DNA sequencing reaction occurs.

As the Ion PGM™ Dx Sequencer flows nucleotides over the chip surface, bases are incorporated into the strands on the bead in each well, resulting in the release of protons and a concomitant pH change in the well. The change in pH is detected by sensors at the base of each well on the chip. This initial electrical signal is processed for each well and transmitted to the Ion Torrent™ Server associated with the system.

Throughout this procedure, as the sample is prepared and processed by each instrument, sample and reagent information are recorded and tracked by Torrent Suite™ Dx Software.

Data analysis

On the Ion Torrent™ Server, the initial signals are processed and bases are called. These calls are assembled into files representing the reads, which are strings of nucleotide bases in the order found in the original library molecules. The reads are then mapped to the reference files provided with the test. Finally, Torrent Suite™ Dx Software assesses the mapped reads at specific nucleotide locations and looks for variation from the sequence information in the human reference sequence.

Results

Using parameters in the specific Assay Definition File designed for a particular set of targets and therapies, Torrent Suite™ Dx Software generates the following electronic results and reports for each sequenced sample and its associated controls.

Table 4 Electronic results and reports generated by the software

Results/report	Description
View Result screen	Contains QC and reference information, detailed sequencing analytics, and all variant and gene fusion calls.
Test Report	A clinical report that lists the variants associated with the cancer type and detected in the sample that are screened by the Oncomine™ Dx Target Test, and any recommended therapies.
Laboratory Report	Contains all the information in the Test Report, as well as sequencing run details and QC evaluation metrics for the sample and controls.

These reports are subject to approval by a lab manager or administrator via electronic signature.

Assay warnings and limitations

- Use of this product must be limited to personnel trained in the techniques of PCR, NGS, and the use of the Oncomine™ Dx Target Test and the Ion PGM™ Dx System.
- The Oncomine™ Dx Target Test has only been validated for use with FFPE tumor slide specimens.
- The Oncomine™ Dx Target Test has been validated to detect the following somatic mutations: RNA fusions, single-nucleotide variations (SNVs), multi-nucleotide variations (MNVs), and deletions of 3, 6, 9, 12, 15, and 18 base pairs from DNA.
- The Oncomine™ Dx Target Test is only validated for use with the Ion PGM™ Dx System and the Veriti™ Dx 96-well Thermal Cycler, 0.2 mL.
- The Oncomine™ Dx Target Test is only validated for use with 10 ng each of DNA and RNA per sample. Input amounts lower or higher than 10 ng are not recommended.
- Both the DNA and RNA from a single sample extraction must meet the concentration requirements specified in the procedure. Do not use DNA from one extraction with RNA from a different extraction.
- The effects of potential variations in FFPE specimen fixation have not been evaluated.
- Extraction from FFPE sample curls has not been evaluated.
- A potential source of contamination in the procedure is nucleic acid from previous sample processing steps. Follow good laboratory practices and all precautions and guidelines in these user guides to avoid cross-contamination between samples.
- The Oncomine™ Dx Target Test is a qualitative test. The test is not for quantitative measurements of percent mutation.
- The Oncomine™ Dx Target Test assay definition file includes prevalent RET isoforms, but not all rare or newly identified RET isoforms. The Oncomine™ Dx Target Test may miss a subset of patients carrying rare or newly identified RET isoforms who may derive benefit from pralsetinib.

Software compatibility and requirements

The procedures in this guide are designed for use with Torrent Suite™ Dx Software version 5.12.5 or later. To view the current software version, sign in to the software as an Administrator, click the **Settings (⚙)** tab, select **Configuration**, then click the **Software Updates** tab. Version-specific information is provided in the software release notes for the version of the software you are using.

Torrent Suite™ Dx Software is supported on Google™ Chrome™ browser version 64 and later, and is best viewed with 1440 × 900 screen resolution. It has not been tested with other browsers.

The Ion Torrent™ Server operating system is Ubuntu™ 18.04 LTS.

Materials provided

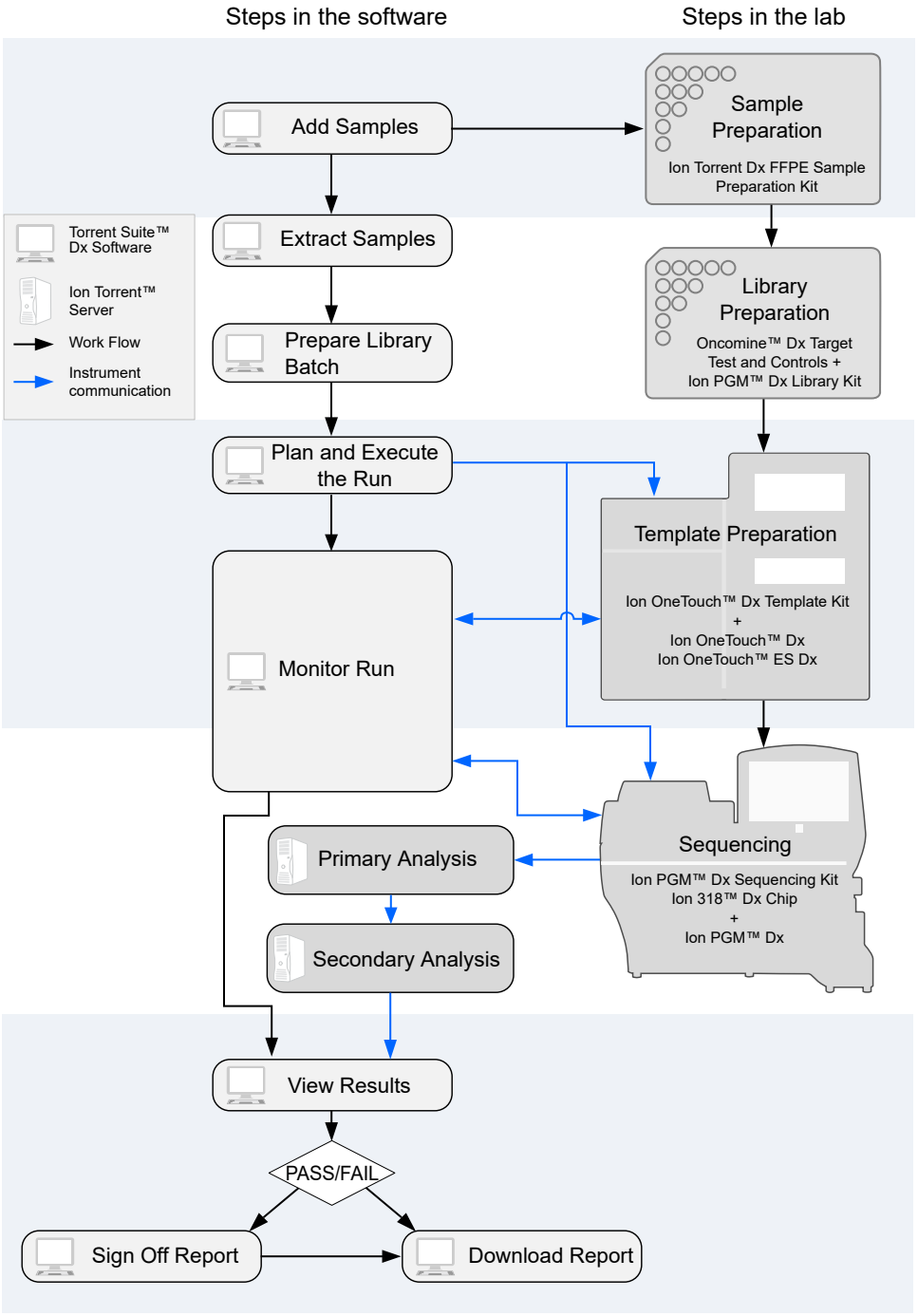
Oncomine™ Dx Target Test Kit

The Oncomine™ Dx Target Test Kit (Cat. No. A49755) includes the following subkits.

IMPORTANT! Refer to the product label for the storage conditions and expiration dates of individual modules and components.

✓	Subkit	Part No.
	Oncomine™ Dx Target Test, Controls, and Diluent Kit	A49756
	Ion Torrent Dx FFPE Sample Preparation Kit	A32445
	Ion PGM™ Dx Library Kit	A49758
	Ion OneTouch™ Dx Template Kit	A49759
	Ion PGM™ Dx Sequencing Kit	A49760
	Ion 318™ Dx Chip Kit	A18937
	Oncomine™ Dx Target Test User Guides and Assay Definition File	A49678

Oncomine™ Dx Target Test system diagram



2 Pass/fail criteria and repeat strategy

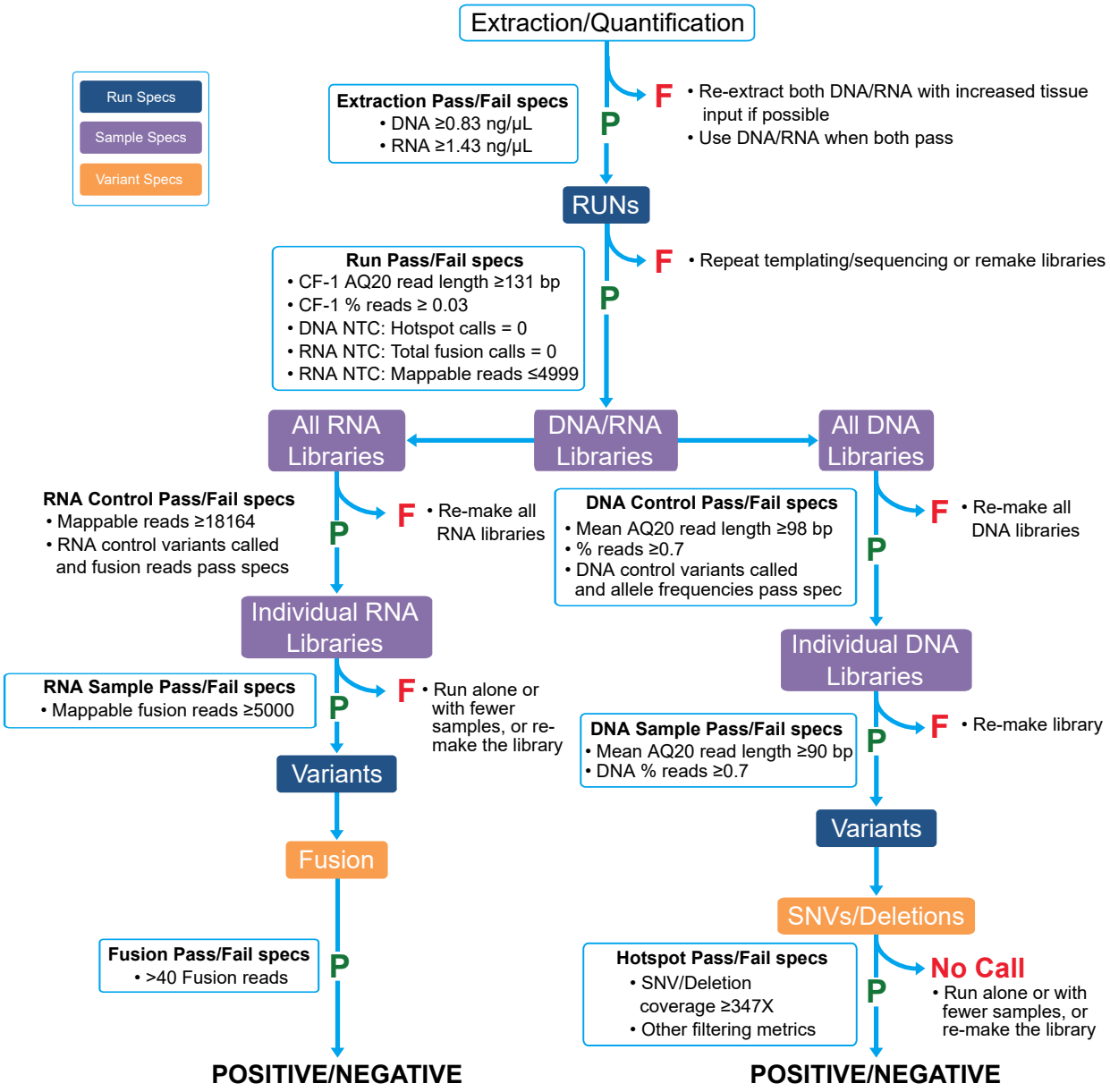
Quality control pass/fail criteria

Metric	Criteria
Run QC	
CF-1 Mean AQ20 Read Length (bp)	≥131
CF-1 Percent Reads (%)	≥0.03
DNA NTC	Hotspot calls = 0
RNA NTC	Total fusion calls = 0
RNA NTC Mappable Reads	≤4999
DNA Library	
Mean AQ20 Read Length (bp)	≥90
Percent Reads (%)	≥0.7
RNA Library	
Mappable Fusion Reads	≥5000
DNA Control	
COSM476_AF	Variant called and AF ≥0.05
COSM521_AF	Variant called and AF ≥0.05
COSM6223_AF	Variant called and AF ≥0.05
COSM6224_AF	Variant called and AF ≥0.05
COSM760_AF	Variant called and AF ≥0.05
Mean AQ20 Read Length (bp)	≥98
Percent Reads (%)	≥0.7
RNA Control	
Mappable Reads	≥18164
ROS1 Fusion Reads	Variant called and fusion reads ≥349

Pass/fail specifications and repeat strategy

In general, if you experience a run or sample failure, you can repeat the run or sample preparation at the workflow step in which the failure occurred. The number of libraries that can be made from an extracted sample for repeat testing depends on the quantity of DNA and RNA from the extraction, which varies from sample to sample.

Based on quality control results, you can determine whether a library requires repeat testing. Refer to the following flowchart to determine the best course of action.



Specification type	Passing criteria	Repeat strategy
Extraction/ Quantification	The following minimum concentrations for DNA and RNA are required: • DNA ≥0.83 ng/µL. • RNA ≥1.43 ng/µL.	If the minimum concentration requirement is not met for either DNA or RNA, the samples must be re-extracted with increased tissue input. Use the set of extracted samples where both the DNA and RNA meet the minimum concentration requirement for the test.
Run	A run must pass the following specifications to have reportable results for any sample within the run: • CF-1 Mean AQ20 Read Length (bp) must be ≥131. • CF-1 Percent Reads must be ≥0.03.	If either CF-1 specification fails, the operator may repeat the templating/sequencing run with the same library pool, or re-pool the libraries if a pooling error is suspected. If the issue persists on the repeat run, remake the libraries.
No Template Control (NTC)	A run must pass the following NTC specifications to have reportable results for any samples within the run: • DNA No Template Control (DNA NTC)—Total "Hotspot Calls" must equal zero (0). • RNA No Template Control (RNA NTC)—Mappable Reads must be ≤4999 and "Total Fusion Calls" must be zero (0).	If only the DNA NTC fails, remake all of the DNA controls and DNA sample libraries, and re-pool with previously made RNA controls and RNA sample libraries. If only the RNA NTC fails, remake all of the RNA controls and RNA sample libraries, and re-pool with previously made DNA controls and DNA sample libraries. If both the DNA NTC and RNA NTC fail, remake all of the DNA and RNA controls and sample libraries.
DNA Control	The DNA control must pass the following specifications in order for any DNA samples within the run to have any reportable results: • AQ20 Mean Read Length (bp) must be ≥98. • Percent Reads must be ≥0.7%. • All variants within the DNA control sample must be called "Present" and pass the allelic frequency range for each variant as specified in the assay definition file.	If any of these specifications fail, the operator must remake all DNA control and DNA sample libraries.

(continued)

Specification type	Passing criteria	Repeat strategy
RNA Control	The RNA control must pass the following specifications for any RNA samples within the run to have any reportable results: • Mappable Reads must meet the minimum threshold required of ≥18164 reads. • All variants within the RNA control sample must be called "Present" and pass the threshold metric set for total fusion reads required for each variant as specified in the assay definition file.	If either of these specifications fails, the operator must remake all the RNA control and RNA sample libraries.
Library DNA Sample	Any individual DNA library sample must meet the following specifications to have reportable results for the DNA library: • Mean AQ20 Read Length (bp) ≥90. • Percent Reads ≥0.7.	Run the DNA library sample alone, or with fewer DNA library samples. If the DNA library sample still fails these specifications, remake the library using the same (previously extracted) DNA, if there is sufficient quantity. If not, re-extract using more tissue input, if possible. The repeat libraries must be prepared and run with new DNA controls. **Note:** Use the original passing RNA control and DNA and RNA sample libraries as placeholders when needed, and re-pool the libraries accordingly in the repeat runs. Any data resulting from the placeholder libraries must be ignored in the repeat run.

(continued)

Specification type	Passing criteria	Repeat strategy
Library RNA Sample	Any individual RNA library sample must have ≥5000 Mappable Fusion Reads to have reportable results for the RNA sample library.	Run the RNA library sample alone, or with fewer RNA library samples. If the RNA library sample still fails this specification, re-make the library using the same (previously extracted) RNA, if there is sufficient quantity. If not, re-extract using more tissue input, if possible. The repeat libraries must be prepared and run with new RNA controls. **Note:** Use the original passing DNA control and DNA and RNA sample libraries as placeholders when needed, and repool the libraries accordingly in the repeat runs. Any data resulting from the placeholder libraries must be ignored in the repeat run.
SNV and Deletion Variant Specifications	All Single Nucleotide Variants (SNVs) and Deletions (Dels) must have coverage ≥347 reads and pass all Variant Caller filtering metrics in order to have a reportable result for the variant.	Any SNVs and deletions that do not meet the coverage criteria will result in a "No Call" for the variant. The operator may run the sample alone or with fewer samples to obtain reportable results for the variant. If the repeat run fails to meet the minimum coverage requirement, the operator may remake the library to obtain reportable results for the variant.

A

Performance characteristics

Analytical studies

Limit of Blank (LoB) study

To ensure that a variant-free ("blank") sample does not generate an analytical signal that might be classified as a mutation, wild-type (WT) samples were evaluated at each variant location that can be detected by the Oncomine™ Dx Target Test. Samples that are WT at all locations should produce a "variant not detected" call at each location. By definition (EP17-A2), the 95th percentile of test results on blank samples equals zero.

Operators extracted and sequenced nucleic acid from 5 WT cell lines prepared as FFPE sections on slides. The cell lines have well-characterized genomes and contain no known cancer biomarker sequences.

The study was conducted using two different lots of the Oncomine™ Dx Target Test Kit. For each lot, each cell-line sample was extracted once and made into 6 DNA and 6 RNA libraries. Operators sequenced each library in duplicate, generating 24 different sets of results across the two reagent lots per sample.

For all 5 samples, there were no positive calls at any of the variant locations analyzed by the test. The false positive rate was therefore zero.

Additionally, operators extracted and sequenced nucleic acid from 3 FFPE clinical samples prepared on slides. Each sample was tested using 24 replicates and 2 reagent lots of the Oncomine™ Dx Target Test Kit, resulting in 144 sequencing replicates each for DNA and RNA. For all replicates, there were no positive calls at any of the variant locations. The false positive rate was therefore zero, and the LoB of the test was determined to be zero.

Finally, nucleic acid from three WT FFPE clinical samples was processed from sample preparation through sequencing using the updated Oncomine™ Dx Target Test Kit RNA workflow. The study was conducted using 2 different lot combinations of kits. Each clinical sample generated 48 different replicates across the 2 reagent lot combinations, for a total of 144 replicates for the entire study. The false positive rate of the test was determined to be zero for the ROS1 fusion target, demonstrating that the LoB of the test was zero when tested with clinical samples.

Tissue input study

Sixty slide-mounted FFPE samples were analyzed to determine if samples extracted using the Ion Torrent Dx Total Nucleic Acid Isolation Kit yield DNA and RNA at the concentrations required by the Oncomine™ Dx Target Test when tissue input requirements are met. The test requires DNA at a concentration of ≥0.83 ng/µL and RNA at a concentration of ≥1.43 ng/µL.

Thirty resection samples with ≥20% tumor content were prepared without macrodissection, 15 resection samples with <20% to ≥10% tumor cell content were macrodissected, and 15 samples were collected by core needle biopsy (CNB). For the resection samples, 2 × 5 µm sections were used per extraction. For CNBs, 9 × 5 µm sections were used per extraction. DNA and RNA concentrations were determined using the Ion Torrent Dx DNA and RNA Quantification Kits, respectively. No sequencing was performed on the extracted samples.

Of the 60 samples tested, 98.3% (59/60) had a DNA concentration of ≥0.83 ng/µL and an RNA concentration of ≥1.43 ng/µL. One CNB sample failed the minimum DNA and RNA concentration specifications, with values of 0.52 ng/µL and 1.23 ng/µL respectively. The low concentrations were likely caused by the small tissue size and low tumor content (5%).

Seven slide-mounted FFPE fine needle aspirate (FNA) samples were analyzed to determine if samples extracted using the Ion Torrent Dx Total Nucleic Acid Isolation Kit yield DNA and RNA at the concentrations required by the Oncomine™ Dx Target Test when tissue input requirements are met.

For FNAs, 7 × 5 µm sections were used per extraction. DNA and RNA concentrations were determined using the Ion Torrent Dx DNA and RNA Quantification Kits, respectively. 100% of the 7 FNA samples extracted using the Ion Torrent Dx FFPE Sample Preparation Kit yielded DNA at a concentration of ≥0.83 ng/µL and RNA at a concentration of ≥1.43 ng/µL.

DNA and RNA input study

Eight cell-line samples were prepared as FFPE sections, and DNA and RNA were extracted and quantified from multiple sections from each cell line for blending and testing. Sample blends were prepared with known variants at various DNA and RNA input-level combinations within the range of 5–15 ng. The DNA and RNA blends had a target allele frequency of 15% for SNVs and deletions and target fusion reads of 300–600 for the ROS1 variant. A total of 540 individual DNA and RNA libraries were tested, including positive controls and NTC controls, with 6 replicate libraries each for DNA and RNA per test condition.

The study demonstrated a 100% positive variant call rate within the input range tested, supporting the specified input amount of 10 ng each for DNA and RNA for the Oncomine™ Dx Target Test.

The negative variant call rate was >95% for all except 4 sample and DNA/RNA input-level combinations. All cases with a negative variant call rate of <95% were due to no calls, 3 of which occurred with a DNA or RNA input amount of 5 ng and 1 of which occurred in a single sample with DNA and RNA inputs of 10 ng each. There were no false-positive calls.

Additionally, 4 clinical samples prepared as FFPE sections were tested: two samples containing DNA variants and two containing the CD74-ROS1 fusion.

The DNA variant samples were paired with wild-type RNA from the same sample at various input combinations within the range of 5–15 ng, and the RNA variant samples were paired with wild-type DNA at input combinations within the same range.

The study demonstrated positive and negative call rates of >95% for the DNA variants at all input combinations, and 100% for one of the CD74-ROS1 fusions at all input combinations. The second CD74-ROS1 clinical sample showed 100% negative call rates for all test conditions, and 100% positive call rates except for Test Condition 4 (8.5 ng RNA/15 ng DNA), where the call rate was 83%, and Test Condition 6 (15 ng RNA/15 ng DNA), where the call rate was 50%. The false negatives for these test conditions were possibly due to operator error during library preparation, since the remaining replicates in these test conditions had both high total mappable reads and fusion reads, but the cause was not definitively determined.

The results support the DNA and RNA 10-ng input requirement for the Oncomine™ Dx Target Test.

In silico specificity study

An *in silico* cross-reactivity analysis was performed that evaluated the 833 primers in the Oncomine™ Dx Target Test Kit DNA and RNA panels to determine the specificity of the primers to their targeted sequences. The primers were checked for specificity to the human genome, the human transcriptome, and genomes from representative bacteria, fungi, and viruses frequently found in human tissue and lung specimens.

Any unintended amplification products were required to have ≥2 base-pair (bp) mismatches to intended amplification product sequences generated by the panels, because mismatches of ≥2 bp prevent mapping to the same location on the genome due to a low mapping score.

For the DNA panel primers, *in silico* analysis predicted 20 unintended potential amplicon-generating primer pairings against the human genome. Nineteen of these had unintended amplification products with ≥2 bp mismatches, and therefore would have low mapping scores and not cause false results. One unintended primer pairing was predicted to amplify regions identical to an intended product, and therefore would detect the same WT and variant locations and not cause false results.

For the RNA panel primers, analysis predicted 63 unintended primer pairings against the human genome and 7 unintended primer pairings against the human transcriptome. All of these predicted amplicons had mismatches of ≥42 bp to intended amplicons, and therefore would not cause false results.

Analysis of representative bacterial, fungi, and viral genomes resulted in one predicted unintended primer pairing with a mismatch of ≥61 bp to intended amplicons, which would not cause false results.

Based on these results, the primers in the Oncomine™ Dx Target Test Kit DNA and RNA panels were deemed specific.

Cross-contamination study

A total of 8 FFPE cell line samples were evaluated to determine the percentage of false positive results caused by cross-contamination (contamination from one sample to another within the same sequencing run) and carryover contamination (contamination from a previous run on the same instrument system). Samples that were WT and variant were tested in consecutive runs on the same instruments, and 5 DNA variant locations and 2 RNA variant locations that were expected to be WT for a sample were evaluated for contamination.

Out of 100 DNA and 80 RNA data points analyzed, no false positive results were reported in the DNA variants, and 1 false positive result was reported in a ROS1 fusion. The false positive was likely caused by sample cross-contamination from an adjacent well. Therefore, the false-positive rate at DNA variant locations was 0% (0/100) and the false-positive rate at RNA variant locations was 1.25% (1/80).

Tissue fixation study

A study was performed to evaluate the effect of 10% neutral buffered formalin (NBF) fixation times on cytosine deamination events at the hotspot locations targeted by the Oncomine™ Dx Target Test, and any effect these potential events would have on assay performance. Pellets from the wild-type cell line GM24385 were fixed with 10% NBF for 12, 24, 48, 72, and 84 hours. Sections from each block were cut, mounted on slides, and tested with the Oncomine™ Dx Target Test. These results were compared to results from cell line GM24385 that had not undergone any fixation with 10% NBF.

The average allelic frequency (AF) observed at each of the 103 cytosine deamination-susceptible hotspots was determined for each fixation time tested. The results showed 2 G>A deamination events as a result of the fixation process, one at a 24-hour fixation time for COSM232755 (AF 0.050%) and the other at a 48-hour fixation time for COSM181063 (AF 0.073%). Each resulted in a "no call". DNA and RNA sequencing quality was evaluated by measuring percent reads, no calls, and total mappable reads for each condition tested. Both DNA and RNA demonstrated valid sequencing results with all NBF fixation times tested in this study. Therefore, it was determined that NBF fixation times did not cause deamination events that negatively impacted sequencing results.

Sample processing reproducibility study

The reproducibility and repeatability of variant detection using the Oncomine™ Dx Target Test were assessed with 2 WT samples and 10 variant-positive samples at 4 testing sites. Each site had 4 Ion PGM™ Dx instrument systems and 4 operators.

Each sample was tested 8 times at each site, for a total of 32 replicates per sample. After repeat testing, the final number of invalid reactions was 15/768 (1.95%), possibly due to low sample quality or lack of sample, though the cause was not definitively determined.

The call rate, no call rate, positive call rate, negative call rate, and within-run repeatability were computed at each variant location of interest. Including no calls and excluding known positive variant locations, the negative call rate at each clinical variant location for all samples was 100%.

The results at positive variant locations are shown in Table 5. Including no calls, all positive call rates from positive variant locations were >84%.

Excluding no calls and combining data across all study samples, the estimate of repeatability was 100% for DNA variants and 87.5% for the RNA variant. The lower limit of the 95% two-sided confidence interval (CI) for repeatability exceeded 96% at all variant locations.

Including no calls from the data, the estimate of repeatability was 100% at 218 out of 605 variant locations, 94–99.9% at 175 out of 605 variant locations, and 71.6–93.9% at 212 out of 605 variant locations. Including no calls, the lower limit of the 95% two-sided confidence interval for repeatability exceeded 64.6% at all variant locations.

Table 5 Call rates at positive variant locations

Sample	Variant identification	Variant location	# of valid sample results (N)	# of positive calls (A)	# of negative calls (B)	# of No Calls (C)	Positive call rate + 95% CI		Negative call rate + 95% CI		Within-run repeatability + 95% CI	
							Including no calls (A/N)	Excluding no calls (A/(A+B))	Including no calls (B/N)	Excluding no calls (B/(A+B))	Including no calls	Excluding no calls
B	COSM6223	EGRF Exon19 del	32	32	0	0	100% (89.1%, 100%)	100% (89.1%, 100%)	0% (0%, 10.9%)	0% (0%, 10.9%)	100% (79.4%, 100%)	100% (79.4%, 100%)
B	COSM763	PIK3CA E545K	32	32	0	0	100% (89.1%, 100%)	100% (89.1%, 100%)	0% (0%, 10.9%)	0% (0%, 10.9%)	100% (79.4%, 100%)	100% (79.4%, 100%)
C	ROS1	N/A	32	30	2	0	93.8% (79.2%, 99.2%)	93.8% (79.2%, 99.2%)	6.3% (0.8%, 20.8%)	6.3% (0.8%, 20.8%)	87.5% (61.7%, 98.4%)	87.5% (61.7%, 98.4%)
D	COSM6225	EGFR Exon19 del	32	32	0	0	100% (89.1%, 100%)	100% (89.1%, 100%)	0% (0%, 10.9%)	0% (0%, 10.9%)	100% (79.4%, 100%)	100% (79.4%, 100%)
E	COSM476	BRAF V600E	32	32	0	0	100% (89.1%, 100%)	100% (89.1%, 100%)	0% (0%, 10.9%)	0% (0%, 10.9%)	100% (79.4%, 100%)	100% (79.4%, 100%)
F	COSM521	KRAS G12D	32	30	0	2	93.8% (79.2%, 99.2%)	100% (88.4%, 100%)	0% (0%, 10.9%)	0% (0%, 11.6%)	87.5% (61.7%, 98.4%)	100% (76.8%, 100%)
F	COSM29313	PIK3CA M1043I	32	30	0	2	93.8% (79.2%, 99.2%)	100% (88.4%, 100%)	0% (0%, 10.9%)	0% (0%, 11.6%)	87.5% (61.7%, 98.4%)	100% (76.8%, 100%)
G	COSM6224	EGFR L858R	32	32	0	0	100% (89.1%, 100%)	100% (89.1%, 100%)	0% (0%, 10.9%)	0% (0%, 10.9%)	100% (79.4%, 100%)	100% (79.4%, 100%)

Table 5 Call rates at positive variant locations *(continued)*

Sample	Variant identification	Variant location	# of valid sample results (N)	# of positive calls (A)	# of negative calls (B)	# of No Calls (C)	Positive call rate + 95% CI		Negative call rate + 95% CI		Within-run repeatability + 95% CI	
							Including no calls (A/N)	Excluding no calls (A/(A+B))	Including no calls (B/N)	Excluding no calls (B/(A+B))	Including no calls	Excluding no calls
J	COSM87298	KRAS Q61K	32	32	0	0	100% (89.1%, 100%)	100% (89.1%, 100%)	0% (0%, 10.9%)	0% (0%, 10.9%)	100% (79.4%, 100%)	100% (79.4%, 100%)
J	COSM172423	ERBB3 V104M	32	32	0	0	100% (89.1%, 100%)	100% (89.1%, 100%)	0% (0%, 10.9%)	0% (0%, 10.9%)	100% (79.4%, 100%)	100% (79.4%, 100%)
K	COSM775	PIK3 H1047R	30[1]	29	0	1	96.7% (82.8%, 99.9%)	100% (88.1%, 100%)	0% (0%, 11.6%)	0% (0%, 11.9%)	93.3% (68.1%, 99.8%)	100% (76.8%, 100%)
M	COSM715	FGR3 S249C	32	32	0	0	100% (89.1%, 100%)	100% (89.1%, 100%)	0% (0%, 10.9%)	0% (0%, 10.9%)	100% (79.4%, 100%)	100% (79.4%, 100%)

[1] Two replicates for sample K were initially invalid and not retested.

Interfering substances studies

Interfering substances—Study I

Six potentially interfering substances that can be found in FFPE tissue samples or carried over from the deparaffinization and nucleic acid extraction process were evaluated using the Oncomine™ Dx Target Test on the Ion PGM™ Dx System.

The guidelines used at the time of testing are defined in section 7.1 of CLSI EP07A2E, which describes testing substances at a relatively high concentration as an interference screen. One potentially interfering endogenous substance, hemoglobin, was tested at twice the concentration recommended in CLSI EP07A2E, Appendix D.

In addition to the substances tested in this study, the data from the Tumor Necrosis Study demonstrated that the performance of the Oncomine™ Dx Target Test was not impacted by the presence of up to 75% necrotic tissue. However, users should macrodissect highly necrotic areas or select alternate samples if possible.

Table 6 Interfering substances and amounts

Potential interfering substance	Step	Amount of substance
Paraffin	At the deparaffinization step, extra paraffin was added to the xylene bath that contained 250 mL of xylene.	4X of normally expected levels
Xylene	Extra xylene was added into the ethanol bath that contained 250 mL of ethanol.	6X of normally expected residual volume
Ethanol	Extra ethanol was added into the Protease digestion step before digestion.	>4X of normally expected residual volume
Hemoglobin	After deparaffinization, hemoglobin was added to the Digestion Buffer used to pre-wet the tissue section.	4 mg/mL
Protease	Extra Protease was added into the reaction after the digestion step and before column purification.	>10X of expected residual Protease after the heat-kill step
Wash buffer	Wash buffer used to isolate DNA and RNA from deparaffinized and digested samples was added into an aliquot of Dilution Solution, which was later used to dilute the RNA and DNA to the appropriate concentration before library preparation.	1% wash buffer (equivalent to ~10% wash buffer carried over into eluate)
Control	Tissue sections were processed using the standard protocol, without the addition of any potentially interfering substances.	N/A

A total of 6 FFPE samples (1 WT and 5 variants) with 6 replicates each were processed through the entire assay workflow. The variant samples included variants from all variant categories that can be detected by the test. The samples were spiked with additional concentrations or amounts of the listed substances at the relevant processing step, as shown in the table. Replicates of a control sample with no spiked substances were also analyzed. The concordance between variant calls in samples with and without interfering substances was computed for each substance under investigation.

With no calls excluded, for each potential interferent used in sample extraction, the positive and negative concordance with the control condition across all samples was 100%, and the overall concordance with the control condition across all samples was 100%.

With no calls excluded, the results of testing with hemoglobin showed positive concordance with the control condition of 100% (only samples with a positive control condition were analyzed), negative concordance of 99.99%, and overall concordance of 99.99%.

Interfering substances—Study II

The interfering substances study was repeated with the updated RNA library preparation workflow to demonstrate that the performance of the Oncomine™ Dx Target Test is not affected by the presence of potentially interfering substances.

The impact on assay performance of the listed interferents (Table 6) was evaluated in this study and the results were compared to the control (no interferents) condition. For the 6 interferents tested, both the positive concordance (no calls excluded) and the overall concordance (no calls excluded) for all samples was 100%. These data support the claim that paraffin, xylene, ethanol, hemoglobin, protease, or wash buffer do not affect assay performance at the level tested.

Interfering substances—Study III

A study was performed to demonstrate that the performance of the Oncomine™ Dx Target Test in detecting RET fusions is not affected by the presence of potentially interfering substances.

A total of 3 FFPE samples (2 RET fusion-positive, and 1 WT) with 3 replicates each were used to evaluate the impact of the listed interferents (Table 6) on assay performance, and the results were compared to the control (no interferents) condition. For the 6 interferents tested, both the positive concordance and the overall concordance for all samples was 100%. These data support the claim that paraffin, xylene, ethanol, hemoglobin, protease, or wash buffer do not affect assay performance at the level tested in detection of the RET fusions.

Limit of Detection (LoD) studies

Limit of Detection (LoD)—Study I

The LoD was evaluated for 14 representative DNA variants detected by the Oncomine™ Dx Target Test in clinical samples. The LoD is the lowest AF of SNV, MNV, or deletion variants that can be detected at least 95% of the time. Variant-containing samples were blended with WT samples at multiple levels and used as the input DNA for the test.

Due to the large number of variants detected by the Oncomine™ Dx Target Test and the rarity of some of the variants, the LoD was established using a representative variant approach. Variants were selected in the following categories:

- Simple SNVs
- Complex SNVs and MNVs (SNVs in di- or tri-nucleotide repeat regions, SNVs in high-GC (>60%) or low-GC (<40%) content regions, and MNVs)
- Deletions (including deletions of 6, 9, 15, and 18 bp)

Clinical specimens were tested for all variants for which clinical claims are being sought. Seven variants for which analytical claims are being sought were unavailable in clinical specimens, and so plasmid constructs were substituted.

A minimum of 120 data points were generated for each representative variant by testing 6 or more titration levels, 2 reagent lots, and 10 replicates (per level per lot). The claimed LoD for all but 1 variant is the maximum of the LoD obtained from testing each of the 2 lots in this study.

Based on 14 representative DNA variants in 6 genes assessed in clinical samples, the LoDs for DNA variants tested in clinical samples (supported by the results from the assay reproducibility study) were determined to have allelic frequencies (AFs) of 6–8%.

Limit of Detection (LoD)—Study II

The LoD of the Oncomine™ Dx Target Test was calculated by testing 2 clinical ROS1 fusion-positive specimens using the updated Oncomine™ Dx Target Test RNA workflow.

RNA extracted from fusion-positive clinical FFPE specimens was blended with RNA extracted from wild-type (WT) clinical FFPE specimens to achieve 6 dilution levels. For each ROS1 fusion isoform, 10 replicates per 6 dilution levels were tested with 2 reagent lots, giving a total of 120 data points. The LoD of the assay for ROS1 fusion detection was determined to be 516 fusion reads (higher of the LoD observed for the 2 isoforms tested).

Limit of Detection (LoD)—Study III

The LoD of the Oncomine™ Dx Target Test was calculated by testing 2 clinical RET fusion-positive specimens using the updated Oncomine™ Dx Target Test RNA workflow.

RNA extracted from fusion-positive clinical FFPE specimens was blended with RNA extracted from wild-type (WT) clinical FFPE specimens to achieve 6 dilution levels. For each RET fusion isoform, 10 replicates per dilution level were tested with 2 reagent lots, giving a total of 120 data points. The LoD of the assay for RET fusion detection was determined to be 405 fusion reads (higher of the LoD observed for the 2 isoforms tested).

Tumor content studies

Tumor content—Study I

To determine the minimum tumor cell content required in FFPE samples used as input material, 55 pre-characterized clinical samples were analyzed using the Oncomine™ Dx Target Test. They contained SNVs, deletions, and fusions confirmed by validated reference methods. The tumor cell content of each specimen and region of interest was estimated before the study by an external pathology lab.

The samples were analyzed with and without macrodissection. Fifty-four samples contained DNA variants and 1 contained an RNA variant. Three samples contained 2 SNV or deletion variants, for a total of 58 variants analyzed. The observed tumor content had the following distribution:

- 10 samples with tumor content <10%
- 16 samples with tumor content 10–19%
- 13 samples with tumor content 20–29%
- 9 samples with tumor content 30–39%
- 3 samples with tumor content 40–49%
- 4 samples with tumor content 50–60%

In the samples without macrodissection, all 58 variants were detected (called positive) by the Oncomine™ Dx Target Test. In the macrodissected samples, there was one "no call" in a BRAF V600E variant sample with a tumor content of 16%.

Additional studies were performed to validate that the minimum tumor cell content required in FFPE input samples is greater than or equal to 20% when using the updated RNA library preparation workflow. As part of the ROS1 study, 9 ROS1-positive samples were identified by both methods. The observed tumor content in the studies ranged from 20–90%, and had the following distribution:

Table 7 Tumor content range in ROS1-positive samples used in clinical studies

ROS1-positive samples	Tumor-content range
1	20–29%
1	30–39%
0	40–49%
7	50–90%

There was no statistically significant association between tumor content and test results. The results confirm that the minimum tumor cell content in FFPE samples used as input material for the Oncomine™ Dx Target Test is greater than or equal to 20%.

Tumor content—Study II

The minimum tumor cell content that is required in FFPE samples used as input material was calculated for 71 pre-characterized FFPE clinical samples. The samples were analyzed using the updated Oncomine™ Dx Target Test RNA workflow. The tumor cell content of each specimen and region of interest was estimated before the study by an external pathology lab. The samples were analyzed with and without macrodissection.

A total of 9 ROS1 positive and 62 ROS1 negative samples were included in the study analysis. All samples gave valid results for both the Oncomine™ Dx Target Test (passing Run, RNA Control, and RNA Sample QC criteria) and the reference method test. These valid samples were used for the tumor content study analysis. The observed tumor content had the following distribution:

- 0 samples with tumor content <20%
- 18 samples with tumor content 20–30%
- 8 samples with tumor content 30–40%
- 10 samples with tumor content 40–50%
- 14 samples with tumor content 60–70%
- 21 samples with tumor content 70–90%

Table 8 Tumor content range in ROS1-positive samples used in clinical studies

ROS1-positive samples	Tumor-content range
0	<20%
1	20–30%
1	30–40%
2	40–50%
4	50–70%
3	70–90%

The corresponding 95% Clopper Pearson Exact CIs of the PPA, NPA, and OPA overlapped between tumor content levels. This shows that the ROS1 fusion detection performance of the Oncomine™ Dx Target Test was similar at all ranges of tumor content level. The tumor content level of the clinical samples had no impact on the performance of the Oncomine™ Dx Target Test.

Assay reproducibility studies

Assay reproducibility—Study I

The reproducibility and repeatability of the Oncomine™ Dx Target Test was evaluated for 30 representative variants from 18 DNA samples.

The study was designed to evaluate within-run precision performance (repeatability) and variability across sites, operators, and instrument platforms (reproducibility). Six of the 18 DNA samples were mixtures of plasmid and clinical DNA. Seven of the 12 deletion variants were represented by these plasmid blends. All other variant types were represented by clinical sample DNA.

Due to the large number of variants detected by the test and the rarity of some of the variants, a representative variant approach was used. Variants were selected in the following categories:

Table 9 Representative variant approach—Study I

Variant category	No. of plasmid blends used	No. of clinical specimens used
6-bp deletion	6	0
9-bp deletion	4	2
15-bp deletion	2	4
18-bp deletion	2	4
Simple SNV	0	8
Complex SNVs[1] and MNVs	0	6

[1] Including SNVs in di- or tri-nucleotide repeat regions and SNVs in high-GC (>60%) or low-GC (<40%) content regions

Two of the 18 DNA samples were WT at all locations, and the remaining 16 contained DNA from one or more DNA variants. Each pre-extracted DNA sample was sequenced at 4 sites by 4 operators on 2 systems at each site.

At each site, operators were grouped into 2 pairs, with each pair assigned to 2 instrument systems and responsible for testing 9 DNA samples. Samples were run in duplicate using 2 different reagent lots at 3 of the study sites and on all 3 reagent lots at one study site. The design resulted in a total of 72 test determinations per DNA sample, and all variant locations were assessed for each sample.

The reproducibility results are summarized in the following table.

Table 10 Reproducibility results—Study I

Description	Variants evaluated across the samples	Call rate excluding no calls[1]		Call rate including no calls[1]	
		Mean	Median	Mean	Median
Variant positive DNA (positive calls)	46	96.6%	97.1%	94.5%	95.8%
WT DNA (negative calls)	872	96.1%	95.0%	96.1%	95.0%

[1] Analysis includes invalid results.

Excluding no calls, the estimate of repeatability at each DNA variant location across all the samples was ≥98.8% (95% CI lower limit of ≥97.5%). The coefficient of variation (CV) across all DNA clinical variants ranged from 9.8% to 39%. The highest CVs (24.9–39.2%) were observed for the BRAF V600E variant. The higher percent CV for this sample was possibly due to poor sample quality, but the cause was not definitively determined. The CVs for the EGFR L858R variant ranged from 9.8% to 11.3%, and the CVs for the EGFR deletion variants ranged from 11.2% to 25.5%.

Assay reproducibility—Study II

An additional study was performed to evaluate the reproducibility and repeatability of the Oncomine™ Dx Target Test for 6 representative variants from 11 DNA samples and 4 RNA samples. All 11 DNA samples and 4 RNA samples were clinical sample blends. In addition, 1 WT DNA sample and 4 WT RNA samples were included in the study.

The study was designed to evaluate within-run precision performance (repeatability) and variability across sites, operators, and instrument platforms (reproducibility). The updated Oncomine™ Dx Target Test RNA workflow was used.

Due to the large number of variants detected by the test and the rarity of some variants, a representative variant approach was used. Variants were selected in the following categories:

Table 11 Representative variant approach—Study II

Variant category	No. of clinical specimens used	No. of levels tested[1]	No. of samples tested
15-bp deletion	1	2	7[2]
Simple SNV	3	2	
Complex SNV[3] and MNV	2	2	4
Fusion	2	2	4
WT RNA	2	N/A	4[4]
WT DNA	1	N/A	1

[1] The number of sample blends with different levels of allele frequency that were tested.

[2] Includes one sample that contained both a 15-bp deletion and a simple SNV, one sample that contained a 15-bp deletion, and three samples that were simple SNVs.

[3] Including SNVs in di- or tri-nucleotide repeat regions and SNVs in high-GC (>60%) or low-GC (<40%) content regions

[4] Each WT RNA sample was tested twice.

One DNA sample was WT at all locations, 1 DNA sample had 2 DNA variants, and the remaining DNA samples had 1 variant each. Four RNA samples were WT for the ROS1 fusion. Each pre-extracted DNA or RNA sample was sequenced at 3 sites by 2 operators on 2 systems at each site.

At each site, 2 operators were assigned to 2 instrument systems and were responsible for testing 12 DNA samples (11 with variants and 1 WT) and 8 RNA samples (4 with variants and 4 WT). Samples were run in duplicate using 3 different reagent lots at all study sites. The study design resulted in a total of 36 test determinations per DNA or RNA sample.

The reproducibility results are summarized in the following table.

Table 12 Reproducibility results (DNA variants)—Study II

Description	Variants evaluated across the samples	Call rate excluding no calls[1]		Call rate including no calls[1]	
		Mean	Median	Mean	Median
Variant positive DNA (positive calls)	12	99%	100%	98%	99%
WT DNA (negative calls)	367	100%	100%	99%	100%

[1] Analysis includes invalid results.

Excluding no calls, the estimate of repeatability at each DNA variant location across all the samples was ≥94.4% (95% CI lower limit of ≥72.7%). The CVs across all DNA clinical variants ranged from 9.1% to 22.6%. The CVs for the BRAF V600E variant ranged from 13.1% to 19%, the CVs for the EGFR L858R variant ranged from 11% to 17.6%, and the CVs for the EGFR deletion variants ranged from 10.1% to 15.9%.

Table 13 Reproducibility results (ROS1 fusion)—Study II

Description	Isoforms evaluated across the samples	Call rate excluding or including unknowns[1]	
		Mean	Median
ROS1 fusion-positive RNA (positive calls)	4	100%	100%
WT RNA (negative calls)	4	99%	100%

[1] Unknowns are defined as invalid or no result using the Oncomine™ Dx Target Test.

The estimate of repeatability at each RNA clinical variant location was 100%. One wild-type sample was found to be contaminated with RNA control during the study, resulting in an NPA estimate of 94.4%. No specific sequencer performed differently between three lots of Oncomine™ Dx Target Test reagents for this sample. The CV across both RNA locations ranged from 47.8% to 76.6%.

Assay reproducibility—Study III

A study was performed to evaluate the reproducibility and repeatability of the Oncomine™ Dx Target Test for detection of RET fusions using FFPE RNA from 4 RET fusion-positive samples (blended with WT clinical samples) and 2 RET fusion-negative (WT) samples.

Table 14 Sample description—Study III

RET fusion	No. of clinical specimens used	No. of levels tested	No. of samples tested
KIF5B-RET.K15R12	1	2	2
CCD6-RET.C1R12	1	2	2
RET-negative/WT RNA	2	N/A	2

The study was designed to evaluate within-run precision performance (repeatability) and variability across sites, operators, and instrument platforms (reproducibility). The updated RNA library preparation workflow was used.

Six pre-extracted RNA sample blends (4 fusion positive blends and 2 WT specimens) and placeholder DNA libraries were sequenced at 3 sites by 6 operators. At each site, 2 operators were assigned to 2 instrument systems and were responsible for testing the 6 RNA sample blends. Samples were run in duplicate using 3 different reagent lots at all study sites. The study design resulted in a total of 36 test determinations per sample blend tested.

The reproducibility results are summarized in the following table.

Table 15 Reproducibility results (RET fusion)—Study III

Description	Isoforms evaluated across the samples	Call rate excluding or including unknowns[1]	
		Mean	Median
RET fusion-positive RNA (positive calls)	4	99%	100%
WT RNA (negative calls)	2	100%	100%

[1] Unknowns are defined as invalid or no result using the Oncomine™ Dx Target Test.

Estimates of the repeatability were reported to be 100% for the CCDC6-RET.C1R12 isoform, and 98.1% for the KIF5B-RET.K15R12 isoform. The CV across both RNA locations ranged from 46.8% to 62.7%.

Panel accuracy study

To evaluate the ability of the Oncomine™ Dx Target Test DNA and RNA panels to identify somatic variants in human specimens, 290 FFPE tumor samples were analyzed using the Oncomine™ Dx Target Test to demonstrate positive percent agreement (PPA) and negative percent agreement (NPA) concordance with validated reference detection methods.

The following reference detection methods were used:

- A validated NGS assay, to detect SNV and deletion hotspot variants
- A ROS1 FISH reference test, to detect ROS1 fusions

Variants detected by the Oncomine™ Dx Target Test that were not covered by the reference methods were not included in the PPA/NPA concordance calculation. Variants detected by the Oncomine™ test for which the reference method testing failed and did not yield a valid result were not included in the PPA/NPA calculation.

Accuracy data was analyzed by the following:

- Each variant location
- Bins (or categories) of variants: RNA fusions, simple SNVs, complex SNVs, and deletions
- Each FFPE sample

The results are shown in the following tables.

Table 16 PPA results

PPA measure	Excluding no calls		Including no calls	
	Percent agreement	95% CI	Percent agreement	95% CI
Variant	98.5% (195/198)	(95.6%, 99.7%)	98.5% (195/198)	(95.6%, 99.7%)
Bin	97.2% (176/181)	(93.7%, 99.1%)	97.2% (176/181)	(93.7%, 99.1%)
Sample	96.9% (158/163)	(93.0%, 99.0%)	96.9% (158/163)	(93.0%, 99.0%)

Table 17 NPA results

NPA measure	Excluding no calls		Including no calls	
	Percent agreement	95% CI	Percent agreement	95% CI
Variant	100.0% (118,155/118,159)	(99.99%,100.0%)	96.8% (118,155/122,012)	(96.7%, 96.9%)
Bin	99.8% (942/944)	(99.2%, 100.0%)	70.0% (657/939)	(66.9%, 72.9%)
Sample	98.4% (124/126)	(94.4%, 99.8%)	23.4% (29/124)	(16.3%, 31.8%)

Table 18 OPA results

OPA measure	Excluding no calls		Including no calls	
	Percent agreement	95% CI	Percent agreement	95% CI
Variant	100.0% (118,350/118,357)	(99.99%, 100.0%)	96.8% (118,350/122,210)	(96.74%, 96.94%)
Bin	99.4% (1,118/1,125)	(98.72%,99.75%)	74.4% (833/1,120)	(71.71%, 76.91%)
Sample	97.6% (282/289)	(95.07%,99.02%)	65.2% (187/287)	(59.34%, 70.66%)

Clinical studies

BRAF clinical study

BRAF study—concordance evaluation

A method comparison evaluated the accuracy of the Oncomine™ Dx Target Test compared to the NSCLC BRAF V600E PCR Assay for the detection of the BRAF V600E mutation in NSCLC samples. Patient samples from the NSCLC BRF113928 clinical trial and an acquired set of negative samples were measured by both assays.

There were a total of 230 samples available for analysis. Of these, 181 samples (67 + 114) provided valid results for both the BRAF V600E PCR assay and the Oncomine™ test. All valid results correlated. Of the remaining samples, 27 samples had invalid results with the Oncomine™ test due to failed control or library QC metrics for the sequencing runs, 9 samples had no calls due to insufficient coverage at the BRAF variant location, and 13 samples were not tested due to insufficient DNA concentration.

The PPA was defined as the proportion of BRAF-positive samples called by the BRAF V600E PCR Assay that were also called by the Oncomine™ Dx Target Test, and the NPA was defined as the proportion of BRAF-negative samples called by the PCR assay that were also identified by the Oncomine™ Dx Target Test. The 95% two-sided exact CIs were determined for PPA, NPA and OPA, and the results are shown in the following table:

Table 19 Concordance between the NSCLC BRAF V600E PCR Assay and the Oncomine™ Dx Target Test

Agreement measure	Excluding no calls		Including no calls	
	Percent agreement	95% CI[1]	Percent agreement	95% CI[1]
PPA	100.0% (67/67)	(94.6%, 100.0%)	91.8%(67/73)	(83.0%, 96.9%)
NPA	100% (114/114)	(96.7%, 100.0%)	97.4%(114/117)	(92.7%, 99.5%)
OPA	100.0% (181/181)	(97.9%, 100.0%)	95.3%(181/190)	(91.2%, 97.8%)

[1] The 95% CI was calculated using the Pearson-Clopper Exact method.

BRAF study—clinical effectiveness

The clinical effectiveness of the Oncomine™ Dx Target Test was evaluated by measuring the objective response rate (ORR) for patients with stage IV NSCLC who tested positive for the BRAF V600E mutation by both the Local Laboratory Tests (LLTs) and the Oncomine™ test. The ORR was calculated for patients in two cohorts (B and C) who were selected for treatment with TAFINLAR® (dabrafenib) administered in combination with MEKINIST® (trametinib).

The ORR for Cohort B was 68.2% (15/22), which is similar to the 63.2% ORR (36/57) observed in the overall population tested as positive by LLTs. The ORR for Cohort C was 60.9% (14/23), which is similar to the 61.1% ORR (22/36) observed in the overall population tested as positive by LLT.

A secondary objective of the bridging study was to determine the clinical effectiveness of the Oncomine™ Dx Target Test in selecting NSCLC patients for treatment with dabrafenib administered as a single agent and in combination with trametinib by evaluating progression-free survival (PFS), duration of response (DoR), and overall survival (OS) by both investigator assessment and independent review.

For the 15 Cohort B patients with a confirmed tumor response based on independent assessment, the median DoR was not estimable, with an event rate less than 50%. The median DoR for the overall LLT(+) population was 12.6 months. PFS was similar between the Oncomine™ Dx Target Test(+)/LLT(+) population (N = 22) and the total LLT(+) population (N = 57) for both independent and investigator review. Also, the ORR observed by independent assessment was similar to that observed by investigator assessment. The median follow-up time for Cohort B was 16.6 months.

For the 14 Cohort C patients with a confirmed tumor response based on independent assessment, the median DoR was not estimable with an event rate less than 50%. The median DoR for the overall LLT(+) was also not estimable with an event rate less than 50%. PFS was similar between the Oncomine™ Dx Target Test(+)/LLT(+) population (N = 23) and the total LLT(+) population (N = 36) for both independent and investigator review. Also, the ORR observed by independent assessment was similar to that observed by investigator assessment. The median follow-up time for Cohort C was 10.4 months.

Refer to the **Drugs@FDA database** for the most recent therapeutic product labeling.

EGFR clinical study

To evaluate the ability of the Oncomine™ Dx Target Test to identify the EGFR biomarker in FFPE NSCLC tumor specimens, 92 specimens from patients that tested positive using the Qiagen *therascreen* EGFR RGQ PCR Kit were analyzed using the Oncomine™ Dx Target Test. In addition, 142 specimens that tested negative using the Qiagen EGFR PCR assay were analyzed using the Oncomine™ Dx Target Test.

Of the EGFR-positive samples, 72 generated valid results from both the Qiagen EGFR PCR assay and the Oncomine™ Dx Target Test. Twenty samples had invalid results due to failed control or library QC metrics for the sequencing runs, or generated no calls due to insufficient coverage.

Of the EGFR-negative samples, 121 generated valid results from both the Qiagen assay and the Oncomine™ test, while 12 had invalid results due to failed QC metrics for the sequencing runs or generated no calls due to insufficient coverage.

In all, 193 samples were used to evaluate concordance between the Oncomine™ test as an investigational method and the Qiagen EGFR PCR assay as the reference method. A total of 70 samples were excluded, and 32 samples were invalid or generated no calls.

The PPA was defined as the proportion of EGFR-positive specimens as called by the EGFR PCR assay that were also EGFR-positive as called by the Oncomine™ Dx Target Test, and the NPA was defined as the proportion of EGFR-negative specimens as called by the EGFR PCR assay that were also EGFR-negative as called by the Oncomine™ test. The concordances by variant and overall concordance are shown in the following tables:

Table 20 Exon 19 deletion—Concordance

Agreement measure	Excluding no calls		Including no calls	
	Percent agreement	95% CI	Percent agreement	95% CI
PPA	97.6% (41/42)	(87.43%, 99.94%)	74.6% (41/55)	(61.00%, 85.33%)
NPA	99.3% (147/148)	(96.29%, 99.98%)	94.2% (147/156)	(89.33%, 97.33%)
OPA	99.0% (188/190) [1]	(96.25%, 99.87%)	89.1% (188/211)	(84.09%, 92.96%)

[1] Two samples were found to be discordant in this analysis, where one was called a false negative and the other a false positive with the Oncomine™ test.

Table 21 EGFR L858R—Concordance

Agreement measure	Excluding no calls		Including no calls	
	Percent agreement	95% CI	Percent agreement	95% CI
PPA	100% (30/30)	(88.43%, 100%)	93.8% (30/32)	(79.19%, 99.23%)
NPA	100% (167/167)	(97.82%, 100%)	93.3% (167/179)	(88.58%, 96.49%)
OPA	100% (197/197)	(98.14%, 100%)	93.4% (197/211)	(89.12%, 96.33%)

Table 22 Overall concordance

Agreement measure	Excluding no calls		Including no calls	
	Percent agreement	95% CI	Percent agreement	95% CI
PPA	98.6% (71/72)	(92.5%, 100.0%)	81.6% (71/87)	(71.86% , 89.11%)
NPA	99.2% (120/121)	(95.5%, 100.0%)	96.8% (120/124)	(91.95% , 99.11%)
OPA	99.0% (191/193)	(96.31%, 99.87%)	90.5% (191/211)	(85.74% , 94.11%)

RET clinical study

RET study—concordance evaluation

To evaluate the ability of the Oncomine™ Dx Target Test RNA panel to identify RET fusions in human specimens, 238 FFPE tumor samples were analyzed using the Oncomine™ Dx Target Test to demonstrate positive percent agreement (PPA) and negative percent agreement (NPA) with a validated reference detection method (Archer assay).

Of the 238 samples were evaluated in the study, 133 were identified by the enrolling LLTs as RET fusion-positive in the BLU-667-1101 trial, and 105 were stage-matched commercially sourced NSCLC samples, screened with either a local laboratory-validated RET FISH assay, or an NGS-based screening assay. After screening 105 NSCLC samples, 2 were identified as RET fusion-positive by the LLTs and were grouped with the clinical trial samples, resulting in 105 LLT-positive and 103 LLT-negative samples.

Of the 135 RET fusion-positive samples by the LLTs, 54 were cancelled before sequencing by Oncomine™ Dx Target Test due to failure to meet test input requirements—22 samples had insufficient tissue available,1 sample had an insufficient number of slides, 8 samples did not meet the tumor content requirement, and 23 samples failed the RNA concentration cutoff. Of the remaining 81 samples, 56 were positive by the Oncomine™ Dx Target Test, 24 samples were called negative, and 1 sample yielded an invalid result.

Of the 103 RET fusion-negative samples (by LLT) available for testing, 102 samples were called negative by the Oncomine™ Dx Target Test, and 1 sample yielded an invalid result.

For testing with the Archer assay, of the 135 RET fusion-positive samples by the LLTs, in addition to the 54 cancelled for the Oncomine™ Dx Target Test, 11 more samples were cancelled due to insufficient RNA concentration, and 4 samples were not tested on the Archer Assay. Of the remaining 66 samples, 43 were positive by the Archer assay, 17 samples were called negative, and 6 samples yielded an

invalid result. Of the 103 RET fusion-negative samples (by LLT), 1 sample had insufficient RNA volume for testing, 93 were negative, 1 was positive, and 8 were invalid by the Archer assay.

The PPA was defined as the proportion of RET fusion-positive specimens as called by the Archer assay that were also RET fusion-positive as called by the Oncomine™ Dx Target Test, and the NPA was defined as the proportion of RET fusion-negative specimens as called by the Archer assay that were also RET fusion-negative as called by the Oncomine™ Dx Target Test. Concordance between the Oncomine™ Dx Target Test and the Archer assay is shown in Table 23.

Table 23 Concordance between the Archer assay and the Oncomine™ Dx Target Test

Agreement measure	Excluding unknowns		Including unknowns[1]	
	Percent agreement	95% CI	Percent agreement	95% CI
PPA	90.9% (40/44)	(78.3%, 97.5%)	90.9% (40/44)	(78.3%, 97.5%)
NPA	91.8% (101/110)	(85.0%, 96.2%)	91.8% (101/110)	(85.0%, 96.2%)
OPA	91.6% (141/154) [2]	(86.0%, 95.4%)	91.6% (141/154)	(86.0%, 95.4%)

[1] The results including and excluding unknowns were identical. Unknowns are defined as invalid or no result using the Oncomine™ Dx Target Test.

[2] Thirteen samples were found to be discordant in this analysis, where four were false negatives and nine were false positives with the Oncomine™ Dx Target Test.

RET study—clinical effectiveness

The clinical effectiveness of the Oncomine™ Dx Target Test was evaluated by measuring the overall response rate (ORR) for patients with NSCLC that tested positive for a RET fusion by both the LLTs and the Oncomine™ Dx Target Test. The ORR was calculated for patients selected for treatment with the RET inhibitor pralsetinib, with prior platinum treatment, and with no prior systemic treatment. The disease control rate (DCR) and clinical benefit rate (CBR) were also calculated for these patients.

In the efficacy population, the ORR for the NSCLC patients with prior platinum treatment was 70.6% (95% CI: 52.5, 84.9). The ORR for NSCLC patients with no prior systemic treatment was 85.7% (95% CI:57.2, 98.2).

In the efficacy population, the DCR was 100.0% (95% CI: 91.6, 100.0) for patients with prior platinum treatment, with 34 patients having complete response (CR), partial response (PR), or stable disease (SD). The DCR was 92.9% (95% CI: 66.1, 99.8) for patients with no prior systemic treatment, with 13 patients having complete response (CR), partial response (PR), or stable disease (SD).

In the efficacy population, the CBR was 82.4% for patients with prior platinum treatment, with 28 patients having CR, PR, or SD for at least 16 weeks with 28 days in a cycle. The CBR was 85.7% for patients with no prior systemic treatment, with 12 patients having CR, PR, or SD for at least 16 weeks with 28 days in a cycle.

Refer to the **Drugs@FDA database** for the most recent therapeutic product labeling.

ROS1 clinical study

ROS1 concordance evaluation—Study I

To evaluate the ability of the Oncomine™ Dx Target Test to identify the ROS1 biomarker in NSCLC tumor samples, 19 specimens from patients in the Phase 1 Pfizer Study A8081001 that tested positive using a ROS1 FISH assay were used, together with 13 archival specimens that also tested positive. These 32 positive specimens and 126 archival specimens that tested negative using the ROS1 FISH assay were analyzed using the Oncomine™ Dx Target Test.

Of the ROS1-positive samples, 25 generated valid results from both the FISH assay and the Oncomine™ Dx Target Test. Of the remaining samples, 4 generated invalid sequencing results due to a control or library QC failure, 2 generated insufficient material for FISH assay analysis, and 1 was subsequently determined to be a false positive for ROS1.

Of the ROS1-negative samples, 119 generated valid results from both the FISH assay and the test, while 7 generated invalid sequencing results due to a control or library QC failure. A total of 144 samples were used to evaluate concordance between the assay and the test. Of these, 139 were FFPE specimens and 5 were extracted RNA samples.

The PPA was defined as the proportion of ROS1-positive specimens called by the ROS1 FISH assay that were also called by the Oncomine™ Dx Target Test, and the NPA was defined as the proportion of ROS1-negative specimens called by the ROS1 FISH assay that were also called by the test. The OPA was defined as the number of calls where the ROS1 FISH assay and the Oncomine™ Dx Target Test agreed, divided by the total number of calls made. The 95% CIs were determined for PPA, NPA, and OPA, and the results are shown in the following table:

Table 24 Concordance between the ROS1 FISH assay and the Oncomine™ Dx Target Test

Agreement measure	Percent agreement	95% CI
PPA	80.0% (20/25)	59.3%, 93.2%
NPA	100% (119/119)	96.9%, 100%
OPA	96.5% (139/144)	92.08%,98.86%

Of the 20 concordant ROS1-positive samples, 17 were from FFPE tissue samples and 3 were from RNA extracts. For FFPE specimens alone, excluding invalids, the PPA was 85.0% (17/20) and the NPA was 100% (119/119). For the extracted RNA specimens alone, excluding invalids, the PPA was 60.0% (3/5) and the NPA was not evaluable because all specimens were ROS1 positive. The results from the 5 RNA extraction specimens should be interpreted with caution due to the limited sample size.

All 5 discordant samples were positive for the FISH assay and negative for the test. Three of these also tested negative using a probe hybridization fusion detection method.

ROS1 concordance evaluation—Study II

An additional concordance study was performed using the updated Oncomine™ Dx Target Test RNA library preparation workflow, to verify that the protocol changes did not impact the effectiveness of the test. In this study, results from the updated test workflow were compared to results from the Kreatech ROS1 FISH assay and to results from the original test workflow.

Of the ROS1-positive samples, 9 generated valid results (excluding unknowns) from both the ROS1 FISH assay and the updated Oncomine™ Dx Target Test workflow. Of the ROS1-negative samples, 62 generated valid results from both, for a total of 71 samples with valid results used to evaluate concordance between the test as an investigational method and the ROS1 FISH assay as the reference method.

Of the ROS1-positive samples, 7 generated valid results (excluding unknowns) from both the ROS1 FISH assay and the original Oncomine™ Dx Target Test workflow. Of the ROS1-negative samples, 59 generated valid results from both, for a total of 67 samples with valid results used to evaluate concordance between the test as an investigational method and the ROS1 FISH assay as the reference method.

Table 25 Overall concordance between the ROS1 FISH assay replicates and the updated Oncomine™ Dx Target Test RNA library preparation workflow

Agreement measure	Excluding unknowns[1]		Including unknowns	
	Percent agreement	95% CI	Percent agreement	95% CI
PPA	100% (9/9)	66.4%, 100.0%	90% (9/10)	55.5%, 99.8%
NPA	100% (62/62)	94.2%, 100.0%	89% (62/70)	78.7%, 94.9%
OPA	100% (71/71)	94.9%, 100.0%	89% (71/80)	79.7%, 94.7%

[1] Unknowns are defined as invalid or no result using the Oncomine™ Dx Target Test

Excluding unknowns, the updated Oncomine™ Dx Target Test workflow showed a 100% PPA, NPA, and OPA with the ROS1 FISH assay.

Table 26 Overall concordance between the updated Oncomine™ Dx Target Test vs original Oncomine™ Dx Target Test workflow

Agreement measure	Excluding unknowns[1]		Including unknowns	
	Percent agreement	95% CI	Percent agreement	95% CI
PPA	100%	71.5%, 100.0%	100%	71.5%, 100.0%
NPA	98%	92.0%, 99.7%	98%	92.0%, 99.7%
OPA	98%	92.9%, 99.8%	98%	92.9%, 99.8%

[1] Unknowns are defined as invalid or no result using the Oncomine™ Dx Target Test

Excluding unknowns, the updated Oncomine™ Dx Target Test workflow showed a 100% PPA, 98% NPA, and 98% OPA agreement with the original Oncomine™ Dx Target Test workflow.

ROS1 study—clinical outcomes evaluation

As part of the Study I concordance evaluation described above, clinical outcomes as measured by objective response rate (ORR) and duration of response (DOR) with XALKORI® (crizotinib) were evaluated for 11 patients whose tumors were designated as ROS1-positive by the ROS1 FISH assay and whose tumors were evaluable by the Oncomine™ Dx Target Test. Of these, 6 samples tested positive by both tests.

The ORR for patients with tumor specimens determined to be ROS1-positive using both tests was 83.3% (5/6) (95% CI: 35.88%, 99.58%).

The mean DOR (N=5) was 17.5 months (95% CI: 10.9, 24.1).

Refer to the **Drugs@FDA database** for the most recent therapeutic product labeling.

B Variants detected by the Oncomine™ Dx Target Test

DNA variants detected by the Oncomine™ Dx Target Test

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
AKT1	3	p.Glu17Lys	c.49G>A	COSM33765
ALK	25	p.Arg1275Leu	c.3824G>T	COSM28060
ALK	25	p.Arg1275Gln	c.3824G>A	COSM28056
ALK	24	p.Phe1245Leu	c.3735C>G	COSM28062
ALK	24	p.Phe1245Leu	c.3735C>A	COSM28493
ALK	24	p.Phe1245Cys	c.3734T>G	COSM28500
ALK	24	p.Phe1245Val	c.3733T>G	COSM28499
ALK	24	p.Phe1245Ile	c.3733T>A	COSM28492
ALK	23	p.Ser1206Tyr	c.3617C>A	COSM144251
ALK	23	p.Gly1202Arg	c.3604G>A	COSM144250
ALK	23	p.Leu1196Gln	c.3587T>A	COSM1169447
ALK	23	p.Leu1196Met	c.3586C>A	COSM99137
ALK	23	p.Val1180Leu	c.3538G>C	COSM4381101
ALK	23	p.Phe1174Leu	c.3522C>G	COSM28061
ALK	23	p.Phe1174Leu	c.3522C>A	COSM28055
ALK	23	p.Phe1174Cys	c.3521T>G	COSM28059
ALK	23	p.Phe1174Ser	c.3521T>C	COSM53063
ALK	23	p.Phe1174Val	c.3520T>G	COSM28054
ALK	23	p.Phe1174Leu	c.3520T>C	COSM28057
ALK	23	p.Phe1174Ile	c.3520T>A	COSM28491

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
ALK	22	p.Ile1171Thr	c.3512T>C	COSM4381100 **Note:** Some "no calls" were observed for this analytical variant due to strand bias with plasmid targets. This does not impact clinical test results.
ALK	22	p.Ile1171Asn	c.3512T>A	COSM28498
ALK	22	p.Cys1156Tyr	c.3467G>A	COSM99136 **Note:** Some "no calls" were observed for this analytical variant due to strand bias with plasmid targets. This does not impact clinical test results.
ALK	22	p.Leu1152Arg	c.3455T>G	COSM97185
ALK	22	p.Leu1152Pro	c.3455T>C	COSM1407659
ALK	21	p.Gly1128Ala	c.3383G>C	COSM98475
BRAF	15	p.Val600_Lys601delinsGlu	c.1799_1801delTGA	COSM1133
BRAF	15	p.Lys601Glu	c.1801A>G	COSM478
BRAF	15	p.Val600Glu	c.1799T>A	COSM476
BRAF	15	p.Val600Glu	c.1799_1800delTGinsAA	COSM475
BRAF	15	p.Val600Arg	c.1798_1799delGTinsAG	COSM474
BRAF	15	p.Val600Lys	c.1798_1799delGTinsAA	COSM473
BRAF	15	p.Asp594Gly	c.1781A>G	COSM467
BRAF	15	p.Asp594Asn	c.1780G>A	COSM27639
BRAF	11	p.Gly469Val	c.1406G>T	COSM459
BRAF	11	p.Gly469Ala	c.1406G>C	COSM460
BRAF	11	p.Gly469Arg	c.1405G>A	COSM457
BRAF	11	p.Gly466Val	c.1397G>T	COSM451
BRAF	11	p.Gly466Glu	c.1397G>A	COSM453
CDK4	2	p.Arg24Leu	c.71G>T	COSM363684

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
CDK4	2	p.Arg24His	c.71G>A	COSM1989836
CDK4	2	p.Arg24Cys	c.70C>T	COSM1677139
CDK4	2	p.Arg24Ser	c.70C>A	COSM3463914
CDK4	2	p.Lys22Met	c.65A>T	COSM3463915
CDK4	2	p.Lys22Arg	c.65A>G	COSM232013
CDK4	2	p.Lys22Gln	c.64A>C	OM3153
DDR2	5	p.Arg124Trp	c.370C>T	COSM4024594
DDR2	5	p.Arg124Leu	c.371G>T	COSM400880
EGFR	3	p.Arg108Gly	c.322A>G	COSM1451536
EGFR	3	p.Arg108Lys	c.323G>A	COSM21683
EGFR	7	p.Ala289Thr	c.865G>A	COSM21686
EGFR	7	p.Ala289Asp	c.866C>A	COSM21685
EGFR	7	p.Ala289Val	c.866C>T	COSM21687
EGFR	12	p.Ser492Arg	c.1474A>C	COSM236671
EGFR	12	p.Ser492Arg	c.1476C>A	COSM236670
EGFR	15	p.Gly598Ala	c.1793G>C	COSM3412196
EGFR	15	p.Gly598Val	c.1793G>T	COSM21690
EGFR	18	p.Glu709Lys	c.2125G>A	COSM12988
EGFR	18	p.Glu709Ala	c.2126A>C	COSM13427
EGFR	18	p.Glu709Gly	c.2126A>G	COSM13009
EGFR	18	p.Glu709Val	c.2126A>T	COSM12371
EGFR	18	p.Gly719Ser	c.2155G>A	COSM6252
EGFR	18	p.Gly719Cys	c.2155G>T	COSM6253
EGFR	18	p.Gly719Asp	c.2156G>A	COSM18425
EGFR	18	p.Gly719Ala	c.2156G>C	COSM6239
EGFR	19	p.Lys745_Glu749del	c.2233_2247delAAGGAATTAAGAGAA	COSM26038
EGFR	19	p.Lys745_Ala750delinsThr	c.2234_2248delAGGAATTAAGAGAAG	COSM1190791

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
EGFR	19	p.Glu746_Glu749del	c.2235_2246delGGAATTAAGAGA	COSM28517
EGFR	19	p.Glu746_Ala750del	c.2235_2249delGGAATTAAGAGAAGC	COSM6223
EGFR	19	p.Glu746_Thr751delinsIle	c.2235_2252delGGAATTAAGAGAAGCAACinsAAT	COSM13551 **Note:** The nucleotide change of COSM13551 overlaps that of COSM6223, so a positive COSM13551 sample will also result in a positive call for COSM6223.
EGFR	19	p.Glu746_Ala750del	c.2236_2250delGAATTAAGAGAAGCA	COSM6225
EGFR	19	p.Glu746_Arg748del	c.2239_2247delTTAAGAGAA	COSM6218
EGFR	19	p.Glu746_Thr751del	c.2236_2253delGAATTAAGAGAAGCAACA	COSM12728
EGFR	19	p.Leu747_Ala750delinsPro	c.2239_2248delTTAAGAGAAGinsC	COSM12382
EGFR	19	p.Leu747_Thr751delinsPro	c.2239_2251delTTAAGAGAAGCAAinsC	COSM12383
EGFR	19	p.Glu746_Thr751delinsAla	c.2237_2251delAATTAAGAGAAGCAA	COSM12678 **Note:** A false negative call was observed for this variant when tested with plasmid targets for 1 out of 4 of the replicates tested.
EGFR	19	p.Leu747_Thr751del	c.2240_2254delTAAGAGAAGCAACAT	COSM12369
EGFR	19	p.Glu746_Ser752delinsAsp	c.2238_2255delATTAAGAGAAGCAACATC	COSM6220
EGFR	19	p.Glu746_Ser752delinsVal	c.2237_2255delAATTAAGAGAAGCAACATCinsT	COSM12384
EGFR	19	p.Glu746_Thr751delinsValAla	c.2237_2253delAATTAAGAGAAGCAACAinsTTGCT	COSM12416
EGFR	19	p.Leu747_Ser752del	c.2239_2256delTTAAGAGAAGCAACATCT	COSM6255

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
EGFR	19	p.Leu747_Thr751delinsGln	c.2238_2252delATTAAGAGAAGCAACinsGCA	COSM12419
EGFR	19	p.Leu747_Ala750delinsPro	c.2238_2248delATTAAGAGAAGinsGC	COSM12422
EGFR	19	p.Leu747_Pro753delinsGln	c.2239_2258delTTAAGAGAAGCAACATCTCCinsCA	COSM12387 **Note:** The nucleotide change of COSM12387 overlaps that of COSM6255, so a positive COSM12387 sample will also result in a positive call for COSM6255.
EGFR	19	p.Leu747_Thr751delinsSer	c.2240_2251delTAAGAGAAGCAA	COSM6210
EGFR	19	p.Leu747_Pro753delinsSer	c.2240_2257delTAAGAGAAGCAACATCTC	COSM12370
EGFR	20	p.Ser768Ile	c.2303G>T	COSM6241
EGFR	20	p.Cys797Ser	c.2389T>A	COSM6493937
EGFR	20	p.Cys797Ser	c.2390G>C	COSM5945664
EGFR	21	p.Leu858Met	c.2572C>A	COSM12366
EGFR	21	p.Leu858Arg	c.2573T>G	COSM6224
EGFR	21	p.Leu861Gln	c.2582T>A	COSM6213
EGFR	21	p.Leu861Arg	c.2582T>G	COSM12374
ERBB2	8	p.Ser310Tyr	c.929C>A	COSM94225
ERBB2	8	p.Ser310Phe	c.929C>T	COSM48358
ERBB2	17	p.Arg678Gln	c.2033G>A	COSM436498
ERBB2	18	p.Thr733Ile	c.2198C>T	COSM14059
ERBB2	19	p.Leu755Met	c.2263T>A	COSM1205571
ERBB2	19	p.Leu755Pro	c.2263_2264delTTinsCC	COSM683
ERBB2	19	p.Asp769His	c.2305G>C	COSM13170
ERBB2	19	p.Asp769Tyr	c.2305G>T	COSM1251412
ERBB2	20	p.Gly776Val	c.2327G>T	COSM18609
ERBB2	20	p.Val777Leu	c.2329G>T	COSM14062

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
ERBB2	21	p.Val842Ile	c.2524G>A	COSM14065
ERBB2	22	p.Arg896Cys	c.2686C>T	COSM14066
ERBB2	22	p.Arg896His	c.2687G>A	COSM119971
ERBB3	2	p.Met60Leu	c.178A>T	COSM1606366
ERBB3	2	p.Met60Lys	c.179T>A	COSM254678
ERBB3	2	p.Met60Arg	c.179T>G	COSM941484
ERBB3	3	p.Met91Ile	c.273G>A	COSM122890
ERBB3	3	p.Met91Ile	c.273G>C	COSM1299636
ERBB3	3	p.Val104Met	c.310G>A	COSM172423
ERBB3	3	p.Val104Leu	c.310G>C	COSM160824
ERBB3	3	p.Val104Leu	c.310G>T	COSM191840
ERBB3	6	p.Ala232Thr	c.694G>A	COSM4043440
ERBB3	6	p.Ala232Val	c.695C>T	COSM1242239
ERBB3	8	p.Asp297Tyr	c.889G>T	COSM160822
ERBB3	8	p.Asp297Val	c.890A>T	COSM941490
ERBB3	9	p.Glu332Lys	c.994G>A	COSM254677
FGFR2	14	p.Lys659Asn	c.1977G>T	COSM49173
FGFR2	14	p.Lys659Asn	c.1977G>C	COSM683054
FGFR2	14	p.Lys659Met	c.1976A>T	COSM49175
FGFR2	14	p.Lys659Glu	c.1975A>G	COSM36909
FGFR2	12	p.Asn549Lys	c.1647T>G	COSM36902
FGFR2	12	p.Asn549Lys	c.1647T>A	COSM36912
FGFR2	12	p.Asn549Ser	c.1646A>G	COSM3665553
FGFR2	12	p.Asn549His	c.1645A>C	COSM250083
FGFR2	9	p.Cys382Tyr	c.1145G>A	COSM915493
FGFR2	9	p.Cys382Arg	c.1144T>C	COSM36906
FGFR2	9	p.Tyr375Cys	c.1124A>G	COSM36904
FGFR2	9	p.Tyr375His	c.1123T>C	COSM1560916

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
FGFR2	8	p.Ala314Asp	c.941C>A	COSM49171
FGFR2	7	p.Pro253Leu	c.758C>T	COSM537801
FGFR2	7	p.Pro253Arg	c.758C>G	COSM49170
FGFR2	7	p.Ser252Trp	c.755C>G	COSM36903
FGFR3	7	p.Arg248Cys	c.742C>T	COSM714
FGFR3	7	p.Ser249Cys	c.746C>G	COSM715
FGFR3	14	p.Lys650Gln	c.1948A>C	COSM726
FGFR3	14	p.Lys650Glu	c.1948A>G	COSM719
FGFR3	14	p.Lys650Asn	c.1950G>T	COSM1428730
FGFR3	16	p.Gly697Cys	c.2089G>T	COSM24802
HRAS	3	p.Gln61His	c.183G>T	COSM502
HRAS	3	p.Gln61His	c.183G>C	COSM503
HRAS	3	p.Gln61Leu	c.182A>T	COSM498
HRAS	3	p.Gln61Arg	c.182A>G	COSM499
HRAS	3	p.Gln61Pro	c.182A>C	COSM500
HRAS	3	p.Gln61Lys	c.181C>A	COSM496
HRAS	2	p.Gly13Val	c.38G>T	COSM489
HRAS	2	p.Gly13Asp	c.38G>A	COSM490
HRAS	2	p.Gly13Cys	c.37G>T	COSM488
HRAS	2	p.Gly13Arg	c.37G>C	COSM486
HRAS	2	p.Gly13Ser	c.37G>A	COSM487
HRAS	2	p.Gly12Val	c.35G>T	COSM483
HRAS	2	p.Gly12Ala	c.35G>C	COSM485
HRAS	2	p.Gly12Asp	c.35G>A	COSM484
HRAS	2	p.Gly12Cys	c.34G>T	COSM481
HRAS	2	p.Gly12Arg	c.34G>C	COSM482
HRAS	2	p.Gly12Ser	c.34G>A	COSM480
KIT	8	p.Asp419del	c.1255_1257delGAC	COSM29014

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
KIT	8	p.Asp419_Arg420del	c.1255_1260delGACAGG	COSM1578132
KIT	11	p.Trp557_Lys558del	c.1669_1674delTGGAAG	COSM1217
KIT	11	p.Trp557Arg	c.1669T>A	COSM1216
KIT	11	p.Trp557Arg	c.1669T>C	COSM1219
KIT	11	p.Trp557Gly	c.1669T>G	COSM1221
KIT	11	p.Trp557_Val559delinsPhe	c.1670_1675delGGAAGG	COSM1226
KIT	11	p.Val559del	c.1679_1681delTTG	COSM1247
KIT	11	p.Val559Asp	c.1676T>A	COSM1252
KIT	11	p.Val559Ala	c.1676T>C	COSM1255
KIT	11	p.Val559Gly	c.1676T>G	COSM1253
KIT	11	p.Val560Asp	c.1679T>A	COSM1257
KIT	11	p.Leu576Pro	c.1727T>C	COSM1290
KIT	11	p.Asp579del	c.1735_1737delGAT	COSM1294
KIT	13	p.Lys642Glu	c.1924A>G	COSM1304
KIT	13	p.Val654Ala	c.1961T>C	COSM12706
KIT	17	p.Arg796Lys	c.2387G>A	COSM1600411
KIT	17	p.Asp816His	c.2446G>C	COSM1311
KIT	17	p.Asp816Tyr	c.2446G>T	COSM1310
KIT	17	p.Asp816Val	c.2447A>T	COSM1314
KIT	17	p.Asn822Lys	c.2466T>A	COSM1321
KIT	17	p.Asn822Lys	c.2466T>G	COSM1322
KIT	17	p.Val825Ala	c.2474T>C	COSM1323
KRAS	4	p.Ala146Val	c.437C>T	COSM19900
KRAS	4	p.Ala146Pro	c.436G>C	COSM19905
KRAS	4	p.Ala146Thr	c.436G>A	COSM19404
KRAS	4	p.Lys117Asn	c.351A>T	COSM28519
KRAS	4	p.Lys117Asn	c.351A>C	COSM19940
KRAS	3	p.Gln61His	c.183A>T	COSM555

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
KRAS	3	p.Gln61His	c.183A>C	COSM554
KRAS	3	p.Gln61Leu	c.182A>T	COSM553
KRAS	3	p.Gln61Arg	c.182A>G	COSM552
KRAS	3	p.Gln61Pro	c.182A>C	COSM551
KRAS	3	p.Gln61Glu	c.181C>G	COSM550
KRAS	3	p.Gln61Lys	c.181C>A	COSM549
KRAS	3	p.Gln61Lys	c.180_181delTCinsAA	COSM87298
KRAS	3	p.Ala59Gly	c.176C>G	COSM28518
KRAS	3	p.Ala59Glu	c.176C>A	COSM547
KRAS	3	p.Ala59Thr	c.175G>A	COSM546
KRAS	2	p.Gly13Asp	c.38_39delGCinsAT	COSM531 **Note:** The nucleotide change of COSM531 overlaps that of COSM532, so a positive COSM531 sample will also result in a positive call for COSM532.
KRAS	2	p.Gly13Val	c.38G>T	COSM534
KRAS	2	p.Gly13Ala	c.38G>C	COSM533
KRAS	2	p.Gly13Asp	c.38G>A	COSM532
KRAS	2	p.Gly13Cys	c.37G>T	COSM527
KRAS	2	p.Gly13Arg	c.37G>C	COSM529
KRAS	2	p.Gly13Ser	c.37G>A	COSM528
KRAS	2	p.Gly12Phe	c.34_35delGGinsTT	COSM512 **Note:** The nucleotide change of COSM512 overlaps that of COSM516, so a positive COSM512 sample will also result in a positive call for COSM516.
KRAS	2	p.Gly12Val	c.35G>T	COSM520
KRAS	2	p.Gly12Ala	c.35G>C	COSM522

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
KRAS	2	p.Gly12Asp	c.35G>A	COSM521
KRAS	2	p.Gly12Cys	c.34G>T	COSM516
KRAS	2	p.Gly12Arg	c.34G>C	COSM518
KRAS	2	p.Gly12Ser	c.34G>A	COSM517
MAP2K1	2	p.Phe53Ile	c.157T>A	COSM3503329
MAP2K1	2	p.Phe53Leu	c.157T>C	COSM555604
MAP2K1	2	p.Phe53Val	c.157T>G	COSM1562837 **Note:** The base change c.157T>G in MAP2K1 is associated with Mutation ID COSM5077832 in the COSMIC v.76 database, even though it has been given the Variant HotSpot ID COSM1562837 in the software. This does not impact the test results.
MAP2K1	2	p.Phe53Leu	c.159T>A	COSM1725008
MAP2K1	2	p.Phe53Leu	c.159T>G	COSM2257208
MAP2K1	2	p.Lys57Thr	c.170A>C	COSM4756761
MAP2K1	2	p.Lys57Met	c.170A>T	MAN124
MAP2K1	2	p.Lys57Asn	c.171G>C	COSM5520914
MAP2K1	2	p.Lys57Asn	c.171G>T	COSM1235478
MAP2K1	3	p.Pro124Ser	c.370C>T	COSM235614
MAP2K1	3	p.Pro124Gln	c.371C>A	COSM1167912
MAP2K1	3	p.Pro124Leu	c.371C>T	COSM1315861
MAP2K1	6	p.Glu203Lys	c.607G>A	COSM232755
MAP2K1	6	p.Glu203Val	c.608A>T	COSM3386991
MAP2K2	2	p.Gln60Pro	c.179A>C	COSM145610
MAP2K2	2	p.Phe57Leu	c.171T>G	OM3158
MAP2K2	2	p.Phe57Leu	c.171T>A	COSM3389034
MAP2K2	2	p.Phe57Val	c.169T>G	COSM3534171

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
MAP2K2	2	p.Phe57Leu	c.169T>C	COSM1235618
MET	14	p.Thr1010Ile	c.3029C>T	COSM707
MET	14	p.Tyr1021Asn	c.3061T>A	COSM48564
MET	14	p.Tyr1021Phe	c.3062A>T	COSM339515
MET	—	NA	NA	COSM29633[1]
MET	—	NA	NA	COSM24687[1]
MET	—	NA	NA	COSM35468[1]
MET	16	p.His1112Tyr	c.3334C>T	COSM696
MET	16	p.His1112Arg	c.3335A>G	COSM703
MET	16	p.His1112Leu	c.3335A>T	COSM698
MET	19	p.Tyr1248His	c.3742T>C	COSM690
MET	19	p.Tyr1248Cys	c.3743A>G	COSM699
MET	19	p.Tyr1253Asp	c.3757T>G	COSM700
MET	19	p.Met1268Thr	c.3803T>C	COSM691
MET	19	p.Met1268Ile	c.3804G>A	COSM694
MTOR	53	p.Leu2427Arg	c.7280T>G	COSM2119114
MTOR	53	p.Leu2427Gln	c.7280T>A	COSM1185313
MTOR	47	p.Ser2215Phe	c.6644C>T	COSM1686998
MTOR	47	p.Ser2215Tyr	c.6644C>A	COSM20417
MTOR	47	p.Ser2215Pro	c.6643T>C	COSM1560108
MTOR	43	p.Val2006Phe	c.6016G>T	COSM249481
MTOR	43	p.Val2006Leu	c.6016G>C	COSM1134662
MTOR	43	p.Val2006Ile	c.6016G>A	COSM893804
MTOR	43	p.Thr1977Arg	c.5930C>G	COSM462602 **Note:** Some "no calls" were observed for this analytical variant due to strand bias with plasmid targets. This does not impact clinical test results.

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
MTOR	43	p.Thr1977Lys	c.5930C>A	COSM462601
MTOR	43	p.Thr1977Ser	c.5929A>T	COSM1289945 **Note:** Some "no calls" were observed for this analytical variant due to strand bias with plasmid targets. This does not impact clinical test results.
MTOR	40	p.Phe1888Leu	c.5664C>G	COSM462604
MTOR	40	p.Phe1888Leu	c.5664C>A	COSM893813
MTOR	40	p.Phe1888Val	c.5662T>G	COSM893814
MTOR	40	p.Phe1888Leu	c.5662T>C	COSM3358967
MTOR	40	p.Phe1888Ile	c.5662T>A	COSM3358968
MTOR	39	p.Glu1799Lys	c.5395G>A	COSM180789
MTOR	30	p.Cys1483Trp	c.4449C>G	OM3149
MTOR	30	p.Cys1483Phe	c.4448G>T	COSM462616
MTOR	30	p.Cys1483Tyr	c.4448G>A	COSM462615
MTOR	30	p.Cys1483Arg	c.4447T>C	COSM3747775
NRAS	4	p.Ala146Val	c.437C>T	COSM4170228
NRAS	4	p.Ala146Thr	c.436G>A	COSM27174
NRAS	4	p.Lys117Asn	c.351G>T	MAN13
NRAS	3	p.Gln61His	c.183A>T	COSM585
NRAS	3	p.Gln61His	c.183A>C	COSM586
NRAS	3	p.Gln61Leu	c.182A>T	COSM583
NRAS	3	p.Gln61Arg	c.182A>G	COSM584
NRAS	3	p.Gln61Pro	c.182A>C	COSM582
NRAS	3	p.Gln61Glu	c.181C>G	COSM581
NRAS	3	p.Gln61Lys	c.181C>A	COSM580
NRAS	3	p.Ala59Thr	c.175G>A	COSM578
NRAS	2	p.Gly13Val	c.38G>T	COSM574

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
NRAS	2	p.Gly13Ala	c.38G>C	COSM575
NRAS	2	p.Gly13Asp	c.38G>A	COSM573
NRAS	2	p.Gly13Cys	c.37G>T	COSM570
NRAS	2	p.Gly13Arg	c.37G>C	COSM569
NRAS	2	p.Gly13Ser	c.37G>A	COSM571
NRAS	2	p.Gly12Val	c.35G>T	COSM566
NRAS	2	p.Gly12Ala	c.35G>C	COSM565
NRAS	2	p.Gly12Asp	c.35G>A	COSM564
NRAS	2	p.Gly12Cys	c.34G>T	COSM562
NRAS	2	p.Gly12Arg	c.34G>C	COSM561
NRAS	2	p.Gly12Ser	c.34G>A	COSM563
PDGFRA	12	p.Val561Asp	c.1682T>A	COSM739
PDGFRA	14	p.Asn659Tyr	c.1975A>T	COSM22416
PDGFRA	14	p.Asn659Lys	c.1977C>A	COSM22415
PDGFRA	14	p.Asn659Lys	c.1977C>G	COSM22414
PDGFRA	18	p.Asp842_His845del	c.2526_2537delCATCATGCATGA	COSM737
PDGFRA	18	p.Asp842_Met844del	c.2524_2532delGACATCATG	COSM12401
PDGFRA	18	p.Asp842Tyr	c.2524G>T	COSM12396
PDGFRA	18	p.Asp842Val	c.2525A>T	COSM736
PDGFRA	18	p.Ile843_Asp846del	c.2527_2538delATCATGCATGAT	COSM12400
PDGFRA	18	p.Ile843_Ser847delinsThr	c.2528_2539delTCATGCATGATT	COSM12407
PIK3CA	2	p.Arg38Ser	c.112C>A	COSM87310
PIK3CA	2	p.Arg38Gly	c.112C>G	COSM40945
PIK3CA	2	p.Arg38Cys	c.112C>T	COSM744
PIK3CA	2	p.Arg38His	c.113G>A	COSM745
PIK3CA	2	p.Glu39Lys	c.115G>A	COSM30625

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
PIK3CA	2	p.Glu81Lys	c.241G>A	COSM27502
PIK3CA	2	p.Arg88Gln	c.263G>A	COSM746
PIK3CA	2	p.Arg93Trp	c.277C>T	COSM27493
PIK3CA	2	p.Arg93Gln	c.278G>A	COSM86041
PIK3CA	2	p.Gly106Val	c.317G>T	COSM748
PIK3CA	2	p.Arg108His	c.323G>A	COSM27497
PIK3CA	2	p.Lys111Glu	c.331A>G	COSM13570
PIK3CA	5	p.Val344Ala	c.1031T>C	COSM86951
PIK3CA	5	p.Val344Gly	c.1031T>G	COSM22540
PIK3CA	5	p.Asn345Ile	c.1034A>T	COSM94978
PIK3CA	5	p.Asn345Lys	c.1035T>A	COSM754
PIK3CA	6	p.Glu365Lys	c.1093G>A	COSM86044
PIK3CA	6	p.Glu365Gly	c.1094A>G	COSM1420797
PIK3CA	6	p.Glu365Val	c.1094A>T	COSM1484860
PIK3CA	6	p.Cys378Arg	c.1132T>C	COSM756
PIK3CA	6	p.Cys378Tyr	c.1133G>A	COSM1041478
PIK3CA	6	p.Cys378Phe	c.1133G>T	COSM21450
PIK3CA	8	p.Cys420Arg	c.1258T>C	COSM757
PIK3CA	10	p.Pro539Arg	c.1616C>G	COSM759
PIK3CA	10	p.Glu542Lys	c.1624G>A	COSM760
PIK3CA	10	p.Glu542Val	c.1625A>T	COSM762
PIK3CA	10	p.Glu545Lys	c.1633G>A	COSM763
PIK3CA	10	p.Glu545Gln	c.1633G>C	COSM27133
PIK3CA	10	p.Glu545Ala	c.1634A>C	COSM12458
PIK3CA	10	p.Glu545Gly	c.1634A>G	COSM764
PIK3CA	10	p.Glu545Asp	c.1635G>C	COSM27374
PIK3CA	10	p.Glu545Asp	c.1635G>T	COSM765
PIK3CA	10	p.Gln546Lys	c.1636C>A	COSM766

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
PIK3CA	10	p.Gln546Glu	c.1636C>G	COSM6147
PIK3CA	10	p.Gln546Pro	c.1637A>C	COSM767
PIK3CA	10	p.Gln546Arg	c.1637A>G	COSM12459
PIK3CA	10	p.Glu547Lys	c.1639G>A	COSM29315
PIK3CA	14	p.His701Pro	c.2102A>C	COSM778
PIK3CA	14	p.His701Arg	c.2102A>G	COSM1420881
PIK3CA	14	p.Glu726Lys	c.2176G>A	COSM87306
PIK3CA	14	p.Glu726Gly	c.2177A>G	COSM1420887
PIK3CA	19	p.Cys901Arg	c.2701T>C	COSM1420899
PIK3CA	19	p.Cys901Tyr	c.2702G>A	COSM1420901
PIK3CA	19	p.Cys901Phe	c.2702G>T	COSM769
PIK3CA	21	p.Tyr1021Cys	c.3062A>G	COSM12461
PIK3CA	21	p.Thr1025Ala	c.3073A>G	COSM771
PIK3CA	21	p.Met1043Val	c.3127A>G	COSM12591
PIK3CA	21	p.Met1043Ile	c.3129G>A	COSM29313
PIK3CA	21	p.Met1043Ile	c.3129G>T	COSM773
PIK3CA	21	p.Asn1044Lys	c.3132T>A	COSM12592
PIK3CA	21	p.His1047Tyr	c.3139C>T	COSM774
PIK3CA	21	p.His1047Arg	c.3140A>G	COSM775
PIK3CA	21	p.His1047Leu	c.3140A>T	COSM776
PIK3CA	21	p.Gly1049Ser	c.3145G>A	COSM777
PIK3CA	21	p.Gly1049Arg	c.3145G>C	COSM12597
RAF1	12	p.Thr421Met	c.1262_1263delCCinsTG	MAN9
RAF1	7	p.Ser257Leu	c.770C>T	COSM181063
RAF1	7	p.Ser257Trp	c.770C>G	COSM581519
RET	10	p.Cys618Arg	c.1852T>C	COSM29803
RET	10	p.Cys618Tyr	c.1853G>A	COSM980
RET	10	p.Cys620Arg	c.1858T>C	COSM29804

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
RET	11	p.Cys634Arg	c.1900T>C	COSM966
RET	13	p.Glu768Gly	c.2303A>G	COSM1347811
RET	13	p.Glu768Asp	c.2304G>C	COSM21338
RET	15	p.Ala883Phe	c.2646_2648delAGCinsTTT	COSM981 **Note:** The nucleotide change of COSM981 overlaps that of COSM133167, so a positive COSM981 sample will also result in a positive call for COSM133167.
RET	15	p.Ala883Ser	c.2647G>T	COSM133167
RET	15	p.Asp898_Glu901del	c.2694_2705delTGTTTATGAAGA	COSM962
RET	16	p.Met918Thr	c.2753T>C	COSM965
ROS1	38	p.Gly2032Arg	c.6094G>C	MAN11
ROS1	38	p.Gly2032Arg	c.6094G>A	MAN10
ROS1	36	p.Leu1951Met	c.5851C>A	COSM1072521

[1] Annotations for this variant are not available for reporting.

Fusion isoforms detected by the Oncomine™ Dx Target Test

RET fusion isoforms (N=40)	
ACBD5-RET.A11R12	KIF5B-RET.K15R11.COSF1255.1
AFAP1-RET.A3R12	KIF5B-RET.K15R12.COSF1232
AKAP13-RET.A35R12	KIF5B-RET.K16R12.COSF1230
AKAP13-RET.A36R12	KIF5B-RET.K22R12.COSF1253
CCDC6-RET.C1R11	KIF5B-RET.K23R12.COSF1234
CCDC6-RET.C1R11.1	KIF5B-RET.K24R11.COSF1262
CCDC6-RET.C1R12	KIF5B-RET.K24R8.COSF1236
CCDC6-RET.C1R12.COSF1271	KTN1-RET.K29R12.COSF1513
CCDC6-RET.C2R12	NCOA4-RET.N7R12
CCDC6-RET.C8R11	NCOA4_ELE1-RET.E6R12
CCDC6-RET.C8R12full	PCM1-RET.P29R12
CUX1-RET.C10R12	PRKAR1A-RET.P7R12
ERC1-RET.E12R12	RUFY2-RET.R9R12
ERC1-RET.E17R12	SPECC1L-RET.S10R11.NGS.1
ERC1-RET.E7R12	SPECC1L-RET.S10R12
ERC1_ELKS-RET.E11R12.COSF1507	TBL1XR1-RET.T9R11.NGS.1
FKBP15-RET.F25R12	TBL1XR1-RET.T9R12
GOLGA5-RET.G7R12	TRIM24-RET.T9R12
HOOK3-RET.H11R12	TRIM27-RET.T3R12
KIAA1468-RET.K10R12	TRIM33-RET.T16R12

ROS1 fusion isoforms (N=34)	
CCDC6-ROS1.C5R35	LRIG3-ROS1.L16R35.COSF1269
CD74-ROS1.C4R33.NGS	MSN-ROS1.M9R34
CD74-ROS1.C6R32.COSF1202	MYO5A-ROS1.M23R35
CD74-ROS1.C6R34.COSF1200	PPFIBP1-ROS1.P9R35
CD74-ROS1.C6R35	PWWP2A-ROS1.P1R36
CD74-ROS1.C7R34	SDC4-ROS1.S2R32.COSF1265
CEP85L-ROS1.C8R36	SDC4-ROS1.S2R34
CLIP1-ROS1.C19R36	SDC4-ROS1.S4R32.COSF1278
CLTC-ROS1.C31R35	SDC4-ROS1.S4R34.COSF1280
ERC1-ROS1.E11R36	SLC34A2-ROS1.S13R32.COSF1259
EZR-ROS1.E10R34.COSF1267	SLC34A2-ROS1.S13R34.COSF1261
EZR-ROS1.E10R35	SLC34A2-ROS1.S4R32.COSF1197
GOPC-ROS1.G4R36.COSF1188	SLC34A2-ROS1.S4R34.COSF1198
GOPC-ROS1.G8R35.COSF1139	TFG-ROS1.T4R35
HLA_A-ROS1.H7R34	TPM3-ROS1.T3R36
KDELR2-ROS1.K5R35	TPM3-ROS1.T7R35.COSF1273
KIAA1598-ROS1.K11R36	ZCCHC8-ROS1.Z2R36

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support
- Order and web support
- Safety Data Sheets (SDSs; also known as MSDSs)

Additional product documentation, including user guides and Certificates of Analysis, are available by contacting Customer Support.

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are printed and shipped with the product.

Obtaining Certificates of Conformance

The Certificate of Conformance provides information on conformance testing of each instrument provided with the system. Certificates of Conformance are shipped with the instrument, and are also available by contacting Customer Support at **thermofisher.com/support**.

ion torrent

Oncomine™ Dx Target Test Part II: Sample and Library Preparation

USER GUIDE

Publication Number MAN0018811

Revision A.0

IVD

For In Vitro Diagnostic Use.

ThermoFisher SCIENTIFIC

Life Technologies Holdings Pte Ltd |
Block 33 |
Marsiling Industrial Estate Road 3 |
#07-06, Singapore 739256

Products manufactured in Singapore:

Ion PGM™ Dx Instrument System
Ion PGM™ Dx Sequencer
Ion OneTouch™ Dx Instrument
Ion OneTouch™ ES Dx Instrument
Ion PGM™ Dx Chip Minifuge (120V)
Ion PGM™ Wireless Scanner
Ion Torrent™ Server
Torrent Suite™ Dx Software
Veriti™ Dx 96-well Thermal Cycler, 0.2 mL

Life Technologies Corporation |
7335 Executive Way |
Frederick, MD 21704 | USA

Products manufactured in Frederick:

Oncomine™ Dx Target Test Kit
Ion Torrent Dx FFPE Sample Preparation Kit
Ion PGM™ Dx Library Kit
Ion OneTouch™ Dx Template Kit
Ion PGM™ Dx Sequencing Kit
Ion 318™ Dx Chip
Ion OneTouch™ Rack Kit
DynaMag™ Dx 96-Well Plate Magnet
DynaMag™ Dx 16 2-mL Magnet

The information in this guide is subject to change without notice.

DISCLAIMER

TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0018811

Revision	Date	Description
A.0	2 September 2020	Oncomine™ Dx Target Test user guide for FDA submission—updated for Torrent Suite™ Dx Software 5.12.5

Contents

About this guide

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Purpose of this guide

This user guide provides instructions for sample preparation, sample quantification, and library preparation using the Oncomine™ Dx Target Test. The resulting libraries are ready for template preparation and sequencing on the Ion PGM™ Dx System.

Oncomine™ Dx Target Test Kit user guides

This user guide is part of a five-guide set.

- *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide*
- *Oncomine™ Dx Target Test Part II: Sample and Library Preparation User Guide*
- *Oncomine™ Dx Target Test Part III: Template Preparation User Guide*
- *Oncomine™ Dx Target Test Part IV: Sequencing and Results Reports User Guide*
- *Oncomine™ Dx Target Test Part V: Torrent Suite™ Dx Software 5.12.5 Reference User Guide*

All five guides are required to complete the entire Oncomine™ Dx Target Test workflow.

Note: The procedures in these guides supersede the instructions in the *Ion PGM™ Dx System User Guide* when using the Ion PGM™ Dx System with the Oncomine™ Dx Target Test.

1 Product information

Product description

Oncomine™ Dx Target Test

The Oncomine™ Dx Target Test is an *in vitro* diagnostic next-generation sequencing test to detect somatic alterations in human DNA and RNA isolated from formalin-fixed, paraffin-embedded (FFPE) tissue samples. Detection of these variants is performed using the Ion PGM™ Dx System.

For a complete product description of the Oncomine™ Dx Target Test, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide* (Pub. No. MAN0018810).

Sample preparation components

The Ion Torrent Dx FFPE Sample Preparation Kit, included as part of the Oncomine™ Dx Target Test Kit, provides the following components for isolating and quantifying DNA and RNA from FFPE tissue samples that are mounted on slides:

- Ion Torrent Dx Total Nucleic Acid Isolation Kit, for extracting and isolating DNA and RNA from FFPE tissue samples
- Ion Torrent Dx DNA Quantification Kit, for quantifying DNA using a fluorometer/fluorescence reader
- Ion Torrent Dx RNA Quantification Kit, for quantifying RNA using a fluorometer/fluorescence reader

The Ion Torrent Dx cDNA Synthesis Kit and the Oncomine™ Dx Target Test, Controls, and Diluent Kit are used to reverse transcribe the quantified RNA into cDNA.

Library preparation components

The Oncomine™ Dx Target Test Kit includes the following components for preparing barcoded libraries from DNA and cDNA for sequencing on the Ion PGM™ Dx System:

- Oncomine™ Dx Target Test, Controls, and Diluent Kit, which includes primer panels for amplifying DNA- and RNA-specific target regions as well as controls
- Ion PGM™ Dx Library Kit, which includes 16 unique barcode adapters (BC 1–BC 16) as well as enzymes and other reagents for library preparation
- Ion PGM™ Dx Library Equalizer™ Reagents, to normalize the concentration of the resulting libraries to ~100 pM without the need for quantification

The library preparation procedure requires 10 ng of DNA and RNA.

Intended use

For the Intended Use statement for the Oncomine™ Dx Target Test, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide* (Pub. No. MAN0018810).

Theory of operation

For a complete description of the Theory of Operation of the system, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide* (Pub. No. MAN0018810).

Software compatibility and requirements

The procedures in this guide are designed for use with Torrent Suite™ Dx Software version 5.12.5 or later. For a complete description of software compatibility and requirements, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide* (Pub. No. MAN0018810).

Materials provided

Oncomine™ Dx Target Test Kit

The Oncomine™ Dx Target Test Kit (Cat. No. A49755) includes the following subkits.

IMPORTANT! Refer to the product label for the storage conditions and expiration dates of individual modules and components.

✓	Subkit	Part No.
	Oncomine™ Dx Target Test, Controls, and Diluent Kit	A49756
	Ion Torrent Dx FFPE Sample Preparation Kit	A32445
	Ion PGM™ Dx Library Kit	A49758
	Ion OneTouch™ Dx Template Kit	A49759
	Ion PGM™ Dx Sequencing Kit	A49760
	Ion 318™ Dx Chip Kit	A18937
	Oncomine™ Dx Target Test User Guides and Assay Definition File	A49678

Subkits used in this guide

The procedures in this user guide use the following subkits from the Oncomine™ Dx Target Test Kit.

Ion Torrent Dx FFPE Sample Preparation Kit

The Ion Torrent Dx FFPE Sample Preparation Kit (Cat. No. A32445) provides reagents for nucleic acid isolation, quantification, and cDNA synthesis from FFPE samples.

✓	Component	Amount	Storage
Ion Torrent Dx Total Nucleic Acid Isolation Kit box 1 of 2 (36 reactions; Part No. A32434)			
	10X DNase Buffer (white cap)	6 × 46 µL	–30°C to –10°C
	Protease (blue cap)	6 × 34 µL	
	DNase (purple cap)	6 × 34 µL	
Ion Torrent Dx Total Nucleic Acid Isolation Kit box 2 of 2 (36 reactions; Part No. A32435)			
	Elution Solution (red cap)	6 × 900 µL	15°C to 30°C
	Isolation Additive (brown cap)	6 × 1 mL	
	Wash 1 Concentrate (amber cap)	6 × 5.9 mL	
	Wash 2 Concentrate (clear cap)	6 × 3.4 mL	
	Digestion Buffer (green cap)	6 × 200 µL	
	Dilution Solution (black cap)	6 × 1.5 mL	
	Collection Tubes	6 × 6 tubes	
	Filter Cartridges[1]	6 × 12 cartridges	
	Low-bind Elution Tubes	6 × 14 tubes	
Ion Torrent Dx DNA Quantification Kit (72 reactions; Part No. A32437)			
	DNA Dye Reagent (blue cap)	6 × 70 µL	2°C to 8°C
	DNA Buffer (white cap)	6 × 14.3 mL	
	DNA Std - 0 ng/µL(white cap)	6 × 150 µL	
	DNA Std - 0.5 ng/µL (green cap)	6 × 150 µL	
	DNA Std - 4 ng/µL (red cap)	6 × 150 µL	
	DNA Std - 10 ng/µL (yellow cap)	6 × 150 µL	

✓	Component	Amount	Storage
Ion Torrent Dx RNA Quantification Kit (72 reactions; Part No. A32438)			
	RNA Dye Reagent (green cap)	6 × 70 µL	2°C to 8°C
	RNA Buffer (blue cap)	6 × 14.3 mL	
	RNA Std - 0 ng/µL (teal cap)	6 × 150 µL	
	RNA Std - 0.5 ng/µL (tan cap)	6 × 150 µL	
	RNA Std - 4 ng/µL (purple cap)	6 × 150 µL	
	RNA Std - 10 ng/µL (orange cap)	6 × 150 µL	
Ion Torrent Dx cDNA Synthesis Kit (48 reactions; Part No. A32436)			
	10X Enzyme Mix (green cap)	6 × 13 µL	–30°C to –10°C
	5X Reaction Mix (red cap)	6 × 22 µL	
Ion Torrent Dx Sample Dilution Kit (Part No. A32439)			
	Dilution Solution (black cap)	8 × 1.5 mL	15°C to 30°C

[1] Includes a filter column pre-inserted in a Collection Tube.

Oncomine™ Dx Target Test, Controls, and Diluent Kit

The Oncomine™ Dx Target Test, Controls, and Diluent Kit (Part No. A49756) provides the following panels and controls.

✓	Component	Amount	Storage
Oncomine™ Dx Target Test DNA and RNA Panel (Part No. A32441)			
	Oncomine™ Dx Target Test—DNA panel (blue cap)	6 × 32 µL	–30°C to –10°C
	Oncomine™ Dx Target Test—RNA panel (yellow cap)	6 × 32 µL	
Oncomine™ Dx Target DNA Control (Part No. A44913)			
	Oncomine™ Dx Target DNA Control (brown cap)	8 × 7 µL (single-use tubes)	–30°C to –10°C
Oncomine™ Dx Target RNA Control (Part No. A32443)			
	Oncomine™ Dx Target RNA Control (white cap)	8 × 7 µL (single-use tubes)	–90°C to –60°C
Oncomine™ Dx Target RNA Control Diluent (Part No. A38872)			
	Oncomine™ Dx Target RNA Control Diluent (blue cap)	8 × 88 µL (single-use tubes)	–90°C to –60°C

✓	Component	Amount	Storage
Ion Torrent Dx No Template Control Kit (Part No. A32444)			
	No Template Control (purple cap)	8 × 30 µL	15°C to 30°C

Ion PGM™ Dx Library Kit

The Ion PGM™ Dx Library Kit (Cat. No. A49758) provides reagents for preparing up to 96 sample libraries.

IMPORTANT! Do not mix components from other library kits.

✓	Component	Amount	Storage
Ion PGM™ Dx Library Reagents (Part No. A18928)			
	LIB HiFi Mix (red cap)	6 × 252 µL	–30°C to –10°C
	LIB FuPa (green cap)	6 × 32 µL	
	LIB Switch Soln (orange cap)	6 × 64 µL	
	LIB DNA Ligase (clear cap)	6 × 32 µL	
	BC 1 through BC 16 (16 unique barcode adapters, numbered 1–16, white cap)	16 × 12 µL	
Ion PGM™ Dx Library Equalizer™ Reagents (Part No. A18929)			
	LIB AMPure™ Reagent (clear cap)	4.4 mL	2°C to 8°C
	LIB Beads (yellow cap)	6 × 48 µL	
	LIB Primers (blue cap)	6 × 36 µL	
	LIB Capture (violet cap)	6 × 160 µL	
	LIB Wash Soln (clear cap)	30 mL	
	LIB Elution Soln (clear cap)	9.6 mL	

Materials and equipment required but not provided

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Description	Source
Veriti™ Dx 96-well Thermal Cycler, 0.2 mL	4452300
Laminar flow hood	MLS
Dry-bath heaters and aluminum heat blocks (quantity = 3), for use with 1.5-mL tubes	MLS
1.5-mL snap-cap low-retention polypropylene microcentrifuge tubes	MLS
1.5-mL tube rack	MLS
Aluminum cold blocks for use with 96-well plates	MLS
Benchtop cold box for use with 1.5-mL tubes	MLS
Microcentrifuge (must accommodate standard 1.5-mL and 0.2-mL microcentrifuge tubes, and generate 20,000 rcf)	MLS
0.2-mL tube adapters	MLS
Mini centrifuge	MLS
96-well plate centrifuge	MLS
Vortex mixer with a rubber platform	MLS
Fluorometer/fluorescence reader (see additional specifications following)	MLS
Tubes or plates for the fluorometer/fluorescence reader	MLS
DynaMag™ Dx 96-Well Plate Magnet magnet	A31347
DynaMag™ Dx 16 2-mL Magnet	A31346
Slide rack, able to hold standard 3″ × 1″ (75 × 25 mm) slides	MLS
Staining dish or jar, able to hold sufficient liquid to fully submerge the slide rack	MLS
Disposable scalpel with a sterile #10 blade	MLS
RNase decontamination solution	MLS
Absolute ethanol (ACS grade)	MLS
Xylene (ACS grade, ≥98.5%)	MLS
Nuclease-free water	MLS
Single- and multi-channel pipettes (2-, 20-, 200-, 1000-µL)	MLS

(continued)

Description	Source
Aerosol-barrier pipette tips (2-, 10-, 20-, 200-, 1000-µL)	MLS
Troughs for multi-channel pipettors	MLS
MicroAmp™ Optical 96-well Reaction Plates	4481191 4481192 (with barcode)
Adhesive PCR Plate Seals	AB0558
15-mL and 50-mL conical tubes and tube holders (for preparing bulk solutions)	MLS
5-mL and 25-mL serological pipettes, and pipette controller (for preparing bulk solutions)	MLS

Fluorometer/ fluorescence reader specifications

For the DNA and RNA quantification procedure, you can use any qualified fluorometer/fluorescence reader that can accommodate the use of a 2–4-point standard curve and is able to operate at the excitation and emission wavelengths listed below:

Dye reagent	Excitation (nm)	Emission (nm)
RNA Dye Reagent	620/15	680/30
DNA Dye Reagent	485/20	528/20

DynaMag™ Dx 96-Well Plate Magnet and DynaMag™ Dx 16 2-mL Magnet

Note: Do not substitute non-IVD labeled magnets for the DynaMag™ Dx 96-Well Plate Magnet and DynaMag™ Dx 16 2-mL Magnet.

The DynaMag™ Dx 96-Well Plate Magnet and DynaMag™ Dx 16 2-mL Magnet, provided with Ion PGM™ Dx System, contain high-energy neodymium magnets and are used as part of the procedure for purifying sample libraries bound to LIB AMPure™ Reagent and LIB Beads. The DynaMag™ Dx 16 2-mL Magnet is also used to prepare TMPL ES Beads as part of template preparation.

The DynaMag™ Dx 96-Well Plate Magnet has 7 bar magnets with a hard plastic top to fit 96-well PCR plates. When you insert a plate, the magnets collect bead-bound biomolecules in suspension at the sides of the plate wells, allowing removal of fluid without disturbing the bead pellets. An extra column in the magnet enables sample mixing by shifting the plate back and forth in the magnet.

The DynaMag™ Dx 16 2-mL Magnet holds 16 standard 1.5-mL or 2-mL microcentrifuge tubes, and collects bead-bound biomolecules in suspension at the sides of the tubes, allowing removal of fluid without disturbing the bead pellets.

Do not use the magnets above 50°C (122°F) and store in a cool, dry environment.

2 Before you begin

Tissue input requirements for FFPE sample extraction

The starting material for the extraction procedure is an FFPE tissue sample that is unstained and mounted on a slide. Confirm the tumor content of each sample based on the area of a hematoxylin and eosin (H&E) stained section.

The recommended number of slide-mounted 5-micron FFPE sections used in extraction varies depending on the sample collection method:

Sample collection method	Recommended number of sections
Resection or surgical biopsies	2 × 5-micron sections
Core needle biopsies	9 × 5-micron sections
Fine needle aspirates	7 × 5-micron sections

Note: Extraction from FFPE sample curls has not been evaluated.

Sample storage and stability

Store FFPE blocks and slides at room temperature (15–30°C).

Blocks and slides (paraffin-dipped or undipped) are stable for up to 5 months at 15–30°C.

Extracted DNA can be stored at –30°C to –10°C for up to 12 months, including 3 freeze-thaw cycles. Extracted RNA can be stored at –90°C to –60°C for up to 5 months, including 3 freeze-thaw cycles.

Procedural guidelines

Definitions

Throughout this guide:

- Room temperature is defined as the temperature range 15–30°C.
- A pulse centrifugation consists of a 3–5 second centrifugation at maximum speed in a mini centrifuge.

Guidelines to prevent cross-contamination

CAUTION! A primary source of contamination is nucleic acid from previous sample processing steps. Do not introduce amplified DNA into the target amplification preparation area.

- When designing the laboratory layout, dedicate separate areas for pre- and post-amplification activities. Dedicate laboratory supplies and/or equipment to the appropriate area.
- Use a laminar flow hood in the dedicated pre-amplification area for target amplification reaction setup.
- Before and after use, clean all surfaces and equipment in the laminar flow hood with 10% bleach followed by two water rinses.
- Turn on the UV light in the hood for 10 minutes before and after use.
- Use fresh gloves before entering the hood.
- Change tips between pipetting steps.
- Prepare a waste container containing 10% bleach solution for disposing of used tips after pipetting libraries.

Reagent contamination

Before use, verify that any nuclease-free water used in the procedure is not cloudy, a potential indication of contamination. If the water is cloudy, use a different vial.

Guidelines for FFPE samples

- For core needle biopsies, macrodissection is not recommended due to the limiting tissue section surface areas.
- For resection or surgical biopsies, macrodissect and enrich the sample for tumor content if the tumor content is less than 20% and the tumor content in the region of interest is greater than or equal to 10%. Following tumor enrichment, proceed with the extraction protocol.
- Necrotic samples: Up to 75% necrotic tissue in the region of interest does not appear to interfere with the assay. However, we recommend that you macrodissect highly necrotic areas or select alternate samples if possible.
- Nucleic acid integrity is important for sample performance. Factors such as age of the block, fixation process used, and sample source can impact the quality of the extracted nucleic acid.
- Nucleic acid yield can be impacted by overall tissue area. Should an initial extraction lead to insufficient concentrations for DNA and RNA, repeat the extractions with more material whenever possible.

Guidelines for RNA

- Wear clean gloves and a clean lab coat.
- Change gloves whenever they may be contaminated.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Clean lab benches and equipment (including gloves, tube racks, pipettes, centrifuges, and vortexers) with an RNase decontamination solution before and after use.
- Work in a designated RNase-free pre-PCR area.
- Keep RNA on ice or in a –30°C to –10°C chilled benchtop cold box during use.
- Never vortex RNA. Flick 4 times to mix, then pulse centrifuge to collect.

Guidelines for mixing reagents

Immediately before each use:

- Mix enzyme solutions (e.g., Protease and DNase) by flicking the tubes 4 times, followed by a pulse centrifugation.
- Vortex non-enzyme-containing reagents for ~5 seconds, followed by a pulse centrifugation.
- Mix reagent bottles by inverting them 5 times.

Guidelines for pipetting

- Use aerosol-barrier pipette tips. Change pipette tips between samples.
- Avoid introducing air bubbles when pipetting by keeping the pipette tip at the bottom of the solution in the wells.
- Set the pipette to the recommended volume for mixing, and insert tip into the solution with the pipette plunger depressed to avoid introducing air bubbles.
- Visually inspect multi-channel pipette tips to ensure volumes are equivalent during pipeting.

- Touch tip to the side of well and slowly pipet reagent on the side of the well to form a droplet. This enables small volumes to be pipetted accurately and to ensure that the reagent has been added to the well.
- Inspect the pipette tips to verify that the reagent has been adequately dispensed.

Guidelines for freezing and thawing samples

There are stopping points throughout this procedure where you can freeze samples overnight or longer and then thaw the samples before proceeding. If you cannot perform the complete procedure in a day, proceed to a designated stopping point and freeze the samples overnight.

IMPORTANT! Freeze-thaw samples no more than 3 times.

Guidelines for library preparation

- Up to 16 barcode adapters may be used in a single sequencing run.
- Freeze-thaw barcode adapters no more than 6 times.
- Verify that the correct program is selected before starting the Veriti™ Dx program.
- To avoid cross-contamination between samples, skip wells or columns when setting up reactions in a 96-well plate. Circle wells that are used with ethanol-resistant marker to help indicate where the samples are located.
- Because cDNA and DNA amplification reactions require a different number of cycles, they must be set up and run on separate 96-well plates.

Equilibrate materials

Equilibrate the following materials for at least 24 hours before use:

- Equilibrate two 96-well aluminum cold blocks to 2–8°C in a refrigerator.
- Equilibrate a benchtop cold box to –30°C to –10°C in a freezer.
- Equilibrate a separate benchtop cold box to 2–8°C in a refrigerator, or use ice to keep reaction tubes chilled on the bench.

Note: A cold box holds temperature for up to 1 hour on the bench.

Reagent management

Follow the guidelines below for proper reagent storage and use.

Storage

Reagents must be stored under appropriate conditions. Refer to the Product Information section in each user guide for the storage conditions of the kit components used in the procedures in that guide. The Oncomine™ Dx Target Test Kit includes kits with multiple component boxes that require different storage conditions. For example, the Oncomine™ Dx Target Test, Controls, and Diluent Kit includes four boxes, which are stored at different temperatures. To use the Oncomine™ Dx Target Test, Controls, and Diluent Kit, retrieve all boxes from their different storage areas and confirm that they are from the same master lot.

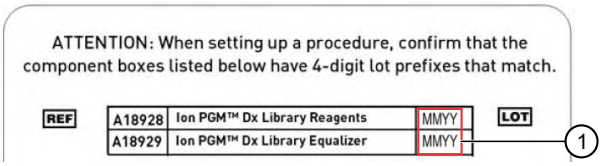
Kit interchangeability and component box lot matching

The top-level kits used for sample preparation, library preparation, template preparation, and sequencing can be mixed and matched. For example, an Ion PGM™ Dx Library Kit can be used with any Ion PGM™ Dx Sequencing Kit. However, the component boxes in a particular kit must be lot-matched with the other boxes in that kit.

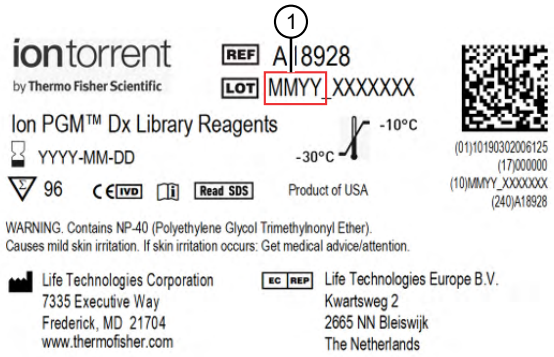
Note: The controls and diluent in the Oncomine™ Dx Target Test, Controls, and Diluent Kit must be lot-matched. However, the controls do not require lot-matching with the Oncomine™ Dx Target Test DNA and RNA Panel.

Each component box lists the 4-digit lot prefixes of the compatible component boxes inside the box lid. Before using a particular kit, check the inside lid of each box to confirm that it is compatible with the other boxes.

An example inside box lid label is shown below:

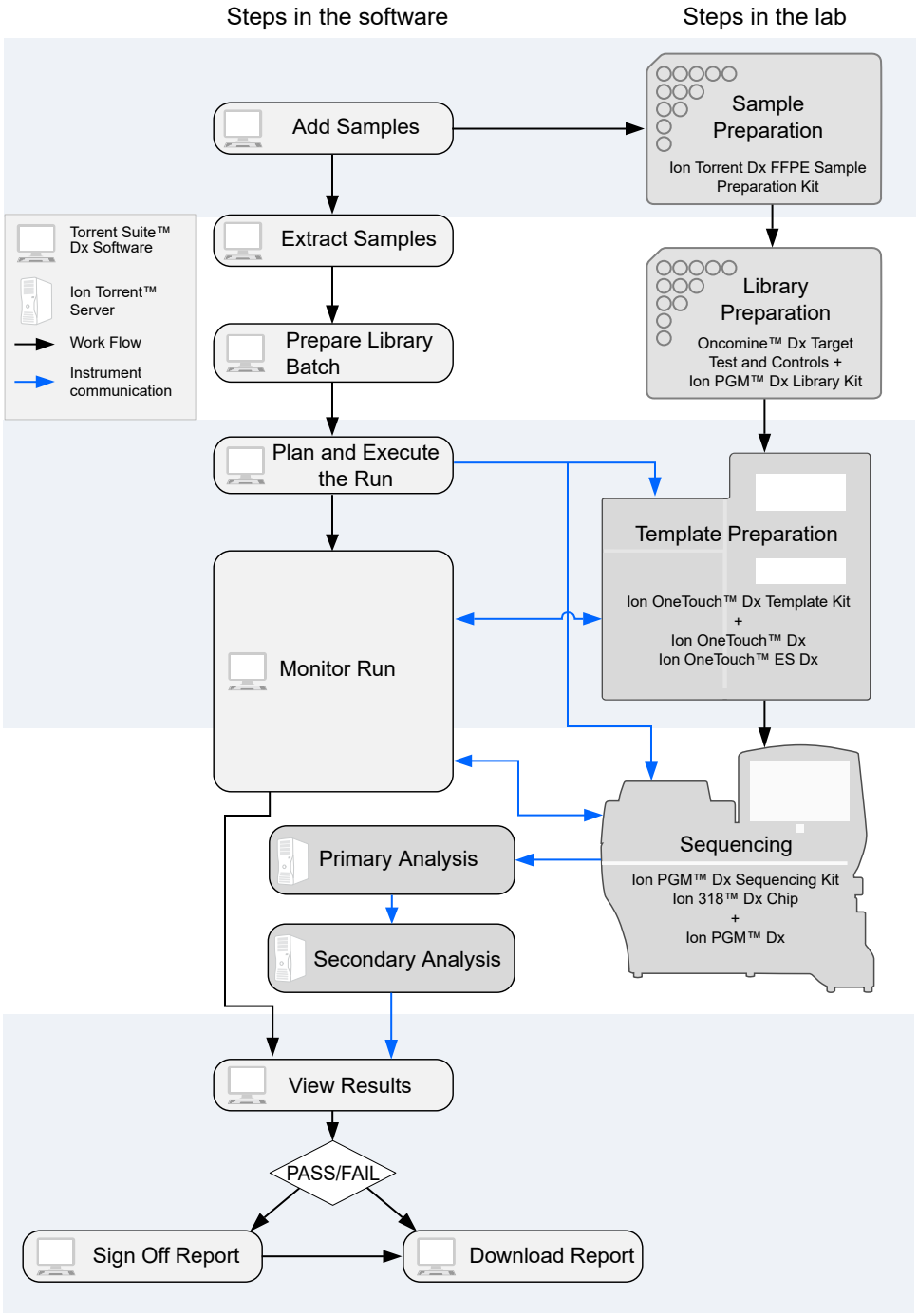


An example box label with lot information is shown below:



1 Lot prefix

Oncomine™ Dx Target Test system diagram



Sample preparation workflow

The following workflow summarizes the steps for isolating DNA and RNA from FFPE tumor samples, and preparing cDNA.

Sample setup in Torrent Suite™ Dx Software (page 23)

▼

Deparaffinize and digest samples (page 29)

▼

Separate RNA from DNA (page 33)

▼

Recover the RNA from the flow-through (page 36)

▼

Recover the DNA from the Filter Cartridge (page 40)

▼

DNA and RNA quantification (page 43)

▼

Prepare the cDNA (page 55)

▼

Proceed to library preparation

Library preparation workflow

"Prepare a library batch in Torrent Suite™ Dx Software" on page 60

▼

"Prepare reagents and equipment" on page 63

▼

"Amplify the cDNA" on page 64

▼

"Amplify the DNA" on page 67

▼

"Transfer the cDNA amplicons" on page 70

▼

"Partially digest amplicons" on page 71

▼

"Ligate barcode adapters" on page 73

▼

"Prepare the LIB HiFi Mix plus LIB Primers mix" on page 74

▼

"Purify the barcode-adapted library" on page 75

▼

"Amplify the barcode-adapted library" on page 76

▼

"Prepare the LIB Beads" on page 76

▼

"Add LIB Capture to the amplified sample library" on page 77

▼

"Add the LIB Beads and wash" on page 77

▼

"Elute the library" on page 78

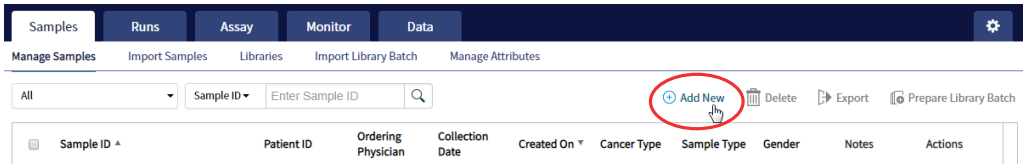
▼

Proceed to *Oncomine™ Dx Target Test Part III: Template Preparation User Guide* (Pub. No. MAN0018812)

3 Sample setup in Torrent Suite™ Dx Software

Add a new sample

1. Under the **Samples** tab, in the **Manage Samples** screen, click ⊕ **Add New**.



2. Complete the **Add New Sample** dialog box. Fields identified with an asterisk (*) are required. If no information is available, substitute dummy data to complete the required fields.

Field	Description
Sample ID*	A unique identifier representing the sample, containing only alphanumeric characters (0–9 and A to Z), full stops/periods (.), underscores (_), or hyphens (-). The Sample ID cannot contain spaces and is limited to a maximum of 20 characters. After a Sample ID is entered into the system, it cannot be edited. It can be deleted unless it has already been used in a library. The software checks all Sample IDs entered or imported to prevent duplication and returns an error message if a non-unique Sample ID is detected.
Patient ID*	An identifier representing the patient. This field accepts all characters including spaces.
Date of Birth*	The patient's date of birth. Click the button to select the date in the correct format.
Ordering Physician*	The name of the ordering physician. This field accepts all characters including spaces.
Collection Date*	The date the sample was collected from the patient. Click the button to select the date in the correct format.
Sample Source	Open-entry field that accepts all characters, including spaces. **Example entry:** Name of the clinic or hospital ordering the test.
Sample Condition*	Open-entry field that accepts all characters, including spaces. **Example entry:** FFPE surgical resection.
Sample Type*	Open-entry field that accepts all characters, including spaces. **Example entry:** Lung tumor, invasive adenocarcinoma.

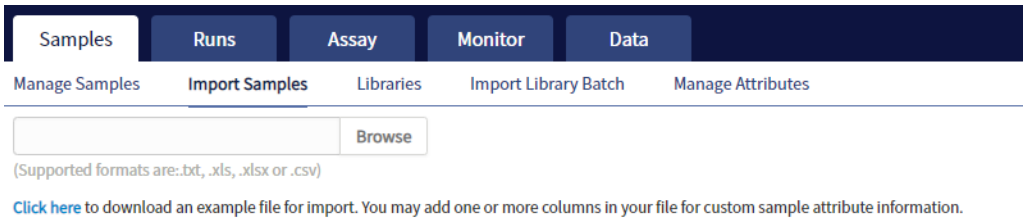
(continued)

Field	Description
Gender*	The biological gender of the sample. This must be Male, Female, or Unknown.
Cancer Type*	Select the cancer type of the sample from the dropdown list.
%Cellularity	The percentage of tumor cellularity in the sample.
%Necrosis	The percentage of cellular necrosis in the sample.
Reference Interval	A normal range of measure for the sample.
Notes	An open-entry field.

3. Click **Save**.
   The sample is listed in the **Manage Samples** screen.

Import samples

Under the **Samples** tab in the **Import Samples** screen, you can import sample data in the form of a TXT, XLS, XLSX, or CSV file. The import file includes the same sample attributes that are listed in the **Add New Sample** dialog box.



1. In the **Import Samples** screen, below the **Browse** field, click **Click here** to download a Microsoft™ Excel™ template file.

   Note: The template file contains default sample attributes as columns. If additional custom sample attributes have been configured in the software, add these attributes as columns to the template file.

2. In the template file, fill in the information for each sample, one sample per row. See "Predefined sample attributes" on page 25 for more information.

3. Save the file.

4. Click **Browse**, navigate to the saved file, then select it.

5. Click **Import**.
   A progress bar followed by an import report displays. If the import process fails, an error message indicates the reason for failure (for example, an invalid character was used). For additional troubleshooting, see "Batch sample import fails" on page 81.

6. Click **Manage Samples** to return to the sample list. Successfully imported samples are listed.

Predefined sample attributes

The software has the following predefined sample attributes, which are listed in the **Add New Sample** dialog box and in the template file for importing samples.

- Sample ID*
- Patient ID*
- Date Of Birth*
- Ordering Physician*
- Collection Date*
- Sample Source
- Sample Condition*
- Sample Type*
- Gender*
- Cancer Type*
- %Cellularity
- %Necrosis
- Reference Interval
- Notes

*Indicates a field required to be filled in during sample creation.

Predefined attributes are locked and cannot be edited. You can create and manage custom sample attributes using the tools in the **Manage Attributes** screen.

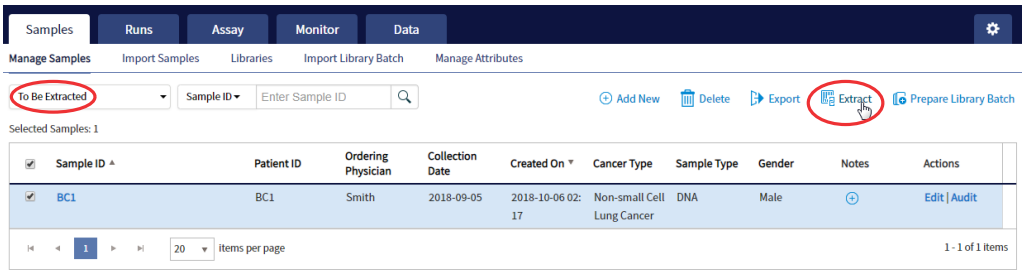
Note: LIMS users must create custom attributes before importing sample and Planned Run information from LIMS for the attributes to be propagated through to output files. The software ignores all input file content that is not a recognized attribute.

Enter the Ion Torrent Dx Total Nucleic Acid Isolation Kit barcode

Under the **Samples** tab, in the **Manage Samples** screen, scan the barcode of the Ion Torrent Dx Total Nucleic Acid Isolation Kit used in the extraction process for a particular sample. This barcode is saved with the sample and can be viewed by clicking the Sample ID.

1. Above the samples list, select **To Be Extracted** from the **Filter Samples by...** dropdown list to display only those samples that do not have a kit barcode that is associated with them.

2. Select the checkbox of the sample to be extracted. Select multiple samples if you are using the same kit to process them.



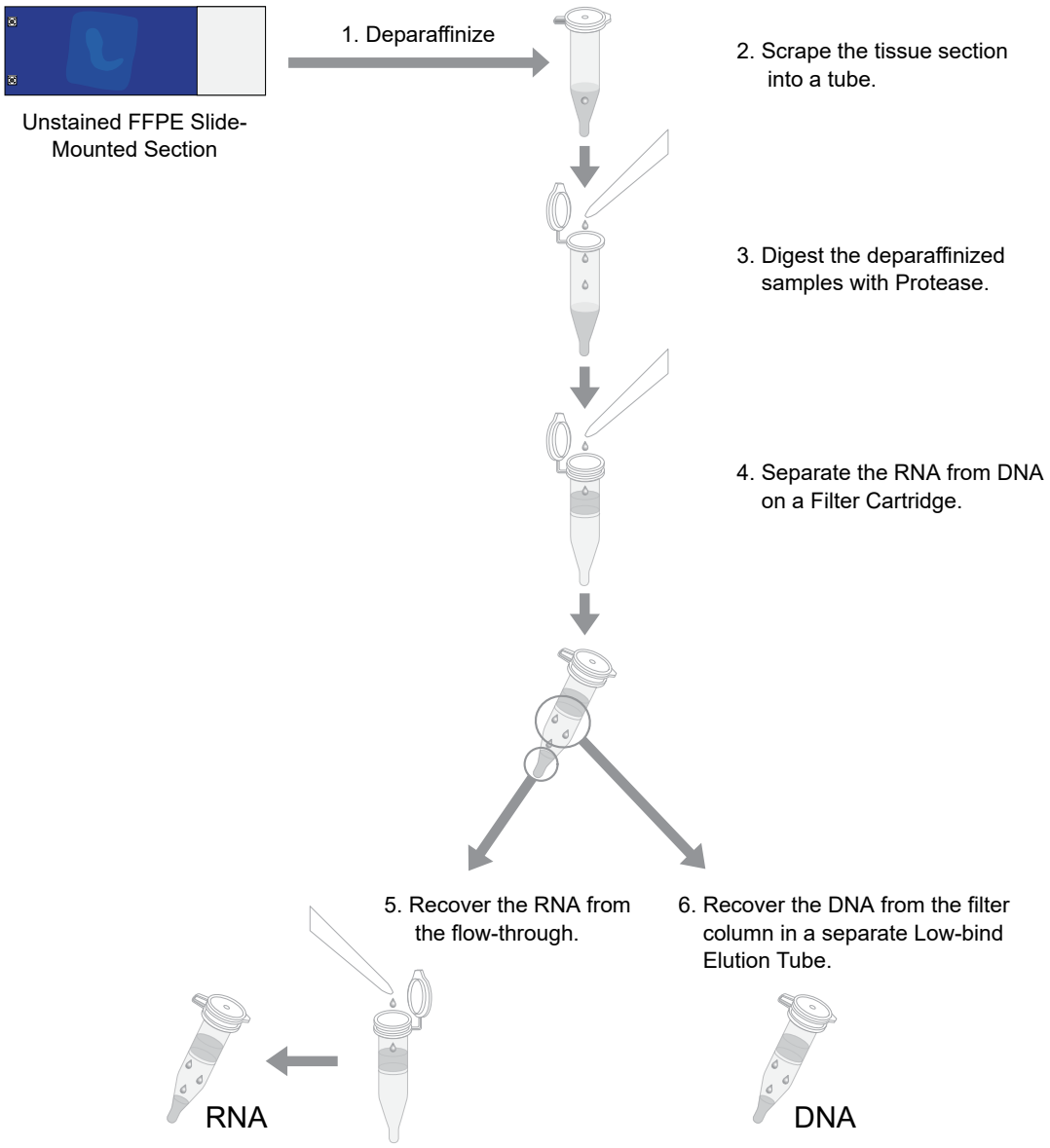
3. Click **Extract**. In the dialog box, scan the barcode that is printed on the Ion Torrent Dx Total Nucleic Acid Isolation Kit (box 1 of 2, Part No. A32434).

IMPORTANT! Check the expiration date on the box. If the kit is expired, select another kit.

4. Click **Save**.
The sample is no longer listed in the **To Be Extracted** list.

4 Isolate RNA and DNA from FFPE samples

Sample extraction workflow



Review the procedural guidelines

Before you begin the procedures in this section, review the procedural guidelines (see "Procedural guidelines" on page 16).

Ion Torrent Dx FFPE Sample Preparation Kit component lot matching

The six components of the Ion Torrent Dx FFPE Sample Preparation Kit must be lot-matched with each other for use.

Component	Part No.	Storage
Ion Torrent Dx Total Nucleic Acid Isolation Kit (box 1 of 2)	A32434	–30°C to –10°C
Ion Torrent Dx Total Nucleic Acid Isolation Kit (box 2 of 2)	A32435	15°C to 30°C
Ion Torrent Dx DNA Quantification Kit	A32437	2°C to 8°C
Ion Torrent Dx RNA Quantification Kit	A32438	2°C to 8°C
Ion Torrent Dx cDNA Synthesis Kit	A32436	–30°C to –10°C
Ion Torrent Dx Sample Dilution Kit	A32439	15°C to 30°C

Equilibrate the equipment and reagents

Before starting the following procedure:

- Equilibrate a benchtop cold box in a –30°C to –10°C freezer for at least 24 hours before use.

  Note: The cold box holds temperature for up to 1 hour on the bench.

- Equilibrate aluminum cold blocks for 96-well plates at 2–8°C before use.
- Power on three dry-bath heaters with aluminum heat blocks 45 minutes before starting the procedure. Set the heaters to 55°C, 90°C, and 95°C.

CAUTION! Use care when working near the heat block to avoid being burned.

Note: Ensure that the heaters are calibrated.

- Remove the Protease and DNase from the freezer, then place them in a cold box equilibrated at –30°C to –10°C.

- Thaw the 10X DNase Buffer at room temperature (15°C to 30°C) and hold at room temperature until use.
- If you plan to quantify the DNA and RNA immediately after the extraction, equilibrate the quantification kit reagents (except the standards) to room temperature for at least 30 minutes before performing the assays.

Prepare wash buffers

Prepare the following buffers before using the Ion Torrent Dx FFPE Sample Preparation Kit. These buffers only need to be prepared once for each kit.

1. To prepare Wash 1 Buffer, add 14 mL of ACS grade 100% ethanol (EtOH) to the bottle labeled "Wash 1 Concentrate". Cap the bottle tightly and mix well by inverting the bottle 5 times.
2. To prepare Wash 2 Buffer, add 14 mL of ACS grade 100% ethanol to the bottle labeled "Wash 2 Concentrate". Cap the bottle tightly and mix well by inverting the bottle 5 times.
3. Mark the bottle labels to indicate that ethanol has been added ("+EtOH," initials, and date). Store the reconstituted Wash 1 and 2 Buffers at room temperature.

Deparaffinize and digest samples

Kit components used in this procedure

Kit component	Box
Digestion Buffer (green cap) Dilution Solution (black cap)	Ion Torrent Dx Total Nucleic Acid Isolation Kit box 2 of 2 (Part No. A32435, stored at 15°C to 30°C)
Protease (blue cap)	Ion Torrent Dx Total Nucleic Acid Isolation Kit box 1 of 2 (Part No. A32434, stored at –30°C to –10°C)

Prepare 1X Digestion Buffer

1. Label a nuclease-free 1.5-mL low-retention microcentrifuge tube for each FFPE tissue sample. Label each tube (cap and side) with its Sample ID using a marker that is resistant to xylene and ethanol.
2. Vortex the Digestion Buffer (green cap) and Dilution Solution (black cap) supplied in the kit for ~5 seconds each, then pulse centrifuge to collect the contents.

3. In a separate 1.5-mL low-retention microcentrifuge tube, prepare a master mix of the 1X Digestion Buffer as follows, where "n" is the number of tissue samples:

	Component	Volume per reaction	
		For ≤6 samples	For ≥7 samples
☐	Digestion Buffer (green cap)	(n+1) × 25 µL	(n+2) × 25 µL
☐	Dilution Solution (black cap)	(n+1) × 75 µL	(n+2) × 75 µL
☐	**Total 1X Digestion Buffer**	**(n+1) × 100 µL**	**(n+2) × 100 µL**

4. Vortex the 1X Digestion Buffer for ~5 seconds to mix, then pulse centrifuge to collect.

5. Add 100 µL of 1X Digestion Buffer to each labeled tube from step 1.

Deparaffinize dipped FFPE slides

WARNING! Xylene is a toxic substance. Read the safety data sheet provided by the manufacturer. Handle it only in a well-ventilated area using personal protection equipment, and discard the waste according to regulations.

IMPORTANT! These instructions are only for paraffin-dipped FFPE slides. For slides that have not been dipped in paraffin, see "Deparaffinize undipped FFPE slides" on page 31.

Note:
- Use fresh xylene and fresh ACS-grade 100% ethanol after two rounds of deparaffinization with dipped slides. Each jar should have ~400 mL of either xylene or ethanol and be clearly marked with the date and initials after replacing the solutions.
- Perform the following steps carefully to avoid tissue loss.

1. Scrape any excess paraffin from each slide.
   a. Grasp the slide at the slide label, and firmly hold the slide in an upright vertical position with the bottom oriented on the lab-bench paper.
   b. Using a sterile disposable scalpel, scrape the layer of paraffin from the back of the slide. Use even pressure to scrape the back from top to bottom. Repeat if necessary to remove all the paraffin.

      IMPORTANT! Use light pressure to prevent cracking the slide.

   c. If the tissue section cannot be visualized, do not perform this step. Turn the slide so the label and tissue face the operator. Carefully scrape around the tissue section to remove the paraffin.

      Note: Scrape away from the tissue section to avoid accidentally removing the section itself.

   d. Repeat steps a–c for each slide, using a new scalpel for each unique sample.

      Note: Properly discard used scalpels.

2. Fill a staining dish or jar with ~400 mL of xylene.
3. Place the slides in a slide rack, then completely submerge the rack in the xylene for 5 minutes at room temperature.
4. Incubate the slides for 30 minutes at room temperature. At ~10-minute intervals, lift the rack up and down 3 times to mix.
5. Remove the rack, then drain any excess xylene solution by tilting the rack.
6. Fill a staining dish or jar with ~400 mL of fresh xylene, then completely submerge the slide rack.
7. Incubate the slides for 15 minutes at room temperature. After ~7.5 minutes, lift the rack up and down 3 times to mix.
8. Remove the slides, then drain any excess xylene solution by tilting the slide holder.
9. Inspect the slides. If any paraffin remains, repeat steps 6–8 one more time.
10. Fill a staining dish or jar with ~400 mL of 100% ethanol.
11. Completely submerge the slides in the rack in the 100% ethanol for 5 minutes at room temperature.
12. Remove the rack, then drain any excess ethanol by tilting the rack.
13. Touch the edge of each slide with a clean laboratory wipe to wick any remaining ethanol from the surface, then lay the slide (section-side up) on a clean laboratory wipe.
14. Air dry each slide for at least 15 minutes.

    Note: The drying time can vary depending on the section size. Ensure that there are no droplets on the tissue section before scraping.

15. Proceed to "Collect the tissue" on page 32.

Deparaffinize undipped FFPE slides

WARNING! Xylene is a toxic substance. Read the safety data sheet provided by the manufacturer. Handle it only in a well-ventilated area using personal protection equipment, and discard the waste according to regulations.

IMPORTANT! These instructions are only for FFPE slides that have not been dipped in paraffin. For slides that have been dipped in paraffin, see "Deparaffinize dipped FFPE slides" on page 30.

Note:

- Use fresh xylene and fresh ACS-grade 100% ethanol each day. Each jar should have ~400 mL of either xylene or ethanol and be clearly marked with the date and initials after replacing the solutions.
- Perform the following steps carefully to avoid tissue loss.

1. Fill a staining dish or jar with ~400 mL of xylene.
2. Place the slides with the unstained FFPE tissue sections in a slide rack, then completely submerge the rack in the xylene for 5 minutes at room temperature.
3. Remove the rack, then drain any excess xylene solution by tilting the rack.
4. Inspect the slides. If any paraffin remains, repeat steps 1–2 one more time.
5. Fill a staining dish or jar with ~400 mL of 100% ethanol.
6. Completely submerge the slides in the rack in the 100% ethanol for 5 minutes at room temperature.
7. Remove the rack, then drain any excess ethanol by tilting the rack.
8. Touch the edge of each slide to a clean laboratory wipe to wick any remaining ethanol from the surface, then lay the slide (section-side up) on a clean laboratory wipe.
9. Air dry each slide for at least 15 minutes.

   Note: The drying time can vary depending on the section size. Ensure that there are no droplets on the tissue section before scraping.

10. Proceed to "Collect the tissue".

Collect the tissue

IMPORTANT! Before proceeding, review the tissue input requirements in "Tissue input requirements for FFPE sample extraction" on page 15 and "Guidelines for FFPE samples" on page 17.

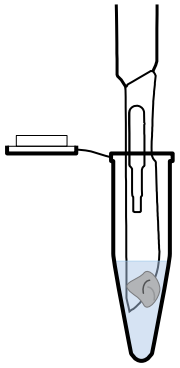
In this procedure, scrape each tissue section into the appropriate labeled 1.5-mL low-retention microcentrifuge tube containing 1X Digestion Buffer (prepared in "Prepare 1X Digestion Buffer" on page 29).

1. Pipet 4 µL of 1X Digestion Buffer from the labeled tube evenly across the fixed tissue section on the slide to pre-wet the tissue section.

   Note: Larger sections may need an additional 4 µL of Digestion Buffer.

2. Using a sterile disposable scalpel, scrape the tissue in a single direction, then collect the tissue into a cohesive mass on the tip of the scalpel blade.

3. Carefully insert the scalpel blade with the tissue mass into the 1X Digestion Buffer in the 1.5-mL low-retention microcentrifuge tube. Rinse the tissue from the blade into the buffer, then ensure that the entire mass is in solution.



4. Remove and inspect the blade to ensure that no tissue remains on it.

5. Inspect the slide to ensure that all the tissue has been removed (the slide should be translucent). Discard the scalpel in a waste container for sharp objects.

Digest the deparaffinized samples

1. Flick-mix the Protease (blue cap) 4 times with your finger, then pulse centrifuge to collect the contents.

2. Add 4 µL of Protease to each tissue sample tube.

3. Flick-mix each sample tube 4 times, then pulse centrifuge.

4. Incubate the samples at 55°C in a calibrated heat block for 1 hour.

   Note: During incubation, proceed to "Label the Filter Cartridges and Collection Tubes" and "Preheat the Elution Solution" to save time.

5. Pulse centrifuge to collect any condensation droplets.

6. Incubate the samples at 90°C in a calibrated heat block for 1 hour.

7. Pulse centrifuge to collect any condensation droplets, then proceed immediately to "Separate RNA from DNA on a Filter Cartridge" on page 35.

Separate RNA from DNA

Kit components used in this procedure

Kit component	Box
Filter Cartridges[1] Collection Tubes Low-bind Elution Tubes Elution Solution (red cap) Isolation Additive (brown cap)	Ion Torrent Dx Total Nucleic Acid Isolation Kit box 2 of 2 (Part No. A32435, stored at 15°C to 30°C)

[1] Includes a filter column pre-inserted in a Collection Tube.

Label the Filter Cartridges and Collection Tubes

Note: To save time, label sets of Filter Cartridges (filter column + Collection Tube) and Collection Tubes (tube only) in advance. Use ethanol-resistant markers for labeling. Do not write on the side of the filter column, because the ink may bleed into the sample.

For each FFPE tissue sample, label the following cartridges and tubes as indicated for use in the subsequent DNA and RNA extraction steps:

- Filter Cartridges (2)
- Collection Tubes (1)
- Low-bind Elution Tubes (2)

Component	Label		Material collected	
	Filter column cap	Tube	Filter	Tube
Filter Cartridge (A)	Sample ID and "DNA"	Sample ID and "RNA"	Bound DNA	Flow-through RNA
Collection Tube (B)	—	Sample ID and "DNA Wash"	—	DNA wash
Filter Cartridge (C)	Sample ID and "RNA"	Sample ID and "RNA Wash"	Bound RNA	RNA wash
Low-bind Elution Tube (D)	—	Sample ID, "RNA," date, and operator initials	—	Eluted RNA
Low-bind Elution Tube (E)	—	Sample ID, "DNA," date, and operator initials	—	Eluted DNA

Preheat the Elution Solution

1. For each sample, pipet 125 µL of Elution Solution (red cap) into a 1.5-mL low-retention microcentrifuge tube.
2. Place the tube(s) of Elution Solution in the 95°C heat block for at least 5 minutes. Keep the Elution Solution in the heat block throughout the following procedure.

CAUTION! Use care when working near the heat block to avoid being burned.

Note: A tube rack may be placed on top of the tubes to prevent the tubes from popping open.

Separate RNA from DNA on a Filter Cartridge

Use the following previously labeled Filter Cartridges and Collection Tubes for the following procedure:

Component	Label		Material collected	
	Filter column cap	Tube	Filter	Tube
Filter Cartridge (A)	Sample ID and "DNA"	Sample ID and "RNA"	Bound DNA	Flow-through RNA
Collection Tube (B)	—	Sample ID and "DNA Wash"	—	DNA wash

1. Place the labeled Filter Cartridge (A) in a tube rack.
2. Add 120 µL of the Isolation Additive (brown cap) to the digested sample, then mix by pipetting up and down 5 times. The sample appears slightly cloudy.
3. Transfer the digested sample and Isolation Additive mix (~224 µL) to the Filter Cartridge, then close the lid.
4. Centrifuge the Filter Cartridge at 10,000 rcf for 30 seconds in a microcentrifuge.

   IMPORTANT! Do not discard the flow-through in the Collection Tube (labeled with Sample ID and "RNA"). The flow-through contains the RNA.
5. Place the filter column with the bound DNA in a new Collection Tube (B), then store it at 2–8°C for later DNA purification in "Recover the DNA from the Filter Cartridge" on page 40.
6. Proceed to "Recover the RNA from the flow-through" on page 36.

Recover the RNA from the flow-through

Kit components used in this procedure

Kit component	Source/Box
Filter Cartridges Collection Tubes Low-bind Elution Tubes	Previously labeled
Wash 1 Buffer Wash 2 Buffer	Previously prepared from concentrate
Dilution Solution (black cap)	Ion Torrent Dx Total Nucleic Acid Isolation Kit box 2 of 2 (Part No. A32435, stored at 15°C to 30°C)
10X DNase Buffer (white cap) DNase (purple cap)	Ion Torrent Dx Total Nucleic Acid Isolation Kit box 1 of 2 (Part No. A32434, stored at –30°C to –10°C)

Bind the RNA to the Filter Cartridge

Use the following previously labeled Filter Cartridge for the following procedure:

Component	Label		Material collected	
	Filter column cap	Tube	Filter	Tube
Filter Cartridge (C)	Sample ID and "RNA"	Sample ID and "RNA Wash"	Bound RNA	RNA wash

1. Place the new Filter Cartridge (C) in a tube rack.
2. Add 275 µL of ACS-grade 100% ethanol to the flow-through containing RNA (the tube labeled with the Sample ID and "RNA") from “Separate RNA from DNA on a Filter Cartridge” on page 35.
3. Mix well by pipetting up and down 5 times, then transfer the sample (~450 µL) to the new Filter Cartridge (C).
4. Centrifuge the Filter Cartridge at 10,000 rcf for 30 seconds in a microcentrifuge.
5. Discard the flow-through in the Collection Tube, then reinsert the filter column into the same Collection Tube.
6. Add 600 µL of Wash 1 Buffer (prepared in “Prepare wash buffers” on page 29) to the Filter Cartridge.
7. Centrifuge the Filter Cartridge at 10,000 rcf for 30 seconds.

8. Discard the flow-through, then reinsert the filter column in the same Collection Tube.

9. Centrifuge the Filter Cartridge at 10,000 rcf for 30 seconds to remove any remaining fluid.

Treat the RNA bound to the Filter Cartridge with DNase

1. In a 1.5-mL low-retention microcentrifuge tube, prepare a master mix of 1X DNase Solution as follows, where "n" is the number of samples you are preparing.

	Component	Volume per reaction	
		For ≤6 samples	For ≥7 samples
☐	Dilution Solution (black cap)	(n+1) × 50 µL	(n+2) × 50 µL
☐	10X DNase Buffer (white cap)	(n+1) × 6 µL	(n+2) × 6 µL
☐	DNase (purple cap)	(n+1) × 4 µL	(n+2) × 4 µL
☐	**Total Volume**	**(n+1) × 60 µL**	**(n+2) × 60 µL**

2. Flick the 1X DNase Solution tube 4 times to mix, then pulse centrifuge to collect.

3. Pipet 60 µL of the 1X DNase Solution into the center of each filter column (previously labeled with Sample ID and "RNA").

IMPORTANT! To avoid puncturing, do **NOT** touch the pipette tip to the filter.

4. Hold the Filter Cartridge at room temperature for 30 minutes.

Note: If you plan to quantify the DNA and RNA immediately after the extraction, begin equilibrating the following reagents from the quantification kits at room temperature for at least 30 minutes:

- DNA Dye Reagent (blue cap)
- DNA Buffer (white cap)
- RNA Dye Reagent (green cap)
- RNA Buffer (blue cap)

Wash the RNA bound to the Filter Cartridge

Use the following previously labeled Filter Cartridges and Low-bind Elution Tubes for the following procedure.

Component	Label		Material collected	
	Filter column cap	Tube	Filter	Tube
Filter Cartridge (C)	Sample ID and "RNA"	Sample ID and "RNA Wash"	Bound RNA	RNA wash
Low-bind Elution Tube (D)	—	Sample ID, "RNA," date, and operator initials	—	Eluted RNA

1. Add 600 µL of Wash 1 Buffer to the Filter Cartridge (C).
2. Hold the Filter Cartridge for 30 seconds at room temperature, then centrifuge the Filter Cartridge at 10,000 rcf for 30 seconds.
3. Discard the flow-through, then reinsert the filter column in the same Collection Tube.
4. Add 500 µL of Wash 2 Buffer (prepared in "Prepare wash buffers" on page 29) to the Filter Cartridge, then centrifuge the Filter Cartridge at 10,000 rcf for 30 seconds.
5. Discard the flow-through, then reinsert the filter column into the same Collection Tube.
6. Repeat steps 4 and 5 for a second wash.
7. Centrifuge the Filter Cartridge at 20,000–21,000 rcf for 2 minutes to remove any remaining fluid.
8. Remove the filter column from the tube, then touch the bottom of the column with a clean laboratory wipe to wick off any remaining wash buffer.
9. Transfer the filter column to the pre-labeled Low-bind Elution Tube (D).

Elute the RNA

Use the following components for this procedure.

Component	Label		Material collected	
	Filter column cap	Tube	Filter	Tube
Filter column with bound RNA from Filter Cartridge (C)	Sample ID and "RNA"	—	Bound RNA	—
Low-bind Elution Tube (D)	—	Sample ID, "RNA," date, and operator initials	—	Eluted RNA

IMPORTANT!

- Keep the 1.5-mL low-retention microcentrifuge tube containing preheated Elution Solution in the heat block throughout the procedure to maintain a 95°C temperature.
- Change pipette tips between samples when pipetting Elution Solution across multiple samples.

1. Remove the Elution Solution from the heat block, and pulse centrifuge the tube to collect the contents. Return the tube to the heat block.

CAUTION! The heat block and Elution Solution are hot. Use care when handling tubes and tube contents to avoid being burned.

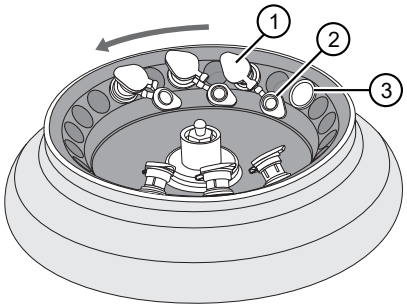
2. Wet the pipette tip by slowly pipetting up and down 3 times in the pre-heated Elution Solution.

3. Slowly pipet up 30 µL of Elution Solution, then confirm that there are no large air gaps in the tip (a small air gap at the bottom of the tip is acceptable). Pipet the solution into the center of the filter column in the Low-bind Elution Tube (D) (from step 9 in "Wash the RNA bound to the Filter Cartridge").

   IMPORTANT! To avoid puncturing the filter, do not touch it with the pipette tip.

4. Close the cap on the filter column, then hold the filter column/Low-bind Elution Tube assembly at room temperature for 1 minute. Close the cap on the Elution Solution tube in the heat block.

5. Insert the filter column/Low-bind Elution Tube assembly in the microcentrifuge in the orientation shown below. To prevent the Low-bind Elution Tube caps from breaking, place a 0.2-mL tube adapter in the position shown.



① Filter column cap (closed)
② Low-bind Elution Tube cap (open)
③ 0.2-mL tube adapter

6. Centrifuge at 20,000–21,000 rcf for 1 minute.

   Note: The eluted RNA is in the Low-bind Elution Tube. If the tube cap breaks in the centrifuge, transfer the sample to a new labeled Low-bind Elution Tube.

7. Discard the filter column.

8. Temporarily store the sample at 2–8°C if quantifying on the same day.

   STOPPING POINT If you are not quantifying on the same day, store the recovered RNA aliquots at –90°C to –60°C for up to 12 months.

Recover the DNA from the Filter Cartridge

Wash the DNA bound to the Filter Cartridge

Use the following pre-labeled Filter Cartridges and tubes for the following protocol:

Component	Label		Material collected	
	Filter column cap	Tube	Filter	Tube
Filter Cartridge (A)	Sample ID and "DNA"	Sample ID and "RNA"	Bound DNA	Flow-through RNA
Collection Tube (B)	—	Sample ID and "DNA Wash"	—	DNA wash
Low-bind Elution Tube (E)	—	Sample ID, "DNA," date, and operator initials	—	Eluted DNA

1. Retrieve the Filter Cartridge (A) with bound DNA and Collection Tube (B) from 2–8°C storage (previously stored in "Separate RNA from DNA on a Filter Cartridge" on page 35).
2. Add 600 µL of Wash 1 Buffer to the filter column.
3. Hold the Filter Cartridge for 30 seconds at room temperature, then centrifuge at 10,000 rcf for 30 seconds.
4. Discard the flow-through, then reinsert the filter column into the same Collection Tube (B).
5. Add 500 µL of Wash 2 Buffer to the filter column, then centrifuge at 10,000 rcf for 30 seconds.
6. Discard the flow-through, then reinsert the filter column into the same Collection Tube (B).
7. Repeat steps 5 and 6 for a second wash.
8. Centrifuge the Filter Cartridge at 20,000–21,000 rcf for 2 minutes to remove any remaining fluid.
9. Remove the filter column from the tube, then touch the bottom of the column with a clean laboratory wipe to wick off any remaining wash buffer.
10. Transfer the filter column to the pre-labeled Low-bind Elution Tube (E).

Elute the DNA

Use the following components for this procedure.

Component	Label		Material collected	
	Filter column cap	Tube	Filter	Tube
Filter column with bound DNA from Filter Cartridge (A)	Sample ID and "DNA"	—	Bound DNA	—
Low-bind Elution Tube (E)	—	Sample ID, "DNA," date, and operator initials	—	Eluted DNA

IMPORTANT!

- Keep the 1.5-mL low-retention microcentrifuge tube containing preheated Elution Solution in the heat block throughout the procedure to maintain a 95°C temperature.
- Change pipette tips between samples when pipetting Elution Solution across multiple samples.

1. Remove the Elution Solution from the heat block, and pulse centrifuge the tube to collect the contents. Return the tube to the heat block.

CAUTION! The heat block and Elution Solution are hot. Use care when handling tubes and tube contents to avoid being burned.

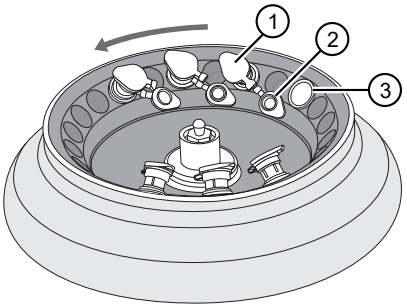
2. Wet the pipette tip by slowly pipetting up and down 3 times in the pre-heated Elution Solution.

3. Slowly pipet up 30 µL of Elution Solution, then confirm that there are no large air gaps in the tip (a small air gap at the bottom of the tip is acceptable). Pipet the solution into the center of the filter column in the Low-bind Elution Tube (E) (from step 10 in "Wash the DNA bound to the Filter Cartridge").

   IMPORTANT! To avoid puncturing the filter, do not touch it with the pipette tip.

4. Close the cap on the filter column, then hold the filter column/Low-bind Elution Tube assembly at room temperature for 1 minute. Close the cap on the Elution Solution tube in the heat block.

5. Insert the filter column/Low-bind Elution Tube assembly in the microcentrifuge in the orientation shown below. To prevent the Low-bind Elution Tube caps from breaking, place a 0.2-mL tube adapter in the position shown.



① Filter column cap (closed)
② Low-bind Elution Tube cap (open)
③ 0.2-mL tube adapter

6. Centrifuge at 20,000–21,000 rcf for 1 minute.

Note: The eluted DNA is in the Low-bind Elution Tube. If the tube cap breaks in the centrifuge, transfer the sample to a new labeled Low-bind Elution Tube.

7. Discard the filter column.

8. Temporarily store the sample at 2–8°C if quantifying on the same day.

STOPPING POINT If you are not quantifying on the same day, store the recovered DNA aliquots at –30°C to –10°C for up to 12 months.

5 DNA and RNA quantification

Review the procedural guidelines

Before you begin the procedures in this section, review the procedural guidelines (see "Procedural guidelines" on page 16).

Prepare the reagents and equipment

- If the DNA and RNA samples were frozen for storage, thaw them at room temperature until no ice crystals are present, then transfer them to 2–8°C storage until use.
- Equilibrate a benchtop cold box at –30°C to –10°C for at least 24 hours before use.

  Note: The cold box holds temperature for up to 1 hour on the bench.

- Equilibrate the DNA Dye Reagent, RNA Dye Reagent, DNA Buffer , and RNA Buffer to room temperature for at least 30 minutes before use.
- Keep the DNA Std and RNA Std at 2–8°C, until the working plate has been set up and the standards are ready to be added.
- If necessary, set up the fluorometer/fluorescence reader to read the appropriate excitation and emission wavelengths:

Dye reagent	Excitation (nm)	Emission (nm)
RNA Dye Reagent	620/15	680/30
DNA Dye Reagent	485/20	528/20

Set up the DNA quantification assay

Kit components used in this procedure

Kit component	Box
DNA Dye Reagent (blue cap) DNA Buffer (white cap) DNA Std - 0 ng/µL(white cap) DNA Std - 0.5 ng/µL (green cap) DNA Std - 4 ng/µL (red cap) DNA Std - 10 ng/µL (yellow cap)	Ion Torrent Dx DNA Quantification Kit (Part No. A32437, stored at 2°C to 8°C)

Prepare the DNA Working Solution

1. Determine the number of DNA standards to use with your quantification system.

   IMPORTANT! We recommend using 4 standards. If your quantification system does not allow the use of 4 standards, use the maximum allowed by the system. At a minimum, you must use the 0 ng/µL and 10 ng/µL DNA standards. Note that R^2 values should only be evaluated when 3 or more standards are used.

2. Calculate the number of reactions using the following formula:

   S (# of standards) + N (# of samples) +1 = # of reactions

3. Calculate the total volume of DNA Dye Reagent and DNA Buffer required for the number of reactions:

   # reactions × 1 µL (DNA Dye Reagent) = total volume of DNA Dye Reagent

   # reactions × 199 µL (DNA Buffer) = total volume of DNA Buffer

4. Mix the DNA Buffer and DNA Dye Reagent bottles by inverting 5 times.

5. Prepare the DNA Working Solution: Pipet the calculated volume of DNA Buffer into a pre-labeled tube, then add the calculated volume of DNA Dye Reagent into the same tube.

6. Vortex the tube for ~5 seconds, then proceed to the next steps.

   IMPORTANT! If you are not immediately proceeding to the next steps, protect the DNA Working Solution from light. The DNA Working Solution must be used within 3 hours.

Prepare the DNA standards

1. Add 190 µL of DNA Working Solution to each well or tube that will contain a DNA standard.
2. Vortex each DNA standard for ~5 seconds, then pulse centrifuge. Refer to the following table of DNA standards and concentrations.

Note: If you are using fewer than four standards, at a minimum you must use the 0 ng/µL and 10 ng/µL DNA standards.

Standard	Concentration
DNA STD 1 (white cap)	0 ng/µL
DNA STD 2 (green cap)	0.5 ng/µL
DNA STD 3 (red cap)	4.0 ng/µL
DNA STD 4 (yellow cap)	10 ng/µL

3. Pipet 10 µL of each DNA standard into its designated well or tube.

Prepare the DNA samples

1. Add 196 µL of DNA Working Solution to each well or tube that will contain a DNA sample.
2. Vortex each DNA sample for ~5 seconds, then pulse centrifuge.
3. Pipet 4 µL of each DNA sample into its designated well or tube, then proceed to set up the RNA quantification assay.

Set up the RNA quantification assay

IMPORTANT! Wipe down your work surface and pipettes with an RNase decontamination solution. Change gloves before starting and as needed to maintain RNase-free conditions.

Kit components used in this procedure

Kit component	Box
RNA Dye Reagent (green cap) RNA Buffer (blue cap) RNA Std - 0 ng/µL (teal cap) RNA Std - 0.5 ng/µL (tan cap) RNA Std - 4 ng/µL (purple cap) RNA Std - 10 ng/µL (orange cap)	Ion Torrent Dx RNA Quantification Kit (Part No. A32438, stored at 2°C to 8°C)

Prepare the RNA working solution

1. Determine the number of RNA standards to use with your quantification system.

   IMPORTANT! We recommend using 4 standards. If your quantification system does not allow the use of 4 standards, use the maximum allowed by the system. At a minimum, you must use the 0 ng/µL and 10 ng/µL RNA standards. Note that R^2 values should only be evaluated when 3 or more standards are used.

2. Calculate the number of reactions using the following formula:

   S (# of standards) + N (# of samples) +1 = # of reactions

3. Calculate the total volume of RNA Dye Reagent and RNA Buffer required for the number of reactions:

   # reactions × 1 µL (RNA Dye Reagent) = total volume of RNA Dye Reagent

   # reactions × 199 µL (RNA Buffer) = total volume of RNA Buffer

4. Mix the RNA Buffer and RNA Dye Reagent bottles by inverting five times.

5. Prepare the RNA Working Solution: Pipet the total volume of RNA Buffer into a pre-labeled tube, then add the total volume of RNA Dye Reagent into the same tube.

6. Vortex the tube for ~5 seconds, then proceed to the next steps.

   IMPORTANT! If you are not immediately proceeding to the next steps, protect the RNA Working Solution from light. The RNA Working Solution must be used within 3 hours.

Prepare the RNA standards

1. Add 190 µL of RNA Working Solution to each well or tube that will contain an RNA standard.

2. Flick mix each RNA Standard 4 times, then pulse centrifuge. Refer to the following table of RNA Standards and concentrations.

   Note: If you are using fewer than four standards, at a minimum you must use RNA STD 1 (0 ng/µL) and RNA STD 4 (10 ng/µL).

Standard	Concentration
RNA STD 1 (teal cap)	0 ng/µL
RNA STD 2 (tan cap)	0.5 ng/µL
RNA STD 3 (purple cap)	4.0 ng/µL
RNA STD 4 (orange cap)	10 ng/µL

3. Pipet 10 µL of each RNA Standard into its designated well or tube.

Prepare the RNA samples

1. Add 196 µL of RNA Working Solution to each well or tube that will contain an RNA sample.
2. Flick mix each RNA sample 4 times, then pulse centrifuge.
3. Pipet 4 µL of each RNA sample into its designated well or tube, then proceed to quantification.

Run the quantification assays

1. Incubate the prepared DNA and RNA standards and samples for at least 2 minutes at room temperature before reading.
2. Determine the concentration of the DNA and RNA samples in ng/µL using a fluorometer/fluorescence reader and linear regression of the standards for DNA and RNA respectively.

   The required minimum values for the Oncomine™ Dx Target Test are shown in the following table:

   Table 1 Required sample concentrations and R^2 values from the linear regression of the standards

Sample type	Required concentration	Required R^2 value[1]
DNA	≥0.83 ng/µL	≥0.99
RNA	≥1.43 ng/µL	≥0.98

[1] R^2 values should be evaluated only if the standard curve includes 3 or more points.

IMPORTANT! To proceed with library preparation, both the DNA and RNA concentrations from a single sample extraction must meet the minimum requirements. Do not use DNA from one extraction with RNA from a different extraction.

3. If the samples do not meet the minimum concentration requirements, repeat the extraction with increased tissue input (i.e., more sections) if possible.

STOPPING POINT If you do not dilute the sample on the same day, store the quantified DNA sample at –30°C to –10°C and the quantified RNA sample at –90°C to –60°C (for up to 9 months).

Dilute the samples

Kit components used in this procedure

Kit component	Box
Dilution Solution (black cap)	Ion Torrent Dx Sample Dilution Kit (Part No. A32439, stored at 15°C to 30°C)

Sample Dilution Calculator

The Sample Dilution Calculator is a locked Microsoft™ Excel™ spreadsheet that is provided as an aide to prepare samples for library preparation. The Sample Dilution Calculator provides a uniform method for diluting DNA samples to 0.83 ng/µL and RNA samples to 1.43 ng/µL, including a 10% overage of the final sample dilution volume.

Copy the **SFW0000786_ODxTT_SampleDilution_Calculator_3.02.xlsm** file from the Oncomine™ Dx Target Test media storage device onto your hard drive before use.

IMPORTANT!

- The Sample Dilution Calculator (Part. No. SFW0000786) requires Microsoft™ Excel™ 2010 or later.
- When a security warning appears after opening the Sample Dilution Calculator file, click **Enable Content** in the message bar to enable the dilution calculator macro.

Dilution volume guidelines

- Unless the test or study protocol dictates that multiple libraries must be made from a single sample (for example, replicate libraries), dilute only sufficient sample to prepare a single library.
- The Sample Dilution Calculator includes a 10% overage in its calculations. For manually calculated dilutions, include a 10% overage, except for low-concentration DNA (0.83–0.97 ng/µL) and RNA (1.43–1.93 ng/µL) samples where there is insufficient sample to include an overage.
- The default **Sample Volume** (**X1**) in the Sample Dilution Calculator is 2 µL. If needed, increase the **Sample Volume** (**X1**) until the **Volume of Dilution Solution** (**Y1**) is ≥2 µL. When all volumes are ≥2 µL, red highlighted table cells turn white, indicating sufficient volumes for accurate pipetting.
- In the following procedure, we recommend that you do not pipet volumes <2.0 µL. For samples that are ≥6.01 ng/ µL, perform a normalization dilution to an intermediate concentration of 5 ng/µL, then further dilute the sample to a final concentration of 0.83 ng/µL for DNA and 1.43 ng/µL for RNA.
- Low-concentration DNA (0.83–0.97 ng/µL) and RNA (1.43–1.93 ng/µL) samples require pipetting volumes <2 µL to achieve the correct concentration. When necessary, only pipet volumes <2 µL with a pipette designed and calibrated to dispense volumes <2 µL.
- Do not use the Sample Dilution Calculator for low-concentration DNA (0.83–0.97 ng/µL) and RNA (1.43–1.93 ng/µL) samples. Instead, follow the detailed procedures that are provided in the second row of the tables on page 49 and page 52.
- Samples that do not meet the minimum concentration specification (DNA samples <0.83 ng/µL and RNA samples <1.43 ng/µL) must not be used for library preparation.

Thaw frozen samples

If DNA and RNA samples were frozen for storage, thaw them at room temperature until no ice crystals are present before proceeding. Transfer samples to 2–8°C storage until use.

Note: Freeze-thaw samples no more than 3 times.

Dilute DNA samples

IMPORTANT! Do not perform the following dilution procedures until you are ready to proceed directly to library preparation. Library preparation requires accurate input of 10 ng DNA. Pipetting volumes <2 µL is not recommended. When necessary, only pipet volumes <2 µL with a pipette designed and calibrated to dispense volumes <2 µL.

Library preparation requires dilution of DNA samples to a final concentration of 0.83 ng/µL. See the following table for sample dilution instructions that are based on the starting sample concentration.

DNA concentration	Dilution procedure
<0.83 ng/µL	Samples do not meet the minimum concentration specification and must not be used for library preparation.
0.83–0.97 ng/µL	Dilute the DNA sample to 0.83 ng/µL in a total volume of 12 µL. 1. Determine the volume of DNA sample required (10 ng ÷ Sample concentration ng/µL = N µL). 2. Add Dilution Solution if required: • If N is <12 µL, pipet N µL of DNA sample into a new labeled 1.5-mL low-retention microcentrifuge tube, then add (12 – N µL) of Dilution Solution, for a total volume of 12 µL. • If N = 12 µL, skip to step 4. 3. Pipet up and down 5 times to mix, then pulse centrifuge. 4. Proceed directly to "Dilute RNA samples" on page 52. Do not store the diluted DNA samples for longer than necessary. **Note:** Store the remaining undiluted DNA sample at –30°C to –10°C for up to 9 months.
0.98–6.00 ng/µL	Perform the Direct Dilution to 0.83 ng/µL. 1. Open **SFW0000786_ODxTT_SampleDilution_Calculator_3.02.xlsm** in Microsoft™ Excel™, select the **Calculator** worksheet tab, then enter the **Operator** and **Date Completed** information. 2. In column **B**, enter the **Sample ID**. **Note:** Only alphanumeric characters (0–9 and A to Z), full stops/periods (.), underscores (_), or hyphens (-) are allowed. 3. In column **C**, enter the DNA sample concentration (**C1**) in ng/µL determined in the quantification assay. 4. Pipet the indicated volume (**X3**) of undiluted DNA sample into a new labeled 1.5-mL low-retention microcentrifuge tube, then add the indicated volume of Dilution Solution (**Y3**). 5. Pipet up and down 5 times to mix, then pulse centrifuge. 6. Proceed directly to "Dilute RNA samples" on page 52. Do not store the diluted DNA samples for longer than necessary. **Note:** Store the remaining undiluted DNA sample at –30°C to –10°C for up to 9 months.

(continued)

DNA concentration	Dilution procedure
6.01–9.99 ng/µL	Perform Normalization Dilution #1 and Normalization Dilution #2 to a final concentration of 0.83 ng/µL. **Note:** The default Sample Volume (**X1**) is 2 µL. When all volumes are ≥2 µL, red highlighted table cells turn white, indicating sufficient volumes for accurate pipetting have been met. **1.** Open **SFW0000786_ODxTT_SampleDilution_Calculator_3.02.xlsm** in Microsoft™ Excel™, select the **Calculator** worksheet tab, then enter the **Operator** and **Date Completed** information. **2.** In column **B**, enter the **Sample ID**. **Note:** Only alphanumeric characters (0–9 and A to Z), full stops/periods (.), underscores (_), or hyphens (-) are allowed. **3.** In column **C**, enter the DNA sample concentration (**C1**) in ng/µL determined in the quantification assay. **4.** Increase the value for **Sample Volume** (**X1**) until the **Volume of Dilution Solution** (**Y1**) is ≥2 µL. **5.** Pipet the indicated volume (**X1**) of undiluted DNA sample into a new labeled 1.5-mL low-retention microcentrifuge tube, then add the indicated volume of Dilution Solution (**Y1**). **6.** Pipet up and down 5 times to mix, then pulse centrifuge. **7.** Pipet the indicated volume (**X2**) of diluted DNA sample (5 ng/µL) into a new labeled 1.5-mL low-retention microcentrifuge tube, then add the indicated volume of Dilution Solution (**Y2**). **8.** Pipet up and down 5 times to mix, then pulse centrifuge. **9.** Proceed directly to "Dilute RNA samples" on page 52. Do not store the diluted DNA samples for longer than necessary. **Note:** Store the remaining Normalization Dilution #1 (5 ng/µL) and undiluted DNA sample at –30°C to –10°C for up to 9 months.

(continued)

DNA concentration	Dilution procedure
≥10 ng/µL	Perform Normalization Dilution #1 and Normalization Dilution #2 to a final concentration of 0.83 ng/µL. **Note:** The default Sample Volume (**X1**) is 2 µL. When all volumes are ≥2 µL, red highlighted table cells turn white, indicating sufficient volumes for accurate pipetting have been met. **1.** Open **SFW0000786_ODxTT_SampleDilution_Calculator_3.02.xlsm** in Microsoft™ Excel™, select the **Calculator** worksheet tab, then enter the **Operator** and **Date Completed** information. **2.** In column **B**, enter the **Sample ID**. **Note:** Only alphanumeric characters (0–9 and A to Z), full stops/periods (.), underscores (_), or hyphens (-) are allowed. **3.** In column **C**, enter the DNA sample concentration (**C1**) in ng/µL determined in the quantification assay. **4.** Pipet the indicated volume (**X1**) of undiluted DNA sample into a new labeled 1.5-mL low-retention microcentrifuge tube, then add the indicated volume of Dilution Solution (**Y1**). **5.** Pipet up and down 5 times to mix, then pulse centrifuge. **6.** Pipet the indicated volume (**X2**) of diluted DNA sample (5 ng/µL) into a new labeled 1.5-mL low-retention microcentrifuge tube, then add the indicated volume of Dilution Solution (**Y2**). **7.** Pipet up and down 5 times to mix, then pulse centrifuge. **8.** Proceed directly to "Dilute RNA samples" on page 52. Do not store the diluted DNA samples for longer than necessary. **Note:** Store the remaining Normalization Dilution #1 (5 ng/µL) and undiluted DNA sample at –30°C to –10°C for up to 9 months.

Dilute RNA samples

IMPORTANT! Do not perform the following dilution procedures until you are ready to proceed directly to library preparation. Library preparation requires accurate input of 10 ng RNA. Pipetting volumes <2 µL is not recommended. When necessary, only pipet volumes <2 µL with a pipette designed and calibrated to dispense volumes <2 µL.

Library preparation requires dilution of RNA samples to a final concentration of 1.43 ng/µL. See the following table for sample dilution instructions that are based on the sample starting concentration.

RNA concentration	Dilution procedure
<1.43 ng/µL	Samples do not meet the minimum concentration specification and must not be used for library preparation.
1.43–1.93 ng/µL	Dilute the RNA sample to 1.43 ng/µL in a total volume of 7 µL. **1.** Determine the volume of RNA sample required (10 ng ÷ Sample concentration ng/µL = N µL). **2.** Add Dilution Solution if required: • If N is <7 µL, pipet N µL of RNA sample into a new labeled 1.5-mL low-retention microcentrifuge tube, then add (7 – N µL) of Dilution Solution, for a total volume of 7 µL. • If N = 7 µL, skip to step 4. **3.** Pipet up and down 5 times to mix, then pulse centrifuge. **4.** Proceed directly to "Reverse transcribe the RNA" on page 56. Do not store the diluted RNA samples for longer than necessary. **Note:** Store the remaining undiluted RNA sample at –30°C to –10°C for up to 9 months.
1.94–6.00 ng/µL	Perform the Direct Dilution to 1.43 ng/µL. **1.** Open **SFW0000786_ODxTT_SampleDilution_Calculator_3.02.xlsm** in Microsoft™ Excel™, select the **Calculator** worksheet tab, then enter the **Operator** and **Date Completed** information. **2.** In column **B**, enter the **Sample ID**. **Note:** Only alphanumeric characters (0–9 and A to Z), full stops/periods (.), underscores (_), or hyphens (-) are allowed. **3.** In column **C**, enter the RNA sample concentration (**C1**) in ng/µL determined in the quantification assay. **4.** Pipet the indicated volume (**X3**) of undiluted RNA sample into a new labeled 1.5-mL low-retention microcentrifuge tube, then add the indicated volume of Dilution Solution (**Y3**). **5.** Pipet up and down 5 times to mix, then pulse centrifuge. **6.** Proceed directly to "Reverse transcribe the RNA" on page 56. Do not store the diluted RNA samples for longer than necessary. **Note:** Store the remaining undiluted RNA sample at –30°C to –10°C for up to 9 months.

(continued)

RNA concentration	Dilution procedure
6.01–9.99 ng/µL	Perform Normalization Dilution #1 and Normalization Dilution #2 to a final concentration of 1.43 ng/µL. **Note:** The default Sample Volume (**X1**) is 2 µL. When all volumes are ≥2 µL, red highlighted table cells turn white, indicating sufficient volumes for accurate pipetting have been met. **1.** Open **SFW0000786_ODxTT_SampleDilution_Calculator_3.02.xlsm** in Microsoft™ Excel™, select the **Calculator** worksheet tab, then enter the **Operator** and **Date Completed** information. **2.** In column **B**, enter the **Sample ID**. **Note:** Only alphanumeric characters (0–9 and A to Z), full stops/periods (.), underscores (_), or hyphens (-) are allowed. **3.** In column **C**, enter the RNA sample concentration (**C1**) in ng/µL determined in the quantification assay. **4.** Increase the value for **Sample Volume** (**X1**) until the **Volume of Dilution Solution** (**Y1**) is ≥2 µL. **5.** Pipet the indicated volume (**X1**) of undiluted RNA sample into a new labeled 1.5-mL low-retention microcentrifuge tube, then add the indicated volume of Dilution Solution (**Y1**). **6.** Pipet up and down 5 times to mix, then pulse centrifuge. **7.** Pipet the indicated volume (**X2**) of diluted RNA sample (5 ng/µL) into a new labeled 1.5-mL low-retention microcentrifuge tube, then add the indicated volume of Dilution Solution (**Y2**). **8.** Pipet up and down 5 times to mix, then pulse centrifuge. **9.** Proceed directly to "Reverse transcribe the RNA" on page 56. Do not store the diluted RNA samples for longer than necessary. **Note:** Store the remaining Normalization Dilution #1 (5 ng/µL) and undiluted RNA sample at –30°C to –10°C for up to 9 months.

(continued)

RNA concentration	Dilution procedure
≥10 ng/µL	Perform Normalization Dilution #1 and Normalization Dilution #2 to a final concentration of 1.43 ng/µL. **Note:** The default Sample Volume (**X1**) is 2 µL. When all volumes are ≥2 µL, red highlighted table cells turn white, indicating sufficient volumes for accurate pipetting have been met. **1.** Open **SFW0000786_ODxTT_SampleDilution_Calculator_3.02.xlsm** in Microsoft™ Excel™, select the **Calculator** worksheet tab, then enter the **Operator** and **Date Completed** information. **2.** In column **B**, enter the **Sample ID**. **Note:** Only alphanumeric characters (0–9 and A to Z), full stops/periods (.), underscores (_), or hyphens (-) are allowed. **3.** In column **C**, enter the RNA sample concentration (**C1**) in ng/µL determined in the quantification assay. **4.** Pipet the indicated volume (**X1**) of undiluted RNA sample into a new labeled 1.5-mL low-retention microcentrifuge tube, then add the indicated volume of Dilution Solution (**Y1**). **5.** Pipet up and down 5 times to mix, then pulse centrifuge. **6.** Pipet the indicated volume (**X2**) of diluted RNA sample (5 ng/µL) into a new labeled 1.5-mL low-retention microcentrifuge tube, then add the indicated volume of Dilution Solution (**Y2**). **7.** Pipet up and down 5 times to mix, then pulse centrifuge. **8.** Proceed directly to "Reverse transcribe the RNA" on page 56. Do not store the diluted RNA samples for longer than necessary. **Note:** Store the remaining Normalization Dilution #1 (5 ng/µL) and undiluted RNA sample at –30°C to –10°C for up to 9 months.

6 Prepare the cDNA

Review the procedural guidelines

Before you begin the procedures in this section, review the procedural guidelines (see "Procedural guidelines" on page 16).

Kit components used in this procedure

Kit component	Box
5X Reaction Mix (red cap) 10X Enzyme Mix (green cap)	Ion Torrent Dx cDNA Synthesis Kit (Part No. A32436, stored at –30°C to –10°C)
Oncomine™ Dx Target RNA Control (white cap; single-use tubes)	Oncomine™ Dx Target RNA Control (Part No. A32443, stored at –90°C to –60°C)
Oncomine™ Dx Target RNA Control Diluent (blue cap; single-use tubes)	Oncomine™ Dx Target RNA Control Diluent (Part No. A38872, stored at –90°C to –60°C)
No Template Control (purple cap)	Ion Torrent Dx No Template Control Kit (Part No. A32444, stored at 15°C to 30°C)

Oncomine™ Dx Target Test Controls lot matching

The following controls and diluent must be lot-matched with each other for use.

Component	Part No.	Storage
Oncomine™ Dx Target DNA Control	A44913	–30°C to –10°C
Oncomine™ Dx Target RNA Control	A32443	–90°C to –60°C
Oncomine™ Dx Target RNA Control Diluent	A38872	–90°C to –60°C
Ion Torrent Dx No Template Control Kit	A32444	15°C to 30°C

Thaw then dilute frozen samples

If DNA and RNA samples were frozen for storage, thaw them at room temperature until no ice crystals are present, then dilute them as described in "Dilute the samples" on page 47 before proceeding. Transfer diluted samples to 2–8°C storage until use.

Note: Freeze-thaw samples no more than 3 times.

Reverse transcribe the RNA

Perform the following steps in a laminar flow hood.

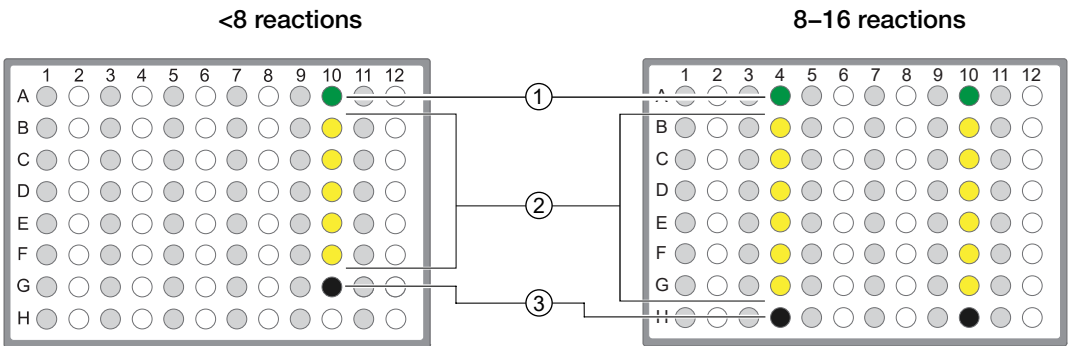
Prepare a master mix for up to 16 cDNA synthesis reactions.

IMPORTANT! For every 6 samples, there must be one No Template Control (NTC) and one Oncomine™ Dx Target RNA Control.

1. Vortex the No Template Control (purple cap) and Dilution Solution (black cap) for ~5 seconds each, then pulse centrifuge.
2. Flick the prediluted RNA sample (1.43 ng/µL), the single-use Oncomine™ Dx Target RNA Control tube (white cap), and the 10X Enzyme Mix (green cap) 4 times each to mix, then pulse centrifuge.

   IMPORTANT! Do not vortex the prediluted RNA sample.
3. Label a MicroAmp™ Optical 96-well Reaction Plate with "RNA/cDNA".
4. Place the labeled 96-well plate on a 2–8°C aluminum cold block, then set up the reactions in the designated wells of the plate. Configure the plate for <8 reactions or 8–16 reactions as shown in the figure.

   IMPORTANT! Include one No Template Control and one Oncomine™ Dx Target RNA Control well for each column, as illustrated.



1. Diluted Oncomine™ Dx Target RNA Control
2. Prediluted RNA samples
3. No Template Control

Note: If you are preparing >8 reactions, skip columns to prevent cross-contamination.

5. To each No Template Control well, add 7 µL of No Template Control.

6. Flick the Oncomine™ Dx Target RNA Control and Oncomine™ Dx Target RNA Control Diluent tubes 4 times to mix, then pulse centrifuge to collect.

7. Dilute the Oncomine™ Dx Target RNA Control. Add the following components to a 1.5-mL low-retention microcentrifuge tube in the order indicated

Note:
- When preparing multiple replicates of the control, create a separate dilution for each replicate.
- The Oncomine™ Dx Target RNA Control and Oncomine™ Dx Target RNA Control Diluent tubes are single-use only. Discard unused volume.

	Order	Component	Volume per reaction
☐	1	Oncomine™ Dx Target RNA Control (white cap)	3 µL
☐	2	Oncomine™ Dx Target RNA Control Diluent (blue cap)	72 µL

8. Flick the diluted Oncomine™ Dx Target RNA Control tube 4 times to mix, then pulse centrifuge to collect.

9. To each Oncomine™ Dx Target RNA Control well, add the following components in the order indicated:

	Order	Component	Volume per reaction
☐	1	Diluted Oncomine™ Dx Target RNA Control	3 µL
☐	2	Dilution Solution (black cap)	4 µL

10. For each RNA sample reaction, add 7 µL of prediluted RNA sample into the designated well.

Note: Do not exceed 7 µL of prediluted RNA, which is equivalent to 10 ng.

11. Prepare a master mix for n+1 reactions. Add the following components to a 1.5-mL low-retention microcentrifuge tube:

	Component	Volume per reaction
☐	5X Reaction Mix (red cap)	(n+1) × 2 µL
☐	10X Enzyme Mix (green cap)	(n+1) × 1 µL
☐	**Total**	**(n+1) × 3 µL**

12. Flick the master mix tube 4 times to mix, then pulse centrifuge to collect.

13. Pipet 3 µL of the master mix into each RNA sample, No Template Control, and Oncomine™ Dx Target RNA Control well in the 96-well plate.

14. Set a pipette to 8 µL, then pipet the contents of each reaction well up and down 5 times to mix.

15. Seal the plate with an Adhesive PCR Plate Seal, then centrifuge the plate at 100 rcf for 30 seconds.

16. Load the plate in the Veriti™ Dx 96-well Thermal Cycler, then select the **1 ODxTT cDNA Synthesis** program. Select **View**, then confirm that the steps in the program match those in the table below.

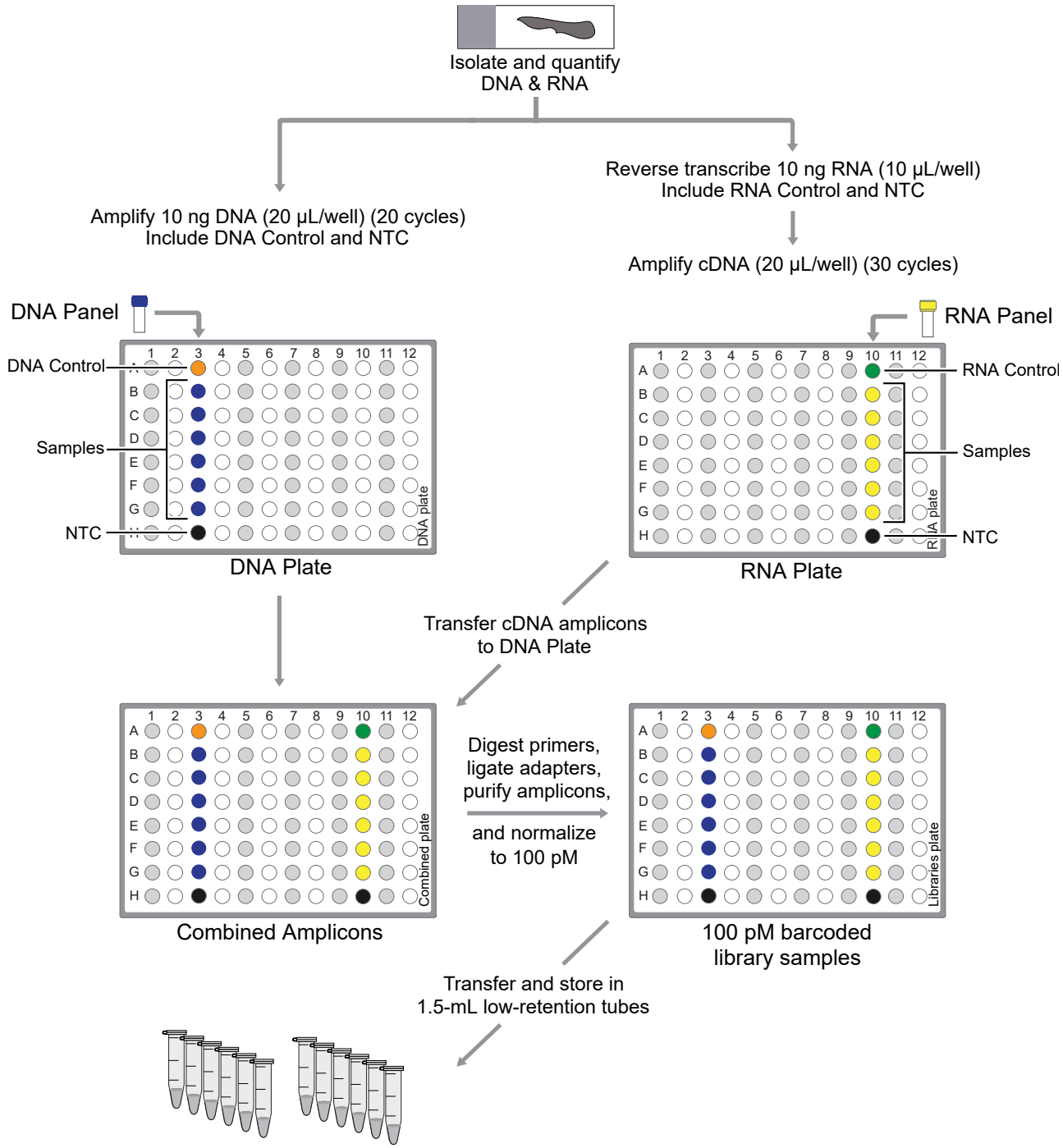
	Temperature	Time
☐	42°C	30 minutes
☐	85°C	5 minutes
☐	10°C	Hold (up to 1 hour)

17. When you have confirmed the steps, run the program.

STOPPING POINT The cDNA can be held on the thermal cycler up to 1 hour. Store at –30°C to –10°C for up to 7 days.

7 Prepare libraries

Library preparation workflow diagram



Prepare a library batch in Torrent Suite™ Dx Software

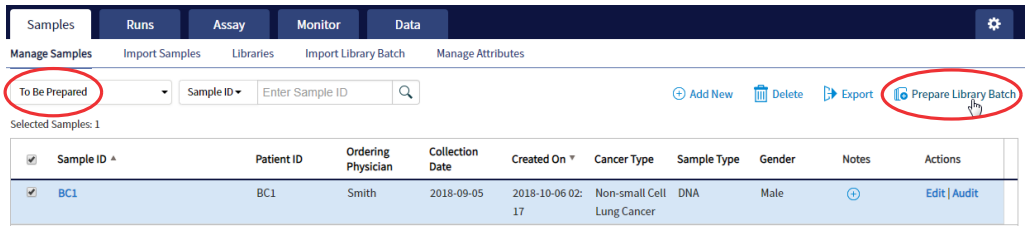
In Torrent Suite™ Dx Software, samples that are entered into the software are placed in library batches for processing and tracking. A library batch consists of a group of libraries that are prepared at the same time.

Note:

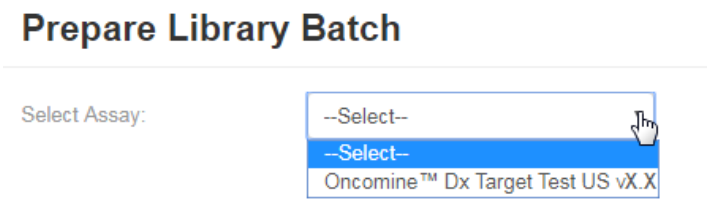
- Each library in a library batch must have a unique library name. When combining libraries in the same run, each must also have a unique barcode.
- Control libraries must be included in the same library batch as the sample library they control for.
- Fields identified with a red asterisk (*) are required.

1. Sign in to Torrent Suite™ Dx Software.
2. Under the **Samples** tab, in the **Manage Samples** screen, select **To Be Prepared** from the **Filter Samples by...** dropdown list to display only those samples that have not been placed in a library batch.

   IMPORTANT! Samples that have not been queued for extraction in the software also appear on this tab. Ensure that the samples have been queued for extraction before queueing them for library batch preparation.



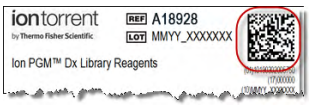
3. Select up to 6 samples in the list, then click **Prepare Library Batch**.
   The **Prepare Library Batch** dialog opens. Required fields are indicated with a red asterisk(*).
4. In the **Select Assay** dropdown list, select **Oncomine™ Dx Target Test**. The assay determines specific parameters of the run, including required controls and post-run data analysis settings.



5. In the following screen, enter a unique identifier for the library batch in the **Library Batch ID** field. Library Batch IDs can only contain alphanumeric characters (0–9 and A to Z), full stop/period (.), underscore (_), and hyphen (-).

6. Scan the barcodes from their respective kit boxes into the appropriate fields. Each library batch is associated with a kit lot by scanning the 2D barcode on the appropriate kit box.

IMPORTANT! Check the expiration date on each box. If the kit is expired, select another kit.

Barcode field	Kit	Kit box	Storage	Label scanned
Library Kit Barcode	Ion PGM™ Dx Library Kit	Ion PGM™ Dx Library Reagents	–30°C to –10°C	
Panel Kit Barcode	Oncomine™ Dx Target Test Panel	Oncomine™ Dx Target Test DNA and RNA Panel (box 1 of 3)	–30°C to –10°C	
Control Kit Barcode	Oncomine™ Dx Target Test, Controls, and Diluent Kit	Oncomine™ Dx Target DNA Control (box 2 of 3)	–30°C to –10°C	

7. Type a unique library name for each DNA and RNA library in the appropriate field. Library names can only contain alphanumeric characters (0–9 and A to Z), full stop/period (.), underscore (_), and hyphen (-).

Note: The Oncomine™ Dx Target Test Kit requires specific controls, which are automatically listed in the **Prepare Library Batch** dialog.

8. Select the Barcode ID of the adapter used to prepare each library. Swap the default barcodes in the dialog between DNA and RNA using the DNA ⇄ RNA button.

Note: Each library in a library batch must have a different Barcode ID. When preparing the physical libraries, we recommend swapping barcodes between DNA and RNA libraries in consecutive sequencing runs to prevent carryover contamination. See "Alternating barcodes" on page 72.

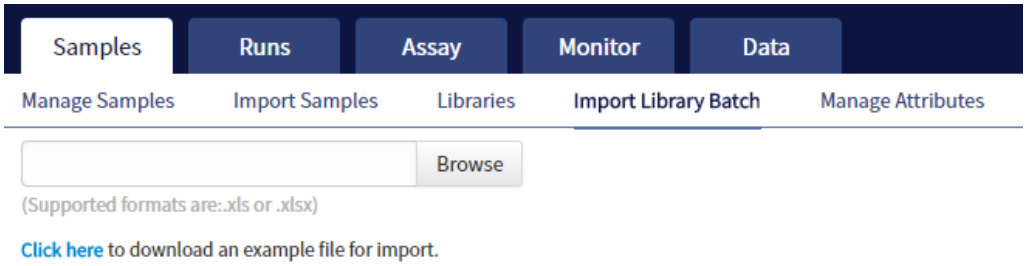
IMPORTANT! Be careful to ensure that the actual barcodes used to create the libraries match the barcodes that are entered in the **Prepare Library Batch** dialog.

9. In the **Input Quantity** field, enter 10 ng for each library.

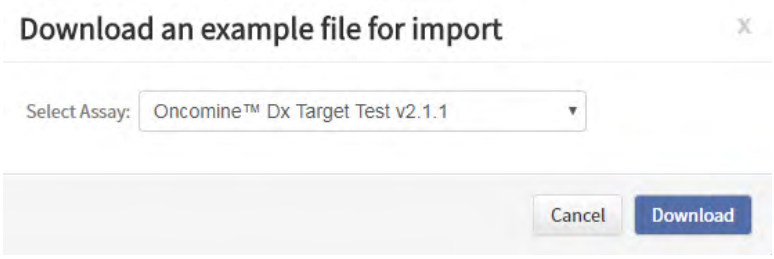
10. Click **Save** to save your selections and close the dialog.
The **Libraries** screen opens, listing the libraries that you created. Libraries that are prepared in the same batch have the same **Library Batch ID**.

Import a library batch

Under the **Samples** tab in the **Import Library Batch** screen, you can import library batch information in the form of an XLS or XLSX file. The import file must include all of the library and kit information that you enter in the **Prepare Library Batch** dialog box.



1. In the **Import Library Batch** screen, below the **Browse** field, click **Click here** to download an example file for import.
2. In the dialog box that opens, select the assay that you are using from the dropdown list, then click **Download**.



   The assay name is auto-populated in the Microsoft™ Excel™ template file that downloads to your drive.

3. In the template file, enter or confirm the library batch information.
   - Assay used (auto-populated)
   - Unique Library Batch ID
   - Library kit, control kit, and panel kit barcodes
   - Sample IDs
   - Library names
   - Barcode IDs used for each library and control
   - Nucleic acid type (DNA or RNA)
   - Library input quantity
4. Save the file.
5. Click **Browse**, navigate to the saved file, then select it.

6. Click **Import**.
   A progress bar followed by an import report displays. If the import process fails, an error message indicates the reason for failure (for example, an invalid character was used). For additional troubleshooting, see "Library batch import fails" on page 81.

7. Click **Libraries** to return to the library batch screen. Your successfully imported library batch is listed.

Prepare reagents and equipment

- See "Procedural guidelines" on page 16 before setting up the reactions.
- Equilibrate the reagents listed below at room temperature for at least 30 minutes.
  - LIB AMPure™ Reagent
  - LIB Beads
  - LIB Primers
  - LIB Capture
  - LIB Wash Soln
  - LIB Elution Soln
- Place kit components that contain enzymes (LIB HiFi Mix, LIB FuPa, and LIB DNA Ligase) on ice or in a –30°C to –10°C chilled benchtop cold box throughout the procedure until needed. Before use, flick each tube 4 times to mix, then pulse centrifuge.
- Thaw the remaining kit components (except enzymes) at room temperature until no ice is present in the tubes. Vortex for ~5 seconds, then pulse centrifuge before use.
- If there is visible precipitate in the LIB Switch Soln after thawing, vortex for ~5 seconds at room temperature, and pulse centrifuge to collect. Repeat if needed until the solution is clear.

Ion PGM™ Dx Library Kit component lot matching

The two components of the Ion PGM™ Dx Library Kit must be lot-matched with each other for use.

Component	Part No.	Storage
Ion PGM™ Dx Library Reagents	A18928	–30°C to –10°C
Ion PGM™ Dx Library Equalizer™ Reagents	A18929	2°C to 8°C

Oncomine™ Dx Target Test Controls lot matching

The following controls and diluent must be lot-matched with each other for use.

Component	Part No.	Storage
Oncomine™ Dx Target DNA Control	A44913	–30°C to –10°C
Oncomine™ Dx Target RNA Control	A32443	–90°C to –60°C
Oncomine™ Dx Target RNA Control Diluent	A38872	–90°C to –60°C
Ion Torrent Dx No Template Control Kit	A32444	15°C to 30°C

Amplify the cDNA

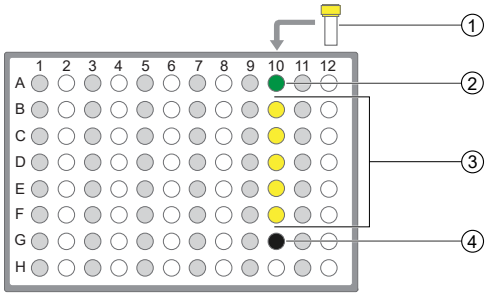
Kit components used in this procedure

Kit component	Box
Oncomine™ Dx Target Test—RNA panel (yellow cap)	Oncomine™ Dx Target Test DNA and RNA Panel (Part No. A32441, stored at –30°C to –10°C)
LIB HiFi Mix (red cap)	Ion PGM™ Dx Library Reagents (Part No. A18928), stored at –30°C to –10°C)

Set up the cDNA amplification reaction (<8 reactions)

If you are preparing <8 reactions, see below. If you are preparing 8–16 reactions, see "Set up the cDNA amplification reaction (8–16 reactions)" on page 65. The number of reactions depends on the configuration of your "RNA/cDNA" plate (prepared as described in "Reverse transcribe the RNA" on page 56).

1. Remove the "RNA/cDNA" plate from the thermal cycler, then centrifuge the plate at 100 rcf for 30 seconds.
2. Transfer the plate to a chilled (2–8°C) 96-well aluminum block.
3. Vortex the Oncomine™ Dx Target Test—RNA panel for ~5 seconds, then pulse centrifuge. Flick the tube of LIB HiFi Mix 4 times to mix, then pulse centrifuge.



① Oncomine™ Dx Target Test—RNA panel
② Oncomine™ Dx Target RNA Control
③ cDNA samples
④ No Template Control

4. Remove the seal from the plate, then add the following components to each well.

IMPORTANT! The volume of Oncomine™ Dx Target Test—RNA panel is critical and must be accurate.

	Component	Volume
☐	Nuclease-free Water	4 µL
☐	Oncomine™ Dx Target Test—RNA panel (yellow cap)	2 µL
☐	LIB HiFi Mix (red cap)	4 µL
☐	**Total volume per well** (includes 10 µL from cDNA synthesis)	**20 µL**

5. With the pipettor set to 15 µL, pipet up and down 5 times to mix the contents of each reaction well.

6. Proceed to "Amplify the cDNA targets" on page 66.

Set up the cDNA amplification reaction (8–16 reactions)

If you are preparing 8–16 reactions, see below. If you are preparing <8 reactions, see "Set up the cDNA amplification reaction (<8 reactions)" on page 64. The number of reactions depends on the configuration of your "RNA/cDNA" plate (prepared as described in "Reverse transcribe the RNA" on page 56).

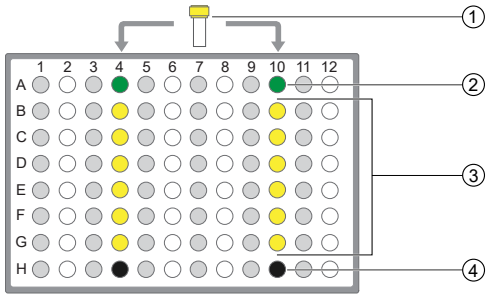
For 8–16 amplification reactions (including controls), make a master mix for n+1 reactions, where "n" is the number of reactions you are preparing.

1. Remove the "RNA/cDNA" plate from the thermal cycler, then centrifuge the plate at 100 rcf for 30 seconds.

2. Transfer the plate to a chilled (2–8°C) 96-well aluminum block.

3. Vortex the Oncomine™ Dx Target Test—RNA panel for ~5 seconds, then pulse centrifuge. Flick the tube of LIB HiFi Mix 4 times to mix, then pulse centrifuge.

4. Calculate the amounts of the following components needed for n+1 reactions, then add the components to a single 1.5-mL low-retention microcentrifuge tube.



① Oncomine™ Dx Target Test—RNA panel
② Oncomine™ Dx Target RNA Control
③ cDNA samples
④ No Template Control

IMPORTANT! IMPORTANT: The volume of Oncomine™ Dx Target Test—RNA panel is critical and must be accurate.

	Component	Volume per reaction
☐	Nuclease-Free Water	(n+1) × 4 µL
☐	Oncomine™ Dx Target Test—RNA panel (yellow cap)	(n+1) × 2 µL
☐	LIB HiFi Mix (red cap)	(n+1) × 4 µL
☐	**Total**	**(n+1) × 10 µL**

5. Vortex the tube for ~5 seconds, then pulse centrifuge to collect.

Note: Keep the master mix on ice or chilled in a 2–8°C benchtop cold box until ready for use.

6. Pipet 10 µL of the master mix into each sample or control well in the 96-well plate.
7. Set the pipettor to 15 µL, then pipet the contents of each well up and down 5 times to mix.
8. Proceed to "Amplify the cDNA targets".

Amplify the cDNA targets

Note: The Veriti™ Dx 96-well Thermal Cycler, 0.2 mL has been validated with this procedure.

1. Seal the 96-well plate with a new adhesive film, then centrifuge the plate at 100 rcf for 30 seconds.
2. Load the 96-well plate in the thermal cycler, then select the **2B ODxTT cDNA Target Amp** program. Select **View**, then confirm that the program steps match those listed in the following table:

Stage	Step	Temperature	Time
Hold	Activate the enzyme	98°C	2 minutes
Cycle (30 cycles)	Denature	98°C	15 seconds
	Anneal and extend	60°C	4 minutes
Hold	—	10°C	Hold (up to 24 hours)

3. After you have confirmed the steps, run the program.

STOPPING POINT Amplicons can be held in the thermal cycler for up to 24 hours or stored at 2–8°C for up to 1 week. If stored longer than 1 week, prepare new amplicons.

Amplify the DNA

Kit components used in this procedure

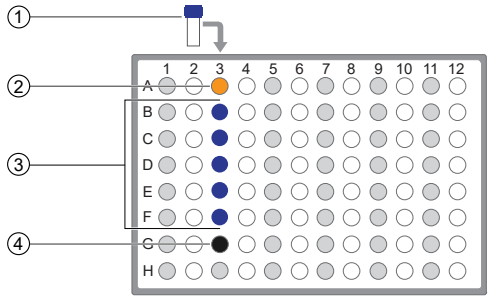
Kit component	Box
No Template Control (purple cap)	Ion Torrent Dx No Template Control Kit (Part No. A32444, stored at 15°C to 30°C)
Oncomine™ Dx Target Test—DNA panel (blue cap)	Oncomine™ Dx Target Test DNA and RNA Panel (Part No. A32441, stored at –30°C to –10°C)
LIB HiFi Mix (red cap)	Ion PGM™ Dx Library Reagents (Part No. A18928, stored at –30°C to –10°C)
Dilution Solution (black cap)	Ion Torrent Dx Sample Dilution Kit (Part No. A32439, stored at 15°C to 30°C)
Oncomine™ Dx Target DNA Control (brown cap)	Oncomine™ Dx Target DNA Control (Part No. A44913, stored at –30°C to –10°C)

Set up the DNA amplification reaction (<8 reactions)

If you are preparing <8 reactions, see below. If you are preparing 8–16 reactions, see page 69.

For <8 reactions, set up individual reactions, including a No Template Control (purple cap), an Oncomine™ Dx Target DNA Control (brown cap), and up to 5 clinical samples.

1. Label a 96-well plate "DNA".
2. Place the labeled 96-well plate on a 2–8°C chilled 96-well aluminum block, then set up individual reactions in an odd-numbered column. For every run, include the No Template Control and the Oncomine™ Dx Target DNA Control.
3. Vortex the Oncomine™ Dx Target Test—DNA panel for ~5 seconds, then pulse centrifuge. Flick the tube of LIB HiFi Mix 4 times to mix, then pulse centrifuge.
4. To the No Template Control well, add the following components in the order indicated:



① Oncomine™ Dx Target Test—DNA panel
② Oncomine™ Dx Target DNA Control
③ Prediluted FFPE DNA sample
④ No Template Control

	Order	Component	Volume
☐	1	No Template Control (purple cap)	12 µL
☐	2	Oncomine™ Dx Target Test—DNA panel (blue cap)	4 µL
☐	3	LIB HiFi Mix (red cap)	4 µL
☐	—	**Total**	**20 µL**

5. To the Oncomine™ Dx Target DNA Control well, add the following components in the order indicated:

	Order	Component	Volume
☐	1	Dilution Solution (black cap)	9 µL
☐	2	Oncomine™ Dx Target DNA Control (brown cap)	3 µL
☐	3	Oncomine™ Dx Target Test—DNA panel (blue cap)	4 µL
☐	4	LIB HiFi Mix (red cap)	4 µL
☐	—	**Total**	**20 µL**

Note: The Oncomine™ Dx Target DNA Control tube is single-use only. Discard unused volume.

6. Vortex the prediluted FFPE DNA sample (0.83 ng/µL) for ~5 seconds, then pulse centrifuge to collect.

7. To each sample well, add the following components in the order indicated.

IMPORTANT! If preparing multiple sample libraries, ensure that the appropriate FFPE DNA sample is added to the correct well to avoid patient sample mix-up.

Note: Do not exceed 12 µL of prediluted FFPE DNA, which is equivalent to 10 ng.

	Order	Component	Volume
☐	1	Prediluted sample FFPE DNA (0.83 ng/µL)	12 µL
☐	2	Oncomine™ Dx Target Test—DNA panel (blue cap)	4 µL
☐	3	LIB HiFi Mix (red cap)	4 µL
☐	—	**Total**	**20 µL**

8. Set a 20-µL pipettor to 15 µL, and pipet the contents of each well up and down 5 times to mix.

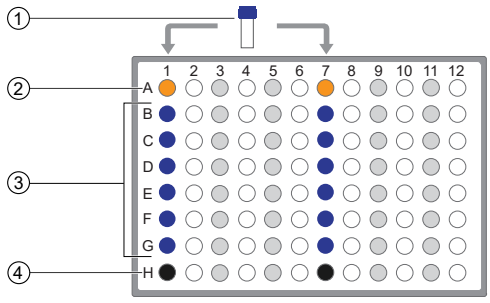
9. Proceed to "Amplify the DNA targets" on page 70.

Set up the DNA amplification reaction (8–16 reactions)

If you are preparing 8–16 reactions, see below. If you are preparing <8 reactions, see page 67.

For 8–16 reactions (for example, 12 clinical samples plus 4 controls), make a master mix containing every component except prediluted FFPE DNA as follows, where "n" is the number of reactions you are preparing. Include one No Template Control (purple cap) and one Oncomine™ Dx Target DNA Control (brown cap) for each column of reactions as illustrated.

1. Label a 96-well plate "DNA".
2. Place the labeled 96-well plate on a 2–8°C chilled aluminum block, then set up reactions in individual wells in odd-numbered columns of the plate. Skip columns to prevent cross-contamination.
3. To each No Template Control well, add 12 µL of No Template Control (purple cap).
4. To each Oncomine™ Dx Target DNA Control well, add the following components in the order indicated:



① Oncomine™ Dx Target Test—DNA panel
② Oncomine™ Dx Target DNA Control
③ Prediluted FFPE DNA samples
④ No Template Control

	Order	Component	Volume per reaction
☐	1	Dilution Solution (black cap)	9 µL
☐	2	Oncomine™ Dx Target DNA Control (brown cap)	3 µL

Note: The Oncomine™ Dx Target DNA Control tube is single-use only. Discard unused volume.

5. Vortex the prediluted FFPE DNA samples (0.83 ng/µL) for ~5 seconds, then pulse centrifuge to collect.
6. To each DNA sample well, add 12 µL prediluted FFPE DNA.

Note: Do not exceed 12 µL of prediluted FFPE DNA, which is equivalent to 10 ng.

7. Vortex the Oncomine™ Dx Target Test—DNA panel for ~5 seconds, then pulse centrifuge. Flick the tube of LIB HiFi Mix 4 times to mix, then pulse centrifuge.
8. Calculate the volume of each component below needed for n+1 reactions, then add that volume to a pre-labeled 1.5-mL low-retention microcentrifuge tube in the order stated:

	Order	Component	Volume
☐	1	Oncomine™ Dx Target Test—DNA panel (blue cap)	(n+1) × 4 µL
☐	2	LIB HiFi Mix (red cap)	(n+1) × 4 µL
☐	—	**Total**	**(n+1) × 8 µL**

9. Vortex for ~5 seconds, then pulse centrifuge.

Note: Keep the master mix at 2–8°C on ice until ready for use.

10. Pipet 8 µL of master mix into each DNA sample, No Template Control, and Oncomine™ Dx Target DNA Control well in the labeled 96-well plate.

11. Set a pipettor to 15 µL, then pipet the contents of each well up and down 5 times to mix.

12. Proceed to "Amplify the DNA targets".

Amplify the DNA targets

Note: The Veriti™ Dx 96-well Thermal Cycler, 0.2 mL has been validated with this procedure.

1. Seal the 96-well plate with a new adhesive film, then centrifuge the plate at 100 rcf for 30 seconds.

2. Load the 96-well plate in the thermal cycler, then select the **3 ODxTT DNA Target Amp** program. Select **View**, and confirm that the program steps match those in the following table:

Stage	Step	Temperature	Time
Hold	Activate the enzyme	99°C	2 minutes
Cycle (20 cycles)	Denature	99°C	15 seconds
	Anneal and extend	60°C	4 minutes
Hold	—	10°C	Hold (up to 24 hours)

3. After you have confirmed the steps, run the program.

STOPPING POINT Amplicons can be held in the thermal cycler for up to 24 hours or stored at 2–8°C for up to 1 week. If stored longer than 1 week, prepare new amplicons.

Transfer the cDNA amplicons

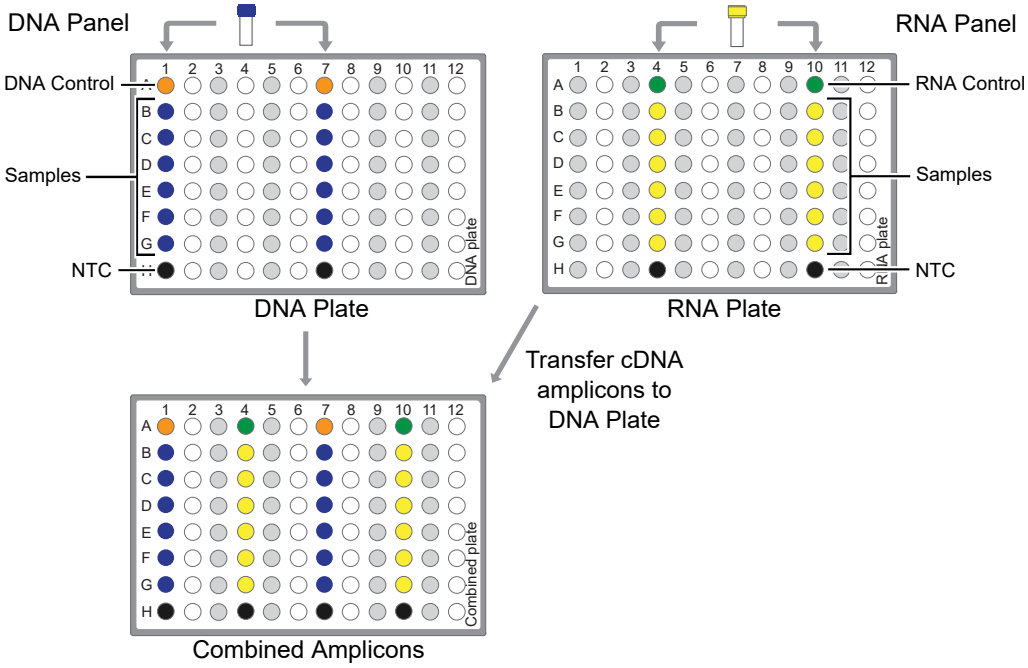
1. After thermal cycling, transfer the 96-well plate to a designated post-PCR preparation area.

2. Centrifuge the plates containing amplified cDNA and DNA at 100 rcf for 30 seconds.

3. Carefully remove the adhesive film from the plates.

IMPORTANT! Be careful when removing the adhesive film from the plate to minimize cross-contamination.

4. Transfer the cDNA amplicons from the cDNA plate to the corresponding empty wells in even-numbered columns of the DNA plate. Skip columns to prevent cross-contamination, as shown in the example below.



Proceed to "Partially digest amplicons" on page 71.

Partially digest amplicons

1. Place the plate with the amplicons on a 2–8°C cold block.
2. Flick the LIB FuPa tube (green cap) 4 times to mix, then pulse centrifuge to collect.
3. Add 2 µL of LIB FuPa to each reaction well. The total volume is 22 µL per well.

   IMPORTANT! LIB FuPa is highly viscous. To avoid carrying over excess enzyme, do not submerge the whole tip in the LIB FuPa solution. Aspirate the solution from just below the surface. The volume is critical and must be accurate. Ensure that no excess solution is added to the sample.

4. Set the pipettor to 15 µL, then slowly pipet the mixture up and down 5 times to mix.
5. Seal the plate with a new adhesive film, then centrifuge at 100 rcf for 30 seconds.

6. Load the plate in the Veriti™ Dx 96-well Thermal Cycler, then select the **4 ODxTT Amplicon Digestion** program. Select **View**, and confirm that the program steps match those listed in the table below:

Temperature	Time
50°C	10 minutes
55°C	10 minutes
60°C	20 minutes
10°C	Hold (for up to 1 hour)

7. After you have confirmed the steps, run the program.

IMPORTANT! Do not leave samples in the thermal cycler for more than 1 hour after cycling.

8. During the run, thaw the LIB Switch Soln (orange cap) and appropriate barcode adapters (white caps) at room temperature for use in subsequent steps.

Alternating barcodes

When preparing libraries, we recommend swapping barcodes between DNA and RNA libraries in consecutive sequencing runs to prevent carryover contamination. The following table provides an example of swapping barcodes between runs.

IMPORTANT! Be careful to confirm that the barcodes used to create the libraries match the barcodes entered in the **Prepare Library Batch** dialog box.

Library type	System Run 1 barcode usage		System Run 2 barcode usage	
	DNA	RNA	DNA	RNA
Positive control	1	9	9	1
Sample	2	10	10	2
Sample	3	11	11	3
Sample	4	12	12	4
Sample	5	13	13	5
Sample	6	14	14	6
Sample	7	15	15	7
No-template control (NTC)	8	16	16	8

Ligate barcode adapters

IMPORTANT! Libraries prepared from DNA and RNA from the same sample must have different barcodes, because the libraries are combined before the amplification reaction on the Ion OneTouch™ Dx Instrument.

1. After thermal cycling, centrifuge the plate at 100 rcf for 30 seconds, then place the plate back on the 2–8°C chilled aluminum block.
2. Vortex the LIB Switch Soln (orange cap) for ~5 seconds, then pulse centrifuge to collect.

   IMPORTANT! LIB Switch Soln is highly viscous and must be thoroughly mixed before use. There should be no visible precipitate after vortexing. Inspect the tube and cap carefully for precipitate. If precipitate is visible, secure the cap, invert the tube, then vortex upside down for ~5 seconds or until no visible precipitate is present. Use caution to ensure that the correct volume is delivered while pipetting.
3. Flick the tube of LIB DNA Ligase (clear cap) 4 times to mix, then pulse centrifuge to collect.
4. Ensure that the barcode adapters (BC 1–16) are thawed such that no visible ice is present. Vortex for ~5 seconds, then pulse centrifuge to collect.
5. Carefully remove the adhesive film from the plate, then add the following components to each well containing digested sample in the order shown:

   IMPORTANT! When preparing barcoded samples, prevent cross-contamination by opening only one tube of barcode adapter at a time during each addition. We recommend that this step be monitored by a co-technician to prevent sample mix-up and/or cross-contamination.

	Order	Component	Volume
☐	1	LIB Switch Soln (orange cap)	4 µL
☐	2	Barcode adapter (white cap)[1]	2 µL
☐	3	LIB DNA Ligase (clear cap)	2 µL
☐	—	**Total volume per well** (includes 22 µL of sample)	**30 µL**

[1] Select from BC 1 through BC 16, based on the sample and your barcode scheme.

6. Set a pipettor to 20 µL, then pipet the volume in each well up and down 5 times.
7. Seal the plate with a new adhesive plate seal, then centrifuge the plate at 100 rcf for 30 seconds.

8. Load the plate in the Veriti™ Dx 96-well Thermal Cycler, then select the **5 ODxTT Adapter Ligation** program. Select **View**, then confirm that the program steps match those listed in the following table.

Temperature	Time
22°C	30 minutes
72°C	10 minutes
10°C	Hold (for up to 1 hour)

9. After you have confirmed the steps, run the program.

IMPORTANT! Do not leave samples in the thermal cycler for more than 1 hour after cycling.

10. During the run, equilibrate the LIB Beads (yellow cap), LIB Capture (violet cap), and LIB Elution Soln to room temperature.

Prepare the LIB HiFi Mix plus LIB Primers mix

1. Flick the LIB HiFi Mix (red cap) 4 times to mix, then pulse centrifuge. Keep the LIB HiFi Mix in a –30°C to –10°C chilled benchtop cold box.

2. Vortex the LIB Primers (blue cap) for ~5 seconds, then pulse centrifuge.

3. Prepare the LIB HiFi Mix plus LIB Primers master mix:

   - <8 libraries—For each library, add components to individual 1.5-mL low-retention microcentrifuge tubes on ice or in a 2–8°C chilled benchtop cold box in the following order:

	Order	Component	Volume
☐	1	Nuclease-free Water	40 µL
☐	2	LIB HiFi Mix (red cap)	10 µL
☐	3	LIB Primers (blue cap)	2 µL
☐	—	**Total**	**52 µL**

   - 8–16 libraries—Calculate the amount of every component needed for n+1 libraries, where "n" is the number of libraries being prepared, then add the components to a single 1.5-mL low-retention microcentrifuge tube on ice or in a 2-8°C chilled benchtop cold box in the following order:

	Order	Component	Volume
☐	1	Nuclease-free Water	(n+1) × 40 µL
☐	2	LIB HiFi Mix (red cap)	(n+1) × 10 µL
☐	3	LIB Primers (blue cap)	(n+1) × 2 µL
☐	—	**Total**	**(n+1) × 52 µL**

4. Flick the master mix 4 times to mix, then pulse centrifuge. Keep at 2–8°C.

   Note: You must use the master mix on the same day it was prepared.

Purify the barcode-adapted library

1. Prepare fresh 70% ethanol: combine 230 µL of ethanol with 100 µL of Nuclease-free Water per library, then vortex for 10 seconds to mix.
2. When thermal cycling is complete, centrifuge the 96-well plate at 100 rcf for 30 seconds.
3. Before use, invert the LIB AMPure™ Reagent 10 times, then vortex for 10 seconds until the beads are thoroughly suspended.
4. Carefully remove the adhesive film from the plate, then add 45 µL of LIB AMPure™ Reagent to each well.

   IMPORTANT! Ensure that an accurate amount of LIB AMPure™ Reagent is dispensed to each sample, and prevent excess carryover from droplets adhering to the tip.

5. With the pipettor set to 45 µL, pipet up and down 5 times to thoroughly mix the beads in each well. The total volume is 75 µL.
6. Hold the mixture for 5 minutes at room temperature.
7. Place the plate in a DynaMag™ Dx 96-Well Plate Magnet for 3 minutes. The solution in each well must be clear, with beads pelleted to one side.
8. Using a 200-µL pipettor, remove and discard ~75 µL of the supernatant without disturbing the pellet. Use a 20-µL pipettor to remove any remaining supernatant.
9. If you see beads in the pipette tip when removing the supernatant, pipet the supernatant and beads back into their respective wells to re-pellet the beads, then remove and discard the supernatant.
10. Add 150 µL of freshly prepared 70% ethanol to each well.
11. Move the plate from left-to-right on the DynaMag™ Dx 96-Well Plate Magnet, then hold for ~5 seconds to wash and re-pellet the beads.
12. Move the plate from right-to-left on the magnet, then hold for ~5 seconds to wash and re-pellet the beads.
13. Repeat steps 11 and 12 two more times. Keep the plate in the final position on the magnet for 3 minutes or until the solution in each well is clear, with the beads in a pellet to one side.
14. Using a 200-µL pipette, remove and discard ~150 µL of the supernatant without disturbing the pellet. Use a 20-µL pipette to remove any remaining supernatant.

15. Repeat steps 10–14 one more time.

16. Ensure that all the ethanol droplets are removed from the wells. Keeping the plate in the magnet, air-dry the beads at room temperature for 5 minutes.

Amplify the barcode-adapted library

1. Flick the LIB HiFi Mix/LIB Primers master mix (prepared in "Prepare the LIB HiFi Mix plus LIB Primers mix" on page 74) 4 times to mix, then pulse centrifuge.

2. Remove the 96-well plate from the DynaMag™ Dx 96-Well Plate Magnet, then add 52 µL of LIB HiFi Mix/LIB Primers master mix to each well.

3. Set the pipettor to 40 µL, then pipet up and down 10 times to mix until the beads are resuspended.

   Note: Visually inspect the sides of the wells to ensure complete resuspension of the beads.

4. Seal the 96-well plate with a new adhesive film. Centrifuge the plate at 100 rcf for 30 seconds.

5. Load the plate in the Veriti™ Dx 96-well Thermal Cycler, then select the **6 ODxTT Library Amplification** program. Select **View**, and confirm that the program steps match those in the table below.

Stage	Temperature	Time
Hold	98°C	2 minutes
7 cycles	98°C	15 seconds
	60°C	1 minute
Hold	10°C	Hold (for up to 30 minutes)

6. After you have confirmed the steps, run the program.

   Note: During thermal cycling, you may start to prepare the LIB Beads as described in the next procedure.

Prepare the LIB Beads

Note: LIB Beads must be freshly prepared before every use.

1. Equilibrate the LIB Beads (yellow cap) to room temperature, vortex for 10 seconds or until resuspended, then pulse centrifuge to collect.

2. For each library, combine 3 µL of LIB Beads and 6 µL of LIB Wash Soln (clear cap) in a 1.5-mL low-retention microcentrifuge tube, as follows:

   - For 1–3 libraries, prepare a separate tube of beads and wash solution per library.

- For ≥4 libraries, prepare a master mix as shown below.

Number of libraries	Number of reactions to prepare in master mix	Volume of LIB Beads to add	Volume of LIB Wash Soln to add
4–7	n + 0.5	(n + 0.5) × 3 µL	(n + 0.5) × 6 µL
8	9	27 µL	54 µL
9–16	n + 2	(n +2) × 3 µL	(n +2) × 6 µL

3. Vortex each tube for ~5 seconds to mix, then pulse centrifuge to collect any beads present on the lid of the tube.
4. Place the tube in the DynaMag™ Dx 16 2-mL Magnet for 1 minute.
5. Carefully remove and discard the supernatant without disturbing the pellet.
6. Remove the tube from the magnet, then add the same volume of LIB Wash Soln as added in step 2.
7. Resuspend by pipetting up and down 5 times.
8. Keep the prepared beads at room temperature and use them on the same day.

Add LIB Capture to the amplified sample library

1. Confirm that the LIB Capture (violet cap) is at room temperature, vortex the tube for ~5 seconds, then pulse centrifuge to collect.
2. When thermal cycling is complete, centrifuge the 96-well plate at 100 rcf for 30 seconds.
3. Carefully remove the adhesive film from the plate, then add 10 µL of LIB Capture (violet cap) to each well.

   IMPORTANT! Accurate volume transfer in this step is critical. Ensure that no excess LIB Capture is carried on the pipette tip by aspirating the solution from just below the surface.

4. Set the pipettor to 40 µL, then pipet the mixture up and down 5 times to mix.
5. Hold at room temperature for 5 minutes.

Add the LIB Beads and wash

1. Mix the prepared LIB Beads by pipetting up and down 5 times, or until the beads are resuspended.
2. Add 6 µL of washed LIB Beads to each well.
3. Set the pipettor to 40 µL, then pipet the mixture up and down 5 times to mix.
4. Hold at room temperature for 5 minutes.

5. Place the 96-well plate in the DynaMag™ Dx 96-Well Plate Magnet for 3 minutes. The solution should be clear.
6. Using a 200-µL pipette, remove, then discard ~68 µL of the supernatant without disturbing the pellet. Use a 20-µL pipette to remove any remaining supernatant.
7. Add 150 µL of LIB Wash Soln to each well.
8. Move the 96-well plate from left-to-right on the DynaMag™ Dx 96-Well Plate Magnet, then hold for 5 seconds to wash and re-pellet the beads.
9. Move the 96-well plate from right-to-left on the magnet, then hold for 5 seconds to wash and re-pellet the beads.
10. Repeat steps 8 and 9 two more times.
11. With the 96-well plate still in the magnet, use a 200-µL pipette to remove and discard ~150 µL of the supernatant without disturbing the pellet.
12. Repeat the bead wash as described in steps 7–11.
13. Use a 20-µL pipette to remove any remaining LIB Wash Soln by pipetting without disturbing the pellet.

Elute the library

1. Remove the plate from the plate magnet, then add 100 µL of LIB Elution Soln to each pellet. Set the pipettor to 80 µL, then pipet up and down at least 10 times until the beads are resuspended.
2. Seal the plate with a new adhesive film, then centrifuge at 100 rcf for 30 seconds.
3. If beads pellet at the bottom of the wells:
   a. Carefully remove the adhesive film, and gently resuspend the pellet by pipetting up and down until resuspended.

      IMPORTANT! Ensure that the sample remains at the bottom of the well. Avoid introducing bubbles while pipetting.

   b. Seal the plate with a new adhesive film.
4. Load the plate in the Veriti™ Dx 96-well Thermal Cycler, then select the **7 ODxTT Library Elution** program. Select **View** and confirm that the program matches the following table.

Temperature	Time
35°C	5 minutes

5. After you have confirmed the steps, run the program.

   IMPORTANT! Remove the plate from the thermal cycler immediately after the 5-minute incubation is complete.

6. During cycling, label a 1.5-mL low-retention microcentrifuge tube for each library. Alternatively, if you are proceeding directly to pooling libraries, label a tube for each library pool.

7. Remove the plate from the thermal cycler, then centrifuge the plate at 100 rcf for 30 seconds.

CAUTION! The sample block and plate are hot. Use care when handling the plate to avoid being burned.

8. Place the plate in the DynaMag™ Dx 96-Well Plate Magnet, then hold at room temperature for 3 minutes. Confirm that the solution is clear.

9. Carefully remove the adhesive film, then transfer the supernatant containing the equalized library (~100-µL total volume) to a labeled 1.5-mL low-retention microcentrifuge tube. The final concentration of each library is ~100 pM.

STOPPING POINT The eluted libraries can be stored at –30°C to –10°C for up to 30 days. If stored for longer than 30 days, prepare new libraries.

A

Troubleshooting

Troubleshooting—Sample preparation

Observation	Possible cause	Recommended action
Slide cracked during scraping	Too much pressure was used during scraping.	Repeat the extraction using a fresh slide if possible. Processing a cracked or broken slide can pose a safety hazard to the operator.
Low-bind Elution tube cap breaks off **Details:**	Low-bind Elution tubes were not properly aligned in the microcentrifuge prior to centrifugation.	Transfer eluted samples to new, prelabeled Low-bind Elution tubes. Extra Low-bind Elution tubes are provided, however 1.5-mL snap-cap low-retention microcentrifuge tubes may also be used.
DNA/RNA quantification values are not returned	The signal for the sample was oversaturated.	Dilute the DNA and RNA samples with Dilution Solution. Prepare new standards and repeat the quantification assay.
	The signal from the sample was too low.	Prepare new standards and repeat the quantification assay. If the low signal persists, repeat the extraction with increased tissue input (for example, more sections) if available.
DNA/RNA samples do not meet the minimum concentration requirement	Quantification assays performed incorrectly.	Prepare new standards and repeat the quantification assay.
	Insufficient tissue was used in the extraction.	Repeat the extraction with increased tissue input (i.e., more sections) if possible. If the tissue was collected via macrodissection of a resection/surgical biopsy sample, repeat the macrodissection and DNA/RNA extraction with more than two 5-micron sections if available. If only two 5-micron sections remain from the sample, repeat the DNA/RNA extraction with the remaining sections without macrodissection. **Note:** To proceed with library preparation, both the DNA and RNA from a single sample extraction must meet the minimum concentration requirement. Do not use DNA from one extraction with RNA from a different extraction.
	Elution Solution cooled below 95°C.	Keep the Elution Solution in a 95°C heat block throughout the procedure, including when pipetting.

Observation	Possible cause	Recommended action
R^2 values do not meet minimum requirement	Standards were not prepared correctly.	Prepare new standards and repeat the quantification assay.
Batch sample import fails	One or more entries in the sample-import spreadsheet contains special characters, lines breaks, unexpected spaces, incorrect entry length, incorrect date formatting, or other formatting errors.	Check each entry for correct formatting, correct any errors, and repeat the import.
	Blank rows were copied into the sample-import template file from a different source.	Rows that appear empty may contain hidden formatting that conflicts with the import function. Start with a clean sample-import template file, and be careful to copy only those rows that contain actual data.
	The sample import spreadsheet contains a nonunique Sample ID.	Every Sample ID in the software must be unique. Make sure the spreadsheet does not contain any duplicate IDs, and repeat the import. Note that the system check is not case-sensitive, so a Sample ID of ABC1 conflicts with abc1.
	The headings in the sample import spreadsheet do not match the sample attributes in the software.	The headings must match the sample attributes in the software exactly. Check the headings for spelling or other errors.
Library batch import fails	One or more entries in the library batch import spreadsheet contains special characters, lines breaks, unexpected spaces, incorrect entry length, incorrect date formatting, or other formatting errors.	Check each entry for correct formatting, correct any errors, and repeat the import.
	Blank rows were copied into the library batch import template file from a different source.	Rows that appear empty can contain hidden formatting that conflicts with the import function. Start with a clean library batch import template file, and be careful to copy only those rows that contain actual data.
	The library batch import spreadsheet contains a nonunique Library Batch ID.	Every Library Batch ID in the software must be unique. Ensure that the spreadsheet does not contain any duplicate IDs, and repeat the import. Note that the system check is not case-sensitive, so a Library Batch ID of ABC1 conflicts with abc1.
	A Sample ID entered in the library batch import spreadsheet does not match a Sample ID listed in the **Manage Samples** screen.	Ensure that the Sample IDs entered into the spreadsheet are correct and match an existing sample ID added to the software.

Observation	Possible cause	Recommended action
Library batch import fails *(continued)*	The Barcode ID name format does not exactly match the format that is used in the **Prepare Library Batch** dialog box.	Use the following Barcode ID name format: IonDx-1 through IonDx-16.
	An invalid library, control, or panel kit barcode has been entered in the spreadsheet.	Ensure that you have correctly entered a valid kit barcode in the appropriate cell of the spreadsheet.

Warnings and alarms—Veriti™ Dx 96-well Thermal Cycler

Observation	Possible cause	Recommended action
"Fatal Error" message displayed by Veriti™ Dx 96-well Thermal Cycler	Various	For assistance, contact Technical Support (see Appendix E, "Customer and technical support"). Refer to the *Veriti™ Dx 96-well Thermal Cycler User Guide* (Pub. No. 4453697) for general troubleshooting information for this instrument.

B Performance characteristics

For performance characteristics of the Oncomine™ Dx Target Test Kit, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide* (Pub. No. MAN0018810).

For performance characteristics of the Ion PGM™ Dx System, see the *Ion PGM™ Dx System Performance Characteristics User Guide* (Pub. No. MAN0018763).

C

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Precaution—strong magnet

Note: Do not substitute non-IVD labeled magnets for the DynaMag™ Dx 96-Well Plate Magnet and DynaMag™ Dx 16 2-mL Magnet, provided with Ion PGM™ Dx System.

The DynaMag™ Dx 96-Well Plate Magnet and DynaMag™ Dx 16 2-mL Magnet contain very strong permanent magnets. People wearing a pacemaker or any other medical magnetized implant should not use this product unless advised by a health professional; the implant could be affected or damaged by exposure to a strong magnetic field. Keep tools and objects that could be damaged by the magnetic field out of the working area. This includes, but is not restricted to, credit cards and other products containing magnetic recording devices. Keep away from delicate instruments, watches, electronic equipment, displays and monitors. The magnet may attract steel or other magnetic material with high mechanical forces. Take care during handling. Avoid contact between two magnets. Do not pull the magnets apart if contact has been made; twist off to prevent damage to the unit or fingers. The Health and Safety Officer should take all necessary steps and full responsibility to ensure that the precautions and statements are followed and adhered to.

Medical device symbols

The following table describes symbols that may be displayed on product labels. The symbols that are used on labels conform to standards BS EN ISO 15223-1:2016 and FDA 21 CFR 809.10 "Labeling for in vitro diagnostic products".

Symbol	Description	Symbol	Description
	MANUFACTURER		CONTAINS SUFFICIENT FOR <n> TESTS
	DATE OF MANUFACTURE		USE BY
	BATCH CODE		CATALOG NUMBER
	SERIAL NUMBER		FRAGILE, HANDLE WITH CARE
	LOWER LIMIT OF TEMPERATURE		PROTECT FROM LIGHT
	UPPER AND LOWER LIMITS OF TEMPERATURE		UPPER LIMIT OF TEMPERATURE
	DO NOT REUSE		BIOLOGICAL RISKS
	CAUTION, CONSULT ACCOMPANYING DOCUMENTS		CONSULT INSTRUCTIONS FOR USE
	UPPER AND LOWER LIMITS OF HUMIDITY		OBSERVE PRECAUTIONS FOR HANDLING ELECTROSTATIC SENSITIVE DEVICES
	IN VITRO DIAGNOSTIC MEDICAL DEVICE		

D Supplemental sample dilution information

We recommend that you use the Sample Dilution Calculator (**SFW0000786_ODxTT_SampleDilution_Calculator_3.02.xlsm**) when preparing libraries (see "Dilute the samples" on page 47).

Dilute the samples (manual calculation)

If you are not using the Sample Dilution Calculator, dilute DNA samples to a final concentration of 0.83 ng/µL and RNA samples to a final concentration of 1.43 ng/µL, by manually calculating dilutions as follows.

IMPORTANT! Do not perform the following dilution procedure until you are ready to proceed directly to reverse transcription and library preparation.

1. Label two new 1.5-mL low-retention microcentrifuge tubes, one for the DNA sample and the other for the RNA sample. Place the tubes in a pre-chilled benchtop cold box until needed.

2. Use the DNA and RNA sample concentrations (ng/µL) determined in the quantification assays to calculate the volume (X) of each RNA and DNA sample required for 10 ng of sample plus 10% overage. Use the following formula:

   1.1 × (10 ng/DNA or RNA sample concentration in ng/µL) = X µL of DNA or RNA

   Note: See "Example dilution calculations" on page 88.

3. Calculate the volume (Y) of Dilution Solution required to yield a correctly diluted sample using the following formulas:

   DNA samples: (11 ng/0.83 ng/µL) – X µL of DNA = Y µL of Dilution Solution

   RNA samples: (11 ng/1.43 ng/µL) – X µL of RNA = Y µL of Dilution Solution

   IMPORTANT!

   - If the sample volume (X) from step 2 is <2.0 µL, use 2.0 µL of the sample and adjust the volume of Dilution Solution accordingly.
   - If volume of Dilution Solution (Y) from step 3 is <2.0 µL, increase the amount of the DNA and/or RNA sample volume until the required volume of Dilution Solution is ≥2 µL. See "Example dilution calculations" on page 88.

4. For each DNA or RNA sample, pipet the calculated Y µL of Dilution Solution into the appropriate labeled 1.5-mL low-retention microcentrifuge tube from step 1.

5. Add the calculated X µL of DNA or RNA sample into the appropriate labeled tube.

6. Pipet up and down 5 times to mix, then pulse centrifuge.

7. Place the diluted DNA and RNA samples back in the chilled benchtop cold box or in a 2–8°C refrigerator, then proceed immediately to "Reverse transcribe the RNA" on page 56.

IMPORTANT! Proceed directly to reverse transcription and then library preparation. Do not store the diluted DNA and RNA samples for longer than necessary.

STOPPING POINT Store the remaining undiluted DNA sample at –30°C to –10°C and the remaining undiluted RNA sample at –90°C to –60°C for up to 9 months.

Example dilution calculations

Table 2 Example calculation if the sample volume is ≥2 µL

		DNA concentration = 3 ng/µL	RNA concentration = 4 ng/µL
1	Sample volume calculation	1.1 × [10 ng/(3 ng/µL)] = **3.67 µL DNA sample volume**	1.1 × [10 ng/(4 ng/µL)] = **2.75 µL RNA sample volume**
2	Dilution Solution calculation	(11 ng/0.83 ng/µL) – 3.67 µL DNA sample = **9.58 µL of Dilution Solution**	(11 ng/1.43 ng/µL) – 2.75 µL RNA sample = **4.90 µL of Dilution Solution**
3	Final concentration check	(3.67 µL × 3 ng/µL) / (3.67 µL + 9.58 µL) = **0.83 ng/µL**	(2.75 µL × 4 ng/µL) / (2.75 µL + 4.94 µL) = **1.43 ng/µL**

Table 3 Example calculation if the sample volume is <2 µL

		DNA concentration = 15 ng/µL	RNA concentration = 14 ng/µL
1	Sample volume calculation	1.1 × [10 ng/(15 ng/µL)] = **0.73 µL DNA sample volume**	1.1 × [10 ng/(14 ng/µL)] = **0.79 µL RNA sample volume**
2	Sample volume adjustment (× 3)	0.73 µL of sample × 3 = **2.19 µL DNA sample volume**	0.79 µL of sample × 3 = **2.37 µL RNA sample volume**
3	Dilution Solution calculation with adjustment	[(11 ng/0.83 ng/µL) × 3] – 2.19 µL DNA sample = **37.6 µL of Dilution Solution**	[(11 ng/1.43 ng/µL) × 3] – 2.37 µL RNA sample = **20.7 µL of Dilution Solution**
4	Final concentration check	(2.19 µL × 15 ng/µL) / (2.19 µL + 37.6 µL) = **0.83 ng/µL**	(2.37 µL × 14 ng/µL) / (2.37 µL + 20.7 µL) = **1.43 ng/µL**

Table 4 Example calculation if the Dilution Solution volume is <2 µL

		DNA concentration = 0.9 ng/µL	RNA concentration = 1.8 ng/µL
1	Sample volume calculation	1.1 × [10 ng/(0.9 ng/µL)] = **12.22 µL DNA sample volume**	1.1 × [10 ng/(1.8 ng/µL)] = **6.11 µL RNA sample volume**
2	Dilution Solution calculation	(11 ng/0.83 ng/µL) – 12.22 µL DNA sample = **1.03 µL of Dilution Solution**	(11 ng/1.43 ng/µL) – 6.11 µL RNA sample = **1.58 µL of Dilution Solution**
3	Dilution Solution adjustment (× 2)	1.03 µL of Dilution Solution × 2 = **2.06 µL of Dilution Solution**	1.58 µL of Dilution Solution × 2 = **3.16 µL of Dilution Solution**
4	Sample volume adjustment (× 2)	12.22 µL of sample × 2 = **24.44 µL DNA sample volume**	6.11 µL of sample × 2 = **12.22 µL RNA sample volume**
5	Dilution Solution calculation with adjustment	[(11 ng/0.83 ng/µL) × 2] – 24.44 µL DNA sample = **2.06 µL of Dilution Solution**	[(11 ng/1.43 ng/µL) × 2] – 12.22 µL RNA sample = **3.16 µL of Dilution Solution**
6	Final concentration check	(24.44 µL × 0.9 ng/µL) / (24.44 µL + 2.06 µL) = **0.83 ng/µL**	(12.22 µL × 1.8 ng/µL) / (12.22 µL + 3.16 µL) = **1.43 ng/µL**

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support
- Order and web support
- Safety Data Sheets (SDSs; also known as MSDSs)

Additional product documentation, including user guides and Certificates of Analysis, are available by contacting Customer Support.

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are printed and shipped with the product.

Obtaining Certificates of Conformance

The Certificate of Conformance provides information on conformance testing of each instrument provided with the system. Certificates of Conformance are shipped with the instrument, and are also available by contacting Customer Support at **thermofisher.com/support**.

ion torrent

Oncomine™ Dx Target Test Part III: Template Preparation

USER GUIDE

Publication Number MAN0018812

Revision A.0

IVD

For In Vitro Diagnostic Use.

ThermoFisher SCIENTIFIC

Life Technologies Holdings Pte Ltd |
Block 33 |
Marsiling Industrial Estate Road 3 |
#07-06, Singapore 739256

Products manufactured in Singapore:

Ion PGM™ Dx Instrument System
Ion PGM™ Dx Sequencer
Ion OneTouch™ Dx Instrument
Ion OneTouch™ ES Dx Instrument
Ion PGM™ Dx Chip Minifuge (120V)
Ion PGM™ Wireless Scanner
Ion Torrent™ Server
Torrent Suite™ Dx Software
Veriti™ Dx 96-well Thermal Cycler, 0.2 mL

Life Technologies Corporation |
7335 Executive Way |
Frederick, MD 21704 | USA

Products manufactured in Frederick:

Oncomine™ Dx Target Test Kit
Ion Torrent Dx FFPE Sample Preparation Kit
Ion PGM™ Dx Library Kit
Ion OneTouch™ Dx Template Kit
Ion PGM™ Dx Sequencing Kit
Ion 318™ Dx Chip
Ion OneTouch™ Rack Kit
DynaMag™ Dx 96-Well Plate Magnet
DynaMag™ Dx 16 2-mL Magnet

The information in this guide is subject to change without notice.

DISCLAIMER

TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0018812

Revision	Date	Description
A.0	2 September 2020	Oncomine™ Dx Target Test user guide for FDA submission—updated for Torrent Suite™ Dx Software 5.12.5

Contents

About this guide

CAUTION! ABBREVIATED SAFETY ALERTS. Hazard symbols and hazard types specified in procedures may be abbreviated in this document. For the complete safety information, see the "Safety" appendix in this document.

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Purpose of this guide

This user guide provides instructions for using the Ion OneTouch™ Dx System to prepare enriched, template-positive Ion PGM™ Dx Ion Sphere™ Particles (ISPs) from sample and control libraries prepared using the Oncomine™ Dx Target Test Kit. The Ion OneTouch™ Dx System includes the Ion OneTouch™ Dx Instrument and the Ion OneTouch™ ES Dx Instrument.

This user guide is organized as follows:

- Prepare template-positive ISPs from Oncomine™ Dx Target Test libraries using the Ion OneTouch™ Dx Template Kit with the Ion OneTouch™ Dx Instrument.
- Enrich the template-positive ISPs with the Ion OneTouch™ ES Dx Instrument.

Oncomine™ Dx Target Test Kit user guides

This user guide is part of a five-guide set.

- *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide*
- *Oncomine™ Dx Target Test Part II: Sample and Library Preparation User Guide*
- *Oncomine™ Dx Target Test Part III: Template Preparation User Guide*
- *Oncomine™ Dx Target Test Part IV: Sequencing and Results Reports User Guide*
- *Oncomine™ Dx Target Test Part V: Torrent Suite™ Dx Software 5.12.5 Reference User Guide*

All five guides are required to complete the entire Oncomine™ Dx Target Test workflow.

Note: The procedures in these guides supersede the instructions in the *Ion PGM™ Dx System User Guide* when using the Ion PGM™ Dx System with the Oncomine™ Dx Target Test.

1 Product information

Product description

Oncomine™ Dx Target Test

The Oncomine™ Dx Target Test is an *in vitro* diagnostic next-generation sequencing test to detect somatic alterations in human DNA and RNA isolated from formalin-fixed, paraffin-embedded (FFPE) tissue samples. Detection of these variants is performed using the Ion PGM™ Dx System.

For a complete product description of the Oncomine™ Dx Target Test, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide* (Pub. No. MAN0018810).

Template preparation components

The Ion OneTouch™ Dx Template Kit, included as part of the Oncomine™ Dx Target Test Kit, is used in conjunction with the Ion OneTouch™ Dx System to prepare template-positive Ion PGM™ Dx ISPs for sequencing on the Ion PGM™ Dx Sequencer.

The Ion OneTouch™ Dx System includes the following components:

- Ion OneTouch™ Dx Instrument and accessories
- Ion OneTouch™ ES Dx Instrument and accessories
- Ion OneTouch™ Solutions Rack
- Ion OneTouch™ Assembly Rack
- Ion OneTouch™ Sample Rack

Intended use

For the Intended Use statement for the Oncomine™ Dx Target Test, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide* (Pub. No. MAN0018810).

Theory of operation

For a complete description of the Theory of Operation of the system, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide* (Pub. No. MAN0018810).

Software compatibility and requirements

The procedures in this guide are designed for use with Torrent Suite™ Dx Software version 5.12.5 or later. For a complete description of software compatibility and requirements, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide* (Pub. No. MAN0018810).

Materials provided

Oncomine™ Dx Target Test Kit

The Oncomine™ Dx Target Test Kit (Cat. No. A49755) includes the following subkits.

IMPORTANT! Refer to the product label for the storage conditions and expiration dates of individual modules and components.

✓	Subkit	Part No.
	Oncomine™ Dx Target Test, Controls, and Diluent Kit	A49756
	Ion Torrent Dx FFPE Sample Preparation Kit	A32445
	Ion PGM™ Dx Library Kit	A49758
	Ion OneTouch™ Dx Template Kit	A49759
	Ion PGM™ Dx Sequencing Kit	A49760
	Ion 318™ Dx Chip Kit	A18937
	Oncomine™ Dx Target Test User Guides and Assay Definition File	A49678

Subkits used in this guide

The procedures in this user guide use the following subkits from the Oncomine™ Dx Target Test Kit.

Ion OneTouch™ Dx Template Kit

The Ion OneTouch™ Dx Template Kit (Cat. No. A49759) includes the following modules and components.

IMPORTANT! Refer to the product label for the expiration date of the kit.

✓	Component	Amount	Storage
	Ion OneTouch™ Dx Template Supplies (Part No. A18933)		
	TMPL Amplification Plate	8	15°C to 30°C
	TMPL Recovery Router	8	
	TMPL Recovery Tube	16	
	TMPL Sipper	2	
	TMPL Reagent Tube	2	
	TMPL ES Tip	8	
	TMPL ES Strip Tube	1 pack of 12	
	TMPL Cleaning Adapter	8	
	TMPL Emulsion Cartridge	8	
	TMPL Reagent Tube Labels	1 set	
	TMPL Sample Collection Tube[1]	1 pack of 50	
	Ion OneTouch™ Dx Template Solutions (Part No. A18932)		
	TMPL Oil (white cap)	450 mL	15°C to 30°C
	TMPL Reaction Oil (white cap)	22 mL	
	TMPL Water (yellow cap)	320 µL	
	TMPL Recovery Solution (brown cap)	280 mL	
	TMPL Wash Solution (white cap)	15.2 mL	
	TMPL Rgnt B (blue cap)	2 × 1.2 mL	
	TMPL ES Rsp Soln (orange cap)	1.04 mL	
	TMPL Neutral Soln (red cap)	80 µL	
	TMPL Tween™ Solution (white cap)	2.24 mL	

✓	Component	Amount	Storage
	Ion OneTouch™ Dx Template Reagents (Part No. A18930)		
	TMPL Enzyme Mix (brown cap)	400 µL	–30°C to –10°C
	TMPL Rgnt Mix (violet cap)	8 × 500 µL (single-use tubes)	
	TMPL ISP (black cap)	800 µL	
	TMPL CF-1 (clear cap)	40 µL	
	Ion OneTouch™ Dx Template ES Beads (Part No. A18931)		
	TMPL ES Beads (green cap)	104 µL	2°C to 8°C

[1] Extra tubes are provided. Use one tube per Ion OneTouch™ ES Dx Instrument run.

Ion PGM™ Dx Instrument System

The Ion PGM™ Dx Instrument System (Cat. No. A25511) includes the following components, which are also sold separately.

✓	Component	Catalog No.
	Ion OneTouch™ Dx Instrument and accessories	A25483
	Ion OneTouch™ ES Dx Instrument and accessories	A25484
	Ion PGM™ Dx Sequencer and accessories	A25485
	Ion PGM™ Wireless Scanner	A25486
	Ion Torrent™ Server (software installed separately)	A28552
	Ion OneTouch™ Rack Kit • Ion OneTouch™ Solutions Rack • Ion OneTouch™ Assembly Rack • Ion OneTouch™ Sample Rack	A24694
	Ion PGM™ Dx Chip Minifuge: • 120 VAC • 230 VAC	A25058 A25482
	DynaMag™ Dx Kit—Tube & Plate • DynaMag™ Dx 96-Well Plate Magnet • DynaMag™ Dx 16 2-mL Magnet	A31755 A31347 A31346

Materials and equipment required but not provided

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

✓	Description	Source
	Laminar flow hood	MLS
	1.5-mL snap-cap low-retention polypropylene microcentrifuge tubes	MLS
	Mini centrifuge	MLS
	Pipettes (2-, 20-, 200-, 1000-µL)	MLS
	Aerosol-barrier pipette tips (10-, 20-, 200-, 1000-µL)	MLS
	Vortex mixer with a rubber platform	MLS
	50-mL conical tubes	MLS
	Holder for 50-mL conical tube	MLS
	DynaMag™ Dx 16 2-mL Magnet	A31346
	NaOH, ACS grade (10 M)	MLS
	Nuclease-free Water	MLS
	Benchtop cold box	MLS

DynaMag™ Dx 96-Well Plate Magnet and DynaMag™ Dx 16 2-mL Magnet

Note: Do not substitute non-IVD labeled magnets for the DynaMag™ Dx 96-Well Plate Magnet and DynaMag™ Dx 16 2-mL Magnet.

The DynaMag™ Dx 96-Well Plate Magnet and DynaMag™ Dx 16 2-mL Magnet, provided with Ion PGM™ Dx System, contain high-energy neodymium magnets and are used as part of the procedure for purifying sample libraries bound to LIB AMPure™ Reagent and LIB Beads. The DynaMag™ Dx 16 2-mL Magnet is also used to prepare TMPL ES Beads as part of template preparation.

The DynaMag™ Dx 96-Well Plate Magnet has 7 bar magnets with a hard plastic top to fit 96-well PCR plates. When you insert a plate, the magnets collect bead-bound biomolecules in suspension at the sides of the plate wells, allowing removal of fluid without disturbing the bead pellets. An extra column in the magnet enables sample mixing by shifting the plate back and forth in the magnet.

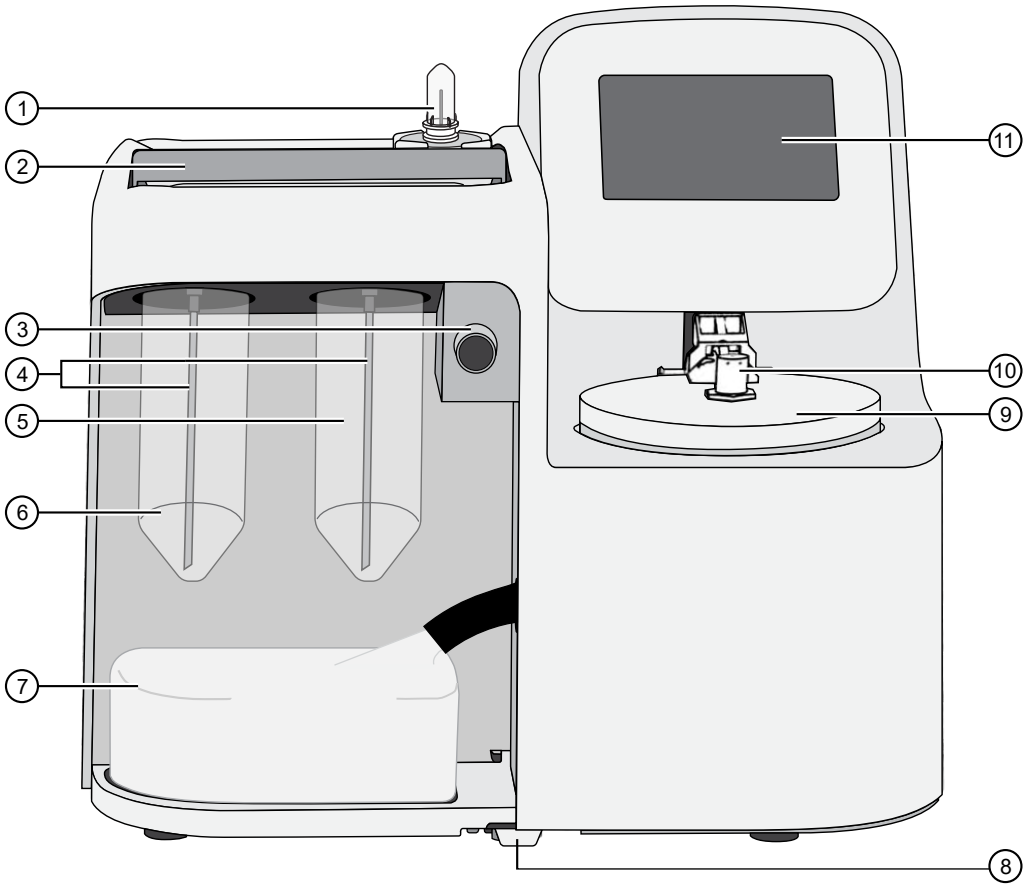
The DynaMag™ Dx 16 2-mL Magnet holds 16 standard 1.5-mL or 2-mL microcentrifuge tubes, and collects bead-bound biomolecules in suspension at the sides of the tubes, allowing removal of fluid without disturbing the bead pellets.

Do not use the magnets above 50°C (122°F) and store in a cool, dry environment.

Recommended materials and equipment

Description	Source
Benchtop absorbent paper or mat	MLS

Ion OneTouch™ Dx Instrument



1. Reaction Filter
2. Clamp handle to access the TMPL Amplification Plate in the heat block
3. Pinch valve to hold disposable tubing
4. TMPL Sippers
5. TMPL Reagent Tube containing TMPL Recovery Solution
6. TMPL Reagent Tube containing TMPL Oil
7. Waste Container
8. Oil waste tray
9. Centrifuge and TMPL Recovery Router
10. Injector hub
11. Touchscreen

2 Before you begin

Electrical and environmental conditions

IMPORTANT! Protection provided by the equipment may be impaired if the instrument is operated outside the environment and use specifications, the user provides inadequate maintenance, or the equipment is used in a manner not specified by the manufacturer.

IMPORTANT! Observe current Good Clinical Laboratory Practices (GCLP) when using this instrument.

See the *Ion PGM™ Dx System Site Preparation Guide* (Pub. No. MAN0016696) for information about instrument location and setup. Instruments should be shielded from excess exposure to dust, vibration, strong magnetic fields, drafts, excessive moisture, or large temperature fluctuations. Surge protectors or line conditioners should be used if the voltage source is not stable. Sudden voltage spikes can cause damage to the electronics inside the instruments.

Ensure that the room where the instruments have been installed is maintained under correct environmental conditions. Avoid placing the instruments adjacent to heaters, cooling ducts, or in direct sunlight. Place the instruments at least a meter away from major sources of electronic noise, such as refrigerators or microwaves.

Electrical requirements

CAUTION! Do not unpack or plug in any components until a field service representative has configured them for the proper operating voltage.

WARNING! For safety, the power outlet used for powering the instrument must be accessible at all times. In case of emergency, you must be able to immediately disconnect the main power supply to all the equipment. Allow adequate space between the wall and the equipment so that the power cords can be disconnected in case of emergency.

- Electric receptacle required: 2-prong with ground pin
- Main AC line voltage tolerances must be at most ±10% percent of nominal voltage.

- Power cords are provided with the instruments. If not suitable for installation in your region, ensure any power cord you do use is:
  - Maximum 10 feet (3 meters) in length
  - Grounding type
  - Compatible with the power supply receptacles used to connect to main power
  - Suitable for the rating of the instrument and main power supply
  - Compliant with local safety requirements (for example, UL Listed for North America, JIS approved for Japan, HAR or agency certified for Europe)
- (Ion OneTouch™ Dx Instrument only) Fuse Rating: 6 A, 250 VAC, Type M. Replace only with the same fuse type and rating.

WARNING! FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

Device	Rated voltage [1,2]	Rated frequency	Rated current [3]
Ion PGM™ Dx Sequencer	110/120VAC 220/240VAC	50/60 Hz	9 A
Ion Torrent™ Server [4]	110/120VAC 220/240VAC	50/60 Hz	11 A
Ion OneTouch™ Dx Instrument with power supply	110/120VAC 220/240VAC	50/60 Hz	5.5 A
Ion OneTouch™ ES Dx Instrument	110/120VAC 220/240VAC	50/60 Hz	375 mA 160 mA
Ion PGM™ Dx Chip Minifuge	120 VAC 220–240 VAC	50/60 Hz	130 mA 65 mA

[1] In Japan, rated voltages of 100 VAC and 200 VAC are acceptable.

[2] If the supplied power fluctuates beyond the rated voltage, a power line regulator may be required. High or low voltages can adversely affect the electronic components of the instrument.

[3] Based on rated current at minimum input voltage.

[4] Minimum Efficiency: 65% (Energy Star Qualified); 85% Efficient Power Supply.

Environmental requirements

Ensure that the room where the instruments have been installed is maintained under the correct environmental conditions. Avoid placing the instruments next to heaters, cooling ducts, or in direct sunlight. Place the sequencer at least a meter away from major sources of electronic noise, such as refrigerators or microwaves.

CAUTION! Use of the instruments in an unspecified manner may result in the protection provided by the instruments to be impaired.

Component	Acceptable range
Altitude	Between sea level and 2,000 meters (6,500 feet) above sea level
Humidity: operating	10–90% relative humidity
Humidity: transportation and storage	20–80% relative humidity
Temperature: operating	15–30°C (59–86°F) At or above 1,800 meters (5,906 feet), the system must not be used if the temperature is above 29.5°C.
Temperature: transportation and storage	–30°C to 60°C (–22°F to 140°F)
Vibration	Ensure that benches where instruments are to be installed are free of vibration and have no contact with equipment that causes vibration (freezers, pumps, and similar equipment). Vibration can reduce the quality of sequencing measurements.
Pollution	The system has a Pollution Degree rating of II (2). The system may only be installed in an environment that has nonconductive pollutants, such as dust particles or wood chips. Typical environments with a Pollution Degree II (2) rating are laboratories, sales, and commercial areas.
Overvoltage category	The instruments have an installation (overvoltage) category of II (2).
Other conditions	For indoor use only. Keep away from any vents that could expel particulate material on the system components.

Precautions before using the Ion OneTouch™ Dx instruments

For additional safety information, see Appendix C, "Safety".

Instrument installation by trained personnel only

IMPORTANT! The Ion PGM™ Dx System is installed by trained service personnel and must not be relocated without assistance from trained service personnel. See Appendix F, "Customer and technical support".

Nucleic acid contamination

IMPORTANT! A primary source of contamination is DNA fragments from previously processed samples. See the notes about avoiding contamination in the procedural guidelines.

Reagent contamination

Before use, verify that the TMPL Water and TMPL Tween™ Solution are not cloudy, a potential indication of contamination. If these reagents are cloudy, use a different vial.

Procedural guidelines

Definitions

Throughout this guide:

- Room temperature is defined as the temperature range 15–30°C.
- A pulse centrifugation consists of a 3–5 second centrifugation at maximum speed in a mini centrifuge.

Guidelines for pipetting

Pipet viscous solutions slowly and ensure complete mixing.

Guidelines to prevent cross-contamination

- Use good laboratory practices to minimize cross-contamination of products and reagents.
- When designing the laboratory layout, dedicate separate areas for pre- and post-amplification activities. Dedicate laboratory supplies and/or equipment to the appropriate area.
- Before and after use, clean all working surfaces with fresh 10% bleach followed by two water rinses.
- Use a laminar flow hood in the dedicated pre-amplification area when combining libraries and preparing the amplification solution.

- Before and after use, clean all surfaces and equipment in the laminar flow hood with fresh 10% bleach followed by two water rinses.
- Turn on the UV light in the hood for 10 minutes before and after use.
- Use fresh gloves before entering the hood.
- Change tips between pipetting steps.
- Prepare a waste container containing fresh 10% bleach solution for disposing of used tips after pipetting libraries.
- To collect the contents before opening, pulse centrifuge tubes containing libraries and library pools.
- When simultaneously preparing more than one amplification solution, only open one library-pool tube at a time.
- Use fresh reagents if a contamination event occurs or is suspected.
- Use fresh gloves when installing new consumables.
- To avoid contamination of TMPL Reagent Tubes (which may be reused), discard waste from the tubes in a separate container from other Ion OneTouch™ Dx Instrument waste and 50-mL conical-tube waste.
- Always change gloves after handling Ion OneTouch™ Dx Instrument waste oil, used amplification plates, and used cleaning adapters.
- To prevent cross-contamination, we do not recommend running the same barcode for the same type of library sample (DNA or RNA) in a consecutive system run.

Guidelines for Ion OneTouch™ Dx Instrument operation

- To prevent debris from entering the system, keep the TMPL Reagent Tubes installed on the Ion OneTouch™ Dx Instrument when not in use.
- After a Planned Run is executed on the Ion Torrent™ Server, the run must be started immediately on the Ion OneTouch™ Dx Instrument.
- A run on the Ion OneTouch™ Dx Instrument can be performed overnight. Enrichment on the Ion OneTouch™ ES Dx Instrument must start within 24 hours after completion of the Ion OneTouch™ Dx Instrument run.
- If a run is aborted for any reason, you must restart the Ion OneTouch™ Dx Instrument by power cycling.
- Ensure that you clean up any spilled oil immediately. Place a nonslip floor mat in front of the instrument to prevent slips.

CAUTION! Spilled oil from the Ion OneTouch™ Dx Instrument can present a slip hazard.

Reagent management

Follow the guidelines below for proper reagent storage and use.

Storage

Reagents must be stored under appropriate conditions. Refer to the Product Information section in each user guide for the storage conditions of the kit components used in the procedures in that guide. The Oncomine™ Dx Target Test Kit includes kits with multiple component boxes that require different storage conditions. For example, the Oncomine™ Dx Target Test, Controls, and Diluent Kit includes four boxes, which are stored at different temperatures. To use the Oncomine™ Dx Target Test, Controls, and Diluent Kit, retrieve all boxes from their different storage areas and confirm that they are from the same master lot.

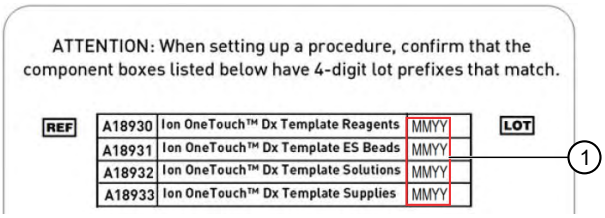
Kit interchangeability and component box lot matching

The top-level kits used for sample preparation, library preparation, template preparation, and sequencing can be mixed and matched. For example, an Ion PGM™ Dx Library Kit can be used with any Ion PGM™ Dx Sequencing Kit. However, the component boxes in a particular kit must be lot-matched with the other boxes in that kit.

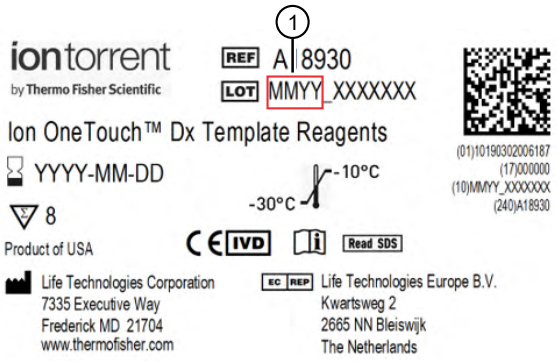
Note: The controls and diluent in the Oncomine™ Dx Target Test, Controls, and Diluent Kit must be lot-matched. However, the controls do not require lot-matching with the Oncomine™ Dx Target Test DNA and RNA Panel.

Each component box lists the 4-digit lot prefixes of the compatible component boxes inside the box lid. Before using a particular kit, check the inside lid of each box to confirm that it is compatible with the other boxes.

An example inside box lid label is shown below:



An example box label with lot information is shown below:

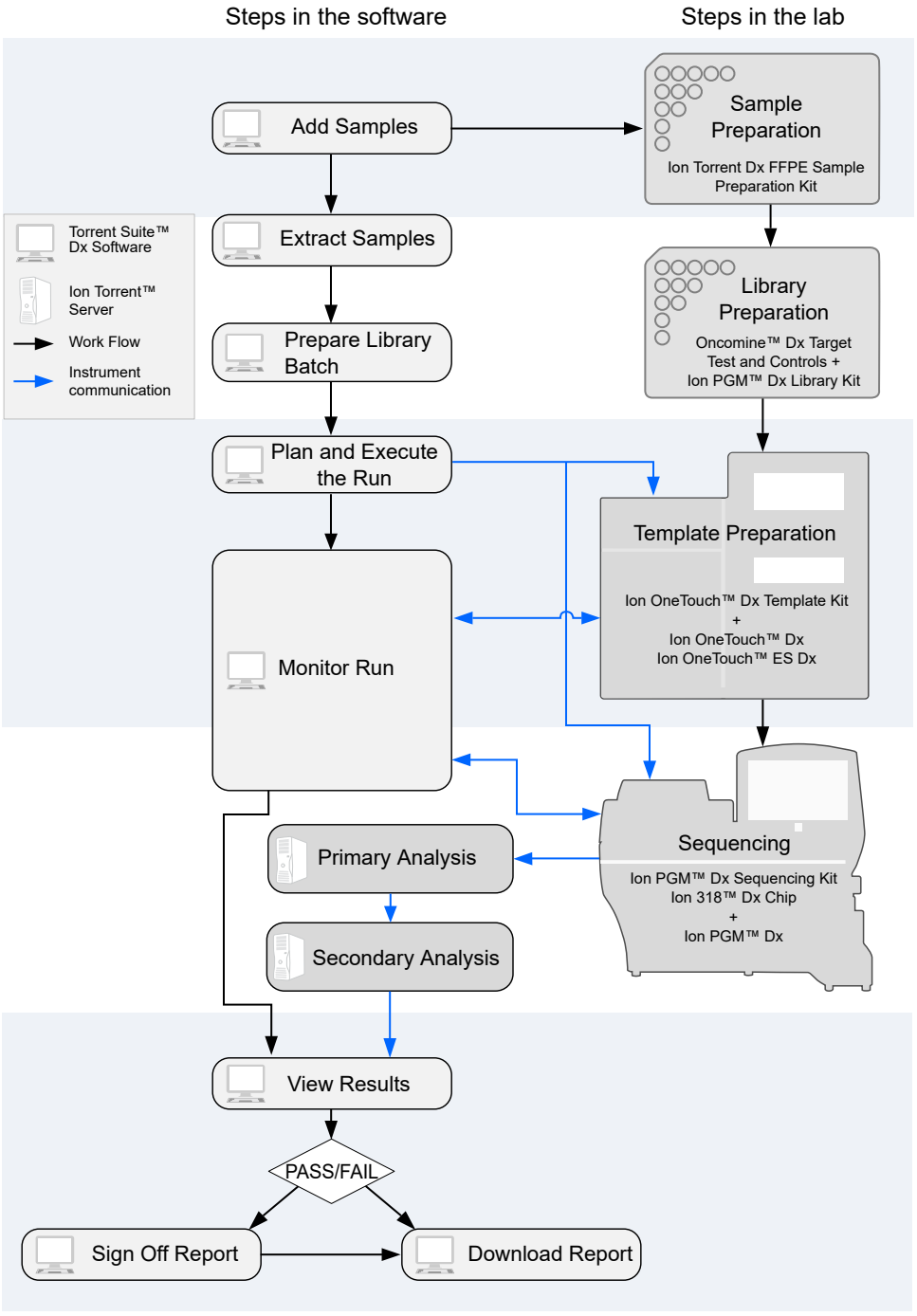


① Lot prefix

Library preparation

Libraries must be prepared as described in the *Oncomine™ Dx Target Test Part II: Sample and Library Preparation User Guide* (Pub. No. MAN0018811).

Oncomine™ Dx Target Test system diagram



Template preparation workflow

The total workflow time is 7 hours, including 1 hour of hands-on time.

Previous guide: *Oncomine™ Dx Target Test Part II: Sample and Library Preparation User Guide* (Pub. No. MAN0018811)

▼

Plan and execute a run on the Torrent Suite™ Dx Software (page 23)

▼

Pool sample and control libraries (page 27)

▼

Clean the Ion OneTouch™ Dx Instrument before a run (page 30)

▼

Run the Ion OneTouch™ Dx Instrument (page 43)

▼

Prepare the Ion OneTouch™ ES Dx Instrument and perform the run (page 49)

▼

Clean the Ion OneTouch™ Dx Instrument after the run (page 51)

▼

Proceed to: *Oncomine™ Dx Target Test Part IV: Sequencing and Results Reports User Guide* (Pub. No. MAN0018813)

3 Plan and execute a run on the Torrent Suite™ Dx Software

Components such as libraries, kits, and chips used in a diagnostic assay must be uniquely identified, and the identification must be stored so that the record can be audited. Torrent Suite™ Dx Software records these components in the Planned Run, which is prepared in the software and then transferred to the system instruments for tracking and verification at each stage of the run.

The software also uses the Planned Run to verify that only unexpired kits and chips are used and the correct assay is performed on the correct sample.

For more information, see the *Oncomine™ Dx Target Test Part V: Torrent Suite™ Dx Software 5.12.5 Reference User Guide* (Pub. No. MAN0018814).

Create a Planned Run

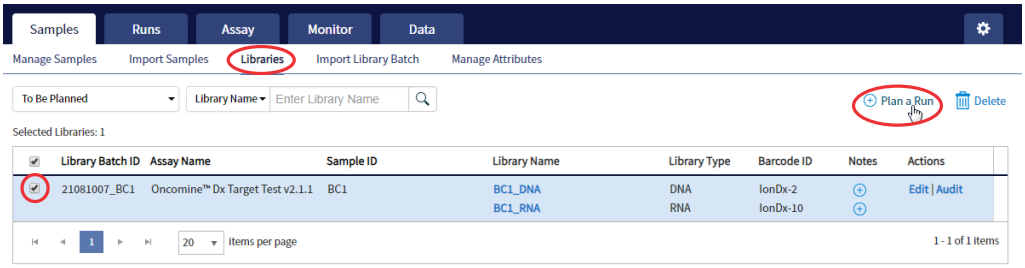
Libraries that are ready to be entered into a Planned Run are listed under the **Samples** tab in the **Libraries** screen.

Note: You can also plan a run from the **Runs** tab in the **Planned Runs** screen (click ⊕ **Add New**).

1. Sign in to Torrent Suite™ Dx Software.
2. In the **Libraries** screen, select the library or libraries to be run by selecting checkboxes in the list. To view only those libraries that have not yet been added to a Planned Run, select **To Be Planned** in the **Filter Libraries by** dropdown list.

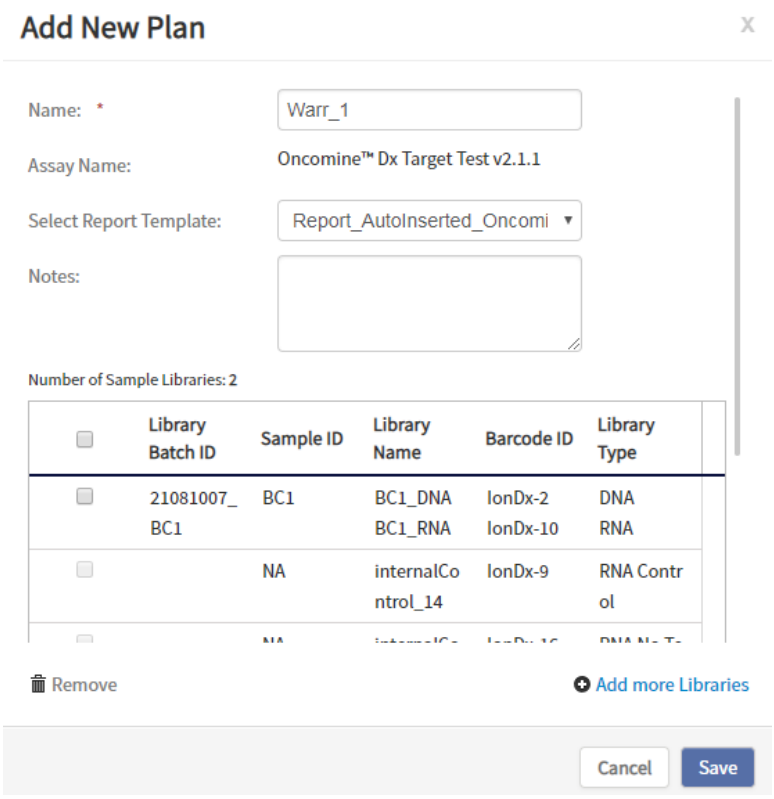
   Note:
   - Libraries prepared with the same assay in the same library batch can be combined and run together, as long as they have unique library names and Barcode IDs.
   - To plan a run with the Oncomine™ Dx Target Test assay, a report template must be created and associated with the assay by an Administrator or Manager. Under the **Assay** tab, go to **Presets ▸ Report Templates** to add a new report template.
   - Up to 16 libraries (including controls) can be sequenced in a single run.
   - If libraries need to be rerun due to a run failure, they can be added to a new Planned Run.

3. Click **Plan a Run**.



4. In the **Add New Plan** dialog box, enter a name for the run, then select the appropriate report template.

   The selected library or libraries are listed in the dialog box, and the control libraries are automatically listed.



5. To remove libraries from the run, select the appropriate checkboxes, then click **Remove**. To add libraries, click ⊕ **Add more Libraries**, then select them from the **Add Libraries** dialog box.

   Any added libraries must be from the same library batch and have unique library names and Barcode IDs.

6. Click **Save**.

   The new Planned Run is automatically assigned a Run Short Code and is displayed at the top of the list under the **Runs** tab in the **Manage Runs** screen.

Execute a Planned Run

Planned Runs are listed under the **Runs** tab in the **Manage Runs** screen. Runs that are ready to be performed have the **Execute** command available in the **Actions** column.

Executing a Planned Run in the software queues the run for initiation on the Ion OneTouch™ Dx Instrument. After a Planned Run has been queued for execution, the operator should immediately start template preparation.

In the **Manage Runs** screen:

1. Click **To Be Started** in the **Filter Run by...** dropdown list to limit the list of Planned Runs to only those runs yet to be started.

2. Find the Planned Run in the list, then under the **Actions** header, click **Execute**.

   The **Execute Planned Run** dialog box opens.

3. In the **Tube Label** field, enter the text that is used to label the tubes that contain the final combined libraries.

   The tube label text can be any combination of letters and numbers. The system uses this text to track the sample throughout the run, so be careful to label each tube legibly at the points noted in the procedure. The software does not allow use of the same Tube Label text within 7 days.

4. Click the **Template Prep Kit barcode** field, then scan the barcode from the Ion OneTouch™ Dx Template Reagents box.

   IMPORTANT! Ensure that you scan the barcode from the actual reagents box that is used in the run.

5. Click **Save** to save your changes.
   The **Review Planned Run** dialog box opens.

6. Write down the **Run Short Code** and/or click **Print** to print the scannable barcode. The code must be entered into the Ion OneTouch™ Dx Instrument and Ion PGM™ Dx Sequencer for tracking and verification before the start of the instrument run.

7. Click **Close** to close the dialog box and send the run to the instrument.

Note: The last 5 executed Planned Runs are listed under the **Monitor** tab in the software.

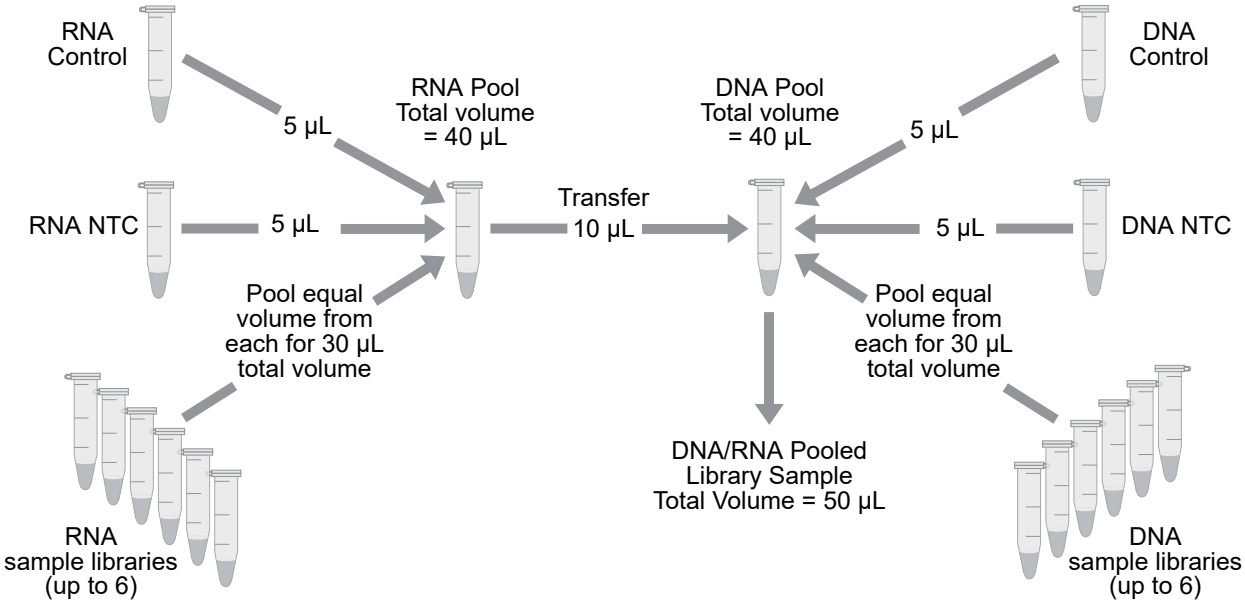
---

4 Pool sample and control libraries

Strategy for combining libraries

You can multiplex up to six RNA sample libraries and six DNA sample libraries into a single Ion PGM™ Dx System run. You must also include separate DNA Control, RNA Control, and No Template Control libraries with each run.

A strategy for combining multiple libraries and controls is diagrammed below.



Create an RNA combined library

Perform the following steps in a laminar flow hood in a designated post-PCR area. Change pipette tips between libraries. See "Guidelines to prevent cross-contamination" on page 17 for additional guidelines to avoid cross-contamination.

1. Before pipetting, vortex each library tube for ~5 seconds, then pulse centrifuge for 3–5 seconds to collect the contents.
2. Label a new, nuclease-free 1.5-mL low-retention microcentrifuge tube with the text "RNA Combined Library" and the date.
3. Add 5 µL of the RNA Control library to the tube.
4. Add 5 µL of the RNA NTC library to the same tube.

5. Based on the number of RNA sample libraries to combine, transfer the volume shown in the following table to the tube.

Note: The total volume of the combined libraries and controls equals 40 µL.

# of RNA sample libraries	Volume per RNA sample library
1	30 µL
2	15 µL
3	10 µL
4	7.5 µL
5	6 µL
6	5 µL

6. Vortex the combined library for ~5 seconds, then pulse centrifuge for 3–5 seconds to collect the contents.

Create a DNA combined library

Perform the following steps in a laminar flow hood. Change pipette tips between libraries. See "Guidelines to prevent cross-contamination" on page 17 for additional guidelines to avoid cross-contamination.

1. Before pipetting, vortex each library tube for ~5 seconds, then pulse centrifuge for 3–5 seconds to collect the contents.
2. Label a new, nuclease-free 1.5-mL low-retention microcentrifuge tube with the text "DNA/RNA Combined Library" and the date.
3. Add 5 µL of the DNA Control library to the tube.
4. Add 5 µL of the DNA NTC library to the tube.
5. Based on the number of DNA sample libraries to combine, add the volume shown in the following table to the tube.

Note: The total volume of the combined libraries and controls equals 40 µL.

# of DNA sample libraries	Volume per DNA sample library
1	30 µL
2	15 µL
3	10 µL
4	7.5 µL

(continued)

# of DNA sample libraries	Volume per DNA sample library
5	6 µL
6	5 µL

6. Vortex the combined library for ~5 seconds, then pulse centrifuge for 3–5 seconds to collect the contents.

Create a DNA/RNA combined library

1. Transfer 10 µL of the RNA combined library (from step 6, “Create an RNA combined library” on page 27) to the tube containing 40 µL of DNA combined library (labeled "DNA/RNA Combined Library").

   Note:
   - The total volume of the DNA/RNA combined library equals 50 µL.
   - The remaining RNA combined library can be stored at –30°C to –10°C for up to 30 days.

2. Vortex the DNA/RNA combined library for ~5 seconds, then pulse centrifuge for 3–5 seconds to collect the contents.

STOPPING POINT Proceed to “Clean the Ion OneTouch™ Dx Instrument before a run” on page 30, or store the DNA/RNA combined library at –30°C to –10°C for up to 30 days.

5 Clean the Ion OneTouch™ Dx Instrument before a run

Track use of TMPL Reagent Tubes and TMPL Sippers

TMPL Reagent Tubes and TMPL Sippers can be used up to 8 times.

1. To track use of the reagent tubes and sippers, label the tubes with the labels that are provided in the kit, then mark the labels after each use.
2. After 8 uses, discard the used reagent tubes and sippers in an appropriate waste container, then label new reagent tubes.

Power cycle the Ion OneTouch™ Dx Instrument

The Ion OneTouch™ Dx Instrument can be left on overnight and on weekends, but should be power cycled under the following conditions:

To power cycle the instrument, turn the instrument off, wait 3 seconds, then turn the instrument back on.

- Power cycle the instrument before installing TMPL Reagent Tubes from a new kit.
- Power cycle the instrument after daylight-saving time changes.

IMPORTANT! Allow up to 20 minutes for the Ion OneTouch™ Dx Instrument to resynchronize with the Ion Torrent™ Server after power cycling. Failure to resynchronize generates an alarm until synchronization is complete.

Clean the Ion OneTouch™ Dx Instrument before a run

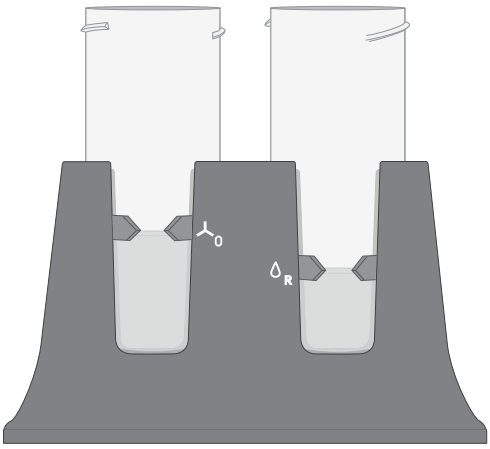
Before you perform a new run on the Ion OneTouch™ Dx Instrument, you must clean the instrument. Until you clean the instrument, the **Run** button on the instrument remains disabled.

IMPORTANT! Change gloves after handling instrument waste during disposal and before handling samples. See the product SDS for guidance regarding proper disposal and handling of Ion OneTouch™ Dx Instrument waste products.

Note: Do not press the instrument touchscreen buttons too rapidly (>1 per second), or the touchscreen may freeze.

1. Log in to the instrument using the touchscreen.
2. Press the **Clean** button, then follow the touchscreen prompts.
3. Remove the TMPL Reagent Tube containing TMPL Oil from the position marked with an "O" on the instrument (the tube on the left when facing the instrument), then discard the contents in an appropriate hazardous waste container.

   IMPORTANT! When removing reagent tubes, do not touch the reagent sippers or allow them to come into contact with any surfaces. Ensure that the reagent tubes do not come into contact with the waste container when emptying contents.
4. Place the TMPL Reagent Tube in the position labeled "O" on the Ion OneTouch™ Solutions Rack.



5. Change gloves, then invert the TMPL Oil bottle (white cap) 5 times to mix. Fill the reagent tube with oil to the level marked on the rack, so the meniscus is within the fill indicator arrows (volume = 50 mL).
6. Confirm that the sipper is securely attached to the instrument, then screw the reagent tube containing the oil back into the position marked with an "O" on the instrument until the tube can no longer rotate. Press **Next**.

CAUTION! Be careful to attach the reagent tube to the correct position on the instrument. Attaching the tube to the wrong position may damage the instrument.

7. Remove the TMPL Reagent Tube containing TMPL Recovery Solution from the position marked with an "R" on the instrument. Discard the contents in an appropriate waste container.

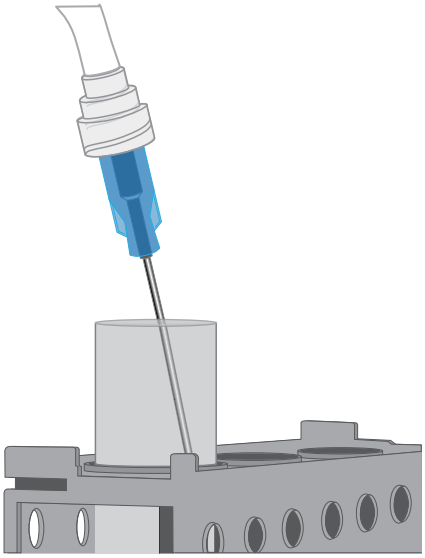
   IMPORTANT! When removing reagent tubes, do not touch the reagent sippers or allow the reagent sippers or reagent tubes to come into contact with any surfaces.
8. Place the TMPL Reagent Tube in the position marked with an "R" on the Ion OneTouch™ Solutions Rack.

9. Change gloves, then invert the TMPL Recovery Solution bottle (brown cap) 5 times to mix. Fill the reagent tube with recovery solution to the level marked with arrows on the "R" position of the rack (volume = 35 mL).

10. Confirm that the sipper is securely attached to the instrument, then screw the reagent tube containing the recovery solution back into the position marked with an "R" on the instrument until the tube can no longer rotate. Press **Next**.

11. Confirm that both the TMPL Cleaning Adapter and TMPL Amplification Plate from the post-run cleaning are in place on the instrument, then check that the tubing from the amplification plate is removed from the pinch valve.

12. Confirm that the injector tip is placed in an empty 50-mL conical tube to collect waste.



13. Press **Next** to start cleaning.

Note: The cleaning procedure takes 13 minutes to complete, and consumes 10 mL of oil.

14. When cleaning is complete, the screen displays "Cleaning Complete". Wearing clean gloves, press **Next**.

15. Remove and appropriately discard the used TMPL Cleaning Adapter, TMPL Amplification Plate, disposable injector, and tubing from the instrument.

CAUTION! Hot Surface. Use care when working near this area to avoid being burned by hot components.

a. Remove the used TMPL Cleaning Adapter, then discard in an appropriate waste container.

b. Lift, then push back the instrument handle to open the heat block.

c. Remove the disposable tubing, remove the needle, then discard each in appropriate waste containers.

CAUTION! Piercing Hazard: Remove the needle from the tubing by unscrewing it or cutting it off with scissors, and discard in an appropriate hazardous waste container for sharp objects.

d. Gently pull back the TMPL Amplification Plate from the inlet and outlet holes of the instrument.

e. Remove the plate from the heat block, then discard in an appropriate waste container.

f. Leave the heat block open.

16. Appropriately discard the 50-mL conical tube waste, then press **Next** to return to the main screen.

IMPORTANT! Always change gloves after handling the waste oil, used amplification plates, and cleaning adapters.

6 Perform an Ion OneTouch™ Dx System run

This chapter describes how to prepare templated ISPs on the Ion OneTouch™ Dx Instrument from a DNA/RNA combined library, and then enrich the templated ISPs on the Ion OneTouch™ ES Dx Instrument.

Prepare reagents and library tube for template preparation

1. Label the library tube with the Tube Label text that you entered into the software.
2. Equilibrate the library, TMPL Rgnt Mix, TMPL ISP, and TMPL CF-1 to room temperature for 30 minutes.

Ion OneTouch™ Dx Template Kit component lot matching

The four components of the Ion OneTouch™ Dx Template Kit must be lot-matched with each other for use.

Component	Part No.	Storage
Ion OneTouch™ Dx Template Supplies	A18933	15°C to 30°C
Ion OneTouch™ Dx Template Solutions	A18932	
Ion OneTouch™ Dx Template Reagents	A18930	–30°C to –10°C
Ion OneTouch™ Dx Template ES Beads	A18931	2°C to 8°C

Select the Planned Run

1. Change gloves, then press the **Run** button on the Ion OneTouch™ Dx Instrument touchscreen.
2. Enter the Run Short Code (generated by the Torrent Suite™ Dx Software when you executed the run). Make sure the Planned Run information on the screen is correct, then press **Next**.

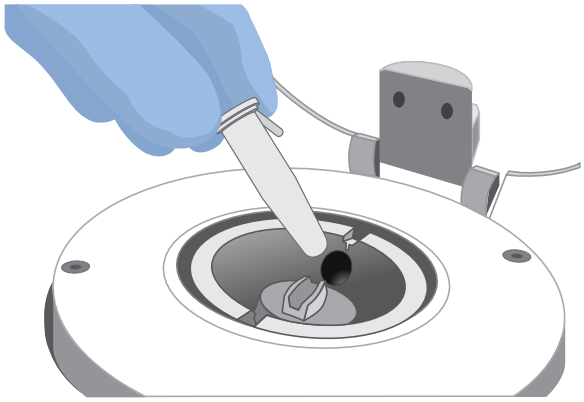
3. Enter the Tube Label text that you entered into the software when you executed the run, then press **Next**.

   Note: For tracking purposes, the Tube Label text must exactly match the text you entered in the **Tube Label** field in the Torrent Suite™ Dx Software.

4. Confirm the run type displayed on the instrument screen, then press **Next**.

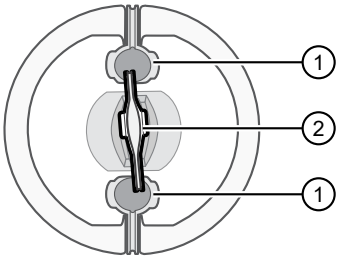
Install the TMPL Recovery Tubes, TMPL Recovery Router, and TMPL Amplification Plate

1. Label two TMPL Recovery Tubes with the Tube Label text entered into the software.
2. Insert the tubes into the holes in the Ion OneTouch™ Dx Instrument centrifuge, making the sure the tube arm is inserted into the slot next to each hole.



3. Pinch the sides of the TMPL Recovery Router, then push the router down into the center slot of the centrifuge until it is seated flat and secure in the center of the rotor.

   Note: The router is not directly aligned with the tubes, but is intentionally positioned at an angle.

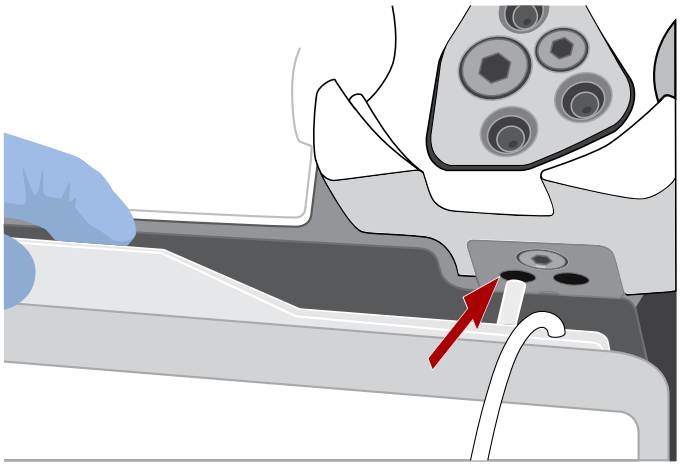


① TMPL Recovery Tube
② TMPL Recovery Router

4. Close the lid of the centrifuge, then press **Next**.

5. Insert a new TMPL Amplification Plate into the open instrument heat block.

Note: Ensure that the plate port is intact, then carefully align the plate port with the left outlet hole on the instrument. The plate includes disposable tubing and a disposable injector.



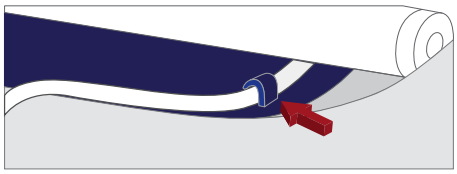
CAUTION! Hot Surface. Use care when working near the heat block to avoid burns from the hot components.

CAUTION! PHYSICAL INJURY HAZARD. The pointed end of the disposable injector can puncture your skin. Keep your hand away from the point of the injector.

6. Pull the handle forward to secure the plate. The tubing should be under the handle. Press **Next**.

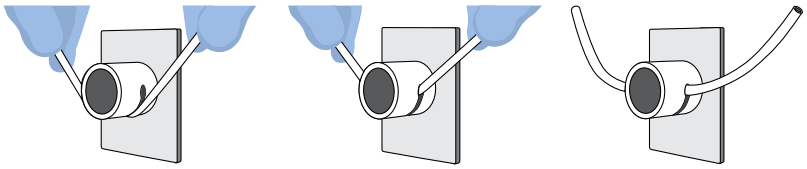
Note: In the following steps, ensure that the tubing is not kinked or twisted at any point along its length.

7. Thread the tubing through the tubing holder.



Note: Examine the tubing to ensure there are no kinks in the tubing before proceeding.

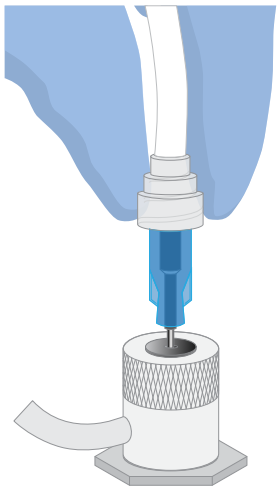
8. Align the tubing with the slot that runs along the bottom of the pinch valve. Gently pull the tubing up into the slot until it is secure in the notch.



9. Adjust the tubing so that it is straight but not too taut. The injector needle should reach the injector hub without stretching the tubing. Press **Next**.

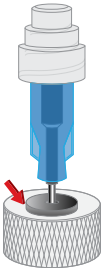
10. Ensure that the injector needle is screwed tightly onto the tubing. Hold the centrifuge lid down with one hand, and with your other hand insert the injector needle straight down into the injector hub. Push down until the injector touches the hub.

Note: The spring-loaded top of the injector hub clicks upon release, which indicates that the tip of the needle is the correct distance from the hub surface.

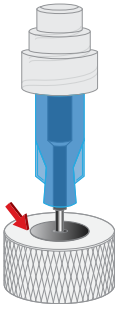


11. Gently push the injector down again and release. You should hear a click from the hub. Then press **Next**.

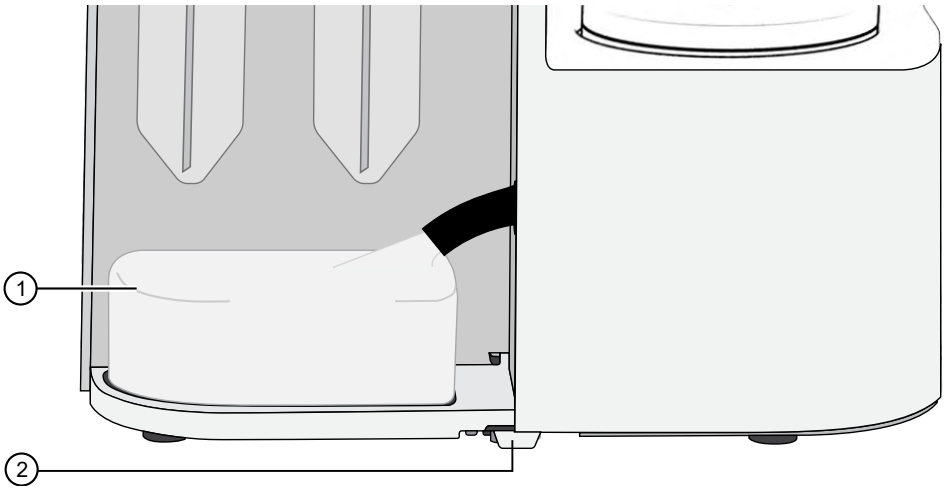
Up position



Down position



12. Pull the waste container from the external waste tubing, and empty the container into an appropriate hazardous waste receptacle.



① Waste container
② Oil waste tray

13. Inspect the oil waste tray on the instrument.

CAUTION! If the oil waste tray is full, do not run the instrument. The instrument must be inspected and repaired by a field application specialist.

14. Put the empty waste container back on the tray, then push the waste tube back into the container port. Press **Next**, then proceed to prepare the amplification solution.

Prepare the amplification solution

Kit components used in this procedure

Kit component	Box
TMPL Water (yellow cap) TMPL Rgnt B (blue cap)	Ion OneTouch™ Dx Template Solutions (Part No. A18932, stored at 15°C to 30°C)
TMPL Rgnt Mix (violet cap) TMPL Enzyme Mix (brown cap) TMPL ISP (black cap) TMPL CF-1 (clear cap)	Ion OneTouch™ Dx Template Reagents (Part No. A18930, stored at –30°C to –10°C)

Prepare the amplification solution

Perform the following steps in a laminar flow hood in a designated preamplification area. See "Guidelines to prevent cross-contamination" on page 17 for additional guidelines to avoid cross-contamination.

IMPORTANT! The volume of TMPL ISP reagent used in the amplification solution is critical and must be accurate.

1. Put on new gloves after emptying the Ion OneTouch™ Dx Instrument waste.
2. Ensure that the library, TMPL Rgnt Mix, TMPL ISP, and TMPL CF-1 are completely thawed.
3. Vortex the TMPL Rgnt B and TMPL Rgnt Mix tubes for 5 seconds each, then pulse centrifuge for 3–5 seconds.
4. Flick the TMPL Enzyme Mix tube with the tip of your finger 4 times, then pulse centrifuge for 3–5 seconds. Place in a benchtop cold box at 2–8°C until needed.
5. In a new nuclease-free 1.5-mL low-retention microcentrifuge tube at room temperature, add the following components in the designated order:

	Order	Reagent	Cap color	Volume
☐	1	TMPL Water	Yellow	40 µL
☐	2	TMPL Rgnt Mix	Purple	500 µL
☐	3	TMPL Rgnt B	Blue	300 µL
☐	4	TMPL Enzyme Mix	Brown	50 µL

6. Cap the 1.5-mL tube and vortex for ~5 seconds, then pulse centrifuge for 3–5 seconds.
7. Vortex the TMPL ISP tube for 30 seconds, then pulse centrifuge for 3–5 seconds.
8. Vortex the library and TMPL CF-1 tubes for 5 seconds each, then pulse centrifuge for 3–5 seconds.
9. Add the following to the tube in the designated order. After each addition, cap the tube, vortex for ~5 seconds, then pulse centrifuge for 3–5 seconds.

	Order	Reagent	Cap color	Volume
☐	1	TMPL ISP	Black	100 µL
☐	2	TMPL CF-1	Clear	5 µL
☐	3	DNA/RNA combined library	—	5 µL

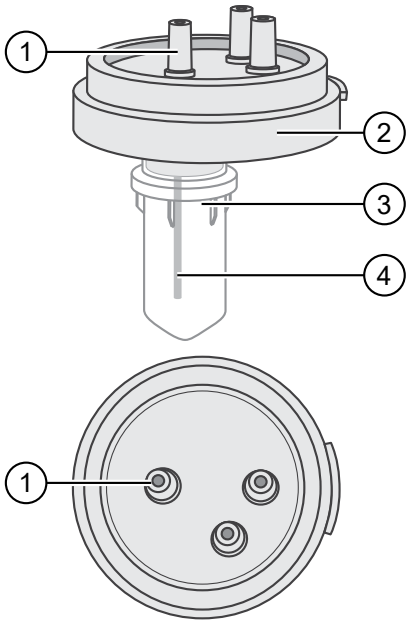
Proceed immediately to "Fill the TMPL Emulsion Cartridge" on page 40.

Fill the TMPL Emulsion Cartridge

1. Clean the Ion OneTouch™ Assembly Rack with fresh 10% bleach followed by two water rinses.
2. Label a pre-assembled TMPL Emulsion Cartridge with the Tube Label text entered into the software.

   Note: Make sure that the short tube is attached to the bottom of the sample port and extends into the Reaction Tube, then push the Reaction Tube firmly onto the cartridge to ensure it is securely fastened to the cartridge.

3. Use a marker to circle the sample port on the top of the TMPL Emulsion Cartridge to distinguish it from the other two ports.

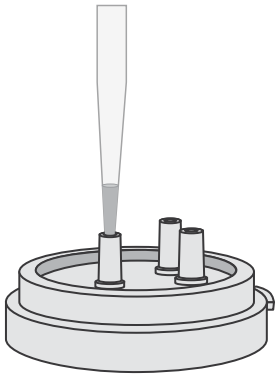


① Sample port (above the Reaction Tube)
② TMPL Emulsion Cartridge
③ Reaction Tube
④ Short tube from the sample port into the Reaction Tube

4. Place the TMPL Emulsion Cartridge into the Ion OneTouch™ Assembly Rack with the ports facing up.

① Sample port (marked as in step 3)

5. Collect the full volume of the amplification solution (~1000 µl) using a P1000 pipettor, then insert the pipette tip vertically into the circled sample port on the cartridge with a snug fit.



6. Slowly pipet the amplification solution into the sample port.

Note: Keep the pipette and pipette tip vertical and the pipette plunger depressed when removing the tip from the port to avoid withdrawing any reaction mix or introducing air bubbles.

7. Invert the TMPL Reaction Oil 5 times to mix.

8. Using a new pipette tip, collect 750 µL of TMPL Reaction Oil using a P1000 pipettor, then insert the pipette tip vertically into the sample port on the cartridge.

9. Slowly pipet the oil into the sample port to layer the oil over the aqueous reaction mix.

Note: Keep the pipette plunger depressed when removing the tip from the port.

10. Replace the pipette tip to avoid contamination, and repeat steps 8–9 one more time, adding another 750 µL of TMPL Reaction Oil (1.5 mL total).

11. Immediately install the TMPL Emulsion Cartridge on the instrument. Do not mix or shake the cartridge.

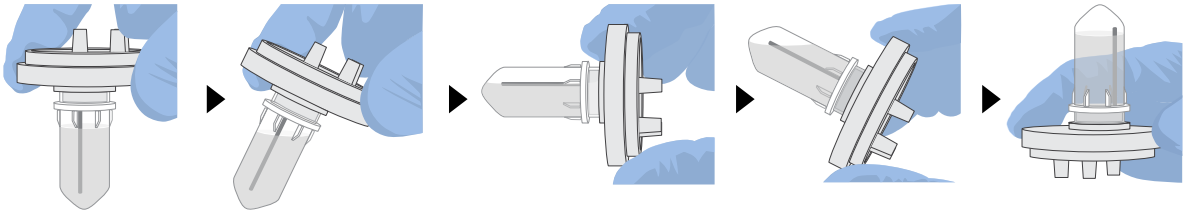
Install the filled TMPL Emulsion Cartridge

IMPORTANT! Do not mix or shake the TMPL Emulsion Cartridge during the following steps.

1. Keep the TMPL Emulsion Cartridge in the Ion OneTouch™ Assembly Rack, then orient the assembly so that the sample port is on your *left*.

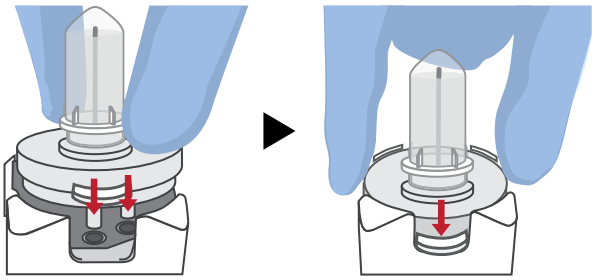
2. Lift the TMPL Emulsion Cartridge straight out of the tube rack.

3. With the short tube in the Reaction Tube to the left, slowly rotate the assembly to your *right* until the Reaction Tube is inverted.



Note: This ensures minimal exposure of the short tubing in the Reaction Tube with the aqueous phase.

4. Insert the three ports of the TMPL Emulsion Cartridge into the three holes on the top of the manifold. Press firmly on all sides to ensure a secure fit on the manifold.



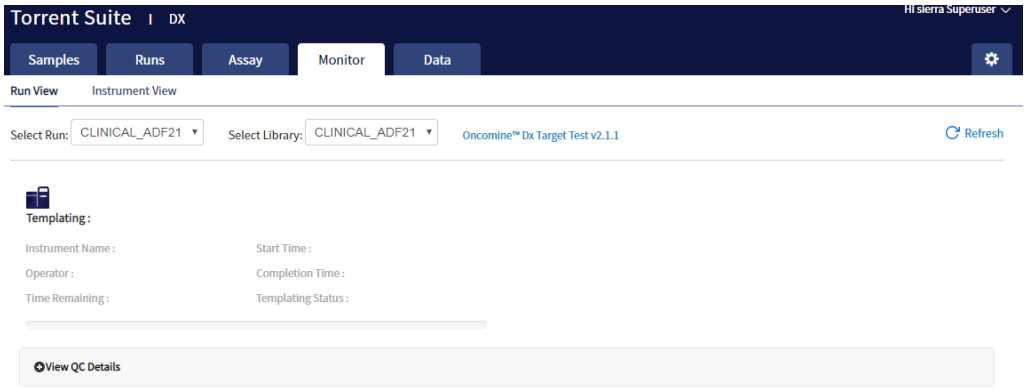
Note: After inserting the TMPL Emulsion Cartridge, bubbles may shoot up into the Reaction Tube. This is normal.

Run the Ion OneTouch™ Dx Instrument

IMPORTANT! ISPs can remain on the Ion OneTouch™ Dx Instrument for up to 24 hours after a run. You must begin enriching the ISPs on the Ion OneTouch™ ES Dx Instrument within 24 hours.

1. Ensure that the centrifuge lid of the Ion OneTouch™ Dx Instrument is closed.
2. Press **Next** on the instrument touchscreen to start the run.

   Note: The run takes approximately 5 hours and 30 minutes to complete. The time remaining and a progress bar is displayed on the instrument screen during the run and under the **Monitor** tab on the Torrent Suite™ Dx Software.



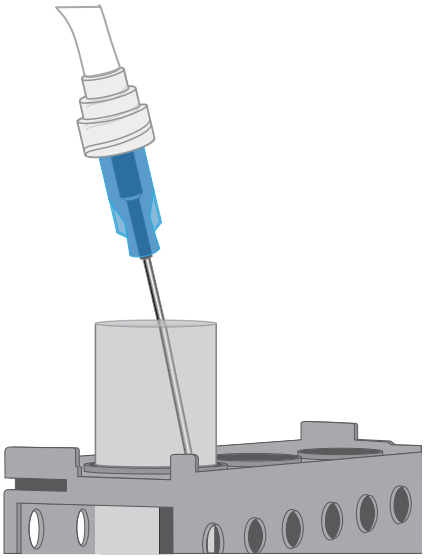
   - To cancel a run, press **Abort**, then press **Yes** to confirm. If there is a high-pressure event on the instrument, the instrument aborts the run automatically.
   - If a run is aborted, you must power-cycle the Ion OneTouch™ Dx Instrument before repeating the run preparation and run. Not doing so can cause the next run to fail.

3. After the run has completed, press **Re-Spin** on the touchscreen. The time since the end of the run is displayed (*hh:mm:ss*).
4. Based on the time displayed, do one of the following:
   - If it has been ≤30 minutes since the end of the run, no re-spin is required. Press **No**, then press **Next** to bypass this step. Proceed to recover the ISPs.
   - If it has been >30 minutes since the end of the run, press **Yes** to repellet the ISPs for 10 minutes. After re-spin is complete, press **Next** and proceed to recover the ISPs.
   - If it has been >30 minutes but the re-spin step was accidentally skipped, repellet the ISPs.
     a. Leave the ISPs in the recovery tube on the instrument, and proceed to "Clean the Ion OneTouch™ Dx Instrument after the run" on page 51. (You can perform the cleaning procedure while the ISPs remain on the instrument.)
     b. After cleaning is complete (~12 minutes), press **Options** on the touchscreen (Managers and Administrators only). Then select **Re-spin** to pellet the ISPs.
     c. When the spin is complete, proceed to recover the ISPs.

Recover the ISPs from the Ion OneTouch™ Dx Instrument

The instrument touchscreen will guide the user through each step in the process. These steps are described below in more detail.

1. Remove the disposable injector from the injector hub, and carefully release the flexible tubing from the pinch valve. Place the injector into an empty 50-mL conical tube to collect waste. Press **Next**.



2. Press **Open Lid** on the Ion OneTouch™ Dx Instrument touchscreen, and wipe up any residue from the inside of the lid with a new disposable wipe.

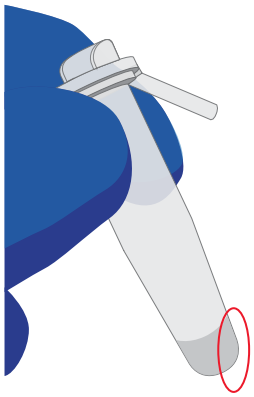
Note: When using multiple instruments, use a new disposable wipe for every instrument.

3. Remove and discard the TMPL Recovery Router.

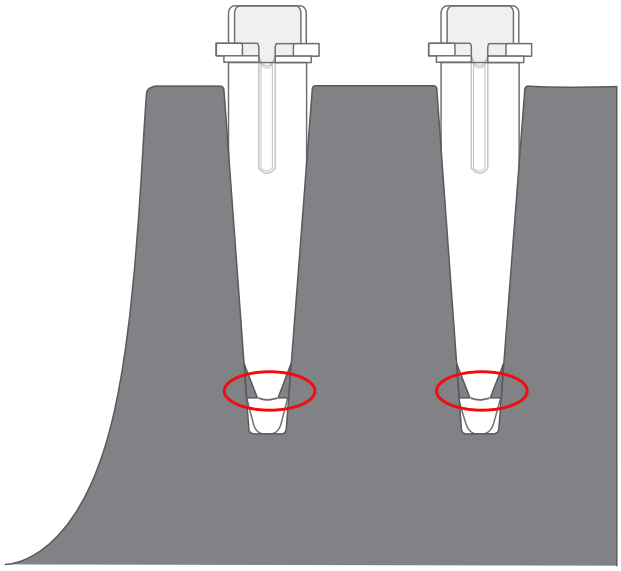
4. Carefully remove the two TMPL Recovery Tubes from the instrument and insert them in the Ion OneTouch™ Sample Rack, with each tube arm inserted into the slot on the back of the rack. Close the instrument lid.

IMPORTANT! Do not agitate the tubes when handling. Make sure the tubes are clearly labeled with the Tube Label text.

Note: The pelleted ISPs are located at the bottom of each tube on the same side as the tube arm, as shown below. The liquid in each tube may appear cloudy and the pellet will not be visible. This is normal.



5. Depress the plunger on a P-1000 pipette and carefully insert the tip into a TMPL Recovery Tube, avoiding the pelleted ISPs. Slowly remove ~1 mL of supernatant from the top down. Switch to a P-200 pipette and use the same procedure to remove the supernatant until the meniscus at the top of the liquid aligns with the bottom of the triangular arrow guides.



IMPORTANT! Top-down removal is essential to avoid loss of ISPs. Avoid the pellet and do not eject liquid back into the tube when pipetting.

Note: The volume remaining in each tube is 50 µL.

Prepare the TMPL ES Strip Tube

Kit components used in this procedure

Kit component	Box
TMPL ES Rsp Soln (orange cap) TMPL Wash Solution (white cap) TMPL Neutral Soln (red cap) TMPL Tween™ Solution (white cap)	Ion OneTouch™ Dx Template Solutions (Part No. A18932, stored at 15°C to 30°C)
TMPL ES Strip Tube TMPL Sample Collection Tube	Ion OneTouch™ Dx Template Supplies (Part No. A18933, stored at 15°C to 30°C)
TMPL ES Beads (green cap)	Ion OneTouch™ Dx Template ES Beads (Part No. A18931, stored at 2°C to 8°C)

Prepare the TMPL ES Beads with TMPL ES Rsp Soln

If you are processing multiple template preparations at the same time, prepare a master mix by increasing the volumes of TMPL ES Beads and TMPL ES Rsp Soln according to the table in step 5.

1. Vortex the TMPL ES Beads (green cap) for 30 seconds to resuspend the beads.
2. In a new 1.5-mL low-retention microcentrifuge tube, add 14.3 µL of TMPL ES Beads per template preparation (13 µL of beads plus 10% extra to mitigate pipetting errors).
3. Fill the tube with 1 mL of TMPL Wash Solution.
4. Cap the tube, vortex for 10 seconds, then place the tube on the DynaMag™ Dx 16 2-mL Magnet for 1 minute to capture the beads. Without disturbing the pellet, carefully remove and discard the supernatant.
5. Add 143 µL of TMPL ES Rsp Soln (orange cap) per template preparation to the tube (130 µL of solution plus 10% extra to mitigate pipetting errors).

Number of template preparations	Volume [1]	
	TMPL ES Beads	TMPL ES Rsp Soln
1	14.3 µL	143 µL
2	28.6 µL	286 µL
3	42.9 µL	429 µL
4	57.2 µL	572 µL
5	71.5 µL	715 µL
6	85.8 µL	858 µL

(continued)

Number of template preparations	Volume [1]	
	TMPL ES Beads	TMPL ES Rsp Soln
7	100.1 µL	1001 µL
8	114.4 µL	1144 µL

[1] Includes 10% extra

6. Cap the tube, then vortex for 30 seconds to resuspend the pellet.

7. If some beads are stuck to the lid of the tube, pulse centrifuge the tube for 3 seconds. Leave the tube at room temperature until ready to use.

Prepare fresh Melt-Off Solution

1. Prepare 1 M NaOH by adding 1 mL of 10 M NaOH to 9 mL Nuclease-Free water. 1 M NaOH must be prepared fresh weekly.

2. In a new 1.5-mL low-retention microcentrifuge tube, combine the following components in order.

	Order	Component	Volume
☐	1	TMPL Tween™ Solution	280 µL
☐	2	1 M NaOH	40 µL
		Total	320 µL

3. Cap the tube, vortex for 10 seconds, then pulse centrifuge for 3 seconds.

Prepare the strip tube

1. Using a marker, label a TMPL ES Strip Tube on the square tab with the Tube Label text, then place it in the Ion OneTouch™ Assembly Rack.

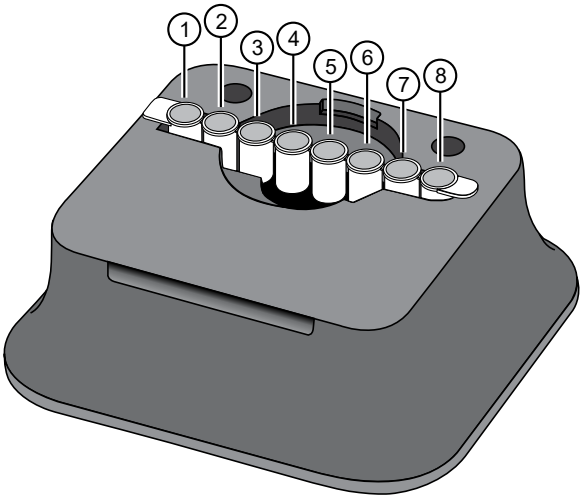
   When facing the rack, make sure that the square tab of the TMPL ES Strip Tube is on the left and the round tab is on the right.

2. Resuspend the contents of each TMPL Recovery Tube containing the templated ISPs in 50 µL (from "Recover the ISPs from the Ion OneTouch™ Dx Instrument" on page 44, step 5) by vigorously pipetting up and down 30 times.

   Note: Set a pipette to 40 µL and keep the pipette tip at the bottom of the tube when mixing to minimize air bubbles, which can lead to the loss of ISPs.

3. Pool the entire volume of templated ISPs from both recovery tubes into well 1 of the TMPL ES Strip Tube. The total pooled volume will be 100 µL.

4. Fill the remaining wells in the strip as follows:



	Well number	Reagent
☐	Well 1	Template-positive ISPs (~100 µL), added in step 3
☐	Well 2	130 µL of TMPL ES Beads in TMPL ES Rsp Soln
☐	Well 3	300 µL of TMPL Wash Solution
☐	Well 4	300 µL of TMPL Wash Solution
☐	Well 5	300 µL of TMPL Wash Solution
☐	Well 6	Empty
☐	Well 7	300 µL of freshly-prepared Melt-Off Solution
☐	Well 8	Empty

5. With a marker, label a 0.2-mL TMPL Sample Collection Tube with the Tube Label text.

6. Add 10 µL of TMPL Neutral Soln (red cap) to the TMPL Sample Collection Tube, then place it in the tube holder in the Ion OneTouch™ Sample Rack.

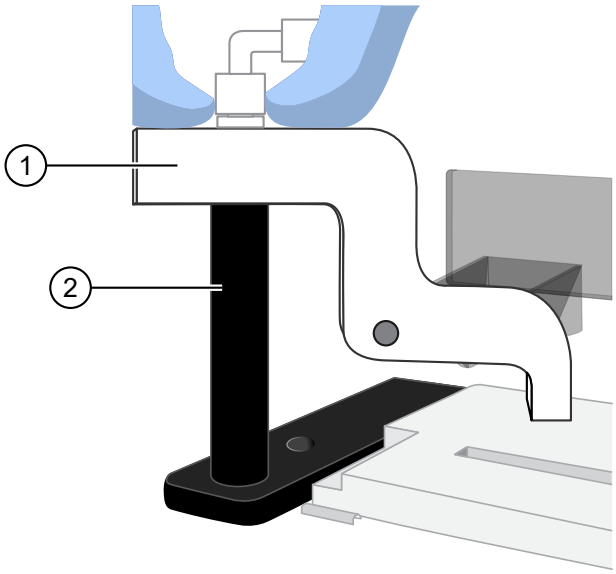
IMPORTANT! The volume of TMPL Neutral Soln added to the TMPL Sample Collection Tube is critical and must be accurate.

Prepare the Ion OneTouch™ ES Dx Instrument and perform the run

1. Place a new TMPL ES Tip in the Tip Loader. Remove the Tip Arm from its cradle on the Ion OneTouch™ ES Dx Instrument.

2. Grip the Tip Arm with two fingers, then align the metal fitting of the Tip Arm with the top of the tip. Firmly press the Tip Arm straight down onto the tip for 3 seconds with even pressure to ensure proper installation.

   IMPORTANT! Do not repeatedly jam the Tip Arm up and down onto the tip.

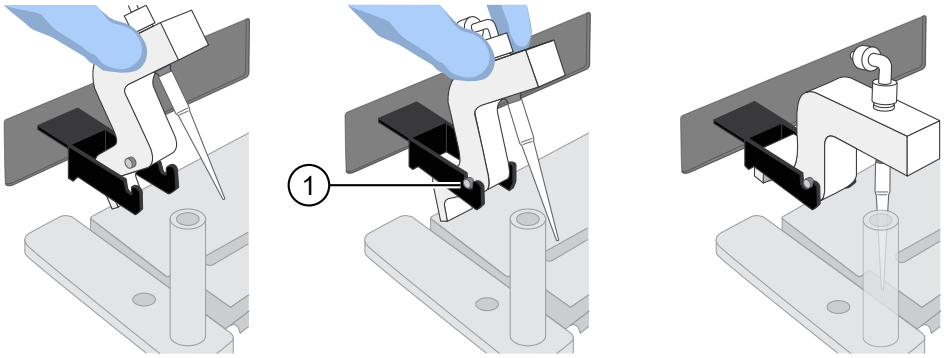


① Tip Arm
② Tip Loader

3. Lift the Tip Arm straight up to pull the installed TMPL ES Tip from the Tip Loader tube.

4. Return the Tip Arm to its cradle on the instrument. Tilt the Tip Arm back and align the pins with the round notches in the cradle, then lower the Tip Arm into the home position.

   Note: Ensure that the back end of the Tip Arm is not resting on top of the thumb screw, causing the Tip Arm to tilt forward.

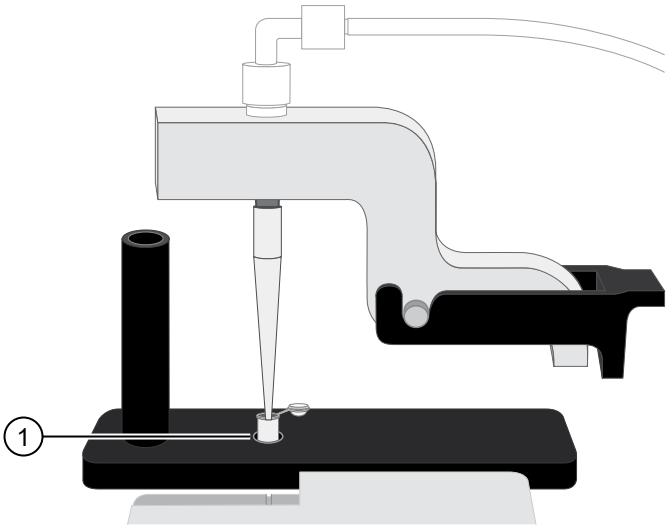


① Tip Arm pins resting in the notches in the cradle

5. Remove the 0.2-mL TMPL Sample Collection Tube containing TMPL Neutral Soln from the Ion OneTouch™ Sample Rack. Place it in the hole in the base of the Tip Loader.

   When the Tip Arm is lowered, the tip will fit inside the TMPL Sample Collection Tube, as shown below.

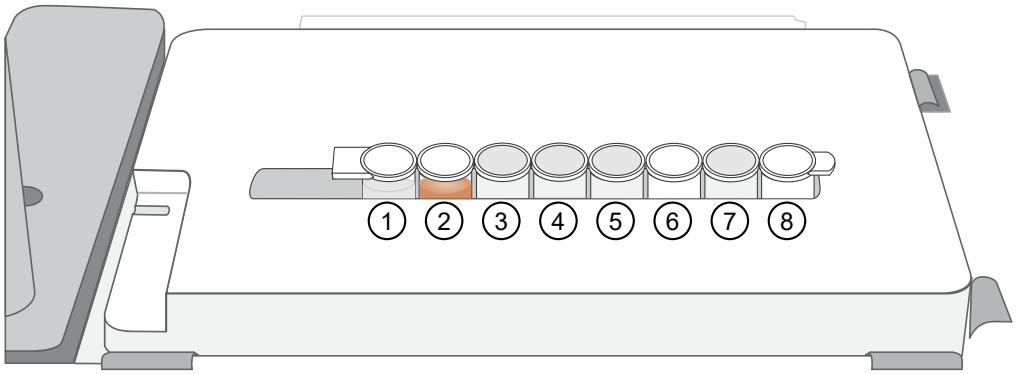
IMPORTANT! Be sure that the Tip Loader is aligned properly in its trough. If the Tip Loader is too far forward or back, the tip will miss the tube and eject sample onto the tip loader.



① TMPL Sample Collection Tube

6. Confirm that the square-shaped tab of the 8-well strip is on the left, then insert the filled strip into the **right end of the slot** on the Ion OneTouch™ ES Dx Instrument tray.

IMPORTANT! Make sure that the strip is pushed all the way to the right in the slot, away from the Tip Arm.



7. Press the **Start/Stop** button on the instrument to start the run.
   The screen displays "Run" during the run, which takes ~35 minutes.

8. If you need to stop the instrument during a run, press **Start/Stop** again. The instrument completes the current step, then stops the run and displays "End". Press **Start/Stop** again to return the Tip Arm to the home position. It is not possible to restart where you left off after stopping a run.

9. During the run, proceed to "Clean the Ion OneTouch™ Dx Instrument after the run" on page 51.

10. At the end of the run, the Ion OneTouch™ ES Dx Instrument displays "End" and beeps every 60 seconds. Press the **Start/Stop** button to silence this alarm, then reset the instrument for the next run. Proceed to "Collect the sample from the Ion OneTouch™ ES Dx Instrument" on page 52.

The enriched ISPs can be left on the instrument for up to 2 hours. The instrument can be left on between runs.

Clean the Ion OneTouch™ Dx Instrument after the run

IMPORTANT! Refer to the product SDS for guidance regarding proper disposal and handling of Ion OneTouch™ Dx Instrument waste products.

1. Return to the Ion OneTouch™ Dx Instrument, then press **Next** on the touchscreen to continue with the post-run cleaning procedure.

2. Wipe any residue from the inside of the lid with a disposable wipe.

3. Remove the used TMPL Emulsion Cartridge and invert it to visually inspect that the aqueous phase was completely injected. Only one phase should be present. Discard the cartridge in the appropriate waste container.

   Note: If more than one phase remains, sample injection from the assembly did not occur. See Appendix A, "Troubleshooting".

4. Temporarily remove the used TMPL Amplification Plate and visually inspect it to check that there is no remaining emulsion or excessive air bubbles in the plate.

   Note:
   - Only clear oil should remain in the plate. Any white or cloudy areas indicate the presence of emulsion.
   - If the TMPL Amplification Plate is filled with emulsion, sample injection from the plate did not occur. See Appendix A, "Troubleshooting". Trace amounts of emulsion are okay.

5. Reinstall the TMPL Amplification Plate.

6. Check the reagent tubes to ensure that the appropriate volume of oil and recovery solution was used. Approximately 20 mL of oil and 11 mL of TMPL Recovery Solution will be left in their respective reagent tubes. Press **Next**.

   Note: Inappropriate volumes may indicate an instrument failure during the run. See Appendix A, "Troubleshooting".

7. Install a new TMPL Cleaning Adapter.

8. Confirm that the disposable injector has been placed into a 50-mL conical tube.

9. Press **Next** on the Ion OneTouch™ Dx Instrument touchscreen to start the cleaning run.

   Note: The cleaning run takes 13 minutes. During cleaning, ensure that oil is flowing from the disposable injector. No flow of oil could indicate a clog in the manifold or in the cleaning adapter. See Appendix A, "Troubleshooting".

10. When cleaning is complete, press **Next**.

    Note: If the touchscreen indicates that cleaning failed, see Appendix A, "Troubleshooting".

11. Remove the disposable injector from the 50-mL conical tube, then discard the tube and its waste into appropriate containers. Place the injector in a new 50-mL conical tube.

    IMPORTANT! Do not remove the TMPL Cleaning Adapter or TMPL Amplification Plate. Leave them on the instrument.

12. Press **Next** to return to the main screen, then log out of the instrument touchscreen.

Collect the sample from the Ion OneTouch™ ES Dx Instrument

1. After the Ion OneTouch™ ES Dx Instrument run ends, remove the TMPL Sample Collection Tube containing the enriched ISPs and securely close the tube.

   Note: The enriched ISPs can be left on the instrument for up to 2 hours.

2. Ensure that the TMPL Sample Collection Tube has >200 µL of solution.

   Note: If the tube has <200 µL of solution, see Appendix A, "Troubleshooting".

3. Remove, then discard the used tip and 8-well strip tube from the Ion OneTouch™ ES Dx Instrument.

STOPPING POINT Proceed to sequencing within 2 hours after the end of the Ion OneTouch™ ES Dx Instrument run, or transfer the template-enriched ISPs to 2–8°C storage. The ISPs may be stored for up to 1 week. If stored longer than 1 week, prepare new template-enriched ISPs from the library.

A Troubleshooting

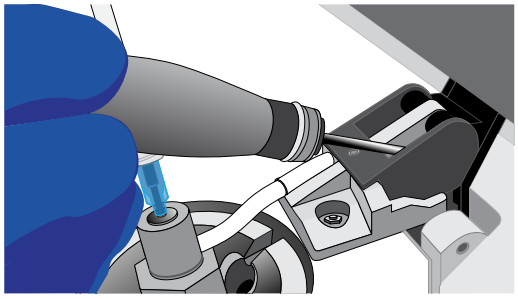
Ion OneTouch™ Dx Instrument

Observation	Possible cause	Recommended action
Display message: Sensor unable to measure pressure.	Hardware issue	Contact Technical Support (see Appendix F, "Customer and technical support"). Reboot the Ion OneTouch™ Dx Instrument to clear the alarm.
Display message: Coolant pump does not flow.	Hardware issue	Contact Technical Support (see Appendix F, "Customer and technical support"). Reboot the Ion OneTouch™ Dx Instrument to clear the alarm.
Display message: Connection failure with Ion Torrent™ Server	Ion OneTouch™ Dx Instrument and Ion Torrent™ Server connection is not established	Check that a network connection to the Ion Torrent™ Server is established, then reboot the Ion OneTouch™ Dx Instrument. **Note:** A sample created during a run with this alarm raised can still be used.
Display message: Failed to connect to the Ion Torrent™ Server. Check your connection.	Ion OneTouch™ Dx Instrument and Ion Torrent™ Server connection is not established during startup	Check your network connection to the Ion Torrent™ Server to make sure the connection is established, then reboot the Ion OneTouch™ Dx Instrument.
Display message: Failed to set up system time at startup. Check your connection to the Ion Torrent™ Server.	Ion OneTouch™ Dx Instrument and Ion Torrent™ Server connection is not established	Check your network connection to the Ion Torrent™ Server to make sure the connection is established, then reboot the Ion OneTouch™ Dx Instrument.
	Instrument is still in the process of establishing a connection	Allow 10 minutes to see if the display message clears.
Display message: Motor current too high. Reboot the instrument to clear the alarm.	Hardware issue	Contact Technical Support (see Appendix F, "Customer and technical support"). Reboot the Ion OneTouch™ Dx Instrument to clear the alarm. **Note:** A sample created during a run with this alarm raised can still be used.
Display message: Pressure too high. Reboot the instrument to clear the alarm.	• Hardware issue • Clogged TMPL Emulsion Cartridge due to contaminated reagents or defective emulsion cartridge	Reboot the Ion OneTouch™ Dx Instrument to clear the alarm. Use a new TMPL Emulsion Cartridge and fresh reagents to repeat the run. Contact Technical Support (see Appendix F, "Customer and technical support") if the issue persists.
Display message: Sensor unable to measure instrument temperature	Hardware issue	Contact Technical Support (see Appendix F, "Customer and technical support"). Reboot the Ion OneTouch™ Dx Instrument to clear the alarm.

Observation	Possible cause	Recommended action
Display message: Set temperature out of range. Reboot the instrument to clear the alarm.	Hardware issue	Contact Technical Support (see Appendix F, "Customer and technical support"). Reboot the Ion OneTouch™ Dx Instrument to clear the alarm. **IMPORTANT!** If this alarm is raised, you cannot use the prepared template.
Display message: Software versions incompatible. Go to the Options menu and update the software.	The system software was updated, but the instrument software was not.	After the system software has been updated, update the instrument software as follows: 1. On the main menu of the instrument, press **Options** and follow the instructions to check for and install updates. 2. When installation is complete, follow the onscreen prompts to restart the instrument. **IMPORTANT!** You must restart the instrument before proceeding.
Display message: TEC current too high. Reboot the instrument to clear the alarm.	Hardware issue	Contact Technical Support (see Appendix F, "Customer and technical support"). Reboot the Ion OneTouch™ Dx Instrument to clear the alarm. **IMPORTANT!** A sample created during a run with this alarm raised must NOT be used.
Ion OneTouch™ Dx Instrument displays a blue or grey screen with folders while the instrument is idle	Instrument issue	Power cycle the Ion OneTouch™ Dx Instrument using the On/Off switch. If alarms appear or the problem persists, contact Technical Support (see Appendix F, "Customer and technical support").
Ion OneTouch™ Dx Instrument displays a blue or grey screen with folders during cleaning or a run	Instrument issue	Power cycle the Ion OneTouch™ Dx Instrument using the On/Off switch, then restart the cleaning or run. If alarms appear or the problem persists, contact Technical Support (see Appendix F, "Customer and technical support").
Ion OneTouch™ Dx Instrument touchscreen freezes	Touchscreen button is pressed more than once per second.	Wait 5 minutes. If still unresponsive: 1. Click on the **Abort** button if displayed, and restart the process. 2. If **Abort** button is not displayed, power-cycle the instrument using the On/Off switch, then restart the process. **Note:** If the touchscreen freezes during Ion OneTouch™ Dx Instrument run setup, the software will remember that the instrument has already been cleaned and will not require the cleaning to be performed a second time.
During cleaning, no liquid comes out of the disposable injector	Loose or damaged reagent tube sipper in the "O" position	Reinstall the sipper in the "O" position, then restart cleaning. If the problem persists, install a new sipper.

Observation	Possible cause	Recommended action
During cleaning, no liquid comes out of the disposable injector *(continued)*	Improper installation of the TMPL Cleaning Adapter and TMPL Amplification Plate	Reinstall the TMPL Cleaning Adapter and TMPL Amplification Plate, then restart cleaning. If the problem persists, use a new TMPL Cleaning Adapter and a new TMPL Amplification Plate.
	Instrument issue	Contact Technical Support (see Appendix F, "Customer and technical support").
Run did not complete	• Operator did not power cycle the Ion OneTouch™ Dx Instrument after an "Abort" operation • Instrument issue	Power cycle the Ion OneTouch™ Dx Instrument using the On/Off switch, then start a new run. If the problem persists, contact Technical Support (see Appendix F, "Customer and technical support").
Cleaning was aborted due to high pressure	Improper installation of the TMPL Cleaning Adapter or TMPL Amplification Plate	Reinstall the TMPL Cleaning Adapter and TMPL Amplification Plate, then restart the cleaning protocol. If the problem persists, contact Technical Support (see Appendix F, "Customer and technical support").
Run was aborted due to high pressure	Clogged TMPL Emulsion Cartridge	Install a new TMPL Emulsion Cartridge, then restart the run. If the problem persists, contact Technical Support (see Appendix F, "Customer and technical support").
	Reagent contamination	Replace the TMPL Reaction Oil, TMPL Reagent Tubes, and TMPL Sippers, then restart the run.
	Instrument issue	Contact Technical Support (see Appendix F, "Customer and technical support").
Oil is leaking from the instrument	Improper installation of consumables	Wipe up any leakage and reinstall TMPL Sippers and TMPL Reagent Tubes.
	Waste was not emptied before the run	Wipe up any leakage and empty the waste container before the next run.
	Instrument issue	Contact Technical Support (see Appendix F, "Customer and technical support").
Excessive oil in waste tray	Instrument issue	Power cycle the Ion OneTouch™ Dx Instrument using the On/Off switch. If the problem persists, contact Technical Support (see Appendix F, "Customer and technical support").
Waste backup observed after completion of Ion OneTouch™ Dx Instrument run, waste leaks from the waste line after removal of the waste container	Filter in waste container clogged causing back-pressure	Remove or clean filter in waste container.
	Improper installation of consumables	Wipe up any leakage and reinstall TMPL Sippers and TMPL Reagent Tubes.
	Waste was not emptied before the run	Wipe up any leakage and empty the waste container before the next run.
	Instrument issue	Contact Technical Support (see Appendix F, "Customer and technical support").

Observation	Possible cause	Recommended action
The centrifuge keeps running and the run never completes	Instrument hardware issue	Press **Abort** and power cycle the Ion OneTouch™ Dx Instrument using the On/Off switch. Press **Open Lid** to remove and discard the recovery tubes, then restart template preparation. If the problem persists, contact Technical Support (see Appendix F, "Customer and technical support").
Two phases are present in the sample cup at the end of the Ion OneTouch™ Dx Instrument run, sample injection from the TMPL Amplification Plate did not occur	• The TMPL Emulsion Cartridge was not inserted properly into the instrument • Problem with the instrument	1. Power cycle the Ion OneTouch™ Dx Instrument using the On/Off switch. 2. Repeat run preparation, then the run, being careful to seat the TMPL Emulsion Cartridge as described. If the problem persists, contact Technical Support (see Appendix F, "Customer and technical support").
Large air gap (1 mL or greater) is present in the reaction cup	• Reagent tube not filled with TMPL Oil to start the run • Problem with the instrument	Repeat run preparation, then the run, being careful to follow all steps as described. If the problem persists, contact Technical Support (see Appendix F, "Customer and technical support").
Emulsion is detected in the TMPL Amplification Plate after completion of the Ion OneTouch™ Dx Instrument run	• Reagent tube not filled with oil to start the run • Improper installation of the consumables • Problem with the instrument	1. Ensure the consumables are installed correctly and not defective. 2. Power cycle the Ion OneTouch™ Dx Instrument using the On/Off switch. 3. Repeat run preparation, then the run, being careful to follow all steps as described. If the problem persists, contact Technical Support (see Appendix F, "Customer and technical support").
Inappropriate volumes of TMPL Oil and TMPL Recovery Solution are left after completion of the Ion OneTouch™ Dx Instrument run	Incorrect volumes of TMPL Oil and TMPL Recovery Solution used to start	Repeat the run, carefully check all volumes during run setup.
	• Improper installation of the consumables • Instrument issue	1. Ensure the consumables are installed correctly and not defective. 2. Power cycle the Ion OneTouch™ Dx Instrument using the On/Off switch. 3. Repeat run preparation, then the run, being careful to follow all steps as described. If the problem persists, contact Technical Support (see Appendix F, "Customer and technical support").
Centrifuge makes a loud sound during centrifugation	There is an obstruction in the centrifuge chassis	Turn off the instrument, then contact Technical Support (see Appendix F, "Customer and technical support").

Observation	Possible cause	Recommended action
Centrifuge lid does not open **Details:**	• Power failure • Software crash	1. Slide a 1/8-inch L-wrench (hex wrench) or equivalent tool into the right-hand hole at the top edge of the centrifuge hinge:  2. Press tool into the hole until there is a slight compression of the tool against the instrument and the centrifuge lid unlocks and opens. 3. Remove the tool from the hole, then open the lid. Do *not* force the lid open. If the problem persists, Contact Technical Support (see Appendix F, "Customer and technical support").
Centrifuge does not spin	Ion OneTouch™ Dx Instrument centrifuge lid was not closed properly **Note:** Centrifuge will not operate unless the lid is fully closed.	1. Open and properly close the centrifuge lid, then press **re-spin**. 2. After re-spin completes, power cycle the Ion OneTouch™ Dx Instrument using the On/Off switch. 3. Repeat run preparation, then the run, beginning with your pooled library sample. Be careful to properly close the centrifuge lid. If the problem persists, contact Technical Support (see Appendix F, "Customer and technical support").
	Instrument failure (fuse, motor driver board, and/or interlock switch failures)	Confirm that the centrifuge is not operating. On the touchscreen press **Options**, then press **respin**. • If the centrifuge does not begin to spin, contact Technical Support (see Appendix F, "Customer and technical support"). • If the centrifuge begins to spin. After re-spin completes: a. Power cycle the Ion OneTouch™ Dx Instrument using the On/Off switch. b. Repeat run preparation, then the run, beginning with your pooled library sample. Be careful to properly close the centrifuge lid.

Ion OneTouch™ ES Dx Instrument

Observation	Possible cause	Recommended action
Final sample volume is <200 uL	Loose or cracked tip or loose tip fitting on the Ion OneTouch™ ES Dx Instrument Tip Arm	Tighten the tip and tip fitting. If the tip is cracked, replace it.
	Improper calibration	Perform a residual volume check; if the residual volume check fails, perform calibration (see "Calibrate the Ion OneTouch™ ES Dx Instrument" on page 64). If the problem persists, contact Technical Support (see Appendix F, "Customer and technical support").
Excessive foaming	Improperly calibrated or inadequate volume in one or more wells of the TMPL ES Strip Tube	Use recommended volumes for all wells. Perform a residual volume check; if the residual volume check fails, perform calibration (see "Calibrate the Ion OneTouch™ ES Dx Instrument" on page 64). If the problem persists, contact Technical Support (see Appendix F, "Customer and technical support").
	Loose or cracked tip, or loose tip fitting on the Ion OneTouch™ ES Dx Instrument Tip Arm	Tighten the tip and tip fitting. If the tip is cracked, replace it.
E4, E12, or E22 error displays when the Ion OneTouch™ ES Dx Instrument is initializing	• Fuse is installed incorrectly • Instrument is below operating temp • Bad program or calibration setting • Tip Arm is not moving	1. Ensure that the fuse module is installed correctly and that the unit is within its recommended operating temperature range of 68°F to 86°F (20°C to 30°C). 2. Reboot the instrument: Power OFF the instrument, wait 3 seconds, then power ON the instrument. 3. If the error persists, restore the factory defaults, then recalibrate the instrument (see"Calibrate the Ion OneTouch™ ES Dx Instrument" on page 64).
Solution overflow during a run	Overloaded reagent volumes in TMPL ES Strip Tube	Repeat enrichment with correct reagent volumes.
Tip is causing the 8-well strip to lift out of its slot during run	Tip is not aligned vertically	Try tightening the tip. If the problem persists, perform vertical axis calibration (see "Vertical axis calibration" on page 64).
Strip lifts up during strip push	Instrument is not calibrated properly	Perform horizontal position calibration.
Strip lifts up when tip is raised from well	Instrument is not calibrated properly	Perform vertical calibration.
Immediately after strip push, the strip is not in contact with the magnet	Instrument is not calibrated properly	Perform horizontal position calibration.

Observation	Possible cause	Recommended action
Tip grinds into the base of the instrument and Code "1999" displays	• Vertical calibration setting too low or out of range • Instrument is not calibrated properly	1. Erase the memory on the instrument: Hold down the vertical-adjust button while powering ON the instrument. The instrument beeps several times. 2. Perform a residual volume test. 3. Recalibrate the instrument if residual volume check failed.
Tip is hitting the top of tray at start of run	• Instrument tray or tip is not properly seated in the instrument • Tip adapter is loose	1. Check for debris between the tray and the instrument, then reinstall the tray and tip. 2. Check the tip adapter to make sure it is tight.
Error displays	Various	1. Power the instrument OFF then ON. 2. If the error continues to display, erase the memory on the instrument. Hold down the vertical adjust button while powering ON the instrument. The instrument beeps several times. 3. Perform residual volume check. 4. Recalibrate the instrument if the residual volume check failed.
Instrument does not aspirate or dispense liquids	Loose fittings	• Ensure that the connections at the elbow on the Tip Arm and at the tubing on the rear syringe pump are finger-tight. • Ensure that the metal tip adapter fitting on the Tip Arm is finger-tight. **IMPORTANT!** After any changes to the metal tip adapter, perform a remaining volume test, and recalibrate the instrument.
Ion OneTouch™ ES Dx Instrument has a blown fuse	Various	Contact Technical Support (see Appendix F, "Customer and technical support").

B

Supplemental procedures and instruments

Fuse replacement on the Ion OneTouch™ ES Dx Instrument

IMPORTANT! The Ion OneTouch™ ES Dx Instrument is supplied with a Fuse Module and two different types of spare fuses, which should only be replaced by trained field service engineers. The Fuse Module is installed by the field service engineer into the Power Entry Module located on the back of the instrument in the proper orientation for the voltage in your area. If you are not sure of the setting that is right for your area, contact your local power company.

Line voltage	Replacement fuse type required
110/120 VAC	375 mA TT (Slow Blow) 1/4 inches × 1--1/4 inches
220/240 VAC	160 mA TT (Slow Blow) 5 × 20 mm

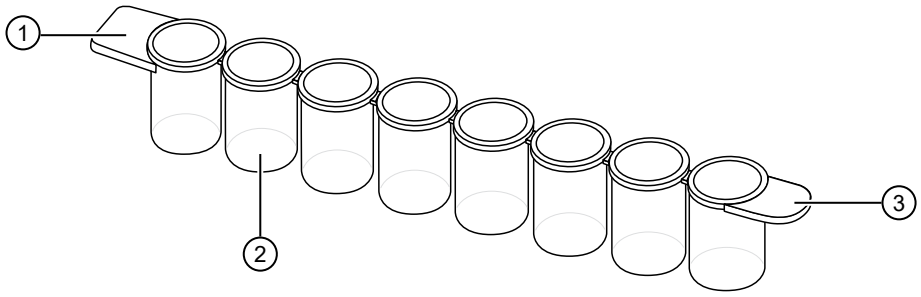
WARNING! ELECTRICAL SHOCK HAZARD. Severe electrical shock, which could cause physical injury or death, can result from working on an instrument when the high voltage power supply is operating. To avoid electrical shock, disconnect the power supply to the instrument, unplug the power cord, and wait at least 1 minute before working on the instrument.

Ion OneTouch™ ES Dx Instrument residual volume test

1. Install a new tip on the Ion OneTouch™ ES Dx Instrument Tip Arm.

   Note: For the residual volume test, you do not need to put a TMPL Sample Collection Tube in the Tip Loader.

2. Load 80 µL water or TMPL Wash Solution into the second well (Well 2) from the square-tab end of the 8-well strip:



① Square tab
② Second well
③ Round tab

3. Load the 8-well strip into the right end of the slot on the Ion OneTouch™ ES Dx Instrument tray so that the square tab is on the left and the rounded tab is on the right.

IMPORTANT! Before proceeding, carefully read and familiarize yourself with the following steps, which require you to manually start and stop the test run and manipulate the strip tube during the run. During the test, confirm that the tip is centered in the wells when moving in or out of a well.

4. Turn the instrument ON.

5. Wait for the instrument to initialize. The screen displays "rdy". The Tip Arm performs a series of movements and returns to the home position (~5 seconds).

6. Press **Start/Stop**.

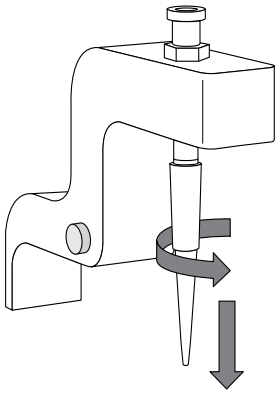
7. Wait for the instrument to aspirate the solution from Well 2 and completely remove the tip from Well 2, then *manually* push the 8-well strip to the left so that Well 4 is positioned directly under the Tip Arm.

8. Wait for the instrument to dispense the tip contents into Well 4.

9. Press **Start/Stop** to stop the test run, then press **Start/Stop** again to return the Tip Arm to the home position.

10. Using a P10 pipette, aspirate the entire residual volume from Well 2, then estimate the residual volume.

11. Remove the used tip: with the Tip Arm in its cradle and while standing above the Tip Arm, twist the tip *counterclockwise* and pull it downward to remove and discard the tip.



IMPORTANT! Improper removal of tips can loosen the metal tip adapter fitting on the Tip Arm and affect instrument operation.

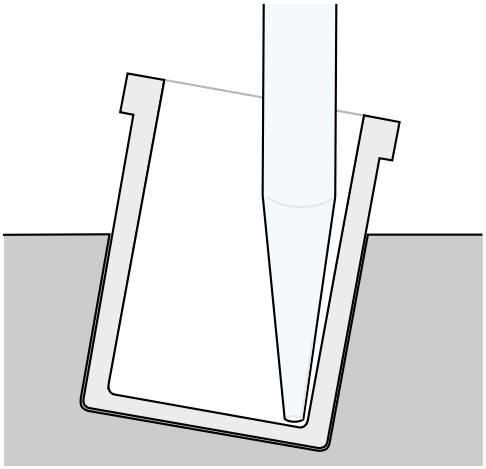
12. Remove and discard the used 8-well strip.

13. After performing the residual volume test, take one or more of the following actions:

Observation	Pass/Fail	Possible cause	Recommended actions
Residual volume in Well 2 is ≤5 µL	Pass	—	—
Residual volume in Well 2 is >5 µL **IMPORTANT!** The volume is measured from the *bottom* of the well, not from the sides.	Fail	The tip height is too high during aspiration.	Calibrate the instrument (see "Calibrate the Ion OneTouch™ ES Dx Instrument" on page 64).
The 8-well strip lifts as the tip rises to the top of the well	Fail	The tip is angled too far forward or the tip height is set too low.	Verify that the tip is vertical and positioned directly over the notch in the calibration shelf. If the tip is positioned correctly, restore defaults, then calibrate the instrument (see "Calibrate the Ion OneTouch™ ES Dx Instrument" on page 64).

Calibrate the Ion OneTouch™ ES Dx Instrument

Perform horizontal and vertical calibrations so that during operation the tip is optimally positioned in the well of the 8-well strip:



Note that the 8-well strip is always tilted at a fixed 10-degree angle in the slot. The pipette tip is vertical. When the tip is aligned properly during calibration so that it is in line with the notch in the calibration shelf, the tip touches the front-bottom edge of the well during the run.

IMPORTANT! If you use more than one Ion OneTouch™ ES Dx Instrument, do not switch Trays or Tip Arms between instruments. Each Tray and Tip Arm is calibrated with a particular instrument. To track the Tray and Tip Arm, each component has a printed label with the matching serial number of the instrument.

Vertical axis calibration

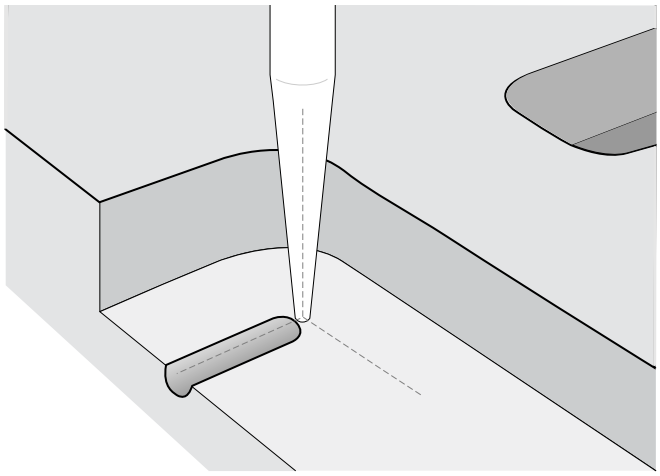
1. Install a new tip.
2. Restore the factory default settings:
   a. Power OFF the instrument, then wait 3 seconds.
   b. While holding down **Vert. Adjust**, power ON the instrument.
3. Put the instrument into calibration mode:
   a. Power OFF the instrument.
   b. While holding down **Select/Calibrate**, power the instrument ON. Keep holding down **Select/Calibrate** until "P1" is displayed.
   c. Press **Select/Calibrate** for ~3 seconds until the instrument beeps 2 times and "CAL" is displayed.

   Note: The instrument will cycle through several values before "CAL" is displayed.

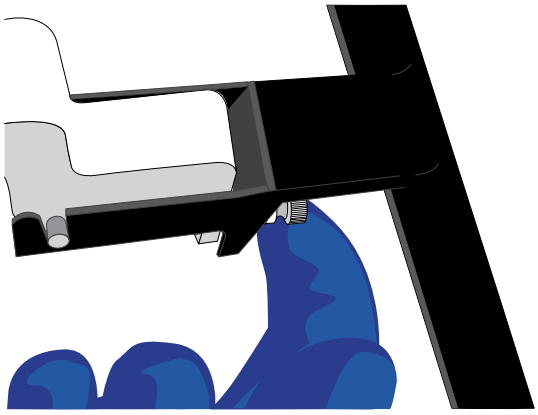
4. Press **Vert. Adjust**. The instrument displays "ASP" (Aspirate or z-bottom position).

5. Press **Start/Stop**. The Tip Arm lowers to bring the tip near the notch in the calibration shelf on the left side of the Tray.

6. The tip should be positioned in line with the slot in the calibration shelf, and the tip should be touching the shelf. If necessary, adjust the tip as follows:



   a. To adjust the alignment of the tip with the slot, turn the thumbscrew at the back of the Tip Arm.



   b. To adjust the height of the tip, press the ▼ (minus) button repeatedly until the tip touches the shelf. Press the button eight more times to lower the tip further. This will account for variations in tip lengths and installation.

   Note: It is better to have the ASP (aspiration) height be too low than too high.

7. Press **Start/Stop**, then wait for the Tip Arm to stop moving and for "P1" to display.

Horizontal axis calibration

1. Press **Select/Calibrate** for ~3 seconds until the instrument beeps 2 times and “CAL” is displayed.

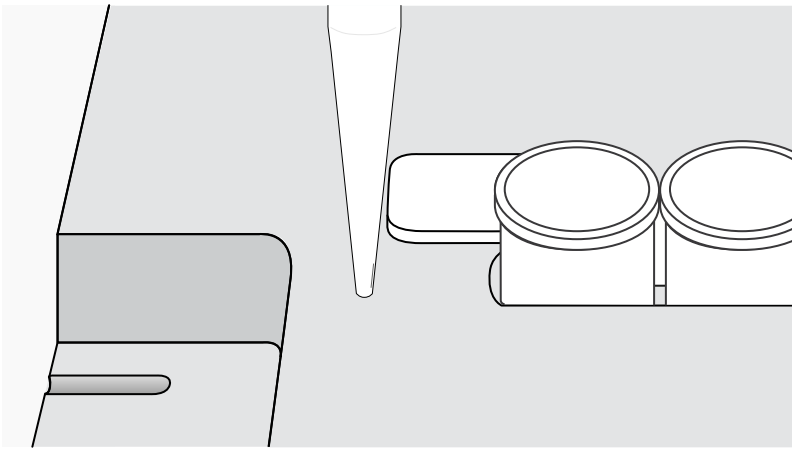
   Note: While you press the button, the instrument cycles through several values before “CAL” is displayed.

2. Press **Horiz. Adjust**. The instrument displays “FLA”. Press **Start/Stop**.

3. Place an empty 8-well strip in the slot in the Tray, with the square tab on the left.

4. Push the 8-well strip as far to the left in the slot as possible.

5. Observe the position of the 8-well strip relative to the position of the tip. When properly calibrated, the 8-well strip is within 1 mm of touching the tip, but not pushing on it. To clearly see the relationship between the pipette tip, calibration shelf, and notch during calibration, mark each of them with a felt-tip pen:



6. Adjust the horizontal position of the Tip Arm so that the tip just touches the square tab on the left of the 8-well strip when the 8-well strip is pushed to the far left of the slot in the Tray*:*
   a. Apply slight pressure to keep the 8-well strip to the far left.
   b. Press the ▲ (plus) button repeatedly until the tip touches the 8-well strip. Each press of the ▲ (plus) key moves the Tip Arm to the *right* by ~0.002 inches (~50 µm), which may be difficult to detect.

7. Press **Start/Stop** to save the setting, then wait for “P1” to display.

8. Power the instrument OFF, wait >3 seconds, then power the instrument ON to return to normal operating mode.

9. Perform a residual volume test (see “Ion OneTouch™ ES Dx Instrument residual volume test” on page 61).

Removal of instruments from use for repair or disposal

To remove the Ion OneTouch™ Dx Instrument or Ion OneTouch™ ES Dx Instrument from use for repair or disposal, perform the following steps:

IMPORTANT! This procedure does not guarantee total decontamination of the Ion OneTouch™ Dx Instrument or Ion OneTouch™ ES Dx Instrument.

1. Wear disposable gloves, safety glasses, and a lab coat.
2. Use a cleaning pad wetted with a solution of 1 part chlorine bleach in 9 parts water (10% bleach solution) to clean all outside surfaces of the Ion OneTouch™ Dx Instrument or Ion OneTouch™ ES Dx Instrument. Use care to avoid getting bleach solution inside the chassis.
3. Dry the surfaces of the instrument with paper towels or other disposable wipes.
4. Use cotton swabs to clean and dry areas that are difficult to access.
5. Properly dispose of all used consumables (including tubes, sippers, amplification plates) and cleaning materials to ensure that no one becomes exposed to contaminants.

If returning the instrument, contact your local Thermo Fisher Scientific representative to schedule a pickup of the instrument.

C

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words:

- **CAUTION!**—Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **WARNING!**—Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- **DANGER!**—Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Symbol	English	Français
	Caution, risk of danger Consult the manual for further safety information.	Attention, risque de danger Consulter le manuel pour d'autres renseignements de sécurité.
	Caution, risk of electrical shock	Attention, risque de choc électrique
	Caution, hot surface	Attention, surface chaude
	Potential biohazard	Danger biologique potentiel
	Potential overhead hazard	Danger en surplomb potentiel
	On	On (marche)
	Off	Off (arrêt)
	Earth (ground) terminal	Borne de (mise à la) terre
	Protective conductor terminal (main ground)	Borne de conducteur de protection (mise à la terre principale)

(continued)

Symbol	English	Français
	Do not dispose of this product in unsorted municipal waste **CAUTION!** To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.	Ne pas éliminer ce produit avec les déchets usuels non soumis au tri sélectif. **MISE EN GARDE !** Pour minimiser les conséquences négatives sur l'environnement à la suite de l'élimination de déchets électroniques, ne pas éliminer ce déchet électronique avec les déchets usuels non soumis au tri sélectif. Se conformer aux ordonnances locales sur les déchets municipaux pour les dispositions d'élimination et communiquer avec le service à la clientèle pour des renseignements sur les options d'élimination responsable.

Conformity symbols on the instrument

Conformity mark	Description
	Indicates conformity with safety requirements for Canada and U.S.A.
	Indicates conformity with European Union requirements for safety and electromagnetic compatibility.
	Indicates conformity with Australian standards for electromagnetic compatibility.

Medical device symbols

The following table describes symbols that may be displayed on product labels. The symbols that are used on labels conform to standards BS EN ISO 15223-1:2016 and FDA 21 CFR 809.10 "Labeling for in vitro diagnostic products".

Symbol	Description	Symbol	Description
	MANUFACTURER		CONTAINS SUFFICIENT FOR <n> TESTS
	DATE OF MANUFACTURE		USE BY

(continued)

Symbol	Description	Symbol	Description
LOT	BATCH CODE	REF	CATALOG NUMBER
SN	SERIAL NUMBER		FRAGILE, HANDLE WITH CARE
	LOWER LIMIT OF TEMPERATURE		PROTECT FROM LIGHT
	UPPER AND LOWER LIMITS OF TEMPERATURE		UPPER LIMIT OF TEMPERATURE
	DO NOT REUSE		BIOLOGICAL RISKS
	CAUTION, CONSULT ACCOMPANYING DOCUMENTS		CONSULT INSTRUCTIONS FOR USE
	UPPER AND LOWER LIMITS OF HUMIDITY		OBSERVE PRECAUTIONS FOR HANDLING ELECTROSTATIC SENSITIVE DEVICES
IVD	IN VITRO DIAGNOSTIC MEDICAL DEVICE		

Safety alerts on this instrument

Additional text may be used with one of the symbols described above when more specific information is needed to avoid exposure to a hazard. See the following table for safety alerts found on the instrument.

English	Français
CAUTION! Hazardous chemicals. Read the Safety Data Sheets (SDSs) before handling.	**MISE EN GARDE ! Produits chimiques dangereux.** Lire les fiches signalétiques (FS) avant de manipuler les produits.
CAUTION! Hazardous waste. Refer to SDS(s) and local regulations for handling and disposal.	**MISE EN GARDE ! Déchets dangereux.** Lire les fiches signalétiques (FS) et la réglementation locale associées à la manipulation et à l'élimination des déchets.

Safety information for third-party instruments

Refer to the manufacturer's documentation for information on the safe use of third-party products provided with the instrument system.

Instrument safety

General

CAUTION! Do not remove instrument protective covers. If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

CAUTION! Solvents and Pressurized fluids. Wear eye protection when working with any pressurized fluids. Use caution when working with any polymeric tubing that is under pressure:

- Extinguish any nearby flames if you use flammable solvents.
- Do not use polymeric tubing that has been severely stressed or kinked.
- Do not use polymeric tubing with tetrahydrofuran or nitric and sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause polymeric tubing to swell and greatly reduce the rupture pressure of the tubing.
- Be aware that high solvent flow rates (~40mL/min) may cause a static charge to build up on the surface of the tubing and electrical sparks may result.

Physical injury

CAUTION! Moving Parts. Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

CAUTION! ROTATION HAZARD. Wait until rotation stops before opening. Rotating parts can cause injury

Electrical safety

WARNING! Fuse Installation. Before installing the instrument, verify that the fuses are properly installed and the fuse voltage matches the supply voltage. Replace fuses only with the type and rating specified for the unit. Improper fuses can damage the instrument wiring system and cause a fire.

WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility.

WARNING! Disconnecting Power. To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

Cleaning and decontamination

CAUTION! Cleaning and Decontamination. Use only the cleaning and decontamination methods specified in the manufacturer's user documentation. It is the responsibility of the operator (or other responsible person) to ensure the following requirements are met:

- No decontamination or cleaning agents are used that could cause a HAZARD as a result of a reaction with parts of the equipment or with material contained in the equipment.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) prior to having the instrument serviced at your facility or sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan (decontamination forms may be requested from customer service).
- Before using any cleaning or decontamination methods (except those recommended by the manufacturer), users should confirm with the manufacturer that the proposed method will not damage the equipment.

Laser

CAUTION! LASER HAZARD, Bar Code Scanner. The bar code scanner included with the instrument system is a Class 2 laser. To avoid damage to eyes, do not stare directly into the beam or point into another person's eyes.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.

Safety

Reference	Description
Directive 2006/95/EC	*European Union "Low Voltage Directive"*
IEC 61010-1 EN 61010-1 CSA C22.2 61010-1	*Safety requirements for electrical equipment for measurement, control, and laboratory use*
IEC 61010-2-010 EN 61010-2-010 CSA C22.2 61010-2-010	*Safety requirements for electrical equipment for measurement, control and laboratory use — Part 2-010: Particular requirements for laboratory equipment for the heating of materials*
IEC 61010-2-020 EN 61010-2-020 CSA C22.2 61010-2-020	*Safety requirements for electrical equipment for measurement, control and laboratory use — Part 2-020: Particular requirements for laboratory centrifuges*
IEC 61010-2-081 EN 61010-2-081 CSA C22.2 61010-2-081	*Safety requirements for electrical equipment for measurement, control and laboratory use — Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes*
IEC 61010-2-101	*Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use — Part 2-101: Particular Requirements for In Vitro Diagnostic (IVD) Medical Equipment*

EMC

Reference	Description
Directive 2004/108/EC	European Union “EMC Directive”
EN 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
EN 61326-2-6	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 26: Particular requirements – In vitro diagnostic (IVD) medical equipment)requirements*
FCC Part 15	U.S. Standard “Industrial, Scientific, and Medical Equipment”
AS/NZS CISPR 22:2009	*Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment*
ICES-003, Issue 5	*Industrial, Scientific and Medical (ISM) Radio Frequency Generators*

Environmental design

Reference	Description
Directive 2012/19/EU	European Union “WEEE Directive” – Waste electrical and electronic equipment
Directive 2011/65/EU	European Union “RoHS Directive” – Restriction of hazardous substances in electrical and electronic equipment

Precaution—strong magnet

Note: Do not substitute non-IVD labeled magnets for the DynaMag™ Dx 96-Well Plate Magnet and DynaMag™ Dx 16 2-mL Magnet, provided with Ion PGM™ Dx System.

The DynaMag™ Dx 96-Well Plate Magnet and DynaMag™ Dx 16 2-mL Magnet contain very strong permanent magnets. People wearing a pacemaker or any other medical magnetized implant should not use this product unless advised by a health professional; the implant could be affected or damaged by exposure to a strong magnetic field. Keep tools and objects that could be damaged by the magnetic field out of the working area. This includes, but is not restricted to, credit cards and other products containing magnetic recording devices. Keep away from delicate instruments, watches, electronic equipment, displays and monitors. The magnet may attract steel or other magnetic material with high mechanical forces. Take care during handling. Avoid contact between two magnets. Do not pull the magnets apart if contact has been made; twist off to prevent damage to the unit or fingers. The Health and Safety Officer should take all necessary steps and full responsibility to ensure that the precautions and statements are followed and adhered to.

Performance characteristics

For performance characteristics of the Oncomine™ Dx Target Test Kit, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide* (Pub. No. MAN0018810).

For performance characteristics of the Ion PGM™ Dx System, see the *Ion PGM™ Dx System Performance Characteristics User Guide* (Pub. No. MAN0018763).

E Instrument warranty

For new Ion Torrent™ instruments, Life Technologies warrants to and only to buyer for twelve (12) months from the date of shipping, that the Ion Torrent™ software and Ion Torrent™ instruments are free from defects in material and workmanship and conform to Life Technologies' published specifications in all material respects. Where a valid and timely claim in respect of breach of Ion Torrent™ warranty is submitted to Life Technologies, Life Technologies may, at its discretion, replace, repair or modify the Ion Torrent™ instrument. Any agreed replacement shall be at 1:1, like-kind basis, at no cost to the buyer. For Ion Torrent™ chips or reagents reasonably determined by Life Technologies to be defective, independent of user error, shall be replaced by Life Technologies on a 1:1, like-kind basis at no cost to buyer, provided that such defective Ion Torrent™ chips or reagents were used by buyer prior to their expiration date, or if there is no expiration date, the Ion Torrent™ chips or reagents were used within six (6) months of receipt, and the defect was promptly reported with appropriate detail to Life Technologies' technical support.

NO OTHER WARRANTIES SHALL BE APPLICABLE TO ION TORRENT PRODUCTS (WHETHER OR NOT ANY FURTHER WARRANTY DOCUMENTATION MAY BE INCLUDED IN THE SHIPMENT), WITH THE EXCEPTION OF THIRD PARTY WARRANTIES WITH RESPECT TO THIRD PARTY PRODUCT. ANY THIRD PARTY PRODUCTS ARE NOT COVERED BY THIS SECTION AND ANY WARRANTIES FOR THIRD PARTY PRODUCTS ARE PROVIDED BY THE ORIGINAL MANUFACTURER OF THE THIRD PARTY PRODUCT. Warranties are made only to buyer purchasing the Ion Torrent™ Product directly from Life Technologies, are not transferable and do not extend to the benefit of any other person or entity, unless otherwise expressly stated in writing by Life Technologies. ANY PRODUCT NOT COVERED BY AN EXPRESS WRITTEN WARRANTY IS SOLD AND PROVIDED "AS IS," WITHOUT WARRANTY OF ANY KIND, STATUTORY, EXPRESS OR IMPLIED. Any description of Ion Torrent™ Product recited in Life Technologies' quotation is for the sole purpose of identifying Ion Torrent™ Product, and any such description is not part of any contract between Life Technologies and buyer and does not constitute a warranty that Ion Torrent™ Product shall conform to that description. Any sample or model used in connection with Life Technologies' quotation is for illustrative purposes only, and is not part of any contract between Life Technologies and buyer and does not constitute a warranty that Ion Torrent™ Product will conform to the sample or model. No affirmation of fact or promise made by Life Technologies, whether or not in Life Technologies' quotation, shall constitute a warranty that Ion Torrent™ Product will conform to the affirmation or promise. Unless otherwise specified in writing in documentation shipped with Ion Torrent™ Product or otherwise agreed by Life Technologies in writing. Life Technologies does not provide service or support for custom products or other products made to buyer's specifications. THE WARRANTIES IDENTIFIED IN THIS CLAUSE ARE LIFE TECHNOLOGIES' SOLE AND EXCLUSIVE WARRANTIES WITH RESPECT TO Ion Torrent™ PRODUCT AND ARE IN LIEU OF ALL OTHER WARRANTIES, STATUTORY, EXPRESS OR IMPLIED, ALL OF WHICH OTHER WARRANTIES ARE EXPRESSLY DISCLAIMED, INCLUDING WITHOUT LIMITATION ANY IMPLIED WARRANTY OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, NON-INFRINGEMENT, OR REGARDING RESULTS OBTAINED THROUGH THE USE OF ANY PRODUCT (INCLUDING, WITHOUT LIMITATION, ANY CLAIM OF INACCURATE, INVALID OR INCOMPLETE RESULTS), WHETHER ARISING FROM A STATUTE OR OTHERWISE IN LAW OR FROM A COURSE OF PERFORMANCE, DEALING OR USAGE OF TRADE.

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support
- Order and web support
- Safety Data Sheets (SDSs; also known as MSDSs)

Additional product documentation, including user guides and Certificates of Analysis, are available by contacting Customer Support.

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are printed and shipped with the product.

Obtaining Certificates of Conformance

The Certificate of Conformance provides information on conformance testing of each instrument provided with the system. Certificates of Conformance are shipped with the instrument, and are also available by contacting Customer Support at **thermofisher.com/support**.

ion torrent

Oncomine™ Dx Target Test Part IV: Sequencing and Results Reports

USER GUIDE

Publication Number MAN0018813

Revision A.0

IVD

For In Vitro Diagnostic Use.

ThermoFisher SCIENTIFIC

Life Technologies Holdings Pte Ltd |
Block 33 |
Marsiling Industrial Estate Road 3 |
#07-06, Singapore 739256

Products manufactured in Singapore:

Ion PGM™ Dx Instrument System
Ion PGM™ Dx Sequencer
Ion OneTouch™ Dx Instrument
Ion OneTouch™ ES Dx Instrument
Ion PGM™ Dx Chip Minifuge (120V)
Ion PGM™ Wireless Scanner
Ion Torrent™ Server
Torrent Suite™ Dx Software
Veriti™ Dx 96-well Thermal Cycler, 0.2 mL

Life Technologies Corporation |
7335 Executive Way |
Frederick, MD 21704 | USA

Products manufactured in Frederick:

Oncomine™ Dx Target Test Kit
Ion Torrent Dx FFPE Sample Preparation Kit
Ion PGM™ Dx Library Kit
Ion OneTouch™ Dx Template Kit
Ion PGM™ Dx Sequencing Kit
Ion 318™ Dx Chip
Ion OneTouch™ Rack Kit
DynaMag™ Dx 96-Well Plate Magnet
DynaMag™ Dx 16 2-mL Magnet

The information in this guide is subject to change without notice.

DISCLAIMER

TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0018811

Revision	Date	Description
A.0	2 September 2020	Oncomine™ Dx Target Test user guide for FDA submission—updated for Torrent Suite™ Dx Software 5.12.5

Contents

About this guide

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Purpose of this guide

This user guide provides instructions for sequencing enriched, template-positive Ion PGM™ Dx Ion Sphere™ Particles (ISPs) that have been prepared from Oncomine™ Dx Target Test libraries. Sequencing is performed using the Ion PGM™ Dx System with the Ion PGM™ Dx Sequencing Kit and the Ion 318™ Dx Chip.

Oncomine™ Dx Target Test Kit user guides

This user guide is part of a five-guide set.

- *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide*
- *Oncomine™ Dx Target Test Part II: Sample and Library Preparation User Guide*
- *Oncomine™ Dx Target Test Part III: Template Preparation User Guide*
- *Oncomine™ Dx Target Test Part IV: Sequencing and Results Reports User Guide*
- *Oncomine™ Dx Target Test Part V: Torrent Suite™ Dx Software 5.12.5 Reference User Guide*

All five guides are required to complete the entire Oncomine™ Dx Target Test workflow.

Note: The procedures in these guides supersede the instructions in the *Ion PGM™ Dx System User Guide* when using the Ion PGM™ Dx System with the Oncomine™ Dx Target Test.

1 Product information

Product description

Oncomine™ Dx Target Test

The Oncomine™ Dx Target Test is an *in vitro* diagnostic next-generation sequencing test to detect somatic alterations in human DNA and RNA isolated from formalin-fixed, paraffin-embedded (FFPE) tissue samples. Detection of these variants is performed using the Ion PGM™ Dx System.

For a complete product description of the Oncomine™ Dx Target Test, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide* (Pub. No. MAN0018810).

Sequencing components

The Ion PGM™ Dx Sequencing Kit, included as part of the Oncomine™ Dx Target Test Kit, is used to prepare template-positive Ion PGM™ Dx ISPs for 200 base-read sequencing on the Ion 318™ Dx Chip and Ion PGM™ Dx System.

Intended use

For the Intended Use statement for the Oncomine™ Dx Target Test, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide* (Pub. No. MAN0018810).

Theory of operation

For a complete description of the Theory of Operation of the system, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide* (Pub. No. MAN0018810).

Software compatibility and requirements

The procedures in this guide are designed for use with Torrent Suite™ Dx Software version 5.12.5 or later. For a complete description of software compatibility and requirements, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide* (Pub. No. MAN0018810).

Materials provided

Oncomine™ Dx Target Test Kit

The Oncomine™ Dx Target Test Kit (Cat. No. A49755) includes the following subkits.

IMPORTANT! Refer to the product label for the storage conditions and expiration dates of individual modules and components.

✓	Subkit	Part No.
	Oncomine™ Dx Target Test, Controls, and Diluent Kit	A49756
	Ion Torrent Dx FFPE Sample Preparation Kit	A32445
	Ion PGM™ Dx Library Kit	A49758
	Ion OneTouch™ Dx Template Kit	A49759
	Ion PGM™ Dx Sequencing Kit	A49760
	Ion 318™ Dx Chip Kit	A18937
	Oncomine™ Dx Target Test User Guides and Assay Definition File	A49678

Subkits used in this guide

The procedures in this user guide use the following subkits from the Oncomine™ Dx Target Test Kit.

Ion PGM™ Dx Sequencing Kit

The Ion PGM™ Dx Sequencing Kit (Cat. No. A49760) includes the following modules and components, and is also included as a part of the Ion PGM™ Dx System consumables bundle:

✓	Component	Amount	Storage
Ion PGM™ Dx Sequencing Supplies (Part No. A18936)			
	SEQ Wash Bottle Sipper	8 long, 16 short	15°C to 30°C
	SEQ Reagent Tube Sipper	32	
	SEQ Reagent Tube plus label	32	
	SEQ Wash 1 Bottle (250-mL bottle)	1	
	SEQ Wash 2 Bottle (2-L bottle)	1	
	SEQ Wash 3 Bottle (250-mL bottle)	1	

✓	Component	Amount	Storage
	Ion PGM™ Dx Sequencing Reagents (Part No. A18934)		
	SEQ dGTP (black cap)	2 × 40 µL	–30°C to –10°C
	SEQ dCTP (blue cap)	2 × 40 µL	
	SEQ dATP (green cap)	2 × 40 µL	
	SEQ dTTP (red cap)	2 × 40 µL	
	SEQ Enzyme (yellow cap)	24 µL	
	SEQ Primer (white cap)	96 µL	
	Ion PGM™ Dx Sequencing Solutions (Part No. A18935)		
	SEQ W2 Solution (white cap)	8 × 126.25 mL	2°C to 8°C (store SEQ W2 Solution bottles in the sealed plastic bag provided)
	SEQ Cleaning Tablet	8 tablets	
	SEQ Sample Buffer (brown cap)	160 µL	
	SEQ W3 Solution (white cap)	4 × 100 mL	

Ion 318™ Dx Chip Kit

The Ion 318™ Dx Chip Kit (Cat. No. A18937) includes the following components and is also included as a part of the Ion PGM™ Dx Consumables bundle:

✓	Component	Amount	Storage
	Ion 318™ Dx Chip	8	15°C to 30°C

Ion PGM™ Dx Instrument System

The Ion PGM™ Dx Instrument System (Cat. No. A25511) includes the following components, which are also sold separately.

✓	Component	Catalog No.
	Ion OneTouch™ Dx Instrument and accessories	A25483
	Ion OneTouch™ ES Dx Instrument and accessories	A25484
	Ion PGM™ Dx Sequencer and accessories	A25485
	Ion PGM™ Wireless Scanner	A25486
	Ion Torrent™ Server (software installed separately)	A28552

(continued)

✓	Component	Catalog No.
	Ion OneTouch™ Rack Kit • Ion OneTouch™ Solutions Rack • Ion OneTouch™ Assembly Rack • Ion OneTouch™ Sample Rack	A24694
	Ion PGM™ Dx Chip Minifuge: • 120 VAC • 230 VAC	 A25058 A25482
	DynaMag™ Dx Kit—Tube & Plate • DynaMag™ Dx 96-Well Plate Magnet • DynaMag™ Dx 16 2-mL Magnet	A31755 A31347 A31346

Materials and equipment required but not provided

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

✓	Description	Source
	Tank of compressed nitrogen (grade 4.8, 99.998% or better)	MLS
	Multistage (dual-stage) gas regulator (0-50 PSI, 2-3 Bar output)	MLS
	18-MΩ water purification system (see the following description)	MLS
	0.45-µm vacuum filtration system and filters (nylon or PVDF filters, 1 L vol.)	MLS
	Pipettes (2-, 20-, 200-, and 1000-µL)	MLS
	Aerosol-barrier pipette tips (10-, 20-, 200-, and 1000- µL)	MLS
	Vortexer with a rubber platform	MLS
	Mini centrifuge	MLS
	Microcentrifuge (must accommodate standard 1.5- and 0.2-mL tubes and generate 15,000 rcf)	MLS
	Veriti™ Dx 96-well Thermal Cycler, 0.2 mL	4452300
	Graduated cylinders (1 L or 2 L volume)	MLS
	Glass bottle (1 L)	MLS
	15-mL conical tubes	MLS
	NaOH, ACS grade (10 M)	MLS

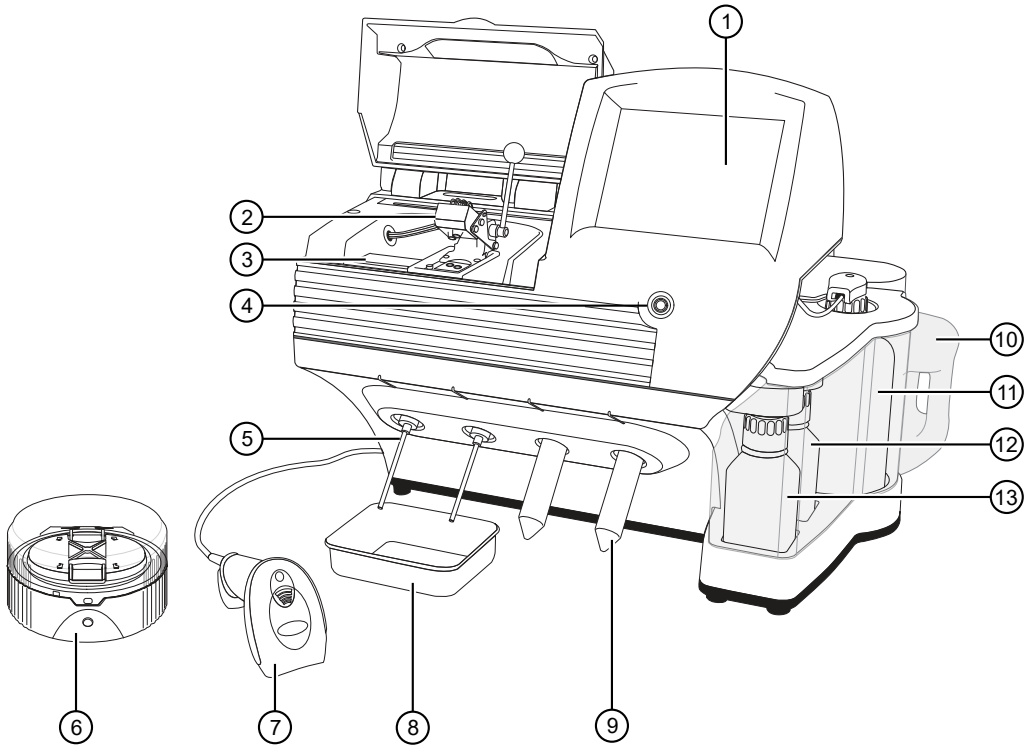
(continued)

✓	Description	Source
	Nuclease-free water	MLS
	Benchtop cold box	MLS
	5-mL and 10-mL serological pipettes, and pipette controller (for preparing the NaOH solution)	MLS

18-MΩ water purification system

The Ion PGM™ Dx Sequencer requires an 18-MΩ water purification system to prepare water for solutions used on the instrument. Such a system is essential to remove ions and organic carbons from the water that might interfere with the chip surface or sequencing enzyme. Water purchased from vendors or stored for any length of time is not acceptable.

Ion PGM™ Dx System with Reagent and Wash Bottles attached



1. Touchscreen
2. Chip clamp
3. Grounding plate
4. Power button
5. Reagent Tube Sipper
6. Ion PGM™ Dx Chip Minifuge
7. Barcode scanner
8. Collection tray
9. Reagent Tube
10. Waste Bottle
11. Wash 2 Bottle (W2 position)
12. Wash 3 Bottle (W3 position)
13. Wash 1 Bottle (W1 position)

2 Before you begin

Installation and special requirements

Special requirements

- The Torrent Suite™ Dx Software requires the use of Google™ Chrome™ browser.
- The Ion PGM™ Dx Sequencer requires the use of an 18-MΩ water purification system installed in the laboratory. Such a system is essential for ensuring that any ions or organic carbons that might interfere with the chip surface or sequencing enzyme are removed from the water immediately before use on the instrument. Water purchased from vendors or stored for any length of time is not acceptable.

Gas cylinders

You must supply the required nitrogen gas cylinder and accessories for the installation. This instrument requires a pressurized house line or one size 1-A nitrogen gas cylinder that holds approximately 7.2 m^3 (257 ft^3) of gas when full. Use only prepurified nitrogen of 99.998% (grade 4.8) or greater purity.

CAUTION! Damage to the instrument and its products can result from using impure gas, gases other than nitrogen, or an inadequate amount of gas.

WARNING! EXPLOSION HAZARD. Pressurized gas cylinders are potentially explosive. Always cap the gas cylinder when it is not in use, and attach it firmly to the wall or gas cylinder cart with approved brackets or chains.

WARNING! Gas cylinders are heavy and may topple over, potentially causing personal injury and tank damage. Cylinders should be firmly secured to a wall or work surface. Please contact your Environmental Health and Safety Coordinator for guidance on the proper installation of a gas cylinder.

Perform a leak test

To perform a leak test on the gas cylinder:

1. Open the main tank shutoff valve. The high-pressure gauge of the gas tank regulator reads approximately 2,000–2,500 psi for a full tank.
2. Adjust the pressure to the instrument by slowly turning the pressure adjustment valve clockwise until the low-pressure gauge reads 30 psi.
3. Close the needle valve, then close the main tank valve.
4. Monitor the high-pressure gauge of the gas tank regulator for 5 minutes. There should be no noticeable drop in pressure.

If the pressure	Action
Drops in 5 minutes	There can be a leak at either the needle valve or the gas tank regulator itself. Check the fittings and resolve any problems, then continue with step 5.
Does not drop in 5 minutes	The instrument passes the leak test. Reopen the main tank valve and skip the following steps.

5. Open the main tank valve and the needle valve for at least 15 seconds to pressurize the instrument.
6. Close the main tank valve.
7. Monitor the high-pressure gas tank regulator gauge. There should be no more than a 100-psi drop in pressure after 5 minutes. Locate, then resolve any leaks. Turn the main tank valve back on.

Electrical and environmental conditions

IMPORTANT! Protection provided by the equipment may be impaired if the instrument is operated outside the environment and use specifications, the user provides inadequate maintenance, or the equipment is used in a manner not specified by the manufacturer.

IMPORTANT! Observe current Good Clinical Laboratory Practices (GCLP) when using this instrument.

See the *Ion PGM™ Dx System Site Preparation Guide* (Pub. No. MAN0016696) for information about instrument location and setup. Instruments should be shielded from excess exposure to dust, vibration, strong magnetic fields, drafts, excessive moisture, or large temperature fluctuations. Surge protectors or line conditioners should be used if the voltage source is not stable. Sudden voltage spikes can cause damage to the electronics inside the instruments.

Ensure that the room where the instruments have been installed is maintained under correct environmental conditions. Avoid placing the instruments adjacent to heaters, cooling ducts, or in direct sunlight. Place the instruments at least a meter away from major sources of electronic noise, such as refrigerators or microwaves.

Electrical requirements

CAUTION! Do not unpack or plug in any components until a field service representative has configured them for the proper operating voltage.

WARNING! For safety, the power outlet used for powering the instrument must be accessible at all times. In case of emergency, you must be able to immediately disconnect the main power supply to all the equipment. Allow adequate space between the wall and the equipment so that the power cords can be disconnected in case of emergency.

- Electric receptacle required: 2-prong with ground pin
- Main AC line voltage tolerances must be at most ±10% percent of nominal voltage.
- Power cords are provided with the instruments. If not suitable for installation in your region, ensure any power cord you do use is:
  - Maximum 10 feet (3 meters) in length
  - Grounding type
  - Compatible with the power supply receptacles used to connect to main power
  - Suitable for the rating of the instrument and main power supply
  - Compliant with local safety requirements (for example, UL Listed for North America, JIS approved for Japan, HAR or agency certified for Europe)
- (Ion OneTouch™ Dx Instrument only) Fuse Rating: 6 A, 250 VAC, Type M. Replace only with the same fuse type and rating.

WARNING! FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

Device	Rated voltage [1,2]	Rated frequency	Rated current [3]
Ion PGM™ Dx Sequencer	110/120VAC 220/240VAC	50/60 Hz	9 A
Ion Torrent™ Server [4]	110/120VAC 220/240VAC	50/60 Hz	11 A
Ion OneTouch™ Dx Instrument with power supply	110/120VAC 220/240VAC	50/60 Hz	5.5 A
Ion OneTouch™ ES Dx Instrument	110/120VAC 220/240VAC	50/60 Hz	375 mA 160 mA
Ion PGM™ Dx Chip Minifuge	120 VAC 220–240 VAC	50/60 Hz	130 mA 65 mA

[1] In Japan, rated voltages of 100 VAC and 200 VAC are acceptable.

[2] If the supplied power fluctuates beyond the rated voltage, a power line regulator may be required. High or low voltages can adversely affect the electronic components of the instrument.

[3] Based on rated current at minimum input voltage.

[4] Minimum Efficiency: 65% (Energy Star Qualified); 85% Efficient Power Supply.

Environmental requirements

Ensure that the room where the instruments have been installed is maintained under the correct environmental conditions. Avoid placing the instruments next to heaters, cooling ducts, or in direct sunlight. Place the sequencer at least a meter away from major sources of electronic noise, such as refrigerators or microwaves.

CAUTION! Use of the instruments in an unspecified manner may result in the protection provided by the instruments to be impaired.

Component	Acceptable range
Altitude	Between sea level and 2,000 meters (6,500 feet) above sea level
Humidity: operating	10–90% relative humidity
Humidity: transportation and storage	20–80% relative humidity
Temperature: operating	15–30°C (59–86°F) At or above 1,800 meters (5,906 feet), the system must not be used if the temperature is above 29.5°C.
Temperature: transportation and storage	–30°C to 60°C (–22°F to 140°F)

(continued)

Component	Acceptable range
Vibration	Ensure that benches where instruments are to be installed are free of vibration and have no contact with equipment that causes vibration (freezers, pumps, and similar equipment). Vibration can reduce the quality of sequencing measurements.
Pollution	The system has a Pollution Degree rating of II (2). The system may only be installed in an environment that has nonconductive pollutants, such as dust particles or wood chips. Typical environments with a Pollution Degree II (2) rating are laboratories, sales, and commercial areas.
Overvoltage category	The instruments have an installation (overvoltage) category of II (2).
Other conditions	For indoor use only. Keep away from any vents that could expel particulate material on the system components.

Precautions before using the Ion PGM™ Dx System

For additional safety information, see Appendix C, "Safety".

Instrument installation by trained personnel only

IMPORTANT! The Ion PGM™ Dx System is installed by trained service personnel and must not be relocated without assistance from trained service personnel. See Appendix F, "Customer and technical support".

Nucleic acid contamination

IMPORTANT! A primary source of contamination is DNA fragments from previously processed samples. Do not introduce amplified DNA into the library preparation laboratory or work area.

IMPORTANT! Possible contamination can occur during the transfer of dNTPs into Reagent Tubes. Be careful to avoid cross contamination of dNTP stocks. Barrier tips are required for all pipetting steps. Change gloves after handling concentrated dNTP stocks.

Reagent contamination

Before use, verify that any nuclease-free water used in the procedure is not cloudy, a potential indication of contamination. If the water is cloudy, use a different vial.

CO_2 contamination

IMPORTANT! Dry ice (solid CO_2) must be kept away from areas where buffers, wash solutions, or sources of molecular biology grade water for the Ion PGM™ Dx System are used. High air concentrations of subliming CO_2 may change the pH of such buffers during or after their preparation. The stability of the pH of these buffers is a critical factor in the performance of the Ion PGM™ Dx System.

Instrument vibration and clearances

IMPORTANT! Significant vibration during sequencing may add noise and reduce the quality of the measurements. The Ion PGM™ Dx Sequencer must be installed on a bench that is free from vibrations or in contact with equipment that can cause vibrations to the bench, such as freezers, pumps, large benchtop centrifuges, and other similar equipment. Mini and microcentrifuges may be used near the sequencer. An air table is not required, nor is securing the sequencer to the bench.

IMPORTANT! Position the Ion PGM™ Dx Sequencer so that the front bezel is a minimum of 12 in. (30.5 cm) and the Reagent Tubes containing dNTPs are a minimum of 8 in. (20.3 cm) from the front of the laboratory bench. Place the instrument at least 40 in. (1 meter) away from major sources of electronic noise such as refrigerators or microwaves.

Static electricity

IMPORTANT! To avoid possible damage to chips from static electricity, see "Guidelines for chip handling and use" on page 20.

Ventilation requirements

WARNING! Instrumentation must be installed and operated in a well-ventilated environment, defined as having a minimum airflow of 6–10 air changes per hour. Assess the need for ventilation or atmospheric monitoring to avoid asphyxiation accidents from inert gases and/or oxygen depletion, and take measures to clearly identify potentially hazardous areas through training or signage. Please contact your Environmental Health and Safety Coordinator to confirm that the instruments will be installed and operated in an environment with sufficient ventilation.

Procedural guidelines

Definitions

Throughout this guide:

- Room temperature is defined as the temperature range 15–30°C.
- A pulse centrifugation consists of a 3–5 second centrifugation at maximum speed in a mini centrifuge.

Guidelines to prevent cross-contamination

- Use good laboratory practices to minimize cross-contamination of products and reagents.
- When designing the laboratory layout, consider separating pre- and post-amplification activities. To significantly reduce the potential for contamination, dedicate laboratory supplies and/or equipment to the appropriate space.

Guidelines for pipetting

- Use aerosol-barrier pipette tips. Change pipette tips between samples.
- Avoid introducing air bubbles when pipetting by keeping the pipette tip at the bottom of the solution in the wells.
- Set the pipette to the recommended volume for mixing, and insert tip into the solution with the pipette plunger depressed to avoid introducing air bubbles.
- Visually inspect multi-channel pipette tips to ensure volumes are equivalent during pipeting.
- Touch tip to the side of well and slowly pipet reagent on the side of the well to form a droplet. This enables small volumes to be pipetted accurately and to ensure that the reagent has been added to the well.
- Inspect the pipette tips to verify that the reagent has been adequately dispensed.

Guidelines for chip handling and use

IMPORTANT! To avoid damage to Ion 318™ Dx Chips or the Ion PGM™ Dx Sequencer due to electrostatic discharge:

- **Remove your gloves when handling chips**, especially before transferring chips on or off the instrument. Follow the steps in the sequencing procedure for taking off and putting on gloves.
- **Hold chips by their edges when handling.**
- **Do not place chips directly on the bench or any other surface.** Always place chips either on the grounding plate on the Ion PGM™ Dx Sequencer or in the Ion PGM™ Dx Chip Minifuge bucket.

Note: Ion 318™ Dx Chips can be handled without gloves during all stages of chip preparation, loading, and sequencing without risk of contamination.

Used chips cannot be reused for sequencing. Used chips must be discarded or clearly marked for cleaning and initialization.

Guidelines for initializing the sequencer

- Handle the SEQ dGTP, SEQ dCTP, SEQ dATP, and SEQ dTTP tubes carefully to avoid cross-contamination. Always change gloves after removing used sippers from the Ion PGM™ Dx System to avoid cross-contamination of the nucleotides. Also change gloves after handling concentrated dNTP stocks.
- Replace the SEQ Reagent Tubes and sippers every time you initialize.
- After 8 sequencing runs, do not use the SEQ Wash 1 Bottle, SEQ Wash 2 Bottle, or SEQ Wash 3 Bottle for initialization or sequencing to avoid possible breakage or leaking. You can continue to use the SEQ Wash 1 Bottle and SEQ Wash 3 Bottle as extra cleaning bottles.

Guidelines for sequencing runs

- One or two sequencing runs may be performed from a single initialization based on the option selected when starting the initialization, but both runs must be started within 27 hours after the start of initialization.
- If you press the **Abort** button on the sequencer touchscreen, the touchscreen may freeze. You may need to restart the sequencer.

IMPORTANT! After aborting a run, do not open the chip clamp, reagent tubes, or wash bottles until a new run or cleaning is initiated. Doing so can cause a fluid or gas leak if the sequencer was in a pressurized state when the run was aborted. From the main menu, select either **Clean** or **Run**, then follow the touchscreen prompts to depressurize the system.

Reagent management

Follow the guidelines below for proper reagent storage and use.

Storage

Reagents must be stored under appropriate conditions. Refer to the Product Information section in each user guide for the storage conditions of the kit components used in the procedures in that guide. The Oncomine™ Dx Target Test Kit includes kits with multiple component boxes that require different storage conditions. For example, the Oncomine™ Dx Target Test, Controls, and Diluent Kit includes four boxes, which are stored at different temperatures. To use the Oncomine™ Dx Target Test, Controls, and Diluent Kit, retrieve all boxes from their different storage areas and confirm that they are from the same master lot.

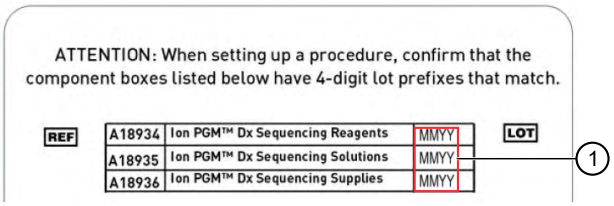
Kit interchangeability and component box lot matching

The top-level kits used for sample preparation, library preparation, template preparation, and sequencing can be mixed and matched. For example, an Ion PGM™ Dx Library Kit can be used with any Ion PGM™ Dx Sequencing Kit. However, the component boxes in a particular kit must be lot-matched with the other boxes in that kit.

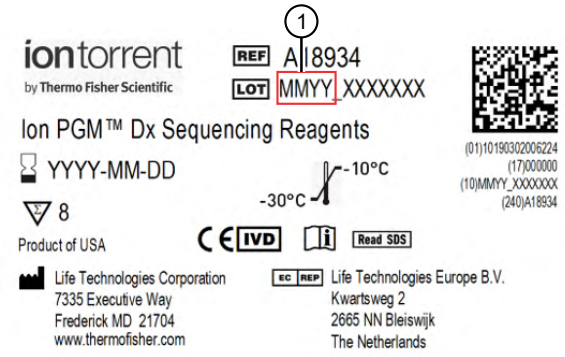
Note: The controls and diluent in the Oncomine™ Dx Target Test, Controls, and Diluent Kit must be lot-matched. However, the controls do not require lot-matching with the Oncomine™ Dx Target Test DNA and RNA Panel.

Each component box lists the 4-digit lot prefixes of the compatible component boxes inside the box lid. Before using a particular kit, check the inside lid of each box to confirm that it is compatible with the other boxes.

An example inside box lid label is shown below:



An example box label with lot information is shown below:



1 Lot prefix

Instrument operation and maintenance

Service and maintenance

You will be alerted by the Ion PGM™ Dx System when annual maintenance service is required. A notification will appear on the instrument touchscreen and in the Torrent Suite™ Dx Software.

Shut down/restart the sequencer

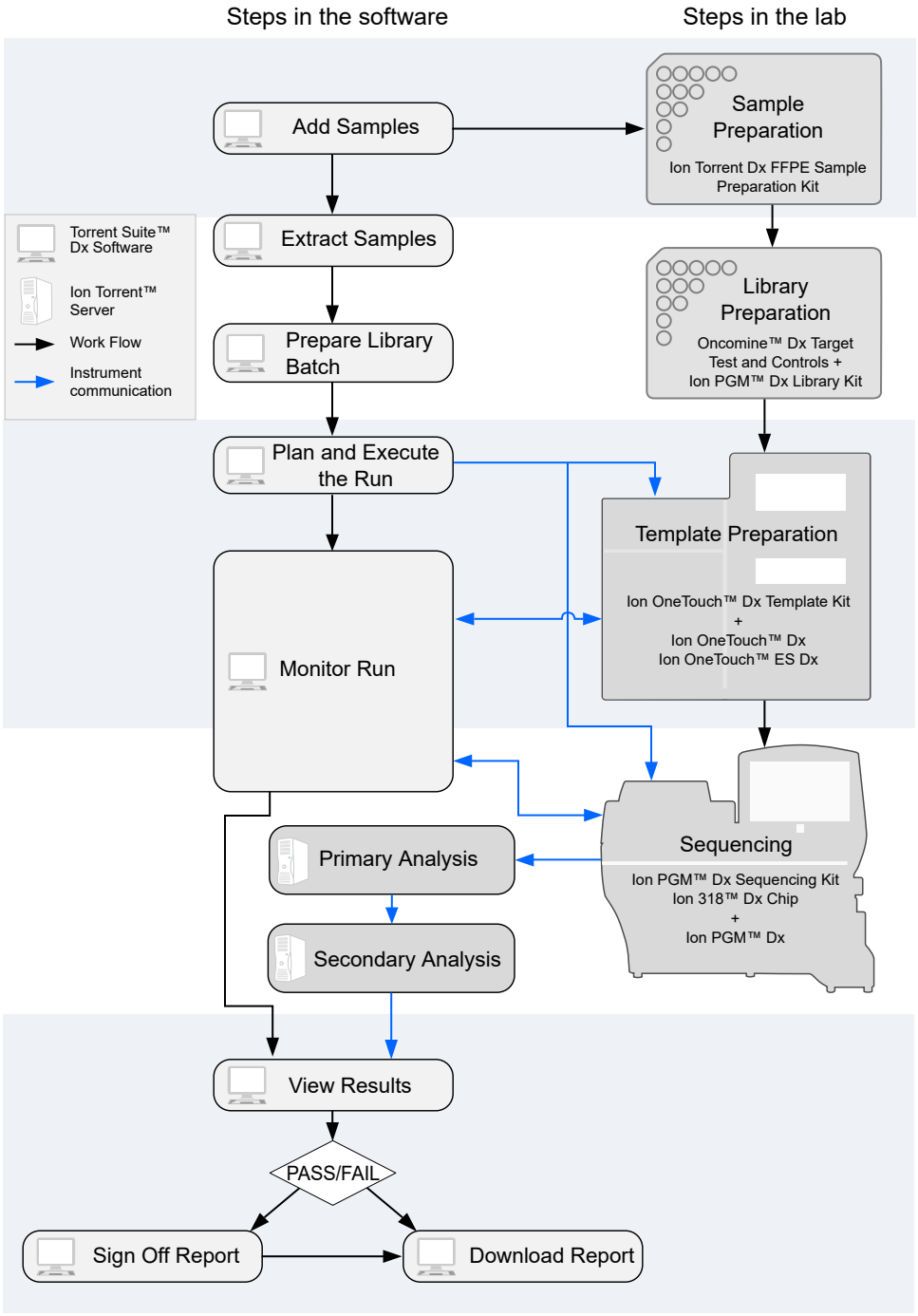
In general, the Ion PGM™ Dx Sequencer can remain on all the time, including overnight and over weekends. If shutdown is necessary, there are two methods, depending on the state of the instrument:

Type of shutdown	Typically used when	Method
Routine shutdown (Managers and Administrators only)	• The instrument will not be used for an extended period of time (> 1-2 weeks). • The instrument needs to be moved or serviced.	1. Press the **Options** button on the main touchscreen and press **Shut Down**. 2. If the instrument will not be used for an extended period of time, select the **Cleaning** checkbox to perform a water clean first, then press **Shut Down**. Otherwise, just press **Shut Down**. 3. In the next screen, press the **Halt** button and then press **OK** to power down the instrument.
Forced shutdown	The instrument isn't responding normally (e.g, the touchscreen is frozen).	Hold down the power button below the touchscreen for ~5 seconds to power down the instrument.

To restart the sequencer:

- Hold down the power button below the touchscreen for ~5 seconds.

Oncomine™ Dx Target Test system diagram



Sequencing workflow

Note: Up to two sequencing runs may be performed from one initialization, if both runs are started within 27 hours after start of initialization.

Previous guide: *Oncomine™ Dx Target Test Part III: Template Preparation User Guide* (Pub. No. MAN0018812)

"Clean the Ion PGM™ System" on page 26

▼

"Initialize the sequencer" on page 34

▼

"Prepare enriched ISPs for sequencing" on page 41

▼

"Set up the sequencing run" on page 43

▼

"Load the sample on the chip" on page 46

▼

"Perform the run" on page 49

Proceed to: *Oncomine™ Dx Target Test Part V: Torrent Suite™ Dx Software 5.12.5 Reference User Guide* (Pub. No. MAN0018814)

3 Clean and initialize

Clean the Ion PGM™ System

Cleaning schedule

The Ion PGM™ Dx Sequencer requires cleaning with either 18 MΩ water or chlorite solution according to the following schedule.

Clean with:	Schedule:
18 MΩ water	The Ion PGM™ Dx Sequencer requires cleaning with 18 MΩ water when one of the following conditions is met: • The last water cleaning was completed more than 27 hours ago, and initialization was performed • If during the last initialization, the **One run per initialization** checkbox was selected, and one run was performed since the last water cleaning • If during the last initialization, the **Two runs per initialization** checkbox was selected, and two runs were performed since the last water cleaning
Chlorite solution	The Ion PGM™ Dx Sequencer requires cleaning with chlorite solution when one of the following conditions is met: • The last chlorite cleaning was completed more than 7 days ago, and one or more runs have been performed since that cleaning • The instrument has been left with reagent for more than 48 hours **Note:** If the Ion PGM™ Dx Sequencer will not be used for more than 3 days, a chlorite cleaning is strongly recommended within 48 hours after run completion.

Before you begin

Mark the cleaning bottles

Three 250-mL bottles are provided as part of instrument installation. Mark these bottles as described below and use them in the cleaning procedures.

Note: After you have used the 250-mL bottles provided in each sequencing kit for 8 runs, you can mark them as extra cleaning bottles.

- Write "Waste" on one 250-mL bottle, to be used for both water and chlorite washes.
- Write "Water" on one 250-mL bottle, to be used for water washes only.
- Write "Chlorite" on one 250-mL bottle, to be used for chlorite solution only.

Mark the cleaning chips

After a sequencing run, used chips can be marked for use in cleaning and initialization. (Chips cannot be reused for sequencing.) Mark used chips as described below.

- To designate a used chip for chlorite washes only, write "CL" on the corner. Do not use this chip for initialization.
- To designate a used chip for water washes and initialization, write "W" on the corner. Do not use this chip for chlorite washes.

Chip marked for chlorite wash only:

Chip marked for water wash/initialization:

Start the cleaning

The sequencer touchscreen will guide you through each step in the cleaning process. The touchscreen provides a brief summary of the steps, which are described below in more detail.

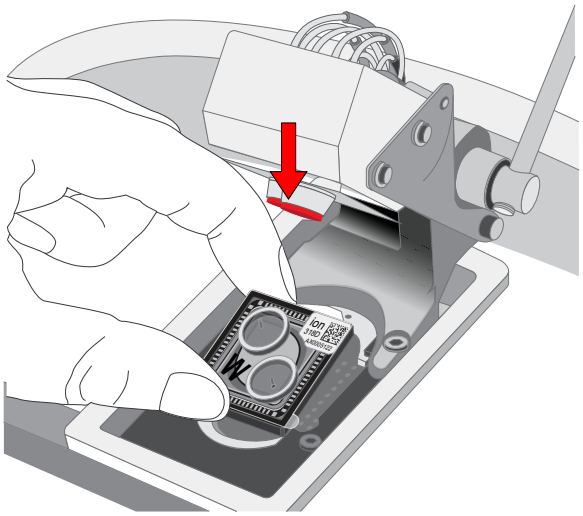
1. Log into the Ion PGM™ Dx Sequencer touchscreen.
2. On the main menu, press the **Clean** button.
   The instrument touchscreen will display the following:
   - **Last water clean: [YYYY-MM-DD] [hh]:[mm]:[ss]**
   - **Last chlorite clean: [YYYY-MM-DD] [hh]:[mm]:[ss]**
3. The type of cleaning required is selected by default (**18 MΩ water cleaning** or **Chlorite cleaning**). Press **Next**.

18-MΩ water cleaning

IMPORTANT! For all the following steps, use 18-MΩ water directly from the purification system. Do not use water that has been collected or stored in any other containers.

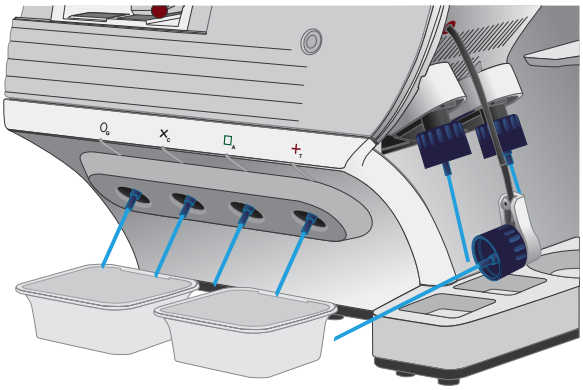
1. Touch the grounding plate on the Ion PGM™ Dx Sequencer with a bare finger, then use ungloved hands to insert the used chip marked "W" into the chip clamp.

   IMPORTANT! Confirm that both red rubber port gaskets are securely in place in the clamp. Loose or missing gaskets can result in a spill hazard and instrument damage.



2. Close the clamp, then press **Next** on the touchscreen.

3. Put on new gloves, then remove all wash bottles and reagent tubes attached to the instrument. Keep the sippers in place at all positions. Press **Next**.



4. Rinse the bottle marked "Water" twice with ~100 mL of 18-MΩ water. Press **Next**.

5. Fill the bottle marked "Water" with 250 mL of 18-MΩ water. Press **Next**.

6. Remove and rinse the W1 sipper with 18-MΩ water, then reattach the sipper. Press **Next**.

7. Attach the bottle marked "Water" to the W1 position, ensuring that the W1 cap is screwed on tightly. Press **Next**.

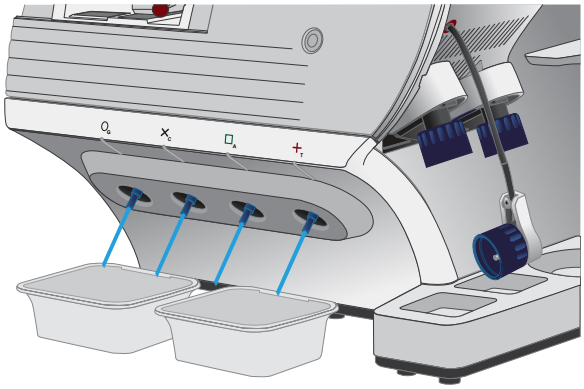
8. With the W3 sipper still in its cap, insert the sipper into the empty bottle marked "Waste". Do not screw on the cap. Insert the W2 sipper into the same bottle. Press **Next**.

9. Place collection trays below the reagent tube sippers in the dNTP positions.

Note: To avoid spills, ensure that the collection trays are properly aligned to catch fluid flowing out from the sippers.

10. Press **Next** to begin cleaning.
The cleaning will take ~15 minutes to complete.

11. When cleaning is complete, leave the reagent sippers and collection trays in place. Remove the bottles and sippers from the W1, W2, and W3 positions. Press **Next** to return to the main menu, then proceed to initialization.



Chlorite cleaning

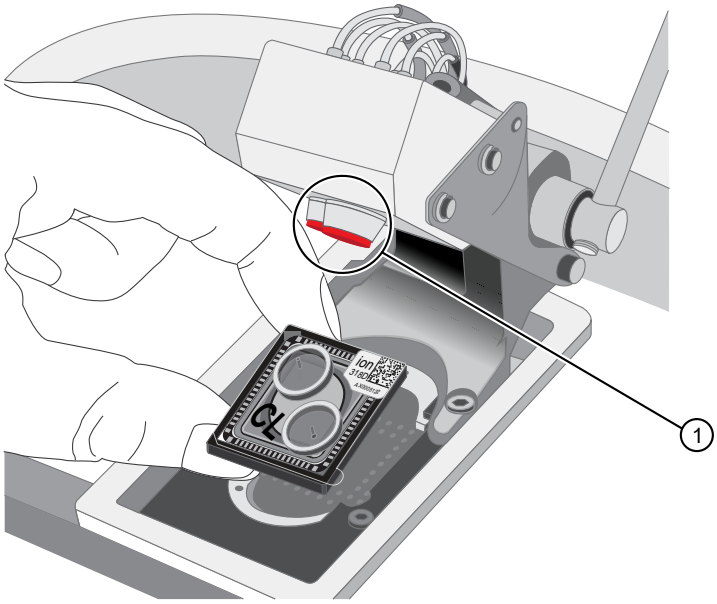
Note: This procedure uses 1 M NaOH, diluted from 10 M NaOH each week using nuclease-free water. You can use the 1 M NaOH that was prepared during template preparation.

1. Fill a glass bottle with 1 L of 18-MΩ water, then add a SEQ Cleaning Tablet (chlorite tablet). Allow the tablet to completely dissolve (~10 minutes). Press **Next** on the touchscreen.
2. Add 1 mL of 1 M NaOH and filter the solution using a 0.22-µm or 0.45-µm filter. Press **Next**.

   Note: Use the chlorite solution within 2–3 hours. Discard the unused solution in an appropriate waste container after this time.

3. Touch the grounding plate on the Ion PGM™ Dx Sequencer with a bare finger, then use ungloved hands to insert the used chip marked "CL" into the chip clamp.

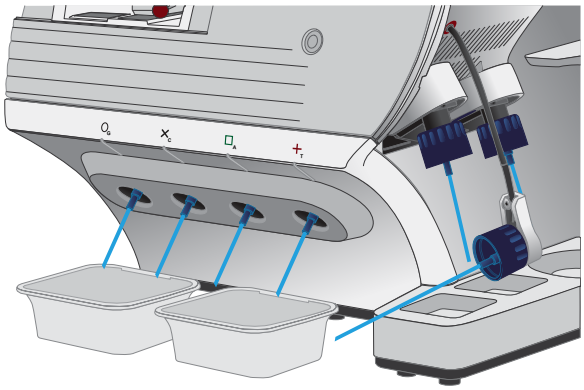
   IMPORTANT! Confirm that both red rubber port gaskets are securely in place in the clamp. Loose or missing gaskets can result in a spill hazard and instrument damage.



(1) Rubber port gaskets

4. Close the clamp, then press **Next**.

5. Put on new gloves, then remove all wash bottles and reagent tubes that are attached to the instrument. Keep the sippers in place at all positions. Press **Next**.



6. Rinse the bottle marked "Chlorite" and the bottle marked "Water" twice each with ~150 mL of 18-MΩ water.

7. Add 250 mL of filtered chlorite solution to the bottle marked "Chlorite". Press **Next**.

8. Remove and rinse the sipper in the W1 position with 18-MΩ water, then reattach it to the instrument. Press **Next**.

9. Attach the bottle marked "Chlorite", containing the filtered chlorite solution, to the W1 position. Make sure that the W1 cap is tight. Press **Next**.

10. With the W3 sipper still in its cap, insert the tube into the empty bottle marked "Waste". Do not screw on the cap. Insert the W2 sipper into the same bottle. Press **Next**.

11. Place collection trays below the reagent tube sippers in the dNTP positions.

    Note: To avoid spills, ensure that the collection trays are properly aligned to catch fluid flowing out from the sippers.

12. Press **Next** to begin cleaning. The cleaning will take ~13 minutes to complete.

13. When prompted, remove the bottle marked "Chlorite" from the W1 position. Press **Next**.

14. Remove and rinse the W1 sipper with 18-MΩ water, then reattach the sipper. Press **Next**.

15. Fill the bottle marked "Water" with 250 mL of 18-MΩ water and attach the bottle in the W1 position. Make sure the cap is tight.

16. Press **Next**. The water rinse will take ~15 minutes to complete.

17. When cleaning is complete, leave the reagent sippers and collection trays in place. Remove the bottles and sippers from the W1, W2, and W3 positions. Press **Next** to return to the main menu.

18. Rinse the "Chlorite" and "Water" bottles twice each with ~150 mL of 18-MΩ water, then proceed to initialization.

Initialization

Initialization guidelines

- When performing one or two sequencing runs from the same initialization, the run(s) must be started within 27 hours after the start of initialization.
- Handle the SEQ dGTP, SEQ dCTP, SEQ dATP, and SEQ dTTP tubes carefully to avoid cross-contamination. Always change gloves after removing used sippers from the Ion PGM™ Dx System to avoid cross-contamination of the nucleotides. Also change gloves after handling concentrated dNTP stocks.
- Replace the SEQ Reagent Tubes and sippers every time you initialize.
- After eight sequencing runs, do not use the SEQ Wash 1 Bottle, SEQ Wash 2 Bottle, or SEQ Wash 3 Bottle for initialization or sequencing to avoid possible breakage or leaking. You can continue to use the SEQ Wash 1 Bottle and SEQ Wash 3 Bottle as extra cleaning bottles, as described in "Mark the cleaning bottles" on page 26.

Ion PGM™ Dx Sequencing Kit component lot matching

The three components of the Ion PGM™ Dx Sequencing Kit must be lot-matched with each other for use.

Component	Part No.	Storage
Ion PGM™ Dx Sequencing Supplies	A18936	15°C to 30°C
Ion PGM™ Dx Sequencing Reagents	A18934	–30°C to –10°C
Ion PGM™ Dx Sequencing Solutions	A18935	2°C to 8°C

Before initialization

Check the tank pressure for the nitrogen gas. When the tank pressure drops below 500 psi, change the tank (see also "Gas cylinders" on page 14).

Initialize the sequencer

The instrument touchscreen provides a summary of the steps in the initialization process, which are described below in more detail.

IMPORTANT! Do not remove the old sippers from the dNTP ports until instructed to do so. **Do not let the new sippers touch any surfaces.**

IMPORTANT! Load the bottles as quickly as possible to prevent atmospheric CO_2 from reducing the pH of the Wash 2 solution.

1. After cleaning is complete, the **Initialize** button on the touchscreen main menu will turn blue. Press the button to start the initialization process.

2. Depending on the number of sequencing runs per initialization, select either the **One 200 bp run per initialization** or the **Two 200 bp runs or One 400 bp run per initialization** checkbox. Press **Next**.

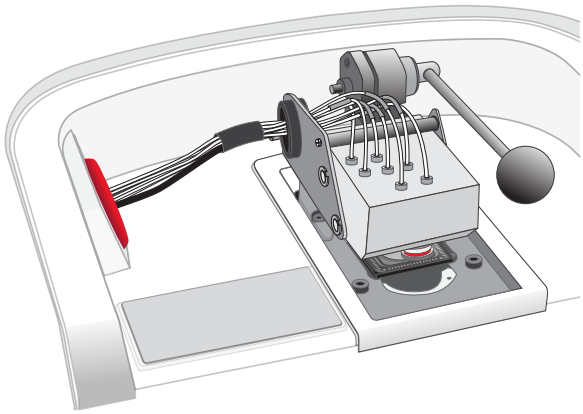
   Note: If you are performing two runs, both must start within 27 hours after start of the initialization.

   Note: 400-bp runs are performed for Assay Development purposes only and are not used with the Oncomine™ Dx Target Test.

3. Press the **Keyboard** button below the **Sequencing kit barcode** field. Using the barcode scanner attached to the sequencer, scan the barcode on the Ion PGM™ Dx Sequencing Reagents box. Press **OK**, then press **Next**.

4. Ensure that the used chip marked "W" (from the cleaning procedure) is secured in the chip clamp on the sequencer.

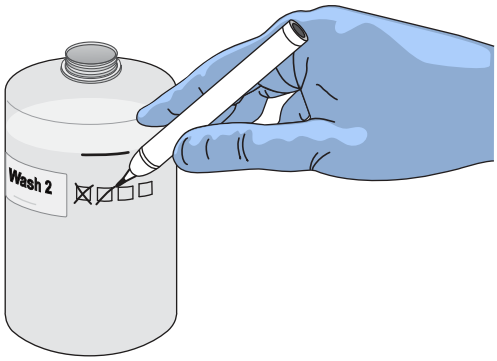
   IMPORTANT! Do *not* use the chip marked "CL" for initialization. (Use it for chlorite cleaning only.)



5. Ensure that the old reagent sippers and collection trays are in place. Press **Next**.

6. Rinse the SEQ Wash 1 Bottle, SEQ Wash 2 Bottle, and SEQ Wash 3 Bottle 3 times each with 18-MΩ water. Use 150 mL for the Wash 1 and Wash 3 bottles, and 500 mL for the Wash 2 bottle.

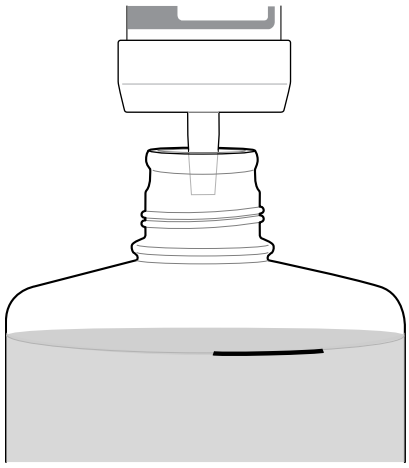
   Note: The bottles can be rinsed and reused for up to 8 sequencing runs. To track the number of uses, mark each bottle each time a run has finished as shown below. After 8 uses, the SEQ Wash 1 Bottle and SEQ Wash 3 Bottle bottles can be marked and used for cleaning only.



7. Prepare 500 µL of fresh 100 mM NaOH daily by diluting 50 µL of 1 M NaOH in 450 µL of nuclease-free water.

   Note: Prepare a stock of fresh 1 M NaOH weekly.

8. If your 18-MΩ water system has a spigot, extend it into **but not below** the neck of the SEQ Wash 2 Bottle. Otherwise, position the nozzle as close to the mouth of the bottle as possible.



IMPORTANT! If your water system has a digital display, verify that it reads "18 MΩ" when filling the bottle. If it does not, see Appendix A, "Troubleshooting".

9. Fill the bottle to the mold line with 18-MΩ water.

Note: The total volume of water is ~2 liters. You can mark the mold line on the bottle for clarity. If you are preparing bottles for multiple sequencers, cap each bottle immediately after filling. Leave them capped until you are ready to add SEQ W2 Solution.

10. Add an entire bottle of SEQ W2 Solution to the SEQ Wash 2 Bottle.

Note: Store the remaining SEQ W2 Solution bottles in the sealed mylar bag they were provided in.

11. Using a P200 pipette, add 70 µL of 100 mM NaOH to the SEQ Wash 2 Bottle.

Note: If the initialization consistently undershoots the pH target (see step 21), add more than the recommended 70 µL of 100 mM NaOH when preparing the Wash 2 Bottle. After adding the NaOH, the Wash 2 Bottle must be between pH 6.0–6.5 at first pH iteration before you start initialization.

12. Cap the bottle and invert 5 times to mix, then immediately proceed through the remainder of the initialization procedure.

IMPORTANT! Do not store the mixed SEQ Wash 2 Bottle.

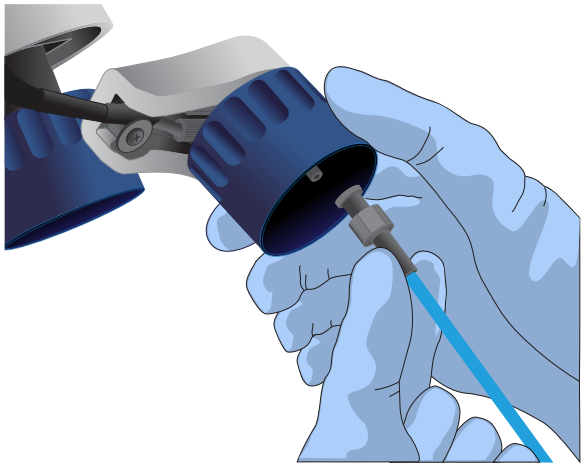
13. SEQ Wash 1 Bottle: Add 350 µL of freshly prepared 100 mM NaOH to a clean SEQ Wash 1 Bottle. Ensure that the pipette tip touches the bottom of the bottle to dispense.

14. SEQ Wash 3 Bottle: Add SEQ W3 Solution to the 50-mL line marked on a clean SEQ Wash 3 Bottle. Press **Next**.

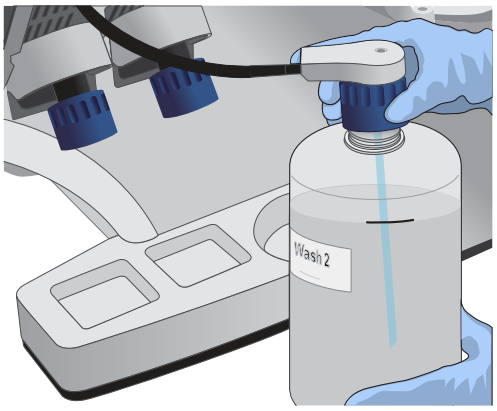
15. Remove the old sippers from the W1, W2, and W3 positions on the instrument, then discard in a waste container.

16. Wearing clean gloves, firmly attach a new, long sipper to the cap in the W2 position. **Do not let the sipper touch any surfaces.**

IMPORTANT! Loosely attached sippers can adversely affect results.

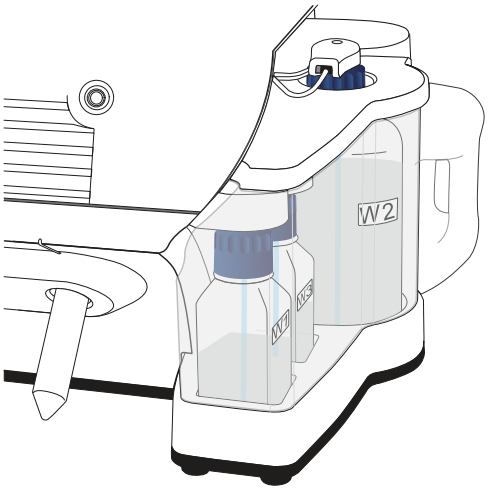


17. Immediately attach the prepared SEQ Wash 2 Bottle to the cap in the W2 position, then tighten the cap. Press **Next**.



18. Change gloves and firmly install new, short sippers to the caps in the W1 and W3 positions.

19. Immediately attach the prepared SEQ Wash 3 Bottle to the cap in the W3 position, then attach the SEQ Wash 1 Bottle to the cap in the W1 position. Tighten the caps.



20. Ensure that the collection trays are properly aligned to catch fluid flowing out of the sippers. Press **Next** to start the auto-pH process.

21. When auto-pH is complete:
    - If auto-pH fails (for example, the instrument overshoots or undershoots the target pH), the touchscreen displays an error message. Do not press **Next**. See Appendix A, "Troubleshooting" for information about specific errors.
    - If auto-PH passes, the touchscreen will display to the next step.

22. Thaw the following at room temperature until no ice crystals are visible in the tubes (~15 minutes). Keep the tubes on ice or in a chilled benchtop cold box until ready to use.
    - SEQ Primer (white cap)
    - SEQ dGTP (black cap)
    - SEQ dCTP (blue cap)
    - SEQ dATP (green cap)
    - SEQ dTTP (red cap)

23. The kit includes labels for each type of dNTP. Attach one of each label type to four SEQ Reagent Tubes.

24. Verify that no ice crystals are visible in each thawed dNTP stock solution tube (SEQ dGTP, SEQ dCTP, etc.) from step 22. Vortex each dNTP for ~5 seconds, then pulse centrifuge to collect.

    Note: To avoid cross-contamination in the next step, open only one dNTP tube at a time, and use a fresh pipette tip for each aliquot.

25. Using a P20 pipette and separate tips, aliquot each dNTP into its appropriately labeled reagent tube as follows:
    - Aliquot 10 µL of dNTP per reagent tube if performing one 200 bp run per initialization.
    - Aliquot 20 µL of dNTP per reagent tube if performing two 200 bp runs per initialization.

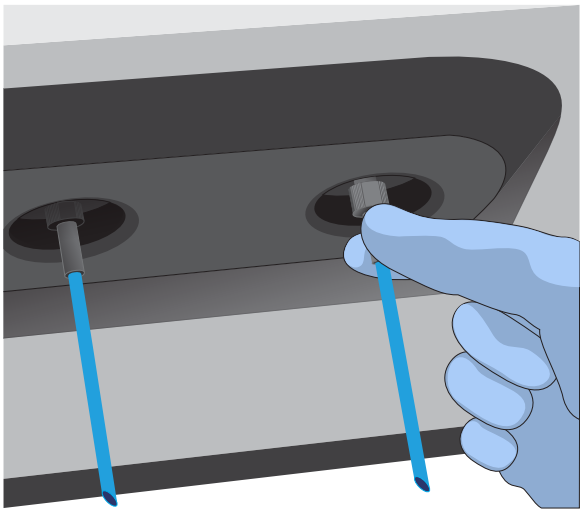
    Note: Reagent tubes can be capped and left at 2–8°C or on ice until ready for use. Place the remaining dNTP stocks back into –30°C to –10°C for storage. The dNTPs can be freeze-thawed up to 4 times. We recommend marking the box with each freeze-thaw to indicate how many have occurred.

26. Press **Next**. Remove the old reagent tube sippers, then discard them in a waste container.

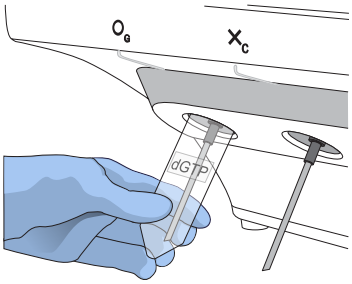
27. Remove the collection trays, discard any waste in a sink, then rinse the trays.

28. Using new gloves, firmly insert a new SEQ Reagent Tube Sipper (blue) into each dNTP port. Do not let the sipper touch any surfaces.

    IMPORTANT! Be careful to push each sipper onto the port firmly. Loosely attached sippers can adversely affect results.



29. Attach each SEQ Reagent Tube to the correct dNTP port (for example, the dGTP tube on the port marked "G"), then tighten firmly by hand until it can no longer rotate.



30. After each reagent tube is securely installed, press **Next**.

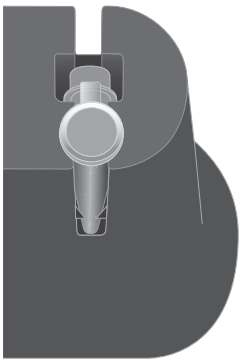
    The instrument automatically completes the rest of the initialization, which takes ~20 minutes. You can monitor the progress on the touchscreen.

    - If initialization is successful, the touchscreen indicates that initialization has "Passed" (highlighted in green). Press **Next** to return to the main menu.
    - If initialization fails, see Appendix A, “Troubleshooting”.

4 ISP preparation

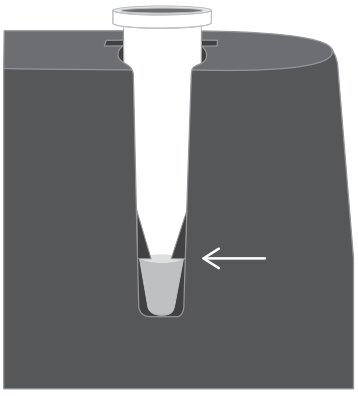
Prepare enriched ISPs for sequencing

1. Confirm that the SEQ Primer is completely thawed before use. No ice crystals should be visible.
2. Vortex the SEQ Primer for ~5 seconds, then pulse centrifuge to collect the contents. Leave the tube on ice or in a chilled benchtop cold box until use.
3. Remove the enriched ISP sample in the TMPL Sample Collection Tube from the Ion OneTouch™ ES Dx Instrument or 2–8°C storage.
4. Place the tube in a microcentrifuge with a 0.2-mL tube adapter. Orient the tab of the tube lid so that it is pointing away from the center of the centrifuge (this will indicate where the sample ISPs are being pelleted).
5. Centrifuge at 15,000 rcf for 2 minutes.
6. Insert the tube into the appropriate slot of the Ion OneTouch™ Sample Rack. Open the tube lid carefully to avoid spillage, then lift the tube out of the slot, fold the lid flat against the back of the tube, and insert the tube and folded lid into the slots in the rack.



7. Use a P200 pipette and keep the pipette plunger depressed as you insert the tip into the tube containing the enriched ISPs. Carefully remove ~200 µL of supernatant from the top down, avoiding the side of the tube where the ISPs are pelleted (i.e., the side with the tab on the tube lid). Discard the supernatant.
8. Change to a P20 pipette. Keep the pipette plunger depressed as you insert the tip into the tube, and carefully remove the remaining supernatant to the volume marked by the arrow guides on the rack.

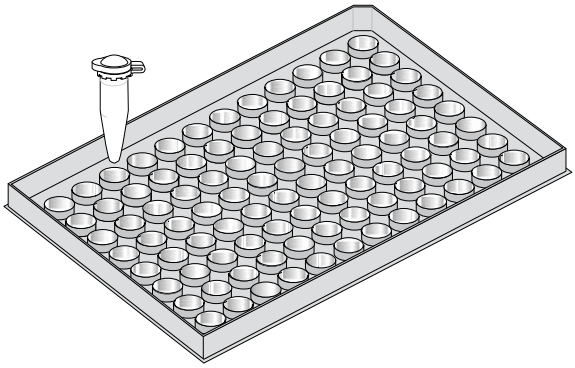
   Again, avoid the side of the tube where the ISPs are pelleted. The final volume in the tube will be ~15 µL.



9. Remove the tube from the sample rack, then add 12 µL of thawed SEQ Primer (white cap) to the ISPs in the tube and vigorously pipet the mixture up and down 30 times.

   IMPORTANT! Make sure that the pipette tip is at the bottom of the tube during mixing to avoid introducing air bubbles into the sample.

10. Insert the tube into the 96-well tray supplied with the Veriti™ Dx 96-well Thermal Cycler.



11. Load the tube and tray assembly into the thermal cycler. Select the **8 ODxTT Seq Primer Hyb** program on the thermal cycler touchscreen. Select **View** and confirm that the program steps match those listed in the table below.

Stage	Temperature	Time
Hold	95°C	2 minutes
Hold	37°C	2 minutes
Hold	25°C	Hold (up to 30 minutes)

12. When you have confirmed the program steps, run the cycling program.

After cycling, keep the tube containing the primer-annealed ISPs at room temperature. Proceed immediately to set up the sequencing run.

5 Sequencing protocol

Set up the sequencing run

The instrument touchscreen provides a summary of the steps in the process, which are described below in more detail.

IMPORTANT!

- To avoid damage to the chips or instrument due to electrostatic discharge:
  - **Remove your gloves when handling chips**, especially before transferring chips on or off the instrument. Hold chips by their edges when handling.
  - **Do not place chips directly on the bench or any other surface.** Always place chips either on the grounding plate on the Ion PGM™ Dx Sequencer or in the Ion PGM™ Dx Chip Minifuge bucket.

  Note: Steps without gloves can be performed without risk of contamination.
- When performing one or two sequencing runs from the same initialization, the runs must be started within 27 hours after the start of initialization.

1. After initialization and sample preparation, go to the main menu of the Ion PGM™ Dx Sequencer, then press the **Run** button to begin the run setup.
2. Remove the waste bottle from the instrument and completely empty the bottle. Return the waste bottle to its position on the instrument. Press **Next**.

   IMPORTANT! Removing any waste before each run is critical. Waste overflow can result in a spill hazard and instrument damage.
3. Make sure the chip used for initialization is still in the chip clamp, then press **Next**. The instrument cleans the fluid lines, then proceeds automatically to the next screen.
4. Press the **Keyboard** button next to the **Planned Run** field. Using the barcode scanner attached to the sequencer, scan the **Run Short Code/Barcode** on the **View Planned Run** printout. Alternatively, type the code (displayed below the barcode) using the touchscreen keyboard. Press **OK**.

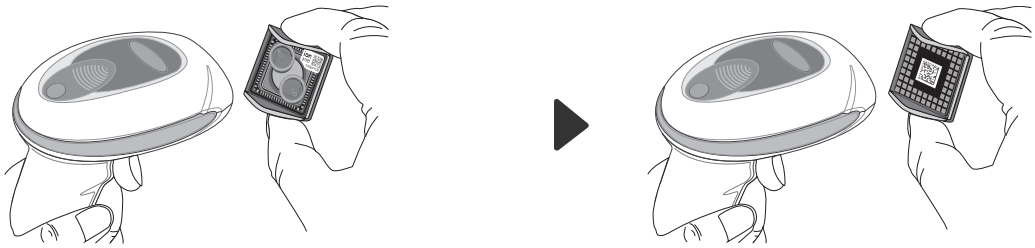
Note: If the Ion Torrent™ Server has ≤1 terabyte (TB) of free disk space, an alert notifies the user that there is insufficient disk space to perform the run. The run cannot proceed until data on the server is archived and deleted. Contact your IT system administrator to manually archive and delete data. An administrator-level user in the software can also change the **Archive Settings** to

reduce the **Auto archive after** interval. See the *Oncomine™ Dx Target Test Part V: Torrent Suite™ Dx Software 5.12.5 Reference User Guide*.

5. Confirm the Planned Run information displayed on the touchscreen, then press **Next**.
   - Planned Run name
   - Sample name if a single sample, or number of samples if multiple samples
   - Assay name
   - Created by [user name]
   - Created date [YYYY-MM-DD]
   - Ion PGM™ Dx Sequencing Kit barcode [scanned during initialization]
6. Remove your gloves.

Note: The following steps can be performed without gloves without risk of contamination.

7. Open the chip clamp, then remove the chip used for initialization. Press **Next**.
8. Touch the grounding plate with a bare finger, then remove a new chip from its packaging.
9. Press the **Keyboard** button next to the **Top barcode** field. Using the barcode scanner attached to the sequencer, scan the barcode on the top of the chip, then press **OK**.

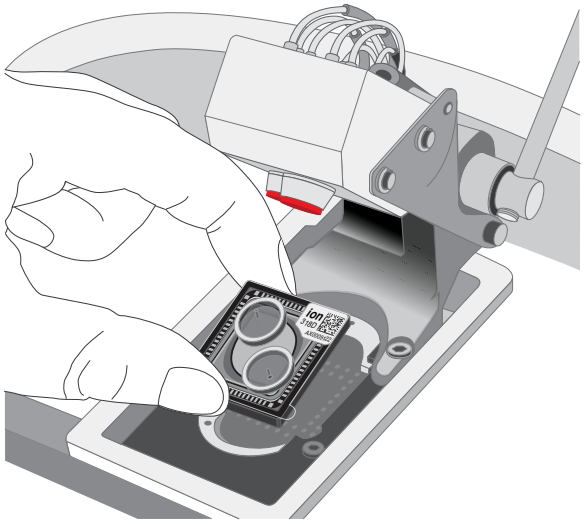


10. Press the **Keyboard** button next to the **Bottom barcode** field. Scan the barcode on the bottom of the chip, then press **OK**.

Note: If the barcode does not scan, use a new chip and contact Technical Support for a replacement.

11. Using a marker, label a corner of the chip with the **Tube Label** text.

12. Secure the new chip in the chip clamp.



13. Press **Next** to calibrate the chip. The screen prompts you to perform certain steps during calibration.

14. When prompted, visually inspect the chip in the clamp for liquid leaking from the chip case into and around the clamp area.

CAUTION! If a leak occurs, press the **Abort** button immediately, then see "Leak of unknown origin during chip calibration (before sample has been loaded on the chip)" on page 85.

15. Close the instrument lid when prompted.

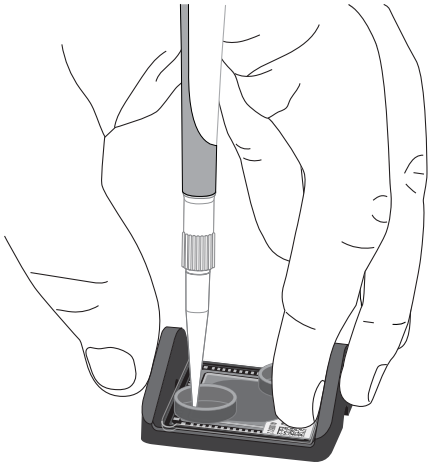
16. When chip calibration is complete, a "Calibration Passed" or "Calibration Failed" message appears.
    - If calibration passes, proceed to chip loading.
    - If calibration fails or you get an error message, see "Chip calibration failure (before sample has been loaded on the chip)" on page 84.

Load the sample on the chip

For additional instructions on using the Ion PGM™ Dx Chip Minifuge, see "Ion PGM™ Dx Chip Minifuge" on page 96.

IMPORTANT! The following steps (including chip loading) should be performed without gloves, except when adding SEQ Enzyme as noted. The steps can be performed without gloves without risk of contamination.

1. Enter the Tube Label text into the Ion PGM™ Dx Sequencer touchscreen. Press **Next**.
2. Touch the grounding plate on the instrument with a bare finger, then remove the new Ion 318™ Dx Chip from the clamp.
3. Insert the used chip from initialization (marked with a "W") back into the chip clamp.
4. Place the new chip in the removable bucket from the Ion PGM™ Dx Chip Minifuge, then place the bucket on a firm, flat surface.
5. Set a P200 pipette to 30 µL. Insert the tip firmly into the chip loading port while holding the bucket and chip steady with your other hand. Remove as much liquid as possible from the port, then discard the liquid.



6. Place the chip **upside-down** in the bucket, then transfer the bucket to the Ion PGM™ Dx Chip Minifuge **with the chip tab pointing out** (away from the center of the minifuge).
7. Balance the minifuge with another upside-down chip in the opposing bucket.

Note: If you have prepared only one loaded chip, balance the minifuge with an empty used chip. Mark the used chip with a marker to differentiate it from the loaded chip.

8. Centrifuge for 3–5 seconds to completely dry the chip surface.

CAUTION! Allow the minifuge to come to a complete stop before opening the lid.

9. Remove the bucket containing the chip from the minifuge. Remove the chip from the bucket, then wipe down the bucket with a disposable wipe to remove residual liquid. Place the chip right-side up in the bucket.

10. Put on new gloves.

11. Remove the SEQ Enzyme (yellow cap) from storage and flick 4 times to mix. Pulse centrifuge the tube to collect the contents, then place the tube on ice or in a chilled benchtop cold box until use.

12. Remove the primer-annealed ISPs from the Veriti™ Dx 96-well Thermal Cycler (from "Prepare enriched ISPs for sequencing" on page 41).

13. Add 3 µL of SEQ Enzyme to the primer-annealed ISPs. Set a P200 pipette with a low-bind tip to 20 µL, then gently pipet the mix up and down 10 times.

Note: If there is condensation on the walls of the tube, push down with the end of the pipette tip to mix the condensation with the sample.

14. Incubate at room temperature for 5 minutes.

15. Remove your gloves.

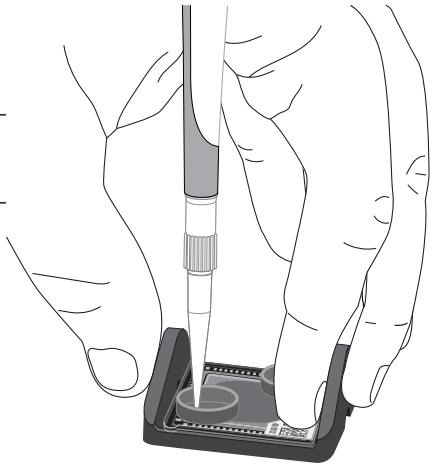
Note: The following steps can be performed without gloves without risk of contamination.

16. Place the chip in the minifuge bucket on a firm, flat surface. Following the 5-minute polymerase incubation, collect 30 µL of sample into a P200 pipette tip. Discard any remaining sample.

Note: If the sample is less than ~30 µL, there will be a visible air gap at the end of the tip. To correct the volume, see "Sample volume is <30 µL" on page 89.

17. Insert the pipette tip firmly into the loading port of the chip. Apply gentle pressure between the tip and chip throughout the loading process.

IMPORTANT! Do not remove the pipette tip from the port or dial up the pipette during loading.

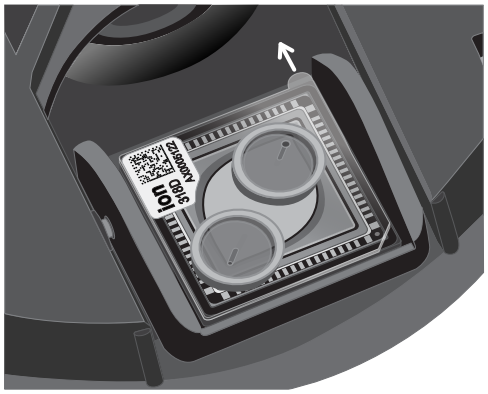


18. With the pipette unlocked, dial down the pipette to deposit the ISPs. Ensure that the entire sample is loaded onto the chip. To avoid introducing air bubbles, leave a small amount of sample in the pipette tip (~0.5 µL).

Note: A small amount of sample may leak from the outlet port, which is acceptable. However, if a large amount of sample leaks from the outlet port (~15 µL or greater), do not continue with the run, and contact Technical Support.

19. Transfer the chip in the bucket to the minifuge **right-side up with the chip tab pointing in** (toward the center of the minifuge).

20. Balance the minifuge with another right-side up chip in the opposing bucket, then centrifuge for 30 seconds.



CAUTION! Allow the minifuge to come to a complete stop before opening the lid.

21. Remove the bucket containing the chip from the minifuge and place it on a flat surface. Set a P200 pipette to 40 µL, then pipet the liquid out through the loading port. Remove as much liquid from the chip as possible.

Note: It is normal to have some liquid remain in the chip after this step.

22. Turn the chip upside-down in the bucket, then place the bucket back in the minifuge with the chip tab pointing out.

23. Balance the minifuge with another upside-down chip in the opposing bucket, then pulse centrifuge for 3–5 seconds at maximum speed to dry the chip surface completely.

24. Remove the chip from the bucket, then wipe down any residual liquid left on the bucket with a disposable wipe.

Proceed immediately to "Perform the run".

Perform the run

1. On the Ion PGM™ Dx Sequencer touchscreen, press **Next**, then press the **Keyboard** button next to the **Top barcode** field. Using the barcode scanner attached to the sequencer, scan the barcode on the top of the new loaded chip, then press **OK**.

2. Remove the used chip from the instrument, then use ungloved hands to insert the loaded chip into the chip clamp.

   IMPORTANT! Confirm that both red rubber port gaskets are securely in place in the clamp.

3. Press **Next** to calibrate the loaded chip. At the start of calibration, visually inspect the chip for leaks before closing the instrument cover. Close the lid when prompted to do so.

CAUTION! If a leak occurs, press the **Abort** button immediately, then see "Leak of unknown origin during chip calibration (after sample has been loaded on the chip)" on page 86.

4. After ~1 minute, the touchscreen indicates if calibration was successful.
   - If the chip fails calibration, reseat the chip and press **Re-try** to recalibrate. If the chip continues to fail calibration, see "Error message: Calibration failed (after sample has been loaded on the chip)" on page 87.
   - If a message says that the chip is not seated correctly, see "Error message: Calibration failed (after sample has been loaded on the chip)" on page 87.

5. When the chip passes calibration, press **Next** to start the sequencing run. The sequencing run takes ~4.5 hrs to complete.

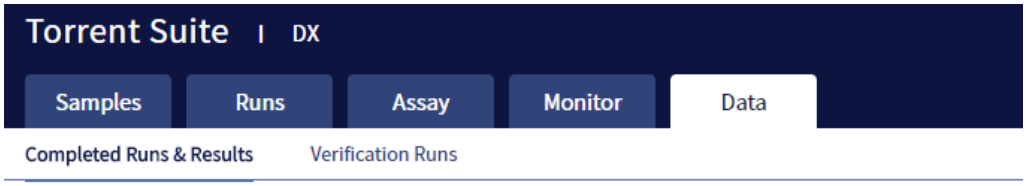
   IMPORTANT! Confirm the run has started before leaving the sequencer. During a run, do not touch the instrument or the attached bottles or tubes, because this can reduce the quality of the measurements.

6. After the run, the touchscreen returns to the main menu.

Review the results and generate reports as described in the next chapter.

Note: Used chips cannot be reused for sequencing. Used chips can be marked for cleaning and initialization as described previously.

6 Review data and results



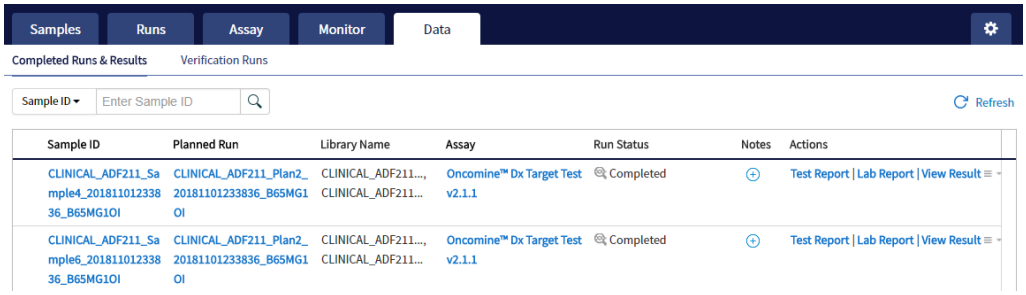
In the **Data** tab, you can review run results and perform data analysis and data management tasks:

- Select **Completed Runs & Results** (the default window) to review completed sample run results and reports. Run results are listed by **Sample ID**.
- Select **Verification Runs** to review data from completed verification runs performed during installation or PQ validation.

Completed Runs & Results screen

Under the **Data** tab, in the **Completed Runs & Results** screen, samples that have been sequenced are listed by Sample ID.

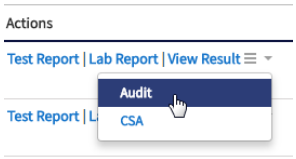
You can search the list of results by Sample ID or Planned Run name. You can also filter and sort the list.



The following information is displayed in the screen:

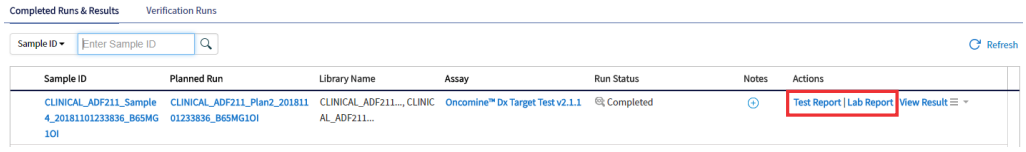
Column	Description
Sample ID	The unique identifier created when the sample was entered into the software. Click on the link to display the details of the sample.
Planned Run	The name of the Planned Run given when it was created in the software, after preparation of the library batch. Click on the name to display details of the Planned Run.

(continued)

Column	Description
Library Name	The names of the DNA and RNA sample libraries given during preparation of the library batch.
Assay	The assay selected when the sample was placed in a library batch prior to creating the Planned Run. Click on the assay name to display the details of the assay.
Run Status	The status of the run (for example: completed, failed, terminated).
Notes	User-entered notes about the sample. To view previously written notes, click . To add new notes, click .
Actions	Click the appropriate link to: • Download the Test Report. • Download the Lab Report. • Open the View Result screen. • View the audit trail for the Planned Run. Click , then click **Audit**.  • Download Customer Support Archive (CSA) log files for the run to aid in troubleshooting. Click , then click **CSA**.

Download the Lab Report or Test Report

1. To download the Lab Report or Test Report for a sample of interest, in the **Completed Runs & Results** screen, click the appropriate link in the **Actions** column for the sample. A ZIP file containing all languages of the report downloads automatically.



2. Extract the downloaded files, then open the PDF file of the desired language in an appropriate viewer.

Test Report

The Test Report (available in the **Completed Runs & Results** screen) is a clinical report generated by the software that can be downloaded in PDF format. It is identical to the Lab Report, except that it does not include the Sequencing Run Details and Control and Sample QC Evaluation Metrics sections. The Test Report contains the following sections and information.

Note: The display of variants in the Test Report depends on cancer type.

Section	Description
Sample Details	The sample and patient information entered into the software.
Results for Sequence Variations for Therapeutic Use	Displays the results for the clinical variants and gene fusions in the sample, and any recommended therapies that are clinically indicated for the selected cancer type.
Results for Analytical Sequence Variations Detected	Displays the results for analytical variants and gene fusions detected in the sample, depending on the variants associated with the cancer type.
Test Description	A description of the test and the gene variants in associated tissue types that it screens for.
Results for Analytical Sequence Variations Not Detected	Displays the results for analytical variants and gene fusions not detected in the sample, depending on the variants associated with the cancer type.

Lab Report

The Lab Report (available in the **Completed Runs & Results** screen) is a report generated by the software that can be downloaded in PDF format. The Lab Report contains the following sections and information.

Note: The display of variants in the Lab Report depends on cancer type.

Section	Description
Sample Details	The sample information entered into the software.
Results for Sequence Variations for Therapeutic Use	The results for the clinical variants and gene fusions in the sample, and any recommended therapies that are clinically indicated for the selected cancer type. Allele frequencies are also reported.
Results for Analytical Sequence Variations Detected	A list of the analytical variants and gene fusions detected by the assay, and associated information for each, depending on the variants associated with the cancer type.
Test Description	A description of the assay.
Analytical Sequence Variations Not Detected	A list of all the analytical variants and gene fusions not detected by the test, and associated information for each, depending on the variants associated with the cancer type.

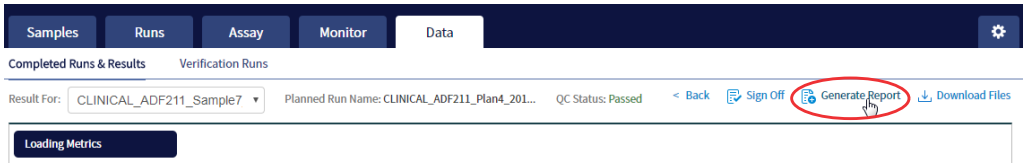
(continued)

Section	Description
Sequencing Run Details	A list of all the kits and instruments used to perform the test.
Control and Sample QC Evaluation Metrics	A summary of the quality control metrics.

Generate customized reports

By default, the Test Report and Lab Report are generated in the language that is selected in the report template. To generate these reports in another language, or change the types of variant calls that are reported, do the following:

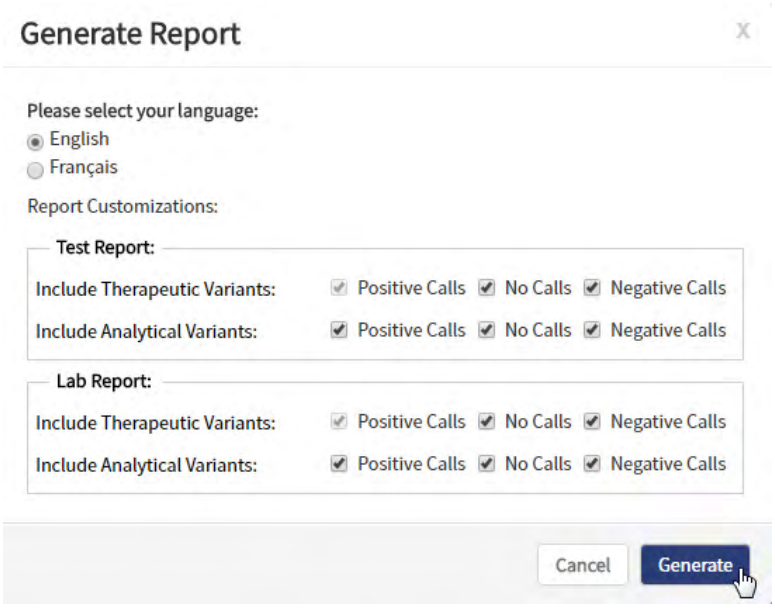
1. In the **Completed Runs and Results** screen, click **View Result**.
2. In the **View Result** screen, click **Generate Report**.



The **Generate Report** dialog box opens.

3. Do one or both of the following:
   - Select the desired language.
   - Select the checkboxes for the positive calls, no calls, or negative calls that you want to report in the Test Report and/or Lab Report.

     Note: These checkboxes are only available in IVD mode if they are enabled for the selected assay.



Note: Positive calls for therapeutic variants cannot be deselected.

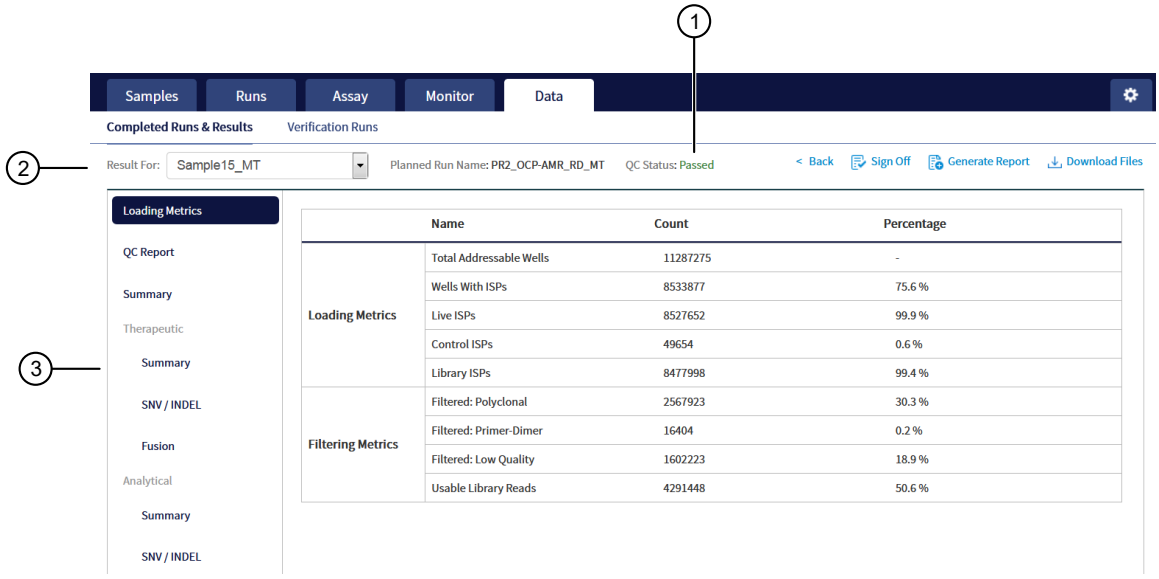
4. Click **Generate**.
   A message displays when the report has been generated. The new report overwrites the existing report.

5. Click **Download Files** to open a dialog box to select the new report and other run-related files for download.

View results

The run results shown in the screens described in this section can also be downloaded in the form of results files (see "Results files" on page 68).

- In the **Completed Runs & Results** screen, click **View Result** to view the sequencing run results for a sample of interest.
  The **Results** screen for the selected sample opens, with the **Loading Metrics** screen displayed. Result categories are arranged vertically on the left side of the screen. The QC status for the run is listed at the top of the screen.

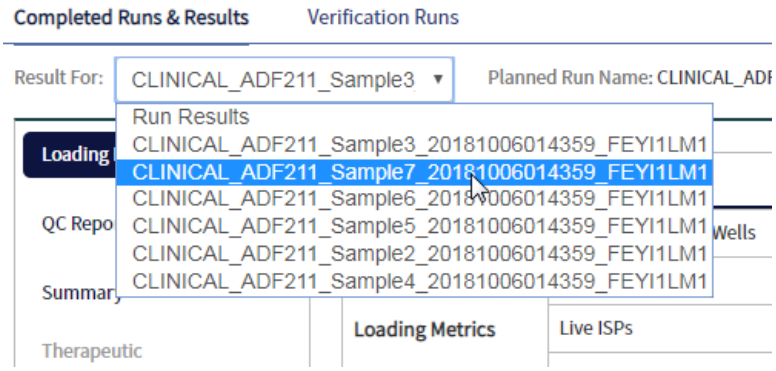


1. QC status
2. Selected sample
3. Result categories

IMPORTANT! A **QC Status** of "Passed" does not guarantee that the genotypes of all analytically relevant variants are determined. See the **Test Result** column in the Lab Report or Test Report for any "No Call" results when interpreting the results.

- Click the links on the left side of the screen to display results of interest, including QC results and results for different categories of variants.

- To view results for another sample in the same run, select the sample from the **Result For:** dropdown list. All samples in a run share the same **Loading Metrics** data. The other result categories are sample-specific.



- To generate the Lab Report and Test Report in other languages, click **Generate Report** (see "Generate customized reports" on page 53).
- To download individual results files for further analysis, click **Download Files** (see "Results files" on page 68).
- To return to the **Completed Runs & Results** screen, click **< Back**.

Loading metrics

The **Loading Metrics** link in the **View Result** screen displays the following loading and filtering metrics for the run:

Metric	Description
Loading Metrics	
Total Addressable Wells	The total number of wells on the chip – excluded wells.
Wells with ISPs	The number (count) and percentage of chip wells that contain ISPs. The percentage is expressed as a percent of total addressable wells.
Live ISPs	The number (count) and percentage of chip wells containing live ISPs (ISP's templated with library or control fragment), with the percentage expressed as a percent of wells with ISPs.
Control ISPs	The number (count) and percentage of ISPs that have a key signal identifying them as internal controls, with the percentage expressed as a percent of live ISPs.
Library ISPs	The number (count) and percentage of ISPs that have a key signal identical to the library key signal, with the percentage expressed as a percent of live ISPs.

(continued)

Metric	Description
Filtering Metrics[1]	
Filtered: Polyclonal	ISPs carrying clones from two or more templates, with the percentage expressed as a percent of library ISPs.
Filtered: Primer-Dimer	ISPs with an insert length of less than 8 bp, with the percentage expressed as a percent of library ISPs.
Filtered: Low Quality	ISPs with low or unrecognizable signal, with the percentage expressed as a percent of library ISPs.
Usable Library Reads[2]	Number (count) and percentage of library ISPs passing all filters.

[1] Filtering Metrics only apply to ISPs templated with library fragments, not control fragment.
[2] Values in the "Filtered:" rows are subtracted from the Library ISPs value (Loading Metrics) to give the Usable Library Reads value.

QC Report

The **QC Report** link displays metrics for the sample libraries and internal controls. This information is also accessible through the **Monitor** tab for the last five runs.

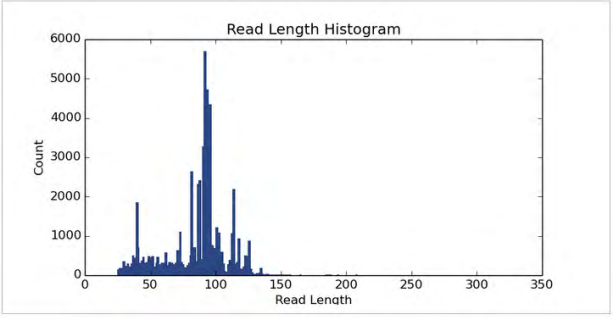
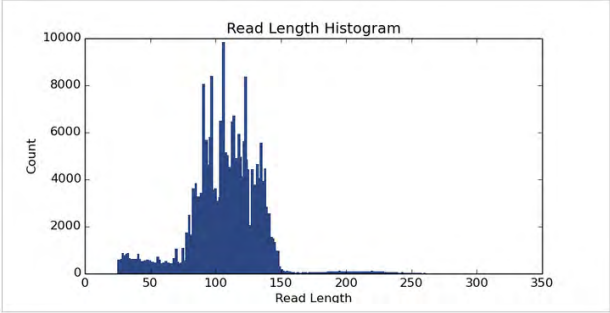
IMPORTANT! The following table describes all the quality control metrics that can be displayed. Whether particular metrics are displayed depends on the assay configuration.

Metric	Description
Library QC Evaluation Metrics	
Library QC: Library RNA	• Mean AQ20 Read Length (bp): The average length, in base pairs, at which the error rate is ≤1% for all aligned reads of a library. • Mean Read Length (bp): The average length, in base pairs, of all reads reported for a given library. • Total Mappable Reads: The number of reads that are mapped to the fusion reference file.[1]
Library QC: Library DNA	• Mean AQ20 Read Length (bp): The average length, in base pairs, at which the error rate is ≤1% for all aligned reads of a library. • Mean Read Length (bp): The average length, in base pairs, of all reads reported for a given library. • Percent Reads: The number of library reads normalized by the total addressable wells in a run.

(continued)

Metric	Description
Control QC Evaluation Metrics	
Control QC: CF-1	• Base Call Accuracy: 1 – (total number of errors for all positions in the control / total number of aligned bases) for Control Fragment-1 (CF-1) reads. • Key Signal: The average peak signal from the incorporation trace measured across the 1-mer incorporations in the CF-1 key sequences. [2] • Mean AQ20 Read Length (bp): The average length, in base pairs, at which the error rate is ≤1% for all aligned reads of CF-1. • Percent Reads: The number of all usable library reads that aligned with the CF-1 sequence divided by the total number of addressable wells.
Control QC: DNA Control	• COSMIC ID variant calls and allelic frequencies: The individual variant positions and wild-type positions that are assessed in the DNA control reagent for presence or absence of the variant. • Mean AQ20 Read Length (bp): The average length, in base pairs, at which the error rate is ≤1% for all aligned reads of a control. • Percent Reads: The number of all usable library reads that aligned with the control fragment sequence divided by the total number of addressable wells.
Control QC: RNA Control	• Fusion calls and number of reads: The individual fusion calls and number of reads that are assessed in the RNA control reagent for the presence or absence of the fusion. • Total Mappable Reads: the number of reads that are mapped to the fusion reference file.
Control QC: DNA NTC	• Hotspot Calls: The total number of hotspots where a call was made in the no-template control.
Control QC: RNA NTC	• Total Fusion Calls: The total number of fusion locations where a call was made in the no-template control. • Total Mappable Reads: The number of reads in the no-template control that are mapped to the fusion reference file.

(continued)

Metric	Description
Histogram of Read Length [3]	• A library-specific read-length histogram: RNA  DNA 

[1] May not equal the sum of the individual fusions, since some detected fusions are not included in the fusion BED file.

[2] The minimum Key Signal value for the CF-1 control (i.e., the Templating Control) is set to 0 in all preinstalled assays and new custom assays in Torrent Suite™ Dx Software version 5.8 and later. Custom assays created in previous versions of the software may have a higher Key Signal value for the control. If a run using an older custom assay fails QC due to this metric, reanalyze the run using a minimum Key Signal of 0 for the control. For future runs, create a new custom assay based on the old assay, changing the minimum Key Signal to 0.

[3] Not displayed in the Monitor tab.

Summary

The **Summary** link displays the following information for the run:

Field	Description
Run and Configuration Summary	
Run Name	The name of the run.
Assay Name	The name of the assay.
Reference Genome	The reference genome used for analysis.
Target Region	The name of the targeted regions BED file used.
Hotspot Regions	The name of the hotspot regions BED file used.
Fusion Reference	The name of the fusion reference used for analysis, when applicable.
Fusion Panel	The name of the fusion panel used, when applicable.
Library Name	The names of the DNA and DNA libraries prepared from the sample, entered during library batch preparation.
Amplicon Summary	
Number of Targets	The number of amplicons in the panel.
On Target Reads	The number of reads mapped to the target amplicons.
Percent On Target Reads	The percentage of reads mapped on target to total reads.
Percent Full Length On Target Reads	The percentage of full length reads mapped on target to total reads.
Average Coverage	The ratio of the depth of coverage at each base in the target region to the length of the target region.
Target Coverage at 20X	The percentage of base positions with depth of coverage ≥20X in the target region.
No Strand Bias	The percentage of bases with a strand bias between 30% and 70%.
Coverage Uniformity	The ratio of passed number of targets to total number of targets, where passed number of targets is the number of amplicons that have at least 0.2X mean coverage.
Variant Summary[1]	
Number of SNVs/MNVs	The number of single- and multi-nucleotide variations (SNVs/MNVs).
Number of INDELs	The number of insertions or deletions.
Number of Fusions	The total number of fusion calls.

[1] Includes Therapeutic, Analytical, and Level2 variants in the **View Result** screen.

Therapeutic variant results

Therapeutic variant results for the selected sample are provided under the **Therapeutic** section of the left navigation bar in the **Results** screen. Depending on the assay, results for these variants may be divided into multiple subscreens, each with a separate link (**SNV/INDEL**, **Fusion**, etc.).

The results are determined by the reference sequences installed on the server, the QC controls used in the run, the assay used for the run, and any Reporting Gene List associated with that assay.

Columns in the screen or subscreens are described in the following table.

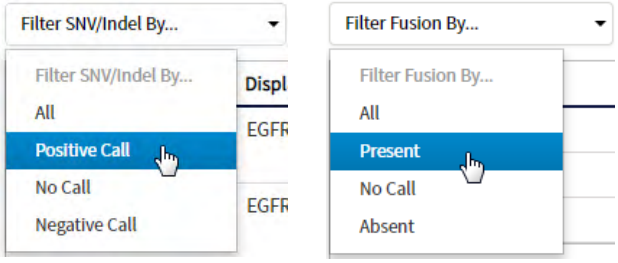
Column	Description
Summary	
Gene Fusion Present Variants Present	The therapeutic gene fusion or SNV/INDEL variant detected in the sample
Associated Therapy	The therapy indicated for each variant listed
SNV / INDEL	
Gene	The gene name, which provides a link to the **View Annotation Sources** popup with additional information about the HotSpot ID (see "View annotation sources" on page 66)
Display Name	The gene name with the exonic location of the deletion or insertion
Amino Acid Change	The amino acid change using HGVS-nomenclature
Nucleotide Change	Identification of the DNA-level nucleotide change using HGVS-nomenclature
Test Result	The presence or absence of the variant
Allele Frequency	The most likely frequency of the variant allele[1]
HotSpot ID	The name of the hotspot as defined in the BED file
Locus	The one-base position of the variant in the reference genome
Type	The type of variation detected: • snv (single nucleotide variation)/mnv (multi-nucleotide variation) • ins (insertion) • del (deletion) • complex
Genotype	The genotype at the locus
Ref	The reference base or bases at the locus

(continued)

Column	Description
Quality Score	The relative probability of either the "reference" hypothesis interval [0,cutoff) or the "variant" hypothesis interval [cutoff,1], on a Phred scale (-10*log10). This provides a measure of the strength of the evidence for the variant call. A higher score means more evidence for the call. Quality scores are capped at 100.
Coverage	The number of reads covering the variant position after down-sampling
Fusion	
Gene	The gene that regulates expression of the gene fusion
Display Name	The gene name of the fusion
Read Count	The number of valid reads aligned to the specific fusion sequence
Test Result	The presence or absence of that fusion variant

[1] The allele frequency is the most likely variant frequency in the reads after corrections are made for probable errors. The software uses this corrected frequency and the uncertainty in the observations to calculate the probability that the variant frequency in the sample falls within defined intervals that can be set by the user. The user sets a threshold frequency for deciding between genotypes: this defines intervals [0,c)[c,1-c)[1-c,1] corresponding to the three diploid genotypes (reference, heterozygous, homozygous) respectively, where “c” is the minimum variant frequency set during the creation of the assay. The software reports the genotype corresponding to the interval with the highest probability of containing the variant frequency in the sample. In most cases, the reads are highly accurate and do not have a high probability of error. In such cases, the observed frequency and the most likely frequency are similar, and are contained within a single interval leading to a high quality genotype. In some cases, if very few reads are observed or the error rate is high, the observed counts and the most likely counts can be different, and the uncertainty in the real sample frequency can be high. When this happens, it is often impossible to exclude a heterozygous population in the sample, and the genotype is assigned to be heterozygous, since a significant portion of the probability falls in that interval.

Note: You can filter the variant lists using options in the **Filter SNV/Indel By...** and **Filter Fusion By...** dropdown lists in the upper left corner of the results report.



Analytical variant results

Analytical variant results for the selected sample are provided under the **Analytical** section of the left navigation bar in the **Results** screen. Depending on the assay, results for these variants may be divided into multiple subscreens, each with a separate link (**SNV/INDEL**, **Fusion**, etc.).

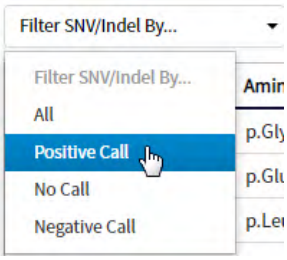
The results are determined by the reference sequences installed on the server, the QC controls used in the run, the assay used for the run, and any Reporting Gene List associated with that assay.

Columns in the screen or subscreens are described in the following table.

Column	Description
Summary	
Variants Present	Lists the analytical SNV or INDEL variants detected in the sample
SNV / INDEL	
Gene	The gene name, which provides a link to the **View Annotation Sources** popup with additional information about the HotSpot ID (see "View annotation sources" on page 66)
Amino Acid Change	The amino acid change using HGVS-nomenclature
Nucleotide Change	The DNA-level nucleotide change using HGVS-nomenclature
Test Result	The presence or absence of the variant
Allele Frequency	The most likely frequency of the variant allele
HotSpot ID	The name of the hotspot as defined in the BED file
Locus	The one-base position of the variant in the reference genome
Type	The type of variation detected: • snv (single nucleotide variation)/mnv (multi-nucleotide variation) • ins (insertion) • del (deletion) • complex
Genotype	The genotype at that locus
Ref	The reference base or bases at the locus
Quality Score	The relative probability of either the "reference" hypothesis interval [0,cutoff) or the "variant" hypothesis interval [cutoff,1], on a Phred scale (-10*log10). This provides a measure of the strength of the evidence for the variant call. A higher score means more evidence for the call. Quality scores are capped at 100.
Coverage	The number of reads covering the variant position after down-sampling
Fusion[1]	
Gene	The gene that regulates expression of the gene fusion
Display Name	The gene name of the fusion
Read Count	The number of valid reads aligned to the specific fusion sequence
Test Result	The presence or absence of that fusion variant

[1] Note that for the Oncomine™ Dx Target Test, no analytical fusions are reported except for expression controls.

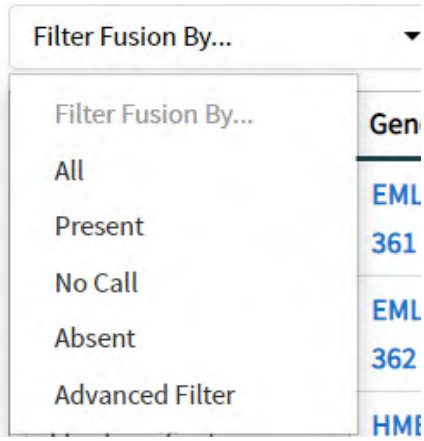
Note: You can filter the variant list using options in the **Filter SNV/Indel By...** dropdown list in the upper left corner of the results report.



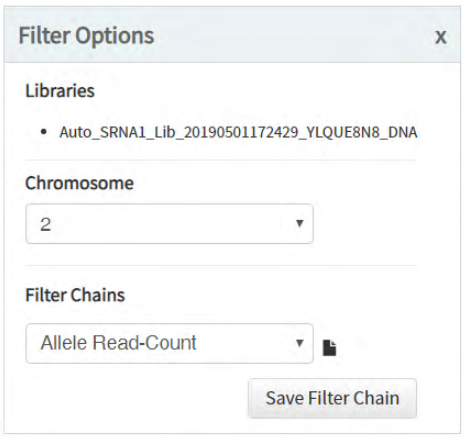
Filter variant results

You can filter the variant results in some subscreens using the **Filter <variant type> By...** dropdown list above the results. The available standard filters depend on the variant type (SNV/INDEL, Fusion, CNV).

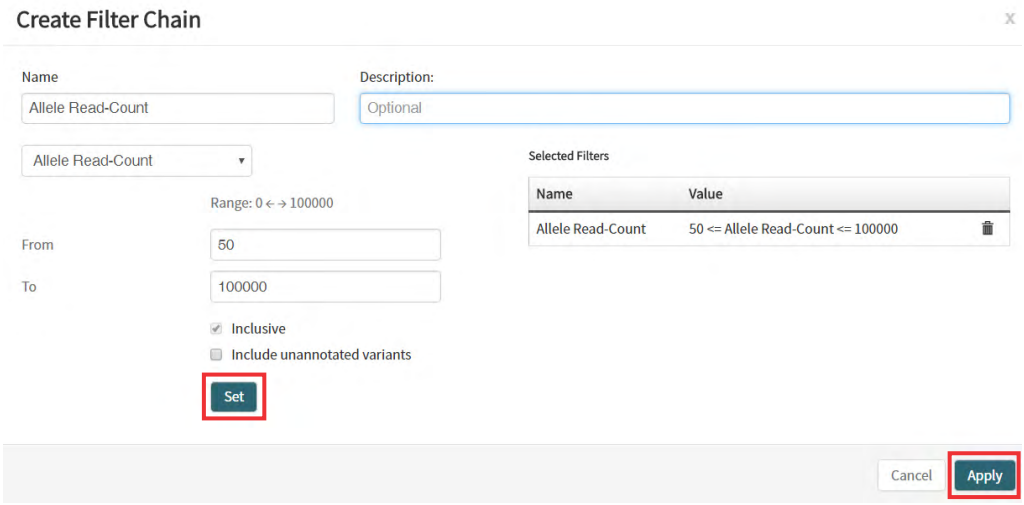
- To filter by a standard preset filter, select the preset filter from the dropdown list.



- To filter by an existing custom filter (Filter Chain), select **Advanced Filter**, then select from the **Filter Chains** list.



- To create a new custom filter (Filter Chain), perform the following steps:
  a. In the **Advanced Filter** dialog box, click **(New)** next to the **Filter Chains** field.
  b. Enter a name and optional description in the **Create Filter Chain** dialog box.
  c. Select a filter from the **Choose Filter** list.
  d. Select the parameters for the filter.
  e. Click **Set** to add the filter to the **Selected Filters** list.

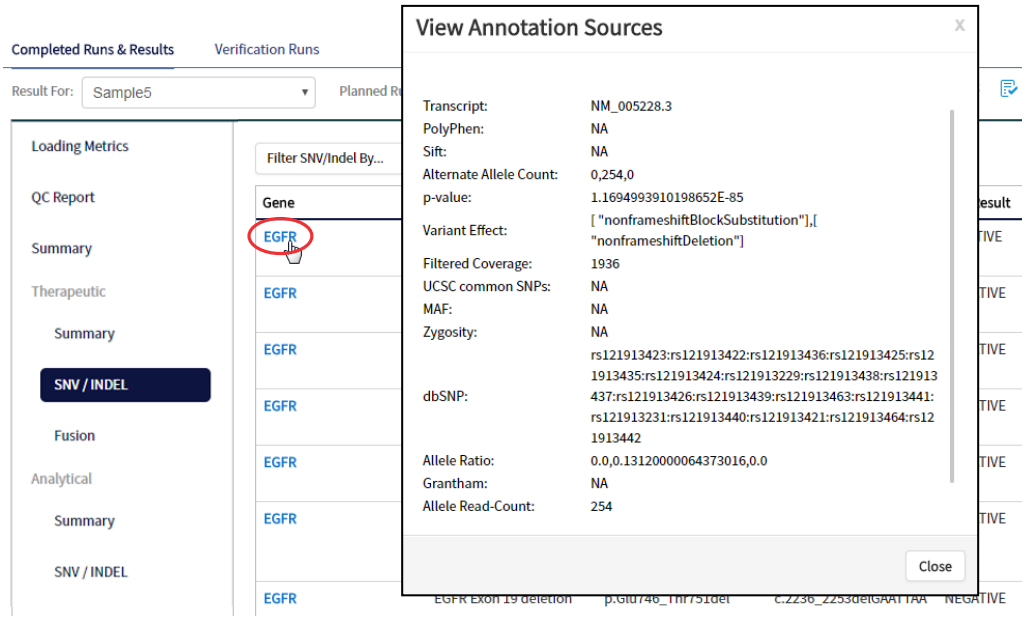


Note: The list of variant results changes to reflect the selected filter.

  f. Select and add additional filters to the Filter Chain as desired, then click **Apply**.
  g. Click **Save Filter Chain**.

View annotation sources

You can view additional information for each hotspot ID listed under the **SNV/Indel** links of the **Therapeutic** and **Analytical** sections in the **View Results** screen. The gene name in the **SNV/Indel** report is a link that opens the **View Annotation Sources** dialog box, which provides information for the particular hotspot.



Example of transcript information in the VCF

The COSM1074639 hotspot ID has multiple transcripts associated with it, which include a mutation from 'A' to 'C'. These are listed in the VCF file as shown below:

chr6 152419923 COSM1074639;COSM1074637 A C,G 122.19 PASS AF=0,0;AO=0,0;DP=1116;FAO=0,0;FDP=1116;FR=.;FRO=1116;FSAF=0,0;FSAR=0,0;FSRF=661;FSRR=455;FWDB=0.00426152,-0.0087378;FXX=0;HRUN=1,1;HS;LEN=1,1;MLLD=103.308,103.438;QD=0.437949;RBI=0.0394298,0.0304316;REFB=-2.22051E-5,-2.32713E-5;REVB=-0.0391988,-0.0291502;RO=1115;SAF=0,0;SAR=0,0;SRF=661;SRR=454;SSEN=0,0;SSEP=0,0;SSSB=-6.44874E-8,-6.44874E-8;STB=0.5,0.5;STBP=1,1;TYPE=snp,snp;VARB=0,0;OID=COSM1074639,COSM1074637;OPOS=152419923,152419923;OREF=A,A;OALT=C,G;OMAPALT=C,G;FUNC=[

{'normalizedRef':'A','transcript':'NM_001122742.1','grantham':'144.0','gene':'ESR1','location':'exonic','origAlt':'C','origPos':'152419923','origRef':'A','normalizedPos':'152419923','exon':'10','function':'missense','protein':'p.Tyr537Ser','normalizedAlt':'C','gt':'neg','codon':'TCT','coding':'c.1610A>C'},

{'normalizedRef':'A','transcript':'NM_001122742.1','grantham':'194.0','gene':'ESR1','location':'exonic','origAlt':'G','origPos':'152419923','origRef':'A','normalizedPos':'152419923','exon':'10','function':'missense','protein':'p.Tyr537Cys','normalizedAlt':'G','gt':'neg','codon':'TGT','coding':'c.1610A>G'},

{'normalizedRef':'A','transcript':'NM_001122741.1','grantham':'144.0','gene':'ESR1','location':'exonic','origAlt':'C','origPos':'152419923','origRef':'A','normalizedPos':'152419923','exon':'9','function':'missense','protein':'p.Tyr537Ser','normalizedAlt':'C','gt':'neg','codon':'TCT','coding':'c.1610A>C'},

{'normalizedRef':'A','transcript':'NM_001122741.1','grantham':'194.0','gene':'ESR1','location':'exonic','origAlt':'G','origPos':'152419923','origRef':'A','normalizedPos':'152419923','exon':'9','function':'missense','protein':'p.Tyr537Cys','normalizedAlt':'G','gt':'neg','codon':'TGT','coding':'c.1610A>G'},

{'normalizedRef':'A','transcript':'NM_001122740.1','grantham':'144.0','gene':'ESR1','location':'exonic','origAlt':'C','origPos':'152419923','origRef':'A','sift':'0.0','normalizedPos':'152419923','exon':'9','function':'missense','protein':'p.Tyr537Ser','gt':'neg','normalizedAlt':'C','codon':'TCT','polyphen':'0.979','coding':'c.1610A>C'},

{'normalizedRef':'A','transcript':'NM_001122740.1','grantham':'194.0','gene':'ESR1','location':'exonic','origAlt':'G','origPos':'152419923','origRef':'A','sift':'0.0','normalizedPos':'152419923','exon':'9','function':'missense','protein':'p.Tyr537Cys','gt':'neg','normalizedAlt':'G','codon':'TGT','polyphen':'0.998','coding':'c.1610A>G'},

Results files

The following files can be downloaded from the **Results Report** window. To download the files, click **Download Files**, select the files to download, then click **Download**.

File name	Description
Test Report	A report of the completed analysis in PDF format
Lab Report	A clinical lab report of the completed analysis in PDF format; includes both clinical and analytical results.
PlannedRun-AuditTrail.pdf	Contains all audit records pertaining to the Planned Run.
Info.csv	Contains information about the run and analysis, such as software, sequencing information, instrument information, analysis information, QC details etc.
<RNABarcode>_rawlib.basecaller.bam	Unmapped RNA BAM File; output of base calling, contains unmapped reads.
Snvindel.tab	A tab-delimited file that contains information about non-targeted SNVs and indels
<RNABarcode>_rawlib.basecaller_alignments.bam	Mapped RNABarcode BAM file; output after reads have been mapped to the fusion reference.
Target_Summary.tab	A tab-delimited file that contains a targeted test results summary
<RNABarcode>_rawlib.basecaller_alignments.bam.bai	Mapped RNABarcode BAM index file
<RNABarcode>_rawlib.basecaller.fastq	FASTQ file generated from unmapped BAM file of the RNA barcode used.
<DNABarcode>_rawlib.basecaller.bam	Unmapped DNA barcode BAM file; output of base calling, contains unmapped reads.
raw_peak_signal	Key signal gives the percentage of LiveISPs with a key signal that is identical to the library key signal.
<LibPrepID>_<analysisID>.final.vcf	A VCF file containing all the variants detected as a result of the analysis, along with information such as test result, read count, gene name, quality scores, etc.
Summary.tab	A tab-delimited file that contains the on-targeted test results summary
<LibPrepID>_rawlib.stats.cov.txt	Amplicon statistics file

(continued)

File name	Description
Fusion.tab	A tab-delimited file that contains non-targeted (analytical) fusion details in a table format. **Note:** The information displayed in the file for each isoform of a particular fusion is identical, because specific isoform and locus information is not included in this table. Detailed isoform and locus information is available in the <LibraryPrepID>_<AnalysisID>.final.vcf file, available under **Download Files**.
readLenHisto.png	Gives the read-length distribution of FASTQ files in the form of a histogram. A thumbnail histogram of the read lengths for a particular barcode.
<LibPrepID>_rawlib.bam.bai	Mapped DNA barcode BAM index file (index file of DNA barcode-mapped BAM file)
Basecaller.log	Base Caller log file
analysis.log	Analysis log file
sigproc.log	Signal processing log file
Bead_density_contour.png	Loading density image; a pseudo-color density image of the Ion Chip plate showing percent loading across the physical surface
<DNABarcode>_rawlib.basecaller.fastq	FASTQ file of the DNA barcode used
Target_cnv.tab	Targeted CNV detail table (CNV results for targeted variants from a sequencing run) **Note:** For use with IVD tests that include CNV reporting.
cnv.tab	Non-targeted CNV detail table (analytical CNV results from a sequencing run) **Note:** For use with IVD tests that include CNV reporting.
Target_fusion.tab	A tab-delimited file that contains targeted (clinical) fusion details in a table format
<LibPrepID>_rawlib.bam	Mapped DNA barcode BAM File; output after mapping reads to reference.
Iontrace_Library.png	Key incorporation trace image showing the average signal readings for flows of the bases T, C, and A in the library key.
rawtf.basecaller.fastq	FASTQ file for the test fragment

Sign the run results

In the **View Result** screen, Managers/Administrators can provide their electronic signature on the run results. The signature information appears in the **QC Report** in the **View Result** screen, and in the downloaded Test Report and Lab Report PDFs.

Multi-language support for PDF report generation is provided. By default reports are generated in the language that is selected in the **Report Template** used. When reports are generated in multiple languages, **Sign Off** occurs only in the report of the default language.

1. At the top of the **View Result** screen, click **Sign Off**, then enter your user name, password, and comments in the dialog box. Fields identified with a red asterisk (*) are required fields.
2. In the **Footer Field**, enter any text.
3. Click **Sign Off** to confirm your electronic signature.

Files in the Reports folder

When a manager- or administrator-level user signs a report, a folder named with the Sample ID is created in the Reports folder on the server (`/results/analysis/output/reports`), and the following files are copied into it:

- Info file (.csv)
- Signal processing log file (.log)
- Targeted Fusion Detail Table (.tab)
- Targeted Test Results Summary (.tab)
- Analysis log file (.log)
- VCF file (.vcf)
- DNA Mapped BAM file (.bam)
- DNA Unmapped BAM file (.bam)
- Key Signal
- Key Incorporation Trace (.png)
- Fusion Detail Table (.tab)
- DNA Mapped BAM Index file (.bam.bai)
- RNA Mapped BAM Index file (.bam.bai)
- DNA FASTQ File (.fastq)
- Base Caller Log File (.log)
- Targeted CNV Detail Table (.tab)[1]
- Non-targeted CNV Detail Table (.tab)[1]
- SNV/INDEL Detail Table (.tab)
- Non-targeted Test Results Summary (.tab)
- Targeted SNV/INDEL Detail Table (.tab)
- Amplicon Stats (_rawlib.stats.cov.txt)
- RNA FASTQ File (.fastq)
- RNA Mapped BAM file (.bam)
- RNA Unmapped BAM file (.bam)
- Test Fragment FASTQ File (.fastq)
- Read Length Histogram (.png)
- Test PDF Report *(optional)* (.pdf)
- Lab PDF Report (.pdf)
- Planned Run Audit (.pdf)
- Basecaller command files (.json)
- checksum file
- Pipeline commands (_pipeline.json)
- Experimental log file (_final.txt)
- Wells with beads (_beadogram.png)
- Bead find stats file (.stats)
- Loading Density Figure (.png)

[1] For use with IVD tests that include CNV reporting.

7 Pass/fail criteria and repeat strategy

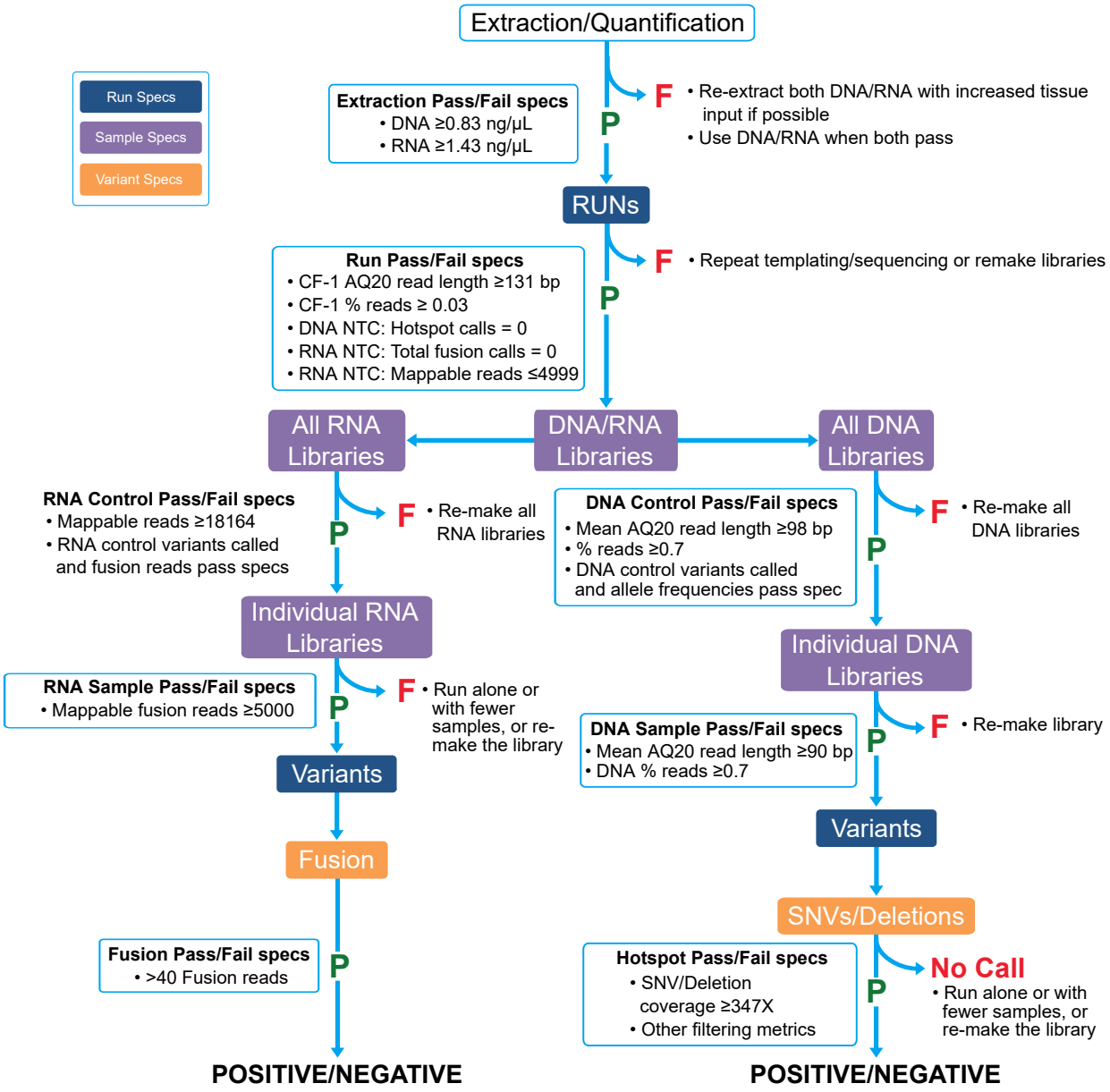
Quality control pass/fail criteria

Metric	Criteria
Run QC	
CF-1 Mean AQ20 Read Length (bp)	≥131
CF-1 Percent Reads (%)	≥0.03
DNA NTC	Hotspot calls = 0
RNA NTC	Total fusion calls = 0
RNA NTC Mappable Reads	≤4999
DNA Library	
Mean AQ20 Read Length (bp)	≥90
Percent Reads (%)	≥0.7
RNA Library	
Mappable Fusion Reads	≥5000
DNA Control	
COSM476_AF	Variant called and AF ≥0.05
COSM521_AF	Variant called and AF ≥0.05
COSM6223_AF	Variant called and AF ≥0.05
COSM6224_AF	Variant called and AF ≥0.05
COSM760_AF	Variant called and AF ≥0.05
Mean AQ20 Read Length (bp)	≥98
Percent Reads (%)	≥0.7
RNA Control	
Mappable Reads	≥18164
ROS1 Fusion Reads	Variant called and fusion reads ≥349

Pass/fail specifications and repeat strategy

In general, if you experience a run or sample failure, you can repeat the run or sample preparation at the workflow step in which the failure occurred. The number of libraries that can be made from an extracted sample for repeat testing depends on the quantity of DNA and RNA from the extraction, which varies from sample to sample.

Based on quality control results, you can determine whether a library requires repeat testing. Refer to the following flowchart to determine the best course of action.



Specification type	Passing criteria	Repeat strategy
Extraction/ Quantification	The following minimum concentrations for DNA and RNA are required: • DNA ≥0.83 ng/µL. • RNA ≥1.43 ng/µL.	If the minimum concentration requirement is not met for either DNA or RNA, the samples must be re-extracted with increased tissue input. Use the set of extracted samples where both the DNA and RNA meet the minimum concentration requirement for the test.
Run	A run must pass the following specifications to have reportable results for any sample within the run: • CF-1 Mean AQ20 Read Length (bp) must be ≥131. • CF-1 Percent Reads must be ≥0.03.	If either CF-1 specification fails, the operator may repeat the templating/sequencing run with the same library pool, or re-pool the libraries if a pooling error is suspected. If the issue persists on the repeat run, remake the libraries.
No Template Control (NTC)	A run must pass the following NTC specifications to have reportable results for any samples within the run: • DNA No Template Control (DNA NTC)—Total "Hotspot Calls" must equal zero (0). • RNA No Template Control (RNA NTC)—Mappable Reads must be ≤4999 and "Total Fusion Calls" must be zero (0).	If only the DNA NTC fails, remake all of the DNA controls and DNA sample libraries, and re-pool with previously made RNA controls and RNA sample libraries. If only the RNA NTC fails, remake all of the RNA controls and RNA sample libraries, and re-pool with previously made DNA controls and DNA sample libraries. If both the DNA NTC and RNA NTC fail, remake all of the DNA and RNA controls and sample libraries.
DNA Control	The DNA control must pass the following specifications in order for any DNA samples within the run to have any reportable results: • AQ20 Mean Read Length (bp) must be ≥98. • Percent Reads must be ≥0.7%. • All variants within the DNA control sample must be called "Present" and pass the allelic frequency range for each variant as specified in the assay definition file.	If any of these specifications fail, the operator must remake all DNA control and DNA sample libraries.

(continued)

Specification type	Passing criteria	Repeat strategy
RNA Control	The RNA control must pass the following specifications for any RNA samples within the run to have any reportable results: • Mappable Reads must meet the minimum threshold required of ≥18164 reads. • All variants within the RNA control sample must be called "Present" and pass the threshold metric set for total fusion reads required for each variant as specified in the assay definition file.	If either of these specifications fails, the operator must remake all the RNA control and RNA sample libraries.
Library DNA Sample	Any individual DNA library sample must meet the following specifications to have reportable results for the DNA library: • Mean AQ20 Read Length (bp) ≥90. • Percent Reads ≥0.7.	Run the DNA library sample alone, or with fewer DNA library samples. If the DNA library sample still fails these specifications, remake the library using the same (previously extracted) DNA, if there is sufficient quantity. If not, re-extract using more tissue input, if possible. The repeat libraries must be prepared and run with new DNA controls. **Note:** Use the original passing RNA control and DNA and RNA sample libraries as placeholders when needed, and re-pool the libraries accordingly in the repeat runs. Any data resulting from the placeholder libraries must be ignored in the repeat run.

(continued)

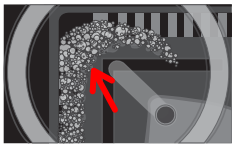
Specification type	Passing criteria	Repeat strategy
Library RNA Sample	Any individual RNA library sample must have ≥5000 Mappable Fusion Reads to have reportable results for the RNA sample library.	Run the RNA library sample alone, or with fewer RNA library samples. If the RNA library sample still fails this specification, re-make the library using the same (previously extracted) RNA, if there is sufficient quantity. If not, re-extract using more tissue input, if possible. The repeat libraries must be prepared and run with new RNA controls. **Note:** Use the original passing DNA control and DNA and RNA sample libraries as placeholders when needed, and repool the libraries accordingly in the repeat runs. Any data resulting from the placeholder libraries must be ignored in the repeat run.
SNV and Deletion Variant Specifications	All Single Nucleotide Variants (SNVs) and Deletions (Dels) must have coverage ≥347 reads and pass all Variant Caller filtering metrics in order to have a reportable result for the variant.	Any SNVs and deletions that do not meet the coverage criteria will result in a "No Call" for the variant. The operator may run the sample alone or with fewer samples to obtain reportable results for the variant. If the repeat run fails to meet the minimum coverage requirement, the operator may remake the library to obtain reportable results for the variant.

A

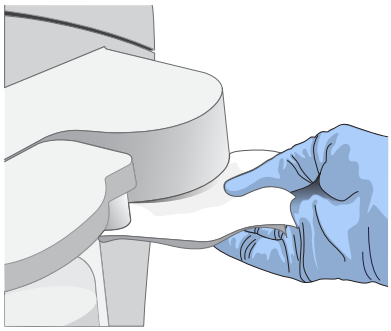
Troubleshooting

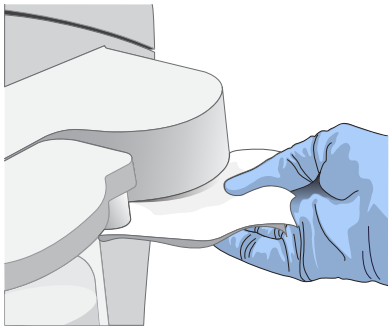
Ion PGM™ Dx Sequencer Initialization

Observation	Possible cause	Recommended action
Pressure alarm	Gas cylinder may be turned off or empty	• Verify that the cylinder has at least 500 PSI and 30 PSI at the outlet of the regulator. Confirm that all valves between the cylinder and the sequencer are open. • Once you confirm gas pressure leading into the instrument, press **Yes** to retry verification of gas pressure. If the test continues to fail, contact Technical Support (see Appendix F, "Customer and technical support").
Error message: Leak check failed, make sure the reagent tubes are not attached to the sequencer and check Wash 1, 2, or 3 for leak	• Caps are not tightened on the Wash 1, 2, or 3 bottles. • Bottles may be damaged or defective.	1. Inspect all the bottles for damage or visible leaks. If a bottle appears damaged, replace it. 2. Finger-tighten all the bottle caps, make sure that the reagent tubes are not attached to the instrument, and then press **Retry**. 3. If leak check continues to fail, contact Technical Support (see Appendix F, "Customer and technical support").
Error message: UNDERSHOT TARGET (instrument touchscreen may also be frozen)	Water used did not meet specifications, or insufficient amount of NaOH added	If the touchscreen is frozen, reboot the sequencer and restart initialization. If you receive the same error and the touchscreen continues to freeze, contact Technical Support (see Appendix F, "Customer and technical support"). Otherwise, proceed to the following steps. 1. Press **Retry** to reattempt pH adjustment. 2. If you receive the same error message after multiple attempts, press **Abort**. 3. Check your water purification system and troubleshoot per the manufacturer's directions, or identify a new source of 18-MΩ water. 4. Restart the initialization procedure and prepare new solutions, including a new aliquot of 100 mM NaOH. 5. If the problem persists, contact Technical Support (see Appendix F, "Customer and technical support").

Observation	Possible cause	Recommended action
Error message: UNDERSHOT TARGET (instrument touchscreen may also be frozen) *(continued)*	Auto-pH did not add enough Wash 1 Solution to the Wash 2 Solution before the maximum iterations occurred.	If the touchscreen is frozen, reboot the sequencer and restart initialization. If you receive the same error and the touchscreen continues to freeze, contact Technical Support (see Appendix F, "Customer and technical support"). Otherwise, proceed to the following steps. 1. A blockage may have occurred. See "Error message: There may be a blockage or no NaOH in W1. Check W1 and run line clear then try again" on page 80. 2. Press **Retry** to restart the pH check. If you still get the "Undershot target pH" error, replace the chip with a new (unused) chip and restart the pH check. **Note:** The new chip can be used for sequencing after initialization completes.
	Loose W1 sipper	Tighten the sipper and retry.
Error message: Please insert a chip and press Start	Instrument cannot detect the chip in chip socket.	1. Open the chip clamp and remove the chip. 2. Check for debris under the chip and in the chip socket. **IMPORTANT!** Never rub or wipe the socket. Rubbing the socket can damage it and cause it to fail. 3. Look for liquid outside the flow cell of the chip:  4. If you see liquid, replace the chip with a new (unused) one. **Note:** The new chip can be used for sequencing after initialization completes. 5. Close the clamp, then press **Start** to restart the process. 6. If the new chip also fails, there could be a problem with the chip socket. Contact Technical Support (see Appendix F, "Customer and technical support").

Observation	Possible cause	Recommended action
Error message: OVERSHOT TARGET	• Wrong amount or concentration of NaOH was added to the Wash 1 Bottle • Auto-pH added more NaOH from the Wash 1 Bottle to the Wash 2 Bottle than was needed	Do not press **Next**. Prepare fresh reagents, then press **Retry** to retry the initialization. If the problem persists, contact Technical Support (see Appendix F, "Customer and technical support").
	Clog in waste line(s)	Do not press **Next**. See "Error message: There may be a blockage or no NaOH in W1. Check W1 and run line clear then try again" on page 80.
	Clog in chip	Do not press **Next**. See "Error message: There may be a blockage or no NaOH in W1. Check W1 and run line clear then try again" on page 80. If the waste lines are not clogged, replace the chip, then click **Retry**.
W2 pH consistently undershoots target	pH of water is too low before any NaOH is added	• Confirm that the 18 MΩ water supply meets specifications. • If necessary, when preparing the Wash 2 Bottle, add more than the recommended 70 µL of 100 mM NaOH. After adding the NaOH, the Wash 2 Bottle must be in the range of pH 6.0–6.5 at first pH iteration before you begin initialization.
Error Message: Leak detected – Check dNTP Conical tubes	• dNTP Reagent Tube seal is not tight. • dNTP Reagent Tube may be damaged or defective.	1. Remove and reinstall each dNTP Reagent Tube. 2. Ensure that each tube is securely tightened (finger-tighten), the press **OK** to re-check pressure. 3. If the error message persists, set up dNTPs in new tubes, secure new tubes on the instrument, then press **OK**. 4. If leak check continues to fail, contact Technical Support (see Appendix F, "Customer and technical support").

Observation	Possible cause	Recommended action
Error message: There may be a blockage or no NaOH in W1. Check W1 and run line clear then try again	The waste lines may be blocked.	1. Remove the waste bottle and place lab wipes under the waste arm. 2. Gently wipe the waste arm with a lab wipe to clear liquid from around the waste line.  3. Press **Flow check** one or more times to observe the flow rates from both lines. One line should drip slightly faster than the other. If one or both lines are blocked (no flow), or the drip rates are significantly different, go to the next step. If the flow rates are normal, see "Error message: There may be a blockage or no NaOH in W1 (continued from previous page)" on page 81 below. 4. Press **Line Clear**. Follow the prompts and use the syringe supplied with the Ion PGM™ Dx System. 5. After Line Clear, press **Flow check**, then check for normal flow rates from the waste lines. 6. If the flow rates are still not normal, perform **Line Clear** one more time. 7. If the line(s) remain blocked, contact Technical Support (see Appendix F, "Customer and technical support"). Otherwise, press **Start** to restart auto-pH.
	Wash 1 or Wash 2 sipper may be loose	1. Loosen the Wash 1 cap and retighten the sipper. Since the gas flows when the cap is loose, tighten the sipper as quickly as possible. (The gas is not harmful to the NaOH solution and is not a hazard.) 2. Loosen the Wash 2 cap and retighten the sipper. Since the gas flows when the cap is loose, tighten the sipper as quickly as possible. (The gas is not harmful to the W2 Solution and is not a hazard.) 3. Press **Start** to restart the auto-pH process.

Observation	Possible cause	Recommended action
Error message: There may be a blockage or no NaOH in W1 (continued from previous page)	Forgot to add NaOH to the Wash 1 Bottle: Chip does not detect a large enough pH difference between the NaOH (W1) and W2 Solutions.	1. If you forgot to add NaOH to the Wash 1 Bottle, loosen the cap and add 350 µL of 100 mM NaOH to the Wash 1 Bottle. (The flowing gas is not harmful to the NaOH solution and is not a hazard.) 2. Recap the bottle and shake gently to mix. 3. Press **Start** to restart auto-pH.
	Damaged chip	1. Replace the chip with a new (unused) one. Insert the chip in the socket, then press **Start**. **Note:** The new chip can be used for sequencing after initialization completes. 2. If the error persists, there could be a problem with the chip clamp. Contact Technical Support (see Appendix F, "Customer and technical support").
Error message: W2 average not stable. Try reseating/replacing chip	Reading for W2 solution is not stabilizing quickly enough	1. Remove the waste bottle and gently wipe excess fluid from the waste lines with a lab wipe.  2. Check for leaks and reseat the chip. Replace the chip with a new (unused) one if needed. **Note:** The new chip can be used for sequencing after initialization completes. 3. Loosen the Wash 2 cap and retighten the sipper. Since the gas flows when the cap is loose, tighten the sipper as quickly as possible. (The gas is not harmful to the W2 Solution and is not a hazard.) 4. After performing one or more above steps, press **Start** to retry for auto-pH. If auto-pH fails even after replacing the chip, contact Technical Support (see Appendix F, "Customer and technical support").
	The waste line may be blocked.	See "Error message: There may be a blockage or no NaOH in W1. Check W1 and run line clear then try again" on page 80.

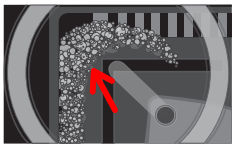
Observation	Possible cause	Recommended action
Error message: W2 out of range	• Chip measurements very unstable • Chip is damaged	See troubleshooting tips for "W2 average not stable" above.
Error message: Chip reading inconsistent. Please replace chip and try again.	• pH response of the chip is not uniform or reliable • Ran out of SEQ W3 Solution or volume too low	1. Verify that there is enough SEQ W3 Solution (>25 mL) in the Wash 3 Bottle and that the sipper is secure. 2. If necessary, loosen the Wash 3 Bottle cap, tighten the sipper, and add more SEQ W3 Solution to fill to 50 mL. Since the gas flows when the cap is loose, perform these operations as quickly as possible. (The gas is not harmful to the SEQ W3 Solution and is not a hazard.) 3. If there is enough SEQ W3 Solution, replace the chip with a new (unused) one. Insert the chip in the socket, then press "re-try". **Note:** The new chip can be used for sequencing after initialization completes.
Error message: Added too much W1 to W2	• Poor water quality • 18-MΩ water exposed to air for too long • Incorrect solution added to the SEQ W2 Solution • Too little NaOH added to SEQ Wash 1 Bottle • Damaged chip	1. Check whether the water meets the 18-MΩ specification and that the 100 mM NaOH and SEQ W2 Solutions were added correctly. 2. If solutions are incorrect or water does not meet specifications, correctly prepare the solution(s) and/or use 18-MΩ water. Abort the initialization and restart using correct solutions/water. 3. If the solutions and water are correct, abort the initialization and try reinitializing with a different chip.
WARNING: AutopH is within expected pH range, 0 milliliters of W1 was added to W2. Press "next" if expected, otherwise press "retry" to restart AutoPH	After auto-pH undershot the target, the user pressed the Retry button to restart auto-pH, but no additional NaOH was added to the Wash 2 bottle.	The pH reading was close enough to the target that no additional NaOH was added. Press Next to proceed with initialization.

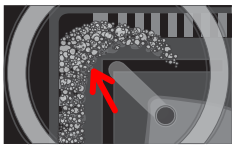
Initialization: Reagent pH verification

Observation	Possible cause	Recommended action
Failure screen	• One or more reagents are not within the target pH range • Chip is damaged	1. Press **Start** to repeat the pH measurement. 2. If the test still fails, replace the chip with a new (unused) chip and press **Start** to repeat. **Note:** The new chip can be used for sequencing after initialization completes. 3. If the test still fails with the new chip, clean and reinitialize the instrument with fresh reagents and a new chip. 4. If the test still fails, contact Technical Support (see Appendix F, "Customer and technical support").

Chip calibration

Observation	Possible cause	Recommended action
Chip calibration failure (before sample has been loaded on the chip)	• Debris on the chip socket • Chip not seated correctly in the chip clamp • Chip is damaged • Problem with chip clamp or socket	1. Open the chip clamp, remove the chip, and check for damage, leaks, and/or debris under the chip and on the chip socket. • If debris or leaks are visible, gently dab the socket with a lab wipe tissue dampened with 18-MΩ water, then reseat the chip in the socket. • If no leaks or debris is visible, reseat the chip in the socket. **IMPORTANT!** Never rub or wipe the socket. Rubbing the socket can damage it and cause it to fail. 2. After reseating the chip, press **Retry**. 3. If the chip passes, press **Next** to start the experiment. If the chip still fails, reseat the chip again, then press **Retry**. 4. If chip calibration continues to fail with the same chip, retry with a new chip. 5. If the new chip fails as well: a. Press **Abort** to abort the run. **IMPORTANT!** Be sure to abort the run before rebooting the sequencer in the subsequent steps. b. Reboot the sequencer by holding down the Power button on the front to shut it down, then press again to restart. c. Restart the run with the new chip. 6. If the new chip continues to fail, there may be an issue with the chip socket. Contact Technical Support (see Appendix F, "Customer and technical support").
Error message: Bad pixels error	Chip not seated correctly in the chip clamp.	1. Make sure the chip is seated correctly and the clamp is closed. 2. Press down on the chip clamp with even pressure until the message instructing to close the lid appears. 3. If the new chip continues to fail, there may be an issue with the chip socket. Contact Technical Support (see Appendix F, "Customer and technical support").

Observation	Possible cause	Recommended action
Leak of unknown origin during chip calibration (before sample has been loaded on the chip)	• Chip clamp not closed properly. • Chip is damaged. • Problem with the chip clamp or socket	1. Press **Abort** to immediately stop the run. **Note:** If the system is pressurized during abort, initiate the clean step or a run to de-pressurize the system and stop the flow of buffer, then abort the clean step. 2. Open the chip clamp, remove the chip, and gently dab the chip socket with a lab wipe tissue to absorb any fluid. **IMPORTANT!** Never rub or wipe the socket. Rubbing the socket can damage it and cause it to fail. 3. Make sure that the rubber gaskets on the chip clamp are properly installed and not loose or out of position. 4. Rinse the socket with 18-MΩ water and gently absorb most of the water with the lab wipe. 5. Repeat the rinse, then gently dab the chip socket with a lab wipe until dry. 6. Place a lab wipe on the grounding plate and dampen it with 18-MΩ water. Wipe the bottom of the chip on this wipe to remove salts from the chip contacts, and then gently dab the bottom of the chip with a dry lab wipe. 7. Remove the lab wipe, dry the grounding plate, and place the chip on it. Confirm that there is no condensation outside the chip flow cell:  8. If there is condensation or fluid, the chip is damaged and cannot be run. If no chip damage is apparent, reseat the chip and press **Run** to restart the experiment. 9. When prompted to install the chip, make certain that the chip clamp is fully closed. 10. If the chip leaks again, press **Abort**, clean the chip socket, then restart the run with a different chip. If the new chip leaks, it may indicate a problem with the chip clamp or socket. Contact Technical Support (see Appendix F, "Customer and technical support").

Observation	Possible cause	Recommended action
Leak of unknown origin during chip calibration (after sample has been loaded on the chip)	• Chip clamp not closed properly • Chip is damaged. • Problem with the chip clamp or socket	1. Press **Abort** to immediately stop the run. **Note:** If the system is pressurized during abort, initiate the clean step to de-pressurize the system to stop the flow of buffer, then abort the clean step. 2. Open the chip clamp, remove the chip, and gently dab the chip socket with a lab wipe tissue to absorb any fluid. **IMPORTANT!** Never rub or wipe the socket. Rubbing the socket can damage it and cause it to fail. 3. Make sure that the rubber gaskets on the chip clamp are tight and in position. 4. Place a lab wipe on the grounding plate and dampen it with 18-MΩ water. Wipe the bottom of the chip on this wipe, and then gently dab with a dry lab wipe. 5. Dry the grounding plate with a lab wipe and place the chip on it. Confirm that there is no condensation outside the chip flow cell:  6. If there is condensation or fluid, the chip is damaged and cannot be run. Prepare a new run starting with template preparation (skip the following troubleshooting steps). 7. If no chip damage is apparent, you can attempt to rerun the loaded chip. Press **Run** on the Main Menu to start a new run. 8. When prompted, place the used chip from initialization in the chip clamp and press **Next** to clean the fluid lines. 9. When prompted, scan or enter the Planned Run Short Code. 10. When prompted, scan the top and bottom barcodes on the loaded chip. 11. Secure the loaded chip in the chip clamp and proceed with initial chip calibration.

Observation	Possible cause	Recommended action
Leak of unknown origin during chip calibration (after sample has been loaded on the chip) *(continued)*		12. If the chip leaks again, press **Abort**, then clean the chip socket. There may be a problem with the chip clamp or socket. Contact Technical Support (see Appendix F, "Customer and technical support"). 13. If the chip does not leak and initial calibration passes, skip the chip loading steps and continue with the run.
Error message: Calibration failed (after sample has been loaded on the chip)	• Debris on the chip socket • Chip clamp not closed properly • Chip is damaged • Problem with chip clamp or socket	1. Open the chip clamp, remove the chip, and check for damage, leaks, or debris under the chip and on the chip socket. • If debris or leaks are visible, gently dab the socket with a lab wipe tissue dampened with 18-MΩ water, then re-clamp the chip. • If no leaks or debris are visible, re-clamp the chip. **IMPORTANT!** Never rub or wipe the socket. Rubbing the socket can damage it and cause it to fail. 2. After re-clamping the chip, press **Retry**. 3. If the chip passes, press **Next** to start the experiment. If the chip still fails, re-clamp the chip and press **Retry**. **Note:** You have a total of 3 attempts to retry the chip before aborting the run. 4. If calibration continues to fail, there may be an issue with the loaded chip, chip clamp, or chip socket. To test the clamp and socket: a. Abort the run, then restart the run. b. Do not use the loaded chip. Instead, place a *used* chip in the chip clamp, then follow the steps for the initial (unloaded) chip calibration. • If the used chip fails initial calibration, there may be an issue with the clamp or socket. Abort the run and contact Technical Support (see Appendix F, "Customer and technical support"). • If the used chip passes calibration, the previously loaded chip may be bad. Abort the run, prepare fresh template, and run with a new chip.

Observation	Possible cause	Recommended action

Error message: Chip not seated correctly. Please reset the chip and click OK to continue	• Debris on the chip socket
	• Chip not seated correctly in the chip clamp
	• Chip is damaged
	• Problem with chip clamp or socket
		1. Open the chip clamp, remove the chip, and check for damage, leaks, and/or debris under the chip and on the chip socket.
		  • If debris or leaks are visible, gently dab the socket with a lab wipe tissue dampened with 18-MΩ water, then reseat the chip in the socket.
		  • If no leaks or debris are visible, reseat the chip in the socket.
		IMPORTANT! Never rub or wipe the socket. Rubbing the socket can damage it and cause it to fail.
		2. Press **Retry**.
		3. If the chip passes, press **Next** to start the experiment. If the chip still fails, try reseating the chip again and pressing **Retry**.
		Note: You have a total of 3 attempts to reseat the chip before aborting the run.
		4. If you continue to receive the "Chip not seated correctly" error message, contact Technical Support (see Appendix F, "Customer and technical support"). There may be an issue with the chip clamp or socket.

Chip calibration status bar does not progress	During chip calibration, the touchscreen status progress bar does not progress.	1. Press the **Abort** button to abort the run. 2. Reboot the sequencer by holding down the **Power** button on the front to shut it down, then press the button again to restart. 3. Restart the run with the same chip. **Note:** Reinitialization is *not* required after rebooting the sequencer if the initialization successfully completed before the run was aborted.
Barcode on the bottom of the chip does not scan	The barcode is printed incorrectly.	Use a new chip and contact Technical Support for a replacement (see Appendix F, "Customer and technical support").

Sample loading

Observation	Possible cause	Recommended action
Sample volume is <30 µL If the sample volume is less than <30 µL, there will be a visible air gap at the end of the pipette tip when you collect the sample for chip loading.	Volume was lost at some point during sample preparation.	**Note:** Equilibrate SEQ Sample Buffer (brown cap) to room temperature for 15 minutes before performing the following steps. 1. With the sample loaded in the pipette tip, dial down the pipette until the liquid reaches the end of the tip. Record the volume shown on the pipette (X). 2. Dispense the sample back into the sample tube. 3. Subtract the volume shown on the pipette from 30 µL (30 – X = Y µL) to determine the missing volume (Y). 4. Change the pipette tip, and add Y µL of SEQ Sample Buffer (brown cap) to the sample. 5. Pipet up and down 4 times to mix the contents, then continue loading the sample into the chip.
Large amount of the sample volume (≥15 µL) leaks from the outlet port during chip loading.	The chip loading port has a clog or obstruction.	Do not continue with the sequencing run. Contact Technical Support.

Warnings and alarms—Ion PGM™ Dx System

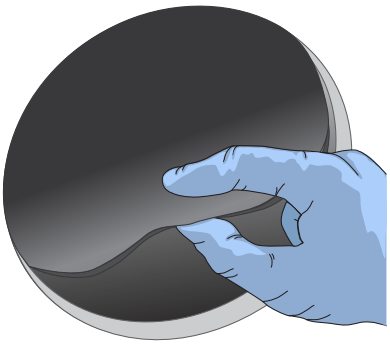
The following warnings and alarms appear on the Ion PGM™ Dx Sequencer touchscreen and in the Torrent Suite™ Dx Software under the **Monitor** tab.

Observation	Possible cause	Recommended action
Display message: Pressure too high	Internal pressure regulator was not set correctly	Contact Technical Support (see Appendix F, "Customer and technical support").
Display message: Pressure too low.	• Gas line is not connected to the instrument • Gas cylinder may be turned off or empty	1. Verify that the gas line is connected to the instrument. 2. Verify that the gas cylinder has at least 500 psi on the pressure gauge inside the tank and 30 psi on the gauge that regulates pressure to the outlet. (Both pressure gauges are mounted on the gas cylinder.) 3. Confirm that the outlet valve on the regulator is turned on. 4. If the problem persists, contact Technical Support (see Appendix F, "Customer and technical support").

Observation	Possible cause	Recommended action
Display message: Instrument idle temperature too high	• Room temperature is too high. • Clogged filter or blocked airway on the instrument • Hardware issue (fan is not running or running too slowly)	**Note:** The data created during a run with this alarm raised may still be used if all the QC metrics are met. See the recommended action for "Display message: Instrument temperature too high" on page 93.
Display message: Instrument idle temperature too low	• Ambient room temperature is below 20°C. • Hardware issue	Bring the ambient temperature up to 20°C. If the problem persists, contact Technical Support (see Appendix F, "Customer and technical support").
Display message: Bad results data drive	• On some machines, the warning appears before the reboot completes. • There is a hardware issue.	Wait for a few minutes to see if the error message disappears. If the error message disappears, data obtained during a run with this alarm raised can still be used. If the problem persists, contact Technical Support (see Appendix F, "Customer and technical support").
Display message: Kernels do not match	Hardware and/or software issue	Contact Technical Support (see Appendix F, "Customer and technical support").
Display message: Failed to set up system time at startup. Check your connection to the Ion Torrent™ Server.	The connection between the Ion PGM™ Dx Sequencer and the Ion Torrent™ Server has been lost.	1. Check the network connection to the Ion Torrent™ Server to make sure the connection is established, then reboot the instrument. 2. If the problem persists, replace the network cable(s) to the instrument and server. 3. If the problem persists, contact Technical Support (see Appendix F, "Customer and technical support").
	Instrument is still in the process of establishing a connection	Allow 10 minutes to see if display message clears.
Display message: Log on failed. Could not authenticate the user.	Incorrect username or password entered by the user.	Enter the correct username and password.
	The connection between the instrument and the server has been lost.	Check the connection to the Ion Torrent™ Server, then reboot the Ion PGM™ Dx Sequencer. Ensure that no alarms involving the connection to the Ion Torrent™ Server appear.
Display message: Lost connection to the Ion Torrent™ Server	The connection between the instrument and the server has been lost.	Check the network connection to the Ion Torrent™ Server, and then reboot the Ion PGM™ Dx Sequencer. If this alarm appears during a run, the data created during that run can still be used.
Display message: UBoots do not match	Hardware issue	Contact Technical Support (see Appendix F, "Customer and technical support").

Observation	Possible cause	Recommended action
Display message: Bad boot drive detected	Hardware issue	Contact Technical Support (see Appendix F, "Customer and technical support"). If this alarm appears during a run and data for the run is generated, that data may still be used.
Display message: Results drive not accessible. Reboot and try again.	• On some machines, the warning appears before the reboot completes • Hardware issue	Wait for a few minutes to see if the error message disappears. If the error message appears and disappears during a run, data obtained during that run can still be used. If the alarm persists, contact Technical Support (see Appendix F, "Customer and technical support").
Display message: Lost chip connection	The instrument cannot detect a chip in the chip clamp	See the instructions under "Chip calibration failure (before sample has been loaded on the chip)" on page 84.
Display message: Sensor unable to measure instrument temperature	Hardware issue	Contact Technical Support (see Appendix F, "Customer and technical support").
Display message: Results drive check failed	Hardware issue	If the error message disappears when you return to the main instrument screen, this alarm can be ignored. Otherwise, contact Technical Support (see Appendix F, "Customer and technical support").
Display message: Software versions incompatible. Go to the Options menu and update the software.	The system software was updated, but the instrument software was not.	After the system software has been updated, update the instrument software as follows: 1. On the main menu of the instrument, press **Options** and follow the instructions to check for and install updates. 2. When installation is complete, follow the onscreen prompts to restart the instrument. **IMPORTANT!** You must restart the instrument before proceeding.
Display message: Sensor unable to measure gas pressure. Check supply gas pressure.	• Gas line is not connected to the instrument • Gas cylinder may be turned off or empty • Hardware issue	1. Verify that the gas line is connected to the instrument. 2. Verify that the gas cylinder has at least 500 psi on the pressure gauge inside the tank and 30 psi on the gauge that regulates pressure to the outlet. (Both pressure gauges are mounted on the gas cylinder.) 3. Confirm that the outlet valve on the regulator is turned on. 4. If the problem persists, contact Technical Support (see Appendix F, "Customer and technical support").

Observation	Possible cause	Recommended action
Display message: Failed to locate the barcode scanner. Check if the scanner is attached.	The connection between the barcode scanner and the Ion PGM™ Dx Sequencer has been lost.	Make sure the scanner is plugged into a USB port on the instrument. If it is connected and the alarm still appears, try plugging the scanner into a second USB port. If the alarm persists, contact Technical Support (see Appendix F, "Customer and technical support").
Display message: Failed to set the pressure to target range. Check the gas connection and try again.	• Gas line is not connected to the instrument • Gas cylinder may be turned off or empty • Hardware issue (regulator malfunction)	1. Verify that the gas line is connected to the instrument. 2. Verify that the gas cylinder has at least 500 psi on the pressure gauge inside the tank and 30 psi on the gauge that regulates pressure to the outlet. (Both pressure gauges are mounted on the gas cylinder.) 3. Confirm that the outlet valve on the regulator is turned on. 4. If the problem persists, contact Technical Support (see Appendix F, "Customer and technical support").
Display message: Failed to set up FTP connection. Check your connection to the Ion Torrent™ Server .	The network connection is not established or an incorrect IP address was used.	Confirm that the server information is correct for the Ion Torrent™ Server. Contact your local network administrator for support if the issue persists.
Display message: Unable to mount the file system	Hardware and/or software issue	1. Reboot the instrument to clear the alarm. 2. If the alarm is not cleared after reboot, contact Technical Support (see Appendix F, "Customer and technical support").
Display message: Instrument temperature too low	• Room temperature is below 20°C. • Hardware issue	**Note:** The data created during a run with this alarm raised may still be used if all the QC metrics are met. If the ambient room temperature is below 20°C, raise it. If the problem persists, contact Technical Support (see Appendix F, "Customer and technical support").

Observation	Possible cause	Recommended action
Display message: Instrument temperature too high	• Room temperature is too high. • Clogged filter or blocked airway on the instrument • Hardware issue (fan is not running or running too slowly)	**Note:** The data created during a run with this alarm raised may still be used if all the QC metrics are met. 1. If the ambient room temperature is above 30°C, lower it. 2. Make sure that the round filter on the back panel of the instrument has unrestricted airflow. If the filter is clogged with dust, clean it as follows: a. Pinch the dirty filter with your fingers, then remove it from the instrument.  b. Shake the filter over a waste container to remove most of the dust. c. Rinse the filter with running water to remove any remaining dust. The water flow should be from the inside-facing surface to the outside-facing surface through the filter. d. Air dry the filter. e. Blot any remaining dust from the filter using tape. f. Reinsert the filter. If the problem persists, contact Technical Support (see Appendix F, "Customer and technical support").
Display message: Chip temperature too low	Hardware issue	**IMPORTANT!** The data created during a run with this alarm raised should *not* be used. Contact Technical Support (see Appendix F, "Customer and technical support").

Observation	Possible cause	Recommended action
Display message: Chip temperature too high	• Room temperature is too high. • Clogged filter or blocked airway on the instrument • Hardware issue (instrument fan is not running or running too slowly)	**IMPORTANT!** The data created during a run with this alarm raised should *not* be used. See the recommended action for "Display message: Instrument temperature too high" on page 93.
Display message: Lost communication with valve board	Hardware issue	Contact Technical Support (see Appendix F, "Customer and technical support").
Display message: Fan current too low	Hardware issue	Contact Technical Support (see Appendix F, "Customer and technical support").
Display message: Heater current too low	Hardware issue	**IMPORTANT!** If the chip temperature is also out of range, data created during a run should *not* be used. Contact Technical Support (see Appendix F, "Customer and technical support"). If no chip temperature alarms are raised, data created during a run may still be used if all the QC metrics are met.
Error message: A non-recoverable error has occurred	Hardware issue	Prepare new template from the same library and plan a new run. If the issue persists, contact Technical Support (see Appendix F, "Customer and technical support").

Sequencer software issues

(For additional software anomalies, see the release notes provided with your version of the software.)

Observation	Possible cause	Recommended action
Sequencer touchscreen is frozen	The user pressed multiple buttons on the screen in rapid succession.	Wait 5 minutes for the touchscreen to unfreeze. If the touchscreen remains frozen, reboot the sequencer by holding down the **Power** button on the front to shut it down, then press again to restart.
	The touchscreen is in a locked state.	Reboot the sequencer by holding down the **Power** button on the front to shut it down, then press again to restart.

Warnings and alarms—Veriti™ Dx 96-well Thermal Cycler

Observation	Possible cause	Recommended action
"Fatal Error" message displayed by Veriti™ Dx 96-well Thermal Cycler	Various	For assistance, contact Technical Support (see Appendix F, "Customer and technical support"). Refer to the *Veriti™ Dx 96-well Thermal Cycler User Guide* (Pub. No. 4453697) for general troubleshooting information for this instrument.

B

Ion PGM™ Dx Chip Minifuge and barcode scanner

Ion PGM™ Dx Chip Minifuge

The Ion PGM™ Dx Chip Minifuge is supplied with one custom rotor and two buckets. The buckets are designed to hold two chips: one in each bucket. The rotor and bucket design enables effective and efficient reagent loading of chips.

Safety precautions

CAUTION!

- Make sure your supply voltage matches the voltage label on the minifuge, i.e., never plug a 120V minifuge into an 220–240 VAC outlet. Operating the minifuge with a supply voltage outside the specified range may cause a fire or electric shock.
- Do not run the minifuge for more than 30 seconds.
- Never operate the minifuge without a rotor properly attached to the shaft.
- Never operate with only one chip in place. A chip must be present in each bucket to balance the rotor. If necessary, you can balance a loaded chip with a used chip of any type.
- Never put hands in the rotor area unless the rotor is completely stopped.
- Never move the minifuge while the rotor is spinning.
- Do not leave the minifuge running when not in use.

Note: The rotor can be balanced with a used chip from a previous reaction without risk of contamination.

Voltage selection

Two different minifuges are available, depending on your supply voltage: 120 VAC and 220–240 VAC. Make sure that the voltage specification on the label of your minifuge matches the supply voltage. If they do not match, change your supply voltage or contact Customer Support to request the appropriate minifuge.

CAUTION! Never plug a 120V minifuge into an 220–240 VAC outlet, or vice versa. Operating the minifuge with a supply voltage outside of the range specified on the label may cause a fire or electric shock.

Voltage, RPM, and RCF

The following tables list the revolutions per minute (RPM) and relative centrifugal force (RCF) at different voltages.

120/50 VAC, 60 Hz	RPM	RCF
90	4100	836
100	4550	1030
110	4960	1224
120	5330	1424
130	5710	1628

230/50 VAC, 60 Hz	RPM	RCF
210	5070	1279
220	5310	1403
230	5515	1513
240	5705	1619
250	5900	1732

Operation

1. Place the Ion PGM™ Dx Chip Minifuge on a level, clean surface near an accessible power outlet so that the cord and outlet are within easy reach of the operator.
2. Make sure the power switch on the minifuge is in the "off" position.
3. Load a chip into each bucket.

   IMPORTANT! A chip must be present in each bucket to balance the rotor. If necessary, you can balance a loaded chip with a used chip of any type.

4. Turn the power switch on.
5. To begin centrifugation, close the lid of the minifuge. (The centrifugation time will vary depending on the step in the chip-loading protocol.)
6. To stop centrifugation, press down on the lid release tab on the front of the minifuge.

CAUTION! Do not attempt to open the lid or remove the chips until the unit has come to a complete stop.

7. After the rotor has stopped, open the lid by grabbing it with the thumb on the front and fingers on the back, then lifting the lid back on the hinge.

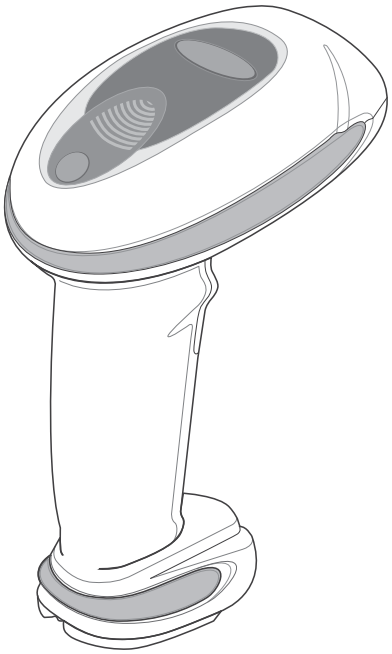
Cleaning

To clean the minifuge, use a damp cloth and a mild, noncorrosive detergent (pH <8). After cleaning, ensure that all parts are dried thoroughly before attempting to operate the unit. Do not immerse the centrifuge in liquid or pour liquids over it.

Note: Use only the cleaning protocol described above.

Barcode scanner

The barcode scanner provided with the Ion PGM™ Dx System uses a low-power, visible-light diode.



CAUTION! As with any bright light source, you should avoid staring directly into the light beam or shining the beam into other people's eyes. Momentary exposure to a Class 2 laser is not known to be harmful.

CAUTION! Use of controls, adjustments, or performance of procedures other than those specified in this guide can result in hazardous laser light exposure.

The barcode scanner specifications are listed below.

Wavelength	Rated Power
630–680 nm	1 mW

C

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words:

- **CAUTION!**—Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **WARNING!**—Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- **DANGER!**—Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Symbol	English	Français
	Caution, risk of danger Consult the manual for further safety information.	Attention, risque de danger Consulter le manuel pour d'autres renseignements de sécurité.
	Caution, risk of electrical shock	Attention, risque de choc électrique

(continued)

Symbol	English	Français
	Caution, piercing hazard	Attention, danger de perforation
	Caution, hot surface	Attention, surface chaude
	Potential biohazard	Danger biologique potentiel
	On	On (marche)
	Off	Off (arrêt)
	Earth (ground) terminal	Borne de (mise à la) terre
	Protective conductor terminal (main ground)	Borne de conducteur de protection (mise à la terre principale)
	Terminal that can receive or supply alternating current or voltage	Borne pouvant recevoir ou envoyer une tension ou un courant de type alternatif
	Do not dispose of this product in unsorted municipal waste **CAUTION!** To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.	Ne pas éliminer ce produit avec les déchets usuels non soumis au tri sélectif. **MISE EN GARDE !** Pour minimiser les conséquences négatives sur l'environnement à la suite de l'élimination de déchets électroniques, ne pas éliminer ce déchet électronique avec les déchets usuels non soumis au tri sélectif. Se conformer aux ordonnances locales sur les déchets municipaux pour les dispositions d'élimination et communiquer avec le service à la clientèle pour des renseignements sur les options d'élimination responsable.

Conformity symbols on the instrument

Conformity mark	Description
	Indicates conformity with safety requirements for Canada and U.S.A.
CE	Indicates conformity with European Union requirements for safety and electromagnetic compatibility.
	Indicates conformity with Australian standards for electromagnetic compatibility.

Medical device symbols

The following table describes symbols that may be displayed on product labels. The symbols that are used on labels conform to standards BS EN ISO 15223-1:2016 and FDA 21 CFR 809.10 "Labeling for in vitro diagnostic products".

Symbol	Description	Symbol	Description
	MANUFACTURER		CONTAINS SUFFICIENT FOR <n> TESTS
	DATE OF MANUFACTURE		USE BY
	BATCH CODE		CATALOG NUMBER
	SERIAL NUMBER		FRAGILE, HANDLE WITH CARE
	LOWER LIMIT OF TEMPERATURE		PROTECT FROM LIGHT
	UPPER AND LOWER LIMITS OF TEMPERATURE		UPPER LIMIT OF TEMPERATURE
	DO NOT REUSE		BIOLOGICAL RISKS
	CAUTION, CONSULT ACCOMPANYING DOCUMENTS		CONSULT INSTRUCTIONS FOR USE
	UPPER AND LOWER LIMITS OF HUMIDITY		OBSERVE PRECAUTIONS FOR HANDLING ELECTROSTATIC SENSITIVE DEVICES
	IN VITRO DIAGNOSTIC MEDICAL DEVICE		

Safety alerts on this instrument

Additional text may be used with one of the symbols described above when more specific information is needed to avoid exposure to a hazard. See the following table for safety alerts found on the instrument.

English	Français
CAUTION! Hazardous chemicals. Read the Safety Data Sheets (SDSs) before handling.	**MISE EN GARDE ! Produits chimiques dangereux.** Lire les fiches signalétiques (FS) avant de manipuler les produits.
CAUTION! Hazardous waste. Refer to SDS(s) and local regulations for handling and disposal.	**MISE EN GARDE ! Déchets dangereux.** Lire les fiches signalétiques (FS) et la réglementation locale associées à la manipulation et à l'élimination des déchets.

Safety information for third-party instruments

Refer to the manufacturer's documentation for information on the safe use of third-party products provided with the instrument system.

Instrument safety

General

CAUTION! Do not remove instrument protective covers. If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

CAUTION! Solvents and Pressurized fluids. Wear eye protection when working with any pressurized fluids. Use caution when working with any polymeric tubing that is under pressure:

- Extinguish any nearby flames if you use flammable solvents.
- Do not use polymeric tubing that has been severely stressed or kinked.
- Do not use polymeric tubing with tetrahydrofuran or nitric and sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause polymeric tubing to swell and greatly reduce the rupture pressure of the tubing.
- Be aware that high solvent flow rates (~40mL/min) may cause a static charge to build up on the surface of the tubing and electrical sparks may result.

Electrical

WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility.

WARNING! Disconnecting Power. To fully disconnect power, either detach or unplug the power cord. Position the instrument such that the power cord is accessible.

WARNING! Radio interference. This equipment has been designed and tested to CISPR 11 Class A. In a domestic environment it may cause radio interference, in which case you may need to take measures to mitigate the interference.

WARNING! Electromagnetic radiation. Do not use this device in close proximity to sources of strong electromagnetic radiation (e.g., unshielded intentional RF sources), as these may interfere with proper operation.

Cleaning and decontamination

CAUTION! Cleaning and Decontamination. Use only the cleaning and decontamination methods specified in the manufacturer's user documentation. It is the responsibility of the operator (or other responsible person) to ensure the following requirements are met:

- No decontamination or cleaning agents are used that could cause a HAZARD as a result of a reaction with parts of the equipment or with material contained in the equipment.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) prior to having the instrument serviced at your facility or sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan (decontamination forms may be requested from customer service).
- Before using any cleaning or decontamination methods (except those recommended by the manufacturer), users should confirm with the manufacturer that the proposed method will not damage the equipment.

Laser

CAUTION! LASER HAZARD, Bar Code Scanner. The bar code scanner included with the instrument system is a Class 2 laser. To avoid damage to eyes, do not stare directly into the beam or point into another person's eyes.

Gas safety

Verify that your installation room can accommodate gas cylinders.

WARNING! Instrumentation must be installed and operated in a well-ventilated environment as defined as having a minimum airflow of 6-10 air changes per hour. Assess the need for ventilation or atmospheric monitoring to avoid asphyxiation accidents from inert gases and/or oxygen depletion, and take measures to clearly identify potentially hazardous areas through training or signage. Please contact your Environmental Health and Safety Coordinator to confirm that the instruments will be installed and operated in an environment with sufficient ventilation.

WARNING! Pressurized gas cylinders are potentially explosive. Always cap the gas cylinder when it is not in use, and attach it firmly to the wall or gas cylinder cart with approved brackets or chains.

WARNING! Gas cylinders are heavy and may topple over, potentially causing personal injury and tank damage. Cylinders should be firmly secured to a wall or work surface. Please contact your Environmental Health and Safety Coordinator for guidance on the proper installation of a gas cylinder.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.

Safety

Reference	Description
EU Directive 2006/95/EC	European Union “Low Voltage Directive”
IEC 61010-1 EN 61010-1 UL 61010-1 CSA C22.2 No. 61010-1	*Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements*

(continued)

Reference	Description
IEC 61010-2-010 EN 61010-2-010	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials*
IEC/EN 61010-2-101	*Safety requirements for electrical equipment for measurement, control and laboratory use - Part 2-101: Particular requirements for in vitro diagnostic (IVD) medical equipment*

EMC

Reference	Description
Directive 2004/108/EC	European Union "EMC Directive"
EN 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
EN 61326-2-6	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 26: Particular requirements – In vitro diagnostic (IVD) medical equipment)requirements*
FCC Part 15	U.S. Standard "Industrial, Scientific, and Medical Equipment"
AS/NZS CISPR 22:2009	*Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment*
ICES-003, Issue 5	*Industrial, Scientific and Medical (ISM) Radio Frequency Generators*

Environmental design

Reference	Description
Directive 2012/19/EU	European Union "WEEE Directive" – Waste electrical and electronic equipment
Directive 2011/65/EU	European Union "RoHS Directive" – Restriction of hazardous substances in electrical and electronic equipment

Precaution—strong magnet

Note: Do not substitute non-IVD labeled magnets for the DynaMag™ Dx 96-Well Plate Magnet and DynaMag™ Dx 16 2-mL Magnet, provided with Ion PGM™ Dx System.

The DynaMag™ Dx 96-Well Plate Magnet and DynaMag™ Dx 16 2-mL Magnet contain very strong permanent magnets. People wearing a pacemaker or any other medical magnetized implant should not use this product unless advised by a health professional; the implant could be affected or damaged by exposure to a strong magnetic field. Keep tools and objects that could be damaged by the magnetic

field out of the working area. This includes, but is not restricted to, credit cards and other products containing magnetic recording devices. Keep away from delicate instruments, watches, electronic equipment, displays and monitors. The magnet may attract steel or other magnetic material with high mechanical forces. Take care during handling. Avoid contact between two magnets. Do not pull the magnets apart if contact has been made; twist off to prevent damage to the unit or fingers. The Health and Safety Officer should take all necessary steps and full responsibility to ensure that the precautions and statements are followed and adhered to.

D Performance characteristics

For performance characteristics of the Oncomine™ Dx Target Test Kit, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide* (Pub. No. MAN0018810).

For performance characteristics of the Ion PGM™ Dx System, see the *Ion PGM™ Dx System Performance Characteristics User Guide* (Pub. No. MAN0018763).

E Instrument warranty

For new Ion Torrent™ instruments, Life Technologies warrants to and only to buyer for twelve (12) months from the date of shipping, that the Ion Torrent™ software and Ion Torrent™ instruments are free from defects in material and workmanship and conform to Life Technologies' published specifications in all material respects. Where a valid and timely claim in respect of breach of Ion Torrent™ warranty is submitted to Life Technologies, Life Technologies may, at its discretion, replace, repair or modify the Ion Torrent™ instrument. Any agreed replacement shall be at 1:1, like-kind basis, at no cost to the buyer. For Ion Torrent™ chips or reagents reasonably determined by Life Technologies to be defective, independent of user error, shall be replaced by Life Technologies on a 1:1, like-kind basis at no cost to buyer, provided that such defective Ion Torrent™ chips or reagents were used by buyer prior to their expiration date, or if there is no expiration date, the Ion Torrent™ chips or reagents were used within six (6) months of receipt, and the defect was promptly reported with appropriate detail to Life Technologies' technical support.

NO OTHER WARRANTIES SHALL BE APPLICABLE TO ION TORRENT PRODUCTS (WHETHER OR NOT ANY FURTHER WARRANTY DOCUMENTATION MAY BE INCLUDED IN THE SHIPMENT), WITH THE EXCEPTION OF THIRD PARTY WARRANTIES WITH RESPECT TO THIRD PARTY PRODUCT. ANY THIRD PARTY PRODUCTS ARE NOT COVERED BY THIS SECTION AND ANY WARRANTIES FOR THIRD PARTY PRODUCTS ARE PROVIDED BY THE ORIGINAL MANUFACTURER OF THE THIRD PARTY PRODUCT. Warranties are made only to buyer purchasing the Ion Torrent™ Product directly from Life Technologies, are not transferable and do not extend to the benefit of any other person or entity, unless otherwise expressly stated in writing by Life Technologies. ANY PRODUCT NOT COVERED BY AN EXPRESS WRITTEN WARRANTY IS SOLD AND PROVIDED "AS IS," WITHOUT WARRANTY OF ANY KIND, STATUTORY, EXPRESS OR IMPLIED. Any description of Ion Torrent™ Product recited in Life Technologies' quotation is for the sole purpose of identifying Ion Torrent™ Product, and any such description is not part of any contract between Life Technologies and buyer and does not constitute a warranty that Ion Torrent™ Product shall conform to that description. Any sample or model used in connection with Life Technologies' quotation is for illustrative purposes only, and is not part of any contract between Life Technologies and buyer and does not constitute a warranty that Ion Torrent™ Product will conform to the sample or model. No affirmation of fact or promise made by Life Technologies, whether or not in Life Technologies' quotation, shall constitute a warranty that Ion Torrent™ Product will conform to the affirmation or promise. Unless otherwise specified in writing in documentation shipped with Ion Torrent™ Product or otherwise agreed by Life Technologies in writing. Life Technologies does not provide service or support for custom products or other products made to buyer's specifications. THE WARRANTIES IDENTIFIED IN THIS CLAUSE ARE LIFE TECHNOLOGIES' SOLE AND EXCLUSIVE WARRANTIES WITH RESPECT TO Ion Torrent™ PRODUCT AND ARE IN LIEU OF ALL OTHER WARRANTIES, STATUTORY, EXPRESS OR IMPLIED, ALL OF WHICH OTHER WARRANTIES ARE EXPRESSLY DISCLAIMED, INCLUDING WITHOUT LIMITATION ANY IMPLIED WARRANTY OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, NON-INFRINGEMENT, OR REGARDING RESULTS OBTAINED THROUGH THE USE OF ANY PRODUCT (INCLUDING, WITHOUT LIMITATION, ANY CLAIM OF INACCURATE, INVALID OR INCOMPLETE RESULTS), WHETHER ARISING FROM A STATUTE OR OTHERWISE IN LAW OR FROM A COURSE OF PERFORMANCE, DEALING OR USAGE OF TRADE.

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support
- Order and web support
- Safety Data Sheets (SDSs; also known as MSDSs)

Additional product documentation, including user guides and Certificates of Analysis, are available by contacting Customer Support.

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are printed and shipped with the product.

Obtaining Certificates of Conformance

The Certificate of Conformance provides information on conformance testing of each instrument provided with the system. Certificates of Conformance are shipped with the instrument, and are also available by contacting Customer Support at **thermofisher.com/support**.

ion torrent

Oncomine™ Dx Target Test Part V: Torrent Suite™ Dx Software 5.12.5 Reference

USER GUIDE

Publication Number MAN0018814

Revision A.0

IVD

For In Vitro Diagnostic Use.

ThermoFisher SCIENTIFIC

Life Technologies Holdings Pte Ltd |
Block 33 |
Marsiling Industrial Estate Road 3 |
#07-06, Singapore 739256

Products manufactured in Singapore:

Ion PGM™ Dx Instrument System
Ion PGM™ Dx Sequencer
Ion OneTouch™ Dx Instrument
Ion OneTouch™ ES Dx Instrument
Ion PGM™ Dx Chip Minifuge (120V)
Ion PGM™ Wireless Scanner
Ion Torrent™ Server
Torrent Suite™ Dx Software
Veriti™ Dx 96-well Thermal Cycler, 0.2 mL

Life Technologies Corporation |
7335 Executive Way |
Frederick, MD 21704 | USA

Products manufactured in Frederick:

Oncomine™ Dx Target Test Kit
Ion Torrent Dx FFPE Sample Preparation Kit
Ion PGM™ Dx Library Kit
Ion OneTouch™ Dx Template Kit
Ion PGM™ Dx Sequencing Kit
Ion 318™ Dx Chip
Ion OneTouch™ Rack Kit
DynaMag™ Dx 96-Well Plate Magnet
DynaMag™ Dx 16 2-mL Magnet

The information in this guide is subject to change without notice.

DISCLAIMER

TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0018810

Revision	Date	Description
A.0	3 September 2020	Oncomine™ Dx Target Test user guide for FDA submission—updated for Torrent Suite™ Dx Software 5.12.5. Changes from April 2020 submission: • Added Repopulate QC function. See "Repopulate QC (Administrator)" on page 44. • Added a troubleshooting topic for QC and run analysis failure conditions. • Added a recommendation for antivirus software. See "Antivirus software" on page 14.

Contents

About this guide

Purpose of this guide

This user guide provides instructions for using Torrent Suite™ Dx Software to analyze sequencing data generated from sample libraries prepared using the Oncomine™ Dx Target Test Kit.

Oncomine™ Dx Target Test Kit user guides

This user guide is part of a five-guide set.

- *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide*
- *Oncomine™ Dx Target Test Part II: Sample and Library Preparation User Guide*
- *Oncomine™ Dx Target Test Part III: Template Preparation User Guide*
- *Oncomine™ Dx Target Test Part IV: Sequencing and Results Reports User Guide*
- *Oncomine™ Dx Target Test Part V: Torrent Suite™ Dx Software 5.12.5 Reference User Guide*

All five guides are required to complete the entire Oncomine™ Dx Target Test workflow.

Note: The procedures in these guides supersede the instructions in the *Ion PGM™ Dx System User Guide* when using the Ion PGM™ Dx System with the Oncomine™ Dx Target Test.

---

1 Product information

Product description

Oncomine™ Dx Target Test

The Oncomine™ Dx Target Test is an *in vitro* diagnostic next-generation sequencing test to detect somatic alterations in human DNA and RNA isolated from formalin-fixed, paraffin-embedded (FFPE) tissue samples. Detection of these variants is performed using the Ion PGM™ Dx System.

For a complete product description of the Oncomine™ Dx Target Test, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide* (Pub. No. MAN0018810).

Software system

Torrent Suite™ Dx Software is a software system for managing samples and libraries, as well as creating, executing, and analyzing templating and sequencing runs on the Ion PGM™ Dx System. The Oncomine™ Dx Target Test assay is a locked assay in the software that provides configuration and analysis settings for using the Oncomine™ Dx Target Test Kit.

Intended use

For the Intended Use statement for the Oncomine™ Dx Target Test, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide* (Pub. No. MAN0018810).

Theory of operation

For a complete description of the Theory of Operation of the system, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide* (Pub. No. MAN0018810).

Software description

Torrent Suite™ Dx Software is hosted on the Ion Torrent™ Server, part of the Ion PGM™ Dx System.

The software is used with the Oncomine™ Dx Target Test Assay Definition File and the Ion PGM™ Dx Instrument System control software to support the system workflow from sample preparation through library preparation, template preparation, sequencing, primary and secondary analysis, and generation of Oncomine™ Dx Target Test reports.

Software component	Main functions
Torrent Suite™ Dx Software	• Generates base calls and quality values (primary analysis) • Filters and trims reads to ensure high sequence accuracy • Performs alignments and variant calling and generates reports using parameters in the Oncomine™ Dx Target Test Assay Definition File (secondary analysis) • Hosts the Torrent Suite™ Dx Software web application
Oncomine™ Dx Target Test Assay Definition File	Installed with the software, this file contains gene lists and parameters for sequencing and analysis of specific DNA and RNA variants targeted by the Oncomine™ Dx Target Test Kit.
Ion OneTouch™ Dx Instrument control software	• Provides control of the Ion OneTouch™ Dx Instrument via a touchscreen interface • Enables users to prepare template-positive Ion PGM™ Dx Ion Sphere™ Particles using the instrument • Enables users to perform instrument maintenance
Ion PGM™ Dx Sequencer control software	• Provides control of the Ion PGM™ Dx Sequencer via a touchscreen interface • Enables users to perform sequencing workflows using the instrument • Collects and compresses sequencing data, then transfers data to the Ion Torrent™ Server for primary and secondary analysis • Enables users to perform instrument maintenance

Features of Torrent Suite™ Dx Software

Torrent Suite™ Dx Software enables users to:

- Generate reports on detected sequence variations in DNA and RNA in genes targeted by the Oncomine™ Dx Target Test
- Enter and manage information about samples and create libraries and library batches
- Plan runs to be executed on the Ion PGM™ Dx System instruments
- Monitor the progress of active instrument runs
- View and download run results and analysis reports
- View QC settings
- View workflow settings
- Manage user information and privileges
- View audit trails
- Configure archiving, reporting, and other administrative functions

Note: This guide provides instructions for using the Torrent Suite™ Dx Software in IVD Mode only.

Limitations and Precautions

Torrent Suite™ Dx Software has the following limitations:

- User names can contain alphanumeric characters and underscores, periods, and hyphens. Passwords can only contain alphanumeric characters.
- Torrent Suite™ Dx Software is intended to be used only with the Ion PGM™ Dx System and does not accept any software plugins.

Software compatibility and requirements

The procedures in this guide are designed for use with Torrent Suite™ Dx Software version 5.12.5 or later. For a complete description of software compatibility and requirements, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide* (Pub. No. MAN0018810).

About screen

Under the tab, the **About** screen contains following links:

- **Support Contact Information** is a link to **thermofisher.com/technical-resources**, where you can access technical support and product information.
- **Instrument Diagnostics** opens a page that lists the active sequencers connected to the Ion Torrent™ Server. For each sequencer, you can click on additional links to download or open the following files:
  - Diagnostic archive file (ZIP file)
  - Init.log file
  - Installation Acceptance Report (PDF file)
- **End User License Agreement** opens the End User License Agreement (EULA) in PDF format.

2 Get started with Torrent Suite™ Dx Software

User-access levels

Users at this level...	Can...
Operator	• Add and import samples • Prepare library batches • Create and save Planned Runs • Execute Planned Runs • Monitor runs • View results and reports
Manager	Operator functions plus: • Create and edit sample attributes • Delete Planned Runs • Approve reports • Amend reports • Manage reference sequences and panel, hotspot, and other sequence files • Access services information
Administrator	Operator and Manager functions plus: • View, export, and print audit records • Configure network settings • View and manage software updates • Configure data archive and storage settings • Manage instrument and software log files • Add and manage users

Ion PGM™ Dx System synchronization requirement

Allow up to 20 minutes for synchronization with the Ion Torrent™ Server after you power on any of the following instruments or the server itself:

- Ion PGM™ Dx Sequencer
- Ion OneTouch™ Dx Instrument

If you attempt to use an instrument before synchronization is complete, it will generate an alarm.

Network and password security requirements

Network configuration and security

The network configuration and security settings of your laboratory or facility (such as firewalls, anti-virus software, network passwords) are the sole responsibility of your facility administrator, IT, and security personnel. This product does not provide any network or security configuration files, utilities, or instructions.

If external or network drives are connected to the software, it is the responsibility of your IT personnel to ensure that such drives are configured and secured correctly to prevent data corruption or loss. It is the responsibility of your facility administrator, IT, and security personnel to prevent the use of any unsecured ports (such as USB, Ethernet) and ensure that the system security is maintained.

Password security

Thermo Fisher Scientific strongly recommends that you maintain unique passwords for all accounts in use on this product. All passwords should be reset upon first sign in to the product. Change passwords according to your organization's password policy.

It is the sole responsibility of your IT personnel to develop and enforce secure use of passwords.

Antivirus software

Thermo Fisher Scientific has tested ClamAV antivirus software from Ubuntu™ on the Ion Torrent™ Server and shown that it does not interfere with the assay or Torrent Suite™ Dx Software. For more information, visit **help.ubuntu.com/community/ClamAV**.

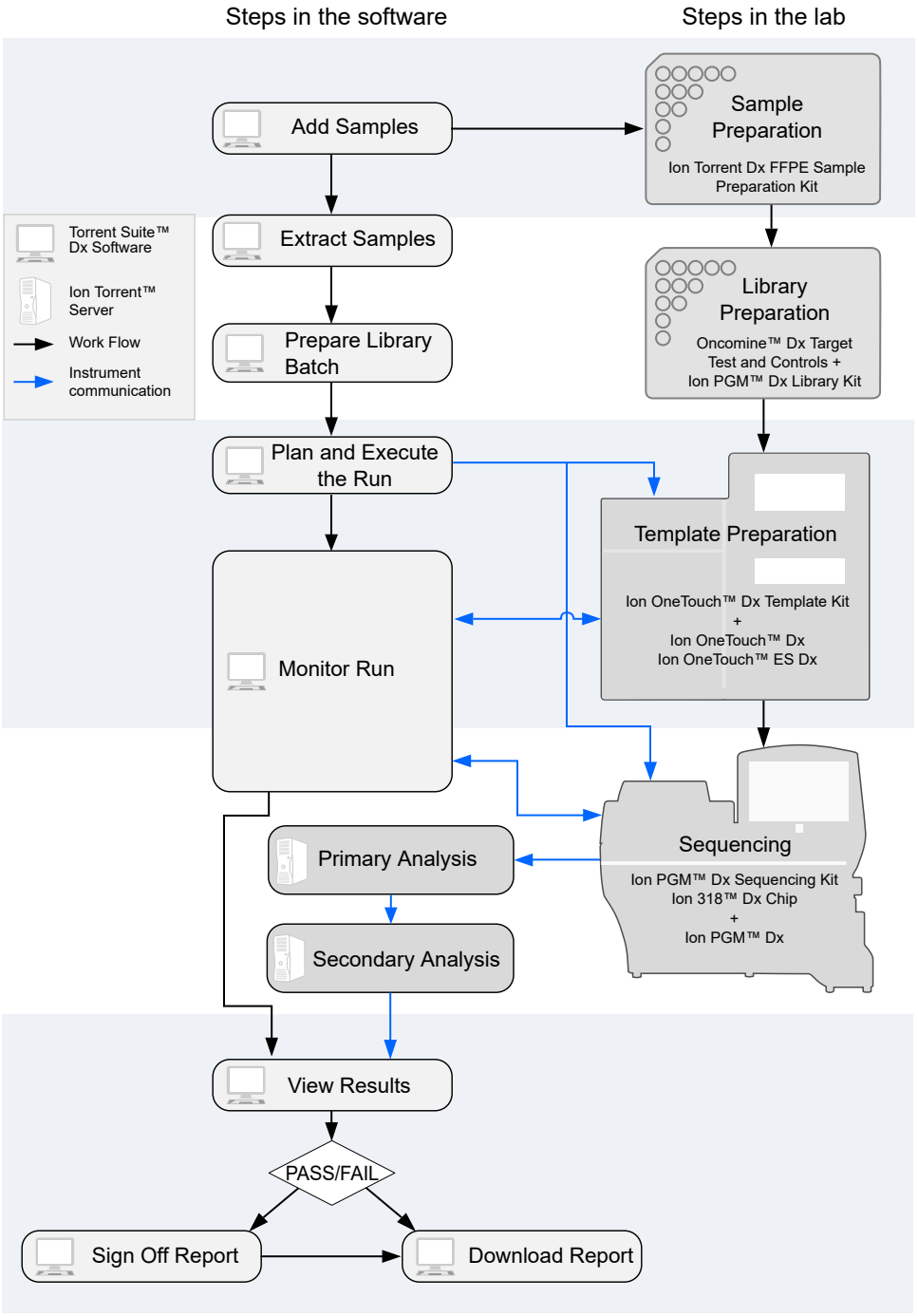
System tracking

The system tracks and checks user, sample, workflow, reagents and QC metrics for auditable records. If the software detects an error at any step—for example, a scanned barcode is inconsistent with the information given for the run—the software alerts the user and does not proceed with the run.

Reagent management

Users should put in place a reagent management system to inventory and track reagent use. Proper use of a reagent management system promotes regulatory compliance and ensures optimal use of kits, chips, and reagents.

Oncomine™ Dx Target Test system diagram



Installation and verification run

Do not attempt to power on any Ion PGM™ Dx System instruments or sign in to Torrent Suite™ Dx Software until a field service engineer (FSE) has installed and performed a performance qualification (PQ) verification run and provided a basic system overview.

The software and all connected instruments are locked until a verification run has been performed and the report is signed by the FSE.

Request and sign in to a new account

Only administrator-level users can create user accounts.

After account creation, the Ion PGM™ Dx System automatically sends an email to the new user with the user name and password information.

- To request a new account, contact your local administrator.
- To sign in to a new account for the first time:
  a. Go to the Torrent Suite™ Dx Software home page, then enter your user name in the **Username** field.
  b. Enter the system-generated password in the **Password** field.
  c. Click **Enter**, or click **Sign In**.
  d. Click **Accept** to accept the End User Software License Agreement.
  e. In the **Change Password** screen, enter your temporary password in the **Current Password** field. Type a new password in the **New Password** field, then confirm the password.
     – Passwords must be between 6 and 10 characters.
     – Passwords must contain at least one alphabetic character (aA–zZ).
     – Passwords must contain at least one numeric character (0–9).
     – Passwords must contain only alphanumeric characters (0–9, aA–zZ), no spaces or special characters.
     – Passwords are case-sensitive.
  f. Click **Change**.

Sign in

To sign in to the software:

1. Open the software home page.
2. Under **Mode Switch** at the bottom of the page, verify that **IVD Mode** is selected.

Note: If you are not in **IVD Mode**, contact a system administrator to switch modes.

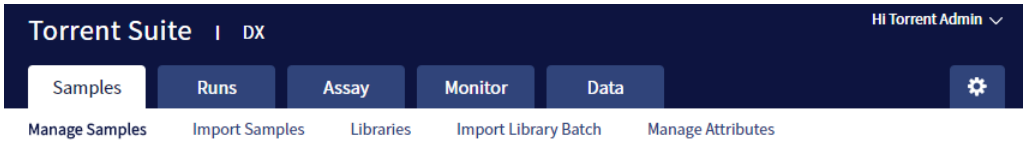
3. Select your preferred language from the dropdown list in the upper right corner of the page.
4. Enter your user name and password, then press **Enter** or click **Sign In**.

   IMPORTANT! Your user name and password must be unique and not shared with other users.

   The software opens to the **Samples** tab and the **Manage Samples** screen.

User interface

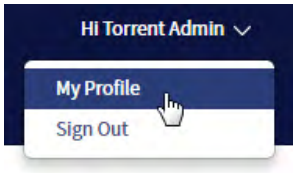
Torrent Suite™ Dx Software has a browser-based interface with five tabs containing the main functions of the software:



- **Samples** tab—Create and manage samples and libraries; plan new runs from libraries.
- **Runs** tab—Create and manage Planned Runs (executable instructions for individual runs). The system uses the Planned Run to verify at each step of the run that the correct kit is used to perform the correct assay on the correct sample.
- **Assay** tab—Manage assays (protocol specifications for templating, sequencing, and data analysis).
- **Monitor** tab—View the status of your system instrument(s) and current runs. Near real-time information is provided on your runs, so that you know early on about any instrument problems.
- **Data** tab—View summaries of completed runs, and detailed run reports; review the run plan settings; download or print output files and run reports.

In addition to the main tabs, the ⚙ **(Settings)** tab enables access to audit records and log files, server configuration and data management settings, reference file information, and user accounts.

Click **My Profile** from the user name dropdown list in the upper right corner to view your account information and change your password and email address. Click **Sign Out** to sign out of the software.



3 Enter sample and library information

Sample and library information is entered into Torrent Suite™ Dx Software under the **Samples** tab. This information is tracked by the software throughout the entire system workflow.

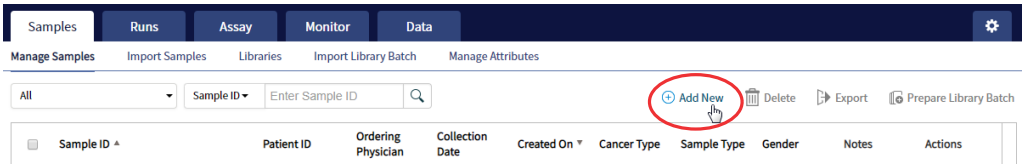
In the **Samples** tab, you can:

- Create, import, and manage samples and their attributes
- Enter the barcode on the kit used to extract each sample
- Prepare libraries and library batches

Add or import samples

Add a new sample

1. Under the **Samples** tab, in the **Manage Samples** screen, click ⊕ **Add New**.



2. Complete the **Add New Sample** dialog box. Fields identified with an asterisk (*) are required. If no information is available, substitute dummy data to complete the required fields.

Field	Description
Sample ID*	A unique identifier representing the sample, containing only alphanumeric characters (0–9 and A to Z), full stops/periods (.), underscores (_), or hyphens (-). The Sample ID cannot contain spaces and is limited to a maximum of 20 characters. After a Sample ID is entered into the system, it cannot be edited. It can be deleted unless it has already been used in a library. The software checks all Sample IDs entered or imported to prevent duplication and returns an error message if a non-unique Sample ID is detected.

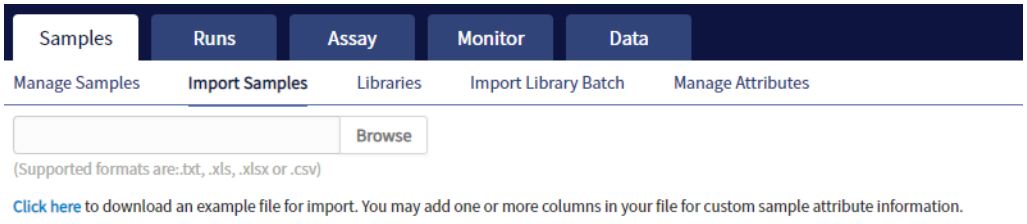
(continued)

Field	Description
Patient ID*	An identifier representing the patient. This field accepts all characters including spaces.
Date of Birth*	The patient's date of birth. Click the button to select the date in the correct format.
Ordering Physician*	The name of the ordering physician. This field accepts all characters including spaces.
Collection Date*	The date the sample was collected from the patient. Click the button to select the date in the correct format.
Sample Source	Open-entry field that accepts all characters, including spaces. **Example entry:** Name of the clinic or hospital ordering the test.
Sample Condition*	Open-entry field that accepts all characters, including spaces. **Example entry:** FFPE surgical resection.
Sample Type*	Open-entry field that accepts all characters, including spaces. **Example entry:** Lung tumor, invasive adenocarcinoma.
Gender*	The biological gender of the sample. This must be Male, Female, or Unknown.
Cancer Type*	Select the cancer type of the sample from the dropdown list.
%Cellularity	The percentage of tumor cellularity in the sample.
%Necrosis	The percentage of cellular necrosis in the sample.
Reference Interval	A normal range of measure for the sample.
Notes	An open-entry field.

3. Click **Save**.
   The sample is listed in the **Manage Samples** screen.

Import samples

Under the **Samples** tab in the **Import Samples** screen, you can import sample data in the form of a TXT, XLS, XLSX, or CSV file. The import file includes the same sample attributes that are listed in the **Add New Sample** dialog box.



1. In the **Import Samples** screen, below the **Browse** field, click **Click here** to download a Microsoft™ Excel™ template file.

   Note: The template file contains default sample attributes as columns. If additional custom sample attributes have been configured in the software, add these attributes as columns to the template file.

2. In the template file, fill in the information for each sample, one sample per row. See "Predefined sample attributes" on page 20 for more information.

3. Save the file.

4. Click **Browse**, navigate to the saved file, then select it.

5. Click **Import**.
   A progress bar followed by an import report displays. If the import process fails, an error message indicates the reason for failure (for example, an invalid character was used). For additional troubleshooting, see "Batch sample import fails" on page 93.

6. Click **Manage Samples** to return to the sample list. Successfully imported samples are listed.

Predefined sample attributes

The software has the following predefined sample attributes, which are listed in the **Add New Sample** dialog box and in the template file for importing samples.

- Sample ID*
- Patient ID*
- Date Of Birth*
- Ordering Physician*
- Collection Date*
- Sample Source
- Sample Condition*
- Sample Type*
- Gender*
- Cancer Type*
- %Cellularity
- %Necrosis
- Reference Interval
- Notes

*Indicates a field required to be filled in during sample creation.

Predefined attributes are locked and cannot be edited. You can create and manage custom sample attributes using the tools in the **Manage Attributes** screen.

Note: LIMS users must create custom attributes before importing sample and Planned Run information from LIMS for the attributes to be propagated through to output files. The software ignores all input file content that is not a recognized attribute.

Create a new sample attribute

1. Under the **Samples** tab in the **Manage Attributes** screen, click ⊕ **Add New Attribute**.
2. Enter an attribute name in the dialog.

   Note: Attribute names are limited to ≤20 alphanumeric characters (0–9 and A–Z), full stops/periods (.), underscores (_), or hyphens (-).

3. Specify whether the attribute is a text or number in the **Data Type** dropdown list.
4. Select the **Required** checkbox to designate the new attribute as required in the **Add New Sample** dialog.

   Note: Users will not be able to create a new sample if this information is not entered in the **Add New Sample** dialog.

5. Click **Save**.
   The new sample attribute is listed in the **Attribute Name** column and is available when you add a new sample. After an attribute has been created, it cannot be deleted. To remove an attribute from use, see "Obsolete an attribute" on page 21.

   Note:
   - When editing samples created before a new attribute was created, the new attribute appears in the **Edit Sample** dialog. If the new attribute was designated as required, a valid entry must be entered into the new attribute field to save the sample information.
   - Samples can be edited after they have been added to a library batch, but samples cannot be edited after execution of a Planned Run that uses the samples in a library batch.

Obsolete an attribute

Under the **Samples** tab in the **Manage Attributes** screen, you can obsolete user-created sample attributes to remove them from use in the software. Obsoleted attributes can be reactivated, and a record of their use is maintained in the audit trail of samples that are created using that attribute.

Note: You can only obsolete user-created attributes, not system-installed attributes.

- To obsolete an attribute, click **Obsolete** in the **Actions** column, then confirm the action. **Reactivate** replaces **Obsolete** in the **Actions** column.
- To reactivate an attribute, click **Reactivate** in the **Actions** column.

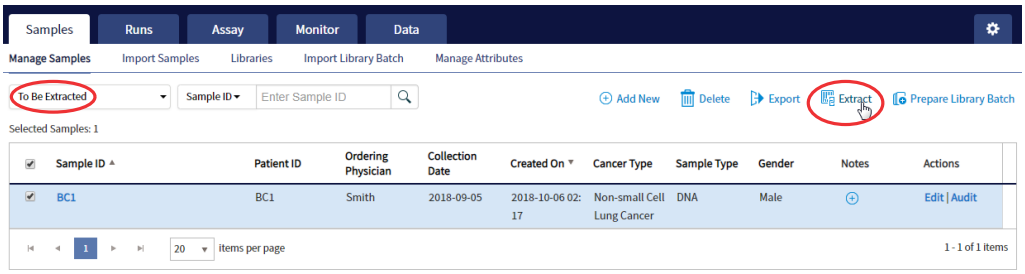
  Note: All active sample attributes are listed in the **Add New Sample** dialog box.

Enter the Ion Torrent Dx Total Nucleic Acid Isolation Kit barcode

Under the **Samples** tab, in the **Manage Samples** screen, scan the barcode of the Ion Torrent Dx Total Nucleic Acid Isolation Kit used in the extraction process for a particular sample. This barcode is saved with the sample and can be viewed by clicking the Sample ID.

1. Above the samples list, select **To Be Extracted** from the **Filter Samples by...** dropdown list to display only those samples that do not have a kit barcode that is associated with them.

2. Select the checkbox of the sample to be extracted. Select multiple samples if you are using the same kit to process them.



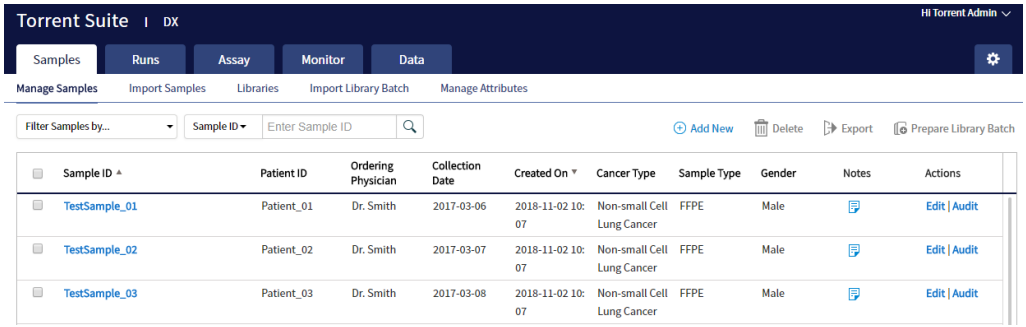
3. Click **Extract**. In the dialog box, scan the barcode that is printed on the Ion Torrent Dx Total Nucleic Acid Isolation Kit (box 1 of 2, Part No. A32434).

IMPORTANT! Check the expiration date on the box. If the kit is expired, select another kit.

4. Click **Save**.
The sample is no longer listed in the **To Be Extracted** list.

Manage samples

You can find tools for creating, searching, sorting, editing, deleting, exporting samples, and viewing the sample history under the **Samples** tab in the **Manage Samples** screen.



Search samples

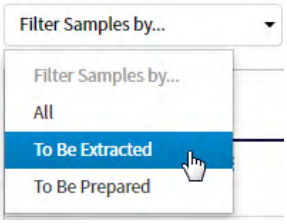
Under the **Samples** tab, in the **Manage Samples** screen:

1. Enter the full or partial sample ID into the **Enter Sample ID** field.
2. Click .
   The sample or samples matching the search parameters are listed.
3. Click × to return to the complete list of samples.

Sort samples

Under the **Samples** tab in the **Manage Samples** screen, the list of samples is displayed with the most recently created sample on top by default. To return to the default display, click **Manage Samples**.

- To list only those samples that have not been extracted, click **To Be Extracted** from the **Filter Samples by** dropdown list.



- To list only those samples that have not been prepared as a library, click **To Be Prepared**.
- To list all samples, click **All**.
- To sort the list:
  a. Click a column header to sort the list by the entries in that column.
  b. Click the column header again to reverse the order.
  c. Click **Manage Samples** to return to the default order (most recently created on top).

Export and print samples

The **Export** function generates a XLS file of the sample details.

1. Under the **Samples** tab in the **Manage Samples** screen, in the **Sample ID** column, select the checkbox next to each sample to be exported. Select the checkbox above the column to select all of the samples.
2. Click **Export**.
   An XLS file is generated. Depending on your browser settings, the software automatically downloads the file or prompts you to open or save the file.
3. Open the XLS file in an appropriate viewer to print.

View notes or add a note to a sample

1. Click in the **Notes** column for a sample to view notes that have been added for the sample.
2. To add a new note, click **Add Note**. Enter the note in the test field, then click **Save**.
3. Click **Close** to return to the **Manage Samples** screen.

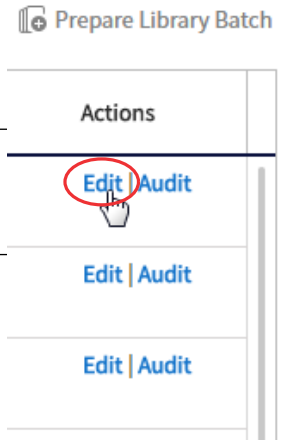
Edit sample

Under the **Samples** tab, in the **Manage Samples** screen, samples that can be edited are identified by the **Edit** link in the **Actions** column.

A sample can be edited at any point before execution of a Planned Run containing that sample, after which the **Edit** link is no longer available. You can edit sample information after a run using the **Edit and Amend Report** command (for more information, see "Edit a sample and amend the report after a run").

1. Click **Edit** in the row of the sample to be edited.
2. In the **Edit Sample** dialog box, click a field to edit the information.

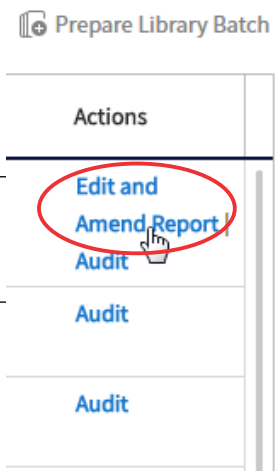
   Note: The Sample ID cannot be edited. For a description of the fields, see "Add a new sample" on page 18.
3. Click **Save**.



Edit a sample and amend the report after a run

After a sequencing run and its analysis have completed, you can edit a sample and amend the Test and Lab Reports for up to 30 days. After 30 days, only the audit link displays. Editing a sample after a run triggers an automatic update of the reports and other files associated with the sample. Under the **Samples** tab, in the **Manage Samples** screen, editable samples are identified by the **Edit and Amend Report** link for the sample in the **Actions** column.

1. To edit a sample after a run, click **Edit and Amend Report** in the row of the sample to be edited.

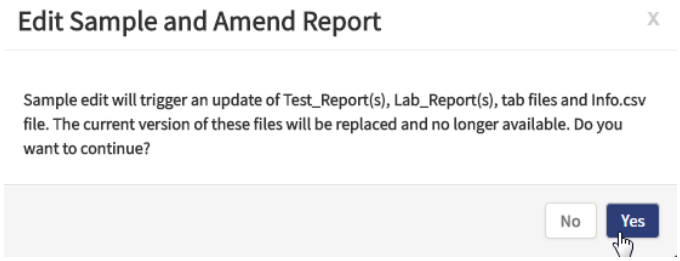


2. In the **Edit Sample and Amend Report** dialog box, click a field to edit the information.

   Note: The Sample ID cannot be edited. For a description of the fields, see "Add a new sample" on page 18.

3. Click **Save**.

4. In the dialog box that appears, click **Yes** to confirm the edit and continue.



The sample information is edited and associated reports and files are updated.

Review sample history

The entire history of a sample is available for review, export, or printing.

1. Under the **Samples** tab in the **Manage Samples** screen, click **Audit** for the sample of interest in the **Actions** column.
   The **Audit Trail** dialog box opens, listing each event that modified the selected sample.

2. Click **(Details)** under the **Record** header to view the details of the change made.

3. In the **Audit Record Details** dialog box, click **Export** to export a PDF of the record.

Delete samples

Under the **Samples** tab, in the **Manage Samples** screen, you can delete samples that have not been assigned to a library.

Note: Samples assigned to libraries are locked and cannot be edited or deleted. Locked samples only display **Audit** under the **Sample ID**.

1. Select the sample or samples to be deleted by selecting the checkbox adjacent to the **Sample ID**. Select all the samples on the page by selecting the checkbox above the column.
2. Click (**Delete**).
   The **Delete Sample** dialog box opens with the message "Are you sure you want to delete selected sample(s)?"
3. Click **Yes** to delete the selected samples. Click **No** to return without deleting.

Prepare a library batch in Torrent Suite™ Dx Software

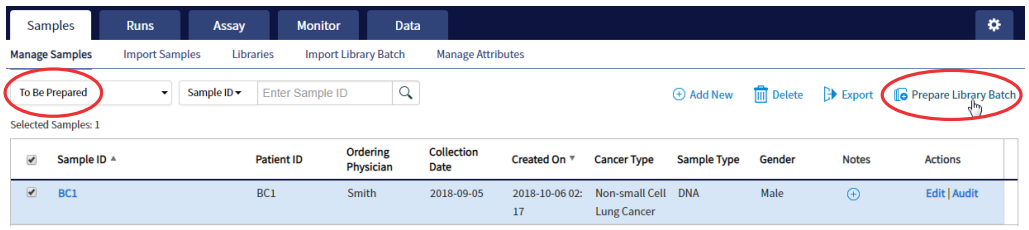
In Torrent Suite™ Dx Software, samples that are entered into the software are placed in library batches for processing and tracking. A library batch consists of a group of libraries that are prepared at the same time.

Note:

- Each library in a library batch must have a unique library name. When combining libraries in the same run, each must also have a unique barcode.
- Control libraries must be included in the same library batch as the sample library they control for.
- Fields identified with a red asterisk (*) are required.

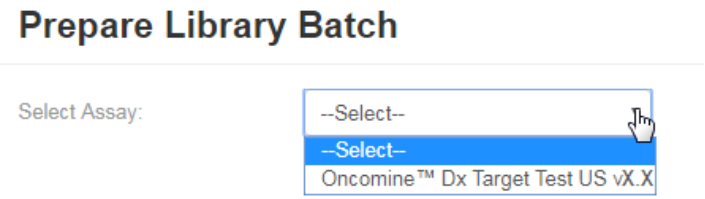
1. Sign in to Torrent Suite™ Dx Software.
2. Under the **Samples** tab, in the **Manage Samples** screen, select **To Be Prepared** from the **Filter Samples by...** dropdown list to display only those samples that have not been placed in a library batch.

   IMPORTANT! Samples that have not been queued for extraction in the software also appear on this tab. Ensure that the samples have been queued for extraction before queueing them for library batch preparation.



3. Select up to 6 samples in the list, then click **Prepare Library Batch**.
   The **Prepare Library Batch** dialog opens. Required fields are indicated with a red asterisk(*).

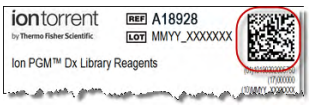
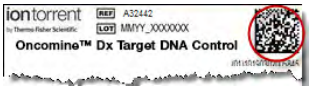
4. In the **Select Assay** dropdown list, select **Oncomine™ Dx Target Test**. The assay determines specific parameters of the run, including required controls and post-run data analysis settings.



5. In the following screen, enter a unique identifier for the library batch in the **Library Batch ID** field. Library Batch IDs can only contain alphanumeric characters (0–9 and A to Z), full stop/period (.), underscore (_), and hyphen (-).

6. Scan the barcodes from their respective kit boxes into the appropriate fields. Each library batch is associated with a kit lot by scanning the 2D barcode on the appropriate kit box.

IMPORTANT! Check the expiration date on each box. If the kit is expired, select another kit.

Barcode field	Kit	Kit box	Storage	Label scanned
Library Kit Barcode	Ion PGM™ Dx Library Kit	Ion PGM™ Dx Library Reagents	–30°C to –10°C	
Panel Kit Barcode	Oncomine™ Dx Target Test Panel	Oncomine™ Dx Target Test DNA and RNA Panel (box 1 of 3)	–30°C to –10°C	
Control Kit Barcode	Oncomine™ Dx Target Test, Controls, and Diluent Kit	Oncomine™ Dx Target DNA Control v2 (box 2 of 3)	–30°C to –10°C	

7. Type a unique library name for each DNA and RNA library in the appropriate field. Library names can only contain alphanumeric characters (0–9 and A to Z), full stop/period (.), underscore (_), and hyphen (-).

Note: The Oncomine™ Dx Target Test Kit requires specific controls, which are automatically listed in the **Prepare Library Batch** dialog.

8. Select the Barcode ID of the adapter used to prepare each library. Swap the default barcodes in the dialog between DNA and RNA using the DNA ⇄ RNA button.

   Note: Each library in a library batch must have a different Barcode ID. When preparing the physical libraries, we recommend swapping barcodes between DNA and RNA libraries in consecutive sequencing runs to prevent carryover contamination. See "Alternating barcodes" on page 28.

   IMPORTANT! Be careful to ensure that the actual barcodes used to create the libraries match the barcodes that are entered in the **Prepare Library Batch** dialog.

9. In the **Input Quantity** field, enter 10 ng for each library.

10. Click **Save** to save your selections and close the dialog.
    The **Libraries** screen opens, listing the libraries that you created. Libraries that are prepared in the same batch have the same **Library Batch ID**.

Alternating barcodes

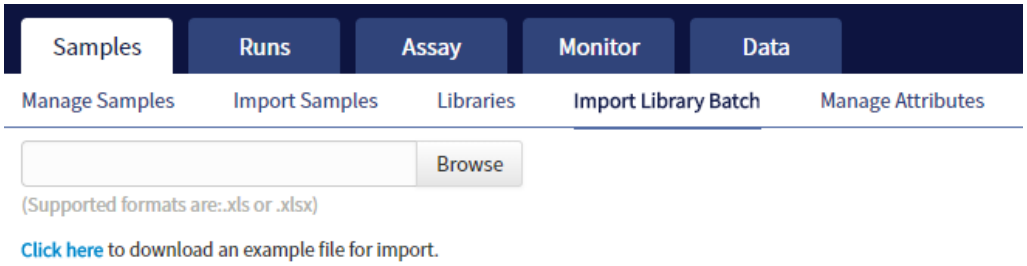
When preparing libraries, we recommend swapping barcodes between DNA and RNA libraries in consecutive sequencing runs to prevent carryover contamination. The following table provides an example of swapping barcodes between runs.

IMPORTANT! Be careful to confirm that the barcodes used to create the libraries match the barcodes entered in the **Prepare Library Batch** dialog box.

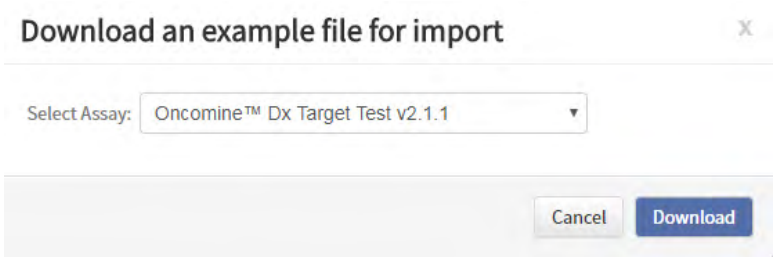
Library type	System Run 1 barcode usage		System Run 2 barcode usage	
	DNA	RNA	DNA	RNA
Positive control	1	9	9	1
Sample	2	10	10	2
Sample	3	11	11	3
Sample	4	12	12	4
Sample	5	13	13	5
Sample	6	14	14	6
Sample	7	15	15	7
No-template control (NTC)	8	16	16	8

Import a library batch

Under the **Samples** tab in the **Import Library Batch** screen, you can import library batch information in the form of an XLS or XLSX file. The import file must include all of the library and kit information that you enter in the **Prepare Library Batch** dialog box.



1. In the **Import Library Batch** screen, below the **Browse** field, click **Click here** to download an example file for import.
2. In the dialog box that opens, select the assay that you are using from the dropdown list, then click **Download**.



   The assay name is auto-populated in the Microsoft™ Excel™ template file that downloads to your drive.

3. In the template file, enter or confirm the library batch information.
   - Assay used (auto-populated)
   - Unique Library Batch ID
   - Library kit, control kit, and panel kit barcodes
   - Sample IDs
   - Library names
   - Barcode IDs used for each library and control
   - Nucleic acid type (DNA or RNA)
   - Library input quantity
4. Save the file.
5. Click **Browse**, navigate to the saved file, then select it.

6. Click **Import**.
   A progress bar followed by an import report displays. If the import process fails, an error message indicates the reason for failure (for example, an invalid character was used). For additional troubleshooting, see "Library batch import fails" on page 93.

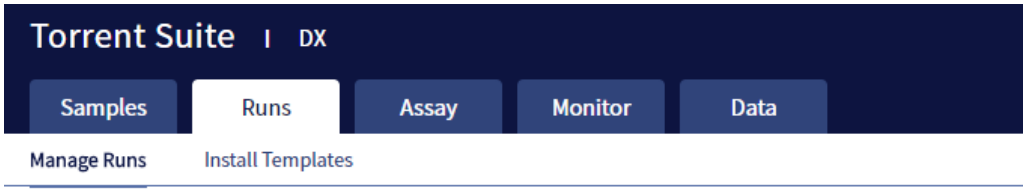
7. Click **Libraries** to return to the library batch screen. Your successfully imported library batch is listed.

Manage libraries and library batches

Use the following tools in the **Libraries** screen under the **Samples** tab to perform the specified actions.

To...	Do the following...
View more library information	Hover over a name in the library list.
View all libraries	Click **All** in the **Filter Libraries by** dropdown list.
View libraries that are not in a Planned Run	Click **To Be Planned** in the **Filter Libraries by** dropdown list.
Search the list	Select search by **Library Name** or **Library Batch ID**, type a full or partial Library Name or Library Batch ID in the search field above the list, then click 🔍. Click **×** to clear the search criteria and display the complete list.
Sort the list	Click a column header to sort the list by the information in that column.
View multiple pages of libraries	Click the scroll buttons below the list.
Delete libraries	Select the checkbox next to the library or related libraries (DNA and RNA), then click 🗑 **Delete**. **Note:** You can only delete a library if none of the libraries in the same library batch have been assigned to a Planned Run.
Edit a library batch	Click **Edit** for the library batch in the **Actions** column, and modify the information in the dialog box.
View or export the audit trail of a library batch	1. Click **Audit** for the library batch in the **Actions** column. The **Audit Trail** dialog box opens, displaying the user, action, and time of each action performed on the batch. 2. To view the action details, click 📄 in the **Record** column of the **Audit Trail** dialog box. The **Audit Record Details** dialog box opens. 3. To export the record as a PDF, click **Export** in the **Audit Record Details** dialog box.
Add or modify notes for a library	Click ⊕ in the **Notes** column.

4 Create and manage Planned Runs



Planned Runs contain all the sample, library, and assay information required to perform a run on template preparation and sequencing instruments. The Planned Run information is tracked by the system from template preparation through sequencing, data analysis, and final results.

Prerequisites to create a Planned Run

Before creating a Planned Run, check that:

- Sample information is correctly entered
- Library batches have been prepared
- Each library has been assigned a unique Barcode ID

The software returns an error message when any of these conditions are not met when creating a Planned Run.

The system identifies and tracks kit components and chips by barcodes. The system includes a cordless barcode reader and a barcode reader that is attached to the Ion PGM™ Dx Sequencer.

Note: All kits and chips that are used in a diagnostic assay must be uniquely identified, and the identification must be stored so that the record can be audited.

Create a Planned Run

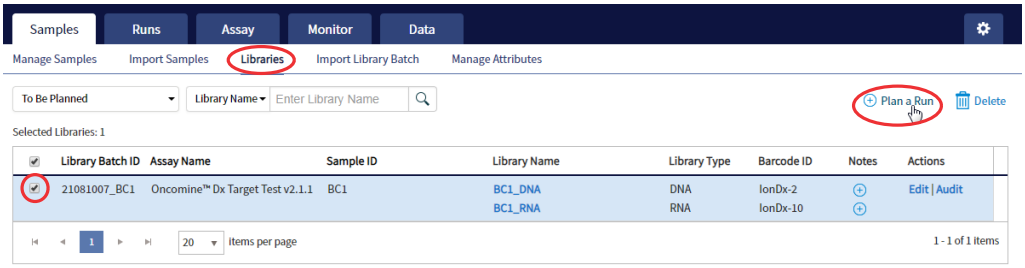
Libraries that are ready to be entered into a Planned Run are listed under the **Samples** tab in the **Libraries** screen.

Note: You can also plan a run from the **Runs** tab in the **Planned Runs** screen (click ⊕ **Add New**).

1. Sign in to Torrent Suite™ Dx Software.
2. In the **Libraries** screen, select the library or libraries to be run by selecting checkboxes in the list. To view only those libraries that have not yet been added to a Planned Run, select **To Be Planned** in the **Filter Libraries by** dropdown list.

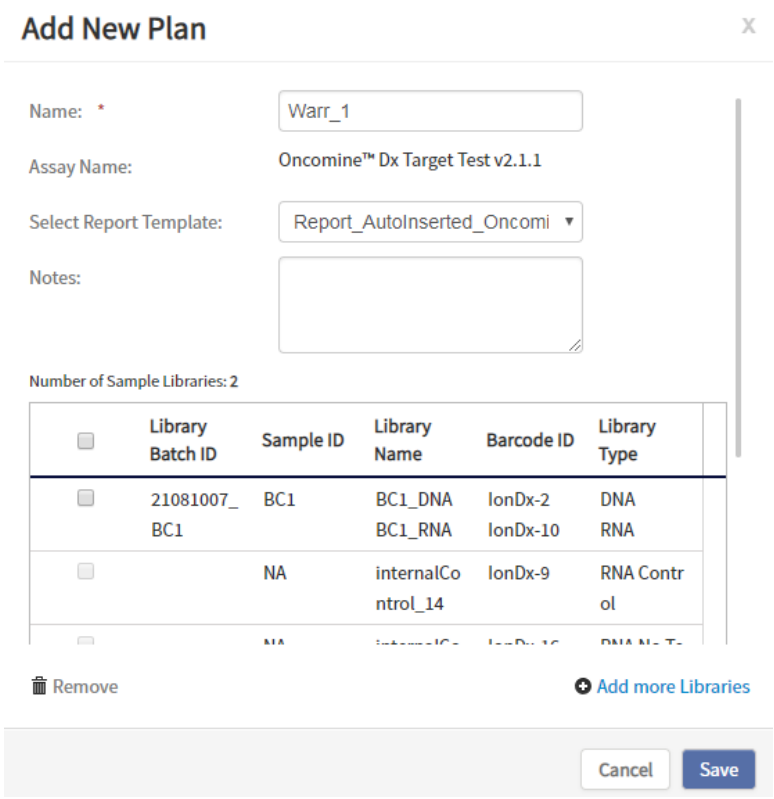
   Note:
   - Libraries prepared with the same assay in the same library batch can be combined and run together, as long as they have unique library names and Barcode IDs.
   - To plan a run with the Oncomine™ Dx Target Test assay, a report template must be created and associated with the assay by an Administrator or Manager. Under the **Assay** tab, go to **Presets ▸ Report Templates** to add a new report template.
   - Up to 16 libraries (including controls) can be sequenced in a single run.
   - If libraries need to be rerun due to a run failure, they can be added to a new Planned Run.

3. Click **Plan a Run**.



4. In the **Add New Plan** dialog box, enter a name for the run, then select the appropriate report template.

   The selected library or libraries are listed in the dialog box, and the control libraries are automatically listed.



5. To remove libraries from the run, select the appropriate checkboxes, then click **Remove**. To add libraries, click ⊕ **Add more Libraries**, then select them from the **Add Libraries** dialog box.

   Any added libraries must be from the same library batch and have unique library names and Barcode IDs.

6. Click **Save**.

   The new Planned Run is automatically assigned a Run Short Code and is displayed at the top of the list under the **Runs** tab in the **Manage Runs** screen.

Execute a Planned Run

Planned Runs are listed under the **Runs** tab in the **Manage Runs** screen. Runs that are ready to be performed have the **Execute** command available in the **Actions** column.

Executing a Planned Run in the software queues the run for initiation on the Ion OneTouch™ Dx Instrument. After a Planned Run has been queued for execution, the operator should immediately start template preparation.

In the **Manage Runs** screen:

1. Click **To Be Started** in the **Filter Run by...** dropdown list to limit the list of Planned Runs to only those runs yet to be started.

2. Find the Planned Run in the list, then under the **Actions** header, click **Execute**.

   The **Execute Planned Run** dialog box opens.

3. In the **Tube Label** field, enter the text that is used to label the tubes that contain the final combined libraries.

   The tube label text can be any combination of letters and numbers. The system uses this text to track the sample throughout the run, so be careful to label each tube legibly at the points noted in the procedure. The software does not allow use of the same Tube Label text within 7 days.

4. Click the **Template Prep Kit barcode** field, then scan the barcode from the Ion OneTouch™ Dx Template Reagents box.

   IMPORTANT! Ensure that you scan the barcode from the actual reagents box that is used in the run.

5. Click **Save** to save your changes.
   The **Review Planned Run** dialog box opens.

6. Write down the **Run Short Code** and/or click **Print** to print the scannable barcode. The code must be entered into the Ion OneTouch™ Dx Instrument and Ion PGM™ Dx Sequencer for tracking and verification before the start of the instrument run.

7. Click **Close** to close the dialog box and send the run to the instrument.

Note: The last 5 executed Planned Runs are listed under the **Monitor** tab in the software.

Manage Planned Runs

Under the **Runs** tab, in the **Planned Runs** screen, a list of all Planned Runs is displayed, with the most recently created run on top. In this screen, you can:

- Sort and search the list of Planned Runs
- Review and print a Planned Run
- View the audit trail of a Planned Run
- Edit a Planned Run
- Delete a Planned Run (Manager/Administrator level users only)

Search and sort Planned Runs

Under the **Run** tab, in the **Manage Runs** screen, the list of Planned Runs is displayed, with the most recently created on top by default.

- To search the Planned Run list:
  a. Enter the full or partial Planned Run name into the **Planned Run** field.

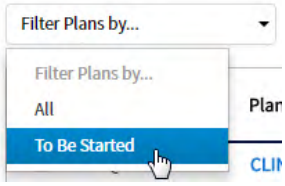
b. Click 🔍.
The Planned Run(s) matching the search parameters are listed.

c. Click × to return to the complete list of Planned Runs.

- To sort the Planned Runs list:
  a. Click the header name of interest.
  The list of Assays reorders alphabetically or numerically based on the header name selected: Run Short Code, Planned Run Name, Assay, and Tube Label.

  b. Click the header name a second time to reverse the order of samples displayed.

- Click **To Be Started** in the **Filter Plans by...** dropdown list to limit the list of Planned Runs to only those runs that are ready to be executed.



Review/Print a Planned Run

You can review and print the information in a **Planned Run** when you execute it. Alternatively:

1. Under the **Runs** tab, in the **Manage Runs** screen, click the Planned Run name in the list.
2. In the **View Planned Run** dialog box, review the information that was entered or generated when you created the run, then click **Print** to print it.

Review a Planned Run audit trail

The entire history of a Planned Run is available for review, export, or printing.

1. Under the **Runs** tab, in the **Manage Runs** screen, click **Audit** in the **Actions** column for the desired run.
   The **Audit Trail** dialog box opens. Each modifying event for the selected Planned Run is listed.
2. Click the details icon under the **Record** header to view the details of the change made.
   The **Audit Record Details** dialog box opens detailing the edits that were made.
3. In the **Audit Record Details** dialog box, click
   a. **Export** to export a Print-Ready PDF of the record.

   b. **Cancel** or the × icon to return to the **Audit Trail** dialog box.
4. Open the downloaded PDF file in an appropriate viewer, then print the record from within the open document.
5. Click **Cancel**, or the × icon, to return to the **Manage Runs** screen.

Edit a Planned Run

You can edit Planned Runs that have not been executed yet.

1. Under the **Runs** tab, in the **Manage Runs** screen, click **Edit** for the Planned Run in the **Actions** column.
2. In the **Edit Planned Run** dialog box, change the settings, then click **Save**.

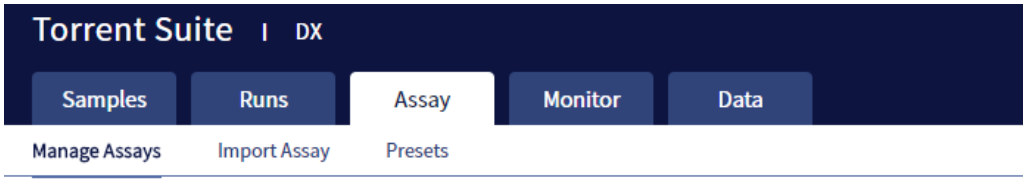
Delete a Planned Run (Manager/Administrator only)

1. Under the **Runs** tab, in the **Manage Runs** screen, select the Planned Run to be deleted by selecting the checkbox next to the **Run Short Code**. Select all of the Planned Runs on the screen by selecting the checkbox above the column.
2. Click **Delete**.
   The **Delete Plan** dialog box opens with the message "Are you sure you want to delete the selected planned run(s)?
3. Click **Yes** to delete the Planned Run, or click **No** to return to the **Manage Runs** screen without deleting.

System installation templates (service engineers only)

IMPORTANT! The templates used by service engineers during Ion PGM™ Dx System installation are listed under the **Runs** tab in the **Install Templates** screen. These should not be used or modified in any way.

5 Assays

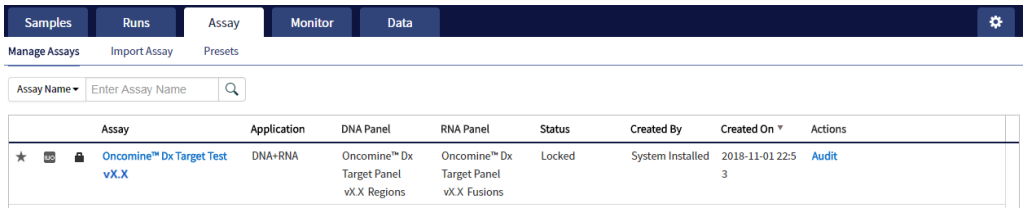


The **Assay** tab has tools for viewing the Oncomine™ Dx Target Test assay and managing and creating report templates.

Oncomine™ Dx Target Test assay

The Oncomine™ Dx Target Test assay is a locked assay that contains the settings and parameters for analyzing sequencing results and determining valid variant calls for the Oncomine™ Dx Target Test. It also defines the kits and chips that can be used with the test, and specifies the threshold values for quality control and variant detection.

IMPORTANT! The Oncomine™ Dx Target Test assay cannot be edited or used as the basis for a Reanalysis assay.



Search and sort assays

Under the **Assay** tab in the **Manage Assays** screen, the most recently created assay is listed first by default. To return to the default display, click **Manage Assays** to refresh the screen.

- To search the assay list:
  a. Enter the full, or partial, assay name into the **Assay Name** field.
  b. Click 🔍.
     The assay or assays matching the search parameters are listed.
  c. Click **×** to return to the complete list of assays.

- To sort the assay list:
  a. Click the desired column header.
     The list of assays reorders based on the header name selected: Assay, Application, Status, Created By, Created On.
  b. Click the header name a second time to reverse the order.

View assay details

The **View Assay Details** dialog displays all the settings for an assay. To open this dialog:

1. In the **Manage Assays** screen, click an assay name in the list.
2. Scroll down to view the all the settings.

Review the assay audit trail

Managers and Administrators can review and export the details of all the actions that are performed on an assay.

1. In the **Manage Assays** screen, click **Audit** for the assay in the **Actions** column.
2. Each action that is performed on the assay is listed in the **Audit Trail** dialog. Click in the **Record** column to view the details of each action.
3. In the **Audit Record Details** dialog, click **Export** to export a PDF of the selected record.

Assay presets

Report templates

You can create and manage custom report templates used to generate PDF reports after a run. You select a report template when you set up a Planned Run.

The tools for setting up report templates are located in the **Report Templates** subtab under the **Assay** tab in the **Presets** screen.

Manage report templates

The tools for managing report templates are found under the **Assay** tab in the **Presets** screen under the **Report Templates** subtab.

- To view the details of a report template, click the template name.
- To preview the template layout, click **Preview** in the **Actions** column.
- To make a template unavailable in the software, click **Obsolete** in the **Actions** column.
- To add notes to a template, click in the **Notes** column.
- To view notes for a template, click in the **Notes** column.

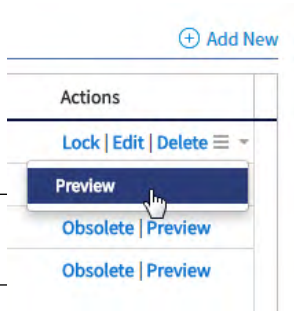
Create a report template

To create a report template:

1. Under the **Assay** tab in the **Presets** screen, click the **Report Templates** subtab.
   Existing report templates are listed on this page, including any system-installed templates.
2. Click ⊕ **Add New**.

   Note: To plan a run with the Oncomine™ Dx Target Test assay, a report template must be created and associated with the assay by an Administrator or Manager.
3. Enter the **Report Name**, then select the **Assay** from the dropdown list.
4. In the **Select Language** dropdown list, select the language for generating the report.
5. Fill out the information in the remaining sections. Fields identified with a red asterisk (*) are required.

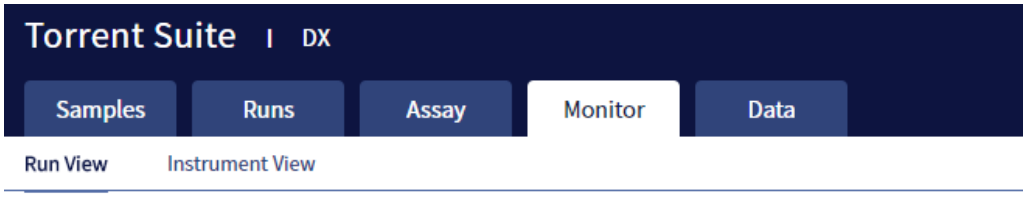
   Note: For locked assays, some fields and attributes cannot be edited and are grayed out.
6. Under **Sample Details**, drag and drop the attributes to display in the report into one of the three column placeholders. You can also drag and drop the additional attributes below the columns into the columns. Select the checkbox next to an attribute to include the attribute in each page header.
7. When you are done, click ⊘ **Save**.
   The new report template appears in **Report Templates** list.
8. To preview the report layout, click ☰ ▾, then click **Preview** in the **Actions** column in the row of the new template. Click **Edit** to make changes.



9. When you are finished, click **Lock**.

   Note: The template must have a locked status to be used in a Planned Run.

6 Monitor runs



In the **Monitor** tab, you can view the status of any jobs running on instruments that are connected to the Ion Torrent™ Server. To monitor the status of runs versus instruments, click **Run View** or **Instrument View** under the tab.

Run information is displayed for the last 5 runs that are in progress, have failed, or have completed data analysis.

Information displayed

The **Run View** and **Instrument View** screens under the **Monitor** tab display the following information:

View	Information displayed
Run View	Ion OneTouch™ Dx Instrument and Ion PGM™ Dx Sequencer • Instrument name • Operator • Start and Completion times • Time remaining • Run Status
	Ion PGM™ Dx Sequencer only • Flow Transfer number • Analysis Status • QC Details (expand to view after analysis is complete) – Run QC – Sample QC – Internal Control QC
Instrument View	Ion OneTouch™ Dx Instrument and Ion PGM™ Dx Sequencer • Current state • Instrument status • Last cleaning • PQ status • PQ due date • Instrument serial number • Any instrument alarms

View a run

Under the **Monitor** tab, in the **Run View** screen, the last 5 sequencing runs (active, completed, or failed) are listed. The instrument type is indicated by either an Ion OneTouch™ Dx Instrument icon  , or an Ion PGM™ Dx Sequencer icon  adjacent to the instrument serial number in the respective panes. To view a run:

1. Click **Refresh** to update the displayed information.
2. In the **Select Run** dropdown list, select the Planned Run to be viewed.
   The instruments used for template preparation and sequencing will be displayed.
3. In the **Select Library** dropdown list, select the library in the run that you want to view.

To view details of the assay used in the designated run, click on the assay name adjacent to the dropdown lists.

View the status and quality control metrics of a run

Under the **Monitor** tab, in the **Run View** screen, you can review the status and quality control metrics of the last 5 template preparation and sequencing runs (active, completed, or failed).

1. In the **Select Run** dropdown list, select the Planned Run.

   The status of the instruments used in the run are displayed. The **Analysis Status** field indicates whether post-sequencing analysis ran to completion or failed.

2. To update the status of the run, click **Refresh**.

3. To display additional information about the run, select from the following options:

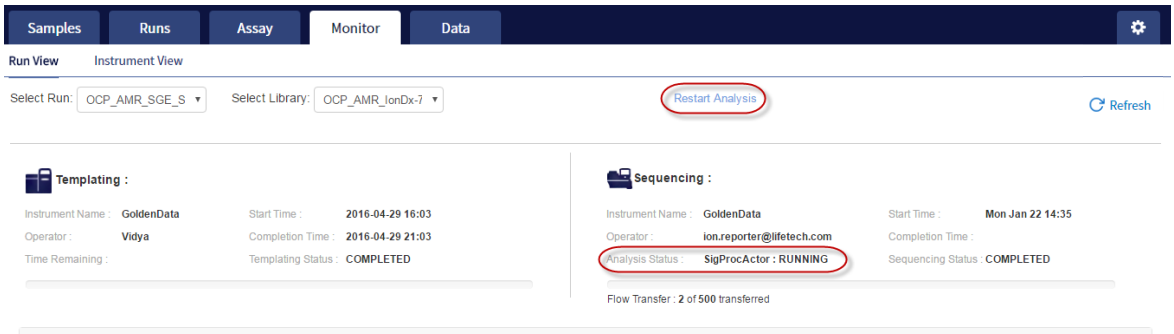
   - To view details of the assay used in the run, click the assay name next to the dropdown lists.
   - To view the quality control information for a library in the run, select the library from the **Select Library** dropdown list, then click **+View QC Details**.

   Note: The QC metrics are described in "Quality control metrics" on page 45.
   See the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide* for detailed pass/fail criteria and a repeat testing strategy.

Restart analysis *(Administrator)*

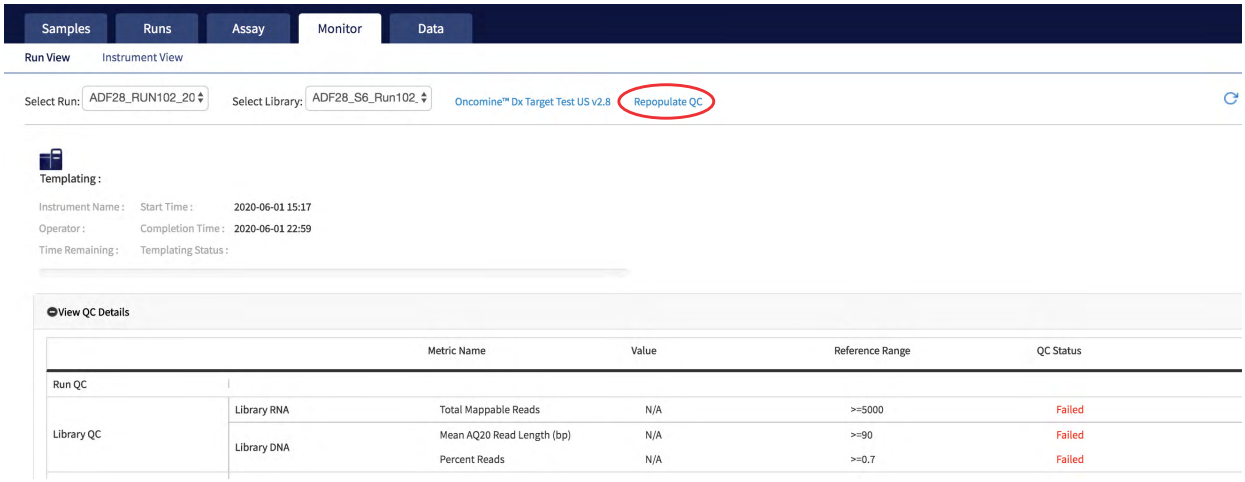
Under the **Monitor** tab, in the **Run View** screen, the **Analysis Status** field indicates whether data analysis has started, is running, or has completed. An analysis listed as "RUNNING" for more than 12 hours may be stuck in the pipeline. After 12 hours, a **Restart Analysis** button appears under the tab for Administrator-level users.

Click the button to restart the analysis from the beginning.



Repopulate QC *(Administrator)*

Under the **Monitor** tab, in the **Run View** screen, the **Repopulate QC** button appears for Administrator-level users after a run is complete. If the **QC Status** of a run is **Failed** and one or more QC metrics are listed as **N/A**, the QC metrics may not have been updated in the database correctly after the run.



Administrator-level users can click **Repopulate QC** to repopulate the QC metrics in the database.

Note: This function does not recalculate or change the QC data from the run. It repopulates the database with the previously calculated metrics and regenerates the Lab and Test Reports. The report status (draft or signed) is retained.

---

1. Under the **Monitor** tab in the **Run View** screen, under **View QC Details**, check the **QC Status** of the run. If the status is **Failed** and any QC metric is listed as **N/A**, proceed to the next step.
2. Click **Repopulate QC** to repopulate all QC metrics in the database.

   Note: Repopulation takes 15–30 minutes.

3. If any metric is still listed as **N/A** and the **QC Status** is still **Failed**, repeat the run starting at template preparation from the DNA/RNA combined library, and ensure that you add all controls. If the problem continues, contact Technical Support.

Quality control metrics

In the **Run View** screen, once analysis of a sequencing run is complete, the following quality control details can be viewed.

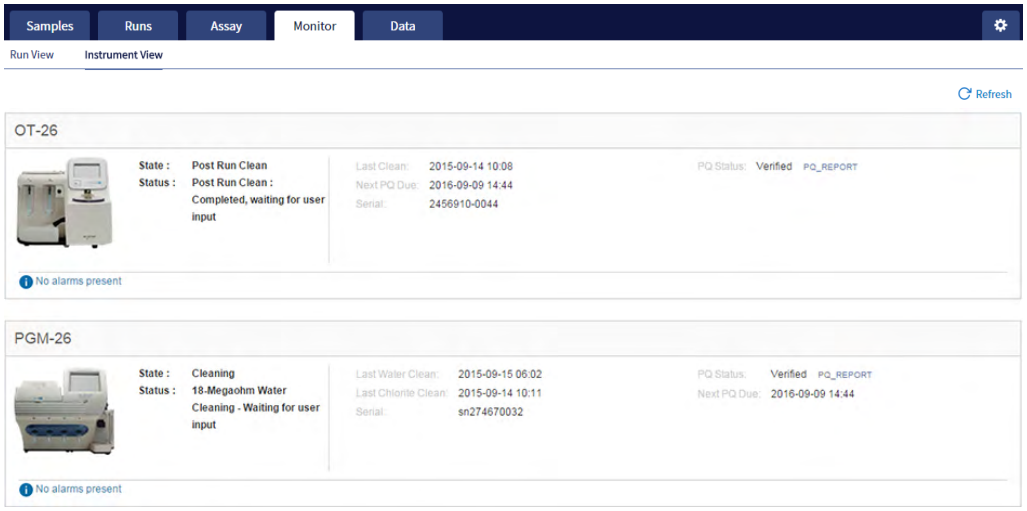
QC detail	QC metric and description
Library QC: DNA	• Mean AQ20 Read Length (bp): The average length, in base pairs, at which the error rate is ≤1% for all aligned reads of a library. • Percent Reads: The number of library reads normalized by the total addressable wells in a run.
Library QC: RNA	• Mappable Fusion Reads: The number of reads that are mapped to the fusion reference file.
Control QC: CF-1	• Mean AQ20 Read Length (bp): The average length, in base pairs, at which the error rate is ≤1% for all aligned reads of CF-1. • Percent Reads: The number of all usable library reads that aligned with the CF-1 sequence divided by the total number of addressable wells.
Control QC: DNA	• Individual COSMIC variant calls and allelic frequencies: Individual variant positions are assessed in the DNA control for presence or absence of the variant. • Mean AQ20 Read Length (bp): The average length, in base pairs, at which the error rate is ≤1% for all aligned reads of a control fragment. • Percent Reads: The number of all usable library reads that aligned with the control fragment sequence divided by the total number of addressable wells.
Control QC: RNA	• Mappable Reads: The number of reads that are mapped to the fusion reference file. • ROS1 Fusion: Detection of ROS1 fusion gene. • ROS1 Fusion Reads: The number of target reads mapping to the ROS1 gene fusion.
Control QC: DNA NTC	• Hotspot Calls: The total number of hotspots where a call was made.
Control QC: RNA NTC	• Mappable Reads: The number of reads that are mapped to the fusion reference file. • Total Fusion Calls: The total number of fusion calls made.

Quality control pass/fail criteria

Metric	Criteria
Run QC	
CF-1 Mean AQ20 Read Length (bp)	≥131
CF-1 Percent Reads (%)	≥0.03
DNA NTC	Hotspot calls = 0
RNA NTC	Total fusion calls = 0
RNA NTC Mappable Reads	≤4999
DNA Library	
Mean AQ20 Read Length (bp)	≥90
Percent Reads (%)	≥0.7
RNA Library	
Mappable Fusion Reads	≥5000
DNA Control	
COSM476_AF	Variant called and AF ≥0.05
COSM521_AF	Variant called and AF ≥0.05
COSM6223_AF	Variant called and AF ≥0.05
COSM6224_AF	Variant called and AF ≥0.05
COSM760_AF	Variant called and AF ≥0.05
Mean AQ20 Read Length (bp)	≥98
Percent Reads (%)	≥0.7
RNA Control	
Mappable Reads	≥18164
ROS1 Fusion Reads	Variant called and fusion reads ≥349

Instrument View screen

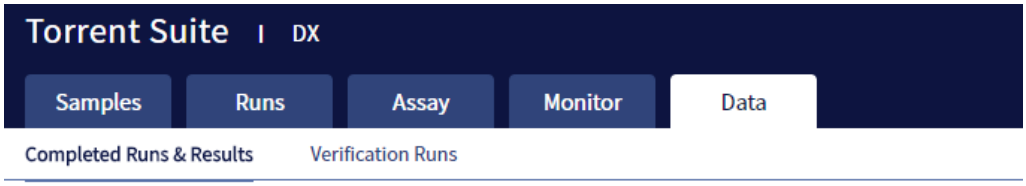
The instruments connected to the Ion Torrent™ Server are listed under the **Monitor** tab in the **Instrument View** screen. To update the information in this screen during a run or following completion of a run, click **Refresh**.



The following information is displayed in this screen:

Label	Description
State	The function that the instrument is currently performing (for example, **Clean**, **Initialize**, or **Run**).
Status	The current status of the active function, including data analysis.
Last Clean	The date and time that the instrument was last cleaned. For the Ion OneTouch™ Dx Instrument, this refers to the pre-run cleaning and is not updated following the post-run cleaning.
Serial	The serial number of the instrument.
PQ Status	The performance qualification (PQ) status of the instrument (**Verified**, **Not Verified**, or **Expired**). Click the link next to the status to download and view the PQ report. **Note:** If the PQ status is **Expired**, users cannot perform a non-PQ run on the instrument until a PQ run is performed and verified.
Next PQ Due	The expiration date of the current PQ run.

7 Review data and results



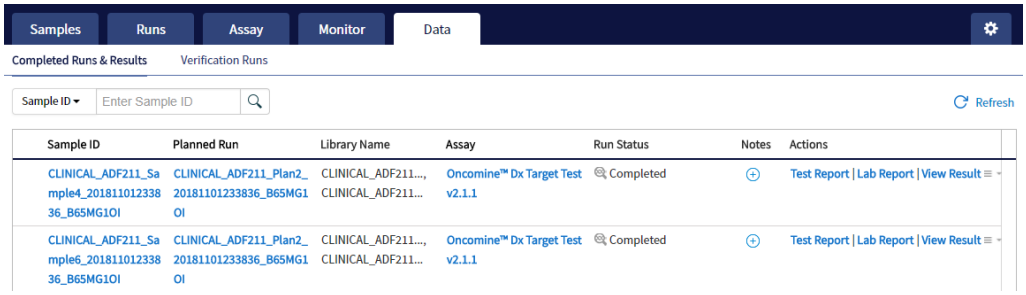
In the **Data** tab, you can review run results and perform data analysis and data management tasks:

- Select **Completed Runs & Results** (the default window) to review completed sample run results and reports. Run results are listed by **Sample ID**.
- Select **Verification Runs** to review data from completed verification runs performed during installation or PQ validation.

Completed Runs & Results screen

Under the **Data** tab, in the **Completed Runs & Results** screen, samples that have been sequenced are listed by Sample ID.

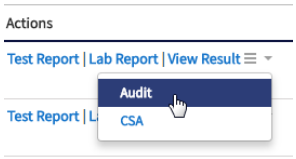
You can search the list of results by Sample ID or Planned Run name. You can also filter and sort the list.



The following information is displayed in the screen:

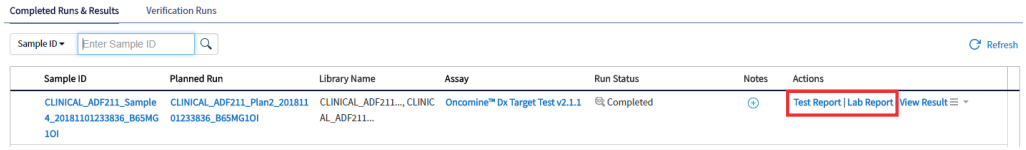
Column	Description
Sample ID	The unique identifier created when the sample was entered into the software. Click on the link to display the details of the sample.
Planned Run	The name of the Planned Run given when it was created in the software, after preparation of the library batch. Click on the name to display details of the Planned Run.

(continued)

Column	Description
Library Name	The names of the DNA and RNA sample libraries given during preparation of the library batch.
Assay	The assay selected when the sample was placed in a library batch prior to creating the Planned Run. Click on the assay name to display the details of the assay.
Run Status	The status of the run (for example: completed, failed, terminated).
Notes	User-entered notes about the sample. To view previously written notes, click . To add new notes, click .
Actions	Click the appropriate link to: • Download the Test Report. • Download the Lab Report. • Open the View Result screen. • View the audit trail for the Planned Run. Click , then click **Audit**.  • Download Customer Support Archive (CSA) log files for the run to aid in troubleshooting. Click , then click **CSA**.

Download the Lab Report or Test Report

1. To download the Lab Report or Test Report for a sample of interest, in the **Completed Runs & Results** screen, click the appropriate link in the **Actions** column for the sample. A ZIP file containing all languages of the report downloads automatically.



2. Extract the downloaded files, then open the PDF file of the desired language in an appropriate viewer.

Test Report

The Test Report (available in the **Completed Runs & Results** screen) is a clinical report generated by the software that can be downloaded in PDF format. It is identical to the Lab Report, except that it does not include the Sequencing Run Details and Control and Sample QC Evaluation Metrics sections. The Test Report contains the following sections and information.

Note: The display of variants in the Test Report depends on cancer type.

Section	Description
Sample Details	The sample and patient information entered into the software.
Results for Sequence Variations for Therapeutic Use	Displays the results for the clinical variants and gene fusions in the sample, and any recommended therapies that are clinically indicated for the selected cancer type.
Results for Analytical Sequence Variations Detected	Displays the results for analytical variants and gene fusions detected in the sample, depending on the variants associated with the cancer type.
Test Description	A description of the test and the gene variants in associated tissue types that it screens for.
Results for Analytical Sequence Variations Not Detected	Displays the results for analytical variants and gene fusions not detected in the sample, depending on the variants associated with the cancer type.

Lab Report

The Lab Report (available in the **Completed Runs & Results** screen) is a report generated by the software that can be downloaded in PDF format. The Lab Report contains the following sections and information.

Note: The display of variants in the Lab Report depends on cancer type.

Section	Description
Sample Details	The sample information entered into the software.
Results for Sequence Variations for Therapeutic Use	The results for the clinical variants and gene fusions in the sample, and any recommended therapies that are clinically indicated for the selected cancer type. Allele frequencies are also reported.
Results for Analytical Sequence Variations Detected	A list of the analytical variants and gene fusions detected by the assay, and associated information for each, depending on the variants associated with the cancer type.
Test Description	A description of the assay.
Analytical Sequence Variations Not Detected	A list of all the analytical variants and gene fusions not detected by the test, and associated information for each, depending on the variants associated with the cancer type.

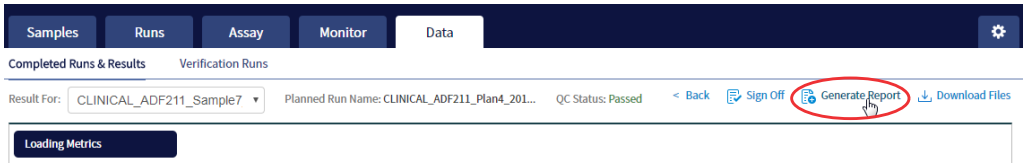
(continued)

Section	Description
Sequencing Run Details	A list of all the kits and instruments used to perform the test.
Control and Sample QC Evaluation Metrics	A summary of the quality control metrics.

Generate customized reports

By default, the Test Report and Lab Report are generated in the language that is selected in the report template. To generate these reports in another language, or change the types of variant calls that are reported, do the following:

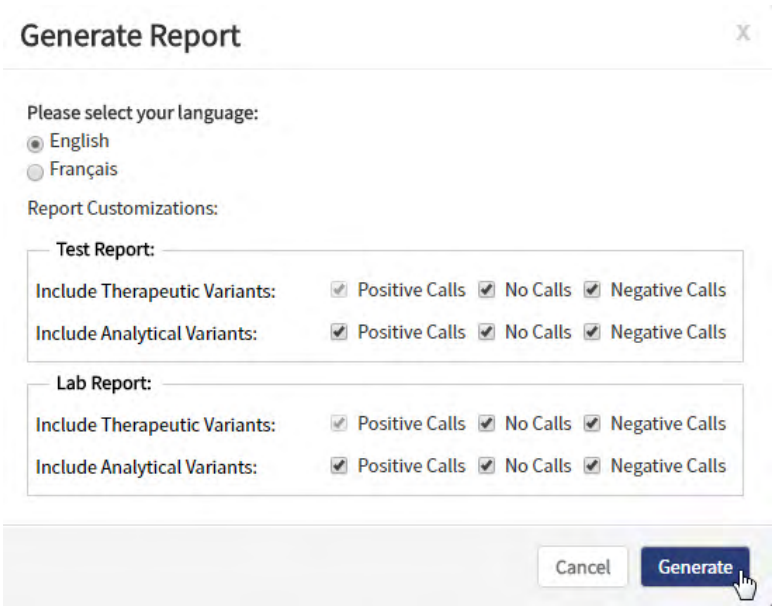
1. In the **Completed Runs and Results** screen, click **View Result**.
2. In the **View Result** screen, click **Generate Report**.



The **Generate Report** dialog box opens.

3. Do one or both of the following:
   - Select the desired language.
   - Select the checkboxes for the positive calls, no calls, or negative calls that you want to report in the Test Report and/or Lab Report.

     Note: These checkboxes are only available in IVD mode if they are enabled for the selected assay.



Note: Positive calls for therapeutic variants cannot be deselected.

4. Click **Generate**.
   A message displays when the report has been generated. The new report overwrites the existing report.

5. Click **Download Files** to open a dialog box to select the new report and other run-related files for download.

Restore archived results

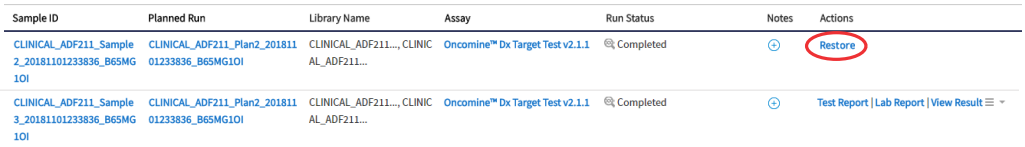
Torrent Suite™ Dx Software can be configured to automatically transfer older run data, results files, and signed reports from an Ion Torrent™ Server to an external server, based on when the results were generated (see "Disk space usage and data archiving" on page 86). Archived results and reports can then be restored to the Ion Torrent™ Server and downloaded from the **Completed Runs & Results** screen.

IMPORTANT!

- If results are restored from runs that were archived in an older version of Torrent Suite™ Dx Software (version 5.6.4 and earlier), the software may not display all the variants from the restored results in the user interface, and reports generated from those restored results may not contain all variants. Carefully review the restored data in the user interface to determine whether all variants are present. Do not generate new reports from results restored from these older archives. Note that all variants are preserved in the restored source files, and can be downloaded using the **Download Files** command. This issue is corrected in version 5.12.5 of the software.
- In older versions of Torrent Suite™ Dx Software (version 5.6.4 and earlier), results reports should be generated and signed before results are archived. See "Sign the run results" on page 67.

Archived results are listed in the **Completed Runs & Results** screen with the **Restore** link active in the **Actions** column. To restore and download archived results:

1. In the **Completed Runs & Results** screen, locate the archived result, then click **Restore** in the **Actions** column.



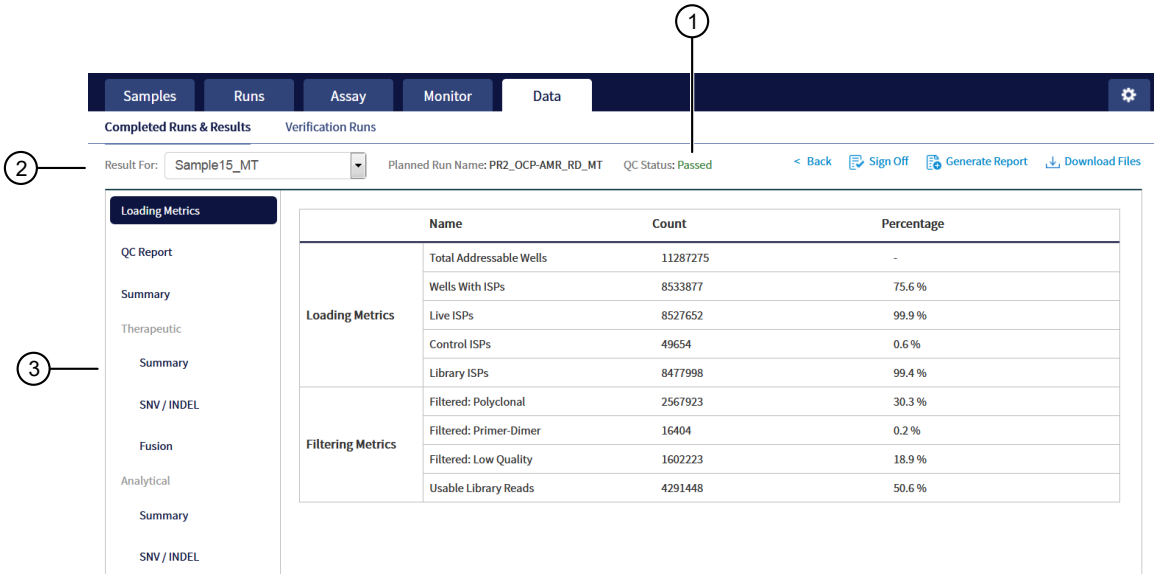
2. Click **OK** in the confirmation dialog box.
   In the **Actions** column, the **View Result** button is now active.

3. Click **View Result**, then click **Download Files** to download and view the restored results files.

View results

The run results shown in the screens described in this section can also be downloaded in the form of results files (see "Results files" on page 65).

- In the **Completed Runs & Results** screen, click **View Result** to view the sequencing run results for a sample of interest.
  The **Results** screen for the selected sample opens, with the **Loading Metrics** screen displayed. Result categories are arranged vertically on the left side of the screen. The QC status for the run is listed at the top of the screen.

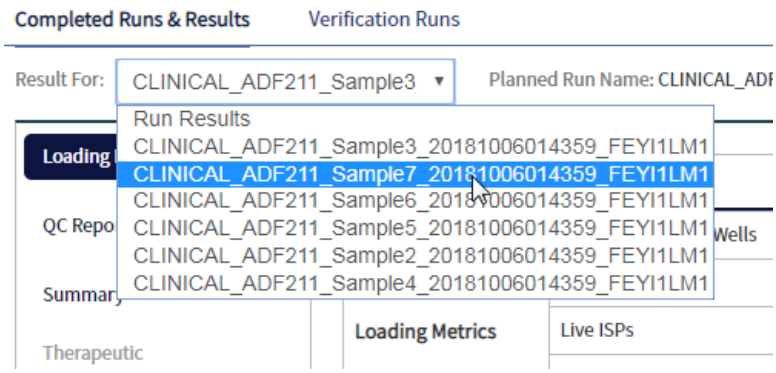


(1) QC status
(2) Selected sample
(3) Result categories

IMPORTANT! A **QC Status** of "Passed" does not guarantee that the genotypes of all analytically relevant variants are determined. See the **Test Result** column in the Lab Report or Test Report for any "No Call" results when interpreting the results.

- Click the links on the left side of the screen to display results of interest, including QC results and results for different categories of variants.

- To view results for another sample in the same run, select the sample from the **Result For:** dropdown list. All samples in a run share the same **Loading Metrics** data. The other result categories are sample-specific.



- To generate the Lab Report and Test Report in other languages, click **Generate Report** (see "Generate customized reports" on page 51).
- To download individual results files for further analysis, click **Download Files** (see "Results files" on page 65).
- To return to the **Completed Runs & Results** screen, click **< Back**.

Loading metrics

The **Loading Metrics** link in the **View Result** screen displays the following loading and filtering metrics for the run:

Metric	Description
Loading Metrics	
Total Addressable Wells	The total number of wells on the chip – excluded wells.
Wells with ISPs	The number (count) and percentage of chip wells that contain ISPs. The percentage is expressed as a percent of total addressable wells.
Live ISPs	The number (count) and percentage of chip wells containing live ISPs (ISP's templated with library or control fragment), with the percentage expressed as a percent of wells with ISPs.
Control ISPs	The number (count) and percentage of ISPs that have a key signal identifying them as internal controls, with the percentage expressed as a percent of live ISPs.
Library ISPs	The number (count) and percentage of ISPs that have a key signal identical to the library key signal, with the percentage expressed as a percent of live ISPs.

(continued)

Metric	Description
Filtering Metrics[1]	
Filtered: Polyclonal	ISPs carrying clones from two or more templates, with the percentage expressed as a percent of library ISPs.
Filtered: Primer-Dimer	ISPs with an insert length of less than 8 bp, with the percentage expressed as a percent of library ISPs.
Filtered: Low Quality	ISPs with low or unrecognizable signal, with the percentage expressed as a percent of library ISPs.
Usable Library Reads[2]	Number (count) and percentage of library ISPs passing all filters.

[1] Filtering Metrics only apply to ISPs templated with library fragments, not control fragment.
[2] Values in the "Filtered:" rows are subtracted from the Library ISPs value (Loading Metrics) to give the Usable Library Reads value.

QC Report

The **QC Report** link displays metrics for the sample libraries and internal controls. This information is also accessible through the **Monitor** tab for the last five runs.

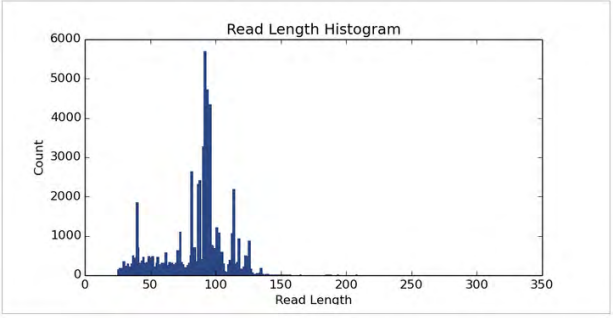
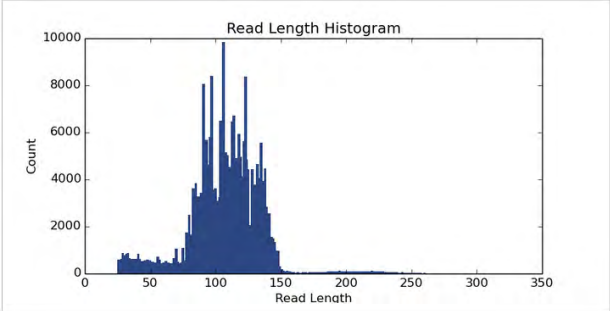
IMPORTANT! The following table describes all the quality control metrics that can be displayed. Whether particular metrics are displayed depends on the assay configuration.

Metric	Description
Library QC Evaluation Metrics	
Library QC: Library RNA	• Mean AQ20 Read Length (bp): The average length, in base pairs, at which the error rate is ≤1% for all aligned reads of a library. • Mean Read Length (bp): The average length, in base pairs, of all reads reported for a given library. • Total Mappable Reads: The number of reads that are mapped to the fusion reference file.[1]
Library QC: Library DNA	• Mean AQ20 Read Length (bp): The average length, in base pairs, at which the error rate is ≤1% for all aligned reads of a library. • Mean Read Length (bp): The average length, in base pairs, of all reads reported for a given library. • Percent Reads: The number of library reads normalized by the total addressable wells in a run.

(continued)

Metric	Description
Control QC Evaluation Metrics	
Control QC: CF-1	• Base Call Accuracy: 1 – (total number of errors for all positions in the control / total number of aligned bases) for Control Fragment-1 (CF-1) reads. • Key Signal: The average peak signal from the incorporation trace measured across the 1-mer incorporations in the CF-1 key sequences. [2] • Mean AQ20 Read Length (bp): The average length, in base pairs, at which the error rate is ≤1% for all aligned reads of CF-1. • Percent Reads: The number of all usable library reads that aligned with the CF-1 sequence divided by the total number of addressable wells.
Control QC: DNA Control	• COSMIC ID variant calls and allelic frequencies: The individual variant positions and wild-type positions that are assessed in the DNA control reagent for presence or absence of the variant. • Mean AQ20 Read Length (bp): The average length, in base pairs, at which the error rate is ≤1% for all aligned reads of a control. • Percent Reads: The number of all usable library reads that aligned with the control fragment sequence divided by the total number of addressable wells.
Control QC: RNA Control	• Fusion calls and number of reads: The individual fusion calls and number of reads that are assessed in the RNA control reagent for the presence or absence of the fusion. • Total Mappable Reads: the number of reads that are mapped to the fusion reference file.
Control QC: DNA NTC	• Hotspot Calls: The total number of hotspots where a call was made in the no-template control.
Control QC: RNA NTC	• Total Fusion Calls: The total number of fusion locations where a call was made in the no-template control. • Total Mappable Reads: The number of reads in the no-template control that are mapped to the fusion reference file.

(continued)

Metric	Description
Histogram of Read Length [3]	• A library-specific read-length histogram: RNA  DNA 

[1] May not equal the sum of the individual fusions, since some detected fusions are not included in the fusion BED file.

[2] The minimum Key Signal value for the CF-1 control (i.e., the Templating Control) is set to 0 in all preinstalled assays and new custom assays in Torrent Suite™ Dx Software version 5.8 and later. Custom assays created in previous versions of the software may have a higher Key Signal value for the control. If a run using an older custom assay fails QC due to this metric, reanalyze the run using a minimum Key Signal of 0 for the control. For future runs, create a new custom assay based on the old assay, changing the minimum Key Signal to 0.

[3] Not displayed in the Monitor tab.

Summary

The **Summary** link displays the following information for the run:

Field	Description
Run and Configuration Summary	
Run Name	The name of the run.
Assay Name	The name of the assay.
Reference Genome	The reference genome used for analysis.
Target Region	The name of the targeted regions BED file used.
Hotspot Regions	The name of the hotspot regions BED file used.
Fusion Reference	The name of the fusion reference used for analysis, when applicable.
Fusion Panel	The name of the fusion panel used, when applicable.

(continued)

Field	Description
Library Name	The names of the DNA and DNA libraries prepared from the sample, entered during library batch preparation.
Amplicon Summary	
Number of Targets	The number of amplicons in the panel.
On Target Reads	The number of reads mapped to the target amplicons.
Percent On Target Reads	The percentage of reads mapped on target to total reads.
Percent Full Length On Target Reads	The percentage of full length reads mapped on target to total reads.
Average Coverage	The ratio of the depth of coverage at each base in the target region to the length of the target region.
Target Coverage at 20X	The percentage of base positions with depth of coverage ≥20X in the target region.
No Strand Bias	The percentage of bases with a strand bias between 30% and 70%.
Coverage Uniformity	The ratio of passed number of targets to total number of targets, where passed number of targets is the number of amplicons that have at least 0.2X mean coverage.
Variant Summary[1]	
Number of SNVs/MNVs	The number of single- and multi-nucleotide variations (SNVs/MNVs).
Number of INDELs	The number of insertions or deletions.
Number of Fusions	The total number of fusion calls.

[1] Includes Therapeutic, Analytical, and Level2 variants in the **View Result** screen.

Therapeutic variant results

Therapeutic variant results for the selected sample are provided under the **Therapeutic** section of the left navigation bar in the **Results** screen. Depending on the assay, results for these variants may be divided into multiple subscreens, each with a separate link (**SNV/INDEL**, **Fusion**, etc.).

The results are determined by the reference sequences installed on the server, the QC controls used in the run, the assay used for the run, and any Reporting Gene List associated with that assay.

Columns in the screen or subscreens are described in the following table.

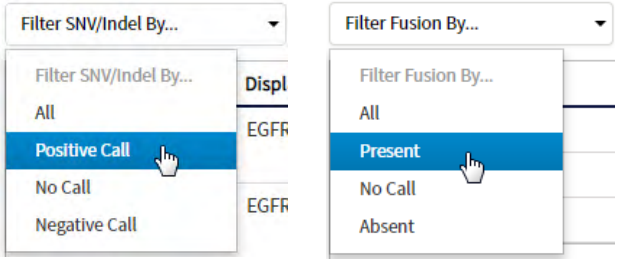
Column	Description
Summary	
Gene Fusion Present Variants Present	The therapeutic gene fusion or SNV/INDEL variant detected in the sample
Associated Therapy	The therapy indicated for each variant listed
SNV / INDEL	
Gene	The gene name, which provides a link to the **View Annotation Sources** popup with additional information about the HotSpot ID (see "View annotation sources" on page 63)
Display Name	The gene name with the exonic location of the deletion or insertion
Amino Acid Change	The amino acid change using HGVS-nomenclature
Nucleotide Change	Identification of the DNA-level nucleotide change using HGVS-nomenclature
Test Result	The presence or absence of the variant
Allele Frequency	The most likely frequency of the variant allele[1]
HotSpot ID	The name of the hotspot as defined in the BED file
Locus	The one-base position of the variant in the reference genome
Type	The type of variation detected: • snv (single nucleotide variation)/mnv (multi-nucleotide variation) • ins (insertion) • del (deletion) • complex
Genotype	The genotype at the locus
Ref	The reference base or bases at the locus
Quality Score	The relative probability of either the "reference" hypothesis interval [0,cutoff) or the "variant" hypothesis interval [cutoff,1], on a Phred scale (-10*log10). This provides a measure of the strength of the evidence for the variant call. A higher score means more evidence for the call. Quality scores are capped at 100.
Coverage	The number of reads covering the variant position after down-sampling
Fusion	
Gene	The gene that regulates expression of the gene fusion
Display Name	The gene name of the fusion

(continued)

Column	Description
Read Count	The number of valid reads aligned to the specific fusion sequence
Test Result	The presence or absence of that fusion variant

[1] The allele frequency is the most likely variant frequency in the reads after corrections are made for probable errors. The software uses this corrected frequency and the uncertainty in the observations to calculate the probability that the variant frequency in the sample falls within defined intervals that can be set by the user. The user sets a threshold frequency for deciding between genotypes: this defines intervals [0,c)[c,1-c)[1-c,1] corresponding to the three diploid genotypes (reference, heterozygous, homozygous) respectively, where "c" is the minimum variant frequency set during the creation of the assay. The software reports the genotype corresponding to the interval with the highest probability of containing the variant frequency in the sample. In most cases, the reads are highly accurate and do not have a high probability of error. In such cases, the observed frequency and the most likely frequency are similar, and are contained within a single interval leading to a high quality genotype. In some cases, if very few reads are observed or the error rate is high, the observed counts and the most likely counts can be different, and the uncertainty in the real sample frequency can be high. When this happens, it is often impossible to exclude a heterozygous population in the sample, and the genotype is assigned to be heterozygous, since a significant portion of the probability falls in that interval.

Note: You can filter the variant lists using options in the **Filter SNV/Indel By...** and **Filter Fusion By...** dropdown lists in the upper left corner of the results report.



Analytical variant results

Analytical variant results for the selected sample are provided under the **Analytical** section of the left navigation bar in the **Results** screen. Depending on the assay, results for these variants may be divided into multiple subscreens, each with a separate link (**SNV/INDEL**, **Fusion**, etc.).

The results are determined by the reference sequences installed on the server, the QC controls used in the run, the assay used for the run, and any Reporting Gene List associated with that assay.

Columns in the screen or subscreens are described in the following table.

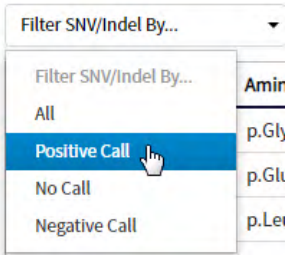
Column	Description
Summary	
Variants Present	Lists the analytical SNV or INDEL variants detected in the sample
SNV / INDEL	
Gene	The gene name, which provides a link to the **View Annotation Sources** popup with additional information about the HotSpot ID (see "View annotation sources" on page 63)
Amino Acid Change	The amino acid change using HGVS-nomenclature

(continued)

Column	Description
Nucleotide Change	The DNA-level nucleotide change using HGVS-nomenclature
Test Result	The presence or absence of the variant
Allele Frequency	The most likely frequency of the variant allele
HotSpot ID	The name of the hotspot as defined in the BED file
Locus	The one-base position of the variant in the reference genome
Type	The type of variation detected: • snv (single nucleotide variation)/mnv (multi-nucleotide variation) • ins (insertion) • del (deletion) • complex
Genotype	The genotype at that locus
Ref	The reference base or bases at the locus
Quality Score	The relative probability of either the "reference" hypothesis interval [0,cutoff) or the "variant" hypothesis interval [cutoff,1], on a Phred scale (-10*log10). This provides a measure of the strength of the evidence for the variant call. A higher score means more evidence for the call. Quality scores are capped at 100.
Coverage	The number of reads covering the variant position after down-sampling
Fusion[1]	
Gene	The gene that regulates expression of the gene fusion
Display Name	The gene name of the fusion
Read Count	The number of valid reads aligned to the specific fusion sequence
Test Result	The presence or absence of that fusion variant

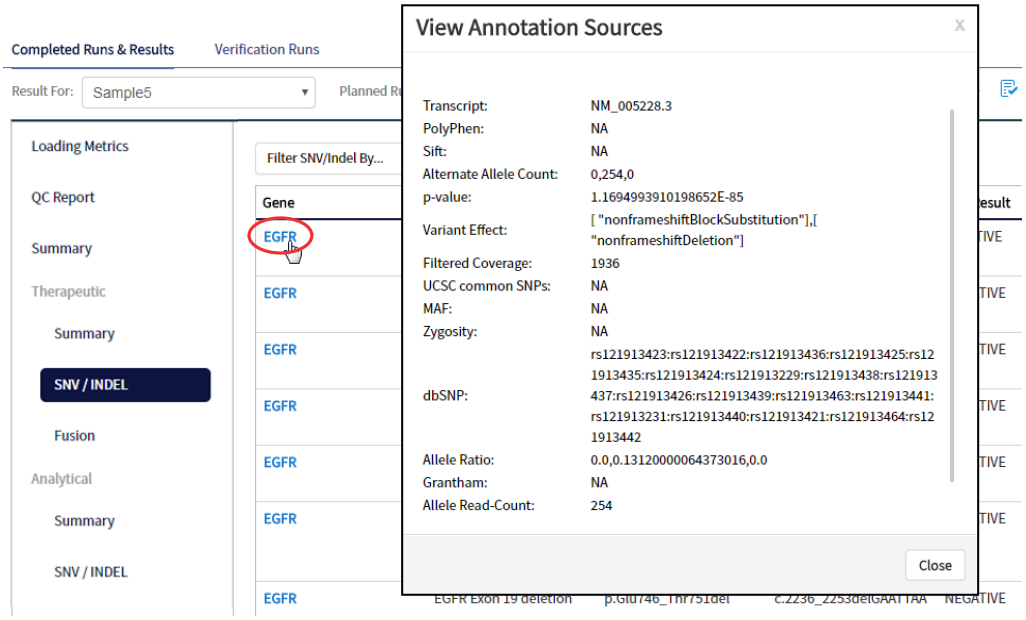
[1] Note that for the Oncomine™ Dx Target Test, no analytical fusions are reported except for expression controls.

Note: You can filter the variant list using options in the **Filter SNV/Indel By...** dropdown list in the upper left corner of the results report.



View annotation sources

You can view additional information for each hotspot ID listed under the **SNV/Indel** links of the **Therapeutic** and **Analytical** sections in the **View Results** screen. The gene name in the **SNV/Indel** report is a link that opens the **View Annotation Sources** dialog box, which provides information for the particular hotspot.



Example of transcript information in the VCF

The COSM1074639 hotspot ID has multiple transcripts associated with it, which include a mutation from 'A' to 'C'. These are listed in the VCF file as shown below:

chr6 152419923 COSM1074639;COSM1074637 A C,G 122.19 PASS
AF=0,0;AO=0,0;DP=1116;FAO=0,0;FDP=1116;FR=.;FRO=1116;FSAF=0,0;FSAR=0,0;FSRF=661;FSRR=455;F
WDB=0.00426152,-
0.0087378;FXX=0;HRUN=1,1;HS;LEN=1,1;MLLD=103.308,103.438;QD=0.437949;RBI=0.0394298,0.03043
16;REFB=-2.22051E-5,-2.32713E-5;REVB=-0.0391988,-
0.0291502;RO=1115;SAF=0,0;SAR=0,0;SRF=661;SRR=454;SSEN=0,0;SSEP=0,0;SSSB=-6.44874E-8,-
6.44874E-
8;STB=0.5,0.5;STBP=1,1;TYPE=snp,snp;VARB=0,0;OID=COSM1074639,COSM1074637;OPOS=152419923,
152419923;OREF=A,A;OALT=C,G;OMAPALT=C,G;FUNC=[

{'normalizedRef':'A','transcript':'NM_001122742.1','grantham':'144.0','gene':'ESR1','location':'exonic','ori
gAlt':'C','origPos':'152419923','origRef':'A','normalizedPos':'152419923','exon':'10','function':'missense','
protein':'p.Tyr537Ser','normalizedAlt':'C','gt':'neg','codon':'TCT','coding':'c.1610A>C'},

{'normalizedRef':'A','transcript':'NM_001122742.1','grantham':'194.0','gene':'ESR1','location':'exonic','ori
gAlt':'G','origPos':'152419923','origRef':'A','normalizedPos':'152419923','exon':'10','function':'missense','
protein':'p.Tyr537Cys','normalizedAlt':'G','gt':'neg','codon':'TGT','coding':'c.1610A>G'},

{'normalizedRef':'A','transcript':'NM_001122741.1','grantham':'144.0','gene':'ESR1','location':'exonic','ori
gAlt':'C','origPos':'152419923','origRef':'A','normalizedPos':'152419923','exon':'9','function':'missense','p
rotein':'p.Tyr537Ser','normalizedAlt':'C','gt':'neg','codon':'TCT','coding':'c.1610A>C'},

{'normalizedRef':'A','transcript':'NM_001122741.1','grantham':'194.0','gene':'ESR1','location':'exonic','ori
gAlt':'G','origPos':'152419923','origRef':'A','normalizedPos':'152419923','exon':'9','function':'missense','p
rotein':'p.Tyr537Cys','normalizedAlt':'G','gt':'neg','codon':'TGT','coding':'c.1610A>G'},

{'normalizedRef':'A','transcript':'NM_001122740.1','grantham':'144.0','gene':'ESR1','location':'exonic','ori
gAlt':'C','origPos':'152419923','origRef':'A','sift':'0.0','normalizedPos':'152419923','exon':'9','function':'mi
ssense','protein':'p.Tyr537Ser','gt':'neg','normalizedAlt':'C','codon':'TCT','polyphen':'0.979','coding':'c.161
0A>C'},

{'normalizedRef':'A','transcript':'NM_001122740.1','grantham':'194.0','gene':'ESR1','location':'exonic','ori
gAlt':'G','origPos':'152419923','origRef':'A','sift':'0.0','normalizedPos':'152419923','exon':'9','function':'mi
ssense','protein':'p.Tyr537Cys','gt':'neg','normalizedAlt':'G','codon':'TGT','polyphen':'0.998','coding':'c.16
10A>G'},

Results files

The following files can be downloaded from the **Results Report** window. To download the files, click **Download Files**, select the files to download, then click **Download**.

File name	Description
Test Report	A report of the completed analysis in PDF format
Lab Report	A clinical lab report of the completed analysis in PDF format; includes both clinical and analytical results.
PlannedRun-AuditTrail.pdf	Contains all audit records pertaining to the Planned Run.
Info.csv	Contains information about the run and analysis, such as software, sequencing information, instrument information, analysis information, QC details etc.
<RNABarcode>_rawlib.basecaller.bam	Unmapped RNA BAM File; output of base calling, contains unmapped reads.
Snvindel.tab	A tab-delimited file that contains information about non-targeted SNVs and indels
<RNABarcode>_rawlib.basecaller_alignments.bam	Mapped RNABarcode BAM file; output after reads have been mapped to the fusion reference.
Target_Summary.tab	A tab-delimited file that contains a targeted test results summary
<RNABarcode>_rawlib.basecaller_alignments.bam.bai	Mapped RNABarcode BAM index file
<RNABarcode>_rawlib.basecaller.fastq	FASTQ file generated from unmapped BAM file of the RNA barcode used.
<DNABarcode>_rawlib.basecaller.bam	Unmapped DNA barcode BAM file; output of base calling, contains unmapped reads.
raw_peak_signal	Key signal gives the percentage of LiveISPs with a key signal that is identical to the library key signal.
<LibPrepID>_<analysisID>.final.vcf	A VCF file containing all the variants detected as a result of the analysis, along with information such as test result, read count, gene name, quality scores, etc.
Summary.tab	A tab-delimited file that contains the on-targeted test results summary
<LibPrepID>_rawlib.stats.cov.txt	Amplicon statistics file

(continued)

File name	Description
Fusion.tab	A tab-delimited file that contains non-targeted (analytical) fusion details in a table format. **Note:** The information displayed in the file for each isoform of a particular fusion is identical, because specific isoform and locus information is not included in this table. Detailed isoform and locus information is available in the <LibraryPrepID>_<AnalysisID>.final.vcf file, available under **Download Files**.
readLenHisto.png	Gives the read-length distribution of FASTQ files in the form of a histogram. A thumbnail histogram of the read lengths for a particular barcode.
<LibPrepID>_rawlib.bam.bai	Mapped DNA barcode BAM index file (index file of DNA barcode-mapped BAM file)
Basecaller.log	Base Caller log file
analysis.log	Analysis log file
sigproc.log	Signal processing log file
Bead_density_contour.png	Loading density image; a pseudo-color density image of the Ion Chip plate showing percent loading across the physical surface
<DNABarcode>_rawlib.basecaller.fastq	FASTQ file of the DNA barcode used
Target_cnv.tab	Targeted CNV detail table (CNV results for targeted variants from a sequencing run) **Note:** For use with IVD tests that include CNV reporting.
cnv.tab	Non-targeted CNV detail table (analytical CNV results from a sequencing run) **Note:** For use with IVD tests that include CNV reporting.
Target_fusion.tab	A tab-delimited file that contains targeted (clinical) fusion details in a table format
<LibPrepID>_rawlib.bam	Mapped DNA barcode BAM File; output after mapping reads to reference.
Iontrace_Library.png	Key incorporation trace image showing the average signal readings for flows of the bases T, C, and A in the library key.
rawtf.basecaller.fastq	FASTQ file for the test fragment

Sign the run results

In the **View Result** screen, Managers/Administrators can provide their electronic signature on the run results. The signature information appears in the **QC Report** in the **View Result** screen, and in the downloaded Test Report and Lab Report PDFs.

Multi-language support for PDF report generation is provided. By default reports are generated in the language that is selected in the **Report Template** used. When reports are generated in multiple languages, **Sign Off** occurs only in the report of the default language.

1. At the top of the **View Result** screen, click **Sign Off**, then enter your user name, password, and comments in the dialog box. Fields identified with a red asterisk (*) are required fields.
2. In the **Footer Field**, enter any text.
3. Click **Sign Off** to confirm your electronic signature.

Files in the Reports folder

When a manager- or administrator-level user signs a report, a folder named with the Sample ID is created in the Reports folder on the server (`/results/analysis/output/reports`), and the following files are copied into it:

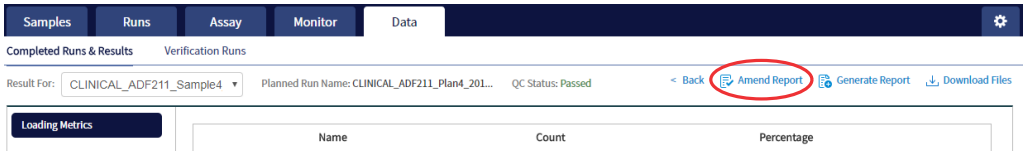
- Info file (.csv)
- Signal processing log file (.log)
- Targeted Fusion Detail Table (.tab)
- Targeted Test Results Summary (.tab)
- Analysis log file (.log)
- VCF file (.vcf)
- DNA Mapped BAM file (.bam)
- DNA Unmapped BAM file (.bam)
- Key Signal
- Key Incorporation Trace (.png)
- Fusion Detail Table (.tab)
- DNA Mapped BAM Index file (.bam.bai)
- RNA Mapped BAM Index file (.bam.bai)
- DNA FASTQ File (.fastq)
- Base Caller Log File (.log)
- Targeted CNV Detail Table (.tab)[1]
- Non-targeted CNV Detail Table (.tab)[1]
- SNV/INDEL Detail Table (.tab)
- Non-targeted Test Results Summary (.tab)
- Targeted SNV/INDEL Detail Table (.tab)
- Amplicon Stats (_rawlib.stats.cov.txt)
- RNA FASTQ File (.fastq)
- RNA Mapped BAM file (.bam)
- RNA Unmapped BAM file (.bam)
- Test Fragment FASTQ File (.fastq)
- Read Length Histogram (.png)
- Test PDF Report *(optional)* (.pdf)
- Lab PDF Report (.pdf)
- Planned Run Audit (.pdf)
- Basecaller command files (.json)
- checksum file
- Pipeline commands (_pipeline.json)
- Experimental log file (_final.txt)
- Wells with beads (_beadogram.png)
- Bead find stats file (.stats)
- Loading Density Figure (.png)

[1] For use with IVD tests that include CNV reporting.

Amend a signed-off report

Managers and administrators can amend a report within 30 days of signing off. A sample that has a signed-off report has a ✔ symbol to the left of the Sample ID in the **Completed Runs & Reports** screen. You can change sign-off or laboratory comments, or the text that appears in the report footer. Follow these steps to amend a signed-off report:

1. In the **Completed Runs & Reports** screen, click **View Result** for the sample of interest.
2. Click **Amend Report**.



3. In the **Electronic Signature** dialog box, make your changes in the appropriate fields, then click **Amend Report**.
4. Click **Test Report** or **Lab Report** to download the amended report.

Repeat testing

Based upon quality control results, users can determine whether a library requires repeat testing. Refer to the following table to determine the best course of action. See the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide* (Pub. No. MAN0018810) for the details of each procedure.

Quality Control			Pooling Error[1]	Retest Sample	Procedures to follow
Library	Run	Control			
Pass	Pass	Pass	No	N/A	Sign off on Library.
Fail	Pass	Pass	No	gDNA	Starting from library preparation.[2]
Fail	Pass	Pass	Yes	Library	Starting from preparing the library pool. If the retest of the sample library fails, retest the gDNA sample starting from library preparation.
Fail	Pass	Fail	No	gDNA	Starting from library preparation.
Fail	Pass	Fail	Yes	Library	Starting from preparing the library pool. If the retest of the library fails, retest the gDNA sample starting from library preparation.
Fail	Fail	Pass	No	gDNA	Starting from library preparation.

(continued)

Quality Control			Pooling Error[1]	Retest Sample	Procedures to follow
Library	Run	Control			
Fail	Fail	Pass	Yes	Library	Starting from preparing the library pool. If the retest of the library fails, retest the gDNA sample starting from library preparation.
Pass (all samples)	Pass	Fail	No	Pooled Library	Starting from the pooled sample libraries, repeat template preparation. If the CF-1 fails QC a second time, reprepare the library pool.
Pass (all samples)	Fail	Fail	No	Pooled Library	Starting from the pooled sample libraries, repeat template preparation. If the CF-1 fails QC a second time, reprepare the library pool.
Pass (all samples)	Fail	Pass	No	Pooled Library	Starting from the pooled sample libraries, repeat template preparation. If the CF-1 fails QC a second time, reprepare the library pool.

[1] Pooling error is defined as no reads detected for a Barcode ID.

[2] **IMPORTANT!** If a library fails Library QC in a second run that passes both Run and Control (CF-1) QC metrics, the sample is considered invalid.

Data files and flow

During an Ion PGM™ Dx Sequencer run, sequence raw data (DAT) files are transferred to the Ion Torrent™ Server via a network cable in a process controlled by the ionCrawler service. After the data from the initial flows on the sequencer are available on the server, the Torrent Suite™ Dx Software begins processing the data, producing the <1> wells file. Basecalling is performed on the <1> wells file data, producing an unmapped BAM (uBAM) file. Subsequent analysis produces mapped reads (BAM) and variant calls (VCF) files.

The following table shows the flow of data and typical file size generated as the Torrent Suite™ Dx Software processes data from an Ion 318™ Dx Chip.

Process	File Type	File Size
		Ion 318™ Dx Chip
Sequencing[1]	DAT	~350 GB
	▼	
Signal Processing	Wells	12 GB
	▼	
Base Calls (reads)	uBAM	1.5 GB
	▼	
Mapped Reads	BAM	4.3 GB
	▼	
Variant Calls	VCF	120 Kb

[1] The sequencing raw data (DAT) files are deleted from the server 72 hours after data analysis to conserve Ion Torrent™ Server disk space.

Note: Data from approximately 200 sequencing runs using an Ion 318™ Dx Chip can be accommodated on an Ion Torrent™ Server before disk space becomes a limiting factor and data archiving is required. See "Data Management (administrator)" on page 84 for more information.

Verification runs on the Ion PGM™ Dx System

Verification runs are sequencing runs performed during Ion PGM™ Dx System installation by Thermo Fisher Scientific support specialists to validate the performance of the instruments.

Verification runs are performed using Torrent Suite™ Dx Software. Verification runs can also be performed using Torrent Suite™ Assay Development Software, but are not required.

You can view verification run reports, but only qualified support specialists can perform the runs. Under the **Data** tab, in the **Verifications Runs** screen, you can search, filter, sort, and view completed verification runs and reports.

The following information is displayed in the **Verification Runs** screen:

Column	Description
Planned Run	The name of the run, created when the run was created. Click the name to open the **Review Planned Run** dialog box. • Click **Audit** to display the list of users who created/edited the Planned Run. You can export and print information from the list from the **Audit Trail** dialog box. • Click **CSA** (Customer Support Archive) to download all the instrument log files, which can be useful for troubleshooting.
Field Engineer Name	The name of the support specialist who performed the run.
Instrument Name	The name of the validated instrument.
Templating Completion	The completion date and time of the Ion OneTouch™ Dx System run. Click the link to open a dialog box that includes additional information about the instrument, operator, and template kit used.
PGM Completion	The completion date and time of the Ion PGM™ Dx System run. Click the link to open a dialog box that includes additional information about the sequencer, operator, sequencing kit, and chip used.
Analysis Completion	The completion date and time of the post-sequencing run analysis.
Run status	The current status of the full run, including analysis.
QC Status	Indicates whether a run passed or failed, based on the QC metrics selected for the assay.
PQ Report	If the results of the run have been approved and signed off by a Manager or Administrator, the approved PQ Report are listed in this column.
Actions	• Click **View Results** to view the results of completed runs that have not yet been approved. Managers and Administrators can also sign off on the results after viewing them. • Click **View Report** to view a report of the run.

Verification run results

Under the **Data** tab, in the **Verifications Runs** screen, click **View Results** in the **Actions** column to view the performance qualification (PQ) report for a verification run.

Note: Manager- and administrator-level users can sign off on this report, at which point it becomes a locked PQ Report. However, we recommend that only qualified support specialists sign off on PQ reports.

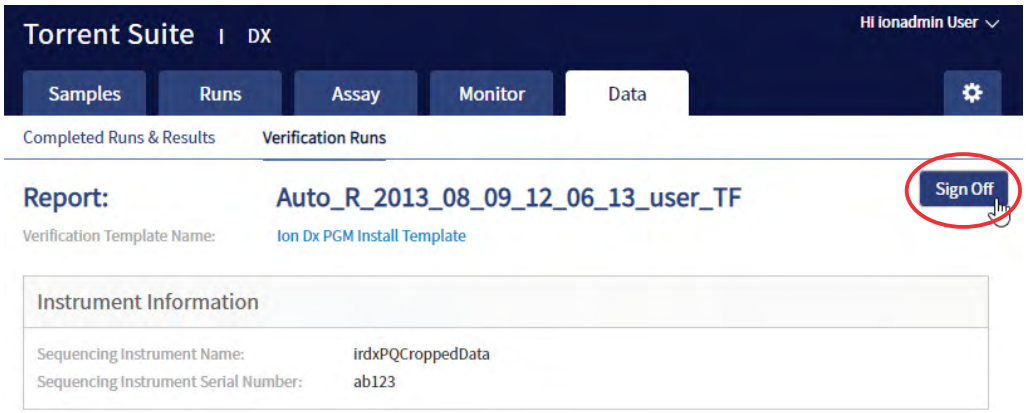
In addition to basic information about the verification run, the following data are reported for the controls that are used in the run:

Metric	Description
Base Call Accuracy	1 – (total number of errors for all positions in the control / total number of aligned bases)
Mean AQ20 read length (bp)	The average length, in base pairs, at which the error rate is ≤1% for all aligned reads of a control fragment
Percent Reads	The number of all usable library reads that align with the control fragment sequence, divided by the total number of addressable wells

Sign verification run reports (manager/administrator)

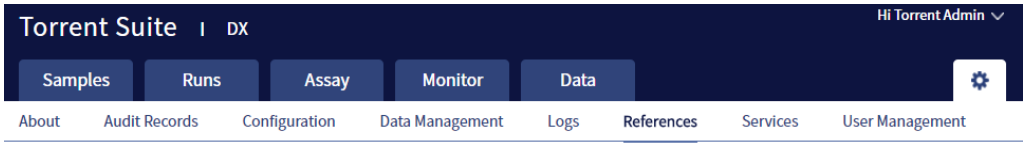
Manager- and administrator-level users can sign results reports for verification runs. However, we recommend that only qualified support specialists sign PQ reports.

1. Click **Data** ▸ **Verification Runs**.
2. In the **Report** window, click **Sign Off** in the upper right corner above the report.



3. In the **Sign off Electronic Signature** dialog box, enter your user name, password, and any comments, then click **Sign Off.**
4. The report is locked and listed in the **PQ Report** field for the run in the **Verification Runs** screen.
5. Click the PQ report name to view, print, or download the report in a PDF file.

8 System administration and management



In the ⚙ **tab**, Managers and Administrators can:

- View, create, and manage reference files, including genome references, panel and hotspot BED files, primers, and others.

  Note: The Oncomine™ Dx Target Test assay is locked. None of the files for this assay can be edited; they can only be viewed.

- View system information and settings.

In addition, Administrators can:

- View and export audit records.
- View and change configuration settings.
- Create and manage user accounts.
- Manage log files.

Access to ⚙ tab functions by user level

In the ⚙ **tab**:

Users at this level…	Can access...
Operator	**About**: More Information and Assistance • Support Contact Information: a link directing users to the **thermofisher.com/technical-resources** website. • Instrument Diagnostics: performs a diagnostic check of the Ion PGM™ Dx Sequencer. • End User License Agreement: opens the End User License Agreement (EULA).

(continued)

Users at this level…	Can access...
Manager	Operator functions plus: **References**: displays and allows management of: • Reference Sequences • Panels • Hotspots • DNA Barcodes • Fusion Reference • Fusion Panel • Reporting Gene List • Control Fragments • Primers **Services**: displays system status report for: • Jobs Server • Active Jobs • ionCrawler Service Details • Smart Monitoring Service Details
Administrator	Manager functions plus: **Audit Records**: perform an audit: search, sort, view, export, and print any record. **Configuration**: Configure settings for the: • Network • Email • Instruments • Lab Information • Software Updates **Data Management**: Manage archiving, view available disk space, and manually delete incomplete runs. **Logs**: Manage retention of instrument logs and Torrent Suite™ Dx Software logs. **User Management**: Add, assign user level, and edit user information.

References (manager/administrator)

Under the ⚙ tab in the **References** screen, manager- and administrator-level users can access the following reference files under the subtabs.

Note: These files are defined by the Oncomine™ Dx Target Test Assay Definition File. They are locked and can only be viewed or exported.

- Reference sequence files
- Panel files (BED format)
- Hotspots files (BED format)
- DNA barcode sequences
- Fusion reference sequences (FASTA format)
- Fusion panel files (BED format)
- Reporting Gene Lists (Microsoft Excel™ format)
- Control fragments
- Primer sequences (FASTA format)

Manage reference sequences

Manager- and administrator-level users manage the DNA reference sequence files that are available in the software

Manage panel files

Manager- and administrator-level users can view and manage the panel BED files that are available in the software.

1. Click ⚙ ▸ **References**
2. Select the **Panels** tab.
3. To export a panel file, in the **Actions** column, click **Export**.

Manage hotspot files

Manager- and administrator-level users can view and manage the hotspot BED files that are available in the software

1. Click ⚙ ▸ **References**
2. Select the **Hotspots** tab.
3. To export a hotspot file, in the **Actions** column click **Export**.

View DNA barcodes

Manager- and administrator-level users can view the barcode IDs and sequences for each barcode set that is available in the software.

1. Click ⚙ ▸ **References**.
2. Select the **DNA Barcodes** tab.

3. In the **DNA Barcodes** screen, click the name of a barcode set in the list.
   A window opens showing the name and sequence of each barcode in the set.

4. Click **Back** to return to list on the **DNA Barcodes** screen.

Manage the Reporting Gene List

Manager- and administrator-level users can view and manage reporting gene lists for the Oncomine™ Dx Target Test. A gene list is the list of the genes and mutations that are used to generate reports for an assay.

1. Click ⚙ ▸ **References**

2. Select the **Reporting Gene List** tab.

3. In the **Reporting Gene List** screen, to view the contents of the gene list:
   a. In the **Name** column, click the name of the gene list.
      A screen opens showing the gene names, mutation IDs, and other information about the genes in the list.

   b. Click **Back** to return to the **Reporting Gene List** screen.

4. To export the gene list, in the **Actions** column, click **Export**.

Control fragments

Manager- and administrator-level users can review information about control fragments. These internal controls are predefined and cannot be modified by users.

View the control fragment information by clicking ⚙ ▸ **Refrences** ▸ **Control Fragments**. The **Control Fragments** screen provides the following information.

Column	Description
Name	The name of the control fragment.
Sequence	The single letter nucleotide sequence of the control fragment.
Control	The process in which the control fragment is used.
Status	Indicates that the control fragment information is locked and cannot be modified.

Manage primers

Under the ⚙ tab, in the **References** screen on the **Primers** tab, manager- and administrator-level users can view the primer sequences in the software.

Services (manager/administrator)

On the **Services** screen, manager- and administrator-level users can view the status and details of active jobs, ionCrawler service, Smart Monitoring service, and the jobs server. Viewing this information can be useful when troubleshooting error messages during an active job or why an analysis failed.

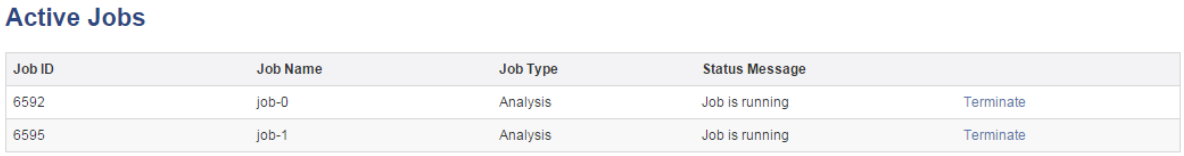
Services

Service	Function
Jobs Server	Lists server information and active (running) software.
Active Jobs	• Lists active and queued analysis jobs on the Ion Torrent™ Server. • When no job is currently active, displays a "No active jobs" message.
ionCrawler Service Details	Displays information about the data transfer process from the Ion PGM™ Dx Sequencer to the Ion Torrent™ Server.
Smart Monitoring Service Details	Allows remote monitoring of Ion PGM™ Dx System.

View or terminate an active job

Manager- and administrator-level users can view jobs that are active on the server and terminate an active job if required. A job is active when the status indicates Job is running.

1. Click ⚙ ▸ **Services**
2. In the **Active Jobs** section, click **Terminate** to stop the active job.



A confirmation message opens, "Are you sure you want to terminate `Job Name` ?"

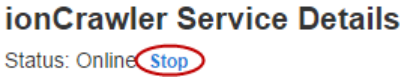
3. Click **Yes** to terminate the active job.

Disable or enable the ionCrawler service

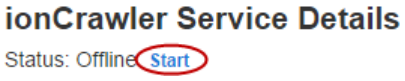
Manager- or adminstrator-level users can manage the IonCrawler service. The ionCrawler service typically remains enabled at all times, but can be disabled for remote service troubleshooting.

- To disable the service:
  a. Click ⚙ ▸ **Services**.

b. In the **ionCrawler Service Details** section, click **Stop**.



c. In the confirmation message, click **Yes** to stop the service.
The **Stop** option changes to **Start**.



- To enable the service:
  a. Click ⚙ ▸ **Services**.
  b. In the **ionCrawler Service Details** section, click **Start**.
  c. In the confirmation message, click **Yes** to start the service.
  The **Start** option changes to **Stop**.

Disable or enable the Smart Monitoring service

Manager- and administrator-level users can manage the Smart Monitoring service.

The Smart Monitoring service allows Thermo Fisher Scientific personnel to remotely monitor the status of the Ion PGM™ Dx System through an internet connection. Smart Monitoring employs multiple layers of security, including a Secure Sockets Layer (SSL) and Lightweight Directory Access Protocol (LDAP) authentication, to provide real-time troubleshooting and problem resolution for the Ion PGM™ Dx System.

The Smart Monitoring service is active by default and can be disabled by manager- or administrator-level users, if required.

- To disable the service:
  a. Click ⚙ ▸ **Services**.
  b. In the **Smart Monitoring Service Details** section, click **Stop**.
  c. In the confirmation message, click **Yes** to disable the Smart Monitoring service.
  The **Stop** option changes to **Start**.

- To enable the service:
  a. Click ⚙ ▸ **Services**.
  b. In the **Smart Monitoring Service Details** section, click **Start**.
  c. In the confirmation message, click **Yes** to enable the Smart Monitoring service.
  The **Start** option changes to **Stop**.

Audit records (administrator)

In the **Audit Records** screen, administrator-level users can use the tools that are provided to review, sort, export, and print audit records.

All components in a diagnostic assay must be uniquely identified, and the identification must be stored so that the record can be audited.

Note: Library Batches and Planned Runs created in a batch with other objects in a single LIMS transaction do not have a "Create" action listed in the **Audit Records** screen. However, you can view their complete audit record from the **Libraries** and **Planned Runs** screens, and any subsequent actions that are performed on them are listed in the **Audit Records** screen. A Library Batch or Planned Run created individually in a LIMS transaction is listed.

Search audit records

Administrator-level users can use the toolbar to search for an existing audit record.

1. Click ⚙ ▸ **Audit Records**.
2. In the **Audit Records** screen, select the search parameters:
   a. To select a date range, click 📅 next to **Start Date** and **End Date**.
      If an end date is not selected, the search results include all records from the start date through the current date.
   b. To limit the results to actions that are performed by a specific user, select the user from the **First Name Last Name** list.
   c. To limit the results to specific actions, select from the **Select Action** list.
   d. To limit the results to a particular sample, select from the **Sample** list.
3. Click **Search**.
4. Click **Clear** to return to the complete list of records.

Sort audit records

In the **Audit Records** screen, the list of audit records is displayed with the oldest record on top by default. Administrator-level users can sort the **Audit Records** screen to find audit records. To return to the default display, click **Audit Records** or **Clear**.

1. Click ⚙ ▸ **Audit Records**.
2. In the **Audit Records**, click the column heading of interest.
   The list of records is reordered based on the headingr name selected. **User**, **Action Performed**, and **Data Object Name** sort alphabetically (**A ▸ Z**).

Click the **Timestamp** heading to reverse the default setting with the most recent record on top.

3. Click the column heading a second time to reverse the order of records displayed.
4. Click **Audit Records** or **Clear** to return to the default display.

Export and print Audit Records

The **Export** function generates a print-ready PDF file of the selected Audit Record.

1. Select the records to be exported by clicking the checkbox next to the record of interest. Select all the records on the screen by selecting the checkbox above the column.
2. Click **Export**.
   A PDF is generated. Depending on your browser settings, the software automatically downloads the file or prompts you to open or save the file.
3. Open the PDF in an appropriate viewer to print.

Update the Audit Configuration

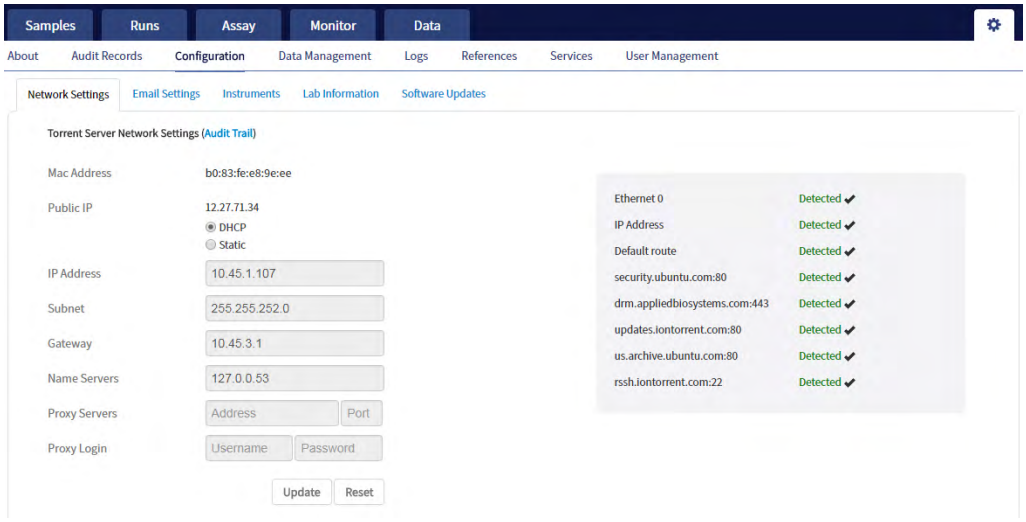
Audit Configuration allows the Administrator to require that a reason for the change is included as part of changing designated objects. To update the Audit Configuration:

1. Click **Audit Configuration**.
2. In the **Audit Configuration** dialog box, click the **Require Reason** checkbox next to the Data Object Name.
3. Click **Save**.
   To remove a reason for change requirement, open the **Audit Configuration** dialog box, deselect the checkbox, then click **Save**.

Configuration *(Administrator)*

Under the tab, in the **Configuration** screen, the settings parameters are grouped by function into 5 subtabs:

- Network Settings
- Email Settings
- Instruments
- Lab Information
- Software Updates



Network Settings

If a problem occurs with the Ion Torrent™ Server, administrator-level users can use network settings information to troubleshoot the cause. Information about the Ion Torrent™ Server is displayed in the **Configuration** screen on the **Network Settings** tab.

An audit trail of the Ion Torrent™ Server network settings is available to view, export, and print by clicking **Audit Trail** on the **Network Settings** tab.

Configure Email Settings

Administrator-level users configure the Ion Torrent™ Server to allow sending email notifications.

Before you begin, obtain SMTP server URL and port information from your IT department.

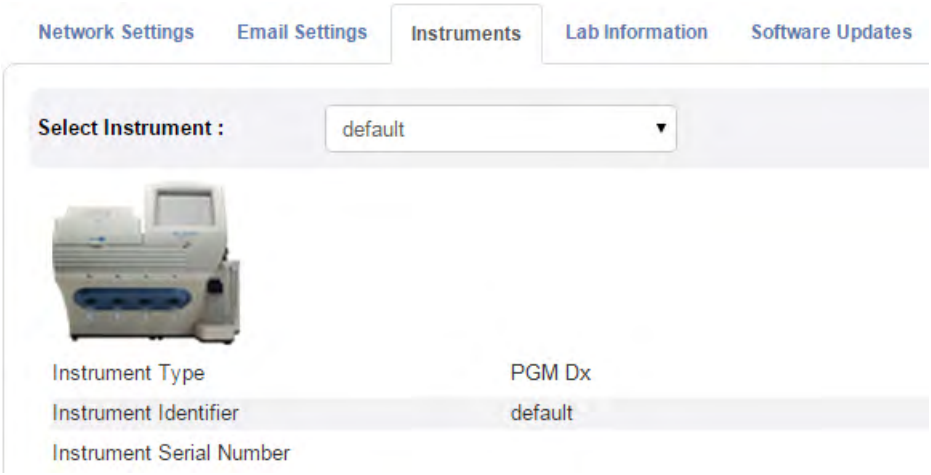
1. Click ⚙ ▸ **Configuration**.
2. Select the **Email Settings** tab.
3. In the **Torrent Server Email Settings** screen, enter the appropriate settings.
   Required fields are indicted with a red asterisk (*).
4. Click **Update**.
5. Click **Send Test Email** to send a test email to the email address provided in the user account.

Instrument configuration

Administrator-level users can view the instrument configuration for an instrument that is connected to the Ion Torrent™ Server, such as:

- **Instrument Type**
- **Instrument Identifier** (name)
- **Instrument State**
- **Last PQ Date**

Click ⚙ ▸ **Configuration** ▸ **Instruments**, then from the **Select Instrument** list, select an instrument and view the instrument details.



Lab Information

IMPORTANT! Your network system administrator should review support personnel contact information periodically to ensure that it is current and accurate.

Administrator-level users can review information about support personnel, listing points of contact if a problem with the Ion Torrent™ Server or a connected Ion PGM™ Dx System instrument occurs.

Click ⚙ ▸ **Configuration** ▸ **Lab Information** to view the lab information.

- **Lab Contact**—This is the person in your organization who should be notified during a support request of problems related to the instrument.
- **IT Contact**—This is the person in your organization who should be notified during a support request of problems related to the Ion Torrent™ Server hardware or the network environment.

Update the software

IMPORTANT! Before updating the software. ensure that all instruments that are connected to the server are idle and no analysis jobs are running on the server or are queued to run.

Administrator-level users can update the software. Use the **Configuration** screen to:

- See the currently installed software version for each software module and component application.
- Check for software updates.
- Install software updates from a USB drive using the following steps.

1. Click ⚙ ▸ **Configuration**.
2. Select the **Software Updates** tab.
3. Click the desired instrument software module link to expand the information.
4. After viewing the software version information, click the software module heading a second time to collapse the information.
5. To check for updates, click **Check for updates**.
   If an available update is found, **Download & Update** appears next to **Check for updates**. If no updates are found, "No updates available" displays.
6. To load an update from a USB drive, connect the USB drive, then click **Check for updates**.
   The software searches the USB drive for updates and lists them.
7. Click **Update Server**.
8. In the confirmation dialog box, click **OK** to start the update.
   After successful completion of the software update, the Ion Torrent™ Server automatically starts rebooting within 2 minutes.
9. After the server has rebooted (~10 minutes), click the browser **Refresh** button to return to the software home page.
   After the update is complete, confirm the software version number in the **Software Updates** tab in the **Configuration screen**.

Data Management (administrator)

Administrator-level users can perform the following functions in the **Data Management** screen under the ⚙ tab.

- Monitor Ion Torrent™ Server disk space usage
- Manage archive settings, and view an audit trail of any changes made

Note: 72 hours after completion of data analysis, raw data (DAT) files are deleted from the server to conserve Ion Torrent™ Server disk space.

About the data output directory

The data output directory is the primary location on the Ion Torrent™ Server where data generated by the Ion PGM™ Dx System is stored. The path to the directory is listed in the **Data Management** screen under the ⚙ tab.

Note: The directory location is locked and cannot be changed. Users should configure their LIMS to access this folder location to receive sequencing output files.

After a sequencing run and data analysis are complete, the software creates a separate results folder for each sample in the data output directory using the following naming convention:

Dx_ <Library Name>_<Assay Name>_<PlannedRunShortCode>_<PlannedRunShortCode>

The following files are added to the results folder:

- Run log files: basecaller.log, sigproc.log, analysis.log
- PlannedRun-AuditTrail.pdf: audit trail of the planned run in PDF format
- Assay-specific variant files in VCF and XLS formats
- Dx_allele_counts.xls for each barcode: allele coverage for bases in hot spot regions
- <barcode>_rawlib.basecaller.bam: unmapped BAM file
- <LibPrepID>_rawlib.bam: mapped BAM file
- <LibPrepID>_rawlib.bam.bai: mapped BAM index file
- A checksum file containing checksums for each output file
- analysis.bfmask.stats: contains analysis statistics of wells in the bead find stage
- BaseCaller.json: a JSON format file of the statistics of basecaller, bead summary, filtering, phasing, and training subset
- datasets_pipeline.json: a JSON format file of the settings needed by the pipeline to run the basecaller module
- explog_final.txt: final run settings needed for analysis
- ion_params_00.json: a JSON format file of the detailed settings of the run and analysis arguments
- TFStats.json: a JSON format file of control statistics
- wells_beadogram.png: a figure of statistics to characterize wells
- Test_Report.pdf: an assay-specific PDF report of targeted clinical and analytical variants; can be generated in multiple languages if selected (filenames are appended with language suffix)
- Lab_Report.pdf: similar to the Test Report, but includes all analytical variants detected; can be generated in multiple languages if selected (filenames are appended with language suffix)
- Summary.tab: non-targeted test results summary
- Snvindel.tab: non-targeted SNV/INDEL detail table
- Fusion.tab: non-targeted fusion detail table
- Cnv.tab: non-targeted CNV detail table
- Target_summary.tab: targeted test results summary
- Target_hotspot.tab: targeted SNV/INDEL detail table
- Target_fusion.tab: targeted fusion detail table
- Target_cnv.tab: targeted CNV detail table
- <LibPrepID>_<AnalysisID>.final.vcf: VCF file
- Info.csv: contains sample attributes, reagent information, QC values, and instrument information
- <barcode>_rawlib.basecaller.bam: unmapped BAM file – DNA
- <barcode>_rawlib.basecaller.fastq: FASTQ file – DNA
- <LibPrepID>_rawlib.bam: mapped BAM file – DNA
- <LibPrepID>_rawlib.bam.bai: mapped BAM index file – DNA
- <barcode>_rawlib.basecaller.bam: unmapped BAM file – RNA
- <barcode>_rawlib.basecaller.fastq: FASTQ file – RNA

- <barcode>_rawlib.basecaller.alignments.bam: mapped BAM file – RNA
- <barcode>_rawlib.basecaller.alignments.bai: mapped BAM index file – RNA
- Bead_density_contour.png: loading density figure
- iontrace_Library.png: key incorporation traces figure
- raw_peak_signal: key signal for controls and library
- readLenHisto.png: histogram of the read length
- <LibPrepID>_rawlib.stats.cov.txt: amplicon statistics
- basecaller.log: base caller log file
- sigproc.log: signal processing log file
- analysis.log: analysis log file
- PlannedRun-AuditTrail.pdf: Planned Run audit file

Disk space usage and data archiving

Ion Torrent™ Server disk space usage is shown in the **Data Management** screen under the ⚙ tab. To maintain sufficient disk space, we recommend implementing a systematic plan to archive older run data, results files, variants information, and signed reports to an external file storage system. Consult your Field Service Engineer to discuss archive and database backup options. Your local system administrator is responsible for establishing your data archive system.

After an archive system has been established, administrator-level users can configure the archive schedule in the **Data Management** screen. Archived results and reports can be restored to the Ion Torrent™ Server and downloaded from the **Completed Runs & Results** screen. See "Restore archived results" on page 53.

Note: If the Ion Torrent™ Server has ≤1 terabyte (TB) of free disk space, an alert notifies the user that there is insufficient disk space when setting up the run on the sequencer. The run cannot proceed until data on the server is archived and deleted. Contact your local system administrator to manually archive and delete data. You should also change the archive schedule.

IMPORTANT!

- If results are restored from runs that were archived in an older version of Torrent Suite™ Dx Software (version 5.6.4 and earlier), the software may not display all the variants from the restored results in the user interface, and reports generated from those restored results may not contain all variants. Carefully review the restored data in the user interface to determine whether all variants are present. Do not generate new reports from results restored from these older archives. Note that all variants are preserved in the restored source files, and can be downloaded using the **Download Files** command. This issue is corrected in version 5.12.5 of the software.
- In older versions of Torrent Suite™ Dx Software (version 5.6.4 and earlier), results reports should be generated and signed before results are archived. See "Sign the run results" on page 67.

Update archive settings

Administrator-level users can view and edit archive settings in the **Data Management** screen. Consult your Field Service Engineer to set up a database archive and backup system.

1. Click ⚙ ▸ **Data Management**.
2. Edit the archive settings.

Setting	Procedure
Auto archive after	Select the number of months for data to remain on the Ion Torrent™ Server before it is archived on an external server. To free up additional space on the Ion Torrent™ Server, reduce this interval.
Archive Directory	Enter the file path to the external archive directory. The default path must be changed before data can be archived.

Note: The **Data Output Directory** is listed in this screen but cannot be changed.

3. Click **Save**.

Archive notifications

Administrator-level users receive email notifications about archives when any of the following conditions occur:

- The archive location has ≤120 gigabytes (GB) of free disk space remaining.
- The archive location has not been changed from the default location (`/results/analysis`). The archive path must be changed to that of the external server before data can be archived.
- The archive location is not accessible when the system tries to archive a run.

To receive email notifications about archives, a valid administrator email address must be entered into Torrent Suite™ Dx Software. For more information, see "User Management (administrator)" on page 88.

Additionally, the Ion Torrent™ Server must be configured to allow sending email notifications. For more information, see "Configure Email Settings" on page 82.

Logs (administrator)

Select a log category

Within the ⚙ ▸ **Logs** screen, administrator-level users choose from the **Select Category** list to view and set auto-deletion rules for **Torrent Suite** and **Instrument** logs.

Manage logs

Administrator-level users can manage logs to set retention times and set up auto-deletion of logs.

1. Click ⚙ ▸ **Logs**.
2. Use the **Select Category** list to view and set auto-deletion rules for **Torrent Suite** or **Instrument** logs.
3. Click **Manage Log**.
4. In the **Manage Log File** dialog box, from the **Retention Period** list, select the number of months that logs are to be retained on the server.
5. Select **Enable Auto Deletion** to automatically delete log files after the designated retention period.
6. Click **Save**.

User Management (administrator)

Administrator-level users can use the ⚙ tab, in the **User Management** screen, to perform the following actions:

- Add users
- Assign user privileges (roles)
- Edit user information
- View user account audit trails
- Manage user account policies

Add a new user

Note: The email settings on the Ion Torrent™ Server must be configured before new users can receive emails. See "Configure Email Settings" on page 82 for more information.

1. To add a new user account, under the ⚙ tab, in the **User Management** screen, click ⊕ **Add New**.
2. In the **Create New User** dialog box, enter the account information.

   Note: Required fields are indicated by a red asterisk (*).
3. Select the user-access level (Administrator, Manager, or Operator) from the **Role** list.
4. To enable the ability to sign reports (Manager- or Administrator-level users only), select the **Electronic Signature** checkbox.
5. Click **Save**.
   The Ion Torrent™ Server sends a temporary password to the email address of the new user.

Set security policies

Administrator-level users set user account security policies. User account security policies include the permissible number of failed sign-in attempts, password lifetime, and the length of inactivity before a user is automatically signed out of the software.

1. Click ⚙ ▸ **User Management**.
2. In the **User Management** screen, click **Policies**.
3. Update the **Policies** dialog box. Accept the default values or select the appropriate values to set the user account suspension, password, and session policies, then click **Save**.
4. Select**Enabled** to enable the session policy settings.
5. Click **Save**.

Sort users

Administrator-level users can sort the list of users to help find a user account to work with.

1. Click ⚙ ▸ **User Management**.
2. Click the header name of interest to sort alphabetically.
3. Click the header name a second time to reverse the order of users displayed.
4. Click **User Management** to return to the default display.

Edit user accounts

Administrator-level users can edit user account information.

1. Click ⚙ ▸ **User Management**.
2. In the **User Name** column, click **Edit** under a user name.
3. In the **Edit User Details** dialog box, enter the desired changes to the account information.
   - From the **Role** list, select the user-access level (**Administrator**, **Manager**, or **Operator**).
   - To allow manager- or administrator-level users to sign reports, select the **Electronic Signature** checkbox.
   - In the **Status** list, select the appropriate status (**Active**, **Suspend**, or **Disable**).
4. Click **Save** to make the changes.

Reset password

Administrator-level users can reset user account passwords.

Note: Operator level users can reset their passwords by following the directions included in the system-generated email notification of a pending password expiration.

1. In the Edit User Details window, c Click **Reset Password**.
2. In the **Reset User Password** dialog box, click **Send password in email**, to email a new password to the email address entered in the user account.
3. Alternatively, enter a new password into the **Password** field.
4. Reenter the new password into the **Re-type Password** field, then click **Save**.

   The confirmation message "Password reset successfully" appears. Click **Back**, then **Save** to return to the **User Management** window.

View user audit trail

The user audit trail is a record of when the user account was created and modified.

1. Under the ⚙ tab, in the **User Management** screen, in the **Actions** column, click **Audit**.
2. In the **Audit Trail** dialog box, click 🗎 **(Details)** under **Record**.
3. In the **Audit Record Details** dialog box, click **Export** to export a PDF of the record.

A Anomalies

Software anomalies

For Torrent Suite™ Dx Software anomalies, refer to the release notes for your version of the software, included on the software USB drive.

B

Troubleshooting

HotSpot ID corrections

The following HotSpot IDs reported in the software are inaccurate, and correspond to the actual COSMIC IDs listed below. These anomalies do not impact test results.

HotSpot ID in the software	Actual COSMIC ID	Gene	Amino acid change	Nucleotide change
OM3157	COSM1235478	MAP2K1	p.Lys57Asn (p.K57N)	c.171G>T
COSM1235478	N/A[1]	MAP2K1	p.Lys57Met (p.K57M)	c.170A>T
COSM1562837	COSM5077832	MAP2K1	p.Phe53Val (p.F53V)	c.157T>G

[1] This variant is not in the COSMIC database.

Torrent Suite™ Dx Software

Observation	Possible cause	Recommended action
Cannot sign in to the Ion Torrent™ Server	You have either forgotten your password or are signed out due to several failed login attempts.	Contact a Torrent Suite™ Dx Software administrator-level user.
Cannot sign in to the Ion PGM™ Dx Sequencer	The Ion PGM™ Dx Sequencer lost its connection to the Ion Torrent™ Server.	Contact a Torrent Suite™ Dx Software administrator-level user.
Data and user profiles are missing from the software	You are signed into the incorrect mode of the software. Data and user profiles created in Torrent Suite™ Dx Software will not be visible in Torrent Suite™ Assay Development Software, and vice versa.	Confirm that you are signed into the correct mode of the software.

Observation	Possible cause	Recommended action
Batch sample import fails	One or more entries in the sample-import spreadsheet contains special characters, lines breaks, unexpected spaces, incorrect entry length, incorrect date formatting, or other formatting errors.	Check each entry for correct formatting, correct any errors, and repeat the import.
	Blank rows were copied into the sample-import template file from a different source.	Rows that appear empty may contain hidden formatting that conflicts with the import function. Start with a clean sample-import template file, and be careful to copy only those rows that contain actual data.
	The sample import spreadsheet contains a nonunique Sample ID.	Every Sample ID in the software must be unique. Make sure the spreadsheet does not contain any duplicate IDs, and repeat the import. Note that the system check is not case-sensitive, so a Sample ID of ABC1 conflicts with abc1.
	The headings in the sample import spreadsheet do not match the sample attributes in the software.	The headings must match the sample attributes in the software exactly. Check the headings for spelling or other errors.
Library batch import fails	One or more entries in the library batch import spreadsheet contains special characters, lines breaks, unexpected spaces, incorrect entry length, incorrect date formatting, or other formatting errors.	Check each entry for correct formatting, correct any errors, and repeat the import.
	Blank rows were copied into the library batch import template file from a different source.	Rows that appear empty can contain hidden formatting that conflicts with the import function. Start with a clean library batch import template file, and be careful to copy only those rows that contain actual data.
	The library batch import spreadsheet contains a nonunique Library Batch ID.	Every Library Batch ID in the software must be unique. Ensure that the spreadsheet does not contain any duplicate IDs, and repeat the import. Note that the system check is not case-sensitive, so a Library Batch ID of ABC1 conflicts with abc1.
	A Sample ID entered in the library batch import spreadsheet does not match a Sample ID listed in the **Manage Samples** screen.	Ensure that the Sample IDs entered into the spreadsheet are correct and match an existing sample ID added to the software.

Observation	Possible cause	Recommended action
Library batch import fails *(continued)*	The Barcode ID name format does not exactly match the format that is used in the **Prepare Library Batch** dialog box.	Use the following Barcode ID name format: IonDx-1 through IonDx-16.
	An invalid library, control, or panel kit barcode has been entered in the spreadsheet.	Ensure that you have correctly entered a valid kit barcode in the appropriate cell of the spreadsheet.
Cannot execute my Planned Run **Details:** "System error" message is displayed	The Tube Label you assigned the **Planned Run** is not unique. **Note:** The Ion PGM™ Dx Instrument Control software does not allow use of the same Tube Label text within 7 days.	Assign the **Planned Run** a new, unique Tube Label.
	Incorrect template prep kit barcode was entered.	Rescan the correct template prep kit barcode.
The results of my run are not showing up in the Data tab	Ion Torrent™ Server disk space is full.	Clear disk space on the Ion Torrent™ Server. After sufficient disk space is cleared, data transfer from the Ion PGM™ Dx Sequencer to the Ion Torrent™ Server recommences and data analysis proceeds.
	Ion PGM™ Dx Sequencer lost connection to the Ion Torrent™ Server.	Reestablish the Ion PGM™ Dx Sequencer Ethernet connection to the Ion Torrent™ Server. Once the connection has been restored, data transfer from the Ion PGM™ Dx Sequencer to the Ion Torrent™ Server recommences and data analysis proceed.
Cannot find file links to the BAM and VCF file	The run failed. Links to BAM and VCF files are not available for runs that fail QC.	Repeat the run.
Loading metrics are reported as 0	• Chip failure • Defective pariposer • Chip leak	Refer to sequencing troubleshooting for possible causes of chip or pariposer failure.
	Sample preparation failure resulting in loss of sample	Refer to troubleshooting for sample, library, and template preparation.

Observation	Possible cause	Recommended action
QC Status is **Failed** due to one or more QC metrics listed as **N/A** **Details:** Under the **Monitor** tab in the **Run View** screen, under **View QC Details**, one or more of the QC metrics (Library RNA, Library DNA, CF-1, DNA Control, RNA Control, DNA NTC, and RNA NTC) have a value of **N/A** and the **QC Status** is **Failed**.	• The control associated with the QC metric was not added to the reaction or otherwise failed to generate a measurable value. • The QC status was not updated properly in the database due to a software issue. This is uncommon.	For Administrator-level users, the **Repopulate QC** button is displayed under the **Monitor** tab in the **Run View** screen after a run. 1. Click **Repopulate QC** to update all QC metrics in the database. **Note:** Repopulation takes 15–30 minutes. 2. If any metric is still listed as **N/A** and the **QC Status** is still **Failed**, repeat the run starting at template preparation from the DNA/RNA combined library, and ensure that you add all controls. If the problem continues, contact Technical Support.
Run analysis does not complete **Details:** If the **Analysis Status** for a run is listed in the **Monitor** tab as "RUNNING" for more than 12 hours, a **Restart Analysis** button appears in the tab (Administrator-level users only).	Run analysis has stalled in the analysis pipeline.	Click **Restart Analysis** to restart the analysis from the beginning.
Run analysis does not complete, and Restart Analysis is not successful	A known software issue exists where the database status of a run is not updated properly. This behavior is uncommon.	If clicking **Restart Analysis** in the **Monitor** tab does not result in a completed analysis, repeat the run starting at template preparation from the DNA/RNA combined library. If the problem continues, contact Technical Support.

Library preparation

Observation	Possible cause	Recommended action
Display message: Invalid kit barcode or Expired kit	Kit is beyond expiration date.	Use a nonexpired kit.
	Incorrect kit scanned.	Rescan the barcode of the correct kit.
Run QC passed but low coverage uniformity (<95%)	Poor purification causes loss of short amplicons	Vortex AMPure™ XP reagent thoroughly before use, and be sure to dispense accurate volume.
Run QC passed, low percent full length On Target Reads, but Control passed	Library prep failure	Reprepare the sample library.
Samples passed but CF-1 Mean AQ20 Read Length failed	Reads filtered out due to high polyclonal ISPs caused by too much library added to the Ion OneTouch™ Dx amplification reaction	Repeat Ion OneTouch™ Dx run using less library in the amplification reaction.
Run QC passed, but one or more sample libraries failed Percent Reads QC metric	Library prep failed due to unwashed beads.	Be sure to wash the library beads prior to use.
	Library preparation failed due to wrong library amplification primers.	Use the LIB Primers provided in the Ion PGM™ Dx Library Kit.
	Library prep failed due to residual salt after wash.	Carefully remove all wash solution prior to elution.
	Library prep failed due to mis-quantification of input DNA.	Requantify input DNA.
	Library prep failed due to inefficient PCR, digestion, or ligation.	Ensure that you properly dispense and mix the viscous components at each step.
		Ensure that you use the correct thermal cycling conditions.
	Library prep failed due to the library being discarded during purification of the amplified library.	Be sure to save the supernatant during first-round purification and save the library pellet during the second round purification of the amplified library.
	Library prep failed due to over-drying of the AMPure™ XP beads.	Do not dry the AMPure™ XP beads more than 5 minutes.
		Ensure that you dispense exactly 10 µL of capture reagent to the amplified library.
	Library failed due to ineffective capture of the amplified library.	Ensure that the library capture reagent is at room temperature before use.
		Ensure that you dispense exactly 10 µL of capture reagent to the amplified library.
		Make sure to mix completely and incubate for 5 minutes at room temperature.

Observation	Possible cause	Recommended action
Run QC passed, but one or more library samples failed Percent Reads QC metric - continued	Reads filtered out due to primer-dimers.	In unamplified library purification, ensure that you use the correct amount of AMPure™ XP reagent.
		Do not combine Barcode Adapters, LIB DNA Ligase, and LIB Switch Soln prior to addition.
		Ensure that Barcode Adapters are diluted properly.
	Reads filtered out due to low quality ISPs.	Repeat the Ion OneTouch™ Dx run. If it still happens, then reprepare the library.
	Reads filtered out due to high polyclonal ISPs caused by failed consumables.	Repeat the Ion OneTouch™ Dx run. If it still happens, then reprepare the library.
	Reads filtered out due to high polyclonal ISPs caused by too much library added to the Ion OneTouch™ Dx amplification reaction.	Requantify input DNA or RNA, and remake the library.
	Forgot to add the library to amplification reaction.	Repeat the Ion OneTouch™ Dx run. If observation recurs, then reprepare the library.

Template preparation

Observation	Possible cause	Recommended action
Display message: Failed to set up system time at startup. Check your connection to the Ion Torrent™ Server.	Ion OneTouch™ Dx Instrument and Ion Torrent™ Server connection is not established	Check your network connection to the Ion Torrent™ Server to make sure the connection is established, then reboot the Ion OneTouch™ Dx Instrument.
	Instrument is still in the process of establishing a connection	Allow 10 minutes to see if the display message clears.
Display message: Failed to connect to the Ion Torrent™ Server. Check your connection.	Ion OneTouch™ Dx Instrument and Ion Torrent™ Server connection is not established during startup	Check your network connection to the Ion Torrent™ Server to make sure the connection is established, then reboot the Ion OneTouch™ Dx Instrument.
Display message: Pressure too high. Reboot the instrument to clear the alarm.	• Hardware issue • Clogged TMPL Emulsion Cartridge due to contaminated reagents or defective emulsion cartridge	Reboot the Ion OneTouch™ Dx Instrument to clear the alarm. Use a new TMPL Emulsion Cartridge and fresh reagents to repeat the run. Contact Technical Support (see Appendix E, "Customer and technical support") if the issue persists.

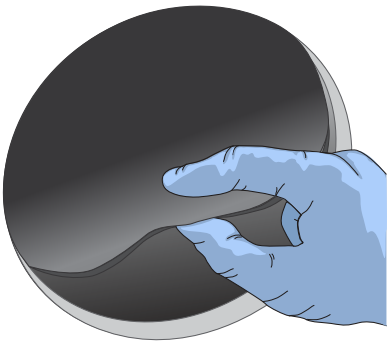
Observation	Possible cause	Recommended action
Display message: TEC current too high. Reboot the instrument to clear the alarm.	Hardware issue	Contact Technical Support (see Appendix E, "Customer and technical support"). Reboot the Ion OneTouch™ Dx Instrument to clear the alarm. **IMPORTANT!** A sample created during a run with this alarm raised must NOT be used.
Display message: Coolant pump does not flow.	Hardware issue	Contact Technical Support (see Appendix E, "Customer and technical support"). Reboot the Ion OneTouch™ Dx Instrument to clear the alarm.
Display message: Sensor unable to measure instrument temperature	Hardware issue	Contact Technical Support (see Appendix E, "Customer and technical support"). Reboot the Ion OneTouch™ Dx Instrument to clear the alarm.
Display message: Software versions incompatible. Go to the Options menu and update the software.	The system software was updated, but the instrument software was not.	After the system software has been updated, update the instrument software as follows: 1. On the main menu of the instrument, press **Options** and follow the instructions to check for and install updates. 2. When installation is complete, follow the onscreen prompts to restart the instrument. **IMPORTANT!** You must restart the instrument before proceeding.
Display message: Sensor unable to measure pressure.	Hardware issue	Contact Technical Support (see Appendix E, "Customer and technical support"). Reboot the Ion OneTouch™ Dx Instrument to clear the alarm.
Display message: Connection failure with Ion Torrent™ Server	Ion OneTouch™ Dx Instrument and Ion Torrent™ Server connection is not established	Check that a network connection to the Ion Torrent™ Server is established, then reboot the Ion OneTouch™ Dx Instrument. **Note:** A sample created during a run with this alarm raised can still be used.
Display message: Motor current too high. Reboot the instrument to clear the alarm.	Hardware issue	Contact Technical Support (see Appendix E, "Customer and technical support"). Reboot the Ion OneTouch™ Dx Instrument to clear the alarm. **Note:** A sample created during a run with this alarm raised can still be used.

Observation	Possible cause	Recommended action
Display message: Set temperature out of range. Reboot the instrument to clear the alarm.	Hardware issue	Contact Technical Support (see Appendix E, "Customer and technical support"). Reboot the Ion OneTouch™ Dx Instrument to clear the alarm. **IMPORTANT!** If this alarm is raised, you cannot use the prepared template.
TMPL CF-1 fails QC metric(s); all sample libraries pass QC metrics	TMPL CF-1 reagent not added	Repeat Ion OneTouch™ Dx Instrument run and Ion PGM™ Dx Sequencer run, and ensure that the TMPL CF-1 reagent is added per procedure description.
	The minimum Key Signal value of the CF-1 control required for the assay is >0.	The minimum Key Signal value for the CF-1 control (i.e., the Templating Control) is set to 0 in all preinstalled assays and new custom assays in Torrent Suite™ Dx Software 5.12.5. Custom assays created in previous versions of the software may have a higher Key Signal value for the control.

Sequencing

Observation	Possible cause	Recommended action
Cannot import my sample	**Sample ID** is greater than 20 characters.	Limit **Sample ID** to 20 characters or fewer.
	Expired library kit.	Use a nonexpired library kit.
	Incorrect library kit barcode.	Rescan the correct library kit barcode.
Run report says run failed	Run failed because one or more QC metrics were not met	
I created a Planned Run but the Ion PGM™ Dx Sequencer does not recognize the Short Code	**Planned Run** was not executed.	In Torrent Suite™ Dx Software: 1. Select the Planned Run, then click **Execute**. 2. Complete the dialog box, then click **Save**.
	The Ion PGM™ Dx Sequencer and Ion Torrent™ Server have lost connection.	Check the network connection to the Ion Torrent™ Server to ensure that the connection is established, then reboot the instrument.
TMPL CF-1 fails QC metric(s); all sample libraries fail QC metrics	• Forgot Enzyme • Forgot Seq primer • Used wrong annealing program	Repeat Ion OneTouch™ Dx Instrument run and Ion PGM™ Dx Sequencer run.
Display message: Lost connection to the Ion Torrent™ Server	The connection between the instrument and the server has been lost.	Check the network connection to the Ion Torrent™ Server, and then reboot the Ion PGM™ Dx Sequencer. If this alarm appears during a run, the data created during that run can still be used.

Observation	Possible cause	Recommended action
Display message: Failed to set up system time at startup. Check your connection to the Ion Torrent™ Server.	The connection between the Ion PGM™ Dx Sequencer and the Ion Torrent™ Server has been lost.	1. Check the network connection to the Ion Torrent™ Server to make sure the connection is established, then reboot the instrument. 2. If the problem persists, replace the network cable(s) to the instrument and server. 3. If the problem persists, contact Technical Support (see Appendix E, "Customer and technical support").
	Instrument is still in the process of establishing a connection	Allow 10 minutes to see if display message clears.
Display message: Bad boot drive detected	Hardware issue	Contact Technical Support (see Appendix E, "Customer and technical support"). If this alarm appears during a run and data for the run is generated, that data may still be used.
Display message: UBoots do not match	Hardware issue	Contact Technical Support (see Appendix E, "Customer and technical support").
Display message: Kernels do not match	Hardware and/or software issue	Contact Technical Support (see Appendix E, "Customer and technical support").
Display message: Bad results data drive	• On some machines, the warning appears before the reboot completes. • There is a hardware issue.	Wait for a few minutes to see if the error message disappears. If the error message disappears, data obtained during a run with this alarm raised can still be used. If the problem persists, contact Technical Support (see Appendix E, "Customer and technical support").
Display message: Lost chip connection	The instrument cannot detect a chip in the chip clamp.	Refer to the *Oncomine™ Dx Target Test Part IV: Sequencing and Results Reports User Guide* troubleshooting section.
Display message: Lost communication with valve board	Hardware issue	Contact Technical Support (see Appendix E, "Customer and technical support").
Run aborted during presequencing step	Critical alarm present on the Ion PGM™ Dx Sequencer	Reboot the Ion PGM™ Dx Sequencer to clear the alarm, then restart the sequencing run.
Display message: Sensor unable to measure instrument temperature	Hardware issue	Contact Technical Support (see Appendix E, "Customer and technical support").

Observation	Possible cause	Recommended action
Display message: Instrument temperature too high	• Room temperature is too high. • Clogged filter or blocked airway on the instrument • Hardware issue (fan is not running or running too slowly)	**Note:** The data created during a run with this alarm raised may still be used if all the QC metrics are met. 1. If the ambient room temperature is above 30°C, lower it. 2. Make sure that the round filter on the back panel of the instrument has unrestricted airflow. If the filter is clogged with dust, clean it as follows: a. Pinch the dirty filter with your fingers, then remove it from the instrument.  b. Shake the filter over a waste container to remove most of the dust. c. Rinse the filter with running water to remove any remaining dust. The water flow should be from the inside-facing surface to the outside-facing surface through the filter. d. Air dry the filter. e. Blot any remaining dust from the filter using tape. f. Reinsert the filter. If the problem persists, contact Technical Support (see Appendix E, "Customer and technical support").
Display message: Instrument temperature too low	• Room temperature is below 20°C. • Hardware issue	**Note:** The data created during a run with this alarm raised may still be used if all the QC metrics are met. If the ambient room temperature is below 20°C, raise it. If the problem persists, contact Technical Support (see Appendix E, "Customer and technical support").

Observation	Possible cause	Recommended action
Display message: Chip temperature too high	• Room temperature is too high. • Clogged filter or blocked airway on the instrument • Hardware issue (instrument fan is not running or running too slowly)	**IMPORTANT!** The data created during a run with this alarm raised should *not* be used. See the recommended action for "Display message: Instrument temperature too high" on page 101.
Display message: Chip temperature too low	Hardware issue	**IMPORTANT!** The data created during a run with this alarm raised should *not* be used. Contact Technical Support (see Appendix E, "Customer and technical support").
Display message: Instrument idle temperature too high	• Room temperature is too high. • Clogged filter or blocked airway on the instrument • Hardware issue (fan is not running or running too slowly)	**Note:** The data created during a run with this alarm raised may still be used if all the QC metrics are met. See the recommended action for "Display message: Instrument temperature too high" on page 101.
Display message: Instrument idle temperature too low	• Ambient room temperature is below 20°C. • Hardware issue	Bring the ambient temperature up to 20°C. If the problem persists, contact Technical Support (see Appendix E, "Customer and technical support").
Display message: Fan current too low	Hardware issue	Contact Technical Support (see Appendix E, "Customer and technical support").
Display message: Heater current too low	Hardware issue	**IMPORTANT!** If the chip temperature is also out of range, data created during a run should *not* be used. Contact Technical Support (see Appendix E, "Customer and technical support"). If no chip temperature alarms are raised, data created during a run may still be used if all the QC metrics are met.
Display message: Pressure too high	Internal pressure regulator was not set correctly	Contact Technical Support (see Appendix E, "Customer and technical support").

Observation	Possible cause	Recommended action
Display message: Pressure too low.	• Gas line is not connected to the instrument • Gas cylinder may be turned off or empty	1. Verify that the gas line is connected to the instrument. 2. Verify that the gas cylinder has at least 500 psi on the pressure gauge inside the tank and 30 psi on the gauge that regulates pressure to the outlet. (Both pressure gauges are mounted on the gas cylinder.) 3. Confirm that the outlet valve on the regulator is turned on. 4. If the problem persists, contact Technical Support (see Appendix E, "Customer and technical support").
Display message: Sensor unable to measure gas pressure. Check supply gas pressure.	• Gas line is not connected to the instrument • Gas cylinder may be turned off or empty • Hardware issue	1. Verify that the gas line is connected to the instrument. 2. Verify that the gas cylinder has at least 500 psi on the pressure gauge inside the tank and 30 psi on the gauge that regulates pressure to the outlet. (Both pressure gauges are mounted on the gas cylinder.) 3. Confirm that the outlet valve on the regulator is turned on. 4. If the problem persists, contact Technical Support (see Appendix E, "Customer and technical support").
Display message: Failed to set the pressure to target range. Check the gas connection and try again.	• Gas line is not connected to the instrument • Gas cylinder may be turned off or empty • Hardware issue (regulator malfunction)	1. Verify that the gas line is connected to the instrument. 2. Verify that the gas cylinder has at least 500 psi on the pressure gauge inside the tank and 30 psi on the gauge that regulates pressure to the outlet. (Both pressure gauges are mounted on the gas cylinder.) 3. Confirm that the outlet valve on the regulator is turned on. 4. If the problem persists, contact Technical Support (see Appendix E, "Customer and technical support").
Display message: Failed to locate the barcode scanner. Check if the scanner is attached.	The connection between the barcode scanner and the Ion PGM™ Dx Sequencer has been lost.	Make sure the scanner is plugged into a USB port on the instrument. If it is connected and the alarm still appears, try plugging the scanner into a second USB port. If the alarm persists, contact Technical Support (see Appendix E, "Customer and technical support").

Observation	Possible cause	Recommended action
Display message: Results drive not accessible. Reboot and try again.	• On some machines, the warning appears before the reboot completes • Hardware issue	Wait for a few minutes to see if the error message disappears. If the error message appears and disappears during a run, data obtained during that run can still be used. If the alarm persists, contact Technical Support (see Appendix E, "Customer and technical support").
Display message: Results drive check failed	Hardware issue	If the error message disappears when you return to the main instrument screen, this alarm can be ignored. Otherwise, contact Technical Support (see Appendix E, "Customer and technical support").
Display message: Unable to mount the file system	Hardware and/or software issue	1. Reboot the instrument to clear the alarm. 2. If the alarm is not cleared after reboot, contact Technical Support (see Appendix E, "Customer and technical support").
Display message: Failed to set up FTP connection. Check your connection to the Ion Torrent™ Server .	The network connection is not established or an incorrect IP address was used.	Confirm that the server information is correct for the Ion Torrent™ Server. Contact your local network administrator for support if the issue persists.

C Performance characteristics

For performance characteristics of the Oncomine™ Dx Target Test Kit, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide* (Pub. No. MAN0018810).

For performance characteristics of the Ion PGM™ Dx System, see the *Ion PGM™ Dx System Performance Characteristics User Guide* (Pub. No. MAN0018763).

D Instrument warranty

For new Ion Torrent™ instruments, Life Technologies warrants to and only to buyer for twelve (12) months from the date of shipping, that the Ion Torrent™ software and Ion Torrent™ instruments are free from defects in material and workmanship and conform to Life Technologies' published specifications in all material respects. Where a valid and timely claim in respect of breach of Ion Torrent™ warranty is submitted to Life Technologies, Life Technologies may, at its discretion, replace, repair or modify the Ion Torrent™ instrument. Any agreed replacement shall be at 1:1, like-kind basis, at no cost to the buyer. For Ion Torrent™ chips or reagents reasonably determined by Life Technologies to be defective, independent of user error, shall be replaced by Life Technologies on a 1:1, like-kind basis at no cost to buyer, provided that such defective Ion Torrent™ chips or reagents were used by buyer prior to their expiration date, or if there is no expiration date, the Ion Torrent™ chips or reagents were used within six (6) months of receipt, and the defect was promptly reported with appropriate detail to Life Technologies' technical support.

NO OTHER WARRANTIES SHALL BE APPLICABLE TO ION TORRENT PRODUCTS (WHETHER OR NOT ANY FURTHER WARRANTY DOCUMENTATION MAY BE INCLUDED IN THE SHIPMENT), WITH THE EXCEPTION OF THIRD PARTY WARRANTIES WITH RESPECT TO THIRD PARTY PRODUCT. ANY THIRD PARTY PRODUCTS ARE NOT COVERED BY THIS SECTION AND ANY WARRANTIES FOR THIRD PARTY PRODUCTS ARE PROVIDED BY THE ORIGINAL MANUFACTURER OF THE THIRD PARTY PRODUCT. Warranties are made only to buyer purchasing the Ion Torrent™ Product directly from Life Technologies, are not transferable and do not extend to the benefit of any other person or entity, unless otherwise expressly stated in writing by Life Technologies. ANY PRODUCT NOT COVERED BY AN EXPRESS WRITTEN WARRANTY IS SOLD AND PROVIDED "AS IS," WITHOUT WARRANTY OF ANY KIND, STATUTORY, EXPRESS OR IMPLIED. Any description of Ion Torrent™ Product recited in Life Technologies' quotation is for the sole purpose of identifying Ion Torrent™ Product, and any such description is not part of any contract between Life Technologies and buyer and does not constitute a warranty that Ion Torrent™ Product shall conform to that description. Any sample or model used in connection with Life Technologies' quotation is for illustrative purposes only, and is not part of any contract between Life Technologies and buyer and does not constitute a warranty that Ion Torrent™ Product will conform to the sample or model. No affirmation of fact or promise made by Life Technologies, whether or not in Life Technologies' quotation, shall constitute a warranty that Ion Torrent™ Product will conform to the affirmation or promise. Unless otherwise specified in writing in documentation shipped with Ion Torrent™ Product or otherwise agreed by Life Technologies in writing. Life Technologies does not provide service or support for custom products or other products made to buyer's specifications. THE WARRANTIES IDENTIFIED IN THIS CLAUSE ARE LIFE TECHNOLOGIES' SOLE AND EXCLUSIVE WARRANTIES WITH RESPECT TO Ion Torrent™ PRODUCT AND ARE IN LIEU OF ALL OTHER WARRANTIES, STATUTORY, EXPRESS OR IMPLIED, ALL OF WHICH OTHER WARRANTIES ARE EXPRESSLY DISCLAIMED, INCLUDING WITHOUT LIMITATION ANY IMPLIED WARRANTY OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, NON-INFRINGEMENT, OR REGARDING RESULTS OBTAINED THROUGH THE USE OF ANY PRODUCT (INCLUDING, WITHOUT LIMITATION, ANY CLAIM OF INACCURATE, INVALID OR INCOMPLETE RESULTS), WHETHER ARISING FROM A STATUTE OR OTHERWISE IN LAW OR FROM A COURSE OF PERFORMANCE, DEALING OR USAGE OF TRADE.

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support
- Order and web support
- Safety Data Sheets (SDSs; also known as MSDSs)

Additional product documentation, including user guides and Certificates of Analysis, are available by contacting Customer Support.

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are printed and shipped with the product.

Obtaining Certificates of Conformance

The Certificate of Conformance provides information on conformance testing of each instrument provided with the system. Certificates of Conformance are shipped with the instrument, and are also available by contacting Customer Support at **thermofisher.com/support**.

iontorrent

Ion PGM™ Dx System

USER GUIDE

Publication Number MAN0018761

Revision A.0

CE IVD

For In Vitro Diagnostic Use.

ThermoFisher SCIENTIFIC

Life Technologies Holdings Pte Ltd |
Block 33 |
Marsiling Industrial Estate Road 3 |
#07-06, Singapore 739256

Life Technologies Corporation |
7335 Executive Way |
Frederick, MD 21704 | USA

EC REP Life Technologies Europe B.V.
Kwartsweg 2, 2665 NN Bleiswijk
The Netherlands

Products manufactured in Singapore:

Ion PGM™ Dx Instrument System
Ion PGM™ Dx Sequencer
Ion OneTouch™ Dx Instrument
Ion OneTouch™ ES Dx Instrument
Ion PGM™ Dx Chip Minifuge (120V or 230V)
Ion PGM™ Wireless Scanner
Ion Torrent™ Server
Torrent Suite™ Dx Software
Torrent Suite™ Assay Development Software
Veriti™ Dx 96-well Thermal Cycler, 0.2 mL

Products manufactured in Frederick:

Ion Torrent Dx FFPE Sample Preparation Kit
Ion PGM™ Dx Library Kit
Ion OneTouch™ Dx Template Kit
Ion PGM™ Dx Sequencing Kit
Ion 318™ Dx Chip
Ion OneTouch™ Rack Kit
DynaMag™ Dx 96-Well Plate Magnet
DynaMag™ Dx 16 2-mL Magnet

The information in this guide is subject to change without notice.

DISCLAIMER

TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN00168761

Revision	Date	Description of change
A.0	2 September 2020	New user guide for Ion PGM™ Dx System updated for Torrent Suite™ Dx Software 5.12.5.

Contents

About this guide

CAUTION! ABBREVIATED SAFETY ALERTS. Hazard symbols and hazard types specified in procedures may be abbreviated in this document. For the complete safety information, see the "Safety" appendix in this document.

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Purpose of this guide

This user guide provides instructions for using the Ion PGM™ Dx System for sample preparation, library preparation, template preparation, and sequencing. This guide is used in conjunction with the following guides:

- The *Torrent Suite™ Dx Software 5.12.5 User Guide* (Pub. No. MAN0018762) provides instructions for using Torrent Suite™ Dx Software to plan and monitor sequencing runs and perform data analysis.
- The *Ion PGM™ Dx System Performance Characteristics User Guide* (Pub. No. MAN0018763) provides summaries and tables of performance results for the Ion PGM™ Dx System based on testing of the system with DNA and RNA from formalin-fixed, paraffin-embedded (FFPE) tissue samples and genomic DNA (gDNA) from whole blood samples.

1 Product information

About the Ion PGM™ Dx System

The Ion PGM™ Dx System is used for detection of human variant sequences from DNA from whole blood samples or RNA and DNA from FFPE tissue samples. Detectable variants include substitutions, insertions, and deletions.

The Ion PGM™ Dx System consists of the following:

- Ion OneTouch™ Dx Instrument
- Ion OneTouch™ ES Dx Instrument
- Ion OneTouch™ Rack Kit
- Ion PGM™ Dx Chip Minifuge
- Ion PGM™ Dx Sequencer
- Ion PGM™ Wireless Scanner
- DynaMag™ Dx Kit—Tube & Plate
- Ion Torrent™ Server
- Torrent Suite™ Dx Software

The Ion PGM™ Dx System is used in conjunction with the following kits:

- Ion PGM™ Dx Library Kit
- Ion OneTouch™ Dx Template Kit
- Ion PGM™ Dx Sequencing Kit
- Ion 318™ Dx Chip Kit

The system should be used only by professionals trained in laboratory techniques and procedures and in the use of the system.

Intended use

Ion PGM™ Dx Instrument System

The Ion PGM™ Dx Instrument System is composed of a sequencing instrument that measures the hydrogen ions that are generated during the incorporation of nucleotides in the DNA sequencing reaction, and the ancillary instrumentation necessary for sample processing. This instrument system is used in conjunction with the instrument-specific Ion PGM™ Dx Library Kit, Ion OneTouch™ Dx Template Kit, Ion PGM™ Dx Sequencing Kit, and Ion 318™ Dx Chip Kit, and data analysis software. The Ion PGM™ Dx Instrument System is intended for targeted sequencing of human genomic DNA (gDNA) from peripheral whole-blood samples and DNA and RNA extracted from formalin-fixed, paraffin-embedded (FFPE) samples.

The Ion PGM™ Dx Instrument System is not intended for whole genome or *de novo* sequencing.

Reagents

The Ion PGM™ Dx Library Kit, Ion OneTouch™ Dx Template Kit, Ion PGM™ Dx Sequencing Kit, and Ion 318™ Dx Chip Kit are a set of reagents and consumables used in the processing and targeted sequencing of human gDNA derived from peripheral whole blood samples and DNA and RNA extracted from FFPE samples. Nucleic acid reagent kits/tests that are manufactured and labeled for diagnostic purposes are required for the preparation of libraries targeting specific genomic regions.

The Ion PGM™ Dx Library Kit, Ion OneTouch™ Dx Template Kit, Ion PGM™ Dx Sequencing Kit, and Ion 318™ Dx Chip Kit are intended for use with the Ion PGM™ Dx System.

Indications for use

Same as intended use.

Special conditions statement

For *in vitro* diagnostic use. For prescription use only.

Special conditions statement for performance derived from DNA from whole blood

1. The Ion PGM™ Dx System has been validated to deliver the following using the System Variant Assay (SVA) panel:
   - Sequencing output >0.7 gigabases
   - Reads >4 million
   - Read length up to 200 base pairs
   - Mean Raw Read Accuracy of 99.0% when compared to hg19

   Note:
   - Mean Raw Read Accuracy is defined as the average raw accuracy across each individual base position in a read, where raw read accuracy is calculated as `100 * (1 - (sum(per base error)/sum(per base depth)))`
   - The 632 primer pairs of the SVA panel are designed to amplify regions across 23 chromosomes in the two well-characterized cell lines. The regions were selected based on the presence of well characterized insertions/deletions (Indels) and single-nucleotide variant (SNV) positions. The amplicons produced range in size from 80 to 200 base pairs, with a GC content of 20–80%.

2. The system has been evaluated for the detection of single-nucleotide variants (SNVs) and insertions and deletions of various lengths on 23 chromosomes. The system identified 440 unique SNV positions in the SVA panel with 100% reproducibility.

The following tables illustrate the lengths and locations of insertions and deletions in the SVA panel that were detected with 100% reproducibility.

Insertion length (base pairs)	Total number of distinct chromosomal locations	Total number of unique chromosomes
1	14	10
2	4	4
3	5	4
4	5	4

Deletion length (base pairs)	Total number of distinct chromosomal locations	Total number of unique chromosomes
1	15	11
2	10	6
3	3	3
4–14[1]	7	7

[1] Deletions of ≥4 bp have been grouped for clarity

3. The system may exhibit a limitation in detecting one-base insertions or deletions in homoploymer tracts (e.g., polyA). Variants in homopolymer runs exceeding 8 bases are called as no calls in the VCF file.
4. The system is designed to deliver qualitative (i.e., genotype) results.
5. As with any hybridization-based workflow, underlying polymorphisms or mutations in primer-binding regions can affect the regions being sequenced and, consequently, the calls made.
6. The recommended minimal coverage per amplicon needed for accurate variant calling for germline DNA is >30X.
7. Special instrument requirements for the Ion PGM™ Dx Library Kit, Ion OneTouch™ Dx Template Kit, Ion PGM™ Dx Sequencing Kit, and Ion 318™ Dx Chip Kit: For use with the Ion PGM™ Dx System.

Special conditions statement for performance derived from a representative assay using RNA and DNA from FFPE samples

1. The Ion PGM™ Dx System has been validated to deliver the following using a representative assay:
   - Sequencing output >0.7 gigabases
   - Reads >3 million
   - Read length up to 141 base pairs

2. A representative assay consisting of two sets of primer panels was used to detect DNA and RNA variants in key regions of cancer-related genes. The Ion PGM™ Dx System has been evaluated for the detection of SNVs, multi-nucleotide variants (MNVs), and deletions of various lengths in FFPE tissue samples using this representative assay. The types and numbers of variants detected by the assay are listed below.

Type of variant	Number of variants detected with a representative assay	Number of samples tested for detection by sample type		
		Plasmid/FFPE Sample Blend	FFPE Cell Line or FFPE Cell Line Blend	FFPE Clinical Sample
MNV	9	9	0	2
SNV	326	329	8	113
3-bp deletion	4	6	0	0
6-bp deletion	4	8	0	0
9-bp deletion	4	8	0	1
12-bp deletion	7	7	0	0
15-bp deletion	7	10	3	23
18-bp deletion	7	8	0	11
ROS1 fusion	34	5	6	20

3. The following studies were used to evaluate the performance of the Ion PGM™ Dx System using a representative assay:
   - Accuracy
   - Sample reproducibility
   - Assay reproducibility
   - Tissue input
   - DNA and RNA input
   - Interfering substances
4. The system is designed to deliver qualitative results.
5. As with any hybridization-based workflow, underlying polymorphisms or mutations in primer-binding regions can affect the regions being sequenced and, consequently, the ability to make calls.
6. The minimal coverage required to call an SNV, MNV or deletion variant is ≥347X. The minimal coverage required to call a fusion variant is ≥41X.
7. Special instrument requirements for the Ion PGM™ Dx Library Kit, Ion OneTouch™ Dx Template Kit, Ion PGM™ Dx Sequencing Kit, and Ion 318™ Dx Chip Kit: For use with the Ion PGM™ Dx System.

Theory of operation

Overview

DNA from whole blood samples is isolated using a commercially available extraction method, or DNA and RNA from FFPE tissue samples are isolated and the RNA is reverse transcribed into cDNA using methods that have been validated with a diagnostic assay. The DNA and/or cDNA is made into amplicon libraries via polymerase chain reaction (PCR) using the Ion PGM™ Dx Library Kit in combination with primer panels targeting the variants of interest. Through this process, up to 16 samples can each be given a unique identifying sequence by the addition of a nucleic acid barcode. Employing emulsion PCR, each sample library is templated onto Ion PGM™ Dx Ion Sphere™ Particles (ISPs) using the Ion OneTouch™ Dx Template Kit and the Ion OneTouch™ Dx Instrument. Templated ISPs are enriched from non-templated ISPs on the Ion OneTouch™ ES Dx Instrument and loaded onto an Ion 318™ Dx Chip with the Ion PGM™ Dx Chip Minifuge. The sequencing reaction is performed using the Ion PGM™ Dx Sequencing Kit on the Ion PGM™ Dx Sequencer, which measures hydrogen ions that are generated during the incorporation of nucleotides into the nascent strand complementary to the template sequence.

The signal generated by the sequencing reaction on the Ion 318™ Dx Chip is translated into base calls and then reads. The reads are mapped to a reference sequence, from which variant calls are determined. This process is executed using the Torrent Suite™ Dx Software, which runs on the Ion Torrent™ Server.

The Torrent Suite™ Dx Software manages the complete end-to-end workflow from sample to variant call. The Ion OneTouch™ Dx Instrument and Ion PGM™ Dx Sequencer are controlled by instrument control software (ICS). The end result of this workflow is a set of variant calls that correspond to the original sample.

Preparing samples

The system has been validated with human gDNA prepared from peripheral whole blood samples, and with DNA or RNA isolated from FFPE tissue samples.

- DNA from whole blood samples can be prepared using any commercially available method that yields at least 10 ng of DNA with an A_{260}/A_{280} ratio of >1.7.
- DNA and RNA can be isolated from FFPE tissue samples using a method that has been validated with a diagnostic assay, and that yields DNA and RNA at the concentrations required for the assay being performed.

Instrument preparation

Both the Ion OneTouch™ Dx Instrument and Ion PGM™ Dx Sequencer must be cleaned prior to running. Following initialization of the sequencer, the operator may elect to perform one or two sequencing runs within a 27-hour period.

During a run

Using the Ion OneTouch™ Dx Instrument and the process of emulsion PCR, the library molecules are bound to ISPs and the DNA sequence is clonally amplified over the ISP surface. Templated beads are enriched and collected using the Ion OneTouch™ ES Dx Instrument. Sequencing primer is annealed to the template, sequencing enzyme is added, and the templated beads are loaded onto the Ion 318™ Dx Chip. Chip loading occurs through use of the Ion PGM™ Dx Chip Minifuge. The chip is then placed onto the Ion PGM™ Dx Sequencer, where the DNA sequencing reaction occurs.

As the Ion PGM™ Dx Sequencer flows nucleotides over the chip surface, bases are incorporated into nascent strands on the bead in each well, resulting in the release of protons and a concomitant pH change in the well. The change in pH is detected by sensors at the base of each well on the chip. This initial electrical trace is processed for each well and transmitted to the Ion Torrent™ Server associated with the system.

Data analysis

On the Ion Torrent™ Server, the initial traces are processed and bases are called. These calls are assembled into files representing the reads, which are strings of nucleotide bases in the order found in the original library molecules. The reads are then mapped to the reference human genome. Finally, Ion Torrent™ Server assesses the mapped reads at specific nucleotide locations and looks for variation from the sequence information in the human reference sequence.

Results

The Torrent Suite™ Dx Software generates an electronic report for each sample sequenced. This report contains a full audit trail relating to the actions within the run and also output files representing reads (uBAM), mapped reads (BAM), and variant calls (VCF). These results are released from the system only upon approval by a lab manager or administrator. Once approved using electronic signature, the output files are made available for export.

Limitations for FFPE samples

The Ion PGM™ Dx System has been validated to detect SNVs, MNVs, and deletions in DNA and fusions in RNA in non-small cell lung cancer (NSCLC) FFPE tumor slide specimens. Results presented for FFPE samples were obtained using the Ion PGM™ Dx System and associated reagents with a representative assay, and are provided for informational purposes only. The validation testing with the representative assay only establishes the instrument's general capabilities and does not establish the instrument's capabilities or suitability with respect to any specific claims. All diagnostic tests developed for use on this instrument require full validation for all aspects of performance.

Kit compatibility

The Ion PGM™ Dx System can be used only with the Ion PGM™ Dx Library Kit, Ion OneTouch™ Dx Template Kit, Ion PGM™ Dx Sequencing Kit, and the Ion 318™ Dx Chip Kit.

Software compatibility and requirements

The procedures in this guide are designed for use with Torrent Suite™ Dx Software version 5.12.5 or later. To view the current software version, sign in to the software as an Administrator, click the **Settings (⚙)** tab, select **Configuration**, then click the **Software Updates** tab. Version-specific information is provided in the software release notes for the version of the software you are using.

Torrent Suite™ Dx Software is supported on Google™ Chrome™ browser version 64 and later, and is best viewed with 1440 × 900 screen resolution. It has not been tested with other browsers.

The Ion Torrent™ Server operating system is Ubuntu™ 18.04 LTS.

For more information about using the software, see the *Torrent Suite™ Dx Software 5.12.5 User Guide* (Pub. No. MAN0018762).

Materials provided

Ion PGM™ Dx Instrument System

The Ion PGM™ Dx Instrument System (Cat. No. A25511) includes the following components, which are also sold separately.

✓	Component	Catalog No.
	Ion OneTouch™ Dx Instrument and accessories	A25483
	Ion OneTouch™ ES Dx Instrument and accessories	A25484
	Ion PGM™ Dx Sequencer and accessories	A25485
	Ion PGM™ Wireless Scanner	A25486
	Ion Torrent™ Server (software installed separately)	A28552
	Ion OneTouch™ Rack Kit • Ion OneTouch™ Solutions Rack • Ion OneTouch™ Assembly Rack • Ion OneTouch™ Sample Rack	A24694
	Ion PGM™ Dx Chip Minifuge: • 120 VAC • 230 VAC	 A25058 A25482
	DynaMag™ Dx Kit—Tube & Plate • DynaMag™ Dx 96-Well Plate Magnet • DynaMag™ Dx 16 2-mL Magnet	A31755 A31347 A31346

Ion PGM™ Dx Consumables

The Ion PGM™ Dx Consumables bundle (Cat. No. A25512) includes the reagents and supplies that are provided in the following kits, which are also sold separately.

✓	Component	Catalog No.
	Ion PGM™ Dx Library Kit	A49758
	Ion OneTouch™ Dx Template Kit	A49759
	Ion PGM™ Dx Sequencing Kit	A49760
	Ion 318™ Dx Chip Kit	A18937

Ion PGM™ Dx Library Kit

The Ion PGM™ Dx Library Kit (Cat. No. A49758) provides reagents for preparing up to 96 sample libraries.

IMPORTANT! Do not mix components from other library kits.

✓	Component	Amount	Storage
Ion PGM™ Dx Library Reagents (Part No. A18928)			
	LIB HiFi Mix (red cap)	6 × 252 µL	–30°C to –10°C
	LIB FuPa (green cap)	6 × 32 µL	
	LIB Switch Soln (orange cap)	6 × 64 µL	
	LIB DNA Ligase (clear cap)	6 × 32 µL	
	BC 1 through BC 16 (16 unique barcode adapters, numbered 1–16, white cap)	16 × 12 µL	
Ion PGM™ Dx Library Equalizer™ Reagents (Part No. A18929)			
	LIB AMPure™ Reagent (clear cap)	4.4 mL	2°C to 8°C
	LIB Beads (yellow cap)	6 × 48 µL	
	LIB Primers (blue cap)	6 × 36 µL	
	LIB Capture (violet cap)	6 × 160 µL	
	LIB Wash Soln (clear cap)	30 mL	
	LIB Elution Soln (clear cap)	9.6 mL	

Ion OneTouch™ Dx Template Kit

The Ion OneTouch™ Dx Template Kit (Cat. No. A49759) includes the following modules and components.

IMPORTANT! Refer to the product label for the expiration date of the kit.

✓	Component	Amount	Storage
Ion OneTouch™ Dx Template Supplies (Part No. A18933)			
	TMPL Amplification Plate	8	15°C to 30°C
	TMPL Recovery Router	8	
	TMPL Recovery Tube	16	
	TMPL Sipper	2	
	TMPL Reagent Tube	2	
	TMPL ES Tip	8	
	TMPL ES Strip Tube	1 pack of 12	
	TMPL Cleaning Adapter	8	
	TMPL Emulsion Cartridge	8	
	TMPL Reagent Tube Labels	1 set	
	TMPL Sample Collection Tube[1]	1 pack of 50	
Ion OneTouch™ Dx Template Solutions (Part No. A18932)			
	TMPL Oil (white cap)	450 mL	15°C to 30°C
	TMPL Reaction Oil (white cap)	22 mL	
	TMPL Water (yellow cap)	320 µL	
	TMPL Recovery Solution (brown cap)	280 mL	
	TMPL Wash Solution (white cap)	15.2 mL	
	TMPL Rgnt B (blue cap)	2 × 1.2 mL	
	TMPL ES Rsp Soln (orange cap)	1.04 mL	
	TMPL Neutral Soln (red cap)	80 µL	
	TMPL Tween™ Solution (white cap)	2.24 mL	

✓	Component	Amount	Storage
	Ion OneTouch™ Dx Template Reagents (Part No. A18930)		
	TMPL Enzyme Mix (brown cap)	400 µL	–30°C to –10°C
	TMPL Rgnt Mix (violet cap)	8 × 500 µL (single-use tubes)	
	TMPL ISP (black cap)	800 µL	
	TMPL CF-1 (clear cap)	40 µL	
	Ion OneTouch™ Dx Template ES Beads (Part No. A18931)		
	TMPL ES Beads (green cap)	104 µL	2°C to 8°C

[1] Extra tubes are provided. Use one tube per Ion OneTouch™ ES Dx Instrument run.

Ion PGM™ Dx Sequencing Kit

The Ion PGM™ Dx Sequencing Kit (Cat. No. A49760) includes the following modules and components, and is also included as a part of the Ion PGM™ Dx System consumables bundle:

✓	Component	Amount	Storage
	Ion PGM™ Dx Sequencing Supplies (Part No. A18936)		
	SEQ Wash Bottle Sipper	8 long, 16 short	15°C to 30°C
	SEQ Reagent Tube Sipper	32	
	SEQ Reagent Tube plus label	32	
	SEQ Wash 1 Bottle (250-mL bottle)	1	
	SEQ Wash 2 Bottle (2-L bottle)	1	
	SEQ Wash 3 Bottle (250-mL bottle)	1	
	Ion PGM™ Dx Sequencing Reagents (Part No. A18934)		
	SEQ dGTP (black cap)	2 × 40 µL	–30°C to –10°C
	SEQ dCTP (blue cap)	2 × 40 µL	
	SEQ dATP (green cap)	2 × 40 µL	
	SEQ dTTP (red cap)	2 × 40 µL	
	SEQ Enzyme (yellow cap)	24 µL	
	SEQ Primer (white cap)	96 µL	

✓	Component	Amount	Storage
	Ion PGM™ Dx Sequencing Solutions (Part No. A18935)		
	SEQ W2 Solution (white cap)	8 × 126.25 mL	2°C to 8°C (store SEQ W2 Solution bottles in the sealed plastic bag provided)
	SEQ Cleaning Tablet	8 tablets	
	SEQ Sample Buffer (brown cap)	160 µL	
	SEQ W3 Solution (white cap)	4 × 100 mL	

Ion 318™ Dx Chip Kit

The Ion 318™ Dx Chip Kit (Cat. No. A18937) includes the following components and is also included as a part of the Ion PGM™ Dx Consumables bundle:

✓	Component	Amount	Storage
	Ion 318™ Dx Chip	8	15°C to 30°C

DynaMag™ Dx 96-Well Plate Magnet and DynaMag™ Dx 16 2-mL Magnet

Note: Do not substitute non-IVD labeled magnets for the DynaMag™ Dx 96-Well Plate Magnet and DynaMag™ Dx 16 2-mL Magnet.

The DynaMag™ Dx 96-Well Plate Magnet and DynaMag™ Dx 16 2-mL Magnet, provided with Ion PGM™ Dx System, contain high-energy neodymium magnets and are used as part of the procedure for purifying sample libraries bound to LIB AMPure™ Reagent and LIB Beads. The DynaMag™ Dx 16 2-mL Magnet is also used to prepare TMPL ES Beads as part of template preparation.

The DynaMag™ Dx 96-Well Plate Magnet has 7 bar magnets with a hard plastic top to fit 96-well PCR plates. When you insert a plate, the magnets collect bead-bound biomolecules in suspension at the sides of the plate wells, allowing removal of fluid without disturbing the bead pellets. An extra column in the magnet enables sample mixing by shifting the plate back and forth in the magnet.

The DynaMag™ Dx 16 2-mL Magnet holds 16 standard 1.5-mL or 2-mL microcentrifuge tubes, and collects bead-bound biomolecules in suspension at the sides of the tubes, allowing removal of fluid without disturbing the bead pellets.

Do not use the magnets above 50°C (122°F) and store in a cool, dry environment.

Materials and equipment required but not provided

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

✓	Description	Source
	Ion Torrent Dx FFPE Sample Preparation Kit (for preparation of DNA and RNA from FFPE tissue samples)	A32445
	IVD primer panels	—
	Veriti™ Dx 96-well Thermal Cycler, 0.2 mL	4452300
	Laminar flow hood	MLS
	1.5-mL snap-cap low-retention polypropylene microcentrifuge tubes	MLS
	Aluminum cold blocks for use with 1.5-mL tubes and 96-well plates	MLS
	Microcentrifuge (must accommodate standard 1.5- and 0.2-mL microcentrifuge tubes, and generate 20,000 rcf)	MLS
	Mini centrifuge	MLS
	96-well plate centrifuge	MLS
	Benchtop cold box	MLS
	Absolute ethanol (ACS grade)	MLS
	Xylene (ACS grade, ≥98.5%)	MLS
	Single- and multi-channel pipettes (2-, 20-, 200-, 1000-µL)	MLS
	Aerosol-barrier pipette tips (2-, 20-, 200-, 1000-µL)	MLS
	MicroAmp™ Optical 96-well Reaction Plates	4481191 4481192 (with barcode)
	Adhesive PCR Plate Seals	AB0558
	Vortex mixer with a rubber platform	MLS
	Tank of compressed nitrogen (grade 4.8, 99.998% or better)	MLS
	Multistage (dual-stage) gas regulator (0-50 PSI, 2-3 Bar output)	MLS
	18-MΩ water purification system (see the following description)	MLS
	0.45-µm vacuum filtration system and filters (nylon or PVDF filters, 1 L vol.)	MLS
	1.5-mL tube rack	MLS
	15-mL conical tubes	MLS

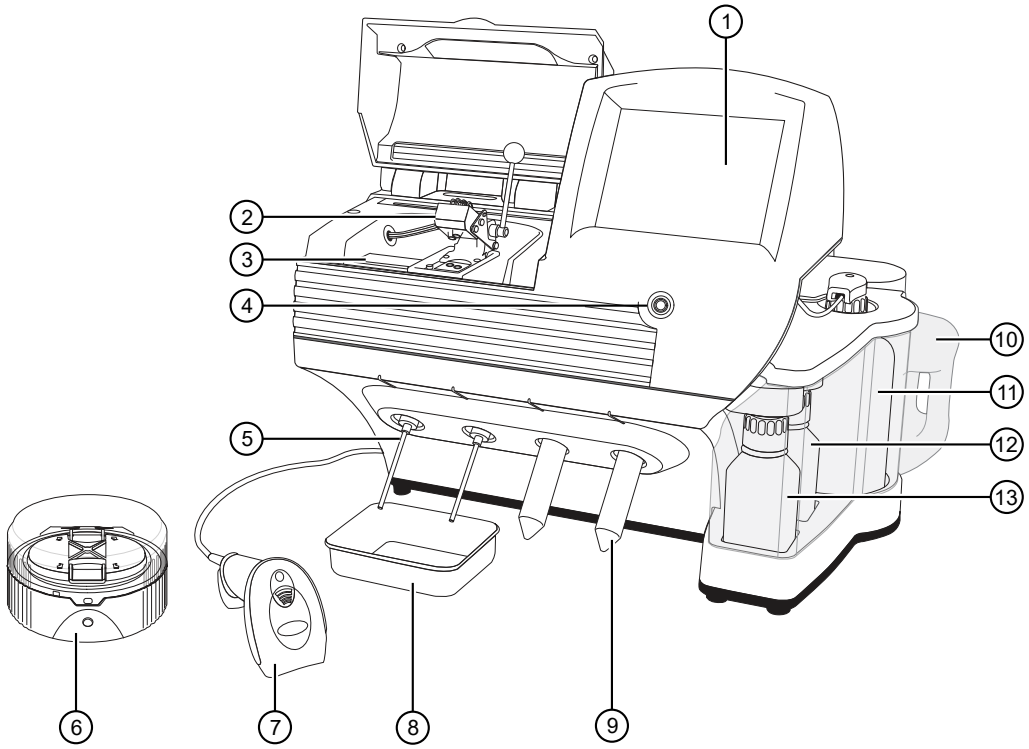
(continued)

✓	Description	Source
	50-mL conical tubes	MLS
	Holder for 50-mL conical tube	MLS
	Graduated cylinders (1 L or 2 L volume)	MLS
	Glass bottle (1 L)	MLS
	Nuclease-free Water	MLS
	NaOH, ACS grade (10 M)	MLS

18-MΩ water purification system

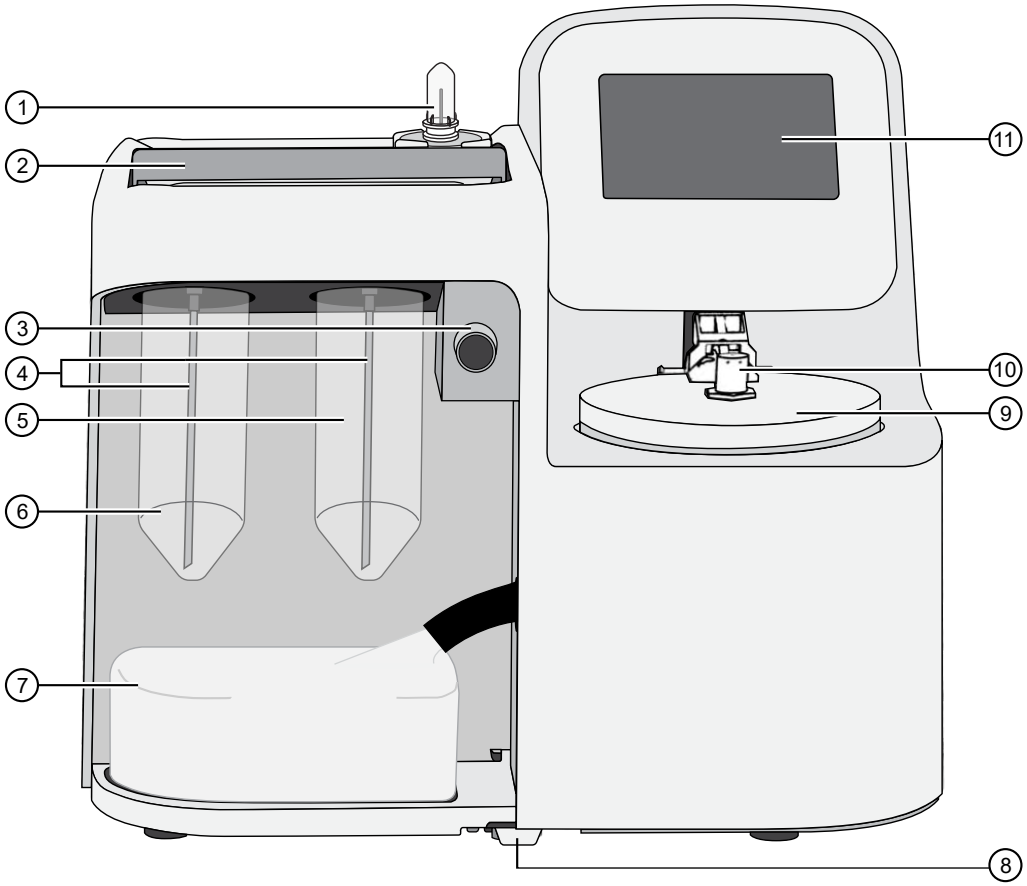
The Ion PGM™ Dx Sequencer requires an 18-MΩ water purification system to prepare water for solutions used on the instrument. Such a system is essential to remove ions and organic carbons from the water that might interfere with the chip surface or sequencing enzyme. Water purchased from vendors or stored for any length of time is not acceptable.

Ion PGM™ Dx System with Reagent and Wash Bottles attached



1. Touchscreen
2. Chip clamp
3. Grounding plate
4. Power button
5. Reagent Tube Sipper
6. Ion PGM™ Dx Chip Minifuge
7. Barcode scanner
8. Collection tray
9. Reagent Tube
10. Waste Bottle
11. Wash 2 Bottle (W2 position)
12. Wash 3 Bottle (W3 position)
13. Wash 1 Bottle (W1 position)

Ion OneTouch™ Dx Instrument



1. Reaction Filter
2. Clamp handle to access the TMPL Amplification Plate in the heat block
3. Pinch valve to hold disposable tubing
4. TMPL Sippers
5. TMPL Reagent Tube containing TMPL Recovery Solution
6. TMPL Reagent Tube containing TMPL Oil
7. Waste Container
8. Oil waste tray
9. Centrifuge and TMPL Recovery Router
10. Injector hub
11. Touchscreen

2 Before you begin

Installation requirements

Refer to the *Ion PGM™ Dx System Pre-Installation Checklist* (Pub. No. MAN0009822) and the *Ion PGM™ Dx System Site Preparation Guide* (Pub. No. MAN0016696) for pre-installation material and training checklists and for a site preparation checklist for customers to complete prior to system installation. Installation of the Ion PGM™ Dx System is performed by your Field Service Engineer and may be postponed if the pre-installation and site preparation checklists have not been completed.

Special requirements

- The Torrent Suite™ Dx Software requires the use of Google™ Chrome™ browser.
- The Ion PGM™ Dx Sequencer requires the use of an 18-MΩ water purification system installed in the laboratory. Such a system is essential for ensuring that any ions or organic carbons that might interfere with the chip surface or sequencing enzyme are removed from the water immediately before use on the instrument. Water purchased from vendors or stored for any length of time is not acceptable.

Gas cylinders

You must supply the required nitrogen gas cylinder and accessories for the installation. This instrument requires a pressurized house line or one size 1-A nitrogen gas cylinder that holds approximately 7.2 m^3 (257 ft^3) of gas when full. Use only prepurified nitrogen of 99.998% (grade 4.8) or greater purity.

CAUTION! Damage to the instrument and its products can result from using impure gas, gases other than nitrogen, or an inadequate amount of gas.

WARNING! EXPLOSION HAZARD. Pressurized gas cylinders are potentially explosive. Always cap the gas cylinder when it is not in use, and attach it firmly to the wall or gas cylinder cart with approved brackets or chains.

WARNING! Gas cylinders are heavy and may topple over, potentially causing personal injury and tank damage. Cylinders should be firmly secured to a wall or work surface. Please contact your Environmental Health and Safety Coordinator for guidance on the proper installation of a gas cylinder.

Perform a leak test

To perform a leak test on the gas cylinder:

1. Open the main tank shutoff valve. The high-pressure gauge of the gas tank regulator reads approximately 2,000–2,500 psi for a full tank.
2. Adjust the pressure to the instrument by slowly turning the pressure adjustment valve clockwise until the low-pressure gauge reads 30 psi.
3. Close the needle valve, then close the main tank valve.
4. Monitor the high-pressure gauge of the gas tank regulator for 5 minutes. There should be no noticeable drop in pressure.

If the pressure	Action
Drops in 5 minutes	There can be a leak at either the needle valve or the gas tank regulator itself. Check the fittings and resolve any problems, then continue with step 5.
Does not drop in 5 minutes	The instrument passes the leak test. Reopen the main tank valve and skip the following steps.

5. Open the main tank valve and the needle valve for at least 15 seconds to pressurize the instrument.
6. Close the main tank valve.
7. Monitor the high-pressure gas tank regulator gauge. There should be no more than a 100-psi drop in pressure after 5 minutes. Locate, then resolve any leaks. Turn the main tank valve back on.

Electrical and environmental conditions

IMPORTANT! Protection provided by the equipment may be impaired if the instrument is operated outside the environment and use specifications, the user provides inadequate maintenance, or the equipment is used in a manner not specified by the manufacturer.

IMPORTANT! Observe current Good Clinical Laboratory Practices (GCLP) when using this instrument.

See the *Ion PGM™ Dx System Site Preparation Guide* (Pub. No. MAN0016696) for information about instrument location and setup. Instruments should be shielded from excess exposure to dust, vibration, strong magnetic fields, drafts, excessive moisture, or large temperature fluctuations. Surge protectors or line conditioners should be used if the voltage source is not stable. Sudden voltage spikes can cause damage to the electronics inside the instruments.

Ensure that the room where the instruments have been installed is maintained under correct environmental conditions. Avoid placing the instruments adjacent to heaters, cooling ducts, or in direct sunlight. Place the instruments at least a meter away from major sources of electronic noise, such as refrigerators or microwaves.

Electrical requirements

CAUTION! Do not unpack or plug in any components until a field service representative has configured them for the proper operating voltage.

WARNING! For safety, the power outlet used for powering the instrument must be accessible at all times. In case of emergency, you must be able to immediately disconnect the main power supply to all the equipment. Allow adequate space between the wall and the equipment so that the power cords can be disconnected in case of emergency.

- Electric receptacle required: 2-prong with ground pin
- Main AC line voltage tolerances must be at most ±10% percent of nominal voltage.
- Power cords are provided with the instruments. If not suitable for installation in your region, ensure any power cord you do use is:
  - Maximum 10 feet (3 meters) in length
  - Grounding type
  - Compatible with the power supply receptacles used to connect to main power
  - Suitable for the rating of the instrument and main power supply
  - Compliant with local safety requirements (for example, UL Listed for North America, JIS approved for Japan, HAR or agency certified for Europe)
- (Ion OneTouch™ Dx Instrument only) Fuse Rating: 6 A, 250 VAC, Type M. Replace only with the same fuse type and rating.

WARNING! FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

Device	Rated voltage [1,2]	Rated frequency	Rated current [3]
Ion PGM™ Dx Sequencer	110/120VAC 220/240VAC	50/60 Hz	9 A
Ion Torrent™ Server [4]	110/120VAC 220/240VAC	50/60 Hz	11 A
Ion OneTouch™ Dx Instrument with power supply	110/120VAC 220/240VAC	50/60 Hz	5.5 A
Ion OneTouch™ ES Dx Instrument	110/120VAC 220/240VAC	50/60 Hz	375 mA 160 mA
Ion PGM™ Dx Chip Minifuge	120 VAC 220–240 VAC	50/60 Hz	130 mA 65 mA

[1] In Japan, rated voltages of 100 VAC and 200 VAC are acceptable.

[2] If the supplied power fluctuates beyond the rated voltage, a power line regulator may be required. High or low voltages can adversely affect the electronic components of the instrument.

[3] Based on rated current at minimum input voltage.

[4] Minimum Efficiency: 65% (Energy Star Qualified); 85% Efficient Power Supply.

Environmental requirements

Ensure that the room where the instruments have been installed is maintained under the correct environmental conditions. Avoid placing the instruments next to heaters, cooling ducts, or in direct sunlight. Place the sequencer at least a meter away from major sources of electronic noise, such as refrigerators or microwaves.

CAUTION! Use of the instruments in an unspecified manner may result in the protection provided by the instruments to be impaired.

Component	Acceptable range
Altitude	Between sea level and 2,000 meters (6,500 feet) above sea level
Humidity: operating	10–90% relative humidity
Humidity: transportation and storage	20–80% relative humidity
Temperature: operating	15–30°C (59–86°F) At or above 1,800 meters (5,906 feet), the system must not be used if the temperature is above 29.5°C.
Temperature: transportation and storage	–30°C to 60°C (–22°F to 140°F)

(continued)

Component	Acceptable range
Vibration	Ensure that benches where instruments are to be installed are free of vibration and have no contact with equipment that causes vibration (freezers, pumps, and similar equipment). Vibration can reduce the quality of sequencing measurements.
Pollution	The system has a Pollution Degree rating of II (2). The system may only be installed in an environment that has nonconductive pollutants, such as dust particles or wood chips. Typical environments with a Pollution Degree II (2) rating are laboratories, sales, and commercial areas.
Overvoltage category	The instruments have an installation (overvoltage) category of II (2).
Other conditions	For indoor use only. Keep away from any vents that could expel particulate material on the system components.

Precautions before using the Ion PGM™ Dx System

For additional safety information, see Appendix E, "Safety".

Instrument installation by trained personnel only

IMPORTANT! The Ion PGM™ Dx System is installed by trained service personnel and must not be relocated without assistance from trained service personnel. See "Customer and technical support" on page 140.

Nucleic acid contamination

IMPORTANT! A primary source of contamination is DNA fragments from previously processed samples. Do not introduce amplified DNA into the library preparation laboratory or work area.

IMPORTANT! Possible contamination can occur during the transfer of dNTPs into Reagent Tubes. Be careful to avoid cross contamination of dNTP stocks. Barrier tips are required for all pipetting steps. Change gloves after handling concentrated dNTP stocks.

Reagent contamination

Before use, verify that the nuclease-free water, TMPL Tween™ Solution, and TMPL Water used in the procedure are not cloudy, a potential indication of contamination. If any of these is cloudy, use a different vial.

CO_2 contamination

IMPORTANT! Dry ice (solid CO_2) must be kept away from areas where buffers, wash solutions, or sources of molecular biology grade water for the Ion PGM™ Dx System are used. High air concentrations of subliming CO_2 may change the pH of such buffers during or after their preparation. The stability of the pH of these buffers is a critical factor in the performance of the Ion PGM™ Dx System.

Instrument vibration and clearances

IMPORTANT! Significant vibration during sequencing may add noise and reduce the quality of the measurements. The Ion PGM™ Dx Sequencer must be installed on a bench that is free from vibrations or in contact with equipment that can cause vibrations to the bench, such as freezers, pumps, large benchtop centrifuges, and other similar equipment. Mini and microcentrifuges may be used near the sequencer. An air table is not required, nor is securing the sequencer to the bench.

IMPORTANT! Position the Ion PGM™ Dx Sequencer so that the front bezel is a minimum of 12 in. (30.5 cm) and the Reagent Tubes containing dNTPs are a minimum of 8 in. (20.3 cm) from the front of the laboratory bench. Place the instrument at least 40 in. (1 meter) away from major sources of electronic noise such as refrigerators or microwaves.

Static electricity

IMPORTANT! To avoid possible damage to chips from static electricity, see "Guidelines for chip handling and use" on page 33.

Ventilation requirements

WARNING! Instrumentation must be installed and operated in a well-ventilated environment, defined as having a minimum airflow of 6–10 air changes per hour. Assess the need for ventilation or atmospheric monitoring to avoid asphyxiation accidents from inert gases and/or oxygen depletion, and take measures to clearly identify potentially hazardous areas through training or signage. Please contact your Environmental Health and Safety Coordinator to confirm that the instruments will be installed and operated in an environment with sufficient ventilation.

Procedural guidelines

Definitions

Throughout this guide:

- Room temperature is defined as the temperature range 15–30°C.
- A pulse centrifugation consists of a 3–5 second centrifugation at maximum speed in a mini centrifuge.

Guidelines to prevent cross-contamination

- Use good laboratory practices to minimize cross-contamination of products and reagents.
- When designing the laboratory layout, dedicate separate areas for pre- and post-amplification activities. Dedicate laboratory supplies and/or equipment to the appropriate area.
- Before and after use, clean all working surfaces with fresh 10% bleach followed by two water rinses.
- Use a laminar flow hood in the dedicated pre-amplification area when combining libraries and preparing the amplification solution.
- Before and after use, clean all surfaces and equipment in the laminar flow hood with fresh 10% bleach followed by two water rinses.
- Turn on the UV light in the hood for 10 minutes before and after use.
- Use fresh gloves before entering the hood.
- Change tips between pipetting steps.
- Prepare a waste container containing fresh 10% bleach solution for disposing of used tips after pipetting libraries.
- To collect the contents before opening, pulse centrifuge tubes containing libraries and library pools.
- When simultaneously preparing more than one amplification solution, only open one library-pool tube at a time.
- Use fresh reagents if a contamination event occurs or is suspected.
- Use fresh gloves when installing new consumables.
- To avoid contamination of TMPL Reagent Tubes (which may be reused), discard waste from the tubes in a separate container from other Ion OneTouch™ Dx Instrument waste and 50-mL conical-tube waste.
- Always change gloves after handling Ion OneTouch™ Dx Instrument waste oil, used amplification plates, and used cleaning adapters.
- To prevent cross-contamination, we do not recommend running the same barcode for the same type of library sample (DNA or RNA) in a consecutive system run.

Guidelines for FFPE samples

- For core needle biopsies, macrodissection is not recommended due to the limiting tissue section surface areas.
- For resection or surgical biopsies, macrodissect and enrich the sample for tumor content if the tumor content is less than 20% and the tumor content in the region of interest is greater than or equal to 10%. Following tumor enrichment, proceed with the extraction protocol.
- Necrotic samples: Up to 75% necrotic tissue in the region of interest does not appear to interfere with the assay. However, we recommend that you macrodissect highly necrotic areas or select alternate samples if possible.
- Nucleic acid integrity is important for sample performance. Factors such as age of the block, fixation process used, and sample source can impact the quality of the extracted nucleic acid.
- Nucleic acid yield can be impacted by overall tissue area. Should an initial extraction lead to insufficient concentrations for DNA and RNA, repeat the extractions with more material whenever possible.

Guidelines for mixing reagents

Immediately before each use:

- Mix enzyme solutions (e.g., Protease and DNase) by flicking the tubes 4 times, followed by a pulse centrifugation.
- Vortex non-enzyme-containing reagents for ~5 seconds, followed by a pulse centrifugation.
- Mix reagent bottles by inverting them 5 times.

Guidelines for RNA

- Wear clean gloves and a clean lab coat.
- Change gloves whenever they may be contaminated.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Clean lab benches and equipment (including gloves, tube racks, pipettes, centrifuges, and vortexers) with an RNase decontamination solution before and after use.
- Work in a designated RNase-free pre-PCR area.
- Keep RNA on ice or in a –30°C to –10°C chilled benchtop cold box during use.
- Never vortex RNA. Flick 4 times to mix, then pulse centrifuge to collect.

Guidelines for pipetting

- Use aerosol-barrier pipette tips. Change pipette tips between samples.
- Avoid introducing air bubbles when pipetting by keeping the pipette tip at the bottom of the solution in the wells.
- Set the pipette to the recommended volume for mixing, and insert tip into the solution with the pipette plunger depressed to avoid introducing air bubbles.
- Visually inspect multi-channel pipette tips to ensure volumes are equivalent during pipeting.
- Touch tip to the side of well and slowly pipet reagent on the side of the well to form a droplet. This enables small volumes to be pipetted accurately and to ensure that the reagent has been added to the well.
- Inspect the pipette tips to verify that the reagent has been adequately dispensed.

Guidelines for freezing and thawing samples

There are stopping points throughout this procedure where you can freeze samples overnight or longer and then thaw the samples before proceeding. If you cannot perform the complete procedure in a day, proceed to a designated stopping point and freeze the samples overnight.

IMPORTANT! Freeze-thaw samples no more than 3 times.

Guidelines for Ion OneTouch™ Dx Instrument operation

- To prevent debris from entering the system, keep the TMPL Reagent Tubes installed on the Ion OneTouch™ Dx Instrument when not in use.
- After a Planned Run is executed on the Ion Torrent™ Server, the run must be started immediately on the Ion OneTouch™ Dx Instrument.
- A run on the Ion OneTouch™ Dx Instrument can be performed overnight. Enrichment on the Ion OneTouch™ ES Dx Instrument must start within 24 hours after completion of the Ion OneTouch™ Dx Instrument run.
- If a run is aborted for any reason, you must restart the Ion OneTouch™ Dx Instrument by power cycling.
- Ensure that you clean up any spilled oil immediately. Place a nonslip floor mat in front of the instrument to prevent slips.

CAUTION! Spilled oil from the Ion OneTouch™ Dx Instrument can present a slip hazard.

Guidelines for chip handling and use

IMPORTANT! To avoid damage to Ion 318™ Dx Chips or the Ion PGM™ Dx Sequencer due to electrostatic discharge:

- **Remove your gloves when handling chips**, especially before transferring chips on or off the instrument. Follow the steps in the sequencing procedure for taking off and putting on gloves.
- **Hold chips by their edges when handling.**
- **Do not place chips directly on the bench or any other surface.** Always place chips either on the grounding plate on the Ion PGM™ Dx Sequencer or in the Ion PGM™ Dx Chip Minifuge bucket.

Note: Ion 318™ Dx Chips can be handled without gloves during all stages of chip preparation, loading, and sequencing without risk of contamination.

Used chips cannot be reused for sequencing. Used chips must be discarded or clearly marked for cleaning and initialization.

Guidelines for initializing the sequencer

- Handle the SEQ dGTP, SEQ dCTP, SEQ dATP, and SEQ dTTP tubes carefully to avoid cross-contamination. Always change gloves after removing used sippers from the Ion PGM™ Dx System to avoid cross-contamination of the nucleotides. Also change gloves after handling concentrated dNTP stocks.
- Replace the SEQ Reagent Tubes and sippers every time you initialize.
- After 8 sequencing runs, do not use the SEQ Wash 1 Bottle, SEQ Wash 2 Bottle, or SEQ Wash 3 Bottle for initialization or sequencing to avoid possible breakage or leaking. You can continue to use the SEQ Wash 1 Bottle and SEQ Wash 3 Bottle as extra cleaning bottles.

Guidelines for sequencing runs

- One or two sequencing runs may be performed from a single initialization based on the option selected when starting the initialization, but both runs must be started within 27 hours after the start of initialization.
- If you press the **Abort** button on the sequencer touchscreen, the touchscreen may freeze. You may need to restart the sequencer.

IMPORTANT! After aborting a run, do not open the chip clamp, reagent tubes, or wash bottles until a new run or cleaning is initiated. Doing so can cause a fluid or gas leak if the sequencer was in a pressurized state when the run was aborted. From the main menu, select either **Clean** or **Run**, then follow the touchscreen prompts to depressurize the system.

Reagent management

Follow the guidelines below for proper reagent storage and use.

Storage

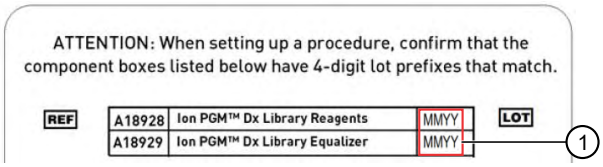
Reagents must be stored under appropriate conditions, as listed in the Product Information section of this user guide. The Ion PGM™ Dx System includes kits with multiple component boxes that require different storage conditions. For example, the Ion PGM™ Dx Library Kit is composed of two boxes: Ion PGM™ Dx Library Reagents and Library Equalizer™ Reagents, which are stored at different temperatures. To use the library kit, retrieve the boxes from their different storage areas and confirm that they are both from the same master lot.

Kit interchangeability and component box lot matching

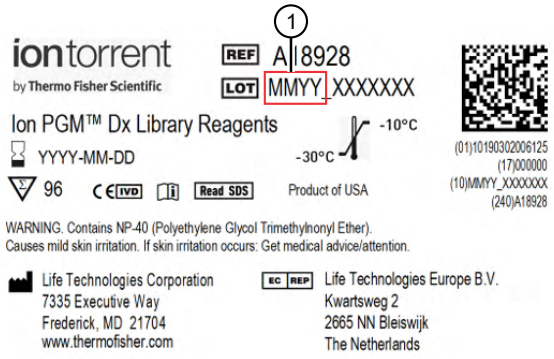
The top-level kits used for sample preparation, library preparation, template preparation, and sequencing can be mixed and matched. For example, an Ion PGM™ Dx Library Kit can be used with any Ion PGM™ Dx Sequencing Kit. However, the component boxes in a particular kit must be lot-matched with the other boxes in that kit.

Each component box lists the 4-digit lot prefixes of the compatible component boxes inside the box lid. Before using a particular kit, check the inside lid of each box to confirm that it is compatible with the other boxes.

An example inside box lid label is shown below:



An example box label with lot information is shown below:



1 Lot prefix

Instrument operation and maintenance

Service and maintenance

You will be alerted by the Ion PGM™ Dx System when annual maintenance service is required. A notification will appear on the instrument touchscreen and in the Torrent Suite™ Dx Software.

Shut down/restart the sequencer

In general, the Ion PGM™ Dx Sequencer can remain on all the time, including overnight and over weekends. If shutdown is necessary, there are two methods, depending on the state of the instrument:

Type of shutdown	Typically used when	Method
Routine shutdown (Managers and Administrators only)	• The instrument will not be used for an extended period of time (> 1-2 weeks). • The instrument needs to be moved or serviced.	1. Press the **Options** button on the main touchscreen and press **Shut Down**. 2. If the instrument will not be used for an extended period of time, select the **Cleaning** checkbox to perform a water clean first, then press **Shut Down**. Otherwise, just press **Shut Down**. 3. In the next screen, press the **Halt** button and then press **OK** to power down the instrument.
Forced shutdown	The instrument isn't responding normally (e.g, the touchscreen is frozen).	Hold down the power button below the touchscreen for ~5 seconds to power down the instrument.

To restart the sequencer:

- Hold down the power button below the touchscreen for ~5 seconds.

3 Workflow overview

This chapter provides a description and diagrams of the system workflow, including library preparation, template preparation, sequencing, and data analysis.

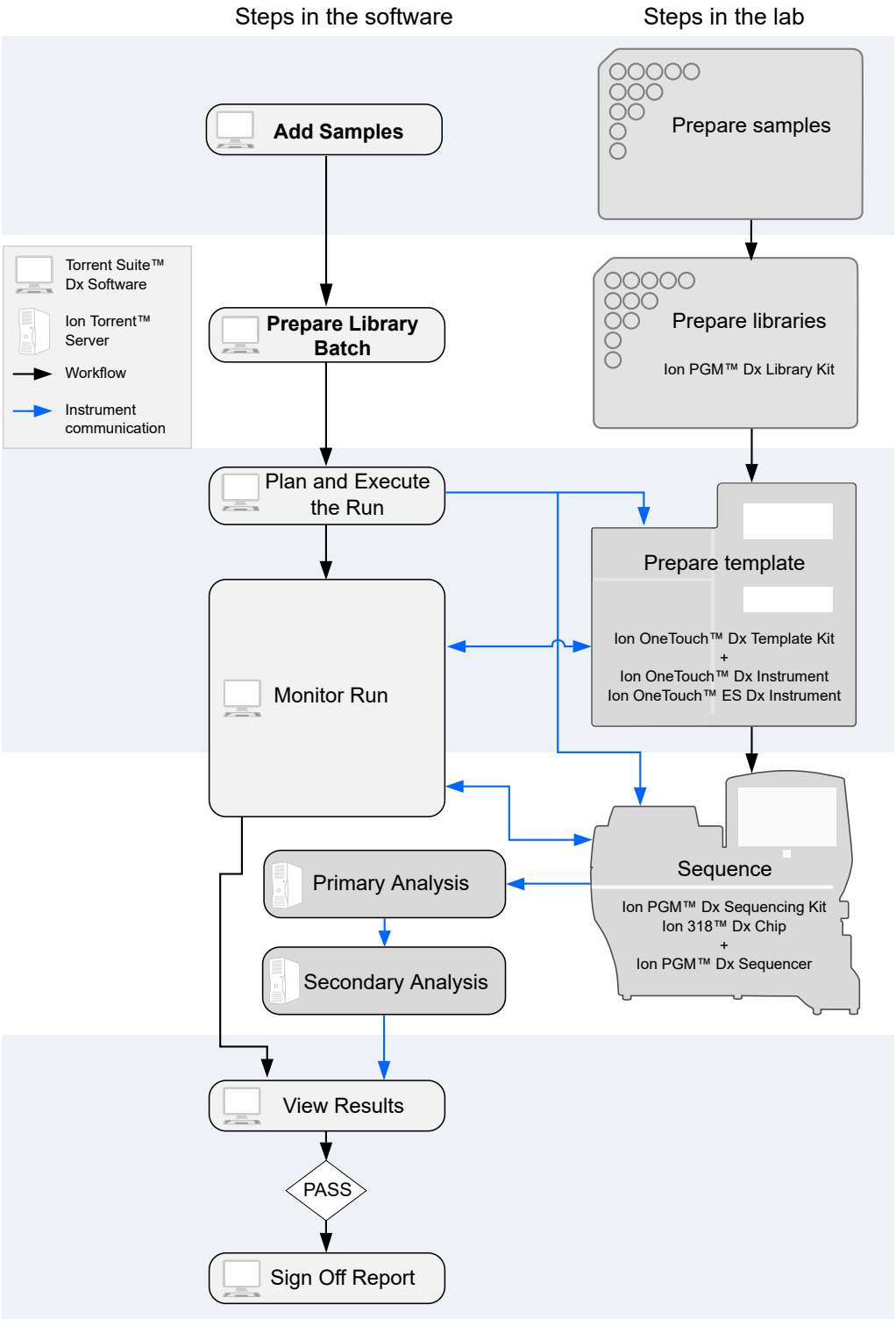
System workflow description

Using the Ion PGM™ Dx Library Kit, barcoded amplicon libraries are generated using IVD primer panels targeting variants of interest in RNA and DNA from FFPE samples or DNA from peripheral whole blood samples. The sample libraries are templated onto Ion PGM™ Dx Ion Sphere™ Particles (ISPs) using the Ion OneTouch™ Dx Template Kit and the Ion OneTouch™ Dx Instrument.

Templated ISPs are enriched from non-templated ISPs on the Ion OneTouch™ ES Dx Instrument and loaded onto an Ion 318™ Dx Chip using the Ion PGM™ Dx Chip Minifuge. The sequencing reaction is performed using the Ion PGM™ Dx Sequencing Kit on the Ion PGM™ Dx Sequencer.

The signals generated by sequencing reactions on the chip are translated into base calls and then reads. The reads are mapped to a reference sequence, and variant calls are determined. This process is executed using the Torrent Suite™ Dx Software.

System workflow diagram



Sample and library preparation workflow

Enter sample and library batch information
Time = ~10 minutes

Isolate and quantify DNA and/or RNA
Time varies

If using RNA, prepare cDNA
Time = ~50 minutes (15 minutes hands-on time)

Amplify targets from DNA or cDNA using an IVD primer panel
Time = 2 hours (10 minutes hands-on time)

Partially digest amplicons
Time = ~50 minutes (10 minutes hands-on time)

Ligate barcode adapters
Time = ~50 minutes (10 minutes hands-on time)

Purify the barcode-adapted library
Time = ~30 minutes

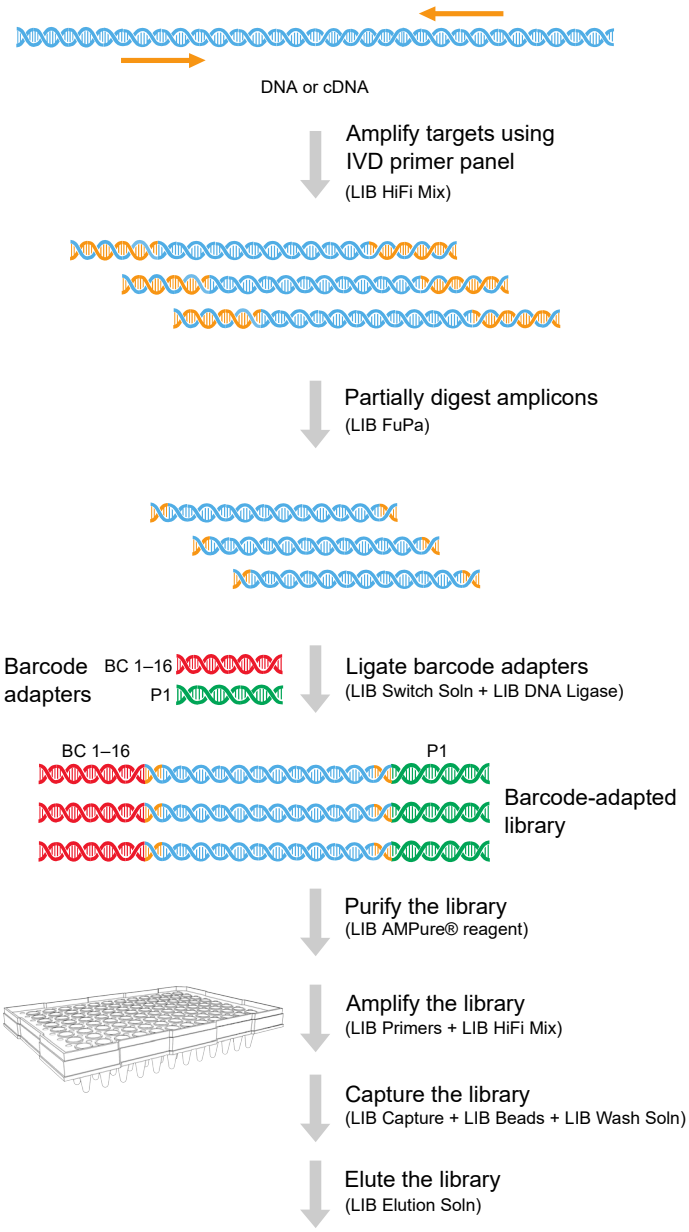
Amplify the purified barcode-adapted sample library
Time = ~30 minues (10 minutes hands-on time)

Prepare the LIB Beads
Time = ~10 minutes

Add LIB Capture to the amplified sample library
Time = ~10 minutes

Add the LIB Beads and wash
Time = ~20 minutes

Elute the library
Time = ~15 minutes (10 minutes hands-on time)



Template preparation workflow

Plan and execute a run
Time = ~15 minutes

Pool libraries and thaw reagents
Time = ~5 minutes

Clean the Ion OneTouch™ Dx Instrument before the run
Time = ~15 minutes (2 minutes hands-on time)

Perform an Ion OneTouch™ Dx run
Time = ~6 hours (45 minutes hands-on time)

Select the Planned Run

Install the Recovery Tubes and Recovery Router

Install the Amplification Plate

Install the disposable injector

Empty the waste container

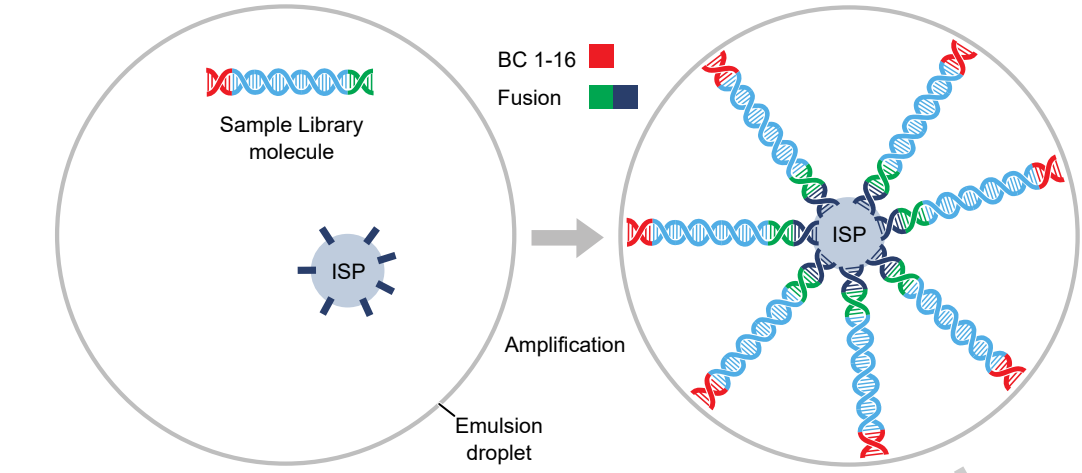
Prepare the Amplification Solution

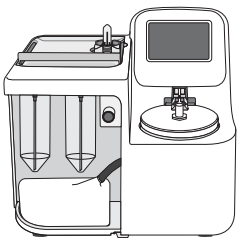
Fill and install the TMPL Emulsion Cartridge

Run the Ion OneTouch™ Dx Instrument (see figure below)

Recover the ISPs

Continued on next page





Ion OneTouch™ Dx Instrument

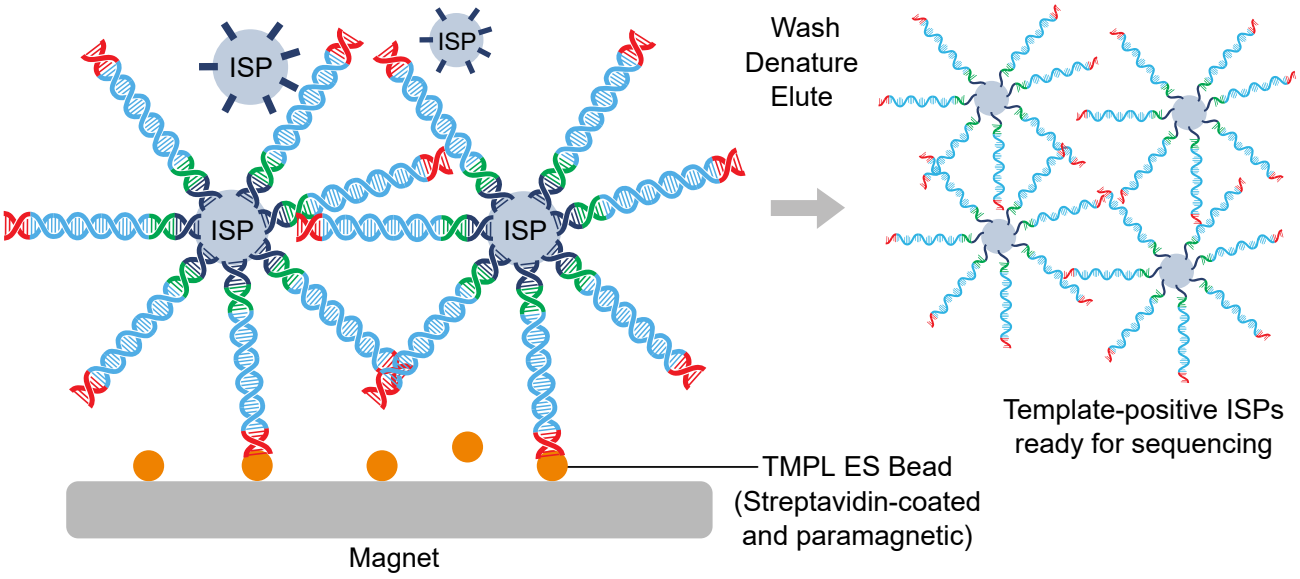
Prepare the Ion OneTouch™ ES Dx Instrument 8-well strip

Perform the ISP enrichment (see figure below)

Collect enriched ISPs from the Ion OneTouch™ ES Dx Instrument

Clean the Ion OneTouch™ Dx Instrument after the run

Ion OneTouch™ ES Dx Instrument



Sequencing workflow

Clean the sequencer
(water or chlorite)
Time = 30–40 minutes (15 minutes hands-on time)

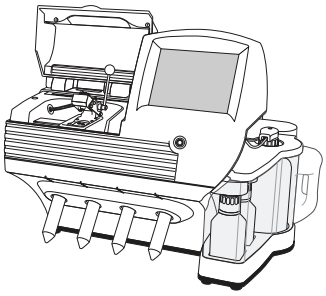
Initialize the sequencer
Time = ~1.5 hours (30 minutes hands-on time)

Prepare enriched ISPs for sequencing
Time = ~15 minutes

Set up the sequencing run
Time = ~5 hours (25 minutes hands-on time)

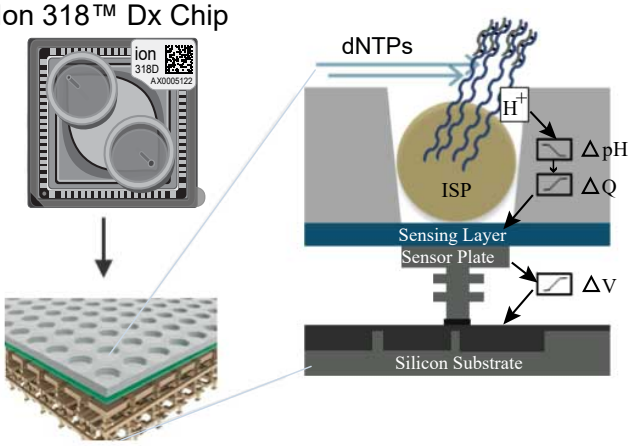
Load the sample on the chip

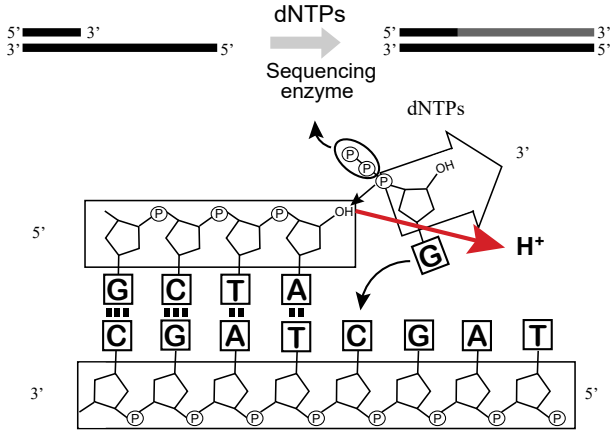
Perform the run



Ion PGM™ Dx Sequencer

Ion 318™ Dx Chip

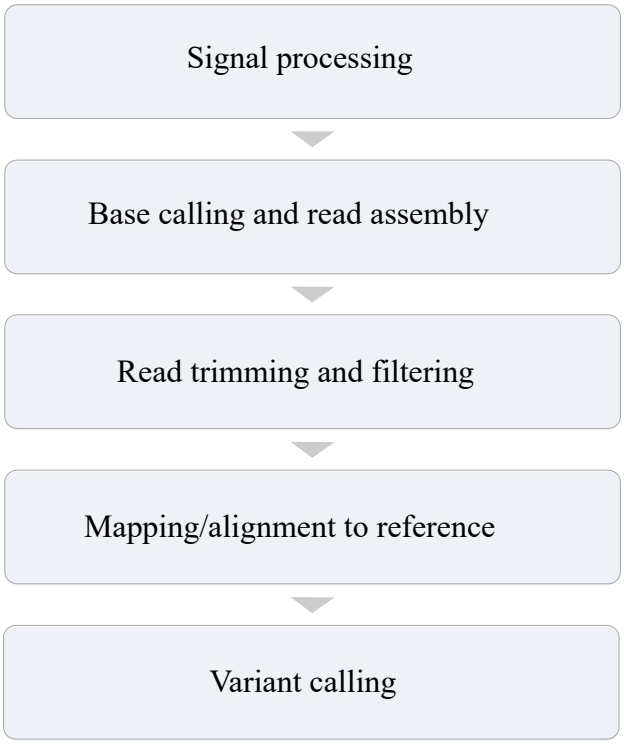




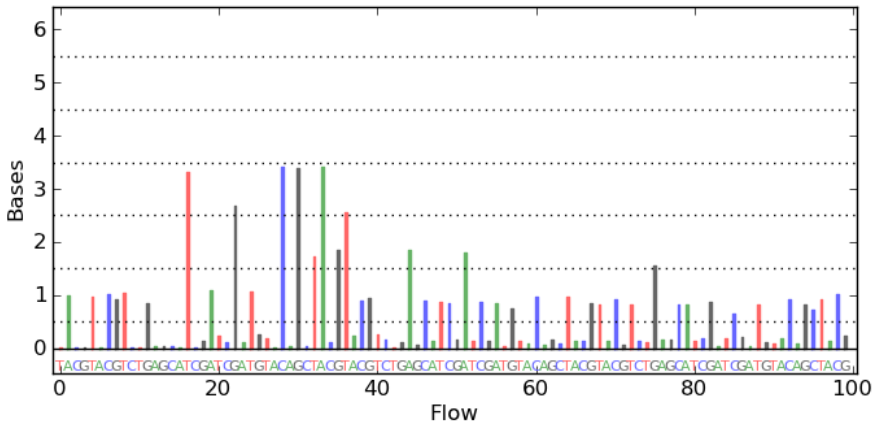
Data Analysis

Data analysis workflow

Primary and secondary analyses of sequencing data are performed by the Torrent Suite™ Dx Software on the Ion Torrent™ Server.



Ion Torrent ™ Server



Base call histogram

4 Sample preparation overview and software setup

Review the procedural guidelines

Before you begin the procedures in this section, review the procedural guidelines (see "Procedural guidelines" on page 30).

Sample preparation from whole-blood or FFPE samples

For whole-blood samples, extract and quantify the DNA using the methods and materials validated for your particular assay.

For FFPE samples, extract and quantify the DNA and RNA using the materials in the Ion Torrent Dx FFPE Sample Preparation Kit (Cat. No. A32445) and follow the procedures in the *Ion Torrent Dx FFPE Sample Preparation Kit User Guide* (Pub. No. MAN0017032).

IMPORTANT! Ion PGM™ Dx System library preparation has been validated with 10 ng of starting material (DNA or RNA) per reaction. You must validate the amount of starting material to use with your assay.

Tissue input requirements for FFPE sample extraction

The starting material for the extraction procedure is an FFPE tissue sample that is unstained and mounted on a slide. Confirm the tumor content of each sample based on the area of a hematoxylin and eosin (H&E) stained section.

We recommend that you macrodissect highly necrotic areas or select alternate samples if possible.

The recommended number of slide-mounted 5-micron FFPE sections used in extraction varies depending on the sample collection method:

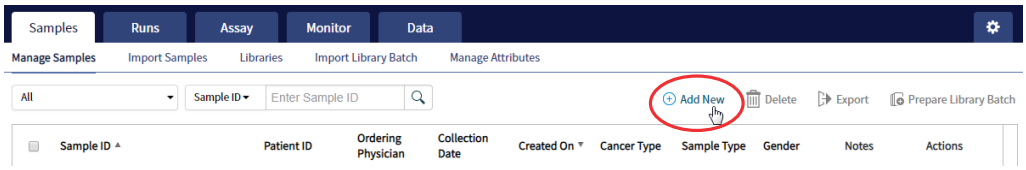
Sample collection method	Recommended number of sections
Resection or surgical biopsies	2 × 5-micron sections
Core needle biopsies	9 × 5-micron sections
Fine needle aspirates	7 × 5-micron sections

Note: Extraction from FFPE sample curls has not been evaluated.

Sample setup in Torrent Suite™ Dx Software

Add a new sample

1. Under the **Samples** tab, in the **Manage Samples** screen, click ⊕ **Add New**.



2. Complete the **Add New Sample** dialog box. Fields identified with an asterisk (*) are required. If no information is available, substitute dummy data to complete the required fields.

Field	Description
Sample ID*	A unique identifier representing the sample, containing only alphanumeric characters (0–9 and A to Z), full stops/periods (.), underscores (_), or hyphens (-). The Sample ID cannot contain spaces and is limited to a maximum of 20 characters. After a Sample ID is entered into the system, it cannot be edited. It can be deleted unless it has already been used in a library. The software checks all Sample IDs entered or imported to prevent duplication and returns an error message if a non-unique Sample ID is detected.
Patient ID*	An identifier representing the patient. This field accepts all characters including spaces.
Date of Birth*	The patient's date of birth. Click the button to select the date in the correct format.
Ordering Physician*	The name of the ordering physician. This field accepts all characters including spaces.
Collection Date*	The date the sample was collected from the patient. Click the button to select the date in the correct format.
Sample Source	Open-entry field that accepts all characters, including spaces. **Example entry:** Name of the clinic or hospital ordering the test.
Sample Condition*	Open-entry field that accepts all characters, including spaces. **Example entry:** FFPE surgical resection.
Sample Type*	Open-entry field that accepts all characters, including spaces. **Example entry:** Lung tumor, invasive adenocarcinoma.
Gender*	The biological gender of the sample. This must be Male, Female, or Unknown.
Cancer Type*	Select the cancer type of the sample from the dropdown list.
%Cellularity	The percentage of tumor cellularity in the sample.
%Necrosis	The percentage of cellular necrosis in the sample.

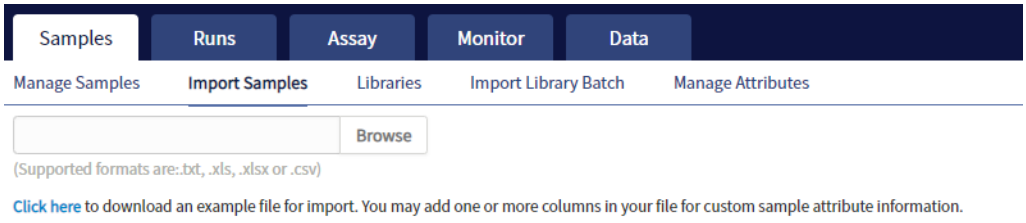
(continued)

Field	Description
Reference Interval	A normal range of measure for the sample.
Notes	An open-entry field.

☐ 3. Click **Save**.
The sample is listed in the **Manage Samples** screen.

Import samples

Under the **Samples** tab in the **Import Samples** screen, you can import sample data in the form of a TXT, XLS, XLSX, or CSV file. The import file includes the same sample attributes that are listed in the **Add New Sample** dialog box.



☐ 1. In the **Import Samples** screen, below the **Browse** field, click **Click here** to download a Microsoft™ Excel™ template file.

Note: The template file contains default sample attributes as columns. If additional custom sample attributes have been configured in the software, add these attributes as columns to the template file.

☐ 2. In the template file, fill in the information for each sample, one sample per row. See "Predefined sample attributes" on page 47 for more information.

☐ 3. Save the file.

☐ 4. Click **Browse**, navigate to the saved file, then select it.

☐ 5. Click **Import**.
A progress bar followed by an import report displays. If the import process fails, an error message indicates the reason for failure (for example, an invalid character was used). For additional troubleshooting, see "Batch sample import fails" on page 89.

☐ 6. Click **Manage Samples** to return to the sample list. Successfully imported samples are listed.

Predefined sample attributes

The software has the following predefined sample attributes, which are listed in the **Add New Sample** dialog box and in the template file for importing samples.

- Sample ID*
- Patient ID*
- Date Of Birth*
- Ordering Physician*
- Collection Date*
- Sample Source
- Sample Condition*
- Sample Type*
- Gender*
- Cancer Type*
- %Cellularity
- %Necrosis
- Reference Interval
- Notes

*Indicates a field required to be filled in during sample creation.

Predefined attributes are locked and cannot be edited. You can create and manage custom sample attributes using the tools in the **Manage Attributes** screen.

Note: LIMS users must create custom attributes before importing sample and Planned Run information from LIMS for the attributes to be propagated through to output files. The software ignores all input file content that is not a recognized attribute.

---

5 Library preparation

Prepare libraries and library batches

In Torrent Suite™ Dx Software, samples that are entered into the software are placed in library batches for processing and tracking. A library batch consists of a group of libraries that are prepared at the same time.

Note:

- Each library in a library batch must have a unique library name. When combining libraries in the same run, each must also have a unique barcode.
- Control libraries must be included in the same library batch as the sample library they control for.
- Fields identified with a red asterisk (*) are required.

1. Sign in to Torrent Suite™ Dx Software.
2. Under the **Samples** tab, in the **Manage Samples** screen, click **To Be Prepared** to display only those samples that have not been placed in a library batch.

   IMPORTANT! Samples that have not been queued for extraction in the software also appear on this tab. Ensure that the samples have been queued for extraction before queueing them for library batch preparation.
3. Select up to 6 samples in the list, then click **Prepare Library Batch**.
4. In the **Prepare Library Batch** dialog box, select the appropriate assay from the **Select Assay** dropdown list. The assay determines specific parameters of the run, including any required controls and post-run data analysis settings.
5. In the following screen, enter a unique identifier for the library batch in the **Library Batch ID** field. Library Batch IDs can only contain alphanumeric characters (0–9 and A to Z), full stop/period (.), underscore (_), and hyphen (-).
6. Click inside the **Library Kit Barcode** field, then scan the barcode from the Ion PGM™ Dx Library Reagents box.

   IMPORTANT! Be sure to scan the barcode from the actual Ion PGM™ Dx Library Reagents box that is used in the run.

☐ **7.** Type a unique library name for each DNA and RNA library in the appropriate field. Library names can only contain alphanumeric characters (0–9 and A to Z), full stop/period (.), underscore (_), and hyphen (-).

Note: If your assay requires specific controls, they are automatically listed in the dialog box. These controls each require a unique Barcode ID within the library batch but do not require library names.

☐ **8.** Select the Barcode ID of the adapter used to prepare each library. Swap the default barcodes in the dialog box between DNA and RNA using the DNA ⇄ RNA button.

Note: Each library in a library batch must have a different Barcode ID. When preparing the physical libraries, we recommend swapping barcodes between DNA and RNA libraries in consecutive sequencing runs to prevent carryover contamination.

IMPORTANT! Be careful to ensure that the actual barcodes used to create the libraries match the barcodes that are entered in the **Prepare Library Batch** dialog.

☐ **9.** Enter the **Input Quantity** for each library.

☐ **10.** Click **Save** to save your selections and close the dialog box.

The **Libraries** screen opens, listing the libraries that you created. Libraries that are prepared in the same batch have the same **Library Batch ID**.

Alternating barcodes

When preparing libraries, we recommend swapping barcodes between DNA and RNA libraries in consecutive sequencing runs to prevent carryover contamination. The following table provides an example of swapping barcodes between runs.

IMPORTANT! Be careful to confirm that the barcodes used to create the libraries match the barcodes entered in the **Prepare Library Batch** dialog box.

Library type	System Run 1 barcode usage		System Run 2 barcode usage	
	DNA	RNA	DNA	RNA
Positive control	1	9	9	1
Sample	2	10	10	2
Sample	3	11	11	3
Sample	4	12	12	4
Sample	5	13	13	5
Sample	6	14	14	6
Sample	7	15	15	7
No-template control (NTC)	8	16	16	8

Prepare reagents and equipment

- ☐ See "Procedural guidelines" on page 30 before setting up the reactions.
- ☐ Equilibrate the reagents listed below at room temperature for at least 30 minutes.
  - LIB AMPure™ Reagent
  - LIB Beads
  - LIB Primers
  - LIB Capture
  - LIB Wash Soln
  - LIB Elution Soln
- ☐ Place kit components that contain enzymes (LIB HiFi Mix, LIB FuPa, and LIB DNA Ligase) on ice or in a –30°C to –10°C chilled benchtop cold box throughout the procedure until needed. Before use, flick each tube 4 times to mix, then pulse centrifuge.

- ☐ • Thaw the remaining kit components (except enzymes) at room temperature until no ice is present in the tubes. Vortex for ~5 seconds, then pulse centrifuge before use.
- ☐ • If there is visible precipitate in the LIB Switch Soln after thawing, vortex for ~5 seconds at room temperature, and pulse centrifuge to collect. Repeat if needed until the solution is clear.

Amplify cDNA from FFPE samples

IMPORTANT! The following procedures have been validated using 10 ng of cDNA generated from RNA from FFPE samples. You must validate the amount of starting material to use for your assay.

Plate layout examples are provided to demonstrate how to arrange sample and control wells to avoid cross-contamination.

Set up the cDNA amplification reaction (<8 reactions)

If you are preparing <8 reactions, follow this procedure. If you are preparing 8–16 reactions, see "Set up the cDNA amplification reaction (8–16 reactions)" on page 52.

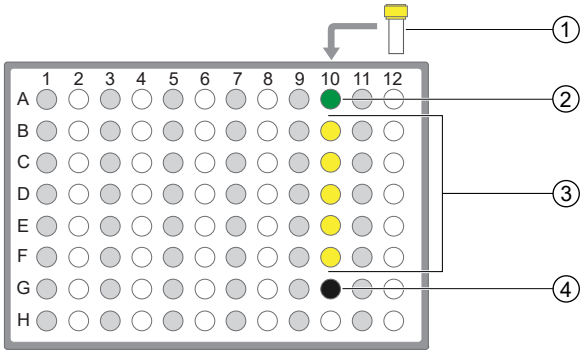


Figure 1 Example plate layout (<8 reactions)

1. IVD primer panel at 5X concentration
2. cDNA control (10 ng/well)
3. cDNA samples, prepared from RNA from FFPE tissue (10 ng/well)
4. No-template control

The following steps assume that you are starting with a plate containing cDNA from a 10-µL reverse transcription reaction, including cDNA from an RNA control, cDNA from RNA samples, and a no-template control.

Note: Do not exceed 10 ng of cDNA per reaction.

☐ **1.** Centrifuge the plate containing the cDNA (labeled "RNA/cDNA") at 100 rcf for 30 seconds.

☐ **2.** Transfer the plate to a chilled (2–8°C) 96-well aluminum block.

☐ **3.** Vortex the 5X primer panel for ~5 seconds, then pulse centrifuge. Flick the tube of LIB HiFi Mix 4 times to mix, then pulse centrifuge.

☐ **4.** Add the following components to each plate well.

	Component	Volume [1]
☐	Nuclease-free Water	2 µL
☐	5X primer panel	4 µL
☐	LIB HiFi Mix (red cap)	4 µL
	Total volume per well (includes 10 µL from cDNA synthesis)	**20 µL**

[1] Use 4 µL of water and 2 µL of 5X primer panel if you are using the Oncomine™ Dx Target Test—RNA panel.

☐ **5.** With the pipettor set to 15 µL, pipet up and down 5 times to mix the contents of each reaction well.

Set up the cDNA amplification reaction (8–16 reactions)

If you are preparing 8–16 reactions, follow this procedure. If you are preparing <8 reactions, see "Set up the cDNA amplification reaction (<8 reactions)" on page 51. For 8–16 amplification reactions (including controls), make a master mix for n+1 reactions, where "n" is the number of reactions you are preparing.

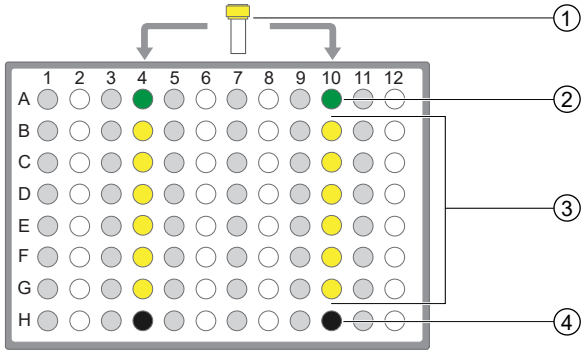


Figure 2 Example plate layout (8–16 samples)

① IVD primer panel at 5X concentration
② cDNA control (10 ng/well)
③ cDNA samples, prepared from RNA from FFPE tissue (10 ng/well)
④ No-template control

The following steps assume that you are starting with a plate containing cDNA from a 10-µL reverse transcription reaction, including cDNA from an RNA control, cDNA from RNA samples, and a no-template control.

Note: Do not exceed 10 ng of cDNA per reaction.

☐ **1.** Centrifuge the plate containing the cDNA (labeled "RNA/cDNA") at 100 rcf for 30 seconds.

☐ **2.** Transfer the plate to a chilled (2–8°C) 96-well aluminum block.

☐ **3.** Vortex the 5X primer panel for ~5 seconds, then pulse centrifuge. Flick the tube of LIB HiFi Mix 4 times to mix, then pulse centrifuge.

☐ **4.** Calculate the amounts of the following components needed for n+1 reactions, then add the components to a single 1.5-mL low-retention microcentrifuge tube.

	Component	Volume per reaction[1]
☐	Nuclease-Free Water	(n+1) × 2 µL
☐	5X primer panel	(n+1) × 4 µL
☐	LIB HiFi Mix (red cap)	(n+1) × 4 µL
	Total	**(n+1) × 10 µL**

[1] Use 4 µL of water and 2 µL of 5X primer panel if you are using the Oncomine™ Dx Target Test—RNA panel.

☐ **5.** Vortex the tube for ~5 seconds, then pulse centrifuge to collect.

Note: Keep the master mix on ice or chilled in a 2–8°C benchtop cold box until ready for use.

☐ **6.** Pipet 10 µL of the master mix into each sample or control well in the 96-well plate.

☐ **7.** Set the pipettor to 15 µL, then pipet the contents of each well up and down 5 times to mix.

Amplify the cDNA

Note: The Veriti™ Dx 96-well Thermal Cycler has been validated with this procedure.

☐ **1.** Seal the 96-well plate with a new adhesive film, then centrifuge the plate at 100 rcf for 30 seconds.

☐ **2.** Load the plate in the thermal cycler, then run the cycling program.

Note: The following example cycling program is for a primer panel containing 287 primers.

Stage	Step	Temperature[1]	Time
Hold	Activate the enzyme	99°C	2 minutes
Cycle (30 cycles)	Denature	99°C	15 seconds
	Anneal and extend	60°C	4 minutes
Hold	—	10°C	Hold (up to 24 hours)

[1] Use 98°C in the enzyme activation and denaturation steps if you are using the Oncomine™ Dx Target Test—RNA panel.

STOPPING POINT Amplicons can be held in the thermal cycler for up to 24 hours or stored at 2–8°C for up to 1 week. If stored longer than 1 week, prepare new amplicons.

Amplify DNA from FFPE samples

IMPORTANT! The following procedures have been validated using 10 ng of DNA from FFPE samples. You must validate the amount of starting material to use for your assay.

Plate layout examples are provided to demonstrate how to arrange sample and control wells to avoid cross-contamination.

Set up the DNA amplification reaction (<8 reactions)

If you are preparing <8 reactions, use the following procedure. If you are preparing 8–16 reactions, see "Set up the DNA amplification reaction (8–16 reactions)" on page 56.

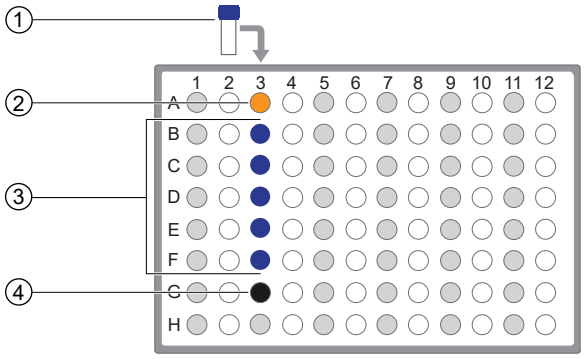


Figure 3 Example plate layout (<8 reactions)

1. IVD primer panel at 5X concentration
2. DNA control (10 ng/well)
3. DNA samples isolated from FFPE tissue (10 ng/well)
4. No-template control

Note: Do not exceed 10 ng of DNA per reaction.

☐ **1.** Label a 96-well plate "DNA".

☐ **2.** Place the labeled 96-well plate on a 2–8°C chilled 96-well aluminum block, then set up individual reactions in an odd-numbered column.

☐ **3.** Vortex the no-template control and 5X primer panel for ~5 seconds, then pulse centrifuge. Flick the tube of LIB HiFi Mix 4 times to mix, then pulse centrifuge.

☐ **4.** To the no-template control well, add the following components in the order indicated:

	Order	Component	Volume
☐	1	No-template control	12 µL
☐	2	5X primer panel	4 µL
☐	3	LIB HiFi Mix (red cap)	4 µL
	—	**Total**	**20 µL**

☐ **5.** In each DNA control well, prepare 10 ng of DNA control in a 12-µL volume by diluting with Dilution Solution as needed:

	Order	Component	Volume per reaction
☐	1	DNA control (10 ng)	X µL
☐	2	Dilution Solution (if needed)	to 12 µL

☐ **6.** To each DNA control well, add the following components in the order indicated:

	Order	Component	Volume
☐	1	5X primer panel	4 µL
☐	2	LIB HiFi Mix (red cap)	4 µL
	—	**Total volume in the well**	**20 µL**

☐ **7.** Vortex each DNA sample for ~5 seconds, then pulse centrifuge to collect.

☐ **8.** To each sample well, add the following components in the order indicated.

IMPORTANT! If you are preparing multiple sample libraries, ensure that the appropriate DNA sample is added to the correct well to avoid sample mix-up.

	Order	Component	Volume
☐	1	DNA sample (10 ng)	12 µL
☐	2	5X primer panel	4 µL
☐	3	LIB HiFi Mix (red cap)	4 µL
	—	**Total**	**20 µL**

☐ **9.** Set a 20-µL pipettor to 15 µL, and pipet the contents of each well up and down 5 times to mix.

Set up the DNA amplification reaction (8–16 reactions)

If you are preparing 8–16 reactions, use the following procedure. If you are preparing <8 reactions, see "Set up the DNA amplification reaction (<8 reactions)" on page 54. For 8–16 amplification reactions (including controls), make a master mix for n+1 reactions, where "n" is the number of reactions you are preparing.

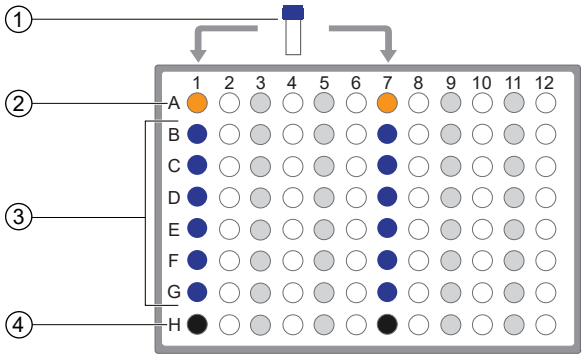


Figure 4 Example plate layout (8–16 reactions)

1. IVD primer panel at 5X concentration
2. DNA control (10 ng/well)
3. DNA samples isolated from FFPE tissue (10 ng/well)
4. No-template control

Note: Do not exceed 10 ng of DNA per reaction.

☐ **1.** Label a 96-well plate "DNA".

☐ **2.** Place the labeled 96-well plate on a 2–8°C chilled aluminum block, then set up reactions in individual wells in odd-numbered columns.

IMPORTANT! Skip columns to prevent cross-contamination.

☐ **3.** Vortex the no-template control for ~5 seconds, then pulse centrifuge.

☐ **4.** To each no-template control well, add 12 µL of no-template control.

☐ **5.** In each DNA control well, prepare 10 ng of DNA control in a 12-µL volume by diluting with Dilution Solution as needed:

	Order	Component	Volume per reaction
☐	1	DNA control (10 ng)	X µL
☐	2	Dilution Solution (if needed)	to 12 µL

☐ **6.** Vortex each DNA sample for ~5 seconds, then pulse centrifuge to collect.

☐ **7.** To each sample well, add 12 µL of DNA sample (10 ng).

☐ **8.** Vortex the 5X primer panel for ~5 seconds, then pulse centrifuge. Flick the tube of LIB HiFi Mix 4 times to mix, then pulse centrifuge.

☐ **9.** Calculate the volume of each component below needed for n+1 reactions, then add that volume to a pre-labeled 1.5-mL low-retention microcentrifuge tube in the order stated:

	Order	Component	Volume
☐	1	5X primer panel	(n+1) × 4 µL
☐	2	LIB HiFi Mix (red cap)	(n+1) × 4 µL
	—	**Total**	**(n+1) × 8 µL**

☐ **10.** Vortex for ~5 seconds, then pulse centrifuge.

Note: Keep the master mix at 2–8°C on ice until ready for use.

☐ **11.** Pipet 8 µL of master mix into each control and sample well.

☐ **12.** Set a pipettor to 15 µL, then pipet the contents of each well up and down 5 times to mix.

Amplify the DNA

Note: The Veriti™ Dx Thermal Cycler has been validated with this procedure.

☐ **1.** Seal the 96-well plate with a new adhesive film, then centrifuge the plate at 100 rcf for 30 seconds.

☐ **2.** Load the plate in the thermal cycler, then run the cycling program.

Note: The example cycling program below is for a primer panel containing 540 primers.

Stage	Step	Temperature	Time
Hold	Activate the enzyme	99°C	2 minutes
Cycle (20 cycles)	Denature	99°C	15 seconds
	Anneal and extend	60°C	4 minutes
Hold	—	10°C	Hold (up to 24 hours)

STOPPING POINT Amplicons can be held in the thermal cycler for up to 24 hours or stored at 2–8°C for up to 1 week. If stored longer than 1 week, prepare new amplicons.

Transfer the cDNA amplicons

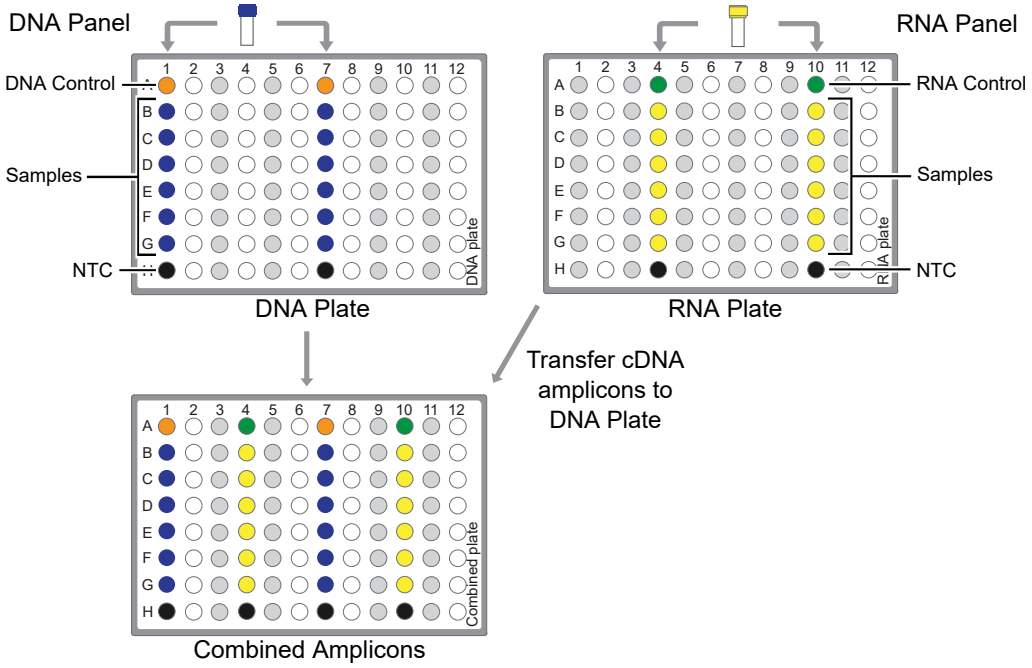
☐ **1.** After thermal cycling, transfer the 96-well plate to a designated post-PCR preparation area.

☐ **2.** Centrifuge the plates containing amplified cDNA and DNA at 100 rcf for 30 seconds.

☐ **3.** Carefully remove the adhesive film from the plates.

IMPORTANT! Be careful when removing the adhesive film from the plate to minimize cross-contamination.

4. Transfer the cDNA amplicons from the cDNA plate to the corresponding empty wells in even-numbered columns of the DNA plate. Skip columns to prevent cross-contamination, as shown in the example below.



Proceed to "Partially digest amplicons" on page 60.

Amplify DNA from whole blood samples

IMPORTANT! The following procedure has been validated using 10 ng of DNA from whole-blood samples. You must validate the amount of starting material to use for your assay.

For whole-blood samples, use the following procedure to amplify DNA with an IVD primer panel at 2X concentration.

Note: Do not exceed 10 ng of DNA per reaction.

1. To create a 2X primer panel, combine and dilute the desired primer pairs to the appropriate 2X working concentration:
   - For panels with an average of ≤96 primer pairs per panel, combine and dilute to 400 nM per primer.
   - For panels with an average of >96 primer pairs per panel, combine and dilute to 100 nM per primer.
2. If necessary, thaw the DNA and 2X primer panel at room temperature in a pre-PCR area. Vortex for ~5 seconds each, then pulse centrifuge.
3. Flick the tube of LIB HiFi Mix 4 times to mix, then pulse centrifuge.

☐ **4.** For each sample, combine the following at room temperature in a 1.5-mL low-retention microcentrifuge tube in the order specified. If you are preparing 8–16 sample libraries, prepare a master mix of components except DNA.

	Order	Component	1X Volume
☐	1	Nuclease-free Water	4 µL
☐	2	2X primer panel	10 µL
☐	3	LIB HiFi Mix (red cap)	4 µL
☐	4	DNA (10 ng at 5 ng/µL)	2 µL
	—	**Total**	**20 µL**

☐ **5.** Vortex the tube for 5 seconds, then pulse centrifuge.

☐ **6.** Label a 96-well plate and place it on a 2–8°C chilled aluminum block. Transfer each 20-µL reaction to a well in a row in the plate. For >8 samples, skip columns to prevent cross-contamination.

☐ **7.** Seal the plate with new adhesive film, then centrifuge the plate at 100 rcf for 30 seconds.

☐ **8.** Load the plate in the thermal cycler, then select or enter the program based on the number of primer pairs in your panel.

Note: The following Veriti™ Dx Thermal Cycler programs are included on the Ion PGM™ Dx System media storage device for amplifying DNA from whole-blood samples. Select **View** and confirm the program steps.

Primer pairs per panel	Veriti™ Dx Thermal Cycler programs
12–24	**01_Target-Amp 21 cycles**
25–48	**01_Target-Amp 20 cycles**
49–96	**01_Target-Amp 19 cycles**
97–192	**01_Target-Amp 18 cycles**
193–384	**01_Target-Amp 17 cycles**
385–768	**01_Target-Amp 16 cycles**

Stage	Step	Temperature	Time
Hold	Activate the enzyme	99°C	2 minutes
Cycle (according to program selected)	Denature	99°C	15 seconds
	Anneal and extend	60°C	4 minutes
Hold	—	10°C	Hold (up to 24 hours)

9. After you have confirmed the steps, run the program.
10. When cycling is complete, remove the plate from the thermal cycler and centrifuge at 100 rcf for 30 seconds.

Partially digest amplicons

1. Place the plate with the amplicons on a 2–8°C cold block.
   Carefully remove the adhesive plate seal, if necessary.
2. Flick the LIB FuPa tube (green cap) 4 times to mix, then pulse centrifuge to collect.
3. Add 2 µL of LIB FuPa to each reaction well. The total volume is 22 µL per well.
4. Set the pipettor to 15 µL, then slowly pipet the mixture up and down 5 times to mix.
5. Seal the plate with a new adhesive film, then centrifuge at 100 rcf for 30 seconds.
6. Load the plate in the Veriti™ Dx 96-well Thermal Cycler, then select the **02_Digestion** program. Select **View**, and confirm that the program steps match those listed in the table below:

Temperature	Time
50°C	10 minutes
55°C	10 minutes
60°C	20 minutes
10°C	Hold (for up to 1 hour)

7. After you have confirmed the steps, run the program.
8. During the run, thaw the LIB Switch Soln (orange cap) and appropriate barcode adapters (white caps) at room temperature for use in subsequent steps.

Ligate barcode adapters

1. After thermal cycling, centrifuge the plate at 100 rcf for 30 seconds, then place the plate back on the 2–8°C chilled aluminum block.
2. Vortex the LIB Switch Soln (orange cap) for ~5 seconds, then pulse centrifuge to collect.
3. Flick the tube of LIB DNA Ligase (clear cap) 4 times to mix, then pulse centrifuge to collect.
4. Ensure that the barcode adapters (BC 1–16) are thawed such that no visible ice is present. Vortex for ~5 seconds, then pulse centrifuge to collect.

☐ 5. Carefully remove the adhesive film from the plate, then add the following components to each well containing digested sample in the order shown:

	Order	Component	Volume
☐	1	LIB Switch Soln (orange cap)	4 µL
☐	2	Barcode adapter (white cap)[1]	2 µL
☐	3	LIB DNA Ligase (clear cap)	2 µL
☐	—	**Total volume per well** (includes 22 µL of sample)	**30 µL**

[1] Select from BC 1 through BC 16, based on the sample and your barcode scheme.

☐ 6. Set a pipettor to 20 µL, then pipet the volume in each well up and down 5 times.

☐ 7. Seal the plate with a new adhesive plate seal, then centrifuge the plate at 100 rcf for 30 seconds.

☐ 8. Load the plate in the Veriti™ Dx 96-well Thermal Cycler, then select the **03_Ligation** program. Select **View**, then confirm that the program steps match those listed in the following table.

Temperature	Time
22°C	30 minutes
72°C	10 minutes
10°C	Hold (for up to 1 hour)

☐ 9. After you have confirmed the steps, run the program.

☐ 10. During the run, equilibrate the LIB Beads (yellow cap), LIB Capture (violet cap), and LIB Elution Soln to room temperature.

Prepare the LIB HiFi Mix plus LIB Primers mix

☐ 1. Flick the LIB HiFi Mix (red cap) 4 times to mix, then pulse centrifuge. Keep the LIB HiFi Mix in a –30°C to –10°C chilled benchtop cold box.

☐ 2. Vortex the LIB Primers (blue cap) for ~5 seconds, then pulse centrifuge.

☐ 3. Prepare the LIB HiFi Mix plus LIB Primers master mix:

- <8 libraries—For each library, add components to individual 1.5-mL low-retention microcentrifuge tubes on ice or in a 2–8°C chilled benchtop cold box in the following order:

	Order	Component	Volume
☐	1	Nuclease-free Water	40 µL
☐	2	LIB HiFi Mix (red cap)	10 µL
☐	3	LIB Primers (blue cap)	2 µL
☐	—	**Total**	**52 µL**

- 8–16 libraries—Calculate the amount of every component needed for n+1 libraries, where "n" is the number of libraries being prepared, then add the components to a single 1.5-mL low-retention microcentrifuge tube on ice or in a 2-8°C chilled benchtop cold box in the following order:

	Order	Component	Volume
☐	1	Nuclease-free Water	(n+1) × 40 µL
☐	2	LIB HiFi Mix (red cap)	(n+1) × 10 µL
☐	3	LIB Primers (blue cap)	(n+1) × 2 µL
☐	—	**Total**	**(n+1) × 52 µL**

☐ **4.** Flick the master mix 4 times to mix, then pulse centrifuge. Keep at 2–8°C.

Note: You must use the master mix on the same day it was prepared.

Purify the barcode-adapted library

☐ **1.** Prepare fresh 70% ethanol: combine 230 µL of ethanol with 100 µL of Nuclease-free Water per library, then vortex for 10 seconds to mix.

☐ **2.** When thermal cycling is complete, centrifuge the 96-well plate at 100 rcf for 30 seconds.

☐ **3.** Before use, invert the LIB AMPure™ Reagent 10 times, then vortex for 10 seconds until the beads are thoroughly suspended.

☐ **4.** Carefully remove the adhesive film from the plate, then add 45 µL of LIB AMPure™ Reagent to each well.

☐ **5.** With the pipettor set to 45 µL, pipet up and down 5 times to thoroughly mix the beads in each well. The total volume is 75 µL.

☐ **6.** Hold the mixture for 5 minutes at room temperature.

☐ **7.** Place the plate in a DynaMag™ Dx 96-Well Plate Magnet for 3 minutes. The solution in each well must be clear, with beads pelleted to one side.

☐ **8.** Using a 200-µL pipettor, remove and discard ~75 µL of the supernatant without disturbing the pellet. Use a 20-µL pipettor to remove any remaining supernatant.

☐ **9.** If you see beads in the pipette tip when removing the supernatant, pipet the supernatant and beads back into their respective wells to re-pellet the beads, then remove and discard the supernatant.

☐ **10.** Add 150 µL of freshly prepared 70% ethanol to each well.

☐ **11.** Move the plate from left-to-right on the DynaMag™ Dx 96-Well Plate Magnet, then hold for ~5 seconds to wash and re-pellet the beads.

12. Move the plate from right-to-left on the magnet, then hold for ~5 seconds to wash and re-pellet the beads.

13. Repeat steps 11 and 12 two more times. Keep the plate in the final position on the magnet for 3 minutes or until the solution in each well is clear, with the beads in a pellet to one side.

14. Using a 200-µL pipette, remove and discard ~150 µL of the supernatant without disturbing the pellet. Use a 20-µL pipette to remove any remaining supernatant.

15. Repeat steps 10–14 one more time.

16. Ensure that all the ethanol droplets are removed from the wells. Keeping the plate in the magnet, air-dry the beads at room temperature for 5 minutes.

Amplify the barcode-adapted library

1. Flick the LIB HiFi Mix/LIB Primers master mix (prepared in "Prepare the LIB HiFi Mix plus LIB Primers mix" on page 61) 4 times to mix, then pulse centrifuge.

2. Remove the 96-well plate from the DynaMag™ Dx 96-Well Plate Magnet, then add 52 µL of LIB HiFi Mix/LIB Primers master mix to each well.

3. Set the pipettor to 40 µL, then pipet up and down 10 times to mix until the beads are resuspended.

   Note: Visually inspect the sides of the wells to ensure complete resuspension of the beads.

4. Seal the 96-well plate with a new adhesive film. Centrifuge the plate at 100 rcf for 30 seconds.

5. Load the plate in the Veriti™ Dx 96-well Thermal Cycler, then select the **04_Amplification** program. Select **View**, and confirm that the program steps match those in the table below.

Stage	Temperature	Time
Hold	98°C	2 minutes
7 cycles	98°C	15 seconds
	60°C	1 minute
Hold	10°C	Hold (for up to 30 minutes)

6. After you have confirmed the steps, run the program.

   Note: During thermal cycling, you may start to prepare the LIB Beads as described in the next procedure.

Prepare the LIB Beads

1. Equilibrate the LIB Beads (yellow cap) to room temperature, vortex for 10 seconds or until resuspended, then pulse centrifuge to collect.
2. For each library, combine 3 µL of LIB Beads and 6 µL of LIB Wash Soln (clear cap) in a 1.5-mL low-retention microcentrifuge tube, as follows:
   - For 1–3 libraries, prepare a separate tube of beads and wash solution per library.
   - For ≥4 libraries, prepare a master mix as shown below.

Number of libraries	Number of reactions to prepare in master mix	Volume of LIB Beads to add	Volume of LIB Wash Soln to add
4–7	n + 0.5	(n + 0.5) × 3 µL	(n + 0.5) × 6 µL
8	9	27 µL	54 µL
9–16	n + 2	(n +2) × 3 µL	(n +2) × 6 µL

3. Vortex each tube for ~5 seconds to mix, then pulse centrifuge to collect any beads present on the lid of the tube.
4. Place the tube in the DynaMag™ Dx 16 2-mL Magnet for 1 minute.
5. Carefully remove and discard the supernatant without disturbing the pellet.
6. Remove the tube from the magnet, then add the same volume of LIB Wash Soln as added in step 2.
7. Resuspend by pipetting up and down 5 times.
8. Keep the prepared beads at room temperature and use them on the same day.

Add LIB Capture to the amplified sample library

1. Confirm that the LIB Capture (violet cap) is at room temperature, vortex the tube for ~5 seconds, then pulse centrifuge to collect.
2. When thermal cycling is complete, centrifuge the 96-well plate at 100 rcf for 30 seconds.
3. Carefully remove the adhesive film from the plate, then add 10 µL of LIB Capture (violet cap) to each well.

   IMPORTANT! Accurate volume transfer in this step is critical. Ensure that no excess LIB Capture is carried on the pipette tip by aspirating the solution from just below the surface.
4. Set the pipettor to 40 µL, then pipet the mixture up and down 5 times to mix.
5. Hold at room temperature for 5 minutes.

Add the LIB Beads and wash

1. Mix the prepared LIB Beads by pipetting up and down 5 times, or until the beads are resuspended.
2. Add 6 µL of washed LIB Beads to each well.
3. Set the pipettor to 40 µL, then pipet the mixture up and down 5 times to mix.
4. Hold at room temperature for 5 minutes.
5. Place the 96-well plate in the DynaMag™ Dx 96-Well Plate Magnet for 3 minutes. The solution should be clear.
6. Using a 200-µL pipette, remove, then discard ~68 µL of the supernatant without disturbing the pellet. Use a 20-µL pipette to remove any remaining supernatant.
7. Add 150 µL of LIB Wash Soln to each well.
8. Move the 96-well plate from left-to-right on the DynaMag™ Dx 96-Well Plate Magnet, then hold for 5 seconds to wash and re-pellet the beads.
9. Move the 96-well plate from right-to-left on the magnet, then hold for 5 seconds to wash and re-pellet the beads.
10. Repeat steps 8 and 9 two more times.
11. With the 96-well plate still in the magnet, use a 200-µL pipette to remove and discard ~150 µL of the supernatant without disturbing the pellet.
12. Repeat the bead wash as described in steps 7–11.
13. Use a 20-µL pipette to remove any remaining LIB Wash Soln by pipetting without disturbing the pellet.

Elute the library

1. Remove the plate from the plate magnet, then add 100 µL of LIB Elution Soln to each pellet. Set the pipettor to 80 µL, then pipet up and down at least 10 times until the beads are resuspended.
2. Seal the plate with a new adhesive film, then centrifuge at 100 rcf for 30 seconds.
3. If beads pellet at the bottom of the wells:
   a. Carefully remove the adhesive film, and gently resuspend the pellet by pipetting up and down until resuspended.
   b. Seal the plate with a new adhesive film.

4. Load the plate in the Veriti™ Dx 96-well Thermal Cycler, then select the **05_Elution** program. Select **View** and confirm that the program matches the following table.

Temperature	Time
35°C	5 minutes

5. After you have confirmed the steps, run the program.

IMPORTANT! Remove the plate from the thermal cycler immediately after the 5-minute incubation is complete.

6. During cycling, label a 1.5-mL low-retention microcentrifuge tube for each library. Alternatively, if you are proceeding directly to pooling libraries, label a tube for each library pool.

7. Remove the plate from the thermal cycler, then centrifuge the plate at 100 rcf for 30 seconds.

8. Place the plate in the DynaMag™ Dx 96-Well Plate Magnet, then hold at room temperature for 3 minutes. Confirm that the solution is clear.

9. Carefully remove the adhesive film, then transfer the supernatant containing the equalized library (~100-µL total volume) to a labeled 1.5-mL low-retention microcentrifuge tube. The final concentration of each library is ~100 pM.

STOPPING POINT The eluted libraries can be stored at –30°C to –10°C for up to 30 days. If stored for longer than 30 days, prepare new libraries.

6 Template preparation

Plan the run

1. Sign in to the Torrent Suite™ Dx Software.
2. Under the **Samples** tab, in the **Libraries** screen, select the library or libraries to be run together, then click **Plan a Run**.
3. In the **Add New Plan** dialog, enter a name for the run, then select the appropriate report template. Add or remove libraries from the run if needed using the controls in the dialog.
4. Click **Save**.
5. Under the **Assay** tab, in the **Planned Runs** screen, find the Planned Run in the list of runs, then click **Execute** in the **Actions** column.
6. In the **Execute Planned Run** dialog, enter the text that you will label each tube with in the **Tube Label** field.

   Tube Label: _____
7. Click inside the **Template Kit Barcode** field, then scan or enter the barcode from the Ion OneTouch™ Dx Template Reagents box.
8. Click **Save**.

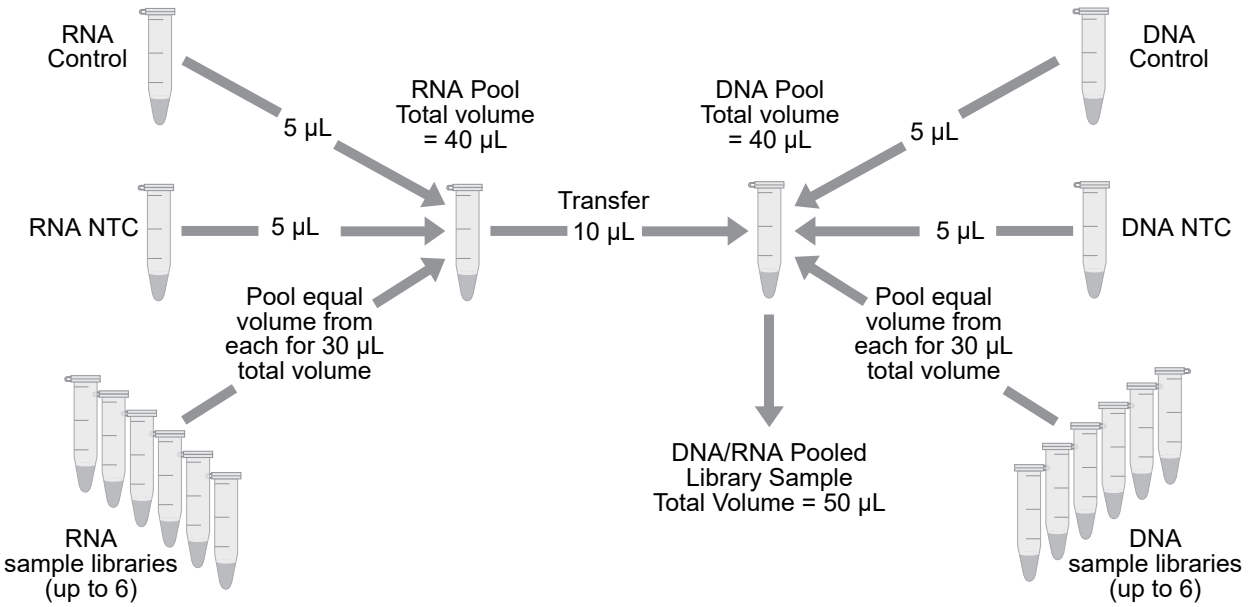
   The **Run Short Code** appears in the **View Planned Run** dialog.
9. Write down the code and/or print the **Planned Run** summary with the barcode.

   Run Short Code: _____
10. Close the **Review Planned Run** dialog.

Strategy for combining libraries

You can multiplex up to 16 barcoded libraries in a single Ion PGM™ Dx System run, including libraries from different samples, paired DNA and RNA libraries from a single sample, controls, or any combination of these. If you are combining libraries, determine the optimal volume of each library to be pooled for your particular assay, then combine them in a single 1.5-mL low-retention microcentrifuge tube. The total volume used in a sequencing run is 5 µL (single library or library pool).

A strategy for combining multiple libraries and controls is diagrammed below.



Prepare reagents and library tube for template preparation

- [ ] 1. Label the library tube with the Tube Label text that you entered into the software.
- [ ] 2. Equilibrate the library, TMPL Rgnt Mix, TMPL ISP, and TMPL CF-1 to room temperature for 30 minutes.

Clean the Ion OneTouch™ Dx Instrument before a run

- [ ] 1. Log in to the instrument using the touchscreen.
- [ ] 2. Press the **Clean** button, then follow the touchscreen prompts.
- [ ] 3. Remove the TMPL Reagent Tube containing TMPL Oil from the position marked with an "O" on the instrument (the tube on the left when facing the instrument), then discard the contents in an appropriate hazardous waste container.
- [ ] 4. Place the TMPL Reagent Tube in the position labeled "O" on the Ion OneTouch™ Solutions Rack.
- [ ] 5. Change gloves, then invert the TMPL Oil bottle (white cap) 5 times to mix. Fill the reagent tube with oil to the level marked on the rack, so the meniscus is within the fill indicator arrows (volume = 50 mL).
- [ ] 6. Confirm that the sipper is securely attached to the instrument, then screw the reagent tube containing the oil back into the position marked with an "O" on the instrument until the tube can no longer rotate. Press **Next**.
- [ ] 7. Remove the TMPL Reagent Tube containing TMPL Recovery Solution from the position marked with an "R" on the instrument. Discard the contents in an appropriate waste container.

8. Place the TMPL Reagent Tube in the position marked with an "R" on the Ion OneTouch™ Solutions Rack.

9. Change gloves, then invert the TMPL Recovery Solution bottle (brown cap) 5 times to mix. Fill the reagent tube with recovery solution to the level marked with arrows on the "R" position of the rack (volume = 35 mL).

10. Confirm that the sipper is securely attached to the instrument, then screw the reagent tube containing the recovery solution back into the position marked with an "R" on the instrument until the tube can no longer rotate. Press **Next**.

11. Confirm that both the TMPL Cleaning Adapter and TMPL Amplification Plate from the post-run cleaning are in place on the instrument, then check that the tubing from the amplification plate is removed from the pinch valve.

12. Confirm that the injector tip is placed in an empty 50-mL conical tube to collect waste.

13. Press **Next** to start cleaning.

14. When cleaning is complete, the screen displays "Cleaning Complete". Wearing clean gloves, press **Next**.

15. Remove and appropriately discard the used TMPL Cleaning Adapter, TMPL Amplification Plate, disposable injector, and tubing from the instrument.

16. Appropriately discard the 50-mL conical tube waste, then press **Next** to return to the main screen.

Select the Planned Run

1. Change gloves, then press the **Run** button on the Ion OneTouch™ Dx Instrument touchscreen.

2. Enter the Run Short Code (generated by the Torrent Suite™ Dx Software when you executed the run). Make sure the Planned Run information on the screen is correct, then press **Next**.

3. Enter the Tube Label text that you entered into the software when you executed the run, then press **Next**.

4. Confirm the run type displayed on the instrument screen, then press **Next**.

Install the TMPL Recovery Tubes, TMPL Recovery Router, and TMPL Amplification Plate

1. Label two TMPL Recovery Tubes with the Tube Label text entered into the software.
2. Insert the tubes into the holes in the Ion OneTouch™ Dx Instrument centrifuge, making the sure the tube arm is inserted into the slot next to each hole.
3. Pinch the sides of the TMPL Recovery Router, then push the router down into the center slot of the centrifuge until it is seated flat and secure in the center of the rotor.
4. Close the lid of the centrifuge, then press **Next**.
5. Insert a new TMPL Amplification Plate into the open instrument heat block.
6. Pull the handle forward to secure the plate. The tubing should be under the handle. Press **Next**.
7. Thread the tubing through the tubing holder.
8. Align the tubing with the slot that runs along the bottom of the pinch valve. Gently pull the tubing up into the slot until it is secure in the notch.
9. Adjust the tubing so that it is straight but not too taut. The injector needle should reach the injector hub without stretching the tubing. Press **Next**.
10. Ensure that the injector needle is screwed tightly onto the tubing. Hold the centrifuge lid down with one hand, and with your other hand insert the injector needle straight down into the injector hub. Push down until the injector touches the hub.
11. Gently push the injector down again and release. You should hear a click from the hub. Then press **Next**.
12. Pull the waste container from the external waste tubing, and empty the container into an appropriate hazardous waste receptacle.
13. Inspect the oil waste tray on the instrument.
14. Put the empty waste container back on the tray, then push the waste tube back into the container port. Press **Next**, then proceed to prepare the amplification solution.

Prepare the amplification solution

1. Put on new gloves after emptying the Ion OneTouch™ Dx Instrument waste.
2. Ensure that the library, TMPL Rgnt Mix, TMPL ISP, and TMPL CF-1 are completely thawed.
3. Vortex the TMPL Rgnt B and TMPL Rgnt Mix tubes for 5 seconds each, then pulse centrifuge for 3–5 seconds.
4. Flick the TMPL Enzyme Mix tube with the tip of your finger 4 times, then pulse centrifuge for 3–5 seconds. Place in a benchtop cold box at 2–8°C until needed.
5. In a new nuclease-free 1.5-mL low-retention microcentrifuge tube at room temperature, add the following components in the designated order:

	Order	Reagent	Cap color	Volume
☐	1	TMPL Water	Yellow	40 µL
☐	2	TMPL Rgnt Mix	Purple	500 µL
☐	3	TMPL Rgnt B	Blue	300 µL
☐	4	TMPL Enzyme Mix	Brown	50 µL

6. Cap the 1.5-mL tube and vortex for ~5 seconds, then pulse centrifuge for 3–5 seconds.
7. Vortex the TMPL ISP tube for 30 seconds, then pulse centrifuge for 3–5 seconds.
8. Vortex the library and TMPL CF-1 tubes for 5 seconds each, then pulse centrifuge for 3–5 seconds.
9. Add the following to the tube in the designated order. After each addition, cap the tube, vortex for ~5 seconds, then pulse centrifuge for 3–5 seconds.

	Order	Reagent	Cap color	Volume
☐	1	TMPL ISP	Black	100 µL
☐	2	TMPL CF-1	Clear	5 µL
☐	3	Library or library pool	—	5 µL

Proceed immediately to "Fill the TMPL Emulsion Cartridge" on page 72.

Fill the TMPL Emulsion Cartridge

1. Clean the Ion OneTouch™ Assembly Rack with fresh 10% bleach followed by two water rinses.
2. Label a pre-assembled TMPL Emulsion Cartridge with the Tube Label text entered into the software.
3. Use a marker to circle the sample port on the top of the TMPL Emulsion Cartridge to distinguish it from the other two ports.
4. Place the TMPL Emulsion Cartridge into the Ion OneTouch™ Assembly Rack with the ports facing up.
5. Collect the full volume of the amplification solution (~1000 µl) using a P1000 pipettor, then insert the pipette tip vertically into the circled sample port on the cartridge with a snug fit.
6. Slowly pipet the amplification solution into the sample port.
7. Invert the TMPL Reaction Oil 5 times to mix.
8. Using a new pipette tip, collect 750 µL of TMPL Reaction Oil using a P1000 pipettor, then insert the pipette tip vertically into the sample port on the cartridge.
9. Slowly pipet the oil into the sample port to layer the oil over the aqueous reaction mix.
10. Replace the pipette tip to avoid contamination, and repeat steps 8–9 one more time, adding another 750 µL of TMPL Reaction Oil (1.5 mL total).
11. Immediately install the TMPL Emulsion Cartridge on the instrument. Do not mix or shake the cartridge.

Install the filled TMPL Emulsion Cartridge

1. Keep the TMPL Emulsion Cartridge in the Ion OneTouch™ Assembly Rack, then orient the assembly so that the sample port is on your *left*.
2. Lift the TMPL Emulsion Cartridge straight out of the tube rack.
3. With the short tube in the Reaction Tube to the left, slowly rotate the assembly to your *right* until the Reaction Tube is inverted.
4. Insert the three ports of the TMPL Emulsion Cartridge into the three holes on the top of the manifold. Press firmly on all sides to ensure a secure fit on the manifold.

Run the Ion OneTouch™ Dx Instrument

1. Ensure that the centrifuge lid of the Ion OneTouch™ Dx Instrument is closed.
2. Press **Next** on the instrument touchscreen to start the run.
3. After the run has completed, press **Re-Spin** on the touchscreen. The time since the end of the run is displayed (*hh:mm:ss*).
4. Based on the time displayed, do one of the following:
   - If it has been ≤30 minutes since the end of the run, no re-spin is required. Press **No**, then press **Next** to bypass this step. Proceed to recover the ISPs.
   - If it has been >30 minutes since the end of the run, press **Yes** to repellet the ISPs for 10 minutes. After re-spin is complete, press **Next** and proceed to recover the ISPs.
   - If it has been >30 minutes but the re-spin step was accidentally skipped, repellet the ISPs.

Recover the ISPs from the Ion OneTouch™ Dx Instrument

1. Remove the disposable injector from the injector hub, and carefully release the flexible tubing from the pinch valve. Place the injector into an empty 50-mL conical tube to collect waste. Press **Next**.
2. Press **Open Lid** on the Ion OneTouch™ Dx Instrument touchscreen, and wipe up any residue from the inside of the lid with a new disposable wipe.
3. Remove and discard the TMPL Recovery Router.
4. Carefully remove the two TMPL Recovery Tubes from the instrument and insert them in the Ion OneTouch™ Sample Rack, with each tube arm inserted into the slot on the back of the rack. Close the instrument lid.
5. Depress the plunger on a P-1000 pipette and carefully insert the tip into a TMPL Recovery Tube, avoiding the pelleted ISPs. Slowly remove ~1 mL of supernatant from the top down. Switch to a P-200 pipette and use the same procedure to remove the supernatant until the meniscus at the top of the liquid aligns with the bottom of the triangular arrow guides.

Prepare the TMPL ES Beads with TMPL ES Rsp Soln

1. Vortex the TMPL ES Beads (green cap) for 30 seconds to resuspend the beads.
2. In a new 1.5-mL low-retention microcentrifuge tube, add 14.3 µL of TMPL ES Beads per template preparation (13 µL of beads plus 10% extra to mitigate pipetting errors).
3. Fill the tube with 1 mL of TMPL Wash Solution.
4. Cap the tube, vortex for 10 seconds, then place the tube on the DynaMag™ Dx 16 2-mL Magnet for 1 minute to capture the beads. Without disturbing the pellet, carefully remove and discard the supernatant.
5. Add 143 µL of TMPL ES Rsp Soln (orange cap) per template preparation to the tube (130 µL of solution plus 10% extra to mitigate pipetting errors).
6. Cap the tube, then vortex for 30 seconds to resuspend the pellet.
7. If some beads are stuck to the lid of the tube, pulse centrifuge the tube for 3 seconds. Leave the tube at room temperature until ready to use.

Prepare fresh Melt-Off Solution

1. Prepare 1 M NaOH by adding 1 mL of 10 M NaOH to 9 mL Nuclease-Free water. 1 M NaOH must be prepared fresh weekly.
2. In a new 1.5-mL low-retention microcentrifuge tube, combine the following components in order.

	Order	Component	Volume
☐	1	TMPL Tween™ Solution	280 µL
☐	2	1 M NaOH	40 µL
		Total	320 µL

3. Cap the tube, vortex for 10 seconds, then pulse centrifuge for 3 seconds.

Prepare the strip tube

1. Using a marker, label a TMPL ES Strip Tube on the square tab with the Tube Label text, then place it in the Ion OneTouch™ Assembly Rack.
2. Resuspend the contents of each TMPL Recovery Tube containing the templated ISPs in 50 µL (from "Recover the ISPs from the Ion OneTouch™ Dx Instrument" on page 73, step 5) by vigorously pipetting up and down 30 times.
3. Pool the entire volume of templated ISPs from both recovery tubes into well 1 of the TMPL ES Strip Tube. The total pooled volume will be 100 µL.

☐ 4. Fill the remaining wells in the strip as follows:

	Well number	Reagent
☐	Well 1	Template-positive ISPs (~100 µL), added in step 3
☐	Well 2	130 µL of TMPL ES Beads in TMPL ES Rsp Soln
☐	Well 3	300 µL of TMPL Wash Solution
☐	Well 4	300 µL of TMPL Wash Solution
☐	Well 5	300 µL of TMPL Wash Solution
☐	Well 6	Empty
☐	Well 7	300 µL of freshly-prepared Melt-Off Solution
☐	Well 8	Empty

☐ 5. With a marker, label a 0.2-mL TMPL Sample Collection Tube with the Tube Label text.

☐ 6. Add 10 µL of TMPL Neutral Soln (red cap) to the TMPL Sample Collection Tube, then place it in the tube holder in the Ion OneTouch™ Sample Rack.

Prepare the Ion OneTouch™ ES Dx Instrument and perform the run

☐ 1. Place a new TMPL ES Tip in the Tip Loader. Remove the Tip Arm from its cradle on the Ion OneTouch™ ES Dx Instrument.

☐ 2. Grip the Tip Arm with two fingers, then align the metal fitting of the Tip Arm with the top of the tip. Firmly press the Tip Arm straight down onto the tip for 3 seconds with even pressure to ensure proper installation.

☐ 3. Lift the Tip Arm straight up to pull the installed TMPL ES Tip from the Tip Loader tube.

☐ 4. Return the Tip Arm to its cradle on the instrument. Tilt the Tip Arm back and align the pins with the round notches in the cradle, then lower the Tip Arm into the home position.

☐ 5. Remove the 0.2-mL TMPL Sample Collection Tube containing TMPL Neutral Soln from the Ion OneTouch™ Sample Rack. Place it in the hole in the base of the Tip Loader.

☐ 6. Confirm that the square-shaped tab of the 8-well strip is on the left, then insert the filled strip into the **right end of the slot** on the Ion OneTouch™ ES Dx Instrument tray.

IMPORTANT! Make sure that the strip is pushed all the way to the right in the slot, away from the Tip Arm.

☐ 7. Press the **Start/Stop** button on the instrument to start the run.

8. During the run, proceed to "Clean the Ion OneTouch™ Dx Instrument after the run" on page 76.

9. At the end of the run, the Ion OneTouch™ ES Dx Instrument displays "End" and beeps every 60 seconds. Press the **Start/Stop** button to silence this alarm, then reset the instrument for the next run. Proceed to "Collect the sample from the Ion OneTouch™ ES Dx Instrument" on page 77.

The enriched ISPs can be left on the instrument for up to 2 hours. The instrument can be left on between runs.

Clean the Ion OneTouch™ Dx Instrument after the run

1. Return to the Ion OneTouch™ Dx Instrument, then press **Next** on the touchscreen to continue with the post-run cleaning procedure.
2. Wipe any residue from the inside of the lid with a disposable wipe.
3. Remove the used TMPL Emulsion Cartridge and invert it to visually inspect that the aqueous phase was completely injected. Only one phase should be present. Discard the cartridge in the appropriate waste container.
4. Temporarily remove the used TMPL Amplification Plate and visually inspect it to check that there is no remaining emulsion or excessive air bubbles in the plate.
5. Reinstall the TMPL Amplification Plate.
6. Check the reagent tubes to ensure that the appropriate volume of oil and recovery solution was used. Approximately 20 mL of oil and 11 mL of TMPL Recovery Solution will be left in their respective reagent tubes. Press **Next**.
7. Install a new TMPL Cleaning Adapter.
8. Confirm that the disposable injector has been placed into a 50-mL conical tube.
9. Press **Next** on the Ion OneTouch™ Dx Instrument touchscreen to start the cleaning run.
10. When cleaning is complete, press **Next**.
11. Remove the disposable injector from the 50-mL conical tube, then discard the tube and its waste into appropriate containers. Place the injector in a new 50-mL conical tube.
12. Press **Next** to return to the main screen, then log out of the instrument touchscreen.

Collect the sample from the Ion OneTouch™ ES Dx Instrument

1. After the Ion OneTouch™ ES Dx Instrument run ends, remove the TMPL Sample Collection Tube containing the enriched ISPs and securely close the tube.
2. Ensure that the TMPL Sample Collection Tube has >200 µL of solution.
3. Remove, then discard the used tip and 8-well strip tube from the Ion OneTouch™ ES Dx Instrument.

STOPPING POINT Proceed to sequencing within 2 hours after the end of the Ion OneTouch™ ES Dx Instrument run, or transfer the template-enriched ISPs to 2–8°C storage. The ISPs may be stored for up to 1 week. If stored longer than 1 week, prepare new template-enriched ISPs from the library.

7 Ion PGM™ Dx Sequencer cleaning and initialization

Cleaning schedule

The Ion PGM™ Dx Sequencer requires cleaning with either 18 MΩ water or chlorite solution according to the following schedule.

Clean with:	Schedule:
18 MΩ water	The Ion PGM™ Dx Sequencer requires cleaning with 18 MΩ water when one of the following conditions is met: • The last water cleaning was completed more than 27 hours ago, and initialization was performed • If during the last initialization, the **One run per initialization** checkbox was selected, and one run was performed since the last water cleaning • If during the last initialization, the **Two runs per initialization** checkbox was selected, and two runs were performed since the last water cleaning
Chlorite solution	The Ion PGM™ Dx Sequencer requires cleaning with chlorite solution when one of the following conditions is met: • The last chlorite cleaning was completed more than 7 days ago, and one or more runs have been performed since that cleaning • The instrument has been left with reagent for more than 48 hours **Note:** If the Ion PGM™ Dx Sequencer will not be used for more than 3 days, a chlorite cleaning is strongly recommended within 48 hours after run completion.

Mark the cleaning bottles

- ☐ Write "Waste" on one 250-mL bottle, to be used for both water and chlorite washes.
- ☐ Write "Water" on one 250-mL bottle, to be used for water washes only.
- ☐ Write "Chlorite" on one 250-mL bottle, to be used for chlorite solution only.

Mark the cleaning chips

- ☐ • To designate a used chip for chlorite washes only, write "CL" on the corner. Do not use this chip for initialization.
- ☐ • To designate a used chip for water washes and initialization, write "W" on the corner. Do not use this chip for chlorite washes.

Start the cleaning

1. ☐ Log into the Ion PGM™ Dx Sequencer touchscreen.
2. ☐ On the main menu, press the **Clean** button.
   The instrument touchscreen will display the following:
   - **Last water clean: [YYYY-MM-DD] [hh]:[mm]:[ss]**
   - **Last chlorite clean: [YYYY-MM-DD] [hh]:[mm]:[ss]**
3. ☐ The type of cleaning required is selected by default (**18 MΩ water cleaning** or **Chlorite cleaning**). Press **Next**.

18-MΩ water cleaning

IMPORTANT! For all the following steps, use 18-MΩ water directly from the purification system. Do not use water that has been collected or stored in any other containers.

1. ☐ Touch the grounding plate on the Ion PGM™ Dx Sequencer with a bare finger, then use ungloved hands to insert the used chip marked "W" into the chip clamp.
2. ☐ Close the clamp, then press **Next** on the touchscreen.
3. ☐ Put on new gloves, then remove all wash bottles and reagent tubes attached to the instrument. Keep the sippers in place at all positions. Press **Next**.
4. ☐ Rinse the bottle marked "Water" twice with ~100 mL of 18-MΩ water. Press **Next**.
5. ☐ Fill the bottle marked "Water" with 250 mL of 18-MΩ water. Press **Next**.
6. ☐ Remove and rinse the W1 sipper with 18-MΩ water, then reattach the sipper. Press **Next**.
7. ☐ Attach the bottle marked "Water" to the W1 position, ensuring that the W1 cap is screwed on tightly. Press **Next**.
8. ☐ With the W3 sipper still in its cap, insert the sipper into the empty bottle marked "Waste". Do not screw on the cap. Insert the W2 sipper into the same bottle. Press **Next**.
9. ☐ Place collection trays below the reagent tube sippers in the dNTP positions.

10. Press **Next** to begin cleaning.

11. When cleaning is complete, leave the reagent sippers and collection trays in place. Remove the bottles and sippers from the W1, W2, and W3 positions. Press **Next** to return to the main menu, then proceed to initialization.

Chlorite cleaning

1. Fill a glass bottle with 1 L of 18-MΩ water, then add a SEQ Cleaning Tablet (chlorite tablet). Allow the tablet to completely dissolve (~10 minutes). Press **Next** on the touchscreen.

2. Add 1 mL of 1 M NaOH and filter the solution using a 0.22-µm or 0.45-µm filter. Press **Next**.

3. Touch the grounding plate on the Ion PGM™ Dx Sequencer with a bare finger, then use ungloved hands to insert the used chip marked "CL" into the chip clamp.

4. Close the clamp, then press **Next**.

5. Put on new gloves, then remove all wash bottles and reagent tubes that are attached to the instrument. Keep the sippers in place at all positions. Press **Next**.

6. Rinse the bottle marked "Chlorite" and the bottle marked "Water" twice each with ~150 mL of 18-MΩ water.

7. Add 250 mL of filtered chlorite solution to the bottle marked "Chlorite". Press **Next**.

8. Remove and rinse the sipper in the W1 position with 18-MΩ water, then reattach it to the instrument. Press **Next**.

9. Attach the bottle marked "Chlorite", containing the filtered chlorite solution, to the W1 position. Make sure that the W1 cap is tight. Press **Next**.

10. With the W3 sipper still in its cap, insert the tube into the empty bottle marked "Waste". Do not screw on the cap. Insert the W2 sipper into the same bottle. Press **Next**.

11. Place collection trays below the reagent tube sippers in the dNTP positions.

12. Press **Next** to begin cleaning. The cleaning will take ~13 minutes to complete.

13. When prompted, remove the bottle marked "Chlorite" from the W1 position. Press **Next**.

14. Remove and rinse the W1 sipper with 18-MΩ water, then reattach the sipper. Press **Next**.

15. Fill the bottle marked "Water" with 250 mL of 18-MΩ water and attach the bottle in the W1 position. Make sure the cap is tight.

16. Press **Next**. The water rinse will take ~15 minutes to complete.

17. When cleaning is complete, leave the reagent sippers and collection trays in place. Remove the bottles and sippers from the W1, W2, and W3 positions. Press **Next** to return to the main menu.

18. Rinse the "Chlorite" and "Water" bottles twice each with ~150 mL of 18-MΩ water, then proceed to initialization.

Before initialization

Check the tank pressure for the nitrogen gas. When the tank pressure drops below 500 psi, change the tank (see also "Gas cylinders" on page 25).

Initialize the sequencer

1. After cleaning is complete, the **Initialize** button on the touchscreen main menu will turn blue. Press the button to start the initialization process.

2. Depending on the number of sequencing runs per initialization, select either the **One 200 bp run per initialization** or the **Two 200 bp runs or One 400 bp run per initialization** checkbox. Press **Next**.

   Note: If you are performing two runs, both must start within 27 hours after start of the initialization.

3. Press the **Keyboard** button below the **Sequencing kit barcode** field. Using the barcode scanner attached to the sequencer, scan the barcode on the Ion PGM™ Dx Sequencing Reagents box. Press **OK**, then press **Next**.

4. Ensure that the used chip marked "W" (from the cleaning procedure) is secured in the chip clamp on the sequencer.

5. Ensure that the old reagent sippers and collection trays are in place. Press **Next**.

6. Rinse the SEQ Wash 1 Bottle, SEQ Wash 2 Bottle, and SEQ Wash 3 Bottle 3 times each with 18-MΩ water. Use 150 mL for the Wash 1 and Wash 3 bottles, and 500 mL for the Wash 2 bottle.

7. Prepare 500 µL of fresh 100 mM NaOH daily by diluting 50 µL of 1 M NaOH in 450 µL of nuclease-free water.

8. If your 18-MΩ water system has a spigot, extend it into **but not below** the neck of the SEQ Wash 2 Bottle. Otherwise, position the nozzle as close to the mouth of the bottle as possible.

9. Fill the bottle to the mold line with 18-MΩ water.

10. Add an entire bottle of SEQ W2 Solution to the SEQ Wash 2 Bottle.

11. Using a P200 pipette, add 70 µL of 100 mM NaOH to the SEQ Wash 2 Bottle.

12. Cap the bottle and invert 5 times to mix, then immediately proceed through the remainder of the initialization procedure.

13. SEQ Wash 1 Bottle: Add 350 µL of freshly prepared 100 mM NaOH to a clean SEQ Wash 1 Bottle. Ensure that the pipette tip touches the bottom of the bottle to dispense.

14. SEQ Wash 3 Bottle: Add SEQ W3 Solution to the 50-mL line marked on a clean SEQ Wash 3 Bottle. Press **Next**.

15. Remove the old sippers from the W1, W2, and W3 positions on the instrument, then discard in a waste container.

16. Wearing clean gloves, firmly attach a new, long sipper to the cap in the W2 position. **Do not let the sipper touch any surfaces.**

17. Immediately attach the prepared SEQ Wash 2 Bottle to the cap in the W2 position, then tighten the cap. Press **Next**.

18. Change gloves and firmly install new, short sippers to the caps in the W1 and W3 positions.

19. Immediately attach the prepared SEQ Wash 3 Bottle to the cap in the W3 position, then attach the SEQ Wash 1 Bottle to the cap in the W1 position. Tighten the caps.

20. Ensure that the collection trays are properly aligned to catch fluid flowing out of the sippers. Press **Next** to start the auto-pH process.

21. Thaw the following at room temperature until no ice crystals are visible in the tubes (~15 minutes). Keep the tubes on ice or in a chilled benchtop cold box until ready to use.
    - SEQ Primer (white cap)
    - SEQ dGTP (black cap)
    - SEQ dCTP (blue cap)
    - SEQ dATP (green cap)
    - SEQ dTTP (red cap)

22. The kit includes labels for each type of dNTP. Attach one of each label type to four SEQ Reagent Tubes.

23. Verify that no ice crystals are visible in each thawed dNTP stock solution tube (SEQ dGTP, SEQ dCTP, etc.) from step 22. Vortex each dNTP for ~5 seconds, then pulse centrifuge to collect.

24. Using a P20 pipette and separate tips, aliquot each dNTP into its appropriately labeled reagent tube as follows:
    - Aliquot 10 µL of dNTP per reagent tube if performing one 200 bp run per initialization.
    - Aliquot 20 µL of dNTP per reagent tube if performing two 200 bp runs per initialization.

25. Press **Next**. Remove the old reagent tube sippers, then discard them in a waste container.

26. Remove the collection trays, discard any waste in a sink, then rinse the trays.

27. Using new gloves, firmly insert a new SEQ Reagent Tube Sipper (blue) into each dNTP port. Do not let the sipper touch any surfaces.

28. Attach each SEQ Reagent Tube to the correct dNTP port (for example, the dGTP tube on the port marked "G"), then tighten firmly by hand until it can no longer rotate.

29. After each reagent tube is securely installed, press **Next**.

8 Sequencing

Prepare enriched ISPs for sequencing

- [ ] 1. Confirm that the SEQ Primer is completely thawed before use. No ice crystals should be visible.
- [ ] 2. Vortex the SEQ Primer for ~5 seconds, then pulse centrifuge to collect the contents. Leave the tube on ice or in a chilled benchtop cold box until use.
- [ ] 3. Remove the enriched ISP sample in the TMPL Sample Collection Tube from the Ion OneTouch™ ES Dx Instrument or 2–8°C storage.
- [ ] 4. Place the tube in a microcentrifuge with a 0.2-mL tube adapter. Orient the tab of the tube lid so that it is pointing away from the center of the centrifuge (this will indicate where the sample ISPs are being pelleted).
- [ ] 5. Centrifuge at 15,000 rcf for 2 minutes.
- [ ] 6. Insert the tube into the appropriate slot of the Ion OneTouch™ Sample Rack. Open the tube lid carefully to avoid spillage, then lift the tube out of the slot, fold the lid flat against the back of the tube, and insert the tube and folded lid into the slots in the rack.
- [ ] 7. Use a P200 pipette and keep the pipette plunger depressed as you insert the tip into the tube containing the enriched ISPs. Carefully remove ~200 µL of supernatant from the top down, avoiding the side of the tube where the ISPs are pelleted (i.e., the side with the tab on the tube lid). Discard the supernatant.
- [ ] 8. Change to a P20 pipette. Keep the pipette plunger depressed as you insert the tip into the tube, and carefully remove the remaining supernatant to the volume marked by the arrow guides on the rack.
- [ ] 9. Remove the tube from the sample rack, then add 12 µL of thawed SEQ Primer (white cap) to the ISPs in the tube and vigorously pipet the mixture up and down 30 times.
- [ ] 10. Insert the tube into the 96-well tray supplied with the Veriti™ Dx 96-well Thermal Cycler.

11. Load the tube and tray assembly into the thermal cycler. Select the **06_Seq Primer Hybridization** program on the thermal cycler touchscreen. Select **View** and confirm that the program steps match those listed in the table below.

Stage	Temperature	Time
Hold	95°C	2 minutes
Hold	37°C	2 minutes
Hold	25°C	Hold (up to 30 minutes)

12. When you have confirmed the program steps, run the cycling program.

After cycling, keep the tube containing the primer-annealed ISPs at room temperature. Proceed immediately to set up the sequencing run.

Set up the sequencing run

IMPORTANT!

- To avoid damage to the chips or instrument due to electrostatic discharge:
  - **Remove your gloves when handling chips**, especially before transferring chips on or off the instrument. Hold chips by their edges when handling.
  - **Do not place chips directly on the bench or any other surface.** Always place chips either on the grounding plate on the Ion PGM™ Dx Sequencer or in the Ion PGM™ Dx Chip Minifuge bucket.

  Note: Steps without gloves can be performed without risk of contamination.
- When performing one or two sequencing runs from the same initialization, the runs must be started within 27 hours after the start of initialization.

1. After initialization and sample preparation, go to the main menu of the Ion PGM™ Dx Sequencer, then press the **Run** button to begin the run setup.
2. Remove the waste bottle from the instrument and completely empty the bottle. Return the waste bottle to its position on the instrument. Press **Next**.
3. Make sure the chip used for initialization is still in the chip clamp, then press **Next**. The instrument cleans the fluid lines, then proceeds automatically to the next screen.
4. Press the **Keyboard** button next to the **Planned Run** field. Using the barcode scanner attached to the sequencer, scan the **Run Short Code/Barcode** on the **View Planned Run** printout. Alternatively, type the code (displayed below the barcode) using the touchscreen keyboard. Press **OK**.
5. Confirm the Planned Run information displayed on the touchscreen, then press **Next**.
6. Remove your gloves.

Note: The following steps can be performed without gloves without risk of contamination.

7. Open the chip clamp, then remove the chip used for initialization. Press **Next**.
8. Touch the grounding plate with a bare finger, then remove a new chip from its packaging.
9. Press the **Keyboard** button next to the **Top barcode** field. Using the barcode scanner attached to the sequencer, scan the barcode on the top of the chip, then press **OK**.
10. Press the **Keyboard** button next to the **Bottom barcode** field. Scan the barcode on the bottom of the chip, then press **OK**.

    Note: If the barcode does not scan, use a new chip and contact Technical Support for a replacement.

11. Using a marker, label a corner of the chip with the **Tube Label** text.
12. Secure the new chip in the chip clamp.
13. Press **Next** to calibrate the chip. The screen prompts you to perform certain steps during calibration.
14. When prompted, visually inspect the chip in the clamp for liquid leaking from the chip case into and around the clamp area.
15. Close the instrument lid when prompted.
16. When chip calibration is complete, a "Calibration Passed" or "Calibration Failed" message appears.

Load the sample on the chip

IMPORTANT! The following steps (including chip loading) should be performed without gloves, except when adding SEQ Enzyme as noted. The steps can be performed without gloves without risk of contamination.

1. Enter the Tube Label text into the Ion PGM™ Dx Sequencer touchscreen. Press **Next**.
2. Touch the grounding plate on the instrument with a bare finger, then remove the new Ion 318™ Dx Chip from the clamp.
3. Insert the used chip from initialization (marked with a "W") back into the chip clamp.
4. Place the new chip in the removable bucket from the Ion PGM™ Dx Chip Minifuge, then place the bucket on a firm, flat surface.
5. Set a P200 pipette to 30 µL. Insert the tip firmly into the chip loading port while holding the bucket and chip steady with your other hand. Remove as much liquid as possible from the port, then discard the liquid.
6. Place the chip **upside-down** in the bucket, then transfer the bucket to the Ion PGM™ Dx Chip Minifuge **with the chip tab pointing out** (away from the center of the minifuge).

7. Balance the minifuge with another upside-down chip in the opposing bucket.
8. Centrifuge for 3–5 seconds to completely dry the chip surface.
9. Remove the bucket containing the chip from the minifuge. Remove the chip from the bucket, then wipe down the bucket with a disposable wipe to remove residual liquid. Place the chip right-side up in the bucket.
10. Put on new gloves.
11. Remove the SEQ Enzyme (yellow cap) from storage and flick 4 times to mix. Pulse centrifuge the tube to collect the contents, then place the tube on ice or in a chilled benchtop cold box until use.
12. Remove the primer-annealed ISPs from the Veriti™ Dx 96-well Thermal Cycler (from "Prepare enriched ISPs for sequencing" on page 84).
13. Add 3 µL of SEQ Enzyme to the primer-annealed ISPs. Set a P200 pipette with a low-bind tip to 20 µL, then gently pipet the mix up and down 10 times.
14. Incubate at room temperature for 5 minutes.
15. Remove your gloves.

Note: The following steps can be performed without gloves without risk of contamination.

16. Place the chip in the minifuge bucket on a firm, flat surface. Following the 5-minute polymerase incubation, collect 30 µL of sample into a P200 pipette tip. Discard any remaining sample.
17. Insert the pipette tip firmly into the loading port of the chip. Apply gentle pressure between the tip and chip throughout the loading process.
18. With the pipette unlocked, dial down the pipette to deposit the ISPs. Ensure that the entire sample is loaded onto the chip. To avoid introducing air bubbles, leave a small amount of sample in the pipette tip (~0.5 µL).

Note: A small amount of sample may leak from the outlet port, which is acceptable. However, if a large amount of sample leaks from the outlet port (~15 µL or greater), do not continue with the run, and contact Technical Support.

19. Transfer the chip in the bucket to the minifuge **right-side up with the chip tab pointing in** (toward the center of the minifuge).
20. Balance the minifuge with another right-side up chip in the opposing bucket, then centrifuge for 30 seconds.

CAUTION! Allow the minifuge to come to a complete stop before opening the lid.

21. Remove the bucket containing the chip from the minifuge and place it on a flat surface. Set a P200 pipette to 40 µL, then pipet the liquid out through the loading port. Remove as much liquid from the chip as possible.

    Note: It is normal to have some liquid remain in the chip after this step.

22. Turn the chip upside-down in the bucket, then place the bucket back in the minifuge with the chip tab pointing out.

23. Balance the minifuge with another upside-down chip in the opposing bucket, then pulse centrifuge for 3–5 seconds at maximum speed to dry the chip surface completely.

24. Remove the chip from the bucket, then wipe down any residual liquid left on the bucket with a disposable wipe.

Proceed immediately to "Perform the run".

Perform the run

1. On the Ion PGM™ Dx Sequencer touchscreen, press **Next**, then press the **Keyboard** button next to the **Top barcode** field. Using the barcode scanner attached to the sequencer, scan the barcode on the top of the new loaded chip, then press **OK**.

2. Remove the used chip from the instrument, then use ungloved hands to insert the loaded chip into the chip clamp.

3. Press **Next** to calibrate the loaded chip. At the start of calibration, visually inspect the chip for leaks before closing the instrument cover. Close the lid when prompted to do so.

4. After ~1 minute, the touchscreen indicates if calibration was successful.

5. When the chip passes calibration, press **Next** to start the sequencing run. The sequencing run takes ~4.5 hrs to complete.

6. After the run, the touchscreen returns to the main menu.

Review the results and generate reports using the Torrent Suite™ Dx Software, as described in the *Torrent Suite™ Dx Software 5.12.5 User Guide* (Pub. No. MAN0018762).

A

Troubleshooting

Troubleshooting—Sample preparation

Observation	Possible cause	Recommended action
Batch sample import fails	One or more entries in the sample-import spreadsheet contains special characters, lines breaks, unexpected spaces, incorrect entry length, incorrect date formatting, or other formatting errors.	Check each entry for correct formatting, correct any errors, and repeat the import.
	Blank rows were copied into the sample-import template file from a different source.	Rows that appear empty may contain hidden formatting that conflicts with the import function. Start with a clean sample-import template file, and be careful to copy only those rows that contain actual data.
	The sample import spreadsheet contains a nonunique Sample ID.	Every Sample ID in the software must be unique. Make sure the spreadsheet does not contain any duplicate IDs, and repeat the import. Note that the system check is not case-sensitive, so a Sample ID of ABC1 conflicts with abc1.
	The headings in the sample import spreadsheet do not match the sample attributes in the software.	The headings must match the sample attributes in the software exactly. Check the headings for spelling or other errors.

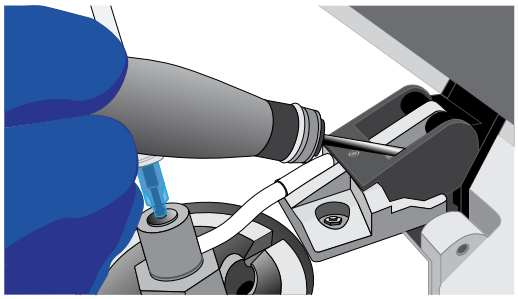
Ion OneTouch™ Dx Instrument

Observation	Possible cause	Recommended action
Display message: Sensor unable to measure pressure.	Hardware issue	Contact Technical Support (see "Customer and technical support" on page 140). Reboot the Ion OneTouch™ Dx Instrument to clear the alarm.
Display message: Coolant pump does not flow.	Hardware issue	Contact Technical Support (see "Customer and technical support" on page 140). Reboot the Ion OneTouch™ Dx Instrument to clear the alarm.

Observation	Possible cause	Recommended action
Display message: Connection failure with Ion Torrent™ Server	Ion OneTouch™ Dx Instrument and Ion Torrent™ Server connection is not established	Check that a network connection to the Ion Torrent™ Server is established, then reboot the Ion OneTouch™ Dx Instrument. **Note:** A sample created during a run with this alarm raised can still be used.
Display message: Failed to connect to the Ion Torrent™ Server. Check your connection.	Ion OneTouch™ Dx Instrument and Ion Torrent™ Server connection is not established during startup	Check your network connection to the Ion Torrent™ Server to make sure the connection is established, then reboot the Ion OneTouch™ Dx Instrument.
Display message: Failed to set up system time at startup. Check your connection to the Ion Torrent™ Server.	Ion OneTouch™ Dx Instrument and Ion Torrent™ Server connection is not established	Check your network connection to the Ion Torrent™ Server to make sure the connection is established, then reboot the Ion OneTouch™ Dx Instrument.
	Instrument is still in the process of establishing a connection	Allow 10 minutes to see if the display message clears.
Display message: Motor current too high. Reboot the instrument to clear the alarm.	Hardware issue	Contact Technical Support (see "Customer and technical support" on page 140). Reboot the Ion OneTouch™ Dx Instrument to clear the alarm. **Note:** A sample created during a run with this alarm raised can still be used.
Display message: Pressure too high. Reboot the instrument to clear the alarm.	• Hardware issue • Clogged TMPL Emulsion Cartridge due to contaminated reagents or defective emulsion cartridge	Reboot the Ion OneTouch™ Dx Instrument to clear the alarm. Use a new TMPL Emulsion Cartridge and fresh reagents to repeat the run. Contact Technical Support (see "Customer and technical support" on page 140) if the issue persists.
Display message: Sensor unable to measure instrument temperature	Hardware issue	Contact Technical Support (see "Customer and technical support" on page 140). Reboot the Ion OneTouch™ Dx Instrument to clear the alarm.
Display message: Set temperature out of range. Reboot the instrument to clear the alarm.	Hardware issue	Contact Technical Support (see "Customer and technical support" on page 140). Reboot the Ion OneTouch™ Dx Instrument to clear the alarm. **IMPORTANT!** If this alarm is raised, you cannot use the prepared template.
Display message: Software versions incompatible. Go to the Options menu and update the software.	The system software was updated, but the instrument software was not.	After the system software has been updated, update the instrument software as follows: 1. On the main menu of the instrument, press Options and follow the instructions to check for and install updates. 2. When installation is complete, follow the onscreen prompts to restart the instrument. **IMPORTANT!** You must restart the instrument before proceeding.

Observation	Possible cause	Recommended action
Display message: TEC current too high. Reboot the instrument to clear the alarm.	Hardware issue	Contact Technical Support (see "Customer and technical support" on page 140). Reboot the Ion OneTouch™ Dx Instrument to clear the alarm. **IMPORTANT!** A sample created during a run with this alarm raised must NOT be used.
Ion OneTouch™ Dx Instrument displays a blue or grey screen with folders while the instrument is idle	Instrument issue	Power cycle the Ion OneTouch™ Dx Instrument using the On/Off switch. If alarms appear or the problem persists, contact Technical Support (see "Customer and technical support" on page 140).
Ion OneTouch™ Dx Instrument displays a blue or grey screen with folders during cleaning or a run	Instrument issue	Power cycle the Ion OneTouch™ Dx Instrument using the On/Off switch, then restart the cleaning or run. If alarms appear or the problem persists, contact Technical Support (see "Customer and technical support" on page 140).
Ion OneTouch™ Dx Instrument touchscreen freezes	Touchscreen button is pressed more than once per second.	Wait 5 minutes. If still unresponsive: 1. Click on the **Abort** button if displayed, and restart the process. 2. If **Abort** button is not displayed, power-cycle the instrument using the On/Off switch, then restart the process. **Note:** If the touchscreen freezes during Ion OneTouch™ Dx Instrument run setup, the software will remember that the instrument has already been cleaned and will not require the cleaning to be performed a second time.
During cleaning, no liquid comes out of the disposable injector	Loose or damaged reagent tube sipper in the "O" position	Reinstall the sipper in the "O" position, then restart cleaning. If the problem persists, install a new sipper.
	Improper installation of the TMPL Cleaning Adapter and TMPL Amplification Plate	Reinstall the TMPL Cleaning Adapter and TMPL Amplification Plate, then restart cleaning. If the problem persists, use a new TMPL Cleaning Adapter and a new TMPL Amplification Plate.
	Instrument issue	Contact Technical Support (see "Customer and technical support" on page 140).
Run did not complete	• Operator did not power cycle the Ion OneTouch™ Dx Instrument after an "Abort" operation • Instrument issue	Power cycle the Ion OneTouch™ Dx Instrument using the On/Off switch, then start a new run. If the problem persists, contact Technical Support (see "Customer and technical support" on page 140).
Cleaning was aborted due to high pressure	Improper installation of the TMPL Cleaning Adapter or TMPL Amplification Plate	Reinstall the TMPL Cleaning Adapter and TMPL Amplification Plate, then restart the cleaning protocol. If the problem persists, contact Technical Support (see "Customer and technical support" on page 140).

Observation	Possible cause	Recommended action
Run was aborted due to high pressure	Clogged TMPL Emulsion Cartridge	Install a new TMPL Emulsion Cartridge, then restart the run. If the problem persists, contact Technical Support (see "Customer and technical support" on page 140).
	Reagent contamination	Replace the TMPL Reaction Oil, TMPL Reagent Tubes, and TMPL Sippers, then restart the run.
	Instrument issue	Contact Technical Support (see "Customer and technical support" on page 140).
Oil is leaking from the instrument	Improper installation of consumables	Wipe up any leakage and reinstall TMPL Sippers and TMPL Reagent Tubes.
	Waste was not emptied before the run	Wipe up any leakage and empty the waste container before the next run.
	Instrument issue	Contact Technical Support (see "Customer and technical support" on page 140).
Excessive oil in waste tray	Instrument issue	Power cycle the Ion OneTouch™ Dx Instrument using the On/Off switch. If the problem persists, contact Technical Support (see "Customer and technical support" on page 140).
Waste backup observed after completion of Ion OneTouch™ Dx Instrument run, waste leaks from the waste line after removal of the waste container	Filter in waste container clogged causing back-pressure	Remove or clean filter in waste container.
	Improper installation of consumables	Wipe up any leakage and reinstall TMPL Sippers and TMPL Reagent Tubes.
	Waste was not emptied before the run	Wipe up any leakage and empty the waste container before the next run.
	Instrument issue	Contact Technical Support (see "Customer and technical support" on page 140).
Two phases are present in the sample cup at the end of the Ion OneTouch™ Dx Instrument run, sample injection from the TMPL Amplification Plate did not occur	• The TMPL Emulsion Cartridge was not inserted properly into the instrument • Problem with the instrument	1. Power cycle the Ion OneTouch™ Dx Instrument using the On/Off switch. 2. Repeat run preparation, then the run, being careful to seat the TMPL Emulsion Cartridge as described. If the problem persists, contact Technical Support (see "Customer and technical support" on page 140).
Large air gap (1 mL or greater) is present in the reaction cup	• Reagent tube not filled with TMPL Oil to start the run • Problem with the instrument	Repeat run preparation, then the run, being careful to follow all steps as described. If the problem persists, contact Technical Support (see "Customer and technical support" on page 140).

Observation	Possible cause	Recommended action
Emulsion is detected in the TMPL Amplification Plate after completion of the Ion OneTouch™ Dx Instrument run	• Reagent tube not filled with oil to start the run • Improper installation of the consumables • Problem with the instrument	1. Ensure the consumables are installed correctly and not defective. 2. Power cycle the Ion OneTouch™ Dx Instrument using the On/Off switch. 3. Repeat run preparation, then the run, being careful to follow all steps as described. If the problem persists, contact Technical Support (see "Customer and technical support" on page 140).
Inappropriate volumes of TMPL Oil and TMPL Recovery Solution are left after completion of the Ion OneTouch™ Dx Instrument run	Incorrect volumes of TMPL Oil and TMPL Recovery Solution used to start	Repeat the run, carefully check all volumes during run setup.
	• Improper installation of the consumables • Instrument issue	1. Ensure the consumables are installed correctly and not defective. 2. Power cycle the Ion OneTouch™ Dx Instrument using the On/Off switch. 3. Repeat run preparation, then the run, being careful to follow all steps as described. If the problem persists, contact Technical Support (see "Customer and technical support" on page 140).
Centrifuge makes a loud sound during centrifugation	There is an obstruction in the centrifuge chassis	Turn off the instrument, then contact Technical Support (see "Customer and technical support" on page 140).
Centrifuge lid does not open **Details:**	• Power failure • Software crash	1. Slide a 1/8-inch L-wrench (hex wrench) or equivalent tool into the right-hand hole at the top edge of the centrifuge hinge:  2. Press tool into the hole until there is a slight compression of the tool against the instrument and the centrifuge lid unlocks and opens. 3. Remove the tool from the hole, then open the lid. Do *not* force the lid open. If the problem persists, Contact Technical Support (see "Customer and technical support" on page 140).

Observation	Possible cause	Recommended action
Centrifuge does not spin	Ion OneTouch™ Dx Instrument centrifuge lid was not closed properly **Note:** Centrifuge will not operate unless the lid is fully closed.	1. Open and properly close the centrifuge lid, then press **re-spin**. 2. After re-spin completes, power cycle the Ion OneTouch™ Dx Instrument using the On/Off switch. 3. Repeat run preparation, then the run, beginning with your pooled library sample. Be careful to properly close the centrifuge lid. If the problem persists, contact Technical Support (see "Customer and technical support" on page 140).
	Instrument failure (fuse, motor driver board, and/or interlock switch failures)	Confirm that the centrifuge is not operating. On the touchscreen press **Options**, then press **respin**. • If the centrifuge does not begin to spin, contact Technical Support (see "Customer and technical support" on page 140). • If the centrifuge begins to spin. After re-spin completes: a. Power cycle the Ion OneTouch™ Dx Instrument using the On/Off switch. b. Repeat run preparation, then the run, beginning with your pooled library sample. Be careful to properly close the centrifuge lid.

Ion OneTouch™ ES Dx Instrument

Observation	Possible cause	Recommended action
Final sample volume is <200 uL	Loose or cracked tip or loose tip fitting on the Ion OneTouch™ ES Dx Instrument Tip Arm	Tighten the tip and tip fitting. If the tip is cracked, replace it.
	Improper calibration	Perform a residual volume check; if the residual volume check fails, perform calibration (see "Calibrate the Ion OneTouch™ ES Dx Instrument" on page 119). If the problem persists, contact Technical Support (see "Customer and technical support" on page 140).
Excessive foaming	Improperly calibrated or inadequate volume in one or more wells of the TMPL ES Strip Tube	Use recommended volumes for all wells. Perform a residual volume check; if the residual volume check fails, perform calibration (see "Calibrate the Ion OneTouch™ ES Dx Instrument" on page 119). If the problem persists, contact Technical Support (see "Customer and technical support" on page 140).

Observation	Possible cause	Recommended action
Excessive foaming *(continued)*	Loose or cracked tip, or loose tip fitting on the Ion OneTouch™ ES Dx Instrument Tip Arm	Tighten the tip and tip fitting. If the tip is cracked, replace it.
E4, E12, or E22 error displays when the Ion OneTouch™ ES Dx Instrument is initializing	• Fuse is installed incorrectly • Instrument is below operating temp • Bad program or calibration setting • Tip Arm is not moving	1. Ensure that the fuse module is installed correctly and that the unit is within its recommended operating temperature range of 68°F to 86°F (20°C to 30°C). 2. Reboot the instrument: Power OFF the instrument, wait 3 seconds, then power ON the instrument. 3. If the error persists, restore the factory defaults, then recalibrate the instrument (see"Calibrate the Ion OneTouch™ ES Dx Instrument" on page 119).
Solution overflow during a run	Overloaded reagent volumes in TMPL ES Strip Tube	Repeat enrichment with correct reagent volumes.
Tip is causing the 8-well strip to lift out of its slot during run	Tip is not aligned vertically	Try tightening the tip. If the problem persists, perform vertical axis calibration (see "Vertical axis calibration" on page 119).
Strip lifts up during strip push	Instrument is not calibrated properly	Perform horizontal position calibration.
Strip lifts up when tip is raised from well	Instrument is not calibrated properly	Perform vertical calibration.
Immediately after strip push, the strip is not in contact with the magnet	Instrument is not calibrated properly	Perform horizontal position calibration.
Tip grinds into the base of the instrument and Code "1999" displays	• Vertical calibration setting too low or out of range • Instrument is not calibrated properly	1. Erase the memory on the instrument: Hold down the vertical-adjust button while powering ON the instrument. The instrument beeps several times. 2. Perform a residual volume test. 3. Recalibrate the instrument if residual volume check failed.
Tip is hitting the top of tray at start of run	• Instrument tray or tip is not properly seated in the instrument • Tip adapter is loose	1. Check for debris between the tray and the instrument, then reinstall the tray and tip. 2. Check the tip adapter to make sure it is tight.
Error displays	Various	1. Power the instrument OFF then ON. 2. If the error continues to display, erase the memory on the instrument. Hold down the vertical adjust button while powering ON the instrument. The instrument beeps several times. 3. Perform residual volume check. 4. Recalibrate the instrument if the residual volume check failed.

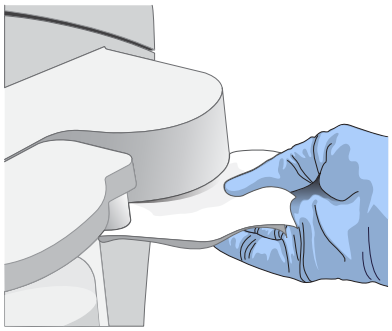
Observation	Possible cause	Recommended action
Instrument does not aspirate or dispense liquids	Loose fittings	• Ensure that the connections at the elbow on the Tip Arm and at the tubing on the rear syringe pump are finger-tight. • Ensure that the metal tip adapter fitting on the Tip Arm is finger-tight. **IMPORTANT!** After any changes to the metal tip adapter, perform a remaining volume test, and recalibrate the instrument.
Ion OneTouch™ ES Dx Instrument has a blown fuse	Various	Contact Technical Support (see "Customer and technical support" on page 140).

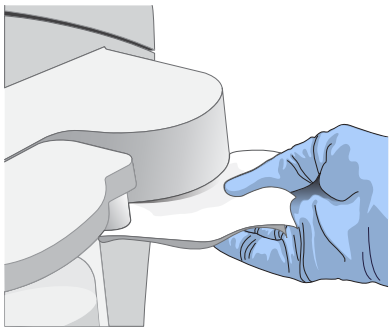
Ion PGM™ Dx Sequencer Initialization

Observation	Possible cause	Recommended action
Pressure alarm	Gas cylinder may be turned off or empty	• Verify that the cylinder has at least 500 PSI and 30 PSI at the outlet of the regulator. Confirm that all valves between the cylinder and the sequencer are open. • Once you confirm gas pressure leading into the instrument, press **Yes** to retry verification of gas pressure. If the test continues to fail, contact Technical Support (see "Customer and technical support" on page 140).
Error message: Leak check failed, make sure the reagent tubes are not attached to the sequencer and check Wash 1, 2, or 3 for leak	• Caps are not tightened on the Wash 1, 2, or 3 bottles. • Bottles may be damaged or defective.	1. Inspect all the bottles for damage or visible leaks. If a bottle appears damaged, replace it. 2. Finger-tighten all the bottle caps, make sure that the reagent tubes are not attached to the instrument, and then press **Retry**. 3. If leak check continues to fail, contact Technical Support (see "Customer and technical support" on page 140).

Observation	Possible cause	Recommended action
Error message: UNDERSHOT TARGET (instrument touchscreen may also be frozen)	Water used did not meet specifications, or insufficient amount of NaOH added	If the touchscreen is frozen, reboot the sequencer and restart initialization. If you receive the same error and the touchscreen continues to freeze, contact Technical Support (see "Customer and technical support" on page 140). Otherwise, proceed to the following steps. 1. Press **Retry** to reattempt pH adjustment. 2. If you receive the same error message after multiple attempts, press **Abort**. 3. Check your water purification system and troubleshoot per the manufacturer's directions, or identify a new source of 18-MΩ water. 4. Restart the initialization procedure and prepare new solutions, including a new aliquot of 100 mM NaOH. 5. If the problem persists, contact Technical Support (see "Customer and technical support" on page 140).
	Auto-pH did not add enough Wash 1 Solution to the Wash 2 Solution before the maximum iterations occurred.	If the touchscreen is frozen, reboot the sequencer and restart initialization. If you receive the same error and the touchscreen continues to freeze, contact Technical Support (see "Customer and technical support" on page 140). Otherwise, proceed to the following steps. 1. A blockage may have occurred. See "Error message: There may be a blockage or no NaOH in W1. Check W1 and run line clear then try again" on page 99. 2. Press **Retry** to restart the pH check. If you still get the "Undershot target pH" error, replace the chip with a new (unused) chip and restart the pH check. **Note:** The new chip can be used for sequencing after initialization completes.
	Loose W1 sipper	Tighten the sipper and retry.

Observation	Possible cause	Recommended action
Error message: Please insert a chip and press Start	Instrument cannot detect the chip in chip socket.	1. Open the chip clamp and remove the chip. 2. Check for debris under the chip and in the chip socket. **IMPORTANT!** Never rub or wipe the socket. Rubbing the socket can damage it and cause it to fail. 3. Look for liquid outside the flow cell of the chip:  4. If you see liquid, replace the chip with a new (unused) one. **Note:** The new chip can be used for sequencing after initialization completes. 5. Close the clamp, then press **Start** to restart the process. 6. If the new chip also fails, there could be a problem with the chip socket. Contact Technical Support (see "Customer and technical support" on page 140).
Error message: OVERSHOT TARGET	• Wrong amount or concentration of NaOH was added to the Wash 1 Bottle • Auto-pH added more NaOH from the Wash 1 Bottle to the Wash 2 Bottle than was needed	Do not press **Next**. Prepare fresh reagents, then press **Retry** to retry the initialization. If the problem persists, contact Technical Support (see "Customer and technical support" on page 140).
	Clog in waste line(s)	Do not press **Next**. See "Error message: There may be a blockage or no NaOH in W1. Check W1 and run line clear then try again" on page 99.
	Clog in chip	Do not press **Next**. See "Error message: There may be a blockage or no NaOH in W1. Check W1 and run line clear then try again" on page 99. If the waste lines are not clogged, replace the chip, then click **Retry**.
W2 pH consistently undershoots target	pH of water is too low before any NaOH is added	• Confirm that the 18 MΩ water supply meets specifications. • If necessary, when preparing the Wash 2 Bottle, add more than the recommended 70 µL of 100 mM NaOH. After adding the NaOH, the Wash 2 Bottle must be in the range of pH 6.0–6.5 at first pH iteration before you begin initialization.

Observation	Possible cause	Recommended action
Error message: There may be a blockage or no NaOH in W1. Check W1 and run line clear then try again	The waste lines may be blocked.	1. Remove the waste bottle and place lab wipes under the waste arm. 2. Gently wipe the waste arm with a lab wipe to clear liquid from around the waste line.  3. Press **Flow check** one or more times to observe the flow rates from both lines. One line should drip slightly faster than the other. If one or both lines are blocked (no flow), or the drip rates are significantly different, go to the next step. If the flow rates are normal, see "Error message: There may be a blockage or no NaOH in W1 (continued from previous page)" on page 100 below. 4. Press **Line Clear**. Follow the prompts and use the syringe supplied with the Ion PGM™ Dx System. 5. After Line Clear, press **Flow check**, then check for normal flow rates from the waste lines. 6. If the flow rates are still not normal, perform **Line Clear** one more time. 7. If the line(s) remain blocked, contact Technical Support (see "Customer and technical support" on page 140). Otherwise, press **Start** to restart auto-pH.
	Wash 1 or Wash 2 sipper may be loose	1. Loosen the Wash 1 cap and retighten the sipper. Since the gas flows when the cap is loose, tighten the sipper as quickly as possible. (The gas is not harmful to the NaOH solution and is not a hazard.) 2. Loosen the Wash 2 cap and retighten the sipper. Since the gas flows when the cap is loose, tighten the sipper as quickly as possible. (The gas is not harmful to the W2 Solution and is not a hazard.) 3. Press **Start** to restart the auto-pH process.

Observation	Possible cause	Recommended action
Error message: There may be a blockage or no NaOH in W1 (continued from previous page)	Forgot to add NaOH to the Wash 1 Bottle: Chip does not detect a large enough pH difference between the NaOH (W1) and W2 Solutions.	1. If you forgot to add NaOH to the Wash 1 Bottle, loosen the cap and add 350 µL of 100 mM NaOH to the Wash 1 Bottle. (The flowing gas is not harmful to the NaOH solution and is not a hazard.) 2. Recap the bottle and shake gently to mix. 3. Press **Start** to restart auto-pH.
	Damaged chip	1. Replace the chip with a new (unused) one. Insert the chip in the socket, then press **Start**. **Note:** The new chip can be used for sequencing after initialization completes. 2. If the error persists, there could be a problem with the chip clamp. Contact Technical Support (see "Customer and technical support" on page 140).
Error message: W2 average not stable. Try reseating/replacing chip	Reading for W2 solution is not stabilizing quickly enough	1. Remove the waste bottle and gently wipe excess fluid from the waste lines with a lab wipe.  2. Check for leaks and reseat the chip. Replace the chip with a new (unused) one if needed. **Note:** The new chip can be used for sequencing after initialization completes. 3. Loosen the Wash 2 cap and retighten the sipper. Since the gas flows when the cap is loose, tighten the sipper as quickly as possible. (The gas is not harmful to the W2 Solution and is not a hazard.) 4. After performing one or more above steps, press **Start** to retry for auto-pH. If auto-pH fails even after replacing the chip, contact Technical Support (see "Customer and technical support" on page 140).
	The waste line may be blocked.	See "Error message: There may be a blockage or no NaOH in W1. Check W1 and run line clear then try again" on page 99.

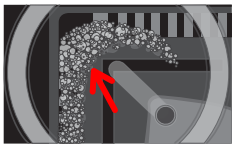
Observation	Possible cause	Recommended action
Error message: W2 out of range	• Chip measurements very unstable • Chip is damaged	See troubleshooting tips for "W2 average not stable" above.
Error message: Chip reading inconsistent. Please replace chip and try again.	• pH response of the chip is not uniform or reliable • Ran out of SEQ W3 Solution or volume too low	1. Verify that there is enough SEQ W3 Solution (>25 mL) in the Wash 3 Bottle and that the sipper is secure. 2. If necessary, loosen the Wash 3 Bottle cap, tighten the sipper, and add more SEQ W3 Solution to fill to 50 mL. Since the gas flows when the cap is loose, perform these operations as quickly as possible. (The gas is not harmful to the SEQ W3 Solution and is not a hazard.) 3. If there is enough SEQ W3 Solution, replace the chip with a new (unused) one. Insert the chip in the socket, then press "**re-try**". **Note:** The new chip can be used for sequencing after initialization completes.
Error message: Added too much W1 to W2	• Poor water quality • 18-MΩ water exposed to air for too long • Incorrect solution added to the SEQ W2 Solution • Too little NaOH added to SEQ Wash 1 Bottle • Damaged chip	1. Check whether the water meets the 18-MΩ specification and that the 100 mM NaOH and SEQ W2 Solutions were added correctly. 2. If solutions are incorrect or water does not meet specifications, correctly prepare the solution(s) and/or use 18-MΩ water. Abort the initialization and restart using correct solutions/water. 3. If the solutions and water are correct, abort the initialization and try reinitializing with a different chip.
WARNING: AutopH is within expected pH range, 0 milliliters of W1 was added to W2. Press "next" if expected, otherwise press "retry" to restart AutoPH	After auto-pH undershot the target, the user pressed the **Retry** button to restart auto-pH, but no additional NaOH was added to the Wash 2 bottle.	The pH reading was close enough to the target that no additional NaOH was added. Press **Next** to proceed with initialization.

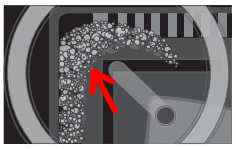
Initialization: Reagent pH verification

Observation	Possible cause	Recommended action
Failure screen	• One or more reagents are not within the target pH range • Chip is damaged	1. Press **Start** to repeat the pH measurement. 2. If the test still fails, replace the chip with a new (unused) chip and press **Start** to repeat. **Note:** The new chip can be used for sequencing after initialization completes. 3. If the test still fails with the new chip, clean and reinitialize the instrument with fresh reagents and a new chip. 4. If the test still fails, contact Technical Support (see "Customer and technical support" on page 140).

Chip calibration

Observation	Possible cause	Recommended action
Chip calibration failure (before sample has been loaded on the chip)	• Debris on the chip socket • Chip not seated correctly in the chip clamp • Chip is damaged • Problem with chip clamp or socket	1. Open the chip clamp, remove the chip, and check for damage, leaks, and/or debris under the chip and on the chip socket. • If debris or leaks are visible, gently dab the socket with a lab wipe tissue dampened with 18-MΩ water, then reseat the chip in the socket. • If no leaks or debris is visible, reseat the chip in the socket. **IMPORTANT!** Never rub or wipe the socket. Rubbing the socket can damage it and cause it to fail. 2. After reseating the chip, press **Retry**. 3. If the chip passes, press **Next** to start the experiment. If the chip still fails, reseat the chip again, then press **Retry**. 4. If chip calibration continues to fail with the same chip, retry with a new chip. 5. If the new chip fails as well: a. Press **Abort** to abort the run. **IMPORTANT!** Be sure to abort the run before rebooting the sequencer in the subsequent steps. b. Reboot the sequencer by holding down the Power button on the front to shut it down, then press again to restart. c. Restart the run with the new chip. 6. If the new chip continues to fail, there may be an issue with the chip socket. Contact Technical Support (see "Customer and technical support" on page 140).
Error message: Bad pixels error	Chip not seated correctly in the chip clamp.	1. Make sure the chip is seated correctly and the clamp is closed. 2. Press down on the chip clamp with even pressure until the message instructing to close the lid appears. 3. If the new chip continues to fail, there may be an issue with the chip socket. Contact Technical Support (see "Customer and technical support" on page 140).

Observation	Possible cause	Recommended action
Leak of unknown origin during chip calibration (before sample has been loaded on the chip)	• Chip clamp not closed properly. • Chip is damaged. • Problem with the chip clamp or socket	1. Press **Abort** to immediately stop the run. **Note:** If the system is pressurized during abort, initiate the clean step or a run to de-pressurize the system and stop the flow of buffer, then abort the clean step. 2. Open the chip clamp, remove the chip, and gently dab the chip socket with a lab wipe tissue to absorb any fluid. **IMPORTANT!** Never rub or wipe the socket. Rubbing the socket can damage it and cause it to fail. 3. Make sure that the rubber gaskets on the chip clamp are properly installed and not loose or out of position. 4. Rinse the socket with 18-MΩ water and gently absorb most of the water with the lab wipe. 5. Repeat the rinse, then gently dab the chip socket with a lab wipe until dry. 6. Place a lab wipe on the grounding plate and dampen it with 18-MΩ water. Wipe the bottom of the chip on this wipe to remove salts from the chip contacts, and then gently dab the bottom of the chip with a dry lab wipe. 7. Remove the lab wipe, dry the grounding plate, and place the chip on it. Confirm that there is no condensation outside the chip flow cell:  8. If there is condensation or fluid, the chip is damaged and cannot be run. If no chip damage is apparent, reseat the chip and press **Run** to restart the experiment. 9. When prompted to install the chip, make certain that the chip clamp is fully closed. 10. If the chip leaks again, press **Abort**, clean the chip socket, then restart the run with a different chip. If the new chip leaks, it may indicate a problem with the chip clamp or socket. Contact Technical Support (see "Customer and technical support" on page 140).

Observation	Possible cause	Recommended action
Leak of unknown origin during chip calibration (after sample has been loaded on the chip)	• Chip clamp not closed properly • Chip is damaged. • Problem with the chip clamp or socket	1. Press **Abort** to immediately stop the run. **Note:** If the system is pressurized during abort, initiate the clean step to de-pressurize the system to stop the flow of buffer, then abort the clean step. 2. Open the chip clamp, remove the chip, and gently dab the chip socket with a lab wipe tissue to absorb any fluid. **IMPORTANT!** Never rub or wipe the socket. Rubbing the socket can damage it and cause it to fail. 3. Make sure that the rubber gaskets on the chip clamp are tight and in position. 4. Place a lab wipe on the grounding plate and dampen it with 18-MΩ water. Wipe the bottom of the chip on this wipe, and then gently dab with a dry lab wipe. 5. Dry the grounding plate with a lab wipe and place the chip on it. Confirm that there is no condensation outside the chip flow cell:  6. If there is condensation or fluid, the chip is damaged and cannot be run. Prepare a new run starting with template preparation (skip the following troubleshooting steps). 7. If no chip damage is apparent, you can attempt to rerun the loaded chip. Press **Run** on the Main Menu to start a new run. 8. When prompted, place the used chip from initialization in the chip clamp and press **Next** to clean the fluid lines. 9. When prompted, scan or enter the Planned Run Short Code. 10. When prompted, scan the top and bottom barcodes on the loaded chip. 11. Secure the loaded chip in the chip clamp and proceed with initial chip calibration.

Observation	Possible cause	Recommended action
Leak of unknown origin during chip calibration (after sample has been loaded on the chip) *(continued)*		12. If the chip leaks again, press **Abort**, then clean the chip socket. There may be a problem with the chip clamp or socket. Contact Technical Support (see "Customer and technical support" on page 140). 13. If the chip does not leak and initial calibration passes, skip the chip loading steps and continue with the run.
Error message: Calibration failed (after sample has been loaded on the chip)	• Debris on the chip socket • Chip clamp not closed properly • Chip is damaged • Problem with chip clamp or socket	1. Open the chip clamp, remove the chip, and check for damage, leaks, or debris under the chip and on the chip socket. • If debris or leaks are visible, gently dab the socket with a lab wipe tissue dampened with 18-MΩ water, then re-clamp the chip. • If no leaks or debris are visible, re-clamp the chip. **IMPORTANT!** Never rub or wipe the socket. Rubbing the socket can damage it and cause it to fail. 2. After re-clamping the chip, press **Retry**. 3. If the chip passes, press **Next** to start the experiment. If the chip still fails, re-clamp the chip and press **Retry**. **Note:** You have a total of 3 attempts to retry the chip before aborting the run. 4. If calibration continues to fail, there may be an issue with the loaded chip, chip clamp, or chip socket. To test the clamp and socket: a. Abort the run, then restart the run. b. Do not use the loaded chip. Instead, place a *used* chip in the chip clamp, then follow the steps for the initial (unloaded) chip calibration. • If the used chip fails initial calibration, there may be an issue with the clamp or socket. Abort the run and contact Technical Support (see "Customer and technical support" on page 140). • If the used chip passes calibration, the previously loaded chip may be bad. Abort the run, prepare fresh template, and run with a new chip.

Observation	Possible cause	Recommended action

Error message: Chip not seated correctly. Please reset the chip and click OK to continue	• Debris on the chip socket
	• Chip not seated correctly in the chip clamp
	• Chip is damaged
	• Problem with chip clamp or socket
		1. Open the chip clamp, remove the chip, and check for damage, leaks, and/or debris under the chip and on the chip socket.
		  • If debris or leaks are visible, gently dab the socket with a lab wipe tissue dampened with 18-MΩ water, then reseat the chip in the socket.
		  • If no leaks or debris are visible, reseat the chip in the socket.
		  IMPORTANT! Never rub or wipe the socket. Rubbing the socket can damage it and cause it to fail.
		2. Press **Retry**.
		3. If the chip passes, press **Next** to start the experiment. If the chip still fails, try reseating the chip again and pressing **Retry**.
		  Note: You have a total of 3 attempts to reseat the chip before aborting the run.
		4. If you continue to receive the "Chip not seated correctly" error message, contact Technical Support (see "Customer and technical support" on page 140). There may be an issue with the chip clamp or socket.

Chip calibration status bar does not progress	During chip calibration, the touchscreen status progress bar does not progress.	1. Press the **Abort** button to abort the run. 2. Reboot the sequencer by holding down the **Power** button on the front to shut it down, then press the button again to restart. 3. Restart the run with the same chip. **Note:** Reinitialization is *not* required after rebooting the sequencer if the initialization successfully completed before the run was aborted.
Barcode on the bottom of the chip does not scan	The barcode is printed incorrectly.	Use a new chip and contact Technical Support for a replacement (see "Customer and technical support" on page 140).

Sample loading

Observation	Possible cause	Recommended action
Sample volume is <30 µL If the sample volume is less than <30 µL, there will be a visible air gap at the end of the pipette tip when you collect the sample for chip loading.	Volume was lost at some point during sample preparation.	**Note:** Equilibrate SEQ Sample Buffer (brown cap) to room temperature for 15 minutes before performing the following steps. 1. With the sample loaded in the pipette tip, dial down the pipette until the liquid reaches the end of the tip. Record the volume shown on the pipette (X). 2. Dispense the sample back into the sample tube. 3. Subtract the volume shown on the pipette from 30 µL (30 – X = Y µL) to determine the missing volume (Y). 4. Change the pipette tip, and add Y µL of SEQ Sample Buffer (brown cap) to the sample. 5. Pipet up and down 4 times to mix the contents, then continue loading the sample into the chip.
Large amount of the sample volume (≥15 µL) leaks from the outlet port during chip loading.	The chip loading port has a clog or obstruction.	Do not continue with the sequencing run. Contact Technical Support.

Warnings and alarms—Ion PGM™ Dx System

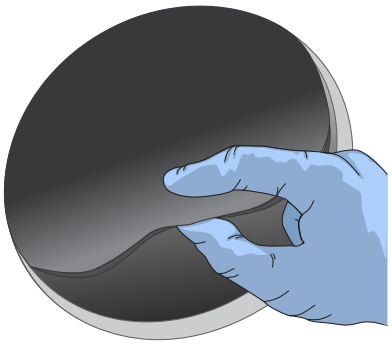
The following warnings and alarms appear on the Ion PGM™ Dx Sequencer touchscreen and in the Torrent Suite™ Dx Software under the **Monitor** tab.

Observation	Possible cause	Recommended action
Display message: Pressure too high	Internal pressure regulator was not set correctly	Contact Technical Support (see "Customer and technical support" on page 140).
Display message: Pressure too low.	• Gas line is not connected to the instrument • Gas cylinder may be turned off or empty	1. Verify that the gas line is connected to the instrument. 2. Verify that the gas cylinder has at least 500 psi on the pressure gauge inside the tank and 30 psi on the gauge that regulates pressure to the outlet. (Both pressure gauges are mounted on the gas cylinder.) 3. Confirm that the outlet valve on the regulator is turned on. 4. If the problem persists, contact Technical Support (see "Customer and technical support" on page 140).

Observation	Possible cause	Recommended action
Display message: Instrument idle temperature too high	• Room temperature is too high. • Clogged filter or blocked airway on the instrument • Hardware issue (fan is not running or running too slowly)	**Note:** The data created during a run with this alarm raised may still be used if all the QC metrics are met. See the recommended action for "Display message: Instrument temperature too high" on page 112.
Display message: Instrument idle temperature too low	• Ambient room temperature is below 20°C. • Hardware issue	Bring the ambient temperature up to 20°C. If the problem persists, contact Technical Support (see "Customer and technical support" on page 140).
Display message: Bad results data drive	• On some machines, the warning appears before the reboot completes. • There is a hardware issue.	Wait for a few minutes to see if the error message disappears. If the error message disappears, data obtained during a run with this alarm raised can still be used. If the problem persists, contact Technical Support (see "Customer and technical support" on page 140).
Display message: Kernels do not match	Hardware and/or software issue	Contact Technical Support (see "Customer and technical support" on page 140).
Display message: Failed to set up system time at startup. Check your connection to the Ion Torrent™ Server.	The connection between the Ion PGM™ Dx Sequencer and the Ion Torrent™ Server has been lost.	1. Check the network connection to the Ion Torrent™ Server to make sure the connection is established, then reboot the instrument. 2. If the problem persists, replace the network cable(s) to the instrument and server. 3. If the problem persists, contact Technical Support (see "Customer and technical support" on page 140).
	Instrument is still in the process of establishing a connection	Allow 10 minutes to see if display message clears.
Display message: Lost connection to the Ion Torrent™ Server	The connection between the instrument and the server has been lost.	Check the network connection to the Ion Torrent™ Server, and then reboot the Ion PGM™ Dx Sequencer. If this alarm appears during a run, the data created during that run can still be used.
Display message: UBoots do not match	Hardware issue	Contact Technical Support (see "Customer and technical support" on page 140).
Display message: Bad boot drive detected	Hardware issue	Contact Technical Support (see "Customer and technical support" on page 140). If this alarm appears during a run and data for the run is generated, that data may still be used.
Display message: Results drive not accessible. Reboot and try again.	• On some machines, the warning appears before the reboot completes • Hardware issue	Wait for a few minutes to see if the error message disappears. If the error message appears and disappears during a run, data obtained during that run can still be used. If the alarm persists, contact Technical Support (see "Customer and technical support" on page 140).

Observation	Possible cause	Recommended action
Display message: Lost chip connection	The instrument cannot detect a chip in the chip clamp	See the instructions under "Chip calibration failure (before sample has been loaded on the chip)" on page 103.
Display message: Sensor unable to measure instrument temperature	Hardware issue	Contact Technical Support (see "Customer and technical support" on page 140).
Display message: Results drive check failed	Hardware issue	If the error message disappears when you return to the main instrument screen, this alarm can be ignored. Otherwise, contact Technical Support (see "Customer and technical support" on page 140).
Display message: Software versions incompatible. Go to the Options menu and update the software.	The system software was updated, but the instrument software was not.	After the system software has been updated, update the instrument software as follows: 1. On the main menu of the instrument, press **Options** and follow the instructions to check for and install updates. 2. When installation is complete, follow the onscreen prompts to restart the instrument. **IMPORTANT!** You must restart the instrument before proceeding.
Display message: Sensor unable to measure gas pressure. Check supply gas pressure.	• Gas line is not connected to the instrument • Gas cylinder may be turned off or empty • Hardware issue	1. Verify that the gas line is connected to the instrument. 2. Verify that the gas cylinder has at least 500 psi on the pressure gauge inside the tank and 30 psi on the gauge that regulates pressure to the outlet. (Both pressure gauges are mounted on the gas cylinder.) 3. Confirm that the outlet valve on the regulator is turned on. 4. If the problem persists, contact Technical Support (see "Customer and technical support" on page 140).
Display message: Failed to locate the barcode scanner. Check if the scanner is attached.	The connection between the barcode scanner and the Ion PGM™ Dx Sequencer has been lost.	Make sure the scanner is plugged into a USB port on the instrument. If it is connected and the alarm still appears, try plugging the scanner into a second USB port. If the alarm persists, contact Technical Support (see "Customer and technical support" on page 140).

Observation	Possible cause	Recommended action
Display message: Failed to set the pressure to target range. Check the gas connection and try again.	• Gas line is not connected to the instrument • Gas cylinder may be turned off or empty • Hardware issue (regulator malfunction)	1. Verify that the gas line is connected to the instrument. 2. Verify that the gas cylinder has at least 500 psi on the pressure gauge inside the tank and 30 psi on the gauge that regulates pressure to the outlet. (Both pressure gauges are mounted on the gas cylinder.) 3. Confirm that the outlet valve on the regulator is turned on. 4. If the problem persists, contact Technical Support (see "Customer and technical support" on page 140).
Display message: Failed to set up FTP connection. Check your connection to the Ion Torrent™ Server .	The network connection is not established or an incorrect IP address was used.	Confirm that the server information is correct for the Ion Torrent™ Server. Contact your local network administrator for support if the issue persists.
Display message: Unable to mount the file system	Hardware and/or software issue	1. Reboot the instrument to clear the alarm. 2. If the alarm is not cleared after reboot, contact Technical Support (see "Customer and technical support" on page 140).
Display message: Instrument temperature too low	• Room temperature is below 20°C. • Hardware issue	**Note:** The data created during a run with this alarm raised may still be used if all the QC metrics are met. If the ambient room temperature is below 20°C, raise it. If the problem persists, contact Technical Support (see "Customer and technical support" on page 140).

Observation	Possible cause	Recommended action
Display message: Instrument temperature too high	• Room temperature is too high. • Clogged filter or blocked airway on the instrument • Hardware issue (fan is not running or running too slowly)	**Note:** The data created during a run with this alarm raised may still be used if all the QC metrics are met. 1. If the ambient room temperature is above 30°C, lower it. 2. Make sure that the round filter on the back panel of the instrument has unrestricted airflow. If the filter is clogged with dust, clean it as follows: a. Pinch the dirty filter with your fingers, then remove it from the instrument.  b. Shake the filter over a waste container to remove most of the dust. c. Rinse the filter with running water to remove any remaining dust. The water flow should be from the inside-facing surface to the outside-facing surface through the filter. d. Air dry the filter. e. Blot any remaining dust from the filter using tape. f. Reinsert the filter. If the problem persists, contact Technical Support (see "Customer and technical support" on page 140).
Display message: Chip temperature too low	Hardware issue	**IMPORTANT!** The data created during a run with this alarm raised should *not* be used. Contact Technical Support (see "Customer and technical support" on page 140).

Observation	Possible cause	Recommended action
Display message: Chip temperature too high	• Room temperature is too high. • Clogged filter or blocked airway on the instrument • Hardware issue (instrument fan is not running or running too slowly)	**IMPORTANT!** The data created during a run with this alarm raised should *not* be used. See the recommended action for "Display message: Instrument temperature too high" on page 112.
Display message: Lost communication with valve board	Hardware issue	Contact Technical Support (see "Customer and technical support" on page 140).
Display message: Fan current too low	Hardware issue	Contact Technical Support (see "Customer and technical support" on page 140).
Display message: Heater current too low	Hardware issue	**IMPORTANT!** If the chip temperature is also out of range, data created during a run should *not* be used. Contact Technical Support (see "Customer and technical support" on page 140). If no chip temperature alarms are raised, data created during a run may still be used if all the QC metrics are met.
Error message: A non-recoverable error has occurred	Hardware issue	Prepare new template from the same library and plan a new run. If the issue persists, contact Technical Support (see "Customer and technical support" on page 140).

Sequencer software issues

(For additional software anomalies, see the release notes provided with your version of the software.)

Observation	Possible cause	Recommended action
Sequencer touchscreen is frozen	The user pressed multiple buttons on the screen in rapid succession.	Wait 5 minutes for the touchscreen to unfreeze. If the touchscreen remains frozen, reboot the sequencer by holding down the **Power** button on the front to shut it down, then press again to restart.
	The touchscreen is in a locked state.	Reboot the sequencer by holding down the **Power** button on the front to shut it down, then press again to restart.

Warnings and alarms—Veriti™ Dx 96-well Thermal Cycler

Observation	Possible cause	Recommended action
"Fatal Error" message displayed by Veriti™ Dx 96-well Thermal Cycler	Various	For assistance, contact Technical Support (see "Customer and technical support" on page 140). Refer to the *Veriti™ Dx 96-well Thermal Cycler User Guide* (Pub. No. 4453697) for general troubleshooting information for this instrument.

B Performance characteristics

For performance characteristics of the Ion PGM™ Dx System, see the *Ion PGM™ Dx System Performance Characteristics User Guide* (Pub. No. MAN0018763).

C Supplemental procedures and instruments

Fuse replacement on the Ion OneTouch™ ES Dx Instrument

IMPORTANT! The Ion OneTouch™ ES Dx Instrument is supplied with a Fuse Module and two different types of spare fuses, which should only be replaced by trained field service engineers. The Fuse Module is installed by the field service engineer into the Power Entry Module located on the back of the instrument in the proper orientation for the voltage in your area. If you are not sure of the setting that is right for your area, contact your local power company.

Line voltage	Replacement fuse type required
110/120 VAC	375 mA TT (Slow Blow) 1/4 inches × 1-–1/4 inches
220/240 VAC	160 mA TT (Slow Blow) 5 × 20 mm

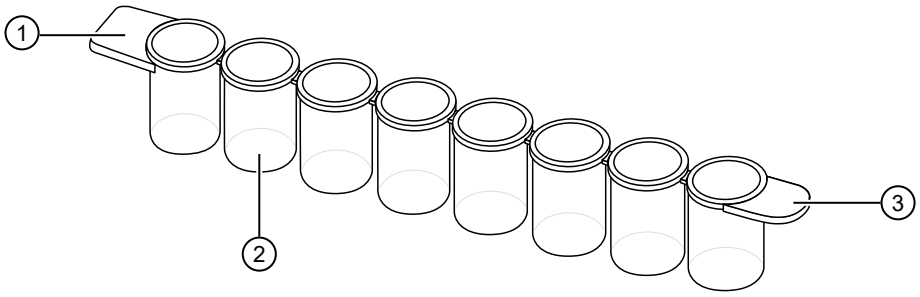
WARNING! ELECTRICAL SHOCK HAZARD. Severe electrical shock, which could cause physical injury or death, can result from working on an instrument when the high voltage power supply is operating. To avoid electrical shock, disconnect the power supply to the instrument, unplug the power cord, and wait at least 1 minute before working on the instrument.

Ion OneTouch™ ES Dx Instrument residual volume test

1. Install a new tip on the Ion OneTouch™ ES Dx Instrument Tip Arm.

   Note: For the residual volume test, you do not need to put a TMPL Sample Collection Tube in the Tip Loader.

2. Load 80 µL water or TMPL Wash Solution into the second well (Well 2) from the square-tab end of the 8-well strip:



① Square tab
② Second well
③ Round tab

3. Load the 8-well strip into the right end of the slot on the Ion OneTouch™ ES Dx Instrument tray so that the square tab is on the left and the rounded tab is on the right.

IMPORTANT! Before proceeding, carefully read and familiarize yourself with the following steps, which require you to manually start and stop the test run and manipulate the strip tube during the run. During the test, confirm that the tip is centered in the wells when moving in or out of a well.

4. Turn the instrument ON.

5. Wait for the instrument to initialize. The screen displays "rdy". The Tip Arm performs a series of movements and returns to the home position (~5 seconds).

6. Press **Start/Stop**.

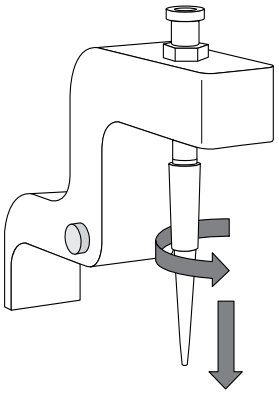
7. Wait for the instrument to aspirate the solution from Well 2 and completely remove the tip from Well 2, then *manually* push the 8-well strip to the left so that Well 4 is positioned directly under the Tip Arm.

8. Wait for the instrument to dispense the tip contents into Well 4.

9. Press **Start/Stop** to stop the test run, then press **Start/Stop** again to return the Tip Arm to the home position.

10. Using a P10 pipette, aspirate the entire residual volume from Well 2, then estimate the residual volume.

11. Remove the used tip: with the Tip Arm in its cradle and while standing above the Tip Arm, twist the tip *counterclockwise* and pull it downward to remove and discard the tip.



IMPORTANT! Improper removal of tips can loosen the metal tip adapter fitting on the Tip Arm and affect instrument operation.

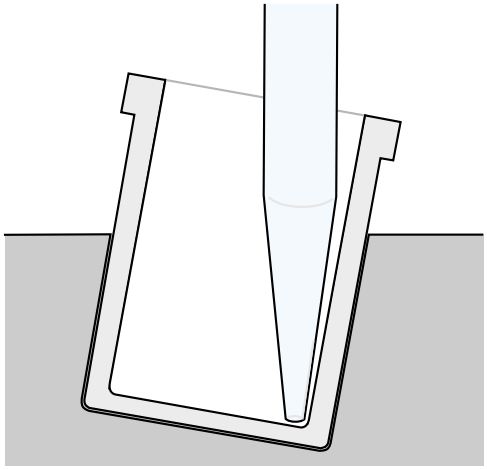
12. Remove and discard the used 8-well strip.

13. After performing the residual volume test, take one or more of the following actions:

Observation	Pass/Fail	Possible cause	Recommended actions
Residual volume in Well 2 is ≤5 µL	Pass	—	—
Residual volume in Well 2 is >5 µL **IMPORTANT!** The volume is measured from the *bottom* of the well, not from the sides.	Fail	The tip height is too high during aspiration.	Calibrate the instrument (see "Calibrate the Ion OneTouch™ ES Dx Instrument" on page 119).
The 8-well strip lifts as the tip rises to the top of the well	Fail	The tip is angled too far forward or the tip height is set too low.	Verify that the tip is vertical and positioned directly over the notch in the calibration shelf. If the tip is positioned correctly, restore defaults, then calibrate the instrument (see "Calibrate the Ion OneTouch™ ES Dx Instrument" on page 119).

Calibrate the Ion OneTouch™ ES Dx Instrument

Perform horizontal and vertical calibrations so that during operation the tip is optimally positioned in the well of the 8-well strip:



Note that the 8-well strip is always tilted at a fixed 10-degree angle in the slot. The pipette tip is vertical. When the tip is aligned properly during calibration so that it is in line with the notch in the calibration shelf, the tip touches the front-bottom edge of the well during the run.

IMPORTANT! If you use more than one Ion OneTouch™ ES Dx Instrument, do not switch Trays or Tip Arms between instruments. Each Tray and Tip Arm is calibrated with a particular instrument. To track the Tray and Tip Arm, each component has a printed label with the matching serial number of the instrument.

Vertical axis calibration

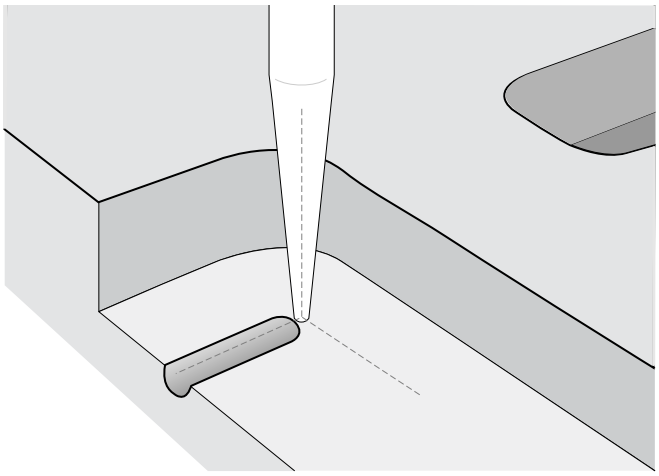
1. Install a new tip.
2. Restore the factory default settings:
   a. Power OFF the instrument, then wait 3 seconds.
   b. While holding down **Vert. Adjust**, power ON the instrument.
3. Put the instrument into calibration mode:
   a. Power OFF the instrument.
   b. While holding down **Select/Calibrate**, power the instrument ON. Keep holding down **Select/Calibrate** until "P1" is displayed.
   c. Press **Select/Calibrate** for ~3 seconds until the instrument beeps 2 times and "CAL" is displayed.

   Note: The instrument will cycle through several values before "CAL" is displayed.

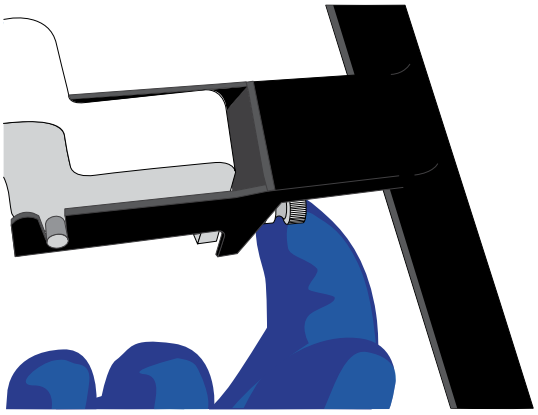
4. Press **Vert. Adjust**. The instrument displays "ASP" (Aspirate or z-bottom position).

5. Press **Start/Stop**. The Tip Arm lowers to bring the tip near the notch in the calibration shelf on the left side of the Tray.

6. The tip should be positioned in line with the slot in the calibration shelf, and the tip should be touching the shelf. If necessary, adjust the tip as follows:



   a. To adjust the alignment of the tip with the slot, turn the thumbscrew at the back of the Tip Arm.



   b. To adjust the height of the tip, press the ▼ (minus) button repeatedly until the tip touches the shelf. Press the button eight more times to lower the tip further. This will account for variations in tip lengths and installation.

   Note: It is better to have the ASP (aspiration) height be too low than too high.

7. Press **Start/Stop**, then wait for the Tip Arm to stop moving and for "P1" to display.

Horizontal axis calibration

1. Press **Select/Calibrate** for ~3 seconds until the instrument beeps 2 times and “CAL” is displayed.

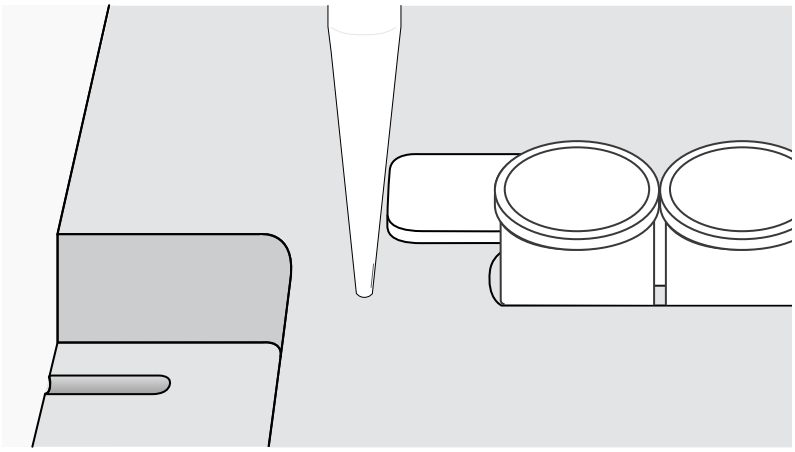
   Note: While you press the button, the instrument cycles through several values before “CAL” is displayed.

2. Press **Horiz. Adjust**. The instrument displays “FLA”. Press **Start/Stop**.

3. Place an empty 8-well strip in the slot in the Tray, with the square tab on the left.

4. Push the 8-well strip as far to the left in the slot as possible.

5. Observe the position of the 8-well strip relative to the position of the tip. When properly calibrated, the 8-well strip is within 1 mm of touching the tip, but not pushing on it. To clearly see the relationship between the pipette tip, calibration shelf, and notch during calibration, mark each of them with a felt-tip pen:



6. Adjust the horizontal position of the Tip Arm so that the tip just touches the square tab on the left of the 8-well strip when the 8-well strip is pushed to the far left of the slot in the Tray*:*
   a. Apply slight pressure to keep the 8-well strip to the far left.
   b. Press the ⚠ (plus) button repeatedly until the tip touches the 8-well strip. Each press of the ⚠ (plus) key moves the Tip Arm to the *right* by ~0.002 inches (~50 µm), which may be difficult to detect.

7. Press **Start/Stop** to save the setting, then wait for “P1” to display.

8. Power the instrument OFF, wait >3 seconds, then power the instrument ON to return to normal operating mode.

9. Perform a residual volume test (see “Ion OneTouch™ ES Dx Instrument residual volume test” on page 116).

Removal of instruments from use for repair or disposal

To remove the Ion OneTouch™ Dx Instrument or Ion OneTouch™ ES Dx Instrument from use for repair or disposal, perform the following steps:

IMPORTANT! This procedure does not guarantee total decontamination of the Ion OneTouch™ Dx Instrument or Ion OneTouch™ ES Dx Instrument.

1. Wear disposable gloves, safety glasses, and a lab coat.
2. Use a cleaning pad wetted with a solution of 1 part chlorine bleach in 9 parts water (10% bleach solution) to clean all outside surfaces of the Ion OneTouch™ Dx Instrument or Ion OneTouch™ ES Dx Instrument. Use care to avoid getting bleach solution inside the chassis.
3. Dry the surfaces of the instrument with paper towels or other disposable wipes.
4. Use cotton swabs to clean and dry areas that are difficult to access.
5. Properly dispose of all used consumables (including tubes, sippers, amplification plates) and cleaning materials to ensure that no one becomes exposed to contaminants.

If returning the instrument, contact your local Thermo Fisher Scientific representative to schedule a pickup of the instrument.

D

Ion PGM™ Dx Chip Minifuge and barcode scanner

Ion PGM™ Dx Chip Minifuge

The Ion PGM™ Dx Chip Minifuge is supplied with one custom rotor and two buckets. The buckets are designed to hold two chips: one in each bucket. The rotor and bucket design enables effective and efficient reagent loading of chips.

Safety precautions

CAUTION!

- Make sure your supply voltage matches the voltage label on the minifuge, i.e., never plug a 120V minifuge into an 220–240 VAC outlet. Operating the minifuge with a supply voltage outside the specified range may cause a fire or electric shock.
- Do not run the minifuge for more than 30 seconds.
- Never operate the minifuge without a rotor properly attached to the shaft.
- Never operate with only one chip in place. A chip must be present in each bucket to balance the rotor. If necessary, you can balance a loaded chip with a used chip of any type.
- Never put hands in the rotor area unless the rotor is completely stopped.
- Never move the minifuge while the rotor is spinning.
- Do not leave the minifuge running when not in use.

Note: The rotor can be balanced with a used chip from a previous reaction without risk of contamination.

Voltage selection

Two different minifuges are available, depending on your supply voltage: 120 VAC and 220–240 VAC. Make sure that the voltage specification on the label of your minifuge matches the supply voltage. If they do not match, change your supply voltage or contact Customer Support to request the appropriate minifuge.

CAUTION! Never plug a 120V minifuge into an 220–240 VAC outlet, or vice versa. Operating the minifuge with a supply voltage outside of the range specified on the label may cause a fire or electric shock.

Voltage, RPM, and RCF

The following tables list the revolutions per minute (RPM) and relative centrifugal force (RCF) at different voltages.

120/50 VAC, 60 Hz	RPM	RCF
90	4100	836
100	4550	1030
110	4960	1224
120	5330	1424
130	5710	1628

230/50 VAC, 60 Hz	RPM	RCF
210	5070	1279
220	5310	1403
230	5515	1513
240	5705	1619
250	5900	1732

Operation

1. Place the Ion PGM™ Dx Chip Minifuge on a level, clean surface near an accessible power outlet so that the cord and outlet are within easy reach of the operator.
2. Make sure the power switch on the minifuge is in the "off" position.
3. Load a chip into each bucket.

   IMPORTANT! A chip must be present in each bucket to balance the rotor. If necessary, you can balance a loaded chip with a used chip of any type.
4. Turn the power switch on.
5. To begin centrifugation, close the lid of the minifuge. (The centrifugation time will vary depending on the step in the chip-loading protocol.)
6. To stop centrifugation, press down on the lid release tab on the front of the minifuge.

CAUTION! Do not attempt to open the lid or remove the chips until the unit has come to a complete stop.

7. After the rotor has stopped, open the lid by grabbing it with the thumb on the front and fingers on the back, then lifting the lid back on the hinge.

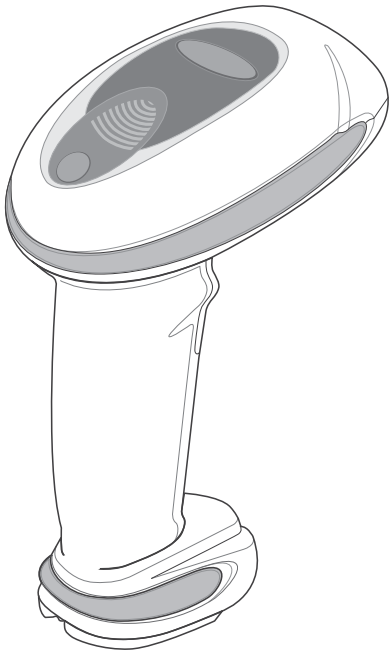
Cleaning

To clean the minifuge, use a damp cloth and a mild, noncorrosive detergent (pH <8). After cleaning, ensure that all parts are dried thoroughly before attempting to operate the unit. Do not immerse the centrifuge in liquid or pour liquids over it.

Note: Use only the cleaning protocol described above.

Barcode scanner

The barcode scanner provided with the Ion PGM™ Dx System uses a low-power, visible-light diode.



CAUTION! As with any bright light source, you should avoid staring directly into the light beam or shining the beam into other people's eyes. Momentary exposure to a Class 2 laser is not known to be harmful.

CAUTION! Use of controls, adjustments, or performance of procedures other than those specified in this guide can result in hazardous laser light exposure.

The barcode scanner specifications are listed below.

Wavelength	Rated Power
630–680 nm	1 mW

E

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the “Documentation and Support” section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l’utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d’utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l’inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu’avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l’absence de fuite ou d’écoulement des produits chimiques. En cas de fuite ou d’écoulement d’un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words:

- **CAUTION!**—Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **WARNING!**—Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- **DANGER!**—Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Symbol	English	Français
	Caution, risk of danger Consult the manual for further safety information.	Attention, risque de danger Consulter le manuel pour d'autres renseignements de sécurité.
	Caution, risk of electrical shock	Attention, risque de choc électrique
	Caution, piercing hazard	Attention, danger de perforation
	Caution, hot surface	Attention, surface chaude
	Potential biohazard	Danger biologique potentiel
	On	On (marche)
	Off	Off (arrêt)
	On/Off	On/Off (marche/arrêt)
	Earth (ground) terminal	Borne de (mise à la) terre
	Protective conductor terminal (main ground)	Borne de conducteur de protection (mise à la terre principale)

(continued)

Symbol	English	Français
	Terminal that can receive or supply alternating current or voltage	Borne pouvant recevoir ou envoyer une tension ou un courant de type alternatif
	Do not dispose of this product in unsorted municipal waste **CAUTION!** To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.	Ne pas éliminer ce produit avec les déchets usuels non soumis au tri sélectif. **MISE EN GARDE !** Pour minimiser les conséquences négatives sur l'environnement à la suite de l'élimination de déchets électroniques, ne pas éliminer ce déchet électronique avec les déchets usuels non soumis au tri sélectif. Se conformer aux ordonnances locales sur les déchets municipaux pour les dispositions d'élimination et communiquer avec le service à la clientèle pour des renseignements sur les options d'élimination responsable.

Conformity symbols on the instrument

Conformity mark	Description
	Indicates conformity with safety requirements for Canada and U.S.A.
	Indicates conformity with European Union requirements for safety and electromagnetic compatibility.
	Indicates conformity with Australian standards for electromagnetic compatibility.

Medical device symbols

The following table describes symbols that may be displayed on product labels. The symbols that are used on labels conform to standards BS EN ISO 15223-1:2016 and FDA 21 CFR 809.10 "Labeling for in vitro diagnostic products".

Symbol	Description	Symbol	Description
	MANUFACTURER		CONTAINS SUFFICIENT FOR <n> TESTS
	DATE OF MANUFACTURE		USE BY
	BATCH CODE		CATALOG NUMBER
	SERIAL NUMBER		FRAGILE, HANDLE WITH CARE
	LOWER LIMIT OF TEMPERATURE		PROTECT FROM LIGHT
	UPPER AND LOWER LIMITS OF TEMPERATURE		UPPER LIMIT OF TEMPERATURE
	DO NOT REUSE		BIOLOGICAL RISKS
	CAUTION, CONSULT ACCOMPANYING DOCUMENTS		CONSULT INSTRUCTIONS FOR USE
	UPPER AND LOWER LIMITS OF HUMIDITY		OBSERVE PRECAUTIONS FOR HANDLING ELECTROSTATIC SENSITIVE DEVICES
	IN VITRO DIAGNOSTIC MEDICAL DEVICE		
	AUTHORISED REPRESENTATIVE IN THE EUROPEAN COMMUNITY		

Safety alerts on this instrument

Additional text may be used with one of the symbols described above when more specific information is needed to avoid exposure to a hazard. See the following table for safety alerts found on the instrument.

English	Français
CAUTION! Hazardous chemicals. Read the Safety Data Sheets (SDSs) before handling.	**MISE EN GARDE ! Produits chimiques dangereux.** Lire les fiches signalétiques (FS) avant de manipuler les produits.
CAUTION! Hazardous waste. Refer to SDS(s) and local regulations for handling and disposal.	**MISE EN GARDE ! Déchets dangereux.** Lire les fiches signalétiques (FS) et la réglementation locale associées à la manipulation et à l'élimination des déchets.

Safety information for third-party instruments

Refer to the manufacturer's documentation for information on the safe use of third-party products provided with the instrument system.

Instrument safety

General

CAUTION! Do not remove instrument protective covers. If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

CAUTION! Solvents and Pressurized fluids. Wear eye protection when working with any pressurized fluids. Use caution when working with any polymeric tubing that is under pressure:

- Extinguish any nearby flames if you use flammable solvents.
- Do not use polymeric tubing that has been severely stressed or kinked.
- Do not use polymeric tubing with tetrahydrofuran or nitric and sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause polymeric tubing to swell and greatly reduce the rupture pressure of the tubing.
- Be aware that high solvent flow rates (~40mL/min) may cause a static charge to build up on the surface of the tubing and electrical sparks may result.

Electrical

WARNING! Fuse Installation. Before installing the instrument, verify that the fuses are properly installed and the fuse voltage matches the supply voltage. Replace fuses only with the type and rating specified for the unit. Improper fuses can damage the instrument wiring system and cause a fire.

WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility.

WARNING! Disconnecting Power. To fully disconnect power, either detach or unplug the power cord. Position the instrument such that the power cord is accessible.

WARNING! Radio interference. This equipment has been designed and tested to CISPR 11 Class A. In a domestic environment it may cause radio interference, in which case you may need to take measures to mitigate the interference.

WARNING! Electromagnetic radiation. Do not use this device in close proximity to sources of strong electromagnetic radiation (e.g., unshielded intentional RF sources), as these may interfere with proper operation.

Cleaning and decontamination

CAUTION! Cleaning and Decontamination. Use only the cleaning and decontamination methods specified in the manufacturer's user documentation. It is the responsibility of the operator (or other responsible person) to ensure the following requirements are met:

- No decontamination or cleaning agents are used that could cause a HAZARD as a result of a reaction with parts of the equipment or with material contained in the equipment.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) prior to having the instrument serviced at your facility or sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan (decontamination forms may be requested from customer service).
- Before using any cleaning or decontamination methods (except those recommended by the manufacturer), users should confirm with the manufacturer that the proposed method will not damage the equipment.

MISE EN GARDE ! Nettoyage et décontamination. Utiliser uniquement les méthodes de nettoyage et de décontamination indiquées dans la documentation du fabricant destinée aux utilisateurs. L'opérateur (ou toute autre personne responsable) est tenu d'assurer le respect des exigences suivantes:

- Ne pas utiliser d'agents de nettoyage ou de décontamination susceptibles de réagir avec certaines parties de l'appareil ou avec les matières qu'il contient et de constituer, de ce fait, un DANGER.
- L'instrument doit être correctement décontaminé a) si des substances dangereuses sont renversées sur ou à l'intérieur de l'équipement, et/ou b) avant de le faire réviser sur site ou de l'envoyer à des fins de réparation, de maintenance, de revente, d'élimination ou à l'expiration d'une période de prêt (des informations sur les formes de décontamination peuvent être demandées auprès du Service clientèle).
- Avant d'utiliser une méthode de nettoyage ou de décontamination (autre que celles recommandées par le fabricant), les utilisateurs doivent vérifier auprès de celui-ci qu'elle ne risque pas d'endommager l'appareil.

Laser

CAUTION! LASER HAZARD, Bar Code Scanner. The bar code scanner included with the instrument system is a Class 2 laser. To avoid damage to eyes, do not stare directly into the beam or point into another person's eyes.

Gas safety

Verify that your installation room can accommodate gas cylinders.

WARNING! Instrumentation must be installed and operated in a well-ventilated environment as defined as having a minimum airflow of 6-10 air changes per hour. Assess the need for ventilation or atmospheric monitoring to avoid asphyxiation accidents from inert gases and/or oxygen depletion, and take measures to clearly identify potentially hazardous areas through training or signage. Please contact your Environmental Health and Safety Coordinator to confirm that the instruments will be installed and operated in an environment with sufficient ventilation.

WARNING! Pressurized gas cylinders are potentially explosive. Always cap the gas cylinder when it is not in use, and attach it firmly to the wall or gas cylinder cart with approved brackets or chains.

WARNING! Gas cylinders are heavy and may topple over, potentially causing personal injury and tank damage. Cylinders should be firmly secured to a wall or work surface. Please contact your Environmental Health and Safety Coordinator for guidance on the proper installation of a gas cylinder.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.

Safety

Reference	Description
EU Directive 2006/95/EC	European Union “Low Voltage Directive”
IEC 61010-1 EN 61010-1 UL 61010-1 CSA C22.2 No. 61010-1	*Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements*
IEC 61010-2-010 EN 61010-2-010	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials*
IEC/EN 61010-2-101	*Safety requirements for electrical equipment for measurement, control and laboratory use - Part 2-101: Particular requirements for in vitro diagnostic (IVD) medical equipment*

EMC

Reference	Description
Directive 2004/108/EC	European Union “EMC Directive”
EN 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
EN 61326-2-6	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 26: Particular requirements – In vitro diagnostic (IVD) medical equipment)requirements*
FCC Part 15	U.S. Standard “Industrial, Scientific, and Medical Equipment”
AS/NZS CISPR 22:2009	*Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment*
ICES-003, Issue 5	*Industrial, Scientific and Medical (ISM) Radio Frequency Generators*

Environmental design

Reference	Description
Directive 2012/19/EU	European Union “WEEE Directive” – Waste electrical and electronic equipment
Directive 2011/65/EU	European Union “RoHS Directive” – Restriction of hazardous substances in electrical and electronic equipment

Precaution—strong magnet

Note: Do not substitute non-IVD labeled magnets for the DynaMag™ Dx 96-Well Plate Magnet and DynaMag™ Dx 16 2-mL Magnet, provided with Ion PGM™ Dx System.

The DynaMag™ Dx 96-Well Plate Magnet and DynaMag™ Dx 16 2-mL Magnet contain very strong permanent magnets. People wearing a pacemaker or any other medical magnetized implant should not use this product unless advised by a health professional; the implant could be affected or damaged by exposure to a strong magnetic field. Keep tools and objects that could be damaged by the magnetic field out of the working area. This includes, but is not restricted to, credit cards and other products containing magnetic recording devices. Keep away from delicate instruments, watches, electronic equipment, displays and monitors. The magnet may attract steel or other magnetic material with high mechanical forces. Take care during handling. Avoid contact between two magnets. Do not pull the magnets apart if contact has been made; twist off to prevent damage to the unit or fingers. The Health and Safety Officer should take all necessary steps and full responsibility to ensure that the precautions and statements are followed and adhered to.

F

Medical device symbols

The following table describes symbols that may be displayed on product labels. The symbols that are used on labels conform to standards BS EN ISO 15223-1:2016 and FDA 21 CFR 809.10 "Labeling for in vitro diagnostic products".

Symbol	Description	Symbol	Description
	MANUFACTURER		CONTAINS SUFFICIENT FOR <n> TESTS
	DATE OF MANUFACTURE		USE BY
	BATCH CODE		CATALOG NUMBER
	SERIAL NUMBER		FRAGILE, HANDLE WITH CARE
	LOWER LIMIT OF TEMPERATURE		PROTECT FROM LIGHT
	UPPER AND LOWER LIMITS OF TEMPERATURE		UPPER LIMIT OF TEMPERATURE
	DO NOT REUSE		BIOLOGICAL RISKS
	CAUTION, CONSULT ACCOMPANYING DOCUMENTS		CONSULT INSTRUCTIONS FOR USE
	UPPER AND LOWER LIMITS OF HUMIDITY		OBSERVE PRECAUTIONS FOR HANDLING ELECTROSTATIC SENSITIVE DEVICES
	IN VITRO DIAGNOSTIC MEDICAL DEVICE		
	AUTHORISED REPRESENTATIVE IN THE EUROPEAN COMMUNITY		

G Instrument warranty

For new Ion Torrent™ instruments, Life Technologies warrants to and only to buyer for twelve (12) months from the date of shipping, that the Ion Torrent™ software and Ion Torrent™ instruments are free from defects in material and workmanship and conform to Life Technologies' published specifications in all material respects. Where a valid and timely claim in respect of breach of Ion Torrent™ warranty is submitted to Life Technologies, Life Technologies may, at its discretion, replace, repair or modify the Ion Torrent™ instrument. Any agreed replacement shall be at 1:1, like-kind basis, at no cost to the buyer. For Ion Torrent™ chips or reagents reasonably determined by Life Technologies to be defective, independent of user error, shall be replaced by Life Technologies on a 1:1, like-kind basis at no cost to buyer, provided that such defective Ion Torrent™ chips or reagents were used by buyer prior to their expiration date, or if there is no expiration date, the Ion Torrent™ chips or reagents were used within six (6) months of receipt, and the defect was promptly reported with appropriate detail to Life Technologies' technical support.

NO OTHER WARRANTIES SHALL BE APPLICABLE TO ION TORRENT PRODUCTS (WHETHER OR NOT ANY FURTHER WARRANTY DOCUMENTATION MAY BE INCLUDED IN THE SHIPMENT), WITH THE EXCEPTION OF THIRD PARTY WARRANTIES WITH RESPECT TO THIRD PARTY PRODUCT. ANY THIRD PARTY PRODUCTS ARE NOT COVERED BY THIS SECTION AND ANY WARRANTIES FOR THIRD PARTY PRODUCTS ARE PROVIDED BY THE ORIGINAL MANUFACTURER OF THE THIRD PARTY PRODUCT. Warranties are made only to buyer purchasing the Ion Torrent™ Product directly from Life Technologies, are not transferable and do not extend to the benefit of any other person or entity, unless otherwise expressly stated in writing by Life Technologies. ANY PRODUCT NOT COVERED BY AN EXPRESS WRITTEN WARRANTY IS SOLD AND PROVIDED "AS IS," WITHOUT WARRANTY OF ANY KIND, STATUTORY, EXPRESS OR IMPLIED. Any description of Ion Torrent™ Product recited in Life Technologies' quotation is for the sole purpose of identifying Ion Torrent™ Product, and any such description is not part of any contract between Life Technologies and buyer and does not constitute a warranty that Ion Torrent™ Product shall conform to that description. Any sample or model used in connection with Life Technologies' quotation is for illustrative purposes only, and is not part of any contract between Life Technologies and buyer and does not constitute a warranty that Ion Torrent™ Product will conform to the sample or model. No affirmation of fact or promise made by Life Technologies, whether or not in Life Technologies' quotation, shall constitute a warranty that Ion Torrent™ Product will conform to the affirmation or promise. Unless otherwise specified in writing in documentation shipped with Ion Torrent™ Product or otherwise agreed by Life Technologies in writing. Life Technologies does not provide service or support for custom products or other products made to buyer's specifications. THE WARRANTIES IDENTIFIED IN THIS CLAUSE ARE LIFE TECHNOLOGIES' SOLE AND EXCLUSIVE WARRANTIES WITH RESPECT TO Ion Torrent™ PRODUCT AND ARE IN LIEU OF ALL OTHER WARRANTIES, STATUTORY, EXPRESS OR IMPLIED, ALL OF WHICH OTHER WARRANTIES ARE EXPRESSLY DISCLAIMED, INCLUDING WITHOUT LIMITATION ANY IMPLIED WARRANTY OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, NON-INFRINGEMENT, OR REGARDING RESULTS OBTAINED THROUGH THE USE OF ANY PRODUCT (INCLUDING, WITHOUT LIMITATION, ANY CLAIM OF INACCURATE, INVALID OR INCOMPLETE RESULTS), WHETHER ARISING FROM A STATUTE OR OTHERWISE IN LAW OR FROM A COURSE OF PERFORMANCE, DEALING OR USAGE OF TRADE.

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support
- Order and web support
- Safety Data Sheets (SDSs; also known as MSDSs)

Additional product documentation, including user guides and Certificates of Analysis, are available by contacting Customer Support.

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are printed and shipped with the product.

Obtaining Certificates of Conformance

The Certificate of Conformance provides information on conformance testing of each instrument provided with the system. Certificates of Conformance are shipped with the instrument, and are also available by contacting Customer Support at **thermofisher.com/support**.

Glossary

Administrator

User with access sufficient to perform Manager and Operator functions, and also to view audit records as well as manage network settings, data storage, logs, reference sequence information, service records, and user access.

Assay

File of test parameters (such as QC thresholds, variant detection parameters, and hotspot region) created by the Manager or Administrator. The information in a Planned Run includes the selected Assay.

Barcode

A unique identifier on a reagent kit box or chip, read with a barcode scanner.

Barcode ID

A DNA sequence containing one of 16 unique sequences. Barcode IDs are adapters ligated to amplicons to identify the DNA in a particular sample library and track the DNA amplification products from that sample library throughout the Ion PGM™ Dx System run and analysis.

ISPs

Ion PGM™ Dx Ion Sphere™ Particles

Manager

User with access sufficient to perform Operator functions and also:

- Sign off a report, allowing the system to release it
- Create an Assay
- Create a new template file for an Assay (changing certain parameters)

Operator

User with access sufficient to:

- Add, import, or select a sample library
- Create and save a Planned Run
- Perform a Planned Run

Planned Run

A file in the Torrent Suite™ Dx Software that contains identification and expiration information for all run components, creates an auditable record, and allows the system to alert the user and stop the run if any components are incorrect or expired.

Sample

1. A sample of whole blood prepared according to laboratory requirements.
2. A Sample file containing all the identifiers of the blood sample, sample library, Library kit, and chemical barcode attached to the target sequence.

Sample library

A collection of target DNA prepared from the sample using the Ion PGM™ Dx Library Kit and barcoded with barcode adapters.

Short Code

The alphanumeric symbols represented by a particular barcode read with a barcode scanner. The Short Code is equivalent to the barcode for audit purposes.

Template

1. DNA template (chemical template): A segment of DNA that is replicated by DNA polymerase in PCR amplification.
2. Template kit: The set of reagents used to prepare DNA templates on Ion PGM™ Dx Ion Sphere™ Particles from a sample library. To be sequenced successfully, an ISP should be populated after templating with many copies of a single sequence of DNA.

iontorrent

Torrent Suite™ Dx Software 5.12.5

USER GUIDE

Publication Number MAN0018762

Revision A.0

CE IVD

For In Vitro Diagnostic Use.

ThermoFisher SCIENTIFIC

Life Technologies Holdings Pte Ltd |
Block 33 |
Marsiling Industrial Estate Road 3 |
#07-06, Singapore 739256

Life Technologies Corporation |
7335 Executive Way |
Frederick, MD 21704 | USA

EC REP Life Technologies Europe B.V.
Kwartsweg 2, 2665 NN Bleiswijk
The Netherlands

Products manufactured in Singapore:

Ion PGM™ Dx Instrument System
Ion PGM™ Dx Sequencer
Ion OneTouch™ Dx Instrument
Ion OneTouch™ ES Dx Instrument
Ion PGM™ Dx Chip Minifuge (120V or 230V)
Ion PGM™ Wireless Scanner
Ion Torrent™ Server
Torrent Suite™ Dx Software
Torrent Suite™ Assay Development Software
Veriti™ Dx 96-well Thermal Cycler, 0.2 mL

Products manufactured in Frederick:

Ion Torrent Dx FFPE Sample Preparation Kit
Ion PGM™ Dx Library Kit
Ion OneTouch™ Dx Template Kit
Ion PGM™ Dx Sequencing Kit
Ion 318™ Dx Chip
Ion OneTouch™ Rack Kit
DynaMag™ Dx 96-Well Plate Magnet
DynaMag™ Dx 16 2-mL Magnet

The information in this guide is subject to change without notice.

DISCLAIMER

TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0018762

Revision	Date	Description
A.0	3 September 2020	New user guide for Torrent Suite™ Dx Software 5.12.5. Changes from April 2020 submission: • **Repopulate QC** function was added. See "Repopulate QC (Administrator)" on page 46. • Additional troubleshooting topics were added for QC and run analysis failure conditions. • Information about a recommended antivirus software program was added. See "Antivirus software" on page 17.

Contents

About this guide

Purpose of the guide

This user guide provides instructions for using Torrent Suite™ Dx Software to plan and monitor sequencing runs and analyze data from the Ion PGM™ Dx System. This guide is used in conjunction with the following guides:

- The *Ion PGM™ Dx System User Guide* (Pub. No. MAN0018761) provides instructions for using system instruments and reagent kits for sample preparation, library preparation, template preparation, and sequencing.
- The *Ion PGM™ Dx System Performance Characteristics User Guide* (Pub. No. MAN0018763) provides summaries and tables of performance results for the Ion PGM™ Dx System based on testing of the system with DNA and RNA from formalin-fixed, paraffin-embedded (FFPE) tissue samples and genomic DNA (gDNA) from whole blood samples

1 Product information

Software description

Torrent Suite™ Dx Software is hosted on the Ion Torrent™ Server, part of the Ion PGM™ Dx System.

The software is used with the Ion PGM™ Dx Instrument System control software to support the system workflow from sample preparation through library preparation, template preparation, sequencing, and primary and secondary analysis.

Software component	Main functions
Torrent Suite™ Dx Software	• Generates base calls and quality values (primary analysis) • Filters and trims reads to ensure high sequence accuracy • Performs alignments and variant calling and generates reports based on user-selected assays (secondary analysis) • Hosts the Torrent Suite™ Dx Software web application
Ion OneTouch™ Dx Instrument or Ion Chef™ Instrument control software	• Provides control of the Ion OneTouch™ Dx Instrument via a touchscreen interface • Enables users to prepare template-positive Ion PGM™ Dx Ion Sphere™ Particles using the instrument • Enables users to perform instrument maintenance
Ion PGM™ Dx Sequencer control software	• Provides control of the Ion PGM™ Dx Sequencer via a touchscreen interface • Enables users to perform sequencing workflows using the instrument • Collects and compresses sequencing data, then transfers data to the Ion Torrent™ Server for primary and secondary analysis • Enables users to perform instrument maintenance

Features of Torrent Suite™ Dx Software

Torrent Suite™ Dx Software enables users to:

- Enter and manage information about samples and create libraries and library batches
- Plan runs to be executed on the Ion PGM™ Dx System instruments
- Monitor the progress of active instrument runs
- View and download run results and analysis reports
- View QC settings
- View workflow settings
- Manage user information and privileges
- View audit trails
- Configure archiving, reporting, and other administrative functions

Note: This guide provides instructions for using the Torrent Suite™ Dx Software in IVD Mode only.

About the Ion PGM™ Dx System

The Ion PGM™ Dx System is used for detection of human variant sequences from DNA from whole blood samples or RNA and DNA from FFPE tissue samples. Detectable variants include substitutions, insertions, and deletions.

The Ion PGM™ Dx System consists of the following:

- Ion OneTouch™ Dx Instrument
- Ion OneTouch™ ES Dx Instrument
- Ion OneTouch™ Rack Kit
- Ion PGM™ Dx Chip Minifuge
- Ion PGM™ Dx Sequencer
- Ion PGM™ Wireless Scanner
- DynaMag™ Dx Kit—Tube & Plate
- Ion Torrent™ Server
- Torrent Suite™ Dx Software

The Ion PGM™ Dx System is used in conjunction with the following kits:

- Ion PGM™ Dx Library Kit
- Ion OneTouch™ Dx Template Kit
- Ion PGM™ Dx Sequencing Kit
- Ion 318™ Dx Chip Kit

The system should be used only by professionals trained in laboratory techniques and procedures and in the use of the system.

Intended use

Ion PGM™ Dx Instrument System

The Ion PGM™ Dx Instrument System is composed of a sequencing instrument that measures the hydrogen ions that are generated during the incorporation of nucleotides in the DNA sequencing reaction, and the ancillary instrumentation necessary for sample processing. This instrument system is used in conjunction with the instrument-specific Ion PGM™ Dx Library Kit, Ion OneTouch™ Dx Template Kit, Ion PGM™ Dx Sequencing Kit, and Ion 318™ Dx Chip Kit, and data analysis software. The Ion PGM™ Dx Instrument System is intended for targeted sequencing of human genomic DNA (gDNA) from peripheral whole-blood samples and DNA and RNA extracted from formalin-fixed, paraffin-embedded (FFPE) samples.

The Ion PGM™ Dx Instrument System is not intended for whole genome or *de novo* sequencing.

Reagents

The Ion PGM™ Dx Library Kit, Ion OneTouch™ Dx Template Kit, Ion PGM™ Dx Sequencing Kit, and Ion 318™ Dx Chip Kit are a set of reagents and consumables used in the processing and targeted sequencing of human gDNA derived from peripheral whole blood samples and DNA and RNA extracted from FFPE samples. Nucleic acid reagent kits/tests that are manufactured and labeled for diagnostic purposes are required for the preparation of libraries targeting specific genomic regions.

The Ion PGM™ Dx Library Kit, Ion OneTouch™ Dx Template Kit, Ion PGM™ Dx Sequencing Kit, and Ion 318™ Dx Chip Kit are intended for use with the Ion PGM™ Dx System.

Indications for use

Same as intended use.

Special conditions statement

For *in vitro* diagnostic use. For prescription use only.

Special conditions statement for performance derived from DNA from whole blood

1. The Ion PGM™ Dx System has been validated to deliver the following using the System Variant Assay (SVA) panel:
    - Sequencing output >0.7 gigabases
    - Reads >4 million
    - Read length up to 200 base pairs
    - Mean Raw Read Accuracy of 99.0% when compared to hg19

Note:

- Mean Raw Read Accuracy is defined as the average raw accuracy across each individual base position in a read, where raw read accuracy is calculated as `100 * (1 - (sum(per base error)/sum(per base depth)))`
- The 632 primer pairs of the SVA panel are designed to amplify regions across 23 chromosomes in the two well-characterized cell lines. The regions were selected based on the presence of well characterized insertions/deletions (Indels) and single-nucleotide variant (SNV) positions. The amplicons produced range in size from 80 to 200 base pairs, with a GC content of 20–80%.

2. The system has been evaluated for the detection of single-nucleotide variants (SNVs) and insertions and deletions of various lengths on 23 chromosomes. The system identified 440 unique SNV positions in the SVA panel with 100% reproducibility.

   The following tables illustrate the lengths and locations of insertions and deletions in the SVA panel that were detected with 100% reproducibility.

Insertion length (base pairs)	Total number of distinct chromosomal locations	Total number of unique chromosomes
1	14	10
2	4	4
3	5	4
4	5	4

Deletion length (base pairs)	Total number of distinct chromosomal locations	Total number of unique chromosomes
1	15	11
2	10	6
3	3	3
4–14[1]	7	7

[1] Deletions of ≥4 bp have been grouped for clarity

3. The system may exhibit a limitation in detecting one-base insertions or deletions in homoploymer tracts (e.g., polyA). Variants in homopolymer runs exceeding 8 bases are called as no calls in the VCF file.
4. The system is designed to deliver qualitative (i.e., genotype) results.
5. As with any hybridization-based workflow, underlying polymorphisms or mutations in primer-binding regions can affect the regions being sequenced and, consequently, the calls made.
6. The recommended minimal coverage per amplicon needed for accurate variant calling for germline DNA is >30X.
7. Special instrument requirements for the Ion PGM™ Dx Library Kit, Ion OneTouch™ Dx Template Kit, Ion PGM™ Dx Sequencing Kit, and Ion 318™ Dx Chip Kit: For use with the Ion PGM™ Dx System.

Special conditions statement for performance derived from a representative assay using RNA and DNA from FFPE samples

1. The Ion PGM™ Dx System has been validated to deliver the following using a representative assay:
   - Sequencing output >0.7 gigabases
   - Reads >3 million
   - Read length up to 141 base pairs
2. A representative assay consisting of two sets of primer panels was used to detect DNA and RNA variants in key regions of cancer-related genes. The Ion PGM™ Dx System has been evaluated for the detection of SNVs, multi-nucleotide variants (MNVs), and deletions of various lengths in FFPE tissue samples using this representative assay. The types and numbers of variants detected by the assay are listed below.

Type of variant	Number of variants detected with a representative assay	Number of samples tested for detection by sample type		
		Plasmid/FFPE Sample Blend	FFPE Cell Line or FFPE Cell Line Blend	FFPE Clinical Sample
MNV	9	9	0	2
SNV	326	329	8	113
3-bp deletion	4	6	0	0
6-bp deletion	4	8	0	0
9-bp deletion	4	8	0	1
12-bp deletion	7	7	0	0
15-bp deletion	7	10	3	23
18-bp deletion	7	8	0	11
ROS1 fusion	34	0	1	21

3. The following studies were used to evaluate the performance of the Ion PGM™ Dx System using a representative assay:
   - Accuracy
   - Sample reproducibility
   - Assay reproducibility
   - Tissue input
   - DNA and RNA input
   - Interfering substances
4. The system is designed to deliver qualitative results.
5. As with any hybridization-based workflow, underlying polymorphisms or mutations in primer-binding regions can affect the regions being sequenced and, consequently, the ability to make calls.

6. The minimal coverage required to call an SNV, MNV or deletion variant is ≥347X. The minimal coverage required to call a fusion variant is ≥41X.
7. Special instrument requirements for the Ion PGM™ Dx Library Kit, Ion OneTouch™ Dx Template Kit, Ion PGM™ Dx Sequencing Kit, and Ion 318™ Dx Chip Kit: For use with the Ion PGM™ Dx System.

Theory of operation

For a complete description of the Theory of Operation of the system, see the *Ion PGM™ Dx System User Guide*.

Limitations and Precautions

Torrent Suite™ Dx Software has the following limitations:

- User names can contain alphanumeric characters and underscores, periods, and hyphens. Passwords can only contain alphanumeric characters.
- Torrent Suite™ Dx Software is intended to be used only with the Ion PGM™ Dx System and does not accept any software plugins.
- Before implementing the Ion PGM™ Dx System in your laboratory, perform quality assurance and quality control for your sequencing panel using known, highly confident genotype samples. Such samples can be obtained from the Coriell Cell Repository.

Limitations for FFPE samples

The Ion PGM™ Dx System has been validated to detect SNVs, MNVs, and deletions in DNA and fusions in RNA in non-small cell lung cancer (NSCLC) FFPE tumor slide specimens. Results presented for FFPE samples were obtained using the Ion PGM™ Dx System and associated reagents with a representative assay, and are provided for informational purposes only. The validation testing with the representative assay only establishes the instrument's general capabilities and does not establish the instrument's capabilities or suitability with respect to any specific claims. All diagnostic tests developed for use on this instrument require full validation for all aspects of performance.

Software compatibility and requirements

The procedures in this guide are designed for use with Torrent Suite™ Dx Software version 5.12.5 or later. To view the current software version, sign in to the software as an Administrator, click the **Settings (⚙)** tab, select **Configuration**, then click the **Software Updates** tab. Version-specific information is provided in the software release notes for the version of the software you are using.

Torrent Suite™ Dx Software is supported on Google™ Chrome™ browser version 64 and later, and is best viewed with 1440 × 900 screen resolution. It has not been tested with other browsers.

The Ion Torrent™ Server operating system is Ubuntu™ 18.04 LTS.

Instrument components and accessories

The following components are included as a part of the Ion PGM™ Dx Instrument System bundle (Cat. no. A25511):

✓	Components	Catalog no.
	Ion Torrent™ Server (Torrent Suite™ Dx Software installed separately)	A28552
	Ion PGM™ Wireless Scanner	A25486

2 Get started with Torrent Suite™ Dx Software

User-access levels

Users at this level...	Can...
Operator	• Add and import samples • Prepare library batches • Create and save Planned Runs • Execute Planned Runs • Monitor runs • View results and reports
Manager	Operator functions plus: • Create and edit sample attributes • Delete Planned Runs • Approve reports • Manage reference sequences and panel, hotspot, and other sequence files • Access services information
Administrator	Operator and Manager functions plus: • View, export, and print audit records • Configure network settings • View and manage software updates • Configure data archive and storage settings • Manage instrument and software log files • Add and manage users

Ion PGM™ Dx System synchronization requirement

Allow up to 20 minutes for synchronization with the Ion Torrent™ Server after you power on any of the following instruments or the server itself:

- Ion PGM™ Dx Sequencer
- Ion OneTouch™ Dx Instrument

If you attempt to use an instrument before synchronization is complete, it will generate an alarm.

Network and password security requirements

Network configuration and security

The network configuration and security settings of your laboratory or facility (such as firewalls, anti-virus software, network passwords) are the sole responsibility of your facility administrator, IT, and security personnel. This product does not provide any network or security configuration files, utilities, or instructions.

If external or network drives are connected to the software, it is the responsibility of your IT personnel to ensure that such drives are configured and secured correctly to prevent data corruption or loss. It is the responsibility of your facility administrator, IT, and security personnel to prevent the use of any unsecured ports (such as USB, Ethernet) and ensure that the system security is maintained.

Password security

Thermo Fisher Scientific strongly recommends that you maintain unique passwords for all accounts in use on this product. All passwords should be reset upon first sign in to the product. Change passwords according to your organization's password policy.

It is the sole responsibility of your IT personnel to develop and enforce secure use of passwords.

Antivirus software

Thermo Fisher Scientific has tested ClamAV antivirus software from Ubuntu™ on the Ion Torrent™ Server and shown that it does not interfere with the assay or Torrent Suite™ Dx Software. For more information, visit **help.ubuntu.com/community/ClamAV**.

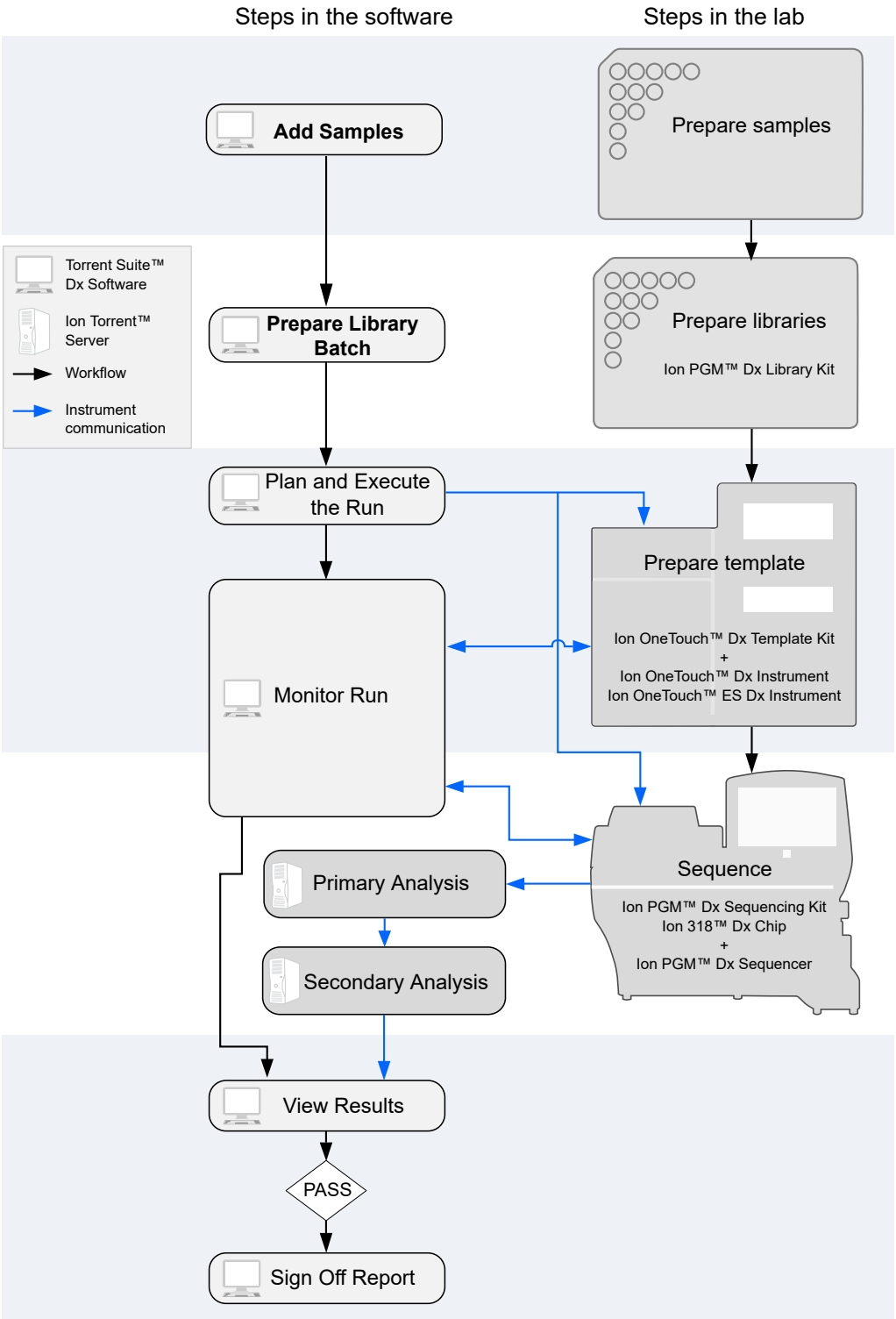
System tracking

The system tracks and checks user, sample, workflow, reagents and QC metrics for auditable records. If the software detects an error at any step—for example, a scanned barcode is inconsistent with the information given for the run—the software alerts the user and does not proceed with the run.

Reagent management

Users should put in place a reagent management system to inventory and track reagent use. Proper use of a reagent management system promotes regulatory compliance and ensures optimal use of kits, chips, and reagents.

System workflow diagram



Installation and verification run

Do not attempt to power on any Ion PGM™ Dx System instruments or sign in to Torrent Suite™ Dx Software until a field service engineer (FSE) has installed and performed a performance qualification (PQ) verification run and provided a basic system overview.

The software and all connected instruments are locked until a verification run has been performed and the report is signed by the FSE.

Request and sign in to a new account

Only administrator-level users can create user accounts.

After account creation, the Ion PGM™ Dx System automatically sends an email to the new user with the user name and password information.

- To request a new account, contact your local administrator.
- To sign in to a new account for the first time:
  a. Go to the Torrent Suite™ Dx Software home page, then enter your user name in the **Username** field.
  b. Enter the system-generated password in the **Password** field.
  c. Click **Enter**, or click **Sign In**.
  d. Click **Accept** to accept the End User Software License Agreement.
  e. In the **Change Password** screen, enter your temporary password in the **Current Password** field. Type a new password in the **New Password** field, then confirm the password.
     – Passwords must be between 6 and 10 characters.
     – Passwords must contain at least one alphabetic character (aA–zZ).
     – Passwords must contain at least one numeric character (0–9).
     – Passwords must contain only alphanumeric characters (0–9, aA–zZ), no spaces or special characters.
     – Passwords are case-sensitive.
  f. Click **Change**.

Switch software mode *(administrator only)*

Administrator-level users can switch between two separate software modes on the Ion Torrent™ Server:

- Assay Development Mode (Torrent Suite™ Assay Development Software)
- IVD mode (Torrent Suite™ Dx Software)

Each software mode has separate user accounts, and you must set up accounts in both to be able to switch between them.

Note: Switching between modes takes several minutes. You cannot switch while an instrument run or data analysis is in progress.

To switch:

1. Click **Sign Out** to sign off of the software you are in.
2. At the bottom of the home screen, under **Mode Switch**, select the mode that you want to switch to.
3. Enter your **Username** and **Password** for the software that you were signed in to, then click **Sign In** and confirm the switch.

   During the switch, a status message appears. If the screen does not automatically refresh in 10 minutes, refresh your browser.
4. Enter your **Username** and **Password** for the new software, then click **Sign In**.

Sign in

To sign in to the software:

1. Open the software home page.
2. Under **Mode Switch** at the bottom of the page, verify that **IVD Mode** is selected.

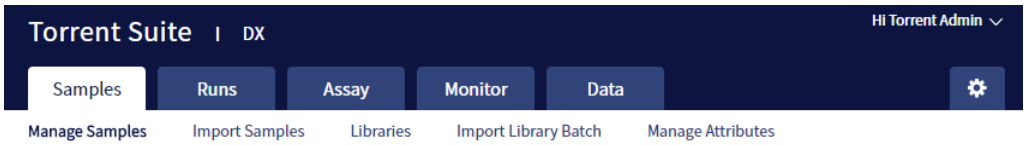
   Note: If you are not in **IVD Mode**, contact a system administrator to switch modes.
3. Select your preferred language from the dropdown list in the upper right corner of the page.
4. Enter your user name and password, then press **Enter** or click **Sign In**.

   IMPORTANT! Your user name and password must be unique and not shared with other users.

   The software opens to the **Samples** tab and the **Manage Samples** screen.

User interface

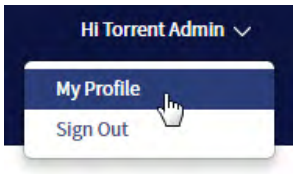
Torrent Suite™ Dx Software has a browser-based interface with five tabs containing the main functions of the software:



- **Samples** tab—Create and manage samples and libraries; plan new runs from libraries.
- **Runs** tab—Create and manage Planned Runs (executable instructions for individual runs). The system uses the Planned Run to verify at each step of the run that the correct kit is used to perform the correct assay on the correct sample.
- **Assay** tab—Manage assays (protocol specifications for templating, sequencing, and data analysis).
- **Monitor** tab—View the status of your system instrument(s) and current runs. Near real-time information is provided on your runs, so that you know early on about any instrument problems.
- **Data** tab—View summaries of completed runs, and detailed run reports; review the run plan settings; download or print output files and run reports.

In addition to the main tabs, the ⚙ **(Settings)** tab enables access to audit records and log files, server configuration and data management settings, reference file information, and user accounts.

Click **My Profile** from the user name dropdown list in the upper right corner to view your account information and change your password and email address. Click **Sign Out** to sign out of the software.



About screen

Under the ⚙ tab, the **About** screen contains following links:

- **Support Contact Information** is a link to **thermofisher.com/technical-resources**, where you can access technical support and product information.
- **Instrument Diagnostics** opens a page that lists the active sequencers connected to the Ion Torrent™ Server. For each sequencer, you can click on additional links to download or open the following files:
  - Diagnostic archive file (ZIP file)
  - Init.log file
  - Installation Acceptance Report (PDF file)
- **End User License Agreement** opens the End User License Agreement (EULA) in PDF format.

3 Enter sample and library information

Sample and library information is entered into Torrent Suite™ Dx Software under the **Samples** tab. This information is tracked by the software throughout the entire system workflow.

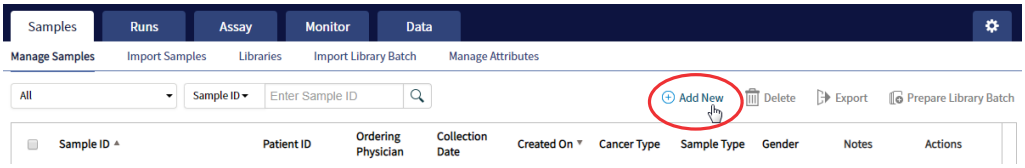
In the **Samples** tab, you can:

- Create, import, and manage samples and their attributes
- Enter the barcode on the kit used to extract each sample
- Prepare libraries and library batches

Add or import samples

Add a new sample

1. Under the **Samples** tab, in the **Manage Samples** screen, click ⊕ **Add New**.



2. Complete the **Add New Sample** dialog box. Fields identified with an asterisk (*) are required. If no information is available, substitute dummy data to complete the required fields.

Field	Description
Sample ID*	A unique identifier representing the sample, containing only alphanumeric characters (0–9 and A to Z), full stops/periods (.), underscores (_), or hyphens (-). The Sample ID cannot contain spaces and is limited to a maximum of 20 characters. After a Sample ID is entered into the system, it cannot be edited. It can be deleted unless it has already been used in a library. The software checks all Sample IDs entered or imported to prevent duplication and returns an error message if a non-unique Sample ID is detected.

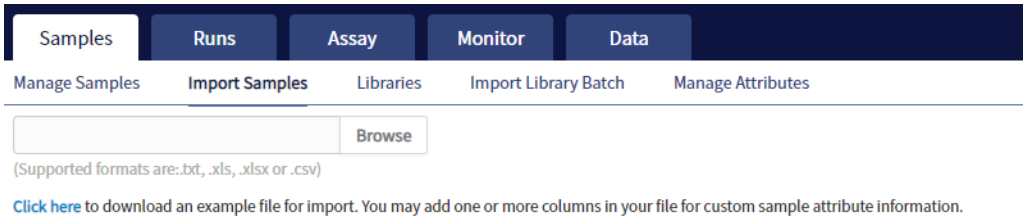
(continued)

Field	Description
Patient ID*	An identifier representing the patient. This field accepts all characters including spaces.
Date of Birth*	The patient's date of birth. Click the button to select the date in the correct format.
Ordering Physician*	The name of the ordering physician. This field accepts all characters including spaces.
Collection Date*	The date the sample was collected from the patient. Click the button to select the date in the correct format.
Sample Source	Open-entry field that accepts all characters, including spaces. **Example entry:** Name of the clinic or hospital ordering the test.
Sample Condition*	Open-entry field that accepts all characters, including spaces. **Example entry:** FFPE surgical resection.
Sample Type*	Open-entry field that accepts all characters, including spaces. **Example entry:** Lung tumor, invasive adenocarcinoma.
Gender*	The biological gender of the sample. This must be Male, Female, or Unknown.
Cancer Type*	Select the cancer type of the sample from the dropdown list.
%Cellularity	The percentage of tumor cellularity in the sample.
%Necrosis	The percentage of cellular necrosis in the sample.
Reference Interval	A normal range of measure for the sample.
Notes	An open-entry field.

3. Click **Save**.
   The sample is listed in the **Manage Samples** screen.

Import samples

Under the **Samples** tab in the **Import Samples** screen, you can import sample data in the form of a TXT, XLS, XLSX, or CSV file. The import file includes the same sample attributes that are listed in the **Add New Sample** dialog box.



1. In the **Import Samples** screen, below the **Browse** field, click **Click here** to download a Microsoft™ Excel™ template file.

   Note: The template file contains default sample attributes as columns. If additional custom sample attributes have been configured in the software, add these attributes as columns to the template file.

2. In the template file, fill in the information for each sample, one sample per row. See "Predefined sample attributes" on page 24 for more information.

3. Save the file.

4. Click **Browse**, navigate to the saved file, then select it.

5. Click **Import**.
   A progress bar followed by an import report displays. If the import process fails, an error message indicates the reason for failure (for example, an invalid character was used). For additional troubleshooting, see "Batch sample import fails" on page 93.

6. Click **Manage Samples** to return to the sample list. Successfully imported samples are listed.

Predefined sample attributes

The software has the following predefined sample attributes, which are listed in the **Add New Sample** dialog box and in the template file for importing samples.

- Sample ID*
- Patient ID*
- Date Of Birth*
- Ordering Physician*
- Collection Date*
- Sample Source
- Sample Condition*
- Sample Type*
- Gender*
- Cancer Type*
- %Cellularity
- %Necrosis
- Reference Interval
- Notes

*Indicates a field required to be filled in during sample creation.

Predefined attributes are locked and cannot be edited. You can create and manage custom sample attributes using the tools in the **Manage Attributes** screen.

Note: LIMS users must create custom attributes before importing sample and Planned Run information from LIMS for the attributes to be propagated through to output files. The software ignores all input file content that is not a recognized attribute.

Create a new sample attribute

1. Under the **Samples** tab in the **Manage Attributes** screen, click **Add New Attribute**.
2. Enter an attribute name in the dialog box.
   Attribute names are limited to ≤20 alphanumeric characters (0–9 and A to Z), full stops/periods (.), underscores (_), or hyphens (-).
3. In the **Data Type** dropdown list, specify whether the attribute is a text or number.
4. To require users to select the new attribute when adding or importing samples, select the **Required** checkbox.
5. Click **Save**.
   The new sample attribute is listed in the **Attribute Name** column and is available when you add a new sample. After an attribute is created, it cannot be deleted. To remove an attribute from use, see "Obsolete an attribute" on page 25.

Note:
- The new attribute will appear in the **Edit Sample** dialog box, even for samples that were created before the new attribute was created. If the new attribute is required, a valid entry must be entered into the **Edit Sample** dialog box to save the changes.
- Samples cannot be edited after they have been added to a library.

Obsolete an attribute

Under the **Samples** tab in the **Manage Attributes** screen, you can obsolete user-created sample attributes to remove them from use in the software. Obsoleted attributes can be reactivated, and a record of their use is maintained in the audit trail of samples that are created using that attribute.

Note: You can only obsolete user-created attributes, not system-installed attributes.

- To obsolete an attribute, click **Obsolete** in the **Actions** column, then confirm the action. **Reactivate** replaces **Obsolete** in the **Actions** column.
- To reactivate an attribute, click **Reactivate** in the **Actions** column.

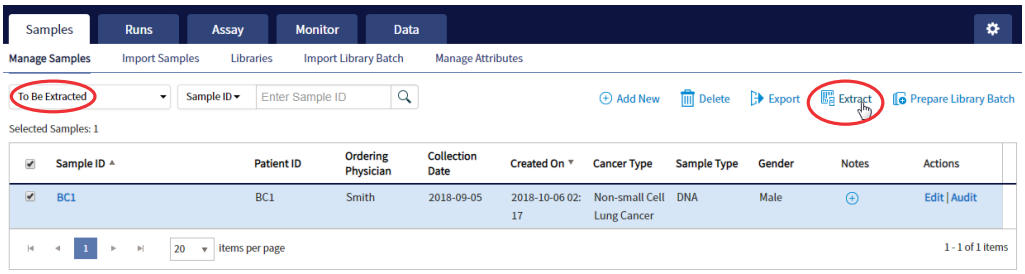
Note: All active sample attributes are listed in the **Add New Sample** dialog box.

Enter the sample extraction kit barcode

Under the **Samples** tab, in the **Manage Samples** screen, scan the barcode of the sample extraction kit that is used in the extraction process for a particular sample. This barcode is saved with the sample and can be viewed by clicking the Sample ID.

1. Above the samples list, select **To Be Extracted** from the **Filter Samples by...** dropdown list to display only those samples that do not have a kit barcode that is associated with them.

2. Select the checkbox of the sample to be extracted. Select multiple samples if you are using the same kit to process them.



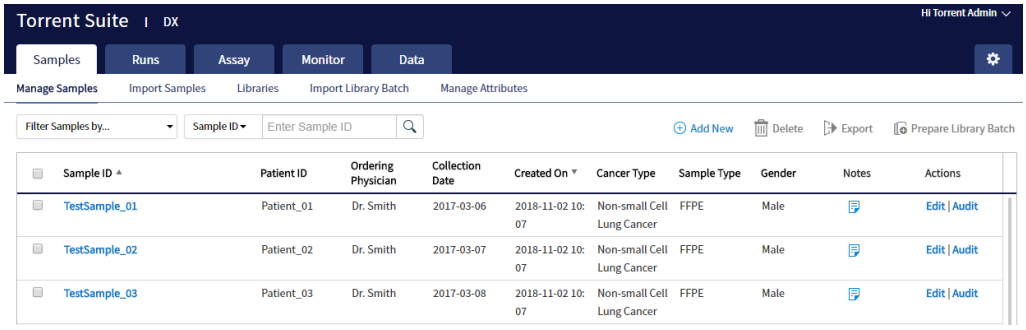
3. Click **Extract**. In the dialog box, scan the barcode that is printed on the box of the sample extraction kit.

   IMPORTANT! Check the expiration date on the box. If the kit is expired, select another kit.

4. Click **Save**.
   The sample is no longer listed in the **To Be Extracted** list.

Manage samples

You can find tools for creating, searching, sorting, editing, deleting, exporting samples, and viewing the sample history under the **Samples** tab in the **Manage Samples** screen.



Search samples

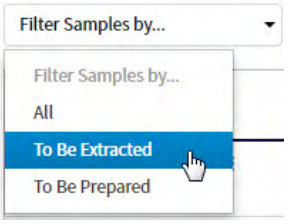
Under the **Samples** tab, in the **Manage Samples** screen:

1. Enter the full or partial sample ID into the **Enter Sample ID** field.
2. Click 🔍.
   The sample or samples matching the search parameters are listed.
3. Click × to return to the complete list of samples.

Sort samples

Under the **Samples** tab in the **Manage Samples** screen, the list of samples is displayed with the most recently created sample on top by default. To return to the default display, click **Manage Samples**.

- To list only those samples that have not been extracted, click **To Be Extracted** from the **Filter Samples by** dropdown list.



- To list only those samples that have not been prepared as a library, click **To Be Prepared**.
- To list all samples, click **All**.
- To sort the list:
  a. Click a column header to sort the list by the entries in that column.
  b. Click the column header again to reverse the order.
  c. Click **Manage Samples** to return to the default order (most recently created on top).

Export and print samples

The **Export** function generates a XLS file of the sample details.

1. Under the **Samples** tab in the **Manage Samples** screen, in the **Sample ID** column, select the checkbox next to each sample to be exported. Select the checkbox above the column to select all of the samples.
2. Click **Export**.
   An XLS file is generated. Depending on your browser settings, the software automatically downloads the file or prompts you to open or save the file.
3. Open the XLS file in an appropriate viewer to print.

View notes or add a note to a sample

1. Click in the **Notes** column for a sample to view notes that have been added for the sample.
2. To add a new note, click **Add Note**. Enter the note in the test field, then click **Save**.
3. Click **Close** to return to the **Manage Samples** screen.

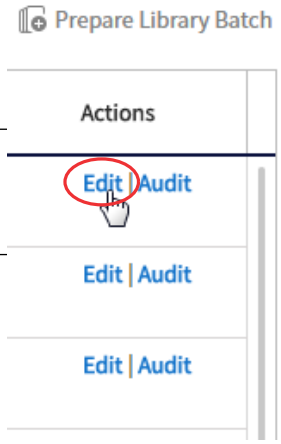
Edit sample

Under the **Samples** tab, in the **Manage Samples** screen, samples that can be edited are identified by the **Edit** link in the **Actions** column.

A sample can be edited at any point before execution of a Planned Run containing that sample, after which the **Edit** link is no longer available. You can edit sample information after a run using the **Edit and Amend Report** command (for more information, see "Edit a sample and amend the report after a run").

1. Click **Edit** in the row of the sample to be edited.
2. In the **Edit Sample** dialog box, click a field to edit the information.

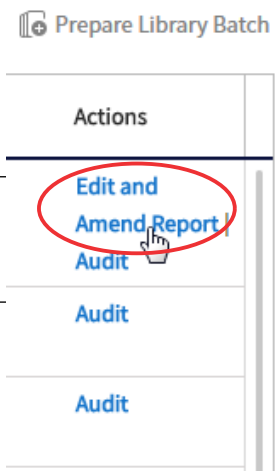
   Note: The Sample ID cannot be edited. For a description of the fields, see "Add a new sample" on page 22.
3. Click **Save**.



Edit a sample and amend the report after a run

After a sequencing run and its analysis have completed, you can edit a sample and amend the Test and Lab Reports for up to 30 days. After 30 days, only the audit link displays. Editing a sample after a run triggers an automatic update of the reports and other files associated with the sample. Under the **Samples** tab, in the **Manage Samples** screen, editable samples are identified by the **Edit and Amend Report** link for the sample in the **Actions** column.

1. To edit a sample after a run, click **Edit and Amend Report** in the row of the sample to be edited.

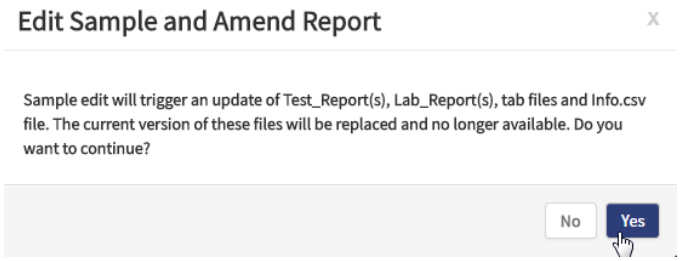


2. In the **Edit Sample and Amend Report** dialog box, click a field to edit the information.

   Note: The Sample ID cannot be edited. For a description of the fields, see "Add a new sample" on page 22.

3. Click **Save**.

4. In the dialog box that appears, click **Yes** to confirm the edit and continue.



The sample information is edited and associated reports and files are updated.

Review sample history

The entire history of a sample is available for review, export, or printing.

1. Under the **Samples** tab in the **Manage Samples** screen, click **Audit** for the sample of interest in the **Actions** column.
   The **Audit Trail** dialog box opens, listing each event that modified the selected sample.

2. Click **(Details)** under the **Record** header to view the details of the change made.

3. In the **Audit Record Details** dialog box, click **Export** to export a PDF of the record.

Delete samples

Under the **Samples** tab, in the **Manage Samples** screen, you can delete samples that have not been assigned to a library.

Note: Samples assigned to libraries are locked and cannot be edited or deleted. Locked samples only display **Audit** under the **Sample ID**.

1. Select the sample or samples to be deleted by selecting the checkbox adjacent to the **Sample ID**. Select all the samples on the page by selecting the checkbox above the column.
2. Click **Delete**.
   The **Delete Sample** dialog box opens with the message "Are you sure you want to delete selected sample(s)?"
3. Click **Yes** to delete the selected samples. Click **No** to return without deleting.

Prepare libraries and library batches

In Torrent Suite™ Dx Software, samples that are entered into the software are placed in library batches for processing and tracking. A library batch consists of a group of libraries that are prepared at the same time.

Note:

- Each library in a library batch must have a unique library name. When combining libraries in the same run, each must also have a unique barcode.
- Control libraries must be included in the same library batch as the sample library they control for.
- Fields identified with a red asterisk (*) are required.

1. Sign in to Torrent Suite™ Dx Software.
2. Under the **Samples** tab, in the **Manage Samples** screen, click **To Be Prepared** to display only those samples that have not been placed in a library batch.

   IMPORTANT! Samples that have not been queued for extraction in the software also appear on this tab. Ensure that the samples have been queued for extraction before queueing them for library batch preparation.
3. Select up to 6 samples in the list, then click **Prepare Library Batch**.
4. In the **Prepare Library Batch** dialog box, select the appropriate assay from the **Select Assay** dropdown list. The assay determines specific parameters of the run, including any required controls and post-run data analysis settings.
5. In the following screen, enter a unique identifier for the library batch in the **Library Batch ID** field. Library Batch IDs can only contain alphanumeric characters (0–9 and A to Z), full stop/period (.), underscore (_), and hyphen (-).

6. Click inside the **Library Kit Barcode** field, then scan the barcode from the Ion PGM™ Dx Library Reagents box.

   IMPORTANT! Be sure to scan the barcode from the actual Ion PGM™ Dx Library Reagents box that is used in the run.

7. Type a unique library name for each DNA and RNA library in the appropriate field. Library names can only contain alphanumeric characters (0–9 and A to Z), full stop/period (.), underscore (_), and hyphen (-).

   Note: If your assay requires specific controls, they are automatically listed in the dialog box. These controls each require a unique Barcode ID within the library batch but do not require library names.

8. Select the Barcode ID of the adapter used to prepare each library. Swap the default barcodes in the dialog box between DNA and RNA using the DNA ⇄ RNA button.

   Note: Each library in a library batch must have a different Barcode ID. When preparing the physical libraries, we recommend swapping barcodes between DNA and RNA libraries in consecutive sequencing runs to prevent carryover contamination.

   IMPORTANT! Be careful to ensure that the actual barcodes used to create the libraries match the barcodes that are entered in the **Prepare Library Batch** dialog.

9. Enter the **Input Quantity** for each library.

10. Click **Save** to save your selections and close the dialog box.
    The **Libraries** screen opens, listing the libraries that you created. Libraries that are prepared in the same batch have the same **Library Batch ID**.

Alternating barcodes

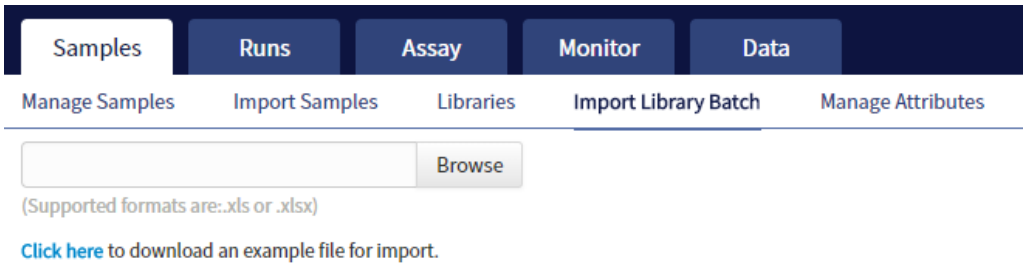
When preparing libraries, we recommend swapping barcodes between DNA and RNA libraries in consecutive sequencing runs to prevent carryover contamination. The following table provides an example of swapping barcodes between runs.

IMPORTANT! Be careful to confirm that the barcodes used to create the libraries match the barcodes entered in the **Prepare Library Batch** dialog box.

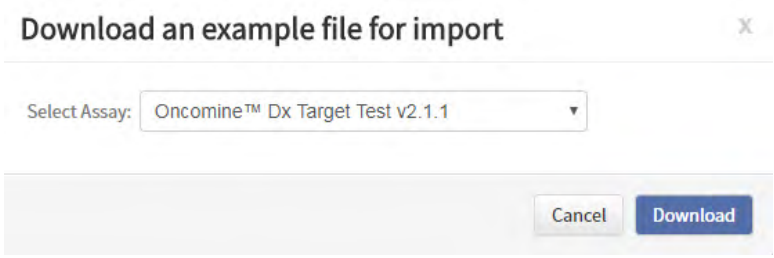
Library type	System Run 1 barcode usage		System Run 2 barcode usage	
	DNA	RNA	DNA	RNA
Positive control	1	9	9	1
Sample	2	10	10	2
Sample	3	11	11	3
Sample	4	12	12	4
Sample	5	13	13	5
Sample	6	14	14	6
Sample	7	15	15	7
No-template control (NTC)	8	16	16	8

Import a library batch

Under the **Samples** tab in the **Import Library Batch** screen, you can import library batch information in the form of an XLS or XLSX file. The import file must include all of the library and kit information that you enter in the **Prepare Library Batch** dialog box.



1. In the **Import Library Batch** screen, below the **Browse** field, click **Click here** to download an example file for import.
2. In the dialog box that opens, select the assay that you are using from the dropdown list, then click **Download**.



   The assay name is auto-populated in the Microsoft™ Excel™ template file that downloads to your drive.

3. In the template file, enter or confirm the library batch information.
   - Assay used (auto-populated)
   - Unique Library Batch ID
   - Library kit, control kit, and panel kit barcodes
   - Sample IDs
   - Library names
   - Barcode IDs used for each library and control
   - Nucleic acid type (DNA or RNA)
   - Library input quantity
4. Save the file.
5. Click **Browse**, navigate to the saved file, then select it.
6. Click **Import**.
   A progress bar followed by an import report displays. If the import process fails, an error message indicates the reason for failure (for example, an invalid character was used). For additional troubleshooting, see "Library batch import fails" on page 94.
7. Click **Libraries** to return to the library batch screen. Your successfully imported library batch is listed.

Manage libraries and library batches

Use the following tools in the **Libraries** screen under the **Samples** tab to perform the specified actions.

To...	Do the following...
View more library information	Hover over a name in the library list.
View all libraries	Click **All** in the **Filter Libraries by** dropdown list.
View libraries that are not in a Planned Run	Click **To Be Planned** in the **Filter Libraries by** dropdown list.

(continued)

To...	Do the following...
Search the list	Select search by **Library Name** or **Library Batch ID**, type a full or partial Library Name or Library Batch ID in the search field above the list, then click 🔍. Click **×** to clear the search criteria and display the complete list.
Sort the list	Click a column header to sort the list by the information in that column.
View multiple pages of libraries	Click the scroll buttons below the list.
Delete libraries	Select the checkbox next to the library or related libraries (DNA and RNA), then click 🗑 **Delete**. **Note:** You can only delete a library if none of the libraries in the same library batch have been assigned to a Planned Run.
Edit a library batch	Click **Edit** for the library batch in the **Actions** column, and modify the information in the dialog box.
View or export the audit trail of a library batch	1. Click **Audit** for the library batch in the **Actions** column. The **Audit Trail** dialog box opens, displaying the user, action, and time of each action performed on the batch. 2. To view the action details, click 📄 in the **Record** column of the **Audit Trail** dialog box. The **Audit Record Details** dialog box opens. 3. To export the record as a PDF, click **Export** in the **Audit Record Details** dialog box.
Add or modify notes for a library	Click ⊕ in the **Notes** column.

LIMS integration

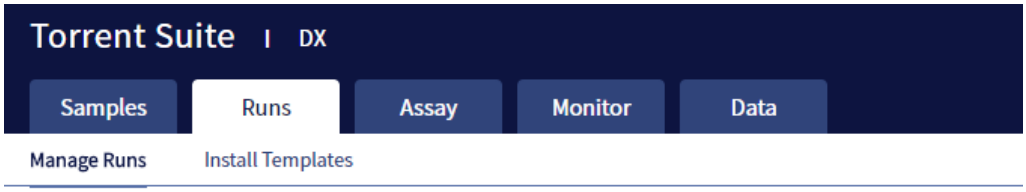
The Torrent Suite™ Dx Software integrates with laboratory information management systems (LIMS) to facilitate Sample, Library and Planned Run creation. The interaction is through LIMS calling a set of RESTful APIs provided by the Torrent Suite™ Dx Software. See Appendix C, "LIMS Integration" for more information and example request and response .xml files.

API	Function	API URL
Sample and Library Batch Creation	To add Sample and sample information, and create a Library Batch with the associated libraries. After the API is called, the software creates Sample, Library Prep, and Library Batch in the database and returns the object IDs.	http://{Torrent Suite IP address}/ir/lims/create-library-batch
Plan Run Creation	To add Planned Run Name, Assay information, Library Batch ID, and Library Prep ID. After the API is called, the software responds with the Run Short Code.	http://{Torrent Suite IP address}/ir/lims/create-plan

(continued)

API	Function	API URL
Analysis Status Check	To query the status of a Planned Run. After the API is called, the software identifies the associated Libraries and sends the response containing output folder path and library prep analysis status.	http://{Torrent Suite IP address}/ir/lims/get-plan-status?plan_name={plan name}
Barcode Set Retrieval	To get a list of Barcode Sets and the barcode names, IDs, and sequences associated with each set.	http://{Torrent Suite IP address}/ir/lims/get-barcode-list
Reporting Template	To get a list of Report templates.	http://{Torrent Suite IP address}/ir/lims/get-reporting-template-list

4 Create and manage Planned Runs



Planned Runs contain all the sample, library, and assay information required to perform a run on template preparation and sequencing instruments. The Planned Run information is tracked by the system from template preparation through sequencing, data analysis, and final results.

Prerequisites to create a Planned Run

Before creating a Planned Run, check that:

- Sample information is correctly entered
- Library batches have been prepared
- Each library has been assigned a unique Barcode ID

The software returns an error message when any of these conditions are not met when creating a Planned Run.

The system identifies and tracks kit components and chips by barcodes. The system includes a cordless barcode reader and a barcode reader that is attached to the Ion PGM™ Dx Sequencer.

Note: All kits and chips that are used in a diagnostic assay must be uniquely identified, and the identification must be stored so that the record can be audited.

Create a Planned Run

Libraries that are ready to be entered into a Planned Run are listed under the **Samples** tab in the **Libraries** screen.

Note: You can also plan a run from the **Runs** tab in the **Planned Runs** screen (click ⊕ **Add New**).

1. Sign in to Torrent Suite™ Dx Software.
2. In the **Libraries** screen, select the library or libraries to be run by selecting checkboxes in the list. To view only those libraries that have not yet been added to a Planned Run, select **To Be Planned** in the **Filter Libraries by** dropdown list.

   Note:
   - Libraries prepared with the same assay in the same or different library batches can be combined and run together, as long as the libraries have unique Barcode IDs and the assays do not include control libraries.
   - To plan a run with the Oncomine™ Dx Target Test assay, a report template must be created and associated with the assay by an Administrator or Manager. Under the **Assay** tab, go to **Presets ▸ Report Templates** to add a new report template.
   - Up to 16 libraries (including controls) can be sequenced in a single run.
   - If libraries need to be rerun due to a run failure, they can be added to a new Planned Run.
3. Click **Plan a Run**.
4. In the **Add New Plan** dialog box, enter a name for the run, then select the appropriate report template.
5. To remove libraries from the run, select the appropriate checkboxes, then click **Remove**. To add libraries, click ⊕ **Add more Libraries**, then select them from the **Add Libraries** dialog box.

   Any added libraries must be from the same library batch and have unique library names and Barcode IDs.
6. Click **Save**.

   The new Planned Run is automatically assigned a Run Short Code and is displayed at the top of the list under the **Runs** tab in the **Manage Runs** screen.

Execute a Planned Run

Planned Runs are listed under the **Runs** tab in the **Manage Runs** screen. Runs that are ready to be performed have the **Execute** command available in the **Actions** column.

Executing a Planned Run in the software queues the run for initiation on the Ion OneTouch™ Dx Instrument or Ion Chef™ Instrument. After a Planned Run has been queued for execution, the operator should immediately start template preparation.

In the **Manage Runs** screen:

1. Click **To Be Started** in the **Filter Run by...** dropdown list to limit the list of Planned Runs to only those runs yet to be started.
2. Find the Planned Run in the list, then under the **Actions** header, click **Execute**.

   The **Execute Planned Run** dialog box opens.
3. In the **Tube Label** field, enter the text that is used to label the tubes that contain the final combined libraries.

   The tube label text can be any combination of letters and numbers. The system uses this text to track the sample throughout the run, so be careful to label each tube legibly at the points noted in the procedure. The software does not allow use of the same Tube Label text within 7 days.
4. Click the **Template Prep Kit barcode** field, then scan the barcode from the Ion OneTouch™ Dx Template Reagents box.

   IMPORTANT! Ensure that you scan the barcode from the actual reagents box that is used in the run.

5. Click **Save** to save your changes.
   The **Review Planned Run** dialog box opens.

6. Write down the **Run Short Code** and/or click **Print** to print the scannable barcode. The code must be entered into the Ion OneTouch™ Dx Instrument or Ion Chef™ Instrument and Ion PGM™ Dx Sequencer for tracking and verification before the start of the instrument run.

7. Click **Close** to close the dialog box and send the run to the instrument.

Note: The last 5 executed Planned Runs are listed under the **Monitor** tab in the software.

Manage Planned Runs

Under the **Runs** tab, in the **Planned Runs** screen, a list of all Planned Runs is displayed, with the most recently created run on top. In this screen, you can:

- Sort and search the list of Planned Runs
- Review and print a Planned Run
- View the audit trail of a Planned Run
- Edit a Planned Run
- Delete a Planned Run (Manager/Administrator level users only)

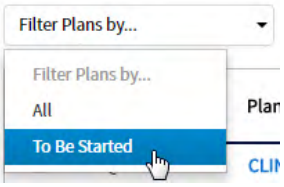
Search and sort Planned Runs

Under the **Run** tab, in the **Manage Runs** screen, the list of Planned Runs is displayed, with the most recently created on top by default.

- To search the Planned Run list:
  a. Enter the full or partial Planned Run name into the **Planned Run** field.
  b. Click 🔍.
  The Planned Run(s) matching the search parameters are listed.
  c. Click × to return to the complete list of Planned Runs.

- To sort the Planned Runs list:
  a. Click the header name of interest.
  The list of Assays reorders alphabetically or numerically based on the header name selected: Run Short Code, Planned Run Name, Assay, and Tube Label.
  b. Click the header name a second time to reverse the order of samples displayed.

- Click **To Be Started** in the **Filter Plans by...** dropdown list to limit the list of Planned Runs to only those runs that are ready to be executed.



Review/Print a Planned Run

You can review and print the information in a **Planned Run** when you execute it. Alternatively:

1. Under the **Runs** tab, in the **Manage Runs** screen, click the Planned Run name in the list.
2. In the **View Planned Run** dialog box, review the information that was entered or generated when you created the run, then click **Print** to print it.

Review a Planned Run audit trail

The entire history of a Planned Run is available for review, export, or printing.

1. Under the **Runs** tab, in the **Manage Runs** screen, click **Audit** in the **Actions** column for the desired run.
   The **Audit Trail** dialog box opens. Each modifying event for the selected Planned Run is listed.
2. Click the details icon under the **Record** header to view the details of the change made.
   The **Audit Record Details** dialog box opens detailing the edits that were made.
3. In the **Audit Record Details** dialog box, click
   a. **Export** to export a Print-Ready PDF of the record.
   b. **Cancel** or the icon to return to the **Audit Trail** dialog box.
4. Open the downloaded PDF file in an appropriate viewer, then print the record from within the open document.
5. Click **Cancel**, or the icon, to return to the **Manage Runs** screen.

Edit a Planned Run

You can edit Planned Runs that have not been executed yet.

1. Under the **Runs** tab, in the **Manage Runs** screen, click **Edit** for the Planned Run in the **Actions** column.
2. In the **Edit Planned Run** dialog box, change the settings, then click **Save**.

Delete a Planned Run (Manager/Administrator only)

1. Under the **Runs** tab, in the **Manage Runs** screen, select the Planned Run to be deleted by selecting the checkbox next to the **Run Short Code**. Select all of the Planned Runs on the screen by selecting the checkbox above the column.
2. Click **Delete**.
   The **Delete Plan** dialog box opens with the message "Are you sure you want to delete the selected planned run(s)?
3. Click **Yes** to delete the Planned Run, or click **No** to return to the **Manage Runs** screen without deleting.

System installation templates (service engineers only)

IMPORTANT! The templates used by service engineers during Ion PGM™ Dx System installation are listed under the **Runs** tab in the **Install Templates** screen. These should not be used or modified in any way.

Calculating coverage for a panel

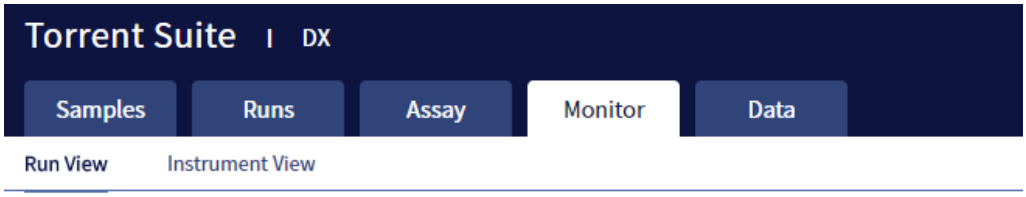
The minimum number of required reads for any sample has been set at greater than or equal to 0.5% of the number of total addressable wells. This minimum number is based on 16 barcoded samples being processed in a single sequencing run. It is important to note that the number of reads per amplicon are a direct function of the number of amplicons in the sequencing panel. As an example, an Ion 318™ Dx Chip, with 11.3 million total addressable wells, would require a minimum of 56,500 reads for a barcoded sample to meet the sequencing run pass criteria. At this level, a panel containing 100 amplicons should have on average 565-fold coverage per amplicon. A panel containing 1,000 amplicons would have on average 10-fold lower coverage per amplicon (56.5-fold). The actual coverage per amplicon varies from the average for various reasons. The design of any panel needs to factor in the required amplicon coverage, the number of addressable wells, the size of the sequencing panel, and the maximum number of samples to be sequenced in the same run.

The formula for calculating the theoretical average coverage per amplicon is:

Total number of addressable wells (for example, the Ion 318™ Dx Chip has on average 11.3 million wells) × expected percentage of wells that generate quality sequences (40–60%, typically)/number of barcoded samples in run (up to a maximum of 16) × number of amplicons in panel.

Example calculation: $(11.3 \times 10^6 \times 0.6) \div (16 \times 1000) = 423.75$ reads per amplicon

5 Monitor runs



In the **Monitor** tab, you can view the status of any jobs running on instruments that are connected to the Ion Torrent™ Server. To monitor the status of runs versus instruments, click **Run View** or **Instrument View** under the tab.

Run information is displayed for the last 5 runs that are in progress, have failed, or have completed data analysis.

Information displayed

The **Run View** and **Instrument View** screens under the **Monitor** tab display the following information:

View	Information displayed
Run View	Ion OneTouch™ Dx Instrument or Ion Chef™ Instrument and Ion PGM™ Dx Sequencer • Instrument name • Operator • Start and Completion times • Time remaining • Run Status
	Ion PGM™ Dx Sequencer only • Flow Transfer number • Analysis Status • QC Details (expand to view after analysis is complete) – Run QC – Sample QC – Internal Control QC
Instrument View	Ion OneTouch™ Dx Instrument or Ion Chef™ Instrument and Ion PGM™ Dx Sequencer • Current state • Instrument status • Last cleaning • PQ status • PQ due date • Instrument serial number • Any instrument alarms

View the status and quality control metrics of a run

Under the **Monitor** tab, in the **Run View** screen, you can review the status and quality control metrics of the last 5 template preparation and sequencing runs (active, completed, or failed).

1. In the **Select Run** dropdown list, select the Planned Run.

   The status of the instruments used in the run are displayed. The **Analysis Status** field indicates whether post-sequencing analysis ran to completion or failed.

2. To update the status of the run, click **Refresh**.

3. To display additional information about the run, select from the following options:

   - To view details of the assay used in the run, click the assay name next to the dropdown lists.
   - To view the quality control information for a library in the run, select the library from the **Select Library** dropdown list, then click **+View QC Details**.

   Note: The QC metrics are described in "QC Details metrics" on page 44.

   An individual **Library QC** metric can fail as long as all **Run QC** and **Control QC** metrics pass. A failed **Run QC** or **Control QC** metric invalidates all library results.

QC Details metrics

After a run is complete, some or all of the following quality control metrics are listed for each library in the run under the **Monitor** tab, in the **Run View** screen under **View QC Details**. The metrics displayed depend on the settings and requirements of the selected assay.

QC category	Description of metrics
Run QC	• Key Signal: The average peak signal from incorporation trace measured across the 1-mer incorporations in the library key sequences. • Percent Loading: The percentage of chip wells that contain an ISP (The percentage value considers only potentially addressable wells).
Library QC: Library DNA	• MAPD: The median of the absolute values of all pairwise differences between log2 ratios per tile for a given run. See "MAPD Copy Number QC metric" on page 111 for more information. • Mean AQ20 Read Length (bp): The average length, in base pairs, at which the error rate is ≤1% for all aligned reads of a library. • Mean Read Length (bp): The average length, in base pairs, of all reads reported for a given library. • Percent Reads: The number of library reads normalized by the total addressable wells in a run.

(continued)

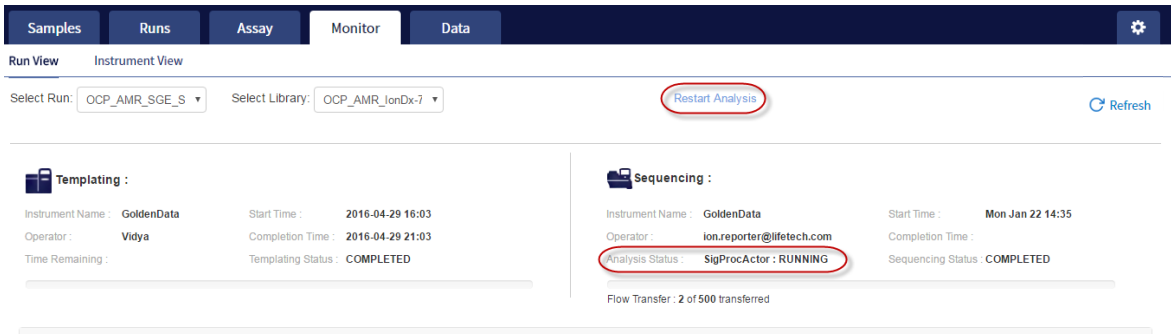
QC category	Description of metrics
Library QC: Library RNA	• Mean AQ20 Read Length (bp): The average length, in base pairs, at which the error rate is ≤1% for all aligned reads of a library. • Mean Read Length (bp): The average length, in base pairs, of all reads reported for a given library. • Total Mappable Reads: The number of reads that are mapped to the fusion reference file.[1]
Control QC: CF-1	• Base Call Accuracy: 1 – (total number of errors for all positions in the control / total number of aligned bases) for Control Fragment-1 (CF-1) reads. • Key Signal: The average peak signal from the incorporation trace measured across the 1-mer incorporations in the CF-1 key sequences. • Mean AQ20 Read Length (bp): The average length, in base pairs, at which the error rate is ≤1% for all aligned reads of CF-1. • Percent Reads: The number of all usable library reads that aligned with the CF-1 sequence divided by the total number of addressable wells.
DNA Control	Assay-specific DNA controls, values, and reference ranges.
RNA Control	Assay-specific RNA controls, values, and reference ranges.
Control QC: DNA NTC	• Hotspot Calls: The total number of hotspots where a call was made in the no-template control.
Control QC: RNA NTC	• Total Fusion Calls: The total number of fusion locations where a call was made in the no-template control. • Total Mappable Reads: The number of reads in the no-template control that are mapped to the fusion reference file.

[1] May not equal the sum of the individual fusions, since some detected fusions are not included in the fusion BED file.

Restart analysis *(Administrator)*

Under the **Monitor** tab, in the **Run View** screen, the **Analysis Status** field indicates whether data analysis has started, is running, or has completed. An analysis listed as "RUNNING" for more than 12 hours may be stuck in the pipeline. After 12 hours, a **Restart Analysis** button appears under the tab for Administrator-level users.

Click the button to restart the analysis from the beginning.



Repopulate QC *(Administrator)*

Under the **Monitor** tab, in the **Run View** screen, the **Repopulate QC** button appears for Administrator-level users after a run is complete. If the **QC Status** of a run is **Failed** and one or more QC metrics are listed as **N/A**, the QC metrics may not have been updated in the database correctly after the run.

Administrator-level users can click **Repopulate QC** to repopulate the QC metrics in the database.

Note: This function does not recalculate or change the QC data from the run. It repopulates the database with the previously calculated metrics and regenerates the Lab and Test Reports. The report status (draft or signed) is retained.

---

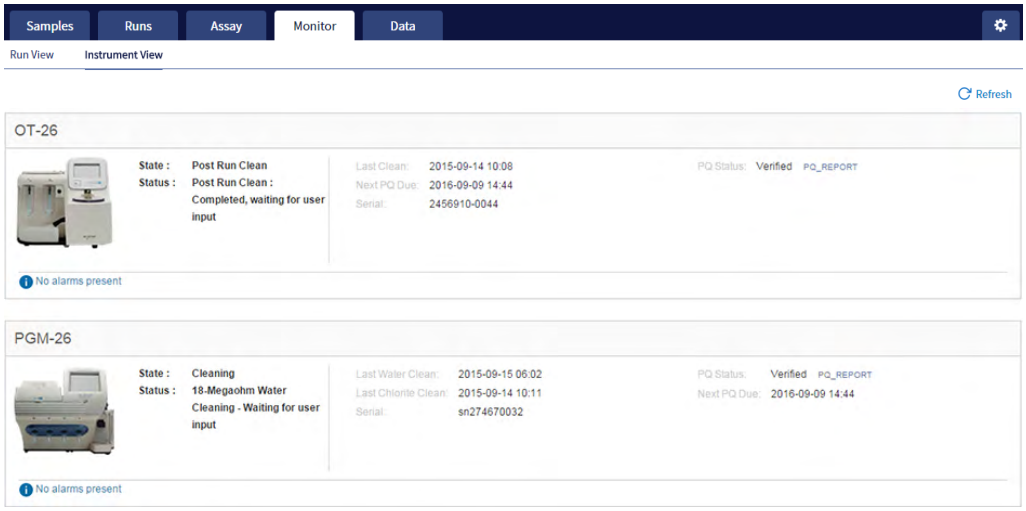
1. Under the **Monitor** tab in the **Run View** screen, under **View QC Details**, check the **QC Status** of the run. If the status is **Failed** and any QC metric is listed as **N/A**, proceed to the next step.
2. Click **Repopulate QC** to repopulate all QC metrics in the database.

   Note: Repopulation takes 15–30 minutes.

3. If any metric is still listed as **N/A** and the **QC Status** is still **Failed**, repeat the run starting at template preparation from the DNA/RNA combined library, and ensure that you add all controls. If the problem continues, contact Technical Support.

Instrument View screen

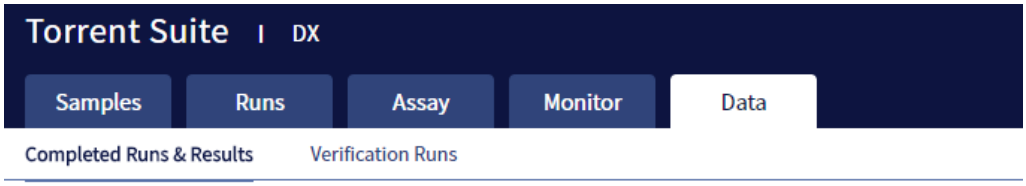
The instruments connected to the Ion Torrent™ Server are listed under the **Monitor** tab in the **Instrument View** screen. To update the information in this screen during a run or following completion of a run, click **Refresh**.



The following information is displayed in this screen:

Label	Description
State	The function that the instrument is currently performing (for example, **Clean**, **Initialize**, or **Run**).
Status	The current status of the active function, including data analysis.
Last Clean	The date and time that the instrument was last cleaned. For the Ion OneTouch™ Dx Instrument or Ion Chef™ Instrument, this refers to the pre-run cleaning and is not updated following the post-run cleaning.
Serial	The serial number of the instrument.
PQ Status	The performance qualification (PQ) status of the instrument (**Verified**, **Not Verified**, or **Expired**). Click the link next to the status to download and view the PQ report. **Note:** If the PQ status is **Expired**, users cannot perform a non-PQ run on the instrument until a PQ run is performed and verified.
Next PQ Due	The expiration date of the current PQ run.

6 Review data and results



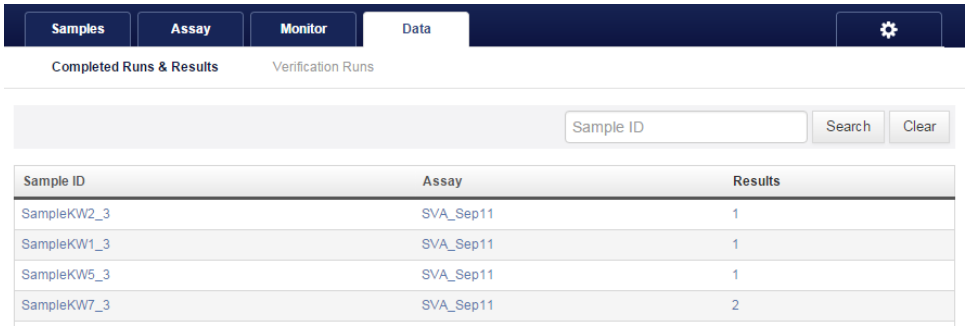
In the **Data** tab, you can review run results and perform data analysis and data management tasks:

- Select **Completed Runs & Results** (the default window) to review completed sample run results and reports. Run results are listed by **Sample ID**.
- Select **Verification Runs** to review data from completed verification runs performed during installation or PQ validation.

Completed Runs & Results screen

Under the **Data** tab, in the **Completed Runs & Results** screen, samples that have been sequenced are listed by Sample ID.

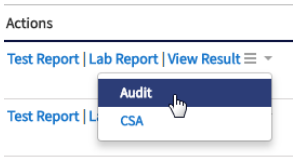
You can search the list of results by Sample ID or Planned Run name. You can also filter and sort the list.



The following information is displayed in the screen:

Column	Description
Sample ID	The unique identifier created when the sample was entered into the software. Click on the link to display the details of the sample.
Planned Run	The name of the Planned Run given when it was created in the software, after preparation of the library batch. Click on the name to display details of the Planned Run.

(continued)

Column	Description
Library Name	The names of the DNA and RNA sample libraries given during preparation of the library batch.
Assay	The assay selected when the sample was placed in a library batch prior to creating the Planned Run. Click on the assay name to display the details of the assay.
Run Status	The status of the run (for example: completed, failed, terminated).
Notes	User-entered notes about the sample. To view previously written notes, click . To add new notes, click .
Actions	Click the appropriate link to: • Download the Test Report. • Download the Lab Report. • Open the View Result screen. • View the audit trail for the Planned Run. Click , then click **Audit**.  • Download Customer Support Archive (CSA) log files for the run to aid in troubleshooting. Click , then click **CSA**.

Download the Lab Report or Test Report

1. To download the Lab Report or Test Report for a sample of interest, in the **Completed Runs & Results** screen, click the appropriate link in the **Actions** column for the sample. A ZIP file containing all languages of the report downloads automatically.

2. Extract the downloaded files, then open the PDF file of the desired language in an appropriate viewer.

Lab Report

The **Lab Report** (available from the **Results List** under the **Completed Runs & Results** screen) is a report generated by the software that can be downloaded in PDF format.

The Lab Report contains the following sections and information. Certain fields of this report may be customized, as described in "Create a report template" on page 72.

Section	Description
Sample Details	The sample information entered into the software.
Analytical Sequence Variations Detected	A list of the analytical variants and gene fusions detected by the assay, and associated information for each.
Laboratory comments regarding sample	Contains laboratory comments (for example, about sample quality) entered when the results were signed in the software.
Test Description	A description of the assay.
Analytical Sequence Variations Not Detected	A list of all the analytical variants and gene fusions not detected by the test, and associated information for each.
Sequencing Run Details	A list of all the kits and instruments used to perform the test.
QC Evaluation Metrics	A summary of the quality control metrics, which may vary by assay.

Test Report

The **Test Report** is available from the **Results List** under the **Completed Runs & Results** screen, if the user selected **Generate Test Report** when creating the assay. The Test Report is identical to the Lab Report except that it reports on only those genes included in the Gene Reporting List selected during assay creation.

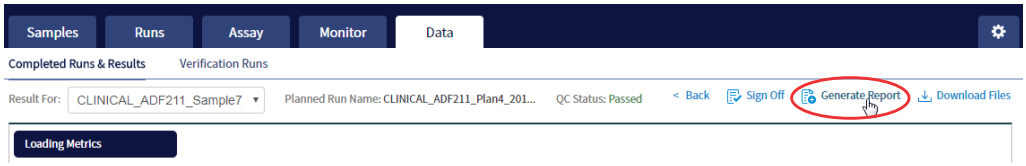
Note: Specific fields of this report can be customized, as described in "Create a report template" on page 72.

Section	Description
Sample Details	The sample information entered into the software.
Test Results Summary	Displays the results for the variants and/or gene fusions in the sample.
Test Description	A description of the test and the gene variants in associated tissue types that it screens for.
Results for Analytical Sequence Variations Detected	Displays the results for analytical variants and gene fusions detected in the sample.
Results for Analytical Sequence Variations Not Detected	Displays the results for analytical variants and gene fusions not detected in the sample.

Generate customized reports

By default, the Test Report and Lab Report are generated in the language that is selected in the report template. To generate these reports in another language, or change the types of variant calls that are reported, do the following:

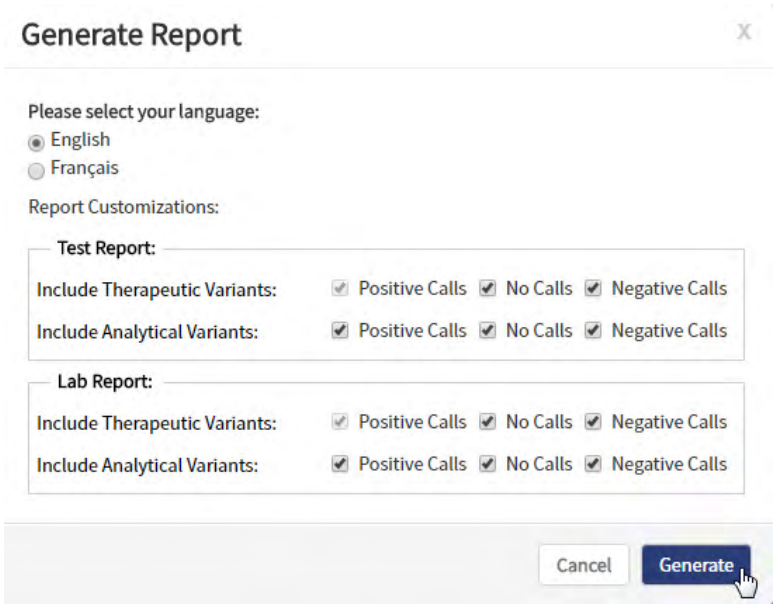
1. In the **Completed Runs and Results** screen, click **View Result**.
2. In the **View Result** screen, click **Generate Report**.



The **Generate Report** dialog box opens.

3. Do one or both of the following:
   - Select the desired language.
   - Select the checkboxes for the positive calls, no calls, or negative calls that you want to report in the Test Report and/or Lab Report.

   Note: These checkboxes are only available in IVD mode if they are enabled for the selected assay.



Note: Positive calls for therapeutic variants cannot be deselected.

4. Click **Generate**.
   A message displays when the report has been generated. The new report overwrites the existing report.

5. Click **Download Files** to open a dialog box to select the new report and other run-related files for download.

Restore archived results

Torrent Suite™ Dx Software can be configured to automatically transfer older run data, results files, and signed reports from an Ion Torrent™ Server to an external server, based on when the results were generated (see "Disk space usage and data archiving" on page 81). Archived results and reports can then be restored to the Ion Torrent™ Server and downloaded from the **Completed Runs & Results** screen.

IMPORTANT!

- If results are restored from runs that were archived in an older version of Torrent Suite™ Dx Software (version 5.6.4 and earlier), the software may not display all the variants from the restored results in the user interface, and reports generated from those restored results may not contain all variants. Carefully review the restored data in the user interface to determine whether all variants are present. Do not generate new reports from results restored from these older archives. Note that all variants are preserved in the restored source files, and can be downloaded using the **Download Files** command. This issue is corrected in version 5.12.5 of the software.
- In older versions of Torrent Suite™ Dx Software (version 5.6.4 and earlier), results reports should be generated and signed before results are archived. See "Sign the run results" on page 64.

Archived results are listed in the **Completed Runs & Results** screen with the **Restore** link active in the **Actions** column. To restore and download archived results:

1. In the **Completed Runs & Results** screen, locate the archived result, then click **Restore** in the **Actions** column.

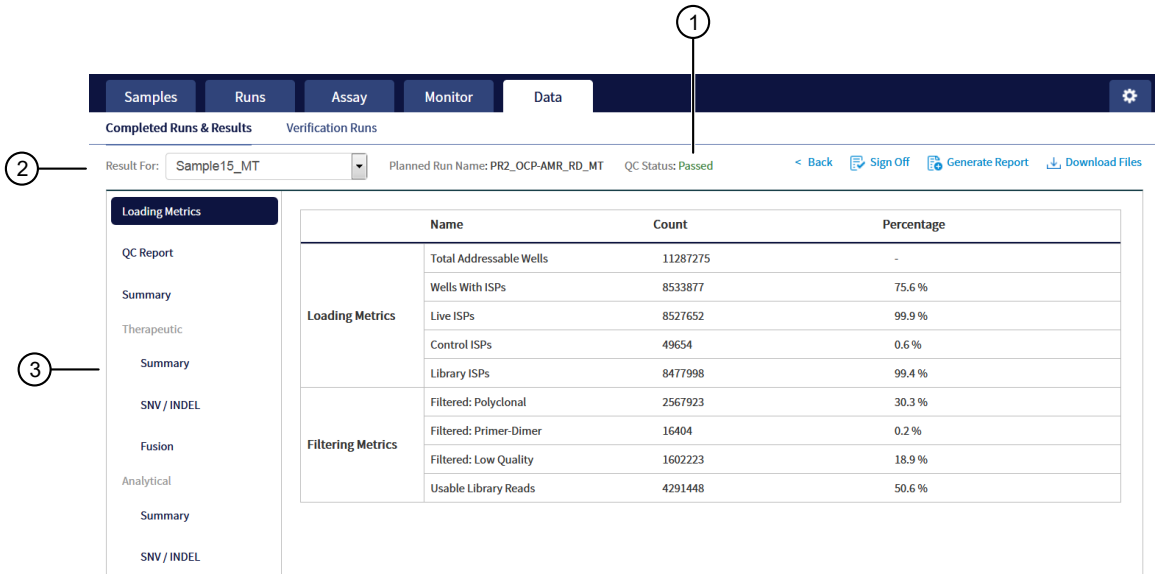
2. Click **OK** in the confirmation dialog box.
   In the **Actions** column, the **View Result** button is now active.

3. Click **View Result**, then click **Download Files** to download and view the restored results files.

View results

The run results shown in the screens described in this section can also be downloaded in the form of results files (see "Results files" on page 62).

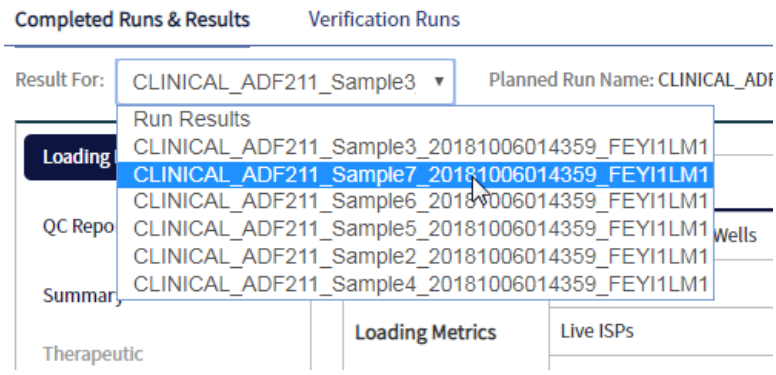
- In the **Completed Runs & Results** screen, click **View Result** to view the sequencing run results for a sample of interest.
  The **Results** screen for the selected sample opens, with the **Loading Metrics** screen displayed. Result categories are arranged vertically on the left side of the screen. The QC status for the run is listed at the top of the screen.



1. QC status
2. Selected sample
3. Result categories

IMPORTANT! A **QC Status** of "Passed" does not guarantee that the genotypes of all analytically relevant variants are determined. See the **Test Result** column in the Lab Report or Test Report for any "No Call" results when interpreting the results.

- Click the links on the left side of the screen to display results of interest, including QC results and results for different categories of variants.
- To view results for another sample in the same run, select the sample from the **Result For:** dropdown list. All samples in a run share the same **Loading Metrics** data. The other result categories are sample-specific.



- To generate the Lab Report and Test Report in other languages, click **Generate Report** (see "Generate customized reports" on page 51).

- To download individual results files for further analysis, click **Download Files** (see "Results files" on page 62).
- To return to the **Completed Runs & Results** screen, click **< Back**.

Loading metrics

The **Loading Metrics** link in the **View Result** screen displays the following loading and filtering metrics for the run:

Metric	Description
Loading Metrics	
Total Addressable Wells	The total number of wells on the chip – excluded wells.
Wells with ISPs	The number (count) and percentage of chip wells that contain ISPs. The percentage is expressed as a percent of total addressable wells.
Live ISPs	The number (count) and percentage of chip wells containing live ISPs (ISP's templated with library or control fragment), with the percentage expressed as a percent of wells with ISPs.
Control ISPs	The number (count) and percentage of ISPs that have a key signal identifying them as internal controls, with the percentage expressed as a percent of live ISPs.
Library ISPs	The number (count) and percentage of ISPs that have a key signal identical to the library key signal, with the percentage expressed as a percent of live ISPs.
Filtering Metrics[1]	
Filtered: Polyclonal	ISPs carrying clones from two or more templates, with the percentage expressed as a percent of library ISPs.
Filtered: Primer-Dimer	ISPs with an insert length of less than 8 bp, with the percentage expressed as a percent of library ISPs.
Filtered: Low Quality	ISPs with low or unrecognizable signal, with the percentage expressed as a percent of library ISPs.
Usable Library Reads[2]	Number (count) and percentage of library ISPs passing all filters.

[1] Filtering Metrics only apply to ISPs templated with library fragments, not control fragment.
[2] Values in the "Filtered:" rows are subtracted from the Library ISPs value (Loading Metrics) to give the Usable Library Reads value.

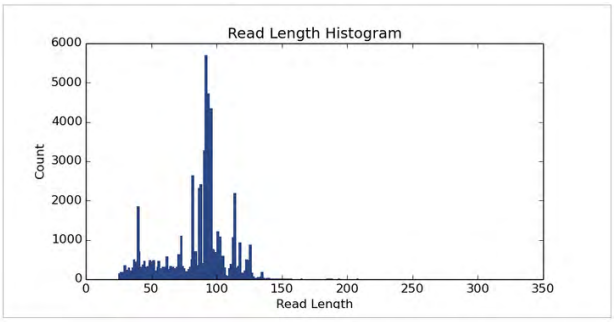
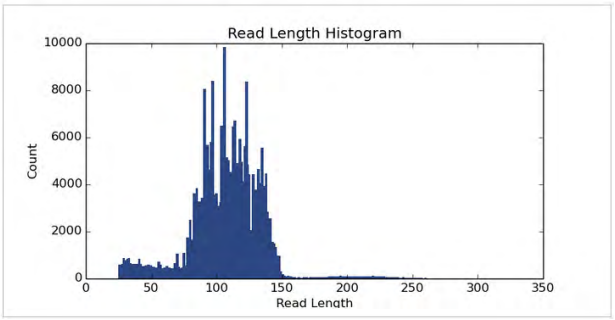
QC Report

The **QC Report** link displays metrics for the sample libraries and internal controls. This information is also accessible through the **Monitor** tab for the last five runs.

IMPORTANT! The following table describes all the quality control metrics that can be displayed. Whether particular metrics are displayed depends on the assay configuration.

Metric	Description
Library QC Evaluation Metrics	
Library QC: Library RNA	• Mean AQ20 Read Length (bp): The average length, in base pairs, at which the error rate is ≤1% for all aligned reads of a library. • Mean Read Length (bp): The average length, in base pairs, of all reads reported for a given library. • Total Mappable Reads: The number of reads that are mapped to the fusion reference file.[1]
Library QC: Library DNA	• Mean AQ20 Read Length (bp): The average length, in base pairs, at which the error rate is ≤1% for all aligned reads of a library. • Mean Read Length (bp): The average length, in base pairs, of all reads reported for a given library. • Percent Reads: The number of library reads normalized by the total addressable wells in a run.
Control QC Evaluation Metrics	
Control QC: CF-1	• Base Call Accuracy: 1 – (total number of errors for all positions in the control / total number of aligned bases) for Control Fragment-1 (CF-1) reads. • Key Signal: The average peak signal from the incorporation trace measured across the 1-mer incorporations in the CF-1 key sequences. [2] • Mean AQ20 Read Length (bp): The average length, in base pairs, at which the error rate is ≤1% for all aligned reads of CF-1. • Percent Reads: The number of all usable library reads that aligned with the CF-1 sequence divided by the total number of addressable wells.
Control QC: DNA Control	• COSMIC ID variant calls and allelic frequencies: The individual variant positions and wild-type positions that are assessed in the DNA control reagent for presence or absence of the variant. • Mean AQ20 Read Length (bp): The average length, in base pairs, at which the error rate is ≤1% for all aligned reads of a control. • Percent Reads: The number of all usable library reads that aligned with the control fragment sequence divided by the total number of addressable wells.

(continued)

Metric	Description
Control QC: RNA Control	• Fusion calls and number of reads: The individual fusion calls and number of reads that are assessed in the RNA control reagent for the presence or absence of the fusion. • Total Mappable Reads: the number of reads that are mapped to the fusion reference file.
Control QC: DNA NTC	• Hotspot Calls: The total number of hotspots where a call was made in the no-template control.
Control QC: RNA NTC	• Total Fusion Calls: The total number of fusion locations where a call was made in the no-template control. • Total Mappable Reads: The number of reads in the no-template control that are mapped to the fusion reference file.
Histogram of Read Length [3]	• A library-specific read-length histogram: RNA  DNA 

[1] May not equal the sum of the individual fusions, since some detected fusions are not included in the fusion BED file.

[2] The minimum Key Signal value for the CF-1 control (i.e., the Templating Control) is set to 0 in all preinstalled assays and new custom assays in Torrent Suite™ Dx Software version 5.8 and later. Custom assays created in previous versions of the software may have a higher Key Signal value for the control. If a run using an older custom assay fails QC due to this metric, reanalyze the run using a minimum Key Signal of 0 for the control. For future runs, create a new custom assay based on the old assay, changing the minimum Key Signal to 0.

[3] Not displayed in the Monitor tab.

Summary

The **Summary** link displays the following information for the run:

Field	Description
Run and Configuration Summary	
Run Name	The name of the run.
Assay Name	The name of the assay.
Reference Genome	The reference genome used for analysis.
Target Region	The name of the targeted regions BED file used.
Hotspot Regions	The name of the hotspot regions BED file used.
Fusion Reference	The name of the fusion reference used for analysis, when applicable.
Fusion Panel	The name of the fusion panel used, when applicable.
Library Name	The names of the DNA and DNA libraries prepared from the sample, entered during library batch preparation.
Amplicon Summary	
Number of Targets	The number of amplicons in the panel.
On Target Reads	The number of reads mapped to the target amplicons.
Percent On Target Reads	The percentage of reads mapped on target to total reads.
Percent Full Length On Target Reads	The percentage of full length reads mapped on target to total reads.
Average Coverage	The ratio of the depth of coverage at each base in the target region to the length of the target region.
Target Coverage at 20X	The percentage of base positions with depth of coverage ≥20X in the target region.
No Strand Bias	The percentage of bases with a strand bias between 30% and 70%.
Coverage Uniformity	The ratio of passed number of targets to total number of targets, where passed number of targets is the number of amplicons that have at least 0.2X mean coverage.
Variant Summary	
Number of SNVs/MNVs	The number of single- and multi-nucleotide variations (SNVs/MNVs).
Number of INDELs	The number of insertions or deletions.
Number of Fusions	The total number of fusion calls.

Therapeutic variant results

Therapeutic variant results for the selected sample are provided under the **Therapeutic** section of the left navigation bar in the **Results** screen. Depending on the assay, results for these variants may be divided into multiple subscreens, each with a separate link (**SNV/INDEL**, **Fusion**, etc.).

The results are determined by the reference sequences installed on the server, the QC controls used in the run, the assay used for the run, and any Reporting Gene List associated with that assay.

Columns in the screen or subscreens are described in the following table.

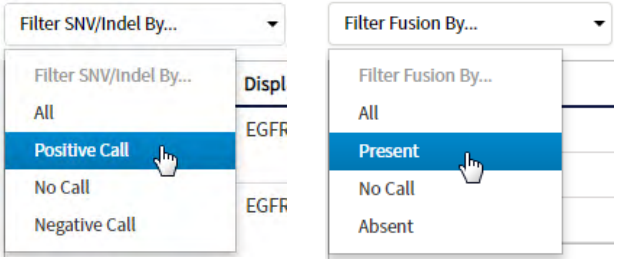
Column	Description
Summary	
Gene Fusion Present Variants Present	The therapeutic gene fusion or SNV/INDEL variant detected in the sample
Associated Therapy	The therapy indicated for each variant listed
SNV / INDEL	
Gene	The gene name, which provides a link to the **View Annotation Sources** popup with additional information about the HotSpot ID (see "View annotation sources" on page 61)
Display Name	The gene name with the exonic location of the deletion or insertion
Amino Acid Change	The amino acid change using HGVS-nomenclature
Nucleotide Change	Identification of the DNA-level nucleotide change using HGVS-nomenclature
Test Result	The presence or absence of the variant
Allele Frequency	The most likely frequency of the variant allele[1]
HotSpot ID	The name of the hotspot as defined in the BED file
Locus	The one-base position of the variant in the reference genome
Type	The type of variation detected: • snv (single nucleotide variation)/mnv (multi-nucleotide variation) • ins (insertion) • del (deletion) • complex
Genotype	The genotype at the locus
Ref	The reference base or bases at the locus

(continued)

Column	Description
Quality Score	The relative probability of either the "reference" hypothesis interval [0,cutoff) or the "variant" hypothesis interval [cutoff,1], on a Phred scale (-10*log10). This provides a measure of the strength of the evidence for the variant call. A higher score means more evidence for the call. Quality scores are capped at 100.
Coverage	The number of reads covering the variant position after down-sampling
Fusion	
Gene	The gene that regulates expression of the gene fusion
Display Name	The gene name of the fusion
Read Count	The number of valid reads aligned to the specific fusion sequence
Test Result	The presence or absence of that fusion variant

[1] The allele frequency is the most likely variant frequency in the reads after corrections are made for probable errors. The software uses this corrected frequency and the uncertainty in the observations to calculate the probability that the variant frequency in the sample falls within defined intervals that can be set by the user. The user sets a threshold frequency for deciding between genotypes: this defines intervals [0,c)[c,1-c)[1-c,1] corresponding to the three diploid genotypes (reference, heterozygous, homozygous) respectively, where “c” is the minimum variant frequency set during the creation of the assay. The software reports the genotype corresponding to the interval with the highest probability of containing the variant frequency in the sample. In most cases, the reads are highly accurate and do not have a high probability of error. In such cases, the observed frequency and the most likely frequency are similar, and are contained within a single interval leading to a high quality genotype. In some cases, if very few reads are observed or the error rate is high, the observed counts and the most likely counts can be different, and the uncertainty in the real sample frequency can be high. When this happens, it is often impossible to exclude a heterozygous population in the sample, and the genotype is assigned to be heterozygous, since a significant portion of the probability falls in that interval.

Note: You can filter the variant lists using options in the **Filter SNV/Indel By...** and **Filter Fusion By...** dropdown lists in the upper left corner of the results report.



Analytical variant results

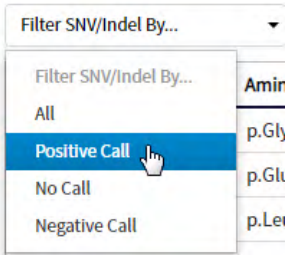
Analytical variant results for the selected sample are provided under the **Analytical** section of the left navigation bar in the **Results** screen. Depending on the assay, results for these variants may be divided into multiple subscreens, each with a separate link (**SNV/INDEL**, **Fusion**, etc.).

The results are determined by the reference sequences installed on the server, the QC controls used in the run, the assay used for the run, and any Reporting Gene List associated with that assay.

Columns in the screen or subscreens are described in the following table.

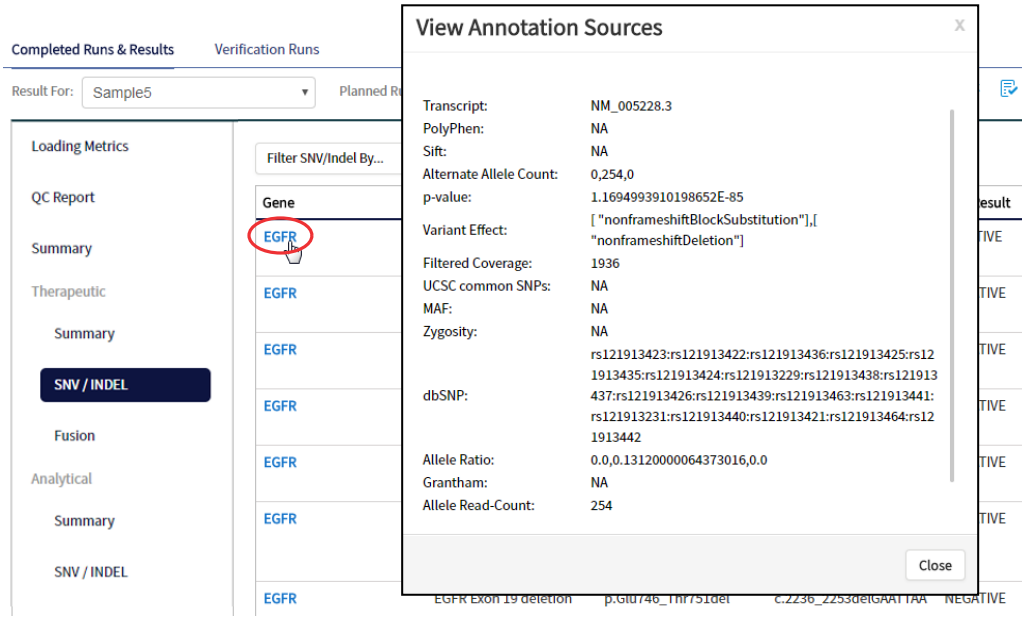
Column	Description
Summary	
Variants Present	Lists the analytical SNV or INDEL variants detected in the sample
SNV / INDEL	
Gene	The gene name, which provides a link to the **View Annotation Sources** popup with additional information about the HotSpot ID (see "View annotation sources" on page 61)
Amino Acid Change	The amino acid change using HGVS-nomenclature
Nucleotide Change	The DNA-level nucleotide change using HGVS-nomenclature
Test Result	The presence or absence of the variant
Allele Frequency	The most likely frequency of the variant allele
HotSpot ID	The name of the hotspot as defined in the BED file
Locus	The one-base position of the variant in the reference genome
Type	The type of variation detected: • snv (single nucleotide variation)/mnv (multi-nucleotide variation) • ins (insertion) • del (deletion) • complex
Genotype	The genotype at that locus
Ref	The reference base or bases at the locus
Quality Score	The relative probability of either the "reference" hypothesis interval [0,cutoff) or the "variant" hypothesis interval [cutoff,1], on a Phred scale (-10*log10). This provides a measure of the strength of the evidence for the variant call. A higher score means more evidence for the call. Quality scores are capped at 100.
Coverage	The number of reads covering the variant position after down-sampling
Fusion	
Gene	The gene that regulates expression of the gene fusion
Display Name	The gene name of the fusion
Read Count	The number of valid reads aligned to the specific fusion sequence
Test Result	The presence or absence of that fusion variant

Note: You can filter the variant list using options in the **Filter SNV/Indel By...** dropdown list in the upper left corner of the results report.



View annotation sources

You can view additional information for each hotspot ID listed under the **SNV/Indel** links of the **Therapeutic** and **Analytical** sections in the **View Results** screen. The gene name in the **SNV/Indel** report is a link that opens the **View Annotation Sources** dialog box, which provides information for the particular hotspot.



Example of transcript information in the VCF

The COSM1074639 hotspot ID has multiple transcripts associated with it, which include a mutation from 'A' to 'C'. These are listed in the VCF file as shown below:

chr6 152419923 COSM1074639;COSM1074637 A C,G 122.19 PASS
AF=0,0;AO=0,0;DP=1116;FAO=0,0;FDP=1116;FR=.;FRO=1116;FSAF=0,0;FSAR=0,0;FSRF=661;FSRR=455;F
WDB=0.00426152,-
0.0087378;FXX=0;HRUN=1,1;HS;LEN=1,1;MLLD=103.308,103.438;QD=0.437949;RBI=0.0394298,0.03043
16;REFB=-2.22051E-5,-2.32713E-5;REVB=-0.0391988,-
0.0291502;RO=1115;SAF=0,0;SAR=0,0;SRF=661;SRR=454;SSEN=0,0;SSEP=0,0;SSSB=-6.44874E-8,-
6.44874E-
8;STB=0.5,0.5;STBP=1,1;TYPE=snp,snp;VARB=0,0;OID=COSM1074639,COSM1074637;OPOS=152419923,
152419923;OREF=A,A;OALT=C,G;OMAPALT=C,G;FUNC=[

{'normalizedRef':'A','transcript':'NM_001122742.1','grantham':'144.0','gene':'ESR1','location':'exonic','ori
gAlt':'C','origPos':'152419923','origRef':'A','normalizedPos':'152419923','exon':'10','function':'missense','
protein':'p.Tyr537Ser','normalizedAlt':'C','gt':'neg','codon':'TCT','coding':'c.1610A>C'},

{'normalizedRef':'A','transcript':'NM_001122742.1','grantham':'194.0','gene':'ESR1','location':'exonic','ori
gAlt':'G','origPos':'152419923','origRef':'A','normalizedPos':'152419923','exon':'10','function':'missense','
protein':'p.Tyr537Cys','normalizedAlt':'G','gt':'neg','codon':'TGT','coding':'c.1610A>G'},

{'normalizedRef':'A','transcript':'NM_001122741.1','grantham':'144.0','gene':'ESR1','location':'exonic','ori
gAlt':'C','origPos':'152419923','origRef':'A','normalizedPos':'152419923','exon':'9','function':'missense','p
rotein':'p.Tyr537Ser','normalizedAlt':'C','gt':'neg','codon':'TCT','coding':'c.1610A>C'},

{'normalizedRef':'A','transcript':'NM_001122741.1','grantham':'194.0','gene':'ESR1','location':'exonic','ori
gAlt':'G','origPos':'152419923','origRef':'A','normalizedPos':'152419923','exon':'9','function':'missense','p
rotein':'p.Tyr537Cys','normalizedAlt':'G','gt':'neg','codon':'TGT','coding':'c.1610A>G'},

{'normalizedRef':'A','transcript':'NM_001122740.1','grantham':'144.0','gene':'ESR1','location':'exonic','ori
gAlt':'C','origPos':'152419923','origRef':'A','sift':'0.0','normalizedPos':'152419923','exon':'9','function':'mi
ssense','protein':'p.Tyr537Ser','gt':'neg','normalizedAlt':'C','codon':'TCT','polyphen':'0.979','coding':'c.161
0A>C'},

{'normalizedRef':'A','transcript':'NM_001122740.1','grantham':'194.0','gene':'ESR1','location':'exonic','ori
gAlt':'G','origPos':'152419923','origRef':'A','sift':'0.0','normalizedPos':'152419923','exon':'9','function':'mi
ssense','protein':'p.Tyr537Cys','gt':'neg','normalizedAlt':'G','codon':'TGT','polyphen':'0.998','coding':'c.16
10A>G'},

Results files

The following files can be downloaded from the **Results Report** window. To download the files, click **Download Files**, select the files to download, then click **Download**.

File name	Description
Test Report	A report of the completed analysis in PDF format
Lab Report	A clinical lab report of the completed analysis in PDF format; includes both clinical and analytical results.
PlannedRun-AuditTrail.pdf	Contains all audit records pertaining to the Planned Run.

(continued)

File name	Description
Info.csv	Contains information about the run and analysis, such as software, sequencing information, instrument information, analysis information, QC details etc.
<RNABarcode>_rawlib.basecaller.bam	Unmapped RNA BAM File; output of base calling, contains unmapped reads.
Snvindel.tab	A tab-delimited file that contains information about non-targeted SNVs and indels
<RNABarcode>_rawlib.basecaller_alignments.bam	Mapped RNABarcode BAM file; output after reads have been mapped to the fusion reference.
Target_Summary.tab	A tab-delimited file that contains a targeted test results summary
<RNABarcode>_rawlib.basecaller_alignments.bam.bai	Mapped RNABarcode BAM index file
<RNABarcode>_rawlib.basecaller.fastq	FASTQ file generated from unmapped BAM file of the RNA barcode used.
<DNABarcode>_rawlib.basecaller.bam	Unmapped DNA barcode BAM file; output of base calling, contains unmapped reads.
raw_peak_signal	Key signal gives the percentage of LiveISPs with a key signal that is identical to the library key signal.
<LibPrepID>_<analysisID>.final.vcf	A VCF file containing all the variants detected as a result of the analysis, along with information such as test result, read count, gene name, quality scores, etc.
Summary.tab	A tab-delimited file that contains the on-targeted test results summary
<LibPrepID>_rawlib.stats.cov.txt	Amplicon statistics file
Fusion.tab	A tab-delimited file that contains non-targeted (analytical) fusion details in a table format. **Note:** The information displayed in the file for each isoform of a particular fusion is identical, because specific isoform and locus information is not included in this table. Detailed isoform and locus information is available in the <LibraryPrepID>_<AnalysisID>.final.vcf file, available under **Download Files**.
readLenHisto.png	Gives the read-length distribution of FASTQ files in the form of a histogram. A thumbnail histogram of the read lengths for a particular barcode.
<LibPrepID>_rawlib.bam.bai	Mapped DNA barcode BAM index file (index file of DNA barcode-mapped BAM file)

(continued)

File name	Description
Basecaller.log	Base Caller log file
analysis.log	Analysis log file
sigproc.log	Signal processing log file
Bead_density_contour.png	Loading density image; a pseudo-color density image of the Ion Chip plate showing percent loading across the physical surface
<DNABarcode>_rawlib.basecaller.fastq	FASTQ file of the DNA barcode used
Target_cnv.tab	Targeted CNV detail table (CNV results for targeted variants from a sequencing run) **Note:** For use with IVD tests that include CNV reporting.
cnv.tab	Non-targeted CNV detail table (analytical CNV results from a sequencing run) **Note:** For use with IVD tests that include CNV reporting.
Target_fusion.tab	A tab-delimited file that contains targeted (clinical) fusion details in a table format
<LibPrepID>_rawlib.bam	Mapped DNA barcode BAM File; output after mapping reads to reference.
Iontrace_Library.png	Key incorporation trace image showing the average signal readings for flows of the bases T, C, and A in the library key.
rawtf.basecaller.fastq	FASTQ file for the test fragment

Sign the run results

In the **View Result** screen, Managers/Administrators can provide their electronic signature on the run results. The signature information appears in the **QC Report** in the **View Result** screen, and in the downloaded Test Report and Lab Report PDFs.

Multi-language support for PDF report generation is provided. By default reports are generated in the language that is selected in the **Report Template** used. When reports are generated in multiple languages, **Sign Off** occurs only in the report of the default language.

1. At the top of the **View Result** screen, click **Sign Off**, then enter your user name, password, and comments in the dialog box. Fields identified with a red asterisk (*) are required fields.
2. In the **Footer Field**, enter any text.
3. Click **Sign Off** to confirm your electronic signature.

Files in the Reports folder

When a manager- or administrator-level user signs a report, a folder named with the Sample ID is created in the Reports folder on the server (`/results/analysis/output/reports`), and the following files are copied into it:

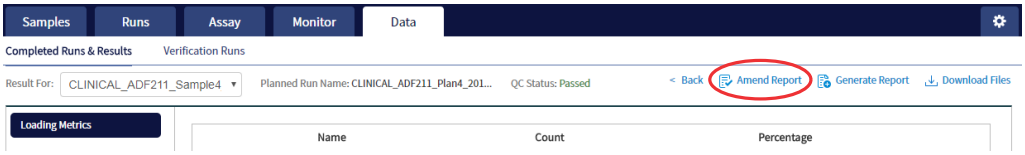
- Info file (.csv)
- Signal processing log file (.log)
- Targeted Fusion Detail Table (.tab)
- Targeted Test Results Summary (.tab)
- Analysis log file (.log)
- VCF file (.vcf)
- DNA Mapped BAM file (.bam)
- DNA Unmapped BAM file (.bam)
- Key Signal
- Key Incorporation Trace (.png)
- Fusion Detail Table (.tab)
- DNA Mapped BAM Index file (.bam.bai)
- RNA Mapped BAM Index file (.bam.bai)
- DNA FASTQ File (.fastq)
- Base Caller Log File (.log)
- Targeted CNV Detail Table (.tab)[1]
- Non-targeted CNV Detail Table (.tab)[1]
- SNV/INDEL Detail Table (.tab)
- Non-targeted Test Results Summary (.tab)
- Targeted SNV/INDEL Detail Table (.tab)
- Amplicon Stats (_rawlib.stats.cov.txt)
- RNA FASTQ File (.fastq)
- RNA Mapped BAM file (.bam)
- RNA Unmapped BAM file (.bam)
- Test Fragment FASTQ File (.fastq)
- Read Length Histogram (.png)
- Test PDF Report *(optional)* (.pdf)
- Lab PDF Report (.pdf)
- Planned Run Audit (.pdf)
- Basecaller command files (.json)
- checksum file
- Pipeline commands (_pipeline.json)
- Experimental log file (_final.txt)
- Wells with beads (_beadogram.png)
- Bead find stats file (.stats)
- Loading Density Figure (.png)

[1] For use with IVD tests that include CNV reporting.

Amend a signed-off report

Managers and administrators can amend a report within 30 days of signing off. A sample that has a signed-off report has a ✔ symbol to the left of the Sample ID in the **Completed Runs & Reports** screen. You can change sign-off or laboratory comments, or the text that appears in the report footer. Follow these steps to amend a signed-off report:

1. In the **Completed Runs & Reports** screen, click **View Result** for the sample of interest.
2. Click **Amend Report**.



3. In the **Electronic Signature** dialog box, make your changes in the appropriate fields, then click **Amend Report**.
4. Click **Test Report** or **Lab Report** to download the amended report.

Repeat testing

Based upon quality control results, users can determine whether a library requires repeat testing. Refer to the following table to determine the best course of action. See the *Ion PGM™ Dx System User Guide* (Pub. No. MAN0018761) for the details of each procedure.

Quality Control			Pooling Error[1]	Retest Sample	Procedures to follow
Library	Run	Control			
Pass	Pass	Pass	No	N/A	Sign off on Library.
Fail	Pass	Pass	No	gDNA	Starting from library preparation.[2]
Fail	Pass	Pass	Yes	Library	Starting from preparing the library pool. If the retest of the sample library fails, retest the gDNA sample starting from library preparation.
Fail	Pass	Fail	No	gDNA	Starting from library preparation.
Fail	Pass	Fail	Yes	Library	Starting from preparing the library pool. If the retest of the library fails, retest the gDNA sample starting from library preparation.
Fail	Fail	Pass	No	gDNA	Starting from library preparation.

(continued)

Quality Control			Pooling Error[1]	Retest Sample	Procedures to follow
Library	Run	Control			
Fail	Fail	Pass	Yes	Library	Starting from preparing the library pool. If the retest of the library fails, retest the gDNA sample starting from library preparation.
Pass (all samples)	Pass	Fail	No	Pooled Library	Starting from the pooled sample libraries, repeat template preparation. If the CF-1 fails QC a second time, reprepare the library pool.
Pass (all samples)	Fail	Fail	No	Pooled Library	Starting from the pooled sample libraries, repeat template preparation. If the CF-1 fails QC a second time, reprepare the library pool.
Pass (all samples)	Fail	Pass	No	Pooled Library	Starting from the pooled sample libraries, repeat template preparation. If the CF-1 fails QC a second time, reprepare the library pool.

[1] Pooling error is defined as no reads detected for a Barcode ID.

[2] **IMPORTANT!** If a library fails Library QC in a second run that passes both Run and Control (CF-1) QC metrics, the sample is considered invalid.

Data files and flow

During an Ion PGM™ Dx Sequencer run, sequence raw data (DAT) files are transferred to the Ion Torrent™ Server via a network cable in a process controlled by the ionCrawler service. After the data from the initial flows on the sequencer are available on the server, the Torrent Suite™ Dx Software begins processing the data, producing the <1> wells file. Basecalling is performed on the <1> wells file data, producing an unmapped BAM (uBAM) file. Subsequent analysis produces mapped reads (BAM) and variant calls (VCF) files.

The following table shows the flow of data and typical file size generated as the Torrent Suite™ Dx Software processes data from an Ion 318™ Dx Chip.

Process	File Type	File Size
		Ion 318™ Dx Chip
Sequencing[1]	DAT	~350 GB
	▼	
Signal Processing	Wells	12 GB
	▼	
Base Calls (reads)	uBAM	1.5 GB
	▼	
Mapped Reads	BAM	4.3 GB

(continued)

Process	File Type	File Size
		Ion 318™ Dx Chip
	▼	
Variant Calls	VCF	120 Kb

[1] The sequencing raw data (DAT) files are deleted from the server 72 hours after data analysis to conserve Ion Torrent™ Server disk space.

Note: Data from approximately 200 sequencing runs using an Ion 318™ Dx Chip can be accommodated on an Ion Torrent™ Server before disk space becomes a limiting factor and data archiving is required. See "Data Management (administrator)" on page 80 for more information.

Verification runs on the Ion PGM™ Dx System

Verification runs are sequencing runs performed during Ion PGM™ Dx System installation by Thermo Fisher Scientific support specialists to validate the performance of the instruments.

Verification runs are performed using Torrent Suite™ Dx Software. Verification runs can also be performed using Torrent Suite™ Assay Development Software, but are not required.

You can view verification run reports, but only qualified support specialists can perform the runs. Under the **Data** tab, in the **Verifications Runs** screen, you can search, filter, sort, and view completed verification runs and reports.

The following information is displayed in the **Verification Runs** screen:

Column	Description
Planned Run	The name of the run, created when the run was created. Click the name to open the **Review Planned Run** dialog box. • Click **Audit** to display the list of users who created/edited the Planned Run. You can export and print information from the list from the **Audit Trail** dialog box. • Click **CSA** (Customer Support Archive) to download all the instrument log files, which can be useful for troubleshooting.
Field Engineer Name	The name of the support specialist who performed the run.
Instrument Name	The name of the validated instrument.
Templating Completion	The completion date and time of the Ion OneTouch™ Dx System run. Click the link to open a dialog box that includes additional information about the instrument, operator, and template kit used.
PGM Completion	The completion date and time of the Ion PGM™ Dx System run. Click the link to open a dialog box that includes additional information about the sequencer, operator, sequencing kit, and chip used.
Analysis Completion	The completion date and time of the post-sequencing run analysis.

(continued)

Column	Description
Run status	The current status of the full run, including analysis.
QC Status	Indicates whether a run passed or failed, based on the QC metrics selected for the assay.
PQ Report	If the results of the run have been approved and signed off by a Manager or Administrator, the approved PQ Report are listed in this column.
Actions	• Click **View Results** to view the results of completed runs that have not yet been approved. Managers and Administrators can also sign off on the results after viewing them. • Click **View Report** to view a report of the run.

Verification run results

Under the **Data** tab, in the **Verifications Runs** screen, click **View Results** in the **Actions** column to view the performance qualification (PQ) report for a verification run.

Note: Manager- and administrator-level users can sign off on this report, at which point it becomes a locked PQ Report. However, we recommend that only qualified support specialists sign off on PQ reports.

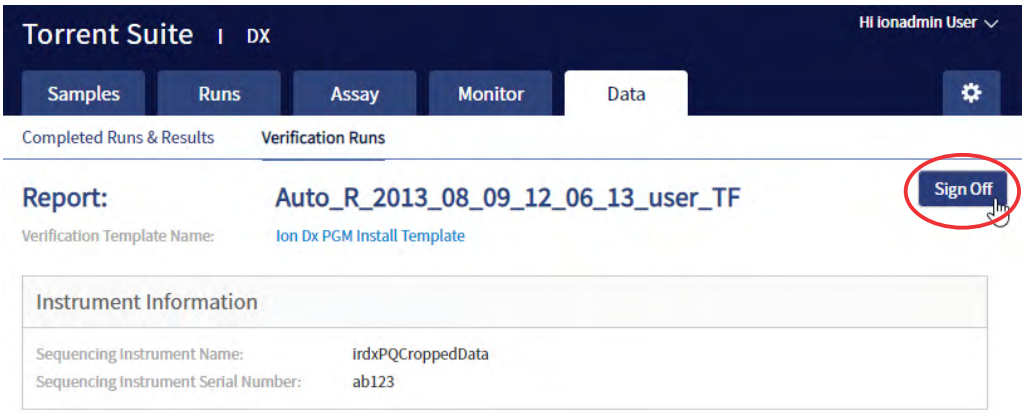
In addition to basic information about the verification run, the following data are reported for the controls that are used in the run:

Metric	Description
Base Call Accuracy	1 – (total number of errors for all positions in the control / total number of aligned bases)
Mean AQ20 read length (bp)	The average length, in base pairs, at which the error rate is ≤1% for all aligned reads of a control fragment
Percent Reads	The number of all usable library reads that align with the control fragment sequence, divided by the total number of addressable wells

Sign verification run reports (manager/administrator)

Manager- and administrator-level users can sign results reports for verification runs. However, we recommend that only qualified support specialists sign PQ reports.

1. Click **Data ▸ Verification Runs**.
2. In the **Report** window, click **Sign Off** in the upper right corner above the report.



3. In the **Sign off Electronic Signature** dialog box, enter your user name, password, and any comments, then click **Sign Off.**
4. The report is locked and listed in the **PQ Report** field for the run in the **Verification Runs** screen.
5. Click the PQ report name to view, print, or download the report in a PDF file.

7 Manage assays

Assays contain the settings and parameters for controlling the sequencing run, analyzing the results, and determining valid variant calls. Assays also define the kits and chips used in a run, and specify the threshold values for quality control and variant detection.

The **Assay** tab contains tools for managing assays.

Manage assays

Click the **Assay** tab to open the **Manage Assays** screen. The following tools are available on this screen:

To...	Do the following...
All users	
Search the assay list	1. Enter the full or partial assay name into the **Assay Name** field. 2. Click **Search**.
Sort the assay list	• Click a column header to sort on that column. • Click the column header again to reverse the order.
Return to the default assay list	Click **Manage Assays** to refresh the screen.
View details of an assay	1. Click an assay name in the list. 2. Scroll down in the **View Assay Details** dialog box to view all the settings.
Manager and Administrator-level users	
View and export the assay audit trail	1. Click **Audit** in the **Actions** column. 2. In the **Audit Trail** dialog box, for each action, click in the **Record** column to view the details of that action. 3. In the **Audit Record Details** dialog box, click **Export** to export a PDF of the selected record.

Import an assay

Under the **Assay** tab, in the **Import Assay** screen, manager-level and administrator-level users can import assays into the software in the form of a ZIP file.

1. In the **Import Assay** screen, click **Select file**, select the ZIP file to import, then click **Open**.
   The file name appears in the **Select file** field.
   - Click **Change** to replace the selected file.
   - Click **Remove** to clear the field.
2. Click **Import** to complete the import.
   The assay appears in the **Manage Assays** screen.

Assay presets

Report templates

You can create and manage custom report templates used to generate PDF reports after a run. You select a report template when you set up a Planned Run.

The tools for setting up report templates are located in the **Report Templates** subtab under the **Assay** tab in the **Presets** screen.

Manage report templates

The tools for managing report templates are located under the **Assay** tab in the **Presets** screen under the **Report Templates** subtab.

- To view the details of a report template, click on the template name.
- To make a template unavailable in the software, click **Obsolete** in the **Actions** column.
- To add notes to a template, click the **+** sign in the **Notes** column.
- To view notes for a template, click the  symbol in the **Notes** column.

Create a report template

1. Under the **Assay** tab in the **Presets** screen, click the **Report Templates** subtab.
   Existing report templates are listed on this page, including any system-installed templates.
2. Click **Add New**.
3. Enter the **Report Name**, then select the **Assay** from the dropdown list.

   Note: Use the **Click here** links to the right of the fields to download an example Lab Report or Test Report.
4. In the **Select Language** dropdown list, select the language for generating the report.

5. Fill out the information in the remaining sections. Fields identified with a red asterisk (*) are required.

   Note: For locked assays, some fields and attributes cannot be edited and are grayed out.

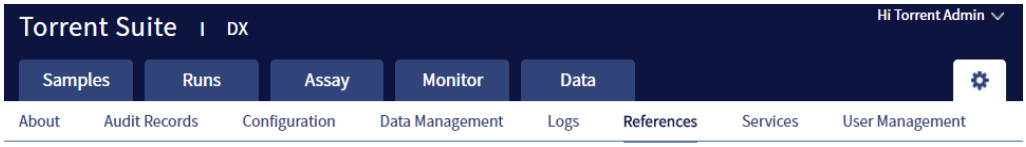
6. Under **Sample Details**, drag and drop the attributes to display in the report into one of the three column placeholders. You can also drag and drop the additional attributes below the columns into the columns. Select the checkbox next to an attribute to include the attribute in each page header.

7. Click **Save**.
   The new report template appears in Report Templates list.

Assay default settings

The parameters specified by an assay are pre-populated with minimum required values.

8 System administration and management



In the ⚙ **tab**, Managers and Administrators can:

- View, create, and manage reference files, including genome references, panel and hotspot BED files, primers, and others.
- View system information and settings.

In addition, Administrators can:

- View and export audit records.
- View and change configuration settings.
- Create and manage user accounts.
- Manage log files.

Access to ⚙ tab functions by user level

In the ⚙ **tab**:

Users at this level…	Can access...
Operator	**About**: More Information and Assistance • Support Contact Information: a link directing users to the **thermofisher.com/technical-resources** website. • Instrument Diagnostics: performs a diagnostic check of the Ion PGM™ Dx Sequencer. • End User License Agreement: opens the End User License Agreement (EULA).

(continued)

Users at this level…	Can access...
Manager	Operator functions plus: **References**: displays and allows management of: • Reference Sequences • Panels • Hotspots • DNA Barcodes • Fusion Reference • Fusion Panel • Reporting Gene List • Control Fragments • Primers **Services**: displays system status report for: • Jobs Server • Active Jobs • ionCrawler Service Details • Smart Monitoring Service Details
Administrator	Manager functions plus: **Audit Records**: perform an audit: search, sort, view, export, and print any record. **Configuration**: Configure settings for the: • Network • Email • Instruments • Lab Information • Software Updates **Data Management**: Manage archiving, view available disk space, and manually delete incomplete runs. **Logs**: Manage retention of instrument logs and Torrent Suite™ Dx Software logs. **User Management**: Add, assign user level, and edit user information.

Audit records (administrator)

In the **Audit Records** screen, administrator-level users can use the tools that are provided to review, sort, export, and print audit records.

All components in a diagnostic assay must be uniquely identified, and the identification must be stored so that the record can be audited.

Note: Library Batches and Planned Runs created in a batch with other objects in a single LIMS transaction do not have a "Create" action listed in the **Audit Records** screen. However, you can view their complete audit record from the **Libraries** and **Planned Runs** screens, and any subsequent actions that are performed on them are listed in the **Audit Records** screen. A Library Batch or Planned Run created individually in a LIMS transaction is listed.

Search audit records

Administrator-level users can use the toolbar to search for an existing audit record.

1. Click ⚙ ▸ **Audit Records**.
2. In the **Audit Records** screen, select the search parameters:
   a. To select a date range, click 📅 next to **Start Date** and **End Date**.
      If an end date is not selected, the search results include all records from the start date through the current date.
   b. To limit the results to actions that are performed by a specific user, select the user from the **First Name Last Name** list.
   c. To limit the results to specific actions, select from the **Select Action** list.
   d. To limit the results to a particular sample, select from the **Sample** list.
3. Click **Search**.
4. Click **Clear** to return to the complete list of records.

Sort audit records

In the **Audit Records** screen, the list of audit records is displayed with the oldest record on top by default. Administrator-level users can sort the **Audit Records** screen to find audit records. To return to the default display, click **Audit Records** or **Clear**.

1. Click ⚙ ▸ **Audit Records**.
2. In the **Audit Records**, click the column heading of interest.
   The list of records is reordered based on the headingr name selected. **User**, **Action Performed**, and **Data Object Name** sort alphabetically (**A ▸ Z**).

Click the **Timestamp** heading to reverse the default setting with the most recent record on top.

3. Click the column heading a second time to reverse the order of records displayed.
4. Click **Audit Records** or **Clear** to return to the default display.

Export and print Audit Records

The **Export** function generates a print-ready PDF file of the selected Audit Record.

1. Select the records to be exported by clicking the checkbox next to the record of interest. Select all the records on the screen by selecting the checkbox above the column.
2. Click **Export**.
   A PDF is generated. Depending on your browser settings, the software automatically downloads the file or prompts you to open or save the file.
3. Open the PDF in an appropriate viewer to print.

Update the Audit Configuration

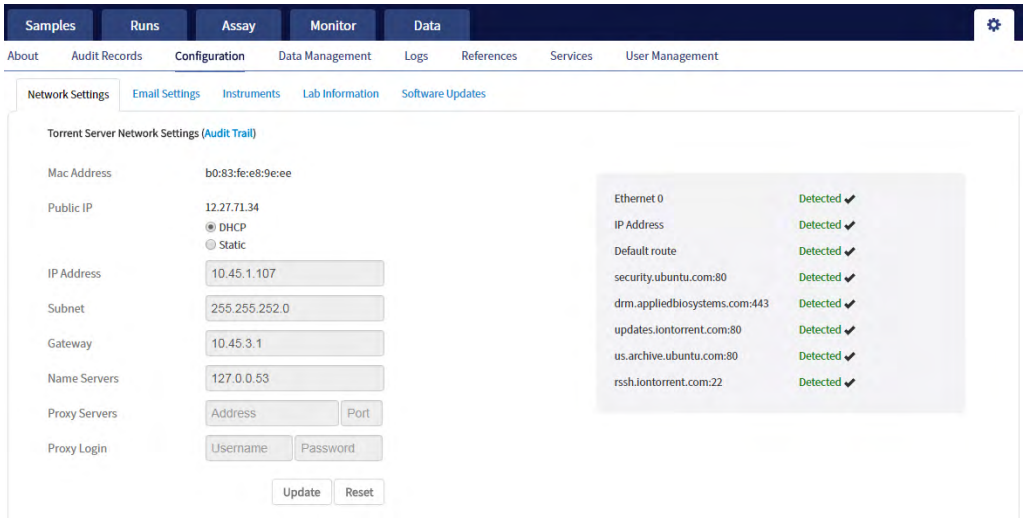
Audit Configuration allows the Administrator to require that a reason for the change is included as part of changing designated objects. To update the Audit Configuration:

1. Click **Audit Configuration**.
2. In the **Audit Configuration** dialog box, click the **Require Reason** checkbox next to the Data Object Name.
3. Click **Save**.
   To remove a reason for change requirement, open the **Audit Configuration** dialog box, deselect the checkbox, then click **Save**.

Configuration *(Administrator)*

Under the tab, in the **Configuration** screen, the settings parameters are grouped by function into 5 subtabs:

- Network Settings
- Email Settings
- Instruments
- Lab Information
- Software Updates



Network Settings

If a problem occurs with the Ion Torrent™ Server, administrator-level users can use network settings information to troubleshoot the cause. Information about the Ion Torrent™ Server is displayed in the **Configuration** screen on the **Network Settings** tab.

An audit trail of the Ion Torrent™ Server network settings is available to view, export, and print by clicking **Audit Trail** on the **Network Settings** tab.

Configure Email Settings

Administrator-level users configure the Ion Torrent™ Server to allow sending email notifications.

Before you begin, obtain SMTP server URL and port information from your IT department.

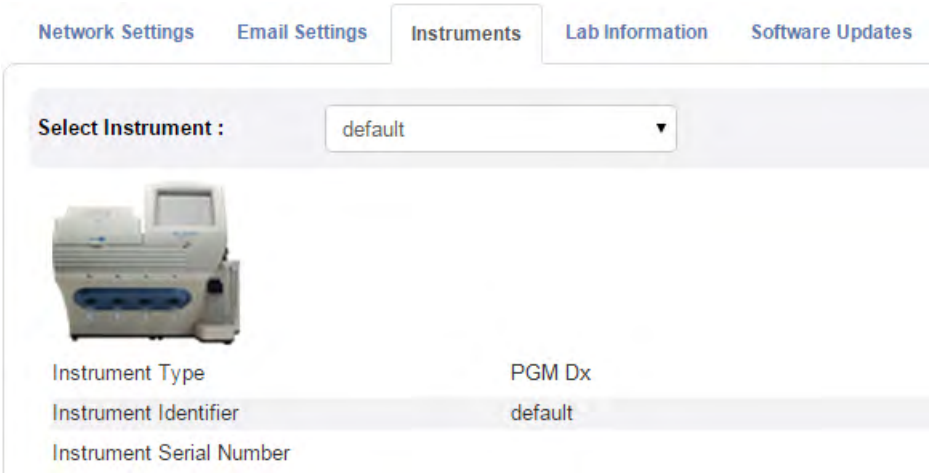
1. Click ⚙ ▸ **Configuration**.
2. Select the **Email Settings** tab.
3. In the **Torrent Server Email Settings** screen, enter the appropriate settings.
   Required fields are indicted with a red asterisk (*).
4. Click **Update**.
5. Click **Send Test Email** to send a test email to the email address provided in the user account.

Instrument configuration

Administrator-level users can view the instrument configuration for an instrument that is connected to the Ion Torrent™ Server, such as:

- **Instrument Type**
- **Instrument Identifier** (name)
- **Instrument State**
- **Last PQ Date**

Click ⚙ ▸ **Configuration** ▸ **Instruments**, then from the **Select Instrument** list, select an instrument and view the instrument details.



Lab Information

IMPORTANT! Your network system administrator should review support personnel contact information periodically to ensure that it is current and accurate.

Administrator-level users can review information about support personnel, listing points of contact if a problem with the Ion Torrent™ Server or a connected Ion PGM™ Dx System instrument occurs.

Click ⚙ ▸ **Configuration** ▸ **Lab Information** to view the lab information.

- **Lab Contact**—This is the person in your organization who should be notified during a support request of problems related to the instrument.
- **IT Contact**—This is the person in your organization who should be notified during a support request of problems related to the Ion Torrent™ Server hardware or the network environment.

Update the software

IMPORTANT! Before updating the software. ensure that all instruments that are connected to the server are idle and no analysis jobs are running on the server or are queued to run.

Administrator-level users can update the software. Use the **Configuration** screen to:

- See the currently installed software version for each software module and component application.
- Check for software updates.
- Install software updates from a USB drive using the following steps.

1. Click ⚙ ▸ **Configuration**.
2. Select the **Software Updates** tab.
3. Click the desired instrument software module link to expand the information.
4. After viewing the software version information, click the software module heading a second time to collapse the information.
5. To check for updates, click **Check for updates**.
   If an available update is found, **Download & Update** appears next to **Check for updates**. If no updates are found, "No updates available" displays.
6. To load an update from a USB drive, connect the USB drive, then click **Check for updates**.
   The software searches the USB drive for updates and lists them.
7. Click **Update Server**.
8. In the confirmation dialog box, click **OK** to start the update.
   After successful completion of the software update, the Ion Torrent™ Server automatically starts rebooting within 2 minutes.
9. After the server has rebooted (~10 minutes), click the browser **Refresh** button to return to the software home page.
   After the update is complete, confirm the software version number in the **Software Updates** tab in the **Configuration screen**.

Data Management (administrator)

Administrator-level users can perform the following functions in the **Data Management** screen under the ⚙ tab.

- Monitor Ion Torrent™ Server disk space usage
- Manage archive settings, and view an audit trail of any changes made

---

Note: 72 hours after completion of data analysis, raw data (DAT) files are deleted from the server to conserve Ion Torrent™ Server disk space.

Disk space usage and data archiving

Ion Torrent™ Server disk space usage is shown in the **Data Management** screen under the ⚙ tab. To maintain sufficient disk space, we recommend implementing a systematic plan to archive older run data, results files, variants information, and signed reports to an external file storage system. Consult your Field Service Engineer to discuss archive and database backup options. Your local system administrator is responsible for establishing your data archive system.

After an archive system has been established, administrator-level users can configure the archive schedule in the **Data Management** screen. Archived results and reports can be restored to the Ion Torrent™ Server and downloaded from the **Completed Runs & Results** screen. See "Restore archived results" on page 52.

Note: If the Ion Torrent™ Server has ≤1 terabyte (TB) of free disk space, an alert notifies the user that there is insufficient disk space when setting up the run on the sequencer. The run cannot proceed until data on the server is archived and deleted. Contact your local system administrator to manually archive and delete data. You should also change the archive schedule.

IMPORTANT!

- If results are restored from runs that were archived in an older version of Torrent Suite™ Dx Software (version 5.6.4 and earlier), the software may not display all the variants from the restored results in the user interface, and reports generated from those restored results may not contain all variants. Carefully review the restored data in the user interface to determine whether all variants are present. Do not generate new reports from results restored from these older archives. Note that all variants are preserved in the restored source files, and can be downloaded using the **Download Files** command. This issue is corrected in version 5.12.5 of the software.
- In older versions of Torrent Suite™ Dx Software (version 5.6.4 and earlier), results reports should be generated and signed before results are archived. See "Sign the run results" on page 64.

Update archive settings

Administrator-level users can view and edit archive settings in the **Data Management** screen. Consult your Field Service Engineer to set up a database archive and backup system.

1. Click ⚙ ▸ **Data Management**.
2. Edit the archive settings.

Setting	Procedure
Auto archive after	Select the number of months for data to remain on the Ion Torrent™ Server before it is archived on an external server. To free up additional space on the Ion Torrent™ Server, reduce this interval.
Archive Directory	Enter the file path to the external archive directory. The default path must be changed before data can be archived.

Note: The **Data Output Directory** is listed in this screen but cannot be changed.

3. Click **Save**.

About the data output directory

The data output directory is the primary location on the Ion Torrent™ Server where data generated by the Ion PGM™ Dx System is stored. The path to the directory is listed in the **Data Management** screen under the ⚙ tab.

Note: The directory location is locked and cannot be changed. Users should configure their LIMS to access this folder location to receive sequencing output files.

After a sequencing run and data analysis are complete, the software creates a separate results folder for each sample in the data output directory using the following naming convention:

Dx_ <Library Name>_<Assay Name>_<PlannedRunShortCode>_<PlannedRunShortCode>

The following files are added to the results folder:

- Run log files: basecaller.log, sigproc.log, analysis.log
- PlannedRun-AuditTrail.pdf: audit trail of the planned run in PDF format
- Assay-specific variant files in VCF and XLS formats
- Dx_allele_counts.xls for each barcode: allele coverage for bases in hot spot regions
- <barcode>_rawlib.basecaller.bam: unmapped BAM file
- <LibPrepID>_rawlib.bam: mapped BAM file
- <LibPrepID>_rawlib.bam.bai: mapped BAM index file
- A checksum file containing checksums for each output file
- analysis.bfmask.stats: contains analysis statistics of wells in the bead find stage
- BaseCaller.json: a JSON format file of the statistics of basecaller, bead summary, filtering, phasing, and training subset
- datasets_pipeline.json: a JSON format file of the settings needed by the pipeline to run the basecaller module
- explog_final.txt: final run settings needed for analysis
- ion_params_00.json: a JSON format file of the detailed settings of the run and analysis arguments
- TFStats.json: a JSON format file of control statistics
- wells_beadogram.png: a figure of statistics to characterize wells
- Test_Report.pdf: an assay-specific PDF report of targeted clinical and analytical variants; can be generated in multiple languages if selected (filenames are appended with language suffix)
- Lab_Report.pdf: similar to the Test Report, but includes all analytical variants detected; can be generated in multiple languages if selected (filenames are appended with language suffix)
- Summary.tab: non-targeted test results summary
- Snvindel.tab: non-targeted SNV/INDEL detail table
- Fusion.tab: non-targeted fusion detail table
- Cnv.tab: non-targeted CNV detail table

- Target_summary.tab: targeted test results summary
- Target_hotspot.tab: targeted SNV/INDEL detail table
- Target_fusion.tab: targeted fusion detail table
- <LibPrepID>_<AnalysisID>.final.vcf: VCF file
- Info.csv: contains sample attributes, reagent information, QC values, and instrument information
- <barcode>_rawlib.basecaller.bam: unmapped BAM file – DNA
- <barcode>_rawlib.basecaller.fastq: FASTQ file – DNA
- <LibPrepID>_rawlib.bam: mapped BAM file – DNA
- <LibPrepID>_rawlib.bam.bai: mapped BAM index file – DNA
- <barcode>_rawlib.basecaller.bam: unmapped BAM file – RNA
- <barcode>_rawlib.basecaller.fastq: FASTQ file – RNA
- <barcode>_rawlib.basecaller.alignments.bam: mapped BAM file – RNA
- <barcode>_rawlib.basecaller.alignments.bai: mapped BAM index file – RNA
- Bead_density_contour.png: loading density figure
- iontrace_Library.png: key incorporation traces figure
- raw_peak_signal: key signal for controls and library
- readLenHisto.png: histogram of the read length
- <LibPrepID>_rawlib.stats.cov.txt: amplicon statistics
- basecaller.log: base caller log file
- sigproc.log: signal processing log file
- analysis.log: analysis log file
- PlannedRun-AuditTrail.pdf: Planned Run audit file

Archive notifications

Administrator-level users receive email notifications about archives when any of the following conditions occur:

- The archive location has ≤120 gigabytes (GB) of free disk space remaining.
- The archive location has not been changed from the default location (`/results/analysis`). The archive path must be changed to that of the external server before data can be archived.
- The archive location is not accessible when the system tries to archive a run.

To receive email notifications about archives, a valid administrator email address must be entered into Torrent Suite™ Dx Software. For more information, see "User Management (administrator)" on page 90.

Additionally, the Ion Torrent™ Server must be configured to allow sending email notifications. For more information, see "Configure Email Settings" on page 78.

Logs (administrator)

Select a log category

Within the ⚙ ▸ **Logs** screen, administrator-level users choose from the **Select Category** list to view and set auto-deletion rules for **Torrent Suite** and **Instrument** logs.

Manage logs

Administrator-level users can manage logs to set retention times and set up auto-deletion of logs.

1. Click ⚙ ▸ **Logs**.
2. Use the **Select Category** list to view and set auto-deletion rules for **Torrent Suite** or **Instrument** logs.
3. Click **Manage Log**.
4. In the **Manage Log File** dialog box, from the **Retention Period** list, select the number of months that logs are to be retained on the server.
5. Select **Enable Auto Deletion** to automatically delete log files after the designated retention period.
6. Click **Save**.

References (manager/administrator)

Under the ⚙ tab in the **References** screen, manager- and administrator-level users can access the following reference files under the subtabs.

Note: Manager- and Administrator-level users can create and obsolete reference files by clicking **Add New** and **Obsolete**, except for those that are system-installed or locked assays.

- Reference sequence files
- Panel files (BED format)
- Hotspots files (BED format)
- DNA barcode sequences
- Fusion reference sequences (FASTA format)
- Fusion panel files (BED format)
- Reporting Gene Lists (Microsoft Excel™ format)
- Control fragments
- Primer sequences (FASTA format)

Manage reference sequences

Manager- and administrator-level users manage the DNA reference sequence files that are available in the software

Manage panel files

Manager- and administrator-level users can view and manage the panel BED files that are available in the software.

1. Click ⚙ ▸ **References**
2. Select the **Panels** tab.
3. To export a panel file, in the **Actions** column, click **Export**.

Manage hotspot files

Manager- and administrator-level users can view and manage the hotspot BED files that are available in the software

1. Click ⚙ ▸ **References**
2. Select the **Hotspots** tab.
3. To export a hotspot file, in the **Actions** column click **Export**.

View DNA barcodes

Manager- and administrator-level users can view the barcode IDs and sequences for each barcode set that is available in the software.

1. Click ⚙ ▸ **References**.
2. Select the **DNA Barcodes** tab.
3. In the **DNA Barcodes** screen, click the name of a barcode set in the list.
   A window opens showing the name and sequence of each barcode in the set.
4. Click **Back** to return to list on the **DNA Barcodes** screen.

Manage fusion references

Manager- and administrator-level users can view and export fusion reference files that are available in the software.

1. Click ⚙ ▸ **References**
2. Select the **Fusion Reference** tab.
3. To export a fusion reference file, in the **Actions** column, click **Export**.

Manage fusion panels

Manager- and administrator-level users can view and export fusion panel BED files that are available in the software.

1. Click ⚙ ▸ **References**
2. Select the **Fusion Panel** tab.
3. To export a fusion panel file, in the **Actions** column, click **Export**.

Reporting Gene List analyses

A Reporting Gene List is a list of specific variants of interest that represent a subset of all the variants that are targeted by a particular assay. The Reporting Gene List is associated with an assay when the assay is created in the software. When the assay is used in a run, the Reporting Gene List determines which variants are reported in the results. A Test Report is only generated after a run if the assay includes a Reporting Gene List. Otherwise, only the Lab Report is generated.

A variant that is detected in a sample is matched with a variant definition in the Reporting Gene List when the following conditions are met:

- The sample origin selected for the sample when it was entered into the software matches the sample origin in the Reporting Gene List.
- A variant that is detected in the sample matches the mutation ID listed in the Reporting Gene List.

The Reporting Gene List has the following features:

- It can be used to group variants of interest into user-defined Reporting Categories in the Test and Lab Reports and in the results screens.
- In addition to Reporting Categories, each variant is assigned a Variant Category of either "blank" or "Analytical" in the Reporting Gene List, and each Variant Category is reported separately in the reports and results.
- Detected variants that are defined with a SNV_Indel prefix in the **Mutation ID** column in the list appear in the **SNV/INDEL** results screen in the software. If the detected variant Test Result is "Positive", it appears in the **Summary** screen.
- Detected variants that are defined with a Fusion prefix in the **Mutation ID** column appear in the **Fusion** results screen. If the detected variant Test Result is "Present", it appears in the **Summary** screen.
- Detected variants that are defined with a CNV prefix in the **Mutation ID** column appear under the CNV subtab. All "GAIN" CNVs (copy number calls with a lower confidence bound value of >4) are displayed in the **Summary** screen.

Note:

- Gene markers that are not defined in the Reporting Gene List are considered analytical gene markers.
- If the Mutation ID prefix (for example, SNV_Indel) and specific Mutation ID (for example, <HotspotID>) are both present, then only the Mutation ID specified in the Reporting Gene List is displayed in the results and reports.

Manage Reporting Gene Lists

The tools for managing Reporting Gene Lists are found under the ⚙ tab in the **References** screen, under the **Reporting Gene List** subtab.

- To view the contents of a Reporting Gene List:
  a. Click the name of the list in the **Name** column.
     A new screen opens showing the gene names, mutation IDs, and other information about the genes in the list.
  b. Click **Back** to return to the list.
- To export the list, click **Export** in the **Actions** column.

Control fragments

Manager- and administrator-level users can review information about control fragments. These internal controls are predefined and cannot be modified by users.

View the control fragment information by clicking ⚙ ▸ **Refrences** ▸ **Control Fragments**. The **Control Fragments** screen provides the following information.

Column	Description
Name	The name of the control fragment.
Sequence	The single letter nucleotide sequence of the control fragment.
Control	The process in which the control fragment is used.
Status	Indicates that the control fragment information is locked and cannot be modified.

Manage primers

Under the ⚙ tab, in the **References** screen on the **Primers** tab, manager- and administrator-level users can view the primer sequences in the software.

Services (manager/administrator)

On the **Services** screen, manager- and administrator-level users can view the status and details of active jobs, ionCrawler service, Smart Monitoring service, and the jobs server. Viewing this information can be useful when troubleshooting error messages during an active job or why an analysis failed.

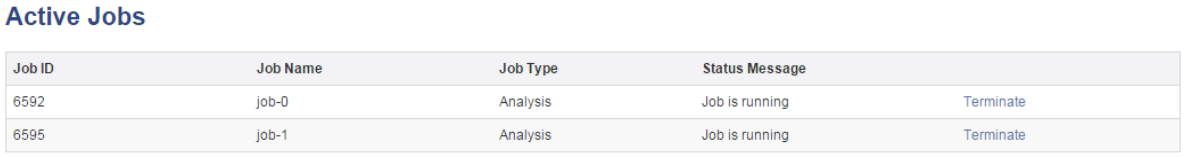
Services

Service	Function
Jobs Server	Lists server information and active (running) software.
Active Jobs	• Lists active and queued analysis jobs on the Ion Torrent™ Server. • When no job is currently active, displays a “No active jobs” message.
ionCrawler Service Details	Displays information about the data transfer process from the Ion PGM™ Dx Sequencer to the Ion Torrent™ Server.
Smart Monitoring Service Details	Allows remote monitoring of Ion PGM™ Dx System.

View or terminate an active job

Manager- and administrator-level users can view jobs that are active on the server and terminate an active job if required. A job is active when the status indicates Job is running.

1. Click ⚙ ▸ **Services**
2. In the **Active Jobs** section, click **Terminate** to stop the active job.



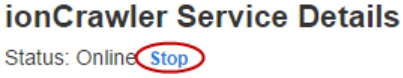
A confirmation message opens, “Are you sure you want to terminate `Job Name` ?”

3. Click **Yes** to terminate the active job.

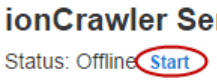
Disable or enable the ionCrawler service

Manager- or adminstrator-level users can manage the IonCrawler service. The ionCrawler service typically remains enabled at all times, but can be disabled for remote service troubleshooting.

- To disable the service:
  a. Click ⚙ ▸ **Services**.
  b. In the **ionCrawler Service Details** section, click **Stop**.



c. In the confirmation message, click **Yes** to stop the service.
The **Stop** option changes to **Start**.



- To enable the service:
  a. Click ⚙ ▸ **Services**.
  b. In the **ionCrawler Service Details** section, click **Start**.
  c. In the confirmation message, click **Yes** to start the service.
  The **Start** option changes to **Stop**.

Disable or enable the Smart Monitoring service

Manager- and administrator-level users can manage the Smart Monitoring service.

The Smart Monitoring service allows Thermo Fisher Scientific personnel to remotely monitor the status of the Ion PGM™ Dx System through an internet connection. Smart Monitoring employs multiple layers of security, including a Secure Sockets Layer (SSL) and Lightweight Directory Access Protocol (LDAP) authentication, to provide real-time troubleshooting and problem resolution for the Ion PGM™ Dx System.

The Smart Monitoring service is active by default and can be disabled by manager- or administrator-level users, if required.

- To disable the service:
  a. Click ⚙ ▸ **Services**.
  b. In the **Smart Monitoring Service Details** section, click **Stop**.
  c. In the confirmation message, click **Yes** to disable the Smart Monitoring service.
  The **Stop** option changes to **Start**.
- To enable the service:
  a. Click ⚙ ▸ **Services**.
  b. In the **Smart Monitoring Service Details** section, click **Start**.
  c. In the confirmation message, click **Yes** to enable the Smart Monitoring service.
  The **Start** option changes to **Stop**.

User Management (administrator)

Administrator-level users can use the ⚙ tab, in the **User Management** screen, to perform the following actions:

- Add users
- Assign user privileges (roles)
- Edit user information
- View user account audit trails
- Manage user account policies

Add a new user

Note: The email settings on the Ion Torrent™ Server must be configured before new users can receive emails. See "Configure Email Settings" on page 78 for more information.

1. To add a new user account, under the ⚙ tab, in the **User Management** screen, click ⊕ **Add New**.
2. In the **Create New User** dialog box, enter the account information.

   Note: Required fields are indicated by a red asterisk (*).
3. Select the user-access level (Administrator, Manager, or Operator) from the **Role** list.
4. To enable the ability to sign reports (Manager- or Administrator-level users only), select the **Electronic Signature** checkbox.
5. Click **Save**.
   The Ion Torrent™ Server sends a temporary password to the email address of the new user.

Set security policies

Administrator-level users set user account security policies. User account security policies include the permissible number of failed sign-in attempts, password lifetime, and the length of inactivity before a user is automatically signed out of the software.

1. Click ⚙ ▸ **User Management**.
2. In the **User Management** screen, click **Policies**.
3. Update the **Policies** dialog box. Accept the default values or select the appropriate values to set the user account suspension, password, and session policies, then click **Save**.
4. Select**Enabled** to enable the session policy settings.
5. Click **Save**.

Sort users

Administrator-level users can sort the list of users to help find a user account to work with.

1. Click ⚙ ▸ **User Management**.
2. Click the header name of interest to sort alphabetically.
3. Click the header name a second time to reverse the order of users displayed.
4. Click **User Management** to return to the default display.

Edit user accounts

Administrator-level users can edit user account information.

1. Click ⚙ ▸ **User Management**.
2. In the **User Name** column, click **Edit** under a user name.
3. In the **Edit User Details** dialog box, enter the desired changes to the account information.
   - From the **Role** list, select the user-access level (**Administrator**, **Manager**, or **Operator**).
   - To allow manager- or administrator-level users to sign reports, select the **Electronic Signature** checkbox.
   - In the **Status** list, select the appropriate status (**Active**, **Suspend**, or **Disable**).
4. Click **Save** to make the changes.

Reset password

Administrator-level users can reset user account passwords.

Note: Operator level users can reset their passwords by following the directions included in the system-generated email notification of a pending password expiration.

---

1. In the Edit User Details window, c Click **Reset Password**.
2. In the **Reset User Password** dialog box, click **Send password in email**, to email a new password to the email address entered in the user account.
3. Alternatively, enter a new password into the **Password** field.
4. Reenter the new password into the **Re-type Password** field, then click **Save**.

   The confirmation message "Password reset successfully" appears. Click **Back**, then **Save** to return to the **User Management** window.

View user audit trail

The user audit trail is a record of when the user account was created and modified.

1. Under the ⚙ tab, in the **User Management** screen, in the **Actions** column, click **Audit**.
2. In the **Audit Trail** dialog box, click 📄 **(Details)** under **Record**.
3. In the **Audit Record Details** dialog box, click **Export** to export a PDF of the record.

A

Troubleshooting

Troubleshooting is divided into the following sections:

Torrent Suite™ Dx Software

Observation	Possible cause	Recommended action
Cannot sign in to the Ion Torrent™ Server	You have either forgotten your password or are signed out due to several failed login attempts.	Contact a Torrent Suite™ Dx Software administrator-level user.
Cannot sign in to the Ion PGM™ Dx Sequencer	The Ion PGM™ Dx Sequencer lost its connection to the Ion Torrent™ Server.	Contact a Torrent Suite™ Dx Software administrator-level user.
Cannot import my sample	Sample ID is greater than 20 characters.	Limit Sample ID to 20 characters or fewer.
	Expired library kit.	Use a nonexpired library kit.
	Incorrect library kit barcode.	Rescan the correct library kit barcode.
Batch sample import fails	One or more entries in the sample-import spreadsheet contains special characters, lines breaks, unexpected spaces, incorrect entry length, incorrect date formatting, or other formatting errors.	Check each entry for correct formatting, correct any errors, and repeat the import.
	Blank rows were copied into the sample-import template file from a different source.	Rows that appear empty may contain hidden formatting that conflicts with the import function. Start with a clean sample-import template file, and be careful to copy only those rows that contain actual data.
	The sample import spreadsheet contains a nonunique Sample ID.	Every Sample ID in the software must be unique. Make sure the spreadsheet does not contain any duplicate IDs, and repeat the import. Note that the system check is not case-sensitive, so a Sample ID of ABC1 conflicts with abc1.

Observation	Possible cause	Recommended action
Batch sample import fails *(continued)*	The headings in the sample import spreadsheet do not match the sample attributes in the software.	The headings must match the sample attributes in the software exactly. Check the headings for spelling or other errors.
Library batch import fails	One or more entries in the library batch import spreadsheet contains special characters, lines breaks, unexpected spaces, incorrect entry length, incorrect date formatting, or other formatting errors.	Check each entry for correct formatting, correct any errors, and repeat the import.
	Blank rows were copied into the library batch import template file from a different source.	Rows that appear empty can contain hidden formatting that conflicts with the import function. Start with a clean library batch import template file, and be careful to copy only those rows that contain actual data.
	The library batch import spreadsheet contains a nonunique Library Batch ID.	Every Library Batch ID in the software must be unique. Ensure that the spreadsheet does not contain any duplicate IDs, and repeat the import. Note that the system check is not case-sensitive, so a Library Batch ID of ABC1 conflicts with abc1.
	A Sample ID entered in the library batch import spreadsheet does not match a Sample ID listed in the **Manage Samples** screen.	Ensure that the Sample IDs entered into the spreadsheet are correct and match an existing sample ID added to the software.
	The Barcode ID name format does not exactly match the format that is used in the **Prepare Library Batch** dialog box.	Use the following Barcode ID name format: IonDx-1 through IonDx-16.
	An invalid library, control, or panel kit barcode has been entered in the spreadsheet.	Ensure that you have correctly entered a valid kit barcode in the appropriate cell of the spreadsheet.
The assay I created does not appear in the menu when I plan a run	Forgot to lock your assay.	Go to **Assay tab** ▸ **Manage Assays** and make sure that the assay is locked.
Cannot upload my panel or hotspots	Issues with BED file format or files do not end in (.bed).	Ensure your file is in the correct BED format and has a (.bed) extension.
Cannot execute my Planned Run **Details:** "System error" message is displayed	The Tube Label you assigned the **Planned Run** is not unique. **Note:** The Ion PGM™ Dx Sequencer Instrument Control software does not allow use of the same Tube Label text within 7 days.	Assign the **Planned Run** a new, unique Tube Label.

Observation	Possible cause	Recommended action
Cannot execute my Planned Run **Details:** "System error" message is displayed *(continued)*	Incorrect template prep kit barcode was entered.	Rescan the correct template prep kit barcode.
The results of my run are not showing up in the Data tab	Ion Torrent™ Server disk space is full.	Clear disk space on the Ion Torrent™ Server. After sufficient disk space is cleared, data transfer from the Ion PGM™ Dx Sequencer to the Ion Torrent™ Server recommences and data analysis proceeds.
	Ion PGM™ Dx Sequencer lost connection to the Ion Torrent™ Server.	Reestablish the Ion PGM™ Dx Sequencer Ethernet connection to the Ion Torrent™ Server. Once the connection has been restored, data transfer from the Ion PGM™ Dx Sequencer to the Ion Torrent™ Server recommences and data analysis proceed.
Cannot find file links to the BAM and VCF file	The run failed. Links to BAM and VCF files are not available for runs that fail QC.	Repeat the run.
Loading metrics are reported as 0	• Chip failure • Defective pariposer • Chip leak	Refer to sequencing troubleshooting for possible causes of chip or pariposer failure.
	Sample preparation failure resulting in loss of sample	Refer to troubleshooting for sample, library, and template preparation.
QC Status is **Failed** due to one or more QC metrics listed as **N/A** **Details:** Under the **Monitor** tab in the **Run View** screen, under **View QC Details**, one or more of the QC metrics (Library RNA, Library DNA, CF-1, DNA Control, RNA Control, DNA NTC, and RNA NTC) have a value of **N/A** and the **QC Status** is **Failed**.	• The control associated with the QC metric was not added to the reaction or otherwise failed to generate a measurable value. • The QC status was not updated properly in the database due to a software issue. This is uncommon.	For Administrator-level users, the **Repopulate QC** button is displayed under the **Monitor** tab in the **Run View** screen after a run. 1. Click **Repopulate QC** to update all QC metrics in the database. **Note:** Repopulation takes 15–30 minutes. 2. If any metric is still listed as **N/A** and the **QC Status** is still **Failed**, repeat the run starting at template preparation from the DNA/RNA combined library, and ensure that you add all controls. If the problem continues, contact Technical Support.
Run analysis does not complete **Details:** If the **Analysis Status** for a run is listed in the **Monitor** tab as "RUNNING" for more than 12 hours, a **Restart Analysis** button appears in the tab (Administrator-level users only).	Run analysis has stalled in the analysis pipeline.	Click **Restart Analysis** to restart the analysis from the beginning.

Observation	Possible cause	Recommended action
Run analysis does not complete, and Restart Analysis is not successful	A known software issue exists where the database status of a run is not updated properly. This behavior is uncommon.	If clicking **Restart Analysis** in the **Monitor** tab does not result in a completed analysis, repeat the run starting at template preparation from the DNA/RNA combined library. If the problem continues, contact Technical Support.
I want more/less stringent variant calling	Parameters chosen while setting up your Assay are not appropriate.	1. Go to **Assays tab ▸ Manage Assays**, click **Reanalysis** to create a new reanalysis assay choosing appropriate parameters. 2. Under the **Data** tab find your **Sample ID**, click on the number under the **Results** column, and click **Reanalyze** under Actions. 3. Select your newly created reanalysis assay, enter a report name, then click **Reanalyze**.
Allele coverage does not match Hotspot coverage	The coverage value reported under the Variants tab and Allele Coverage tab can be different.	No action required. The coverage value reported under the Variants tab is the coverage after down-sampling, while the Allele Coverage tab reports the raw coverage without down-sampling. Down-sampling can speed up variant calling for some over-sampled positions. The down-sampling threshold for Ion PGM™ Dx variant calling is 400.
Variants tab is missing hotspot entries. **Details:** The remaining entries are present.	Hotspot BED file contains entries that are incorrectly formatted.	Check that BED file entry is correctly formatted. See the following examples: SNP entry: chr1 2337276 2337277 SVA_322 0 + REF=C;OBS=T;ANCHOR=G AMPL Deletion entry: chr1 201341175 201341180 SVA_497 0 + REF=AGAAG;OBS=;ANCHOR=C AMPL Insertion entry: chr1 236978992 236978992 SVA_621 0 + REF=;OBS=TCTG;ANCHOR=T AMPL
		Confirm the REF values match the actual reference coordinate of hg19.
Target summary does not meet expectation **Details:** For example: • number of targets don't match up • coverage uniformity is below expected value	Incorrect barcode used for sample prep.	Verify the Sample ID-Patient ID agreement, then repeat the run using the correct sample library. Re-prepare the sample library from the patient sample if necessary.
	Wrong panel was chosen while setting up your assay or wrong assay chosen while setting up your Planned Run.	If you start a run with the wrong panel, there is no way to reanalyze using the software. Use your library to prepare another template reaction.

Observation	Possible cause	Recommended action
No information for my loci of interest in the results	The wrong hotspot or BED file is associated with the assay.	1. Check the hotspot and BED files associated with the assay. If either is incorrect, create a new assay. 2. Set up a new Planned Run for the sample library with the correct assay. 3. Repeat sequencing of the sample library.

Library preparation

Observation	Possible cause	Recommended action
Run QC passed but low coverage uniformity (<95%)	Poor purification causes loss of short amplicons	Vortex AMPure™ XP reagent thoroughly before use, and be sure to dispense accurate volume.
	Inappropriate primer design	Redesign Assay for degraded or low quality samples.
Run QC passed, low percent full length On Target Reads, but Control passed	Inappropriate primer design	Redesign Assay for degraded or low quality samples.
	Library prep failure	Reprepare the sample library.
Run QC passed but low percent on target reads	Inappropriate primer design	Redesign Assay for degraded or low quality samples.
Samples passed but CF-1 Mean AQ20 Read Length failed	Reads filtered out due to high polyclonal ISPs caused by too much library added to the Ion OneTouch™ Dx amplification reaction	Perform library input titration.
		Repeat Ion OneTouch™ Dx run using less library in the amplification reaction.
Run QC passed, but one or more sample libraries failed Percent Reads QC metric	Library prep failed due to unwashed beads.	Be sure to wash the library beads prior to use.
	Library preparation failed due to wrong library amplification primers.	Use the LIB Primers provided in the Ion PGM™ Dx Library Kit.
	Library prep failed due to residual salt after wash.	Carefully remove all wash solution prior to elution.
	Library prep failed due to mis-quantification of input DNA.	Requantify input DNA.
	Library prep failed due to inefficient PCR, digestion, or ligation.	Ensure that you properly dispense and mix the viscous components at each step.
		Ensure that you use the correct thermal cycling conditions.
	Library prep failed due to the library being discarded during purification of the amplified library.	Be sure to save the supernatant during first-round purification and save the library pellet during the second round purification of the amplified library.

Observation	Possible cause	Recommended action
Run QC passed, but one or more sample libraries failed Percent Reads QC metric *(continued)*	Library prep failed due to over-drying of the AMPure™ XP beads.	Do not dry the AMPure™ XP beads more than 5 minutes.
		Ensure that you dispense exactly 10 µL of capture reagent to the amplified library.
	Library failed due to ineffective capture of the amplified library.	Ensure that the library capture reagent is at room temperature before use.
		Ensure that you dispense exactly 10 µL of capture reagent to the amplified library.
		Make sure to mix completely and incubate for 5 minutes at room temperature.
Run QC passed, but one or more library samples failed Percent Reads QC metric - continued	Reads filtered out due to primer-dimers.	In unamplified library purification, ensure that you use the correct amount of AMPure™ XP reagent.
		Do not combine Barcode Adapters, LIB DNA Ligase, and LIB Switch Soln prior to addition.
		Ensure that Barcode Adapters are diluted properly.
	Reads filtered out due to low quality ISPs.	Repeat the Ion OneTouch™ Dx run. If it still happens, then reprepare the library.
	Reads filtered out due to high polyclonal ISPs caused by failed consumables.	Repeat the Ion OneTouch™ Dx run. If it still happens, then reprepare the library.
	Reads filtered out due to high polyclonal ISPs caused by too much library added to the Ion OneTouch™ Dx amplification reaction.	Perform library input titration.
		Repeat Ion OneTouch™ Dx run using less library in the amplification reaction.
	Forgot to add the library to amplification reaction.	Repeat the Ion OneTouch™ Dx run. If observation recurs, then reprepare the library.

Template preparation

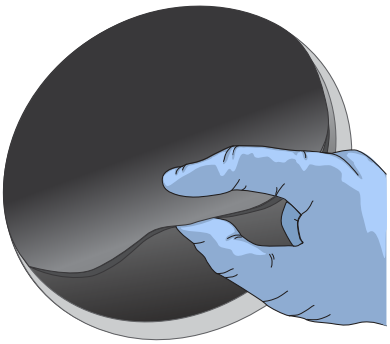
Observation	Possible cause	Recommended action
Display message: Failed to set up system time at startup. Check your connection to the Ion Torrent™ Server.	Ion OneTouch™ Dx Instrument and Ion Torrent™ Server connection is not established	Check your network connection to the Ion Torrent™ Server to make sure the connection is established, then reboot the Ion OneTouch™ Dx Instrument.
	Instrument is still in the process of establishing a connection	Allow 10 minutes to see if the display message clears.
Display message: Failed to connect to the Ion Torrent™ Server. Check your connection.	Ion OneTouch™ Dx Instrument and Ion Torrent™ Server connection is not established during startup	Check your network connection to the Ion Torrent™ Server to make sure the connection is established, then reboot the Ion OneTouch™ Dx Instrument.
Display message: Pressure too high. Reboot the instrument to clear the alarm.	• Hardware issue • Clogged TMPL Emulsion Cartridge due to contaminated reagents or defective emulsion cartridge	Reboot the Ion OneTouch™ Dx Instrument or Ion Chef™ Instrument to clear the alarm. Use a new TMPL Emulsion Cartridge and fresh reagents to repeat the run. Contact Technical Support (see "Customer and technical support" on page 126) if the issue persists.
Display message: TEC current too high. Reboot the instrument to clear the alarm.	Hardware issue	Contact Technical Support (see "Customer and technical support" on page 126). Reboot the Ion OneTouch™ Dx Instrument or Ion Chef™ Instrument to clear the alarm. **IMPORTANT!** A sample created during a run with this alarm raised must NOT be used.
Display message: Coolant pump does not flow.	Hardware issue	Contact Technical Support (see "Customer and technical support" on page 126). Reboot the Ion OneTouch™ Dx Instrument or Ion Chef™ Instrument to clear the alarm.
Display message: Sensor unable to measure instrument temperature	Hardware issue	Contact Technical Support (see "Customer and technical support" on page 126). Reboot the Ion OneTouch™ Dx Instrument or Ion Chef™ Instrument to clear the alarm.
Display message: Software versions incompatible. Go to the Options menu and update the software.	The system software was updated, but the instrument software was not.	After the system software has been updated, update the instrument software as follows: 1. On the main menu of the instrument, press Options and follow the instructions to check for and install updates. 2. When installation is complete, follow the onscreen prompts to restart the instrument. **IMPORTANT!** You must restart the instrument before proceeding.

Observation	Possible cause	Recommended action
Display message: Sensor unable to measure pressure.	Hardware issue	Contact Technical Support (see "Customer and technical support" on page 126). Reboot the Ion OneTouch™ Dx Instrument or Ion Chef™ Instrument to clear the alarm.
Display message: Connection failure with Ion Torrent™ Server	Ion OneTouch™ Dx Instrument and Ion Torrent™ Server connection is not established	Check that a network connection to the Ion Torrent™ Server is established, then reboot the Ion OneTouch™ Dx Instrument. **Note:** A sample created during a run with this alarm raised can still be used.
Display message: Motor current too high. Reboot the instrument to clear the alarm.	Hardware issue	Contact Technical Support (see "Customer and technical support" on page 126). Reboot the Ion OneTouch™ Dx Instrument or Ion Chef™ Instrument to clear the alarm. **Note:** A sample created during a run with this alarm raised can still be used.
Display message: Set temperature out of range. Reboot the instrument to clear the alarm.	Hardware issue	Contact Technical Support (see "Customer and technical support" on page 126). Reboot the Ion OneTouch™ Dx Instrument or Ion Chef™ Instrument to clear the alarm. **IMPORTANT!** If this alarm is raised, you cannot use the prepared template.
TMPL CF-1 fails QC metric(s); all sample libraries pass QC metrics	TMPL CF-1 reagent not added	Repeat Ion OneTouch™ Dx Instrument or Ion Chef™ Instrument run and Ion PGM™ Dx Sequencer run, and ensure that the TMPL CF-1 reagent is added per procedure description.
	The minimum Key Signal value of the CF-1 control required for the assay is >0.	The minimum Key Signal value for the CF-1 control (i.e., the Templating Control) is set to 0 in all preinstalled assays and new custom assays in Torrent Suite™ Dx Software 5.12.5. Custom assays created in previous versions of the software may have a higher Key Signal value for the control.
Ion OneTouch™ Dx Instrument screen hangs and user interface is not responsive after clean or post run clean	• The ambient temperature has gone above its maximum limit (35°C) and the Ion OneTouch™ Dx Instrument is waiting for the ambient temperature to go below 35°C. • The run pump had an error code from a failed operation and the Ion OneTouch™ Dx Instrument was not able to recover from it.	Reboot the Ion OneTouch™ Dx Instrument.

Sequencing

Observation	Possible cause	Recommended action
Run report says run failed	Run failed because one or more QC metrics were not met	Refer to the *Ion PGM™ Dx System User Guide* troubleshooting section.
I created a Planned Run but the Ion PGM™ Dx Sequencer does not recognize the Short Code	**Planned Run** was not executed.	In Torrent Suite™ Dx Software: 1. Select the Planned Run, then click **Execute**. 2. Complete the dialog box, then click **Save**.
	The Ion PGM™ Dx Sequencer and Ion Torrent™ Server have lost connection.	Check the network connection to the Ion Torrent™ Server to ensure that the connection is established, then reboot the instrument.
A QC metric meets the reference range however, the QC status says it failed	The actual value is less than the minimum value in the reference range, however the number is displayed as a value rounded up to two digits (e.g., a QC value is reported as 0.01 but the actual value is 0.0094 and is rounded up to 0.01)	The sample or run did not pass QC, see appropriate troubleshooting section and repeat the run if desired.
TMPL CF-1 fails QC metric(s); all sample libraries fail QC metrics	• Forgot Enzyme • Forgot Seq primer • Used wrong annealing program	Repeat Ion OneTouch™ Dx Instrument or Ion Chef™ Instrument run and Ion PGM™ Dx Sequencer run.
Run was aborted before sequencing due to a critical alarm	The Ion PGM™ Dx Sequencer aborted the run before sequencing began (e.g., during chip calibration) due to a critical alarm.	Reboot the sequencer to clear the alarm, then restart the sequencing run. Rebooting is necessary to ensure that all pre-sequencing steps are completed on the instrument.
Display message: Lost connection to the Ion Torrent™ Server	The connection between the instrument and the server has been lost.	Check the network connection to the Ion Torrent™ Server, and then reboot the Ion PGM™ Dx Sequencer. If this alarm appears during a run, the data created during that run can still be used.
Display message: Failed to set up system time at startup. Check your connection to the Ion Torrent™ Server.	The connection between the Ion PGM™ Dx Sequencer and the Ion Torrent™ Server has been lost.	1. Check the network connection to the Ion Torrent™ Server to make sure the connection is established, then reboot the instrument. 2. If the problem persists, replace the network cable(s) to the instrument and server. 3. If the problem persists, contact Technical Support (see "Customer and technical support" on page 126).
	Instrument is still in the process of establishing a connection	Allow 10 minutes to see if display message clears.

Observation	Possible cause	Recommended action
Display message: Bad boot drive detected	Hardware issue	Contact Technical Support (see "Customer and technical support" on page 126). If this alarm appears during a run and data for the run is generated, that data may still be used.
Display message: UBoots do not match	Hardware issue	Contact Technical Support (see "Customer and technical support" on page 126).
Display message: Kernels do not match	Hardware and/or software issue	Contact Technical Support (see "Customer and technical support" on page 126).
Display message: Bad results data drive	• On some machines, the warning appears before the reboot completes. • There is a hardware issue.	Wait for a few minutes to see if the error message disappears. If the error message disappears, data obtained during a run with this alarm raised can still be used. If the problem persists, contact Technical Support (see "Customer and technical support" on page 126).
Display message: Lost chip connection	The instrument cannot detect a chip in the chip clamp.	Refer to the *Ion PGM™ Dx System User Guide* troubleshooting section.
Display message: Lost communication with valve board	Hardware issue	Contact Technical Support (see "Customer and technical support" on page 126).
Display message: Sensor unable to measure instrument temperature	Hardware issue	Contact Technical Support (see "Customer and technical support" on page 126).

Observation	Possible cause	Recommended action
Display message: Instrument temperature too high	• Room temperature is too high. • Clogged filter or blocked airway on the instrument • Hardware issue (fan is not running or running too slowly)	**Note:** The data created during a run with this alarm raised may still be used if all the QC metrics are met. 1. If the ambient room temperature is above 30°C, lower it. 2. Make sure that the round filter on the back panel of the instrument has unrestricted airflow. If the filter is clogged with dust, clean it as follows: a. Pinch the dirty filter with your fingers, then remove it from the instrument.  b. Shake the filter over a waste container to remove most of the dust. c. Rinse the filter with running water to remove any remaining dust. The water flow should be from the inside-facing surface to the outside-facing surface through the filter. d. Air dry the filter. e. Blot any remaining dust from the filter using tape. f. Reinsert the filter. If the problem persists, contact Technical Support (see "Customer and technical support" on page 126).
Display message: Instrument temperature too low	• Room temperature is below 20°C. • Hardware issue	**Note:** The data created during a run with this alarm raised may still be used if all the QC metrics are met. If the ambient room temperature is below 20°C, raise it. If the problem persists, contact Technical Support (see "Customer and technical support" on page 126).

Observation	Possible cause	Recommended action
Display message: Chip temperature too high	• Room temperature is too high. • Clogged filter or blocked airway on the instrument • Hardware issue (instrument fan is not running or running too slowly)	**IMPORTANT!** The data created during a run with this alarm raised should *not* be used. See the recommended action for "Display message: Instrument temperature too high" on page 103.
Display message: Chip temperature too low	Hardware issue	**IMPORTANT!** The data created during a run with this alarm raised should *not* be used. Contact Technical Support (see "Customer and technical support" on page 126).
Display message: Instrument idle temperature too high	• Room temperature is too high. • Clogged filter or blocked airway on the instrument • Hardware issue (fan is not running or running too slowly)	**Note:** The data created during a run with this alarm raised may still be used if all the QC metrics are met. See the recommended action for "Display message: Instrument temperature too high" on page 103.
Display message: Instrument idle temperature too low	• Ambient room temperature is below 20°C. • Hardware issue	Bring the ambient temperature up to 20°C. If the problem persists, contact Technical Support (see "Customer and technical support" on page 126).
Display message: Fan current too low	Hardware issue	Contact Technical Support (see "Customer and technical support" on page 126).
Display message: Heater current too low	Hardware issue	**IMPORTANT!** If the chip temperature is also out of range, data created during a run should *not* be used. Contact Technical Support (see "Customer and technical support" on page 126). If no chip temperature alarms are raised, data created during a run may still be used if all the QC metrics are met.
Display message: Pressure too high	Internal pressure regulator was not set correctly	Contact Technical Support (see "Customer and technical support" on page 126).

Observation	Possible cause	Recommended action
Display message: Pressure too low.	• Gas line is not connected to the instrument • Gas cylinder may be turned off or empty	1. Verify that the gas line is connected to the instrument. 2. Verify that the gas cylinder has at least 500 psi on the pressure gauge inside the tank and 30 psi on the gauge that regulates pressure to the outlet. (Both pressure gauges are mounted on the gas cylinder.) 3. Confirm that the outlet valve on the regulator is turned on. 4. If the problem persists, contact Technical Support (see "Customer and technical support" on page 126).
Display message: Sensor unable to measure gas pressure. Check supply gas pressure.	• Gas line is not connected to the instrument • Gas cylinder may be turned off or empty • Hardware issue	1. Verify that the gas line is connected to the instrument. 2. Verify that the gas cylinder has at least 500 psi on the pressure gauge inside the tank and 30 psi on the gauge that regulates pressure to the outlet. (Both pressure gauges are mounted on the gas cylinder.) 3. Confirm that the outlet valve on the regulator is turned on. 4. If the problem persists, contact Technical Support (see "Customer and technical support" on page 126).
Display message: Failed to set the pressure to target range. Check the gas connection and try again.	• Gas line is not connected to the instrument • Gas cylinder may be turned off or empty • Hardware issue (regulator malfunction)	1. Verify that the gas line is connected to the instrument. 2. Verify that the gas cylinder has at least 500 psi on the pressure gauge inside the tank and 30 psi on the gauge that regulates pressure to the outlet. (Both pressure gauges are mounted on the gas cylinder.) 3. Confirm that the outlet valve on the regulator is turned on. 4. If the problem persists, contact Technical Support (see "Customer and technical support" on page 126).
Display message: Failed to locate the barcode scanner. Check if the scanner is attached.	The connection between the barcode scanner and the Ion PGM™ Dx Sequencer has been lost.	Make sure the scanner is plugged into a USB port on the instrument. If it is connected and the alarm still appears, try plugging the scanner into a second USB port. If the alarm persists, contact Technical Support (see "Customer and technical support" on page 126).

Observation	Possible cause	Recommended action
Display message: Results drive not accessible. Reboot and try again.	• On some machines, the warning appears before the reboot completes • Hardware issue	Wait for a few minutes to see if the error message disappears. If the error message appears and disappears during a run, data obtained during that run can still be used. If the alarm persists, contact Technical Support (see "Customer and technical support" on page 126).
Display message: Results drive check failed	Hardware issue	If the error message disappears when you return to the main instrument screen, this alarm can be ignored. Otherwise, contact Technical Support (see "Customer and technical support" on page 126).
Display message: Unable to mount the file system	Hardware and/or software issue	1. Reboot the instrument to clear the alarm. 2. If the alarm is not cleared after reboot, contact Technical Support (see "Customer and technical support" on page 126).
Display message: Failed to set up FTP connection. Check your connection to the Ion Torrent™ Server .	The network connection is not established or an incorrect IP address was used.	Confirm that the server information is correct for the Ion Torrent™ Server. Contact your local network administrator for support if the issue persists.

B

References

Variant finding parameters

Parameter	Definition
Hotspot Min Cov Each Strand	The minimum number of reads for each strand overlying a potential variant that allows the calling of the variant (default setting = 3). User can set the minimum coverage for each strand to ≥3.
Hotspot Variant Score	See "Variant Quality Score definition" on page 107.
Hotspot Min Allele Freq	Minimum allele frequency set to 10%. A variant is not called when variant sequences are present at less than 10% of the total.
Hotspot Min Coverage	Minimum number of reads overlying a potential variant which allows the calling of the variant.
Hotspot Strand Bias	Indicates the maximum percentage of reads that can come from one strand and still have a valid variant call.

Variant Quality Score definition

Underlying statistical model

Reads are observed from a population with some (unknown) variant allele frequency. We evaluate the likelihood of each read under the assumption that the read comes from a "reference" allele or a "variant" allele, in the context of the ensemble of all reads. This allows the Torrent Suite™ Dx Software to construct a quality measure (change in log-likelihood) for each read, given the variability observed across all relevant reads. This log-likelihood allows the software to evaluate whether reads evaluated as individuals by the basecaller as variants or reference are mistakes, or real support for reference or variant. Using a uniform likelihood framework allows the software to evaluate insertions, deletions, or SNPs in the same way, so that all variant classes are handled in the same way, and scores can be compared across variants.

This likelihood is evaluated based on the fit of the measured signal values for each read when compared to the predicted signal values for each read. These signal values are used without correction for phase, because this reflects the real observational variation in the data instead of a transformed version. One important aspect of using the uncorrected data is that a variant at a single position can cause changes across many flows: both because of out-of-phase populations, and because the flows in which predicted signal occurs can differ. This generally provides strong statistical support for SNPs, insertions, and deletions that cause such disruption to the flow positions of predicted signal.

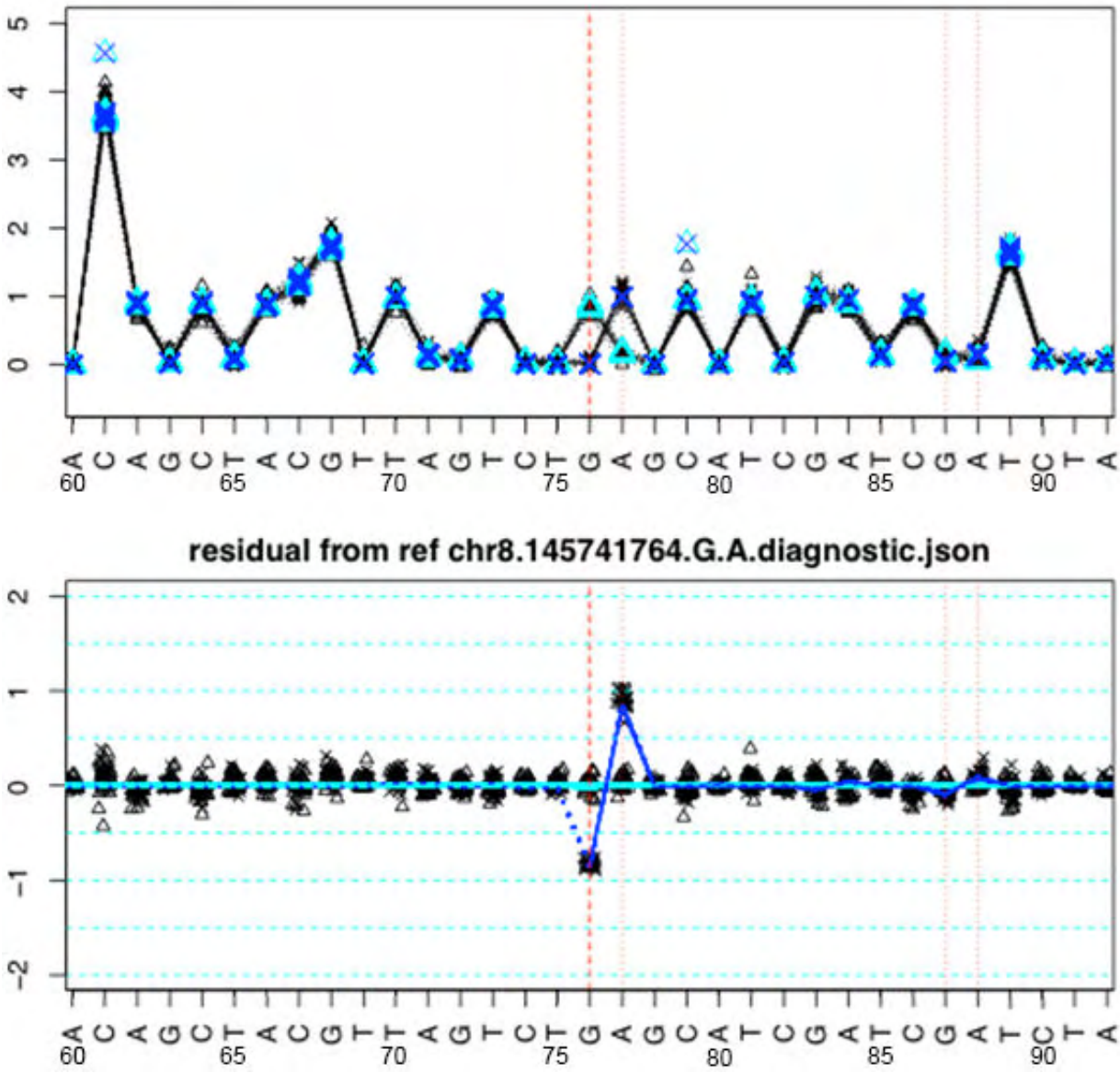


Figure 1 Forward strand measured intensities

Measured intensities on the forward strand for the SNP G->A at chr8, 145741764 (zero based). The primary in-phase population affects the signal strongly at flows 76 and 77, showing a strong difference between states compared to the variability. Predicted values under each hypothesis are shown in cyan and blue. Reference calls are triangles, variant calls are ×'s. Residuals shown below to more clearly show the size of the residuals under the reference predictions. Note the "echo" of out-of-phase population at flows 87 and 88, which also contribute to the evaluation of these reads.

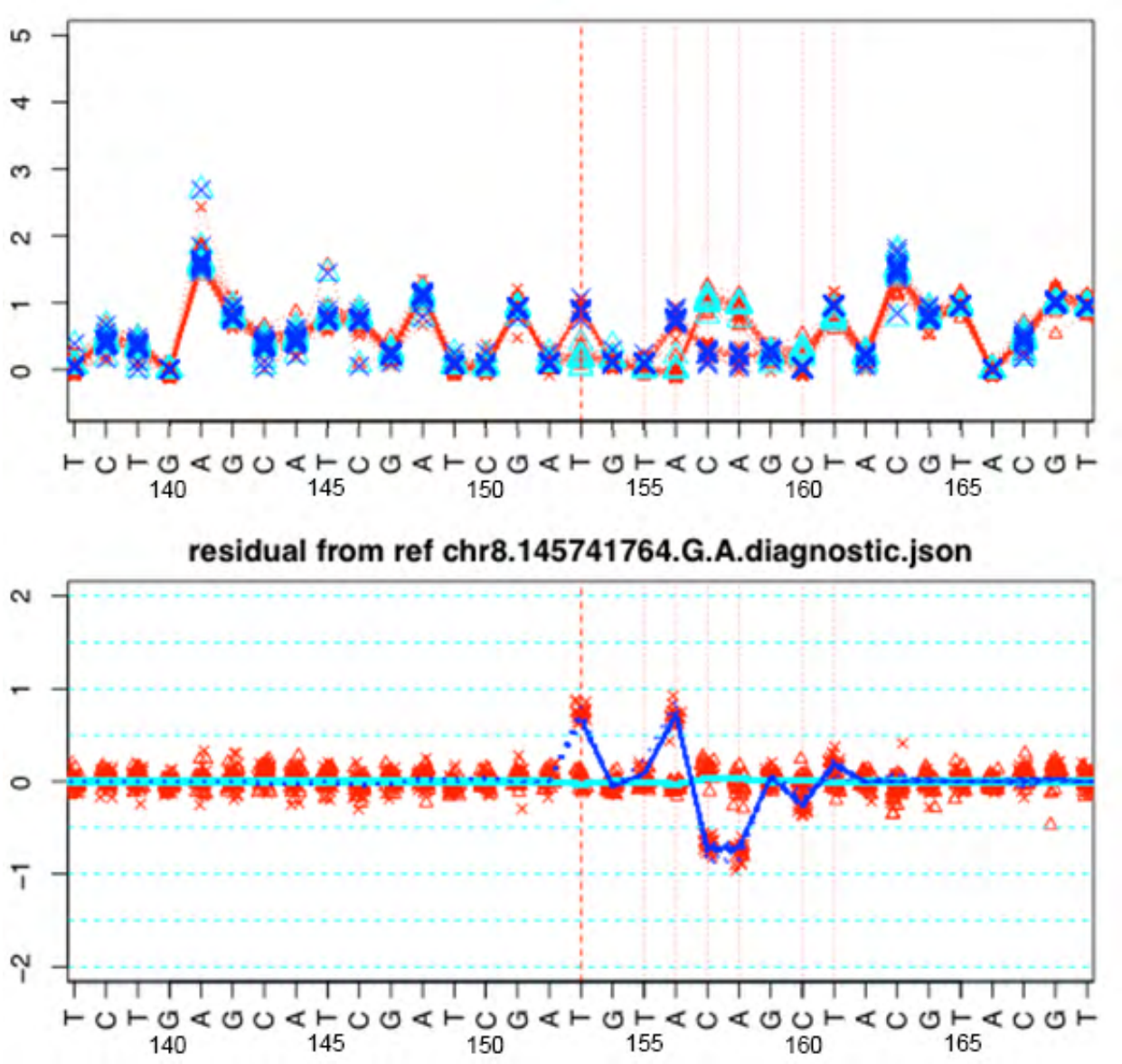


Figure 2 Reverse strand measured intensities

This is the exact same SNP on the reverse strand. On this strand, it is a T->C change in flows 153 and 157. Additional changes are induced at flows 156 and 158 as the "A" signal changes its position in the variant case when compared to the reference case. This provides additional support for the variant or reference, not obtainable by inspecting the bases independently, or deconvolving the flow signal.

To evaluate this likelihood for a given measurement when compared to a prediction, the Torrent Suite™ Dx Software uses a t-distribution (heavy tailed to resist outliers), with a mean and variance fitted to the data. The mean is generally near zero, as predictions are generally accurate, but the system allows a bias term for each strand to adapt the predictions to any small systematic deviations within the data. The software provides an initial precision estimate for the mean to downweight deviations from zero (prediction-precision), but average with the data in standard Bayesian fashion. The variance is estimated based on the predicted intensity: the system software fits the nonlinear trend of variance by intensity to the data in all flows relevant to the variant across the ensemble of reads, then uses the predicted variance for each relevant individual flow in each read for the likelihood evaluation.

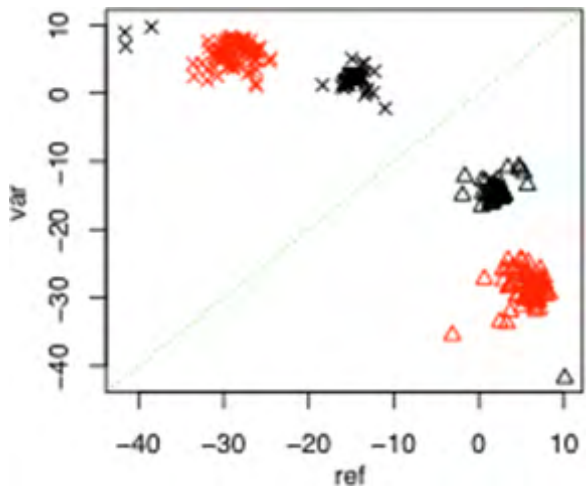


Figure 3 Log-likelihood of variant versus reference

The log-likelihood of each read under the reference or variant hypothesis, under the bias and variance fit to the data. As seen previously, the reverse strand reads in this case provide more statistical support per read than the forward strand (although both are quite well separated).

The software fits these latent variables of bias and variance, as well as the likely probability of each read being a variant or reference using an EM algorithm. Given the current bias, variance and variant allele frequency, the system can assign each read a responsibility for reference or variant. Using the responsibility (soft-classification), improved estimates for bias, variance, and the variant allele frequency are derived. After the software has converged to appropriate bias and variance terms, the posterior distribution of variant frequency given the ensemble of reads are evaluated.

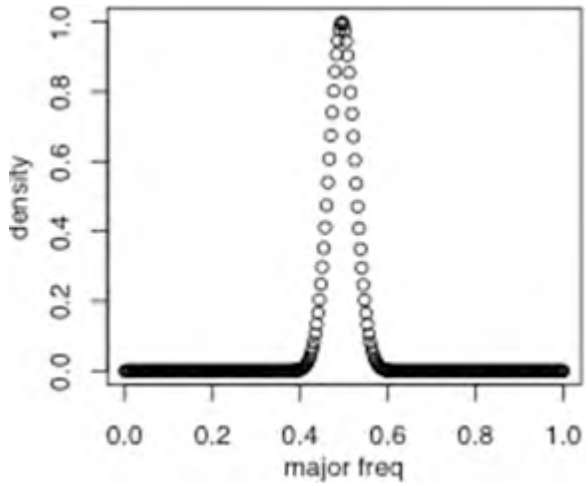


Figure 4 Posterior distribution of variant frequency

Posterior distribution of log-likelihood for the reference allele frequency of this SNP, scaled so the maximum density is 1.0. Note that while each read has extremely strong statistical support, sampling variability still causes some uncertainty in the inference of the allele frequency in the sample.

Variant Quality Score

The variant Quality Score is a measure of how strong the evidence is for the variant call. The preceding statistical model (page 107) provides a posterior distribution for allele frequency given the observations (and latent variables). This posterior observation is translated into a variant Quality Score (the **QUAL** field score according to the VCF standard), which is the Phred-scaled (-10*log10) relative probability under whatever statistical model that the assertion made about the variant is incorrect.

In our case, the assertion made is that the sample variant allele frequency is greater than the specified minimum allele frequency for a nonreference call, or that the sample variant frequency is less than the specified minimum allele frequency for a reference call. This posterior probability is computed by integrating out the posterior likelihood obtained from the observed reads over the two intervals [0,threshold), [threshold,1], and includes sampling variability.

Typically this value is very large for reads strongly distinguishing variants with good depth (that is, under the model assumed, evidence is overwhelming for the variant or for the reference). The variant Quality Scores are capped at 100. Marginal values in this field can mean either that the reads do not distinguish between alleles well or there is insufficient depth of coverage, or the observed frequency is near the cutoff.

As with all variant callers, filters are used to determine cases in which the assumptions behind the statistical model do not match the observed data, and therefore may make no-calls even in the case when there is apparently large statistical support in the reads. Filters used within the variant caller are:

- Reference
- SSE
- Strand bias
- Stringency
- Cross Bias
- Coverage
- Prediction shift
- Prediction hypershift
- Hplen

MAPD Copy Number QC metric

The Median of the Absolute values of all Pairwise Differences (MAPD) score is reported on Aneuploidy analyses and other analyses that detect CNVs.

MAPD is one of the metrics that we define to assess whether the panel data is useful for copy number (CN) analysis.

MAPD is defined as the Median of the Absolute values of all Pairwise Differences between log2 ratios per tile for a given run. Tiles roughly correspond to amplicons in an Ion AmpliSeq™ assay. Each pair is defined as adjacent in terms of genomic distance. Tiles corresponding to Copy Number amplicons and other amplicons are being treated equally as no differences in variability are seen between these types. Then, any two log2 ratios that are adjacent on the genome are a pair. Except at the beginning and the end of a chromosome, every log2 ratio belongs to two pairs.

Formally, if *xi* is the log2 ratio at position *i*, with *i* ordered by genomic position:

```
MAPD = median ( | x(i - 1) - x(i) | )
```

MAPD is a per-sequencing run estimate of copy number variability, similar to standard deviation (SD). If one assumes the log2 ratios are distributed normally with mean 0 and a constant SD, then MAPD/0.95 is approximately equal to SD. However, unlike SD, using MAPD is robust against high biological variability in log2 ratios induced by known conditions such as cancer.

Regardless of the source of the variability, increased variability decreases the quality of CN calls.

Note: MAPD is not shown on the QC Report.

Fusions parameters

Fusion algorithm parameters include:

Parameter Name	UI Group	Allowed Values	Default Value	Description
Sensitivity	Main	Low/Medium/High (Fixed values only one of the three can be applied)	Medium	For High value, the algorithm requires 60% overlap between reads and reference sequence with at-least 50% exact matches in the overlap. For Medium value, the algorithm requires 70% overlap between reads and reference sequence with at-least 66.66% exact matches in the overlap. For Low value, the algorithm requires 80% overlap between reads and reference sequence with at-least 75% exact matches in the overlap.
Minimum Read Counts for Fusions	Main	≥0 Integers only	20	Minimum number of valid reads aligned to a specific fusion isoform sequence required to call the isoform as Present. The normalized read count must also be greater than the threshold.
Minimum Read Counts for Non-Targeted Fusions	Advanced	≥0 Integers only	250	Minimum number of valid reads aligned to a non-targeted fusion sequence required to call the fusion as Present.
Minimum Read Counts for Controls	Advanced	≥0 Integers only	15	Minimum number of valid reads aligned to specific expression control sequence required to call it as Present.

(continued)

Parameter Name	UI Group	Allowed Values	Default Value	Description
Minimum Normalized Read Counts for Fusions	Main	≥0 Float values.	0	Minimum normalized read counts threshold required to call a fusion isoform as present.
Minimum Total Valid mapped reads	Main	≥0 Integers only	20000	Minimum number of total valid mapped reads required to qualify a Sample as Valid and to proceed with the analysis.
Minimum Total Control reads	Advanced	≥0 Integers only	1200	Minimum number of housekeeping control reads required to compute Imbalance scores for 5p3p Assays.
Make calls based on Imbalance Score	Main	True/False Boolean	True	If this flag is set to true, Imbalance scores are used to make Fusion presence, absence, or Nocall calls.
Maximum Imbalance for Negatives	Advanced	Text field String value in specific format as shown in the default value. Verify the user's input using a Regular expression.	ALK=0.001 RET=0.03 ROS1=0.2	If the Imbalance score of any driver gene is less than this value, the sample is called Fusion Negative for that gene.
Minimum Imbalance for Positives	Advanced	Text Field String value in a specific format as shown in the default value. This verifies the user's input using a Regular expression.	ALK=0.015 RET=0.55 ROS1=0.5	If the Imbalance score of any driver gene is greater than this value, the sample is called Fusion Positive for that gene. However, there is grey zone between maximum and minimum values where scores are called NOCALL. If they are equal, there is no grey zone.

(continued)

Parameter Name	UI Group	Allowed Values	Default Value	Description
Minimum Isoform Counts for Imbalance	Advanced	Text Field String value in a specific format as shown in the default value. This verifies the user's input using a Regular expression.	ALK=5 RET=5 ROS1=5	If the sum of counts from all the isoforms of that driver gene is greater than this number, different and thresholds (the two parameters below) are used for the Imbalance scores.
Maximum Imbalance for Negatives with evidence from Isoforms	Advanced	Text Field String value in a specific format as shown in the default value. This verifies the user's input using a Regular expression.	ALK=0.001 RET=0.3 ROS1=0.15	If the Imbalance score of any driver gene is less than this value, the sample is called Fusion Negative for that gene.
Minimum Imbalance for Positives with evidence from Isoforms	Advanced	Text Field String value in a specific format as shown in the default value. This verifies the user's input using a Regular expression.	ALK=0.01 RET=0.25 ROS1=0.5	If the Imbalance score of any driver gene is greater than this value, the sample is called Fusion Positive for that gene. However, there is grey zone between Maximum and minimum values where scores are called NOCALL. If they are equal, there is no grey zone.
Estimate max crosstalk	Advanced	≥0 (% values) Float values	0.5	Maximum percentage of spill-over reads that could be seen in any sample due to reasons like Barcode Crosstalk.

System installation templates (service engineers only)

IMPORTANT! The templates used by service engineers during Ion PGM™ Dx System installation are listed under the **Assay** tab in the **Install Templates** screen. These should not be used or modified in any way.

C

LIMS Integration

Overview of API functions in the software

Note: If a LIMS system is configured to retrieve analysis files from the Ion Torrent™ Server, manual FTP setup or drive mapping is required. This configuration is not provided as part of Ion Torrent™ Server installation, and must be set up by your LIMS system integration or IT group.

Torrent Suite™ Dx Software integrates with laboratory information management systems (LIMS) to facilitate sample, library and Planned Run creation. The interaction is through the LIMS calling a set of RESTful APIs provided by the software (see the following table). The LIMS connects to the software by making an HTTP call to the URL of an API, and sending a user name and a password in the HTTP header. The user name and password must be for a registered active user account of the software. The LIMS passes the input information in XML format within the HTTP body, while the response from the software is returned in XML format as well. Users must configure their LIMS to create XML (.xml) files containing the required attributes for each LIMS request file. See "Predefined sample attributes" on page 24 for more information.

Outlined in the following table are the API name, function, and URL of the five RESTful APIs provided.

API	Function	API URL
Sample and Library Batch Creation	To add sample and library information and create a library batch with the associated libraries. Once the API is called, the software creates samples, libraries, and library batches in the database and returns the object IDs.	http://{Torrent Suite IP address}/ir/lims/create-library-batch
Planned Run Creation	To add a Planned Run name, assay information, Library Batch ID, and Library Prep ID. Once the API is called, the software responds with the Run Short Code.	http://{Torrent Suite IP address}/ir/lims/create-plan
Analysis Status Check	To query the status of a Planned Run. Once the API is called, the software identifies the associated libraries and sends the response containing output folder path and library prep analysis status.	http://{Torrent Suite IP address}/ir/lims/get-plan-status?plan_name={plan name}
Barcode Set Retrieval	To get a list of all barcode sets and the barcode names, IDs, and sequences associated with each set.	http://{Torrent Suite IP address}/ir/lims/get-barcode-list

(continued)

API	Function	API URL
Reporting Template	To get a list of all report templates.	http://{Torrent Suite IP address}/ir/lims/get-reporting-template-list
Signed Off Samples	A function to locate sample results that have been signed off.	http://{Torrent Suite IP Address}/ir/api/ir/samples?offset={Page number}&limit={Page size}&signedOff={Fetch only signed off samples or not}&start_date={Start Date}&end_date={End Date}

LIMS system FTP setup or drive mapping

To enable the LIMS system to retrieve analysis files from the Ion Torrent™ Server, a manual FTP setup or drive mapping is required. This configuration is not provided as part of the Ion Torrent™ Server, and must be set up as part of the LIMS system by the system integration or laboratory IT group. See the sections in this appendix for file paths and other setup information.

Create-Library-Batch API

Format the Library Batch API

Note: All request and response files are in XML format only.

1. Set the Create Library batch API:
   - URL to: `http://{reporterDx_ip}:{port}/ir/lims/create-library-batch`
   - Header to: username={ user name}, password={user password}, Content-Type= application/xml
   - Request Type = PUT
2. Complete the input fields:

Field	Description
Assay	A mandatory field that populates the Assay Display name in Torrent Suite™ Dx Software .
LibraryKitBarcode	This field depends on the assay configuration. If the assay mandates a Library kit barcode to be recorded and validated, then LIMS must send this information. Otherwise it should be blank.
ControlKitBarcode	This field depends on the assay configuration. If the assay mandates a control kit barcode, then LIMS must send this information. Otherwise it should be blank

(continued)

Field	Description
Control	This field depends on the assay configuration. If the assay mandates any controls, controls should be specified. Every control will have two attributes with the names 'cName' and 'cValue'. These will hold the control name, either 'DNA Positive Control' or 'DNA No Template Control (NTC)', and barcode assigned to that control. Control name and values for each element must be present and unique.
BarcodeSet	A mandatory field that holds the barcode set name for the Assay. All barcodes present in the request must be within the given barcode set.
Sample	A mandatory field that holds elements that specify sample information. It can be single nodes or multiple nodes.
SampleID	A mandatory field that holds a unique value within the Torrent Suite™ Dx Software database. SampleID is the unique ID for each sample within Torrent Suite™ Dx Software database.
PatientID	Mandatory field. It does not have to be unique.
DateofBirth	Mandatory field. yyyy-MM-dd format.
OrderingPhysician	Mandatory field. It does not have to be unique.
SampleSource	Optional field.
SampleCondition	Optional field.
Gender	Enter 'Male',' Female' or 'Unknown.
CollectionDate	Mandatory field. yyyy-MM-dd format.
ReferenceInterval	Optional field.
SampleType	Mandatory field.
ExtractionKitBarcode	This field depends on the assay configuration. If the assay mandates an extraction kit barcode to be recorded and validated, then LIMS must send this information. Otherwise it should be blank.
SampleNotes	Optional field.
Attribute	Optional field. All sample attributes are validated against pre-defined sample attribute list in Torrent Suite™ Dx Software database for name and type.
LibraryPrep	Mandatory field. It is a single node for each sample and contains library nodes.
Library	Mandatory field. It can be single node or multiple nodes (max =2).
LibraryType	Library type for each library must be present and the value should be DNA.
LibraryName	Library name for each library must be present and should be unique within Torrent Suite™ Dx Software database.
BarcodeID	Barcode ID for each library must be present. It is validated against the barcode set and must be unique within the Planned Run.
InputQuantity	Optional field. A number.
LimsBatchExternalID	Mandatory and can contain only alphanumeric characters, periods, underscores, and hyphens.

Library Batch Validations

In order for Create Library batch API request to be valid, the following conditions must be met:

- SampleID must be unique within Torrent Suite™ Dx Software database.
- Library name must be unique within Torrent Suite™ Dx Software database.
- Library Kit Barcode, Control Kit Barcode, Panel Kit Barcode and Extraction kit barcode should be provided per Assay configuration. If the assay mandates any or all of these barcodes, they need to be provided otherwise leave the fields blank.
- Controls should be provided as per assay configuration.
- Library type is validated per assay configuration. For example, if the assay is a DNA assay, the software validates that each sample has an associated DNA library.
- Each control in a batch should have a unique barcode ID.

Library Batch Response Fields

Users must configure their LIMS to receive .xml files with the following attributes in response to the Create Library Batch API request.

Field	Description
BatchId	This is the batch ID generated from and provided by LIMS in order for LIMS to identify the entity in LIMS after response from Torrent Suite™ Dx Software comes back.
Status	This field indicates success or failure of the call. In case of success, sample and library batch are created. In case of failure, transaction is rolled back and no entities are created.
LibraryPrepID	This field holds the Library Prep ID generated by Torrent Suite™ Dx Software. It can be multiple nodes. Only one library prep can be created per sample.
LibraryPrepIDs	This element holds one or more LibraryPrepID nodes.
@sampleID	This attribute under LibraryPrepID is used by LIMS to identify which library prep belongs to which sample.
LogMessages	Contains one or more message nodes. It must be a single node.
Message	It can contain multiple nodes that hold success or failure information.
Mode	Set to "Dx".

Example Library Batch API .xml Files

Request type: PUT

```
<?xml version="1.0" encoding="UTF-8"?>
<LibraryBatchRequest>
  <Assay>CustomAssay</Assay>
  <LibraryKitBarcode>0100000123000017101212-123456817191124210010024 0A18928 </
LibraryKitBarcode>
  <PanelKitBarcode>0100000123000017101212-123456817191124210010024 0A28638 </
PanelKitBarcode>
  <ControlKitBarcode>0100000123000017101212-123456817171230210010024 0A18943 </
ControlKitBarcode>
  <LibraryBatchID>OCP_BATCH_TEST_ONE </LibraryBatchID>
  <BarcodeSet>Ion Dx BC Set-1</BarcodeSet>
  <Controls>
    <Control cValue="IonDx-3" cName="DNA No Template Control (NTC)"/>
  </Controls>
  <Samples>
    <Sample>
      <SampleID>Sample2</SampleID>
      <OrderingPhysician>DR NO</OrderingPhysician>
      <CollectionDate>2012-01-01</CollectionDate>
      <SampleSource>Blood</SampleSource>
      <SampleCondition>Good</SampleCondition>
      <SampleType>plasma</SampleType>
      <Gender>Unknown</Gender>
      <RefernceInterval>3</RefernceInterval>
      <SampleNotes>sample test</SampleNotes>
       <ExtractionKitBarcode>0100001230000171012123456817123021001002 40A18942
</ExtractionKitBarcode>
      <LibraryPrep>
        <Library>
          <LibraryType>DNA</LibraryType>
          <BarcodeID>IonDx-4</BarcodeID>
          <LibraryName>LIMS1</LibraryName>
          <InputQuantity>1</InputQuantity>
        </Library>
      </LibraryPrep>
    </Sample>
  </Samples>
</LibraryBatchRequest>
```

Response:

```
<?xml version="1.0" encoding="UTF-8" standalone="yes"?>
<LibraryBatchResponse>
  <Mode>AssayDev</Mode>
  <Status>Success</Status>
  <BatchId>1</BatchId>
  <LibraryPrepIDs>
    <LibraryPrepID specimenID="Sample2">1</LibraryPrepID>
  </LibraryPrepIDs>
  <LogMessages>
    <Message>Specimen Id [Sample2] created Successfully.</Message>
    <Message>Library [LIMS1] Added Successfully.</Message>
    <Message>Library [LIMS2] Added Successfully.</Message>
    <Message>Control [DNA No Template Control(NTC)] Added Successfully.</Message>
    <Message>Library Batch Added Successfully.</Message>
```

```
  </LogMessages>
</LibraryBatchResponse>
```

Create Planned Run API

Format the Planned Run API

Note: All request and response files are in XML format only.

1. To format the Create Planned Run API, set the:
   - URL to: `http://{reporterDx_ip}:{port}/ir/lims/create-plan`
   - Header to: username={ user name}, password={user password}, Content-Type= application/xml
   - Request Type = PUT
2. Complete the input fields:

Field	Description
PlannedRunName	A mandatory field that holds a unique value within theTorrent Suite™ Dx Software database. This is a single node. Once the planned run is created, it can only be modified manually through Torrent Suite™ Dx Software web user interface.
Assay	This is mandatory field. A locked assay with this name must be present in the Torrent Suite™ Dx Software database.
Tubelabel	This is a mandatory single node. Tube labels must be unique among all Planned Runs created or executed in the past 7 days.
TemplateKitBarcode	This field depends on assay configuration. If the assay requires a template kit barcode, it must be provided.
Reporting Template	This field depends on assay configuration.
PlanRunNotes	Optional field.
LibraryPrepIDs	This is a mandatory single node that contains LibraryPrepID.
LibraryPrepID	It can be single or multiple nodes that contain valid library prep IDs within Torrent Suite™ Dx Software.

Planned Run Validations

In order for the Create Plan API request to be valid, the following conditions must be met:

- Planned Run name must be unique within Torrent Suite™ Dx Software database.
- LibraryPrepID's must exist within the Torrent Suite™ Dx Software database.
- Tube labels must be unique among all Planned Runs created or executed in the past 7 days. Tube label must be 10 characters or less in length, and only contain alphanumeric characters (0–9 and A to Z), full stop/period (.), underscore (_), and hyphen (-). Tube labels are case insensitive.

Planned Run Response Fields

Users must configure their LIMS to receive .xml files with the following attributes in response to the Create Plan API request.

Field	Description
Mode	Set to "Dx".
Status	This field indicates success or failure of the call. In case of success, sample and library batch are created. In case of failure, the transaction is rolled back and no entities are created.
PlanShortCode	A single node contains generated Run Short Code.
LogMessages	Contains one or more message nodes. It must be a single node.
Message	It can contain multiple nodes that hold success of failure information.

Example Planned Run .xml Files

Request type: PUT

```
<PlanRunRequest>
  <PlannedRunName>CustomPlan</PlannedRunName>
  <Assay>CustomAssay</Assay>
  <TubeLabel>test</TubeLabel>
  <TemplateKitBarcode>91A18930101212-12345671603172100001301</
TemplateKitBarcode>
  <ReportingTemplate>CustomReport</ReportingTemplate>
  <PlanRunNotes>This is my 1st Plan</PlanRunNotes>
  <LibraryPrepIDs>
    <LibraryPrepID specimenID="">1</LibraryPrepID>
  </LibraryPrepIDs>
</PlanRunRequest>
```

Response:

```
<?xml version="1.0" encoding="UTF-8" standalone="yes"?>
<PlanRunResponse>
  <Mode>Dx</Mode>
  <Status>Success</Status>
  <PlanShortCode>RS2I8</PlanShortCode>
  <LogMessages>
    <Message>Experiment with Planned Run Name [CustomPlan] Added
Successfully.</Message>
    <Message>Planned Run Name [CustomPlan] Executed Successfully.</Message>
  </LogMessages>
</PlanRunResponse>
```

Analysis Status Check API

Format the Analysis Status API

Note: All request and response files are in XML format only.

1. To format the Analysis Status API, set the:
   - URL to: `http://{reporterDx_ip}:{port}/ir/lims/get-plan-status?plan_name={plan_ name}`
   - Header to: username={ user name}, password={user password}, Content-Type= application/xml
   - Query Parameter: LIMS sends a query parameter with name 'plan_name'. This parameter contains the actual Planned Run name for the status to be returned.
   - Body: not required
   - Request Type = GET
2. Complete the fields:

Field	Description
PlannedRunName	The Planned Run name must be a single node.
PlanShortCode	The information about the generated run short code. This must be a single node.
LibraryPrepResult	The status and output path information for each library prep. This may be multiple nodes.
@status	The analysis status of library prep for a given Planned Run.
@outputPath	The output path where analysis results will be stored. LIMS should have access to this path.
@specimenID	The Sample ID for which library prep was created.
LogMessages	The unique node. It will contain Message nodes.
Message	The failure messages. There is no message in case of success. This can be multiple nodes.

Example Analysis Status API .xml Files

Request type: GET, no request body required.

Response:

```
<?xml version="1.0" encoding="UTF-8" standalone="yes"?>
<LimsStatusReponse>
  <Mode>Dx</Mode>
  <PlannedRunName>CustomPlan</PlannedRunName>
  <PlanShortCode>RS2I8</PlanShortCode>
  <LibraryPrepResult libraryPrepId="1" specimenID="Sample2"
status="Not Started" outputPath="/results/analysis/output/reports/Dx_LIMS1-
LIMS2_CustomPlan_RS2I8_RS2I8"/>
  <LogMessages/>
</LimsStatusReponse>
```

Barcode Set API

Format Barcode Set API

Note: All request and response files are in XML format only.

1. To format the Barcode Set API, set the:
   - URL to: `http://{reporterDx_ip}:{port}/ir/lims/get-barcode-list`
   - Header to: username={ user name}, password={user password}, Content-Type= application/xml
   - Body: not required
   - Request Type = GET
2. Configure the LIMS to receive XML files with the following attributes in response to the Barcode Set API request:

Output Field	Description
BarcodeSetList	A unique node that holds a list of barcode sets.
BarcodeSet	The BarcodeSetName and BarcodeSetId. This can be one or more nodes.
Barcode	The barcode name, barcode ID, and barcode sequence. This can be one or more nodes.

Example Barcode Set .xml Files

Request type: GET, no request body required.

Response:

```
<?xml version="1.0" encoding="UTF-8" standalone="yes"?>
<BarcodeSetList>
  <Mode>Dx</Mode>
  <BarcodeSet>
    <BarcodeSetName>Ion Dx BC Set-1</BarcodeSetName>
    <BarcodeSetId>6</BarcodeSetId>
    <Barcode>
      <Name>IonDx-1</Name>
      <BarcodeId>1</BarcodeId>
      <Sequence>CTAAGGTAAC</Sequence>
    </Barcode>
    <Barcode>
      <Name>IonDx-2</Name>
      <BarcodeId>2</BarcodeId>
      <Sequence>TTACAACCTC</Sequence>
    </Barcode>
      …
  </BarcodeSet>
</BarcodeSetList>
```

Reporting Template API

Format the Reporting Template API

Note: All request and response files are in XML format only.

1. To format the Reporting Template API, set the:
   - URL to: `http://{reporterDx_ip}:{port}/ir/lims/get- reporting-template-list`
   - Header to: username={ user name}, password={user password}, Content-Type= application/xml
   - Body: Not required
   - Request Type = GET
2. Configure the LIMS to receive .xml files with the following attributes in response to the Reporting Template API request:

Output Field	Description
ReportingTemplateList	The root element. It is be a unique node that holds ReportingTemplate nodes.
ReportingTemplate	This can be one or more nodes that hold the report template name and ID.

Example Report Template .xml Files

Request type: GET, no request body required.

Response:

```
<?xml version="1.0" encoding="UTF-8" standalone="yes"?>
<ReportingTemplateList>
  <Mode>Dx</Mode>
  <ReportingTemplate>
    <Name>temp1</Name>
    <Id>1</Id>
    <AssayName>DNA Germline Default</AssayName>
  </ReportingTemplate>
  <ReportingTemplate>
    <Name>Install Template</Name>
    <Id>2</Id>
    <AssayName>InstallAssay</AssayName>
  </ReportingTemplate>
  <ReportingTemplate>
    <Name>CustomReport</Name>
    <Id>3</Id>
    <AssayName>CustomAssay</AssayName>
  </ReportingTemplate>
</ReportingTemplateList>
```

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support
- Order and web support
- Safety Data Sheets (SDSs; also known as MSDSs)

Additional product documentation, including user guides and Certificates of Analysis, are available by contacting Customer Support.

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are printed and shipped with the product.

Obtaining Certificates of Conformance

The Certificate of Conformance provides information on conformance testing of each instrument provided with the system. Certificates of Conformance are shipped with the instrument, and are also available by contacting Customer Support at **thermofisher.com/support**.

ion torrent
by Thermo Fisher Scientific

Ion PGM™ Dx System Performance Characteristics

USER GUIDE

Publication Number MAN0018763

Revision A.0

CE IVD

For In Vitro Diagnostic Use.

Life Technologies Holdings Pte Ltd |
Block 33 |
Marsiling Industrial Estate Road 3 |
#07-06, Singapore 739256

Life Technologies Corporation |
7335 Executive Way |
Frederick, MD 21704 | USA

EC REP Life Technologies Europe B.V.
Kwartsweg 2, 2665 NN Bleiswijk
The Netherlands

Products manufactured in Singapore:

Ion PGM™ Dx Instrument System
Ion PGM™ Dx Sequencer
Ion OneTouch™ Dx Instrument
Ion OneTouch™ ES Dx Instrument
Ion PGM™ Dx Chip Minifuge (120V or 230V)
Ion PGM™ Wireless Scanner
Ion Torrent™ Server
Torrent Suite™ Dx Software
Torrent Suite™ Assay Development Software
Veriti™ Dx 96-well Thermal Cycler, 0.2 mL

Products manufactured in Frederick:

Ion Torrent Dx FFPE Sample Preparation Kit
Ion PGM™ Dx Library Kit
Ion OneTouch™ Dx Template Kit
Ion PGM™ Dx Sequencing Kit
Ion 318™ Dx Chip
Ion OneTouch™ Rack Kit
DynaMag™ Dx 96-Well Plate Magnet
DynaMag™ Dx 16 2-mL Magnet

The information in this guide is subject to change without notice.

DISCLAIMER

TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0018763

Revision	Date	Description
A.0	2 September 2020	New user guide with updated results for the tissue input, interfering substances, and assay reproducibility studies

Contents

About this guide

Purpose of this guide

This user guide provides summaries and tables of performance results for the Ion PGM™ Dx System based on testing of the system with DNA and RNA from formalin-fixed, paraffin-embedded (FFPE) tissue samples and genomic DNA (gDNA) from whole blood samples. This guide is used in conjunction with the following guides:

- The *Ion PGM™ Dx System User Guide* (Pub. No. MAN0018761) provides instructions for using the system instruments and kits for sample preparation, library preparation, template preparation, and sequencing.
- The *Torrent Suite™ Dx Software 5.12.5 User Guide* (Pub. No. MAN0018762) provides instructions for using Torrent Suite™ Dx Software to plan and monitor sequencing runs and perform data analysis.

Limitations for FFPE samples

The Ion PGM™ Dx System has been validated to detect SNVs, MNVs, and deletions in DNA and fusions in RNA in non-small cell lung cancer (NSCLC) FFPE tumor slide specimens. Results presented for FFPE samples were obtained using the Ion PGM™ Dx System and associated reagents with a representative assay, and are provided for informational purposes only. The validation testing with the representative assay only establishes the instrument's general capabilities and does not establish the instrument's capabilities or suitability with respect to any specific claims. All diagnostic tests developed for use on this instrument require full validation for all aspects of performance.

1 Performance characteristics of the system with FFPE samples

This section describes studies documenting the performance of the Ion PGM™ Dx System with DNA and RNA from formalin-fixed, paraffin-embedded (FFPE) samples.

Accuracy study

To evaluate the ability of the system to identify somatic variants in human specimens, 290 FFPE tumor samples were analyzed using a representative next-generation sequencing (NGS) assay to demonstrate positive percent agreement (PPA) and negative percent agreement (NPA) concordance with validated reference detection methods.

The following reference detection methods were used:

- A validated NGS assay, to detect SNV and deletion hotspot variants
- A ROS1 FISH reference test, to detect ROS1 fusions

Variants detected by the NGS assay that were not covered by the reference methods were not included in the PPA/NPA concordance calculation. Variants detected by the NGS assay for which the reference method testing failed and did not yield a valid result were not included in the PPA/NPA calculation.

Accuracy data was analyzed by the following:

- Each variant location
- Bins (or categories) of variants: RNA fusions , simple SNVs, complex SNVs, and deletions
- Each FFPE sample

The results are shown in the following tables.

Table 1 PPA results

PPA measure	Excluding no calls		Including no calls	
	Percent agreement	95% CI	Percent agreement	95% CI
Variant	98.5% (195/198)	(95.6%, 99.7%)	98.5% (195/198)	(95.6%, 99.7%)
Bin	97.2% (176/181)	(93.7%, 99.1%)	97.2% (176/181)	(93.7%, 99.1%)
Sample	96.9% (158/163)	(93.0%, 99.0%)	96.9% (158/163)	(93.0%, 99.0%)

Table 2 NPA results

NPA measure	Excluding no calls		Including no calls	
	Percent agreement	95% CI	Percent agreement	95% CI
Variant	100.0% (118,155/118,159)	(99.99%,100.0%)	96.8% (118,155/122,012)	(96.7%, 96.9%)
Bin	99.8% (942/944)	(99.2%, 100.0%)	70.0% (657/939)	(66.9%, 72.9%)
Sample	98.4% (124/126)	(94.4%, 99.8%)	23.4% (29/124)	(16.3%, 31.8%)

Table 3 OPA results

OPA measure	Excluding no calls		Including no calls	
	Percent agreement	95% CI	Percent agreement	95% CI
Variant	100.0% (118,350/118,357)	(99.99%, 100.0%)	96.8% (118,350/122,210)	(96.74%, 96.94%)
Bin	99.4% (1,118/1,125)	(98.72%,99.75%)	74.4% (833/1,120)	(71.71%, 76.91%)
Sample	97.6% (282/289)	(95.07%,99.02%)	65.2% (187/287)	(59.34%, 70.66%)

Sample reproduciblity study

The reproducibility and repeatability of variant detection using a representative NGS assay were assessed with 2 WT samples and 10 variant-positive samples at 4 testing sites. Each site had 4 Ion PGM™ Dx instrument systems and 4 operators.

Each sample was tested 8 times at each site, for a total of 32 replicates per sample. After repeat testing, the final number of invalid reactions was 15/768 (1.95%).

The call rate, no call rate, positive call rate, negative call rate, and within-run repeatability were computed at each variant location of interest. Including no calls and excluding known positive variant locations, the negative call rate at each clinical variant location for all samples was 100%.

Including no calls, all positive call rates from positive variant locations were >84%.

Excluding no calls and combining data across all study samples, the estimate of repeatability was 100% for DNA variants and 98.9% for RNA variants. The lower limit of the 95% two-sided confidence interval (CI) for repeatability exceeded 96% at all variant locations.

Including no calls from the data, the estimate of repeatability was 100% at 218 out of 605 variant locations, 94–99.9% at 175 out of 605 variant locations, and 71.6–93.9% at 184 out of 605 variant locations. Including no calls, the lower limit of the 95% two-sided confidence interval for repeatability exceeded 64.6% at all variant locations.

Assay reproducibility studies

Assay reproducibility—Study I

The reproducibility and repeatability of a representative assay on the Ion PGM™ Dx System was evaluated for 30 representative variants from 18 DNA samples.

The study was designed to evaluate within-run precision performance (repeatability) and variability across sites, operators, and instrument platforms (reproducibility). Six of the 18 DNA samples were mixtures of plasmid and clinical DNA. Seven of the 12 deletion variants were represented by these plasmid blends. All other variant types were represented by clinical sample DNA.

Due to the large number of variants detected by the test and the rarity of some of the variants, a representative variant approach was used. Variants were selected in the following categories:

Table 4 Representative variant approach—Study I

Variant category	No. of plasmid blends used	No. of clinical specimens used
6-bp deletion	6	0
9-bp deletion	4	2
15-bp deletion	2	4
18-bp deletion	2	4
Simple SNV	0	8
Complex SNVs[1] and MNVs	0	6

[1] Including SNVs in di- or tri-nucleotide repeat regions and SNVs in high-GC (>60%) or low-GC (<40%) content regions

Two of the 18 DNA samples were WT at all locations, and the remaining 16 contained DNA from one or more DNA variants. Each pre-extracted DNA sample was sequenced at 4 sites by 4 operators on 2 systems at each site.

At each site, operators were grouped into 2 pairs, with each pair assigned to 2 instrument systems and responsible for testing 9 DNA samples. Samples were run in duplicate using 2 different reagent lots at 3 of the study sites and on all 3 reagent lots at one study site. The design resulted in a total of 72 test determinations per DNA sample, and all variant locations were assessed for each sample.

The reproducibility results are summarized in the following table.

Table 5 Reproducibility results—Study I

Description	Variants evaluated across the samples	Call rate excluding no calls[1]		Call rate including no calls[1]	
		Mean	Median	Mean	Median
Variant positive DNA (positive calls)	46	96.6%	97.1%	94.5%	95.8%
WT DNA (negative calls)	872	96.1%	95.0%	96.1%	95.0%

[1] Analysis includes invalid results.

Excluding no calls, the estimate of repeatability at each DNA variant location across all the samples was ≥98.8% (95% CI lower limit of ≥97.5%). The coefficient of variation (CV) across all DNA clinical variants ranged from 9.8% to 39%. The highest CVs (24.9–39.2%) were observed for the BRAF V600E variant. The CVs for the EGFR L858R variant ranged from 9.8% to 11.3%, and the CVs for the EGFR deletion variants ranged from 11.2% to 25.5%.

Assay reproducibility—Study II

An additional study was performed to evaluate the reproducibility and repeatability of the Ion PGM™ Dx System for 6 representative variants from 11 DNA samples and 4 RNA samples. All 11 DNA samples and 4 RNA samples were clinical sample blends. In addition, 1 WT DNA sample and 4 WT RNA samples were included in the study.

The study was designed to evaluate within-run precision performance (repeatability) and variability across sites, operators, and instrument platforms (reproducibility).

Due to the large number of variants detected by the test and the rarity of some variants, a representative variant approach was used. Variants were selected in the following categories:

Table 6 Representative variant approach—Study II

Variant category	No. of clinical specimens used	No. of levels tested[1]	No. of samples tested
15-bp deletion	1	2	7[2]
Simple SNV	3	2	
Complex SNV[3] and MNV	2	2	4
Fusion	2	2	4
WT RNA	2	N/A	4[4]
WT DNA	1	N/A	1

[1] The number of sample blends with different levels of allele frequency that were tested.

[2] Includes one sample that contained both a 15-bp deletion and a simple SNV, one sample that contained a 15-bp deletion, and three samples that were simple SNVs.

[3] Including SNVs in di- or tri-nucleotide repeat regions and SNVs in high-GC (>60%) or low-GC (<40%) content regions

[4] Each WT RNA sample was tested twice.

One DNA sample was WT at all locations, 1 DNA sample had 2 DNA variants, and the remaining DNA samples had 1 variant each. Four RNA samples were WT for the ROS1 fusion. Each pre-extracted DNA or RNA sample was sequenced at 3 sites by 2 operators on 2 systems at each site.

At each site, 2 operators were assigned to 2 instrument systems and were responsible for testing 12 DNA samples (11 with variants and 1 WT) and 8 RNA samples (4 with variants and 4 WT). Samples were run in duplicate using 3 different reagent lots at all study sites. The study design resulted in a total of 36 test determinations per DNA or RNA sample.

The reproducibility results are summarized in the following table.

Table 7 Reproducibility results (DNA variants)—Study II

Description	Variants evaluated across the samples	Call rate excluding no calls[1]		Call rate including no calls[1]	
		Mean	Median	Mean	Median
Variant positive DNA (positive calls)	12	99%	100%	98%	99%
WT DNA (negative calls)	367	100%	100%	99%	100%

[1] Analysis includes invalid results.

Excluding no calls, the estimate of repeatability at each DNA variant location across all the samples was ≥94.4% (95% CI lower limit of ≥72.7%). The CVs across all DNA clinical variants ranged from 9.1% to 22.6%. The CVs for the BRAF V600E variant ranged from 13.1% to 19%, the CVs for the EGFR L858R variant ranged from 11% to 17.6%, and the CVs for the EGFR deletion variants ranged from 10.1% to 15.9%.

Table 8 Reproducibility results (ROS1 fusion)—Study II

Description	Isoforms evaluated across the samples	Call rate excluding or including unknowns[1]	
		Mean	Median
ROS1 fusion-positive RNA (positive calls)	4	100%	100%
WT RNA (negative calls)	4	99%	100%

[1] Unknowns are defined as invalid or no result using the Oncomine™ Dx Target Test.

The estimate of repeatability at each RNA clinical variant location was 100%. One wild-type sample was found to be contaminated with RNA control during the study, resulting in an NPA estimate of 94.4%. No specific sequencer performed differently between three lots of Oncomine™ Dx Target Test reagents for this sample. The CV across both RNA locations ranged from 47.8% to 76.6%.

Assay reproducibility—Study III

A study was performed to evaluate the reproducibility and repeatability of the Ion PGM™ Dx System for detection of RET fusions using FFPE RNA from 4 RET fusion-positive samples (blended with WT clinical samples) and 2 RET fusion-negative (WT) specimens.

Table 9 Sample description—Study III

RET fusion	No. of clinical specimens used	No. of levels tested	No. of samples tested
KIF5B-RET.K15R12	1	2	2
CCD6-RET.C1R12	1	2	2
RET-negative/WT RNA	2	N/A	2

The study was designed to evaluate within-run precision performance (repeatability) and variability across sites, operators, and instrument platforms (reproducibility). The updated RNA library preparation workflow was used.

Six pre-extracted RNA sample blends (4 fusion positive blends and 2 WT specimens) and placeholder DNA libraries were sequenced at 3 sites by 6 operators. At each site, 2 operators were assigned to 2 instrument systems and were responsible for testing the 6 RNA sample blends. Samples were run in duplicate using 3 different reagent lots at all study sites. The study design resulted in a total of 36 test determinations per sample blend tested.

The reproducibility results are summarized in the following table.

Table 10 Reproducibility results (RET fusion)—Study III

Description	Isoforms evaluated across the samples	Call rate excluding or including unknowns[1]	
		Mean	Median
RET fusion-positive RNA (positive calls)	4	99%	100%
WT RNA (negative calls)	2	100%	100%

[1] Unknowns are defined as invalid or no result using the Oncomine™ Dx Target Test.

Estimates of the repeatability were reported to be 100% for the CCDC6-RET.C1R12 isoform, and 98.1% for the KIF5B-RET.K15R12 isoform. The CV across both RNA locations ranged from 46.8% to 62.7%.

Tissue input study

Sixty slide-mounted FFPE samples were analyzed to determine if they yield DNA and RNA at the concentrations required by a representative NGS assay when tissue input requirements are met. The assay requires DNA at a concentration of ≥0.83 ng/µL and RNA at a concentration of ≥1.43 ng/µL.

Thirty resection samples with ≥20% tumor content were prepared without macrodissection, 15 resection samples with <20% to ≥10% tumor cell content were macrodissected, and 15 samples were collected by core needle biopsy (CNB). For the resection samples, two 5-µm sections were used per extraction. For CNBs, nine 5-µm sections were used per extraction. DNA and RNA concentrations were determined using dye-based quantification kits (no sequencing was performed).

Of the 60 samples tested, 98.3% (59/60) had a DNA concentration of ≥0.83 ng/µL and an RNA concentration of ≥1.43 ng/µL. One CNB sample failed the minimum DNA and RNA concentration specifications, with values of 0.52 ng/µL and 1.23 ng/µL respectively.

Seven slide-mounted FFPE fine needle aspirate (FNA) samples were extracted to determine if they yield DNA and RNA at the concentrations required by the representative NGS assay when tissue input requirements are met.

For FNAs, seven 5-µm sections were used per extraction. DNA and RNA concentrations were determined using dye-based quantification kits (no sequencing was performed). 100% of the 7 FNA samples yielded DNA at a concentration of ≥0.83 ng/µL and RNA at a concentration of ≥1.43 ng/µL.

DNA and RNA input studies

Eight cell-line samples were prepared as FFPE sections, and DNA and RNA were extracted and quantified from multiple sections from each cell line for blending and testing. Sample blends were prepared with known variants at various DNA and RNA input level combinations within the range of 5–15 ng. The DNA and RNA blends had a target allele frequency of 15% for SNVs and deletions, and target fusion reads of 300–600 for a fusion variant. A total of 540 individual DNA and RNA libraries were tested, including positive controls and NTC controls, with a total of 6 replicate libraries each for DNA and RNA per test condition.

The study demonstrated a 100% positive variant call rate within the input range tested, supporting an input amount of 10 ng each for DNA and RNA for a representative NGS assay on the Ion PGM™ Dx System.

The negative variant call rate was >95% for all except 4 sample and DNA/RNA input-level combinations. All cases with a negative variant call rate of <95% were due to no calls, 3 of which occurred with a DNA or RNA input amount of 5 ng and 1 of which occurred in a single sample with DNA and RNA inputs of 10 ng each. There were no false positive calls.

Additionally, 4 clinical samples prepared as FFPE sections were tested: two samples containing DNA variants and two containing an RNA fusion variant.

The DNA variant samples were paired with wild-type RNA from the same sample at various input combinations within the range of 5–15 ng, and the RNA variant samples were paired with wild-type DNA at input combinations within the same range.

This study demonstrated positive and negative call rates of >95% for the DNA variants at all input combinations, and 100% for one of the RNA fusion variants at all input combinations. The second fusion sample showed 100% negative call rates for all test conditions, and 100% positive call rates except for Test Condition 4 (8.5 ng RNA/15 ng DNA), where the call rate was 83%, and Test Condition 6 (15 ng RNA/15 ng DNA), where the call rate was 50%.

Interfering substances studies

Interfering substances—Study I

Six potentially interfering substances that may be found in FFPE tissue samples or carried over from the deparaffinization and nucleic acid extraction process were evaluated using a representative assay on the Ion PGM™ Dx System.

The guidelines used at the time of testing are defined in section 7.1 of CLSI EP07A2E, which describes testing substances at a relatively high concentration as an interference screen. One potentially interfering endogenous substance, hemoglobin, was tested at twice the concentration recommended in CLSI EP07A2E, Appendix D.

In addition to the substances tested in this study, the data from the Tumor Necrosis Study demonstrated that the performance of the Oncomine™ Dx Target Test was not impacted by the presence of up to 75% necrotic tissue. However, users should macrodissect highly necrotic areas or select alternate samples if possible.

Table 11 Interfering substances and amounts

Potential interfering substance	Step	Amount of substance
Paraffin	At the deparaffinization step, extra paraffin was added to the xylene bath that contained 250 mL of xylene.	4X of normally expected levels
Xylene	Extra xylene was added into the ethanol bath that contained 250 mL of ethanol.	6X of normally expected residual volume
Ethanol	Extra ethanol was added into the Protease digestion step before digestion.	>4X of normally expected residual volume
Hemoglobin	After deparaffinization, hemoglobin was added to the Digestion Buffer used to pre-wet the tissue section.	4 mg/mL
Protease	Extra Protease was added into the reaction after the digestion step and before column purification.	>10X of expected residual Protease after the heat-kill step
Wash buffer	Wash buffer used to isolate DNA and RNA from deparaffinized and digested samples was added into an aliquot of Dilution Solution, which was later used to dilute the RNA and DNA to the appropriate concentration before library preparation.	1% wash buffer (equivalent to ~10% wash buffer carried over into eluate)
Control	Tissue sections were processed using the standard protocol, without the addition of any potentially interfering substances.	N/A

A total of 6 FFPE samples (1 WT and 5 variants) with 6 replicates each were processed through the entire assay workflow. The variant samples included variants from all variant categories that can be detected by the test. The samples were spiked with additional concentrations or amounts of the listed substances at the relevant processing step, as shown in the table. Replicates of a control sample with no spiked substances were also analyzed. The concordance between variant calls in samples with and without interfering substances was computed for each substance under investigation.

With no calls excluded, for each potential interferent used in sample extraction, the positive and negative concordance with the control condition across all samples was 100%, and the overall concordance with the control condition across all samples was 100%.

With no calls excluded, the results of testing with hemoglobin showed positive concordance with the control condition of 100% (only samples with a positive control condition were analyzed), negative concordance of 99.99%, and overall concordance of 99.99%.

Interfering substances—Study II

The interfering substances study was repeated with the updated RNA library preparation workflow to demonstrate that the performance of the representative assay on the Ion PGM™ Dx System is not affected by the presence of potentially interfering substances.

The impact on assay performance of the listed interferents (Table 11) was evaluated in this study and the results were compared to the control (no interferents) condition. For the 6 interferents tested, both the positive concordance (no calls excluded) and the overall concordance (no calls excluded) for all samples was 100%. These data support the claim that paraffin, xylene, ethanol, hemoglobin, protease, or wash buffer do not affect assay performance at the level tested.

Interfering substances—Study III

A study was performed to demonstrate that the performance of the representative assay on the Ion PGM™ Dx System in detecting RET fusions is not affected by the presence of potentially interfering substances.

A total of 3 FFPE samples (2 RET fusion-positive, and 1 WT) with 3 replicates each were used to evaluate the impact of the listed interferents (Table 11) on assay performance, and the results were compared to the control (no interferents) condition. For the 6 interferents tested, both the positive concordance and the overall concordance for all samples was 100%. These data support the claim that paraffin, xylene, ethanol, hemoglobin, protease, or wash buffer do not affect assay performance at the level tested in detection of the RET fusions.

Cross-contamination study

Eight FFPE cell line samples were evaluated to determine the percentage of false positive results caused by cross-contamination (contamination from one sample to another within the same sequencing run) and carryover contamination (contamination from a previous run on the same instrument system). Samples that were WT and mutant were tested in consecutive sequencing runs on the same instruments, and 5 DNA variant locations and 2 RNA variant locations that were expected to be WT for a sample were evaluated for contamination.

Out of 100 DNA and 80 RNA data points analyzed, no false positive results were reported in the DNA variants, and 1 false positive result was reported in an RNA variant. Therefore, the false-positive rate at the DNA variant locations was 0% (0/100) and the false-positive rate at the RNA variant locations was 1.25% (1/80).

Sample barcoding

Sample barcode adapters are used by the Ion PGM™ Dx System to assign a unique nucleic acid barcode to each sample DNA, enabling up to 16 barcoded sample libraries in a single sequencing run.

A barcode performance study was conducted with the SVA panel and both genomic DNA samples (NA12878 and NA19240) (see "Sample barcoding (indexing)" on page 29) to determine variant calls (SNVs and deletions). Therefore, an additional barcode performance study was not repeated for FFPE samples. From a chemical standpoint, the barcode adapters did not experience a sequence-specific difference in stability when being used with FFPE samples. Barcode adaptors are appended to PCR-

amplified double-stranded DNA fragments via blunt ligation chemistry. For the FFPE samples tested, libraries were prepared using DNA and cDNA derived from RNA. Amplified cDNA and DNA are both double stranded, and so the chemistry of barcode ligation is the same when preparing libraries from both RNA and DNA libraries. The chemistry and software algorithms used to decipher barcodes have not changed, therefore no further barcode equivalence testing has been conducted.

2 Performance characteristics of the system with whole blood samples

This section describes studies documenting the performance of the Ion PGM™ Dx System with DNA from whole blood samples. For detailed accuracy and reproducibility results tables, see "Performance characteristics tables—whole blood samples" on page 30.

System Variant Assay panel

The sequencing and variant calling performance of the Ion PGM™ Dx System was evaluated using the System Variant Assay (SVA) panel and genomic DNA isolated from two well-characterized female human cell lines, NA12878 and NA19240.

SVA panel description

The 632 primer pairs of the SVA panel are designed to amplify regions across 23 chromosomes in the two well-characterized cell lines used in the study. The regions were selected based on the presence of well characterized insertions/deletions (Indels) and single-nucleotide variant (SNV) positions (including positions known to be problematic). The amplicons produced range in size from 80–200 base pairs, with GC content from 20–80%, and mean Raw Read Accuracy of 99.0% for the SVA panel.

By design, not all variants were identified during validation studies, as the SVA panel was chosen to include some variants that the system detects with a high level of accuracy and others that are problematic (e.g., long homopolymeric regions). The results demonstrate that in the majority of problematic cases, the Ion PGM™ Dx System generates a No Call result rather than an incorrect result. For this reason, the observed sensitivity and specificity when No Call results are factored in may appear lower than would occur with a properly optimized sequencing assay. No efforts were undertaken to improve the sequencing performance for these problematic regions.

In contrast to the SVA panel, customer-designed panels developed for an *in vitro* diagnostic product will be optimized for reproducible detection of the variants of interest. If a panel cannot be optimized for reproducible variant calling of a particular locus, that variant would need to be eliminated from the product hotspot file and from the product variant report.

Raw Read Accuracy description

The Torrent Suite™ Dx Software treats raw read sequences through various stages of processing in order to assess the overall quality of the sequencing data. In the first stage, the software's per-base quality system predicts a Phred scaled probability of the base call being correct. This is the predicted quality value. In the subsequent stage, the reads are aligned to a reference genome and any discrepancy in the alignment to the reference genome is listed as a mismatch. These mismatches are treated as errors when used to calculate the Raw Read Accuracy, which is the overall measure of system performance. Raw Read Accuracy is calculated using the following formula:

Raw Read Accuracy = 1 – (Total errors in the sequenced reads)/Total bases sequenced.

True variants are counted as mismatches and contribute to a lower Raw Read Accuracy.

Genomes referenced

Genomic DNA samples from NA12878 and NA19240, were used as template for these studies. Sequence information for these cell lines is available at:
http://www.1000genomes.org/data or **http://genomeinabottle.org/**.
These sequences were first described in *A map of human genome variation from population-scale sequencing* (Nature **467**, 1061–1073 (2010)).

To obtain NA12878 or NA19240 DNA samples, contact the Coriell Institute for Medical Research at: **http://ccr.coriell.org** .

Reference database sequences

Several sources were used for the generation of each well-characterized variant reference database sequence for the two cell lines:

- NA12878 cell line: Illumina Platinum genome calls, Complete Genomics calls and Gertstein lab variants from the 1000 Genome Project were used to create the variant reference database sequence. A variant was added to the database if it was present in two of the three sources.
- NA19240 cell line: Complete Genomics variant calls, variants from the high-coverage PCR-free Illumina bam files, and Illumina SNP calls from the pilot 2 phase of the 1000 Genome Project were used to create the variant reference database sequence. An Indel variant present in both the Complete Genomics calls as well as the high coverage PCR-free Illumina bam files was added to the NA19240 variant database sequence. A SNP variant was added to the database if it was present in two of the three sources.

These sponsor-generated variant reference database sequences were used when making comparisons at variant positions in the SVA panel, and the reference human genome assembly sequence hg19 (GRCh37) was used to compare sequence data at non-variant positions.

Quality control procedures

Sequence data from each sequencing run was analyzed to determine run validity. Only runs that passed the % Loading and Ion Dx CF-1 Control Fragment QC metrics were analyzed for sample variant calls by the software. Any run that failed a Run QC metric was designated as invalid (fail) and the run and all sample results from that run were excluded from further analysis. Individual sequencing reaction results were also analyzed using Sample QC metrics before that sample result was defined as valid. Only sample data from reactions that passed Run QC, Ion Dx CF-1 QC, and Sample QC metrics were included in the analysis.

Accuracy and reproducibility study design

Two de-identified samples of purified genomic DNA (5 ng/µL) from both cell lines (four DNA samples in total) were sequenced using the SVA panel by two operators at each of three trial sites (two Ion PGM™ Dx instrument systems per site). To understand lot-to-lot, operator-to-operator, and instrument system-to-instrument system reproducibility, the study was designed such that sequencing of each DNA sample was performed at all three locations with:

- Three lots of each reagent kit, in multiple combinations
- Each barcode adapter
- Three chip lots
- Two Ion PGM™ Dx instrument systems

Twenty-eight sequencing runs were performed at each study site, with 14 runs carried out by each operator. The protocol from start to finish (library preparation, templating, and sequencing) in each run was performed by a single operator over two days. Library preparation and templating (using the Ion OneTouch™ Dx Instrument) were performed on the first day, and enrichment (using the Ion OneTouch™ ES Dx Instrument) followed by sequencing on the Ion PGM™ Dx Sequencer on the second day.

Accuracy and reproducibility of the Ion PGM™ Dx System were determined by comparing the study data to sponsor-generated variant reference database sequences for variant positions in each cell line. Non-variant sequences were compared to hg19 (see, “Reference database sequences” on page 17).

Accuracy and reproducibility study results summary

For detailed tables of results from the accuracy and reproducibility study for whole blood samples, see “Performance characteristics tables—whole blood samples” on page 30.

Per base sequencing accuracy (percent correct calls) was calculated by comparing variant positions to the respective sponsor generated variant reference database sequence and by comparing the non-variant positions to hg19 across all amplicons sequenced using the SVA panel. A total of 83 runs were completed, 4 of which were invalid due to a run or QC failure. All 4 runs were repeated with valid results. Summary data is shown in the following table.

Table 12 Per base sequencing accuracy

Sequencing accuracy was determined using the study data from a sequencing run preselected for evaluation of Ion PGM™ Dx System accuracy.

Genome	% Correct Calls	
	Excluding No Calls[1]	Including No Calls[2]
NA12878	99.88%	96.87%
NA19240	99.86%	96.39%

[1] = (# Correct Calls / # Correct Calls + # Miscalls) × 100

[2] = (# Correct Calls / # Correct Calls + # Miscalls + # No Calls) × 100

Per base accuracy for the preselected NA12878 sample was also calculated using the highly confident reference sequence for NA12878 established by the National Institutes of Standards and Technology (NIST). All the bases in the SVA Panel are not covered by the NIST sequence. The regions in SVA not covered by the NIST sequence were ignored from accuracy analysis. Additionally we only looked at SVA amplicons where at least 50% of the amplicon overlapped with NIST highly confident reference sequence.

Table 13 Per base sequencing accuracy of NA12878 when compared to the NIST highly confident reference sequence

Sequencing accuracy was determined using the study data from a sequencing run preselected for evaluation of Ion PGM™ Dx System accuracy.

Genome	% Correct Calls	
	Excluding No Calls[1]	Including No Calls[2]
NA12878	99.98%	97.76%

[1] = (# Correct Calls / # Correct Calls + # Miscalls) × 100

[2] = (# Correct Calls / # Correct Calls + # Miscalls + # No Calls) × 100

The sensitivity (true positive rate) and specificity (true negative rate) of the Ion PGM™ Dx System are presented in the following tables. Results are presented for both SNVs and Indels in each genome studied.

Table 14 SNV sensitivity

Sensitivity is the measure of whether the Ion PGM™ Dx System made the correct variant call (true positive rate) including zygosity at positions where variants are known to occur based on the sponsor-generated variant reference database sequence. SNV sensitivity calculations were performed excluding no calls.

Genome	# Runs	# SNVs	# Correct SNV Calls	# No Calls	# Miscalls	Mean SNV Sensitivity[1]
NA12878	311	302	92934	917	71	99.92%
NA19240	310	373	112917	2064	649	99.43%

[1] The mean SNV sensitivity, excluding No Calls, over all valid runs for a given DNA sample will be calculated using the formula Sensitivity = TP/(TP+FN), where TP = total number of SNV positions called correctly (including zygosity determination) when compared to the variant reference database sequence, and FN = total number of SNVs present in the variant reference database sequence but not called correctly by the system.

Table 15 Indel sensitivity

Indel sensitivity calculations were performed excluding no calls.

Genome	# Runs	# Indels	# Correct Calls	# No Calls	# Miscalls	Mean Indel Sensitivity[1]
NA12878	311	97	22741	5266	2160	91.33%
NA19240	310	100	24142	5547	1311	94.85%

[1] The mean Indel sensitivity, excluding No Calls, over all valid runs for a given DNA sample will be calculated using the formula Sensitivity = TP/(TP+FN), where TP = total number of Indel positions called correctly (including zygosity determination) when compared to the variant reference database sequence, and FN = total number of Indels present in the variant reference database sequence but not called correctly by the system.

Specificity is the measure of how well the Ion PGM™ Dx System correctly identified base positions as reference (true negative rate) when compared to hg19.

Table 16 Specificity

Specificity calculations were performed excluding no calls.

Genome	# Runs	# Reference locations	# Correct Calls	# No Calls	# Miscalls	Mean specificity[1]
NA12878	311	237	66717	6192	798	98.82%
NA19240	310	163	43791	5102	1637	96.40%

[1] The specificity, excluding No Calls, over all valid runs for a given DNA sample will be calculated using the formula Specificity = TN/(TN + FP), where TN = total number of wild type hotspot positions present in the reference database sequence called correctly by the system, and FP = total number of wild type hotspot positions present in the reference database sequence that are not correctly called as WT by the system.

The ability of the Ion PGM™ Dx System to reproducibly make SNV and Indel variant calls is summarized in the following table.

Table 17 Reproducibility

Reproducibility for SNVs and Indels is defined as the number of variant locations called with a minimum of 95% reproducibility across all sample runs. Reproducibility calculations were performed including no calls where a no-call result is counted as not being reproducible.

Genome	SNV reproducibility	Indel reproducibility
NA12878	96.36% (291/302)	59.79% (58/97)
NA19240	94.10% (351/373)	67.00% (67/100)

The ability of the Ion PGM™ Dx System to make correct variant calls was independent of instrument, operator, kit lot, site, and the number of samples sequenced in a single run.

Performance in clinical samples (accuracy)

Accuracy of the Ion PGM™ Dx System was assessed by comparing DNA sequence results between the Ion PGM™ Dx System and bidirectional CE sequencing on a set of 120 genomic DNA samples extracted from human blood. The samples were collected from four equally represented ethnic groups, African-American, Asian, Caucasian, and Hispanic populations to increase positive variant coverage. Both platforms were used to sequence samples amplified with primers of the Clinical Variant Assay (CVA) panel, which creates amplicons for evaluation of 17 variants from CYP2D6 and 3 SNVs from the Y chromosome.

Table 18 CVA panel composition

Variants are listed in the following table.

Amplicon Name	Chr	Start	End	Length	% GC	Hotspot Variant	Type
Hotspot_2.159943	chr22	42522442	42522629	187	58%	*2_4180G>C	SNP
Hotspot_3.335595	chr22	42523503	42523687	184	64%	*29_3183G>A	SNP
Hotspot_3.242432	chr22	42523776	42523959	183	61%	*41_2988G>A	SNP
Hotspot_3.242432	chr22	42523776	42523959	183	61%	*7_2935A>C	SNP
Hotspot_3.242432	chr22	42523776	42523959	183	61%	*2_2850T>C	SNP
Hotspot_4.325650	chr22	42524148	42524321	173	64%	*9_2613delAAG	DEL
Hotspot_4.325650	chr22	42524148	42524321	173	64%	*3_2549A>del	DEL
Hotspot_4.325650	chr22	42524148	42524321	173	64%	*19_2539_2542delAACT	DEL
Hotspot_5.184879	chr22	42524768	42524958	190	66%	*4_1846G>A	SNP
Hotspot_5.167581	chr22	42525012	42525164	152	68%	*8_1758G>T	SNP
Hotspot_5.167581	chr22	42525012	42525164	152	68%	*6_1707T>del	DEL
Hotspot_5.167581	chr22	42525012	42525164	152	68%	Com_1661G>C	SNP
Hotspot_5.167581	chr22	42525012	42525164	152	68%	*29_1659G>A	SNP
Hotspot_6.102387	chr22	42525679	42525864	185	70%	*17_1023C>T	SNP
Hotspot_7.178704	chr22	42526610	42526808	198	64%	*15_138insT	INS
Hotspot_7.178704	chr22	42526610	42526808	198	64%	*10_100C>T	SNP
Hotspot_7.178704	chr22	42526610	42526808	198	64%	*35_31G>A	SNP
556757084	chrY	15581920	15582055	135	35%	chrY3-15581982	SNP
4933406689	chrY	19054826	19054966	140	36%	chrY7-19054888	SNP
556938408	chrY	21867701	21867876	175	40%	chrY10-21867786	SNP

Table 18 CVA panel composition *(continued)*

Amplicon Name	Chr	Start	End	Length	% GC	Hotspot Variant	Type
Hotspot_1.47978	chr19	45411891	45412088	197	73%	*[1]	*
Hotspot_8.10504	chr22	42528358	42528544	186	51%	*	*
Hotspot_9.62292	chr22	42539450	42539632	182	72%	*	*

[1] Not used for variant detection.

Table 19 Concordance of results

A total of 2400 positions were evaluated. The results by variant position are shown in the following table. Results from CE sequencing were used to define truth.

Variants	CE sequencing				Ion PGM™ Dx Sequencing				% Concordance[1]
	Total # Samples analyzed	# of Variant Calls	# of WT Calls	# of No Calls	# of Correct Variant Calls	# of Correct WT Calls	# of Incorrect Calls	# of No Calls	
*10_100C>T	120	52	65	3	52	65	0	0	100
*15_138insT	120	0	117	3	0	113	0	4	96.58
*17_1023C>T	120	10	105	5	10	105	0	0	100
*19_2539_2542delAACT	120	0	118	2	0	118	0	0	100
*2_2850T>C	120	101	16	3	101	16	0	0	100
*2_4180G>C	120	74	45	1	74	44	1[2]	0	99.16
*29_1659G>A	120	5	113	2	5	113	0	0	100
*29_3183G>A	120	5	110	5	5	110	0	0	100
*3_2549A>del	120	0	118	2	0	118	0	0	100
*35_31G>A	120	5	113	2	5	112	0	1	99.15
*41_2988G>A	120	18	98	4	18	98	0	0	100
*4_1846G>A	120	27	91	2	22	91	1[2]	4	95.76

Table 19 Concordance of results *(continued)*

Variants	CE sequencing				Ion PGM™ Dx Sequencing				% Concordance[1]
	Total # Samples analyzed	# of Variant Calls	# of WT Calls	# of No Calls	# of Correct Variant Calls	# of Correct WT Calls	# of Incorrect Calls	# of No Calls	
*6_1707T>del	120	0	118	2	0	118	0	0	100
*7_2935A>C	120	0	117	3	0	117	0	0	100
*8_1758G>T	120	2	116	2	2	116	0	0	100
*9_2613delAAG	120	3	116	1	3	116	0	0	100
chrY10	120	53	28	39	53	27	0	1	98.77
chrY3	120	56	25	39	56	25	0	0	100
chrY7	120	61	20	39	61	20	0	0	100
Com_1661G>C	120	79	39	2	79	39	0	0	100
Total	2400	551	1688	161[3]	546	1681	2	10	99.46

[1] % concordance = 100 × (# correct variant calls + # correct WT calls) / (# CE variant + CE WT calls)

[2] The two discordant calls came from one sample. Both positions were called a homozygous reference position by CE sequencing and as a heterozygous variant by Ion PGM™ Dx. Analysis of the CE data indicated very low background noise for this sample, consistent with a low level of contamination with a second DNA sequence.

[3] Positions called as no calls by CE sequencing were excluded from the analysis.

The positive, negative and overall level of agreement (or concordance) was determined. Of the 2239 positions called by CE, concordance was observed for 2227 locations. The overall concordance between the two platforms was computed as the number of correct Ion PGM™ Dx System variant calls plus the number of correct Ion PGM™ Dx System WT calls divided by the number of CE variant calls plus the number of CE WT calls. Overall concordance was 99.46%.

The level of positive agreement, calculated as the number of correct Ion PGM™ Dx variant calls divided by the number of CE variant calls, was 99.09%. The level of negative agreement, calculated as the number of correct Ion PGM™ Dx WT calls divided by the number of CE WT calls, was 99.59%.

The Ion PGM™ Dx no call rate, calculated as the number of Ion PGM™ Dx System No Calls divided by the number of CE variant calls plus the number of CE WT calls, was 0.45%.

The overall positive and negative agreement from the Ion PGM™ Dx System is shown in the following table.

Table 20 Concordance of Ion PGM™ Dx System results to CE results

Agreement Type	Result
Overall level of agreement (concordance)	99.46%
Positive agreement excluding no-call results	99.82%
Negative agreement excluding no-call results	99.94%
Positive agreement with no calls	99.09%
Negative agreement with no calls	99.59%
No Call rate	0.45%

DNA Extraction

Three different genomic DNA extraction methods, silica column, paramagnetic bead extraction, and 96-well plate membrane isolation were evaluated. Genomic DNA samples were independently prepared using the three methods from four unique, K_2EDTA anticoagulated, whole blood samples by 3 different operators. The DNA concentration and the A_{260}/A_{280} ratio of the extracted genomic DNA samples were determined by spectrophotometry. The total sample size for each extraction method was 12 (4 samples x 3 operators), each sequenced using the SVA panel and evaluated at 637 variant positions. The within-method concordance was computed for each sample and each extraction method as the proportion of variant calls for which the three results (from different operators) for that location were equivalent. Analysis of the 637 variant positions over the large set of variant types showed concordance varied from 93.56% to 96.23%, with an overall concordance rate of 7272/7644 or 95.13%. One hundred (100) percent concordance was observed for 526 of the 637 variant positions. Thirteen (13) positions showed 100% No Calls. Non-concordance was attributed to one or more No Calls for 84 positions, and for 14 positions, to one or more mixed HOM/HET or HET/REF calls. Results are summarized in the following table.

Table 21 Overall concordance

Extraction Method	Sample	Concordance Rate[1]	Overall Concordance Rate for Method	Overall Concordance Rate Across All Methods
Paramagnetic bead extraction	1	613/637 = 96.23%	2432/2548 = 95.45%	7272/7644 = 95.13%
	2	609/637 = 95.60%		
	3	606/637 = 95.13%		
	4	604/637 = 94.82%		
96-well plate membrane isolation	1	605/637 = 94.98%	2430/2548 = 95.37%	
	2	610/637 = 95.76%		
	3	607/637 = 95.29%		
	4	608/637 = 95.45%		
Silica column	1	606/637 = 95.13%	2410/2548 = 94.58%	
	2	608/637 = 95.45%		
	3	600/637 = 94.19%		
	4	596/637 = 93.56%		

[1] Number of variant positions with 100% concordance / total number of variants

No statistically significant difference in the concordance rate was observed across the three methods ($p = 0.113$).

Based on preliminary data generated using the two well-characterized genomic DNA samples, a highly reproducible subset of 523 variant locations (446 SNV and 77 Indel locations) was identified such that the correct call rate for each variant in this subset was significantly greater than 95% when using the Ion PGM™ Dx System. Analysis of the results for this subset of variants, more representative of

variants detected in an optimized clinical assay, showed concordance per sample ranging from 97.90% to 99.43%, with an overall concordance rate of 6181/6276 or 98.49% . One hundred (100) percent concordance was observed for 488 of the 523 variant positions. One (1) position showed 100% No Calls. Non-concordance was attributed to one or more No Calls for 31 positions, and to one or more mixed HOM/HET calls for 3 positions. Results are summarized in the following table.

Table 22 Concordance within the high-reproducibility subset of variants

Extraction Method	Sample	Concordance Rate	Overall Concordance Rate by Method	Overall Concordance Rate Across All Methods
Paramagnetic bead extraction	1	518/523 = 99.04%	2068/2092 = 98.85%	6181/6276 = 98.49% 95% C.I. = (98.15%, 98.77%)
	2	520/523 = 99.43%		
	3	513/523 = 98.09%		
	4	517/523 = 98.85%		
96-well plate membrane isolation	1	512/523 = 97.90%	2057/2092 = 98.33%	
	2	517/523 = 98.85%		
	3	515/523 = 98.47%		
	4	513/523 = 98.09%		
Silica column	1	514/523 = 98.28%	2056/2092 = 98.28%	
	2	517/523 = 98.85%		
	3	513/523 = 98.09%		
	4	512/523 = 97.90%		

No statistically significant differences in concordance rate were observed across the three methods (p = 0.100).

DNA input

To evaluate the amount of input genomic DNA required for the Ion PGM™ Dx System to reproducibly make accurate variant calls, sample libraries were prepared across a range from 0.125 ng to 808 ng (0.125 ng, 0.25 ng, 0.5 ng, 1.0 ng, 5.0 ng, 7.5 ng, 10 ng, 20 ng, 40 ng, 100 ng, 300 ng, and 808 ng) of genomic DNA per library. Ten replicate libraries were prepared for each of the 13 genomic DNA input levels, differing only by the barcode adapter used.

Each genomic DNA input level was sequenced ten times and the correct variant call rates were calculated for each input level assessed against a well-characterized variant reference database sequence and compared to the correct call rate observed with a 10-ng standard input level. The variant call rates were unaffected by genomic DNA input amounts ranging from 0.5 ng to 808 ng when compared to the rate observed with the 10-ng input level. Genomic DNA inputs of 0.25 ng and lower resulted in statistically significant lower correct call rates than were observed with the standard input

amount of 10 ng. No Calls accounted for >91% of the discordant SNV calls and >85% of the discordant Indel calls at the lowest input DNA amount.

Interfering substances

To assess the impact of interfering substances on the Ion PGM™ Dx System performance, the ability to make variant calls with the SVA panel was evaluated in the presence and absence of potential interfering substances.

Four unique, K_2EDTA anticoagulated, whole blood samples were utilized in this study. Four endogenous interfering substances (bilirubin, cholesterol, hemoglobin, and triglyceride) were tested at relatively high concentrations by independently spiking each interferent into the four blood specimens prior to DNA extraction. To assess interference resulting from blood collection (short draw), EDTA was spiked into blood samples at 5X concentration. As a control, aliquots of each blood sample were spiked with PBS. Each control and spiked blood sample was processed using three different DNA extraction methods. To assess interference resulting from sample preparation, 15% (v/v) wash buffer from each of the extraction kits was added to aliquots of the four genomic DNA control samples.

The concentration of each spiked substance is shown in the following table. Concentrations were selected based on CLSI guideline EP-7A2, Appendices C and D.

Variant calls from the test condition and extraction method were compared to variant calls from the control and extraction condition. Concordance of >97% was achieved for all samples tested in the presence of potentially interfering substances when compared to controls. Of locations, 501 locations resulted in concordant calls across runs for all four samples. Fifteen (15) of the locations provided 100% no call results. Of the non-concordant variant locations, non-concordance was attributed to one or more No Calls in 101 locations and to mixed HOM/HET, HET/REF or HOM/REF calls in 20 locations (3 SNPs, 17 Indels).

Table 23 Overall Concordance of Spiked Samples with Control Samples

Interferent	Concentration	Concordance to control			Samples/method
		DNA extraction method			
		1 (QIAamp)	2 (PureLink)	3 (Maxwell)	
Bilirubin	342 µmol/L	97.7%	97.5%	97.3%	4
Cholesterol	13 mmol/L	97.6%	97.0%	97.3%	4
Hemoglobin	2 g/L	97.6%	97.8%	97.4%	4
Triglyceride	37 mmol/L	97.7%	97.7%	97.4%	4
EDTA	17 µmol/L	97.7%	97.3%	97.1%	4
Wash	15% (v/v)	97.9%	97.3%	97.5%	4

Based on preliminary data generated using two well-characterized genomic DNA samples, a highly reproducible subset of 523 variant locations (446 SNV and 77 Indel locations) was identified such that the correct call rate for each variant in this subset was significantly greater than 95% when using

the Ion PGM™ Dx System. Analysis of the results for this subset of variants, more representative of variants detected in an optimized clinical assay, showed concordance ranging from 98.71% to 99.43%. One hundred (100) percent concordance was observed for 471 of the 523 variant locations. One (1) variant position showed 100% No Calls. Of the non-concordant variant positions, non-concordance was attributed to one or more No Calls in 47 locations, and to Mixed Calls in 4 locations.

Table 24 Concordance within the highly reproducible subset of variants

Interferent	Concentration	Concordance to control			Samples/method
		DNA extraction method			
		1 (QIAamp)	2 (PureLink)	3 (Maxwell)	
Bilirubin	342 µmol/L	99.33%	99.00%	99.04%	4
Cholesterol	13 mmol/L	99.43%	98.71%	99.14%	4
Hemoglobin	2 g/L	99.24%	99.24%	98.90%	4
Triglyceride	37 mmol/L	99.14%	99.19%	99.14%	4
EDTA	17 µmol/L	99.33%	98.80%	98.71%	4
Wash	15% (v/v)	99.43%	98.80%	99.04%	4

Sample barcoding (indexing)

Sample barcode adapters are used by the Ion PGM™ Dx System to assign a unique nucleic acid barcode to each sample DNA, allowing the ability to pool up to sixteen (16) barcoded sample libraries into a single Ion PGM™ Dx sequencing run.

All 16 barcode adapters were tested with the SVA panel and both genomic DNA samples (NA12878 and NA19240) to determine variant call (SNV and Indel) reproducibility between adapter sequences. Sample results were compared to reference database sequences for the NA12878 and NA19240 genomes at all variant and non-variant positions. Comparison of data from 60 runs consisting of 4 sequencing reactions (2 DNA samples × 2 barcodes each) and 24 runs consisting of 16 sequencing reactions (2 DNA samples × 8 barcodes each) indicate that the barcode adapter used and the number of samples pooled together had no effect on variant call reproducibility.

Performance characteristics tables—whole blood samples

The following accuracy and reproducibilty results tables are for DNA from whole blood samples.

Glossary of terms

AMPL	Amplicon
Avg	Average
chr	Chromosome
DEL	Deletion
Het	Heterozygous
Homo	Homozygous
Indel	Insertion or Deletion
INS	Insertion
INST	Instrument
LB	Lower Boundary
MB	Megabase
NA	Not Applicable
NIST	National Institutes of Standards and Technology
OP	Operator
REF	Reference
SNP	Single Nucleotide Polymorphism
SVA	System Variant Assay
UB	Upper Boundary

Table 25 System Variant Assay Panel

Amplicon details for the 632 amplicons generated by the System Variant Assay (SVA) panel primers are presented including: location, length, genotype and zygosity for NA12878 and NA19240

Amplicon Information						NA12878				NA19240			
Amplicon Name	Chromosome	Start	End	Length	% GC	Genotype Expected	Zygosity	Variation Type	Variation Length	Genotype Expected	Zygosity	Variation Type	Variation Length
AMPL4394358129	chr1	2337182	2337360	178	65%	C/T	Het	SNP	1	REF	Homo	NA	NA
AMPL4394343000	chr1	2339998	2340166	168	74%	C/G	Het	SNP	1	REF	Homo	NA	NA
AMPL4393046521	chr1	6529112	6529297	185	64%	REF	Homo	NA	NA	TTCCTCC/T	Het	DEL	6
AMPL4392504456	chr1	6530875	6531053	178	76%	CG	Homo	INS	1	REF	Homo	NA	NA
AMPL4393385216	chr1	11194498	11194678	180	54%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4393372520	chr1	11303059	11303232	173	50%	REF	Homo	NA	NA	C/A	Het	SNP	1
AMPL4392440990	chr1	20972212	20972391	179	49%	G	Homo	DEL	14	REF	Homo	NA	NA
AMPL4392440990	chr1	20972212	20972391	179	49%	C	Homo	DEL	1	REF	Homo	NA	NA
AMPL4392338993	chr1	41296732	41296903	171	66%	T/G	Het	SNP	1	REF	Homo	NA	NA
AMPL3200084096	chr1	45797398	45797574	176	65%	C/G	Het	SNP	1	REF	Homo	NA	NA
AMPL959019077	chr1	68894640	68894813	173	23%	G/GCT	Het	INS	2	REF	Homo	NA	NA
AMPL4297944685	chr1	94466568	94466745	177	64%	A/G	Het	SNP	1	G	Homo	SNP	1
AMPL4298066535	chr1	94471000	94471154	154	42%	G	Homo	SNP	1	A/G	Het	SNP	1
AMPL4392347320	chr1	94474216	94474397	181	39%	T/C	Het	SNP	1	T/C	Het	SNP	1
AMPL4392659170	chr1	94481406	94481568	162	49%	REF	Homo	NA	NA	A/G	Het	SNP	1
AMPL4392650892	chr1	94564387	94564567	180	62%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL4373731419	chr1	98015154	98015325	171	40%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4392328096	chr1	103343416	103343570	154	40%	G/A	Het	SNP	1	G/A	Het	SNP	1
AMPL4392651712	chr1	103355122	103355289	167	24%	REF	Homo	NA	NA	A/C	Het	SNP	1
AMPL4392320712	chr1	103461444	103461570	126	39%	A	Homo	SNP	1	T/A	Het	SNP	1
AMPL4392327972	chr1	103467524	103467699	175	30%	T	Homo	SNP	1	C/T	Het	SNP	1
AMPL4322363520	chr1	116243799	116243953	154	38%	G/A	Het	SNP	1	G/A	Het	SNP	1
AMPL3289619107	chr1	116247734	116247912	178	56%	A	Homo	SNP	1	G/A	Het	SNP	1
AMPL3289619107	chr1	116247734	116247912	178	56%	T/C	Het	SNP	1	REF	Homo	NA	NA
AMPL4392073064	chr1	120480311	120480488	177	50%	C	Homo	SNP	1	C	Homo	SNP	1
AMPL4301817011	chr1	120508965	120509131	166	45%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL413175205	chr1	120512227	120512381	154	54%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL1871943647	chr1	144852294	144852453	159	57%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL3840258113	chr1	144854118	144854294	176	43%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL1090715363	chr1	144866566	144866736	170	61%	G/A	Het	SNP	1	G/A	Het	SNP	1
AMPL4392034654	chr1	144871817	144871961	144	43%	A/C	Het	SNP	1	A/C	Het	SNP	1
AMPL4392047471	chr1	144874561	144874730	169	51%	G/C	Het	SNP	1	G/C	Het	SNP	1
AMPL4392040940	chr1	144879204	144879382	178	52%	G	Homo	SNP	1	A/G	Het	SNP	1
AMPL4392033882	chr1	144882483	144882655	172	51%	A/G	Het	SNP	1	A/G	Het	SNP	1
AMPL4392030295	chr1	144882737	144882914	177	51%	C/T	Het	SNP	1	C/T	Het	SNP	1
AMPL4392028734	chr1	144886111	144886285	174	51%	A/T	Het	SNP	1	A/T	Het	SNP	1
AMPL2858925985	chr1	144918861	144919032	171	39%	T/A	Het	SNP	1	T/A	Het	SNP	1
AMPL4392024816	chr1	144921856	144922028	172	49%	G/A	Het	SNP	1	REF	Homo	NA	NA
AMPL4392024816	chr1	144921856	144922028	172	49%	REF	Homo	NA	NA	C/G	Het	SNP	1
AMPL4392025061	chr1	144922086	144922256	170	46%	C/T	Het	SNP	1	C/T	Het	SNP	1
AMPL4394524216	chr1	144923632	144923813	181	44%	GT/G	Het	DEL	1	REF	Homo	NA	NA
AMPL4394090071	chr1	144931242	144931417	175	56%	C/T	Het	SNP	1	C/T	Het	SNP	1
AMPL4394090071	chr1	144931242	144931417	175	56%	G/A	Het	SNP	1	G/A	Het	SNP	1
AMPL4393326327	chr1	144931520	144931695	175	57%	REF	Homo	NA	NA	C/G	Het	SNP	1
AMPL4393326327	chr1	144931520	144931695	175	57%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL4393283848	chr1	144946625	144946800	175	43%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL4394093339	chr1	144994599	144994712	113	56%	C/A	Het	SNP	1	C/A	Het	SNP	1
AMPL4373875155	chr1	145015934	145016100	166	40%	G	Homo	SNP	1	T/G	Het	SNP	1
AMPL4392008110	chr1	145021110	145021225	115	48%	T/C	Het	SNP	1	T/C	Het	SNP	1
AMPL4392008110	chr1	145021110	145021225	115	48%	C/A	Het	SNP	1	C/A	Het	SNP	1
AMPL3819598533	chr1	145075592	145075776	184	73%	C/T	Het	SNP	1	C/T	Het	SNP	1
AMPL398678636	chr1	156106073	156106255	182	62%	REF	Homo	NA	NA	C	Homo	SNP	1
AMPL4392693991	chr1	156107527	156107698	171	59%	REF	Homo	NA	NA	TC/T	Het	DEL	1

Amplicon Information						NA12878				NA19240			
Amplicon Name	Chromosome	Start	End	Length	% GC	Genotype Expected	Zygosity	Variation Type	Variation Length	Genotype Expected	Zygosity	Variation Type	Variation Length
AMPL3731499965	chr1	156128162	156128308	146	54%	T/C	Het	SNP	1	REF	Homo	NA	NA
AMPL4393296666	chr1	156834102	156834269	167	63%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL526439206	chr1	156848829	156849016	187	65%	REF	Homo	NA	NA	CA/C	Het	DEL	1
AMPL4393093192	chr1	164761616	164761793	177	46%	REF	Homo	NA	NA	C/CTT	Het	INS	2
AMPL4392317144	chr1	171076877	171076993	116	42%	G/A	Het	SNP	1	G/A	Het	SNP	1
AMPL4304199970	chr1	171085229	171085400	171	33%	REF	Homo	NA	NA	A/AT	Het	INS	1
AMPL4103839312	chr1	182554478	182554634	156	47%	C/T	Het	SNP	1	REF	Homo	NA	NA
AMPL4394463576	chr1	185069217	185069391	174	28%	C/CT	Het	INS	1	REF	Homo	NA	NA
AMPL4394318742	chr1	197325815	197325987	172	45%	T/G	Het	SNP	1	G	Homo	SNP	1
AMPL4392325406	chr1	197404684	197404859	175	39%	G/T	Het	SNP	1	REF	Homo	NA	NA
AMPL4324701736	chr1	201335861	201335974	113	63%	T	Homo	SNP	1	T	Homo	SNP	1
AMPL3257535118	chr1	201341086	201341261	175	54%	C	Homo	DEL	5	CAGAAG/C	Het	DEL	5
AMPL4392598603	chr1	204433421	204433598	177	55%	TC	Homo	INS	1	T/TC	Het	INS	1
AMPL4394085835	chr1	204501286	204501453	167	37%	C/T	Het	SNP	1	REF	Homo	NA	NA
AMPL4394068372	chr1	204515786	204515939	153	35%	T	Homo	SNP	1	REF	Homo	NA	NA
AMPL4039838351	chr1	206650009	206650165	156	61%	A/G	Het	SNP	1	REF	Homo	NA	NA
AMPL4392315810	chr1	209804041	209804226	185	60%	G/A	Het	SNP	1	A	Homo	SNP	1
AMPL4392611784	chr1	215821868	215822039	171	60%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL4392611784	chr1	215821868	215822039	171	60%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4304947808	chr1	216052266	216052425	159	43%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4392311692	chr1	216258160	216258333	173	30%	T/C	Het	SNP	1	REF	Homo	NA	NA
AMPL3198880818	chr1	226555221	226555389	168	54%	G	Homo	SNP	1	REF	Homo	NA	NA
AMPL4395108860	chr1	226579941	226580101	160	54%	REF	Homo	NA	NA	G/T	Het	SNP	1
AMPL4318413913	chr1	227071401	227071580	179	60%	C/T	Het	SNP	1	T	Homo	SNP	1
AMPL4392298562	chr1	236883308	236883481	173	35%	T	Homo	SNP	1	T	Homo	SNP	1
AMPL461585857	chr1	236925749	236925927	178	62%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4394997727	chr1	236978916	236979075	159	35%	REF	Homo	NA	NA	T/TTCTG	Het	INS	4
AMPL4393286038	chr1	237060788	237060912	124	37%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL4392312449	chr1	237711741	237711872	131	53%	G	Homo	SNP	1	REF	Homo	NA	NA
AMPL4373352228	chr1	237814741	237814908	167	32%	T	Homo	SNP	1	T	Homo	SNP	1
AMPL598421930	chr1	237863653	237863832	179	37%	G	Homo	SNP	1	G	Homo	SNP	1
AMPL4394316952	chr1	237969536	237969709	173	34%	A/G	Het	SNP	1	REF	Homo	NA	NA
AMPL3497995644	chr2	24905826	24905970	144	40%	C	Homo	SNP	1	REF	Homo	NA	NA
AMPL4391994088	chr2	24974867	24975024	157	56%	G	Homo	SNP	1	REF	Homo	NA	NA
AMPL4393073176	chr2	25471099	25471271	172	51%	REF	Homo	NA	NA	GC/G	Het	DEL	1
AMPL3666903404	chr2	29416505	29416665	160	61%	C	Homo	SNP	1	T/C	Het	SNP	1
AMPL735306822	chr2	42509983	42510066	83	35%	G	Homo	SNP	1	G	Homo	SNP	1
AMPL4374432645	chr2	48030745	48030905	160	39%	A/T	Het	SNP	1	REF	Homo	NA	NA
AMPL4394446055	chr2	48032799	48032952	153	29%	ACTAT/A	Het	DEL	4	ACTAT/A	Het	DEL	4
AMPL4394058159	chr2	61760739	61760913	174	29%	G/C	Het	SNP	1	REF	Homo	NA	NA
AMPL3770343132	chr2	71795075	71795244	169	59%	T	Homo	SNP	1	T	Homo	SNP	1
AMPL4394312266	chr2	71829740	71829912	172	57%	T	Homo	SNP	1	T	Homo	SNP	1
AMPL4373941627	chr2	71838434	71838613	179	68%	REF	Homo	NA	NA	TG/T	Het	DEL	1
AMPL3434544210	chr2	112725618	112725782	164	43%	ATGTGTG	Homo	INS	6	A/ATGTGTG	Het	INS	6
AMPL4392293614	chr2	112785960	112786137	177	53%	G/A	Het	SNP	1	REF	Homo	NA	NA
AMPL4391994985	chr2	113999463	113999628	165	56%	G/C	Het	SNP	1	G/C	Het	SNP	1
AMPL4394057009	chr2	141130459	141130633	174	32%	T/C	Het	SNP	1	REF	Homo	NA	NA
AMPL905336021	chr2	141819531	141819648	117	32%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL4394077238	chr2	141945878	141946047	169	34%	T	Homo	SNP	1	T	Homo	SNP	1
AMPL1705717513	chr2	148657075	148657158	83	40%	G/A	Het	SNP	1	A	Homo	SNP	1
AMPL4392286218	chr2	167162956	167163129	173	36%	C	Homo	SNP	1	C	Homo	SNP	1
AMPL4394302264	chr2	167168007	167168182	175	49%	T	Homo	SNP	1	T	Homo	SNP	1
AMPL4394548445	chr2	175613194	175613358	164	46%	REF	Homo	NA	NA	A/T	Het	SNP	1
AMPL4392296241	chr2	175622202	175622278	76	62%	C/T	Het	SNP	1	REF	Homo	NA	NA
AMPL4309761177	chr2	178098049	178098119	70	34%	CA/C	Het	DEL	1	REF	Homo	NA	NA
AMPL556873511	chr2	182413150	182413318	168	29%	C/T	Het	SNP	1	REF	Homo	NA	NA
AMPL556873511	chr2	182413150	182413318	168	29%	A/G	Het	SNP	1	G	Homo	SNP	1

Amplicon Information						NA12878				NA19240			
Amplicon Name	Chromosome	Start	End	Length	% GC	Genotype Expected	Zygosity	Variation Type	Variation Length	Genotype Expected	Zygosity	Variation Type	Variation Length
AMPL4392283526	chr2	182413512	182413690	178	41%	A/T	Het	SNP	1	T	Homo	SNP	1
AMPL4392722819	chr2	189866271	189866446	175	45%	AC/A	Het	DEL	1	AC/A	Het	DEL	1
AMPL4392277638	chr2	189907839	189907972	133	50%	G	Homo	SNP	1	G	Homo	SNP	1
AMPL4373957362	chr2	189953250	189953402	152	27%	AAATG	Homo	INS	4	A/AAATG	Het	INS	4
AMPL4392651475	chr2	191109442	191109617	175	45%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL3379180838	chr2	198257730	198257861	131	44%	T/C	Het	SNP	1	T/C	Het	SNP	1
AMPL3454598707	chr2	198267704	198267860	156	34%	G/GAA	Het	INS	2	G/GAA	Het	INS	2
AMPL3498234957	chr2	209101824	209101995	171	33%	G	Homo	DEL	1	G	Homo	DEL	1
AMPL3073489807	chr2	212812017	212812179	162	29%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL4392487356	chr2	216240070	216240213	143	42%	A	Homo	DEL	2	REF	Homo	NA	NA
AMPL4392495150	chr2	216248637	216248796	159	41%	T	Homo	DEL	2	TAA/T	Het	DEL	2
AMPL4394453234	chr2	216274767	216274941	174	39%	A	Homo	DEL	2	AAC/A	Het	DEL	2
AMPL581765267	chr2	216299491	216299667	176	33%	T/TTAC	Het	INS	3	T/TTAC	Het	INS	3
AMPL4391973685	chr2	219545227	219545394	167	55%	C	Homo	SNP	1	C	Homo	SNP	1
AMPL4392278772	chr2	220284803	220284972	169	60%	C/T	Het	SNP	1	REF	Homo	NA	NA
AMPL4392278508	chr2	223161579	223161725	146	64%	G	Homo	SNP	1	REF	Homo	NA	NA
AMPL4374039730	chr3	3192429	3192614	185	32%	A/ATAAC	Het	INS	4	ATAAC	Homo	INS	4
AMPL4394049619	chr3	10085029	10085181	152	41%	C/G	Het	SNP	1	G	Homo	SNP	1
AMPL4219350794	chr3	10133832	10134000	168	46%	A/G	Het	SNP	1	G	Homo	SNP	1
AMPL969040897	chr3	30733318	30733491	173	25%	TTA/T	Het	DEL	2	REF	Homo	NA	NA
AMPL4392714050	chr3	32188168	32188327	159	47%	REF	Homo	NA	NA	GA/G	Het	DEL	1
AMPL4395093302	chr3	37695087	37695263	176	52%	REF	Homo	NA	NA	A	Homo	SNP	1
AMPL3255695397	chr3	38592316	38592486	170	52%	G	Homo	SNP	1	G	Homo	SNP	1
AMPL4394436054	chr3	46501197	46501370	173	58%	CCTT	Homo	INS	3	CCTT	Homo	INS	3
AMPL4391970287	chr3	47125298	47125479	181	44%	G/A	Het	SNP	1	G/A	Het	SNP	1
AMPL4393295145	chr3	47163254	47163430	176	41%	REF	Homo	NA	NA	G/C	Het	SNP	1
AMPL4393058046	chr3	52584390	52584479	89	45%	REF	Homo	NA	NA	T/TCTC	Het	INS	3
AMPL4395087540	chr3	52584612	52584789	177	52%	REF	Homo	NA	NA	A/G	Het	SNP	1
AMPL4393144102	chr3	52662843	52662925	82	29%	REF	Homo	NA	NA	A/C	Het	SNP	1
AMPL4392648148	chr3	63973642	63973815	173	39%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL4392262531	chr3	63981560	63981737	177	50%	C/T	Het	SNP	1	REF	Homo	NA	NA
AMPL4392638186	chr3	63982141	63982319	178	39%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL4392477090	chr3	65361300	65361451	151	38%	CAGAG/C	Het	DEL	4	REF	Homo	NA	NA
AMPL4394514215	chr3	81627127	81627235	108	40%	REF	Homo	NA	NA	C	Homo	SNP	1
AMPL4392619457	chr3	123419647	123419803	156	66%	REF	Homo	NA	NA	G	Homo	SNP	1
AMPL748437993	chr3	123452970	123453148	178	48%	REF	Homo	NA	NA	G	Homo	SNP	1
AMPL4393315786	chr3	134644590	134644728	138	46%	G/T	Het	SNP	1	T	Homo	SNP	1
AMPL736692473	chr3	138456663	138456775	112	38%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4305635694	chr3	138664978	138665146	168	79%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4395062735	chr3	142222222	142222340	118	31%	REF	Homo	NA	NA	A/G	Het	SNP	1
AMPL4219407492	chr3	142277478	142277603	125	38%	REF	Homo	NA	NA	A/G	Het	SNP	1
AMPL4222168082	chr3	142281539	142281708	169	32%	A/G	Het	SNP	1	A/G	Het	SNP	1
AMPL4301889960	chr3	178922197	178922351	154	35%	REF	Homo	NA	NA	C/A	Het	SNP	1
AMPL4301889960	chr3	178922197	178922351	154	35%	A	Homo	SNP	1	A	Homo	SNP	1
AMPL4392603343	chr3	180365979	180366124	145	37%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL4393227469	chr3	188326831	188327003	172	32%	REF	Homo	NA	NA	A/G	Het	SNP	1
AMPL4392596733	chr4	995219	995382	163	60%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL4392251505	chr4	2834725	2834866	141	59%	A/C	Het	SNP	1	REF	Homo	NA	NA
AMPL4227407944	chr4	16037270	16037423	153	41%	C/T	Het	SNP	1	C/T	Het	SNP	1
AMPL395434724	chr4	55151883	55152058	175	53%	T/TA	Het	INS	1	T/TA	Het	INS	1
AMPL4395087555	chr4	87967145	87967324	179	43%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL4322933975	chr4	88056652	88056800	148	38%	G	Homo	DEL	1	G	Homo	DEL	1
AMPL4394545846	chr4	88959326	88959458	132	49%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4394601334	chr4	90743240	90743392	152	46%	REF	Homo	NA	NA	T/TC	Het	INS	1
AMPL4373689558	chr4	103533541	103533722	181	44%	REF	Homo	NA	NA	G/C	Het	SNP	1
AMPL4391975825	chr4	103534463	103534644	181	57%	C/G	Het	SNP	1	C/G	Het	SNP	1
AMPL4393733107	chr4	106154902	106155085	183	29%	TATAGATAG/T	Het	DEL	8	REF	Homo	NA	NA

Amplicon Information						NA12878				NA19240			
Amplicon Name	Chromosome	Start	End	Length	% GC	Genotype Expected	Zygosity	Variation Type	Variation Length	Genotype Expected	Zygosity	Variation Type	Variation Length
AMPL3498362734	chr4	106155682	106155822	140	41%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL413738456	chr4	106190649	106190764	115	26%	REF	Homo	NA	NA	C/A	Het	SNP	1
AMPL4392997426	chr4	114117355	114117528	173	35%	REF	Homo	NA	NA	A/AT	Het	INS	1
AMPL4392261532	chr4	146576327	146576507	180	48%	G/C	Het	SNP	1	REF	Homo	NA	NA
AMPL2742861328	chr5	7870891	7871058	167	44%	A/G	Het	SNP	1	A/G	Het	SNP	1
AMPL4392257952	chr5	13811672	13811844	172	40%	T/C	Het	SNP	1	REF	Homo	NA	NA
AMPL4308774096	chr5	13829536	13829687	151	41%	G/A	Het	SNP	1	G/A	Het	SNP	1
AMPL4308774096	chr5	13829536	13829687	151	41%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL4392228266	chr5	13864638	13864787	149	49%	C/A	Het	SNP	1	REF	Homo	NA	NA
AMPL3467347768	chr5	13870942	13871106	164	40%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL552238544	chr5	35873501	35873673	172	43%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL551297934	chr5	35874489	35874659	170	45%	C/T	Het	SNP	1	REF	Homo	NA	NA
AMPL3498059646	chr5	35876183	35876358	175	51%	A/G	Het	SNP	1	REF	Homo	NA	NA
AMPL4393190958	chr5	38499689	38499869	180	36%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4392317694	chr5	38528868	38529043	175	43%	G	Homo	DEL	2	GACAC/G	Het	DEL	4
AMPL4393183760	chr5	55250656	55250796	140	42%	REF	Homo	NA	NA	A/G	Het	SNP	1
AMPL1629455589	chr5	55251854	55252024	170	37%	REF	Homo	NA	NA	G/C	Het	SNP	1
AMPL393611061	chr5	112162794	112162913	119	40%	T/C	Het	SNP	1	REF	Homo	NA	NA
AMPL3653082204	chr5	112175680	112175856	176	45%	G/A	Het	SNP	1	G/A	Het	SNP	1
AMPL4391964653	chr5	131915114	131915285	171	31%	G/A	Het	SNP	1	G/A	Het	SNP	1
AMPL1211083733	chr5	149435492	149435677	185	61%	G/C	Het	SNP	1	REF	Homo	NA	NA
AMPL4392743054	chr5	149440913	149441090	177	59%	AC/A	Het	DEL	1	REF	Homo	NA	NA
AMPL407783594	chr5	149456838	149457006	168	52%	REF	Homo	NA	NA	C/A	Het	SNP	1
AMPL1663497431	chr5	149460469	149460647	178	62%	A/G	Het	SNP	1	REF	Homo	NA	NA
AMPL863277015	chr5	149749019	149749201	182	65%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL4394285134	chr5	149776112	149776267	155	58%	T	Homo	SNP	1	REF	Homo	NA	NA
AMPL4393684226	chr5	156190079	156190259	180	41%	G/GA	Het	INS	1	G/GA	Het	INS	1
AMPL3433472167	chr5	176517382	176517555	173	70%	G	Homo	SNP	1	G	Homo	SNP	1
AMPL4394027906	chr5	176517719	176517895	176	56%	T	Homo	SNP	1	T	Homo	SNP	1
AMPL416374362	chr5	176522673	176522786	113	65%	T	Homo	SNP	1	T	Homo	SNP	1
AMPL4393199548	chr5	176636796	176636966	170	41%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL4392452727	chr5	180045634	180045767	133	62%	GGGTTACCCTA	Homo	INS	10	G/GGGTTACCCTA	Het	INS	10
AMPL4394422924	chr5	180046334	180046517	183	74%	REF	Homo	NA	NA	CAG/C	Het	DEL	2
AMPL4392438280	chr5	180047641	180047818	177	62%	C/CGTGT	Het	INS	4	REF	Homo	NA	NA
AMPL3438454613	chr5	180052891	180053005	114	71%	G/A	Het	SNP	1	REF	Homo	NA	NA
AMPL4394498383	chr6	16327236	16327416	180	62%	REF	Homo	NA	NA	C	Homo	SNP	1
AMPL4392232288	chr6	31978571	31978756	185	64%	T	Homo	SNP	1	REF	Homo	NA	NA
AMPL4392232288	chr6	31978571	31978756	185	64%	REF	Homo	NA	NA	C/G	Het	SNP	1
AMPL4392232288	chr6	31978571	31978756	185	64%	A	Homo	SNP	1	A	Homo	SNP	1
AMPL4392247152	chr6	32032664	32032823	159	63%	G/A	Het	SNP	1	G/A	Het	SNP	1
AMPL4392197049	chr6	32064623	32064808	185	69%	T/C	Het	SNP	1	REF	Homo	NA	NA
AMPL4393991123	chr6	32164753	32164935	182	60%	G/C	Het	SNP	1	REF	Homo	NA	NA
AMPL4393991123	chr6	32164753	32164935	182	60%	C/A	Het	SNP	1	REF	Homo	NA	NA
AMPL4393996246	chr6	32188172	32188354	182	62%	G/C	Het	SNP	1	G/C	Het	SNP	1
AMPL4392574004	chr6	33132252	33132386	134	63%	REF	Homo	NA	NA	A	Homo	SNP	1
AMPL4392530564	chr6	33134710	33134897	187	66%	REF	Homo	NA	NA	A	Homo	SNP	1
AMPL4392171571	chr6	33136560	33136713	153	63%	G/A	Het	SNP	1	A	Homo	SNP	1
AMPL4392174571	chr6	33139259	33139411	152	66%	C/T	Het	SNP	1	T	Homo	SNP	1
AMPL3682590436	chr6	33141063	33141229	166	60%	A/G	Het	SNP	1	G	Homo	SNP	1
AMPL4392240361	chr6	33145285	33145461	176	58%	C/A	Het	SNP	1	A	Homo	SNP	1
AMPL4395049446	chr6	41565366	41565551	185	68%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL3518568818	chr6	49425465	49425637	172	33%	C/T	Het	SNP	1	T	Homo	SNP	1
AMPL4394999207	chr6	51618087	51618258	171	46%	G	Homo	SNP	1	G	Homo	DEL	1
AMPL4393163023	chr6	51776448	51776622	174	38%	REF	Homo	NA	NA	C	Homo	SNP	1
AMPL4393980045	chr6	51875164	51875340	176	39%	C	Homo	SNP	1	C	Homo	SNP	1
AMPL4393983602	chr6	51890899	51891075	176	45%	C	Homo	SNP	1	T/C	Het	SNP	1
AMPL4393695146	chr6	51921529	51921703	174	44%	REF	Homo	NA	NA	A/G	Het	SNP	1

Amplicon Information						NA12878				NA19240			
Amplicon Name	Chromosome	Start	End	Length	% GC	Genotype Expected	Zygosity	Variation Type	Variation Length	Genotype Expected	Zygosity	Variation Type	Variation Length
AMPL4343482687	chr6	51935734	51935807	73	33%	T	Homo	SNP	1	C/T	Het	SNP	1
AMPL4393984337	chr6	56373469	56373623	154	44%	C/T	Het	SNP	1	REF	Homo	NA	NA
AMPL4059164024	chr6	56463326	56463484	158	36%	T/C	Het	SNP	1	REF	Homo	NA	NA
AMPL4392391519	chr6	65016904	65017065	161	34%	TGA/T	Het	DEL	2	T	Homo	DEL	2
AMPL4394279241	chr6	65098675	65098847	172	26%	A/G	Het	SNP	1	G	Homo	SNP	1
AMPL4393723902	chr6	65301432	65301608	176	39%	REF	Homo	NA	NA	G	Homo	SNP	1
AMPL4392346671	chr6	65611868	65612035	167	37%	TATTA	Homo	INS	4	TATTA	Homo	INS	4
AMPL4373578084	chr6	66005793	66005970	177	43%	T/A	Het	SNP	1	REF	Homo	NA	NA
AMPL4373578084	chr6	66005793	66005970	177	43%	C/T	Het	SNP	1	REF	Homo	NA	NA
AMPL4393984326	chr6	69666617	69666769	152	41%	G	Homo	SNP	1	G	Homo	SNP	1
AMPL4393961771	chr6	70071073	70071249	176	39%	A	Homo	SNP	1	G/A	Het	SNP	1
AMPL4373985988	chr6	70950260	70950421	161	29%	A	Homo	DEL	2	REF	Homo	NA	NA
AMPL4392235190	chr6	70965029	70965207	178	36%	C	Homo	SNP	1	C	Homo	SNP	1
AMPL4392253940	chr6	70990566	70990736	170	60%	G/A	Het	SNP	1	REF	Homo	NA	NA
AMPL4392992251	chr6	91226228	91226347	119	41%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4374147776	chr6	91266243	91266417	174	37%	REF	Homo	NA	NA	TA	Homo	INS	1
AMPL4373707639	chr6	93964304	93964397	93	33%	REF	Homo	NA	NA	T	Homo	SNP	1
AMPL4392220658	chr6	116950654	116950811	157	35%	G/A	Het	SNP	1	G/A	Het	SNP	1
AMPL4394603296	chr6	116951433	116951585	152	34%	REF	Homo	NA	NA	CAT/C	Het	DEL	2
AMPL4393051580	chr6	117686635	117686804	169	46%	REF	Homo	NA	NA	TAC/T	Het	DEL	2
AMPL4393964460	chr6	117708898	117709048	150	51%	C/T	Het	SNP	1	REF	Homo	NA	NA
AMPL3434408361	chr6	117730746	117730921	175	45%	G/A	Het	SNP	1	G/A	Het	SNP	1
AMPL4392985517	chr6	135515380	135515543	163	42%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL4395015331	chr6	135518020	135518200	180	44%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL4392990319	chr6	135521405	135521571	166	42%	REF	Homo	NA	NA	A/C	Het	SNP	1
AMPL4392441515	chr6	138195863	138196040	177	46%	TCTC/T	Het	DEL	3	REF	Homo	NA	NA
AMPL4392216992	chr6	143792539	143792686	147	27%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL4392216992	chr6	143792539	143792686	147	27%	T/C	Het	SNP	1	REF	Homo	NA	NA
AMPL4393954745	chr6	152453199	152453372	173	49%	G/A	Het	SNP	1	REF	Homo	NA	NA
AMPL4393010759	chr6	152460958	152461120	162	53%	REF	Homo	NA	NA	CTGTT/C	Het	DEL	4
AMPL711877918	chr6	152469224	152469404	180	62%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL4392573539	chr6	152539479	152539651	172	30%	T	Homo	DEL	1	REF	Homo	NA	NA
AMPL4373702125	chr6	152557253	152557403	150	37%	REF	Homo	NA	NA	A/G	Het	SNP	1
AMPL4393942602	chr6	152660191	152660363	172	29%	A/G	Het	SNP	1	REF	Homo	NA	NA
AMPL4393950751	chr6	152671407	152671578	171	43%	C	Homo	SNP	1	REF	Homo	NA	NA
AMPL4392987625	chr6	152711312	152711478	166	45%	REF	Homo	NA	NA	A	Homo	SNP	1
AMPL4316030239	chr6	152793299	152793452	153	37%	TA/T	Het	DEL	1	TA/T	Het	DEL	1
AMPL4394982619	chr6	152826217	152826385	168	42%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL1158164934	chr6	167271627	167271796	169	45%	C	Homo	SNP	1	T/C	Het	SNP	1
AMPL1158164934	chr6	167271627	167271796	169	45%	C	Homo	SNP	1	REF	Homo	NA	NA
AMPL3683333814	chr7	2962263	2962388	125	62%	REF	Homo	NA	NA	C/G	Het	SNP	1
AMPL755591047	chr7	2962694	2962819	125	64%	A	Homo	SNP	1	REF	Homo	NA	NA
AMPL575431534	chr7	21582831	21583016	185	72%	C/T	Het	SNP	1	REF	Homo	NA	NA
AMPL575431534	chr7	21582831	21583016	185	72%	T	Homo	SNP	1	REF	Homo	NA	NA
AMPL575431534	chr7	21582831	21583016	185	72%	T	Homo	SNP	1	REF	Homo	NA	NA
AMPL4394281382	chr7	21640536	21640700	164	33%	C/G	Het	SNP	1	REF	Homo	NA	NA
AMPL575510303	chr7	21659430	21659603	173	28%	ATTAAT	Homo	INS	5	A/ATTAAT	Het	INS	5
AMPL4393743713	chr7	21723474	21723649	175	40%	REF	Homo	NA	NA	A/T	Het	SNP	1
AMPL4392200573	chr7	21778342	21778491	149	41%	T	Homo	SNP	1	REF	Homo	NA	NA
AMPL4393737942	chr7	21784344	21784519	175	50%	REF	Homo	NA	NA	A/C	Het	SNP	1
AMPL4393656617	chr7	33148602	33148786	184	78%	A/G	Het	SNP	1	G	Homo	SNP	1
AMPL4394594066	chr7	37903992	37904159	167	31%	T	Homo	DEL	3	TAAC/T	Het	DEL	3
AMPL571636108	chr7	37916396	37916558	162	31%	A/G	Het	SNP	1	REF	Homo	NA	NA
AMPL868949058	chr7	55228034	55228203	169	30%	T	Homo	SNP	1	T	Homo	SNP	1
AMPL4231728591	chr7	55266356	55266508	152	51%	C	Homo	SNP	1	C	Homo	SNP	1
AMPL692547671	chr7	73471957	73472130	173	63%	C/T	Het	SNP	1	T	Homo	SNP	1
AMPL4323577210	chr7	91632262	91632403	141	34%	T	Homo	SNP	1	T	Homo	SNP	1

Amplicon Information						NA12878				NA19240			
Amplicon Name	Chromosome	Start	End	Length	% GC	Genotype Expected	Zygosity	Variation Type	Variation Length	Genotype Expected	Zygosity	Variation Type	Variation Length
AMPL413836218	chr7	91652076	91652246	170	30%	A/AAAC	Het	INS	3	A/AAAC	Het	INS	3
AMPL4392936584	chr7	91695632	91695804	172	33%	REF	Homo	NA	NA	A/G	Het	SNP	1
AMPL4393915769	chr7	91712634	91712802	168	36%	A/G	Het	SNP	1	REF	Homo	NA	NA
AMPL4022901967	chr7	91713869	91713994	125	32%	C/T	Het	SNP	1	REF	Homo	NA	NA
AMPL415167643	chr7	91714820	91714992	172	41%	T	Homo	SNP	1	T	Homo	SNP	1
AMPL4323505451	chr7	91726854	91726998	144	34%	A/C	Het	SNP	1	REF	Homo	NA	NA
AMPL4393686515	chr7	92146620	92146792	172	35%	REF	Homo	NA	NA	G/T	Het	SNP	1
AMPL4374016890	chr7	92244513	92244693	180	35%	TATACA/T	Het	DEL	5	REF	Homo	NA	NA
AMPL4374230397	chr7	94047040	94047219	179	53%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL4374521900	chr7	98534838	98535012	174	47%	CAT/C	Het	DEL	2	CAT/C	Het	DEL	2
AMPL4392992071	chr7	100411259	100411341	82	50%	C	Homo	SNP	1	C	Homo	SNP	1
AMPL719291358	chr7	106508909	106509082	173	61%	A/G	Het	SNP	1	G	Homo	SNP	1
AMPL719291358	chr7	106508909	106509082	173	61%	T/C	Het	SNP	1	C	Homo	SNP	1
AMPL4393683810	chr7	107353052	107353224	172	31%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL4392932301	chr7	124486862	124487026	164	34%	C	Homo	SNP	1	T/C	Het	SNP	1
AMPL403750326	chr7	124491940	124492058	118	31%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL4374399269	chr7	126079045	126079214	169	37%	A/G	Het	SNP	1	A/G	Het	SNP	1
AMPL4374398779	chr7	126410048	126410214	166	36%	REF	Homo	NA	NA	G/T	Het	SNP	1
AMPL4374398779	chr7	126410048	126410214	166	36%	C	Homo	SNP	1	G/C	Het	SNP	1
AMPL4392203039	chr7	128034570	128034709	139	60%	T	Homo	SNP	1	REF	Homo	NA	NA
AMPL4395001028	chr7	142566626	142566789	163	65%	REF	Homo	NA	NA	CCT	Homo	INS	2
AMPL4395342492	chr7	143016839	143017014	175	46%	REF	Homo	NA	NA	T	Homo	SNP	1
AMPL4392223494	chr7	143036465	143036639	174	45%	T/A	Het	SNP	1	A	Homo	SNP	1
AMPL4392215902	chr7	143043155	143043324	169	58%	C/T	Het	SNP	1	T	Homo	SNP	1
AMPL4393114509	chr7	148504578	148504761	183	38%	REF	Homo	NA	NA	A	Homo	DEL	1
AMPL4394953784	chr7	148543425	148543598	173	38%	A/G	Het	SNP	1	G	Homo	SNP	1
AMPL596607752	chr7	150644353	150644524	171	74%	C/A	Het	SNP	1	REF	Homo	NA	NA
AMPL596173137	chr7	150648588	150648759	171	61%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4320903836	chr7	150654528	150654706	178	68%	AG	Homo	INS	1	AG	Homo	INS	1
AMPL417791888	chr7	151873751	151873921	170	41%	C/T	Het	SNP	1	C/T	Het	SNP	1
AMPL4307576268	chr7	151874427	151874581	154	42%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL413267348	chr7	151896448	151896620	172	30%	REF	Homo	NA	NA	G/GGTTA	Het	INS	4
AMPL3498610579	chr7	151948979	151949154	175	37%	REF	Homo	NA	NA	C/G	Het	SNP	1
AMPL4392211943	chr8	11614501	11614661	160	68%	G	Homo	SNP	1	REF	Homo	NA	NA
AMPL4393613220	chr8	21978984	21979159	175	63%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL2692711234	chr8	30925677	30925808	131	27%	A	Homo	SNP	1	REF	Homo	NA	NA
AMPL4392926980	chr8	30977602	30977770	168	32%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL3676783348	chr8	30999046	30999214	168	38%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL2692395510	chr8	31004917	31005093	176	35%	TC	Homo	INS	1	TC	Homo	INS	1
AMPL3936418821	chr8	48805729	48805903	174	62%	AG	Homo	INS	1	AG	Homo	INS	1
AMPL4374406487	chr8	90967622	90967780	158	35%	A/G	Het	SNP	1	A/G	Het	SNP	1
AMPL4393927397	chr8	90970844	90970970	126	33%	G/T	Het	SNP	1	REF	Homo	NA	NA
AMPL4393927397	chr8	90970844	90970970	126	33%	G/A	Het	SNP	1	REF	Homo	NA	NA
AMPL4373781431	chr8	113364573	113364743	170	35%	A/G	Het	SNP	1	REF	Homo	NA	NA
AMPL4393043045	chr8	114326973	114327141	168	26%	REF	Homo	NA	NA	GATA/G	Het	DEL	3
AMPL4038401239	chr8	118819493	118819662	169	53%	C/T	Het	SNP	1	C/T	Het	SNP	1
AMPL4392423431	chr8	126056111	126056282	171	37%	GC	Homo	INS	1	GC	Homo	INS	1
AMPL4392201407	chr8	144991994	144992172	178	65%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL4392201407	chr8	144991994	144992172	178	65%	C	Homo	SNP	1	C	Homo	SNP	1
AMPL4302499643	chr8	144995441	144995618	177	71%	T	Homo	SNP	1	REF	Homo	NA	NA
AMPL704947325	chr8	144996310	144996498	188	66%	G	Homo	SNP	1	REF	Homo	NA	NA
AMPL4202336805	chr8	144998056	144998244	188	75%	T	Homo	SNP	1	REF	Homo	NA	NA
AMPL4202336805	chr8	144998056	144998244	188	75%	G	Homo	SNP	1	REF	Homo	NA	NA
AMPL4203136737	chr8	144999951	145000136	185	70%	C	Homo	SNP	1	REF	Homo	NA	NA
AMPL4302482618	chr8	145005656	145005749	93	73%	AC	Homo	INS	1	REF	Homo	NA	NA
AMPL4392191781	chr8	145008240	145008422	182	74%	A	Homo	SNP	1	REF	Homo	NA	NA
AMPL3659553353	chr9	5081691	5081848	157	36%	REF	Homo	NA	NA	A	Homo	SNP	1

Amplicon Information						NA12878				NA19240			
Amplicon Name	Chromosome	Start	End	Length	% GC	Genotype Expected	Zygosity	Variation Type	Variation Length	Genotype Expected	Zygosity	Variation Type	Variation Length
AMPL4392890404	chr9	5090614	5090759	145	28%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4374509052	chr9	8331463	8331590	127	31%	A/AAACTTACCATTCCTGAACTGT	Het	INS	21	REF	Homo	NA	NA
AMPL4394429615	chr9	8338750	8338928	178	39%	CAG/C	Het	DEL	2	REF	Homo	NA	NA
AMPL4392895580	chr9	8460509	8460678	169	39%	A/G	Het	SNP	1	A/G	Het	SNP	1
AMPL4373372000	chr9	8484273	8484443	170	34%	TA/T	Het	DEL	1	REF	Homo	NA	NA
AMPL1153902623	chr9	15479561	15479722	161	35%	T/C	Het	SNP	1	REF	Homo	NA	NA
AMPL4393659645	chr9	34485153	34485329	176	52%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL3679236852	chr9	71650709	71650830	121	79%	G	Homo	SNP	1	G	Homo	SNP	1
AMPL3498104622	chr9	93639754	93639932	178	41%	G/A	Het	SNP	1	REF	Homo	NA	NA
AMPL708625773	chr9	94486254	94486426	172	69%	T	Homo	SNP	1	C/T	Het	SNP	1
AMPL4302673454	chr9	97934207	97934378	171	32%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL1705657360	chr9	98229296	98229469	173	46%	REF	Homo	NA	NA	G	Homo	SNP	1
AMPL1702865108	chr9	98232141	98232309	168	41%	GA/G	Het	DEL	1	REF	Homo	NA	NA
AMPL4392692973	chr9	111685015	111685186	171	44%	REF	Homo	NA	NA	C/CAT	Het	INS	2
AMPL4195002255	chr9	130587032	130587125	93	65%	A/AGGGAGG	Het	INS	6	REF	Homo	NA	NA
AMPL3498144888	chr9	135786831	135786980	149	50%	REF	Homo	NA	NA	G	Homo	SNP	1
AMPL3299722549	chr9	137659148	137659326	178	62%	C/T	Het	SNP	1	REF	Homo	NA	NA
AMPL4392675093	chr9	137688702	137688883	181	62%	G	Homo	DEL	20	G	Homo	DEL	20
AMPL4393781331	chr10	3818352	3818532	180	55%	T/TG	Het	INS	1	REF	Homo	NA	NA
AMPL4395333937	chr10	3820069	3820163	94	27%	G/GTT	Het	INS	2	REF	Homo	NA	NA
AMPL4393786655	chr10	27294306	27294476	170	24%	CAT/C	Het	DEL	2	REF	Homo	NA	NA
AMPL4393896650	chr10	51568319	51568428	109	48%	T/G	Het	SNP	1	REF	Homo	NA	NA
AMPL4392914397	chr10	51582812	51582972	160	50%	C/T	Het	SNP	1	C/T	Het	SNP	1
AMPL4395281946	chr10	55591227	55591398	171	29%	G/A	Het	SNP	1	A	Homo	SNP	1
AMPL4395290340	chr10	55755416	55755559	143	40%	REF	Homo	NA	NA	T	Homo	SNP	1
AMPL4393619887	chr10	55826412	55826529	117	25%	REF	Homo	NA	NA	A/G	Het	SNP	1
AMPL4394604242	chr10	55892534	55892708	174	40%	TAC/T	Het	DEL	2	TAC/T	Het	DEL	2
AMPL3710790718	chr10	55943146	55943244	98	36%	T/C	Het	SNP	1	C	Homo	SNP	1
AMPL4395266793	chr10	55955468	55955549	81	44%	REF	Homo	NA	NA	G	Homo	SNP	1
AMPL4392189306	chr10	56423910	56424024	114	43%	C	Homo	SNP	1	A/C	Het	SNP	1
AMPL3671540502	chr10	70405159	70405320	161	47%	G/A	Het	SNP	1	G/A	Het	SNP	1
AMPL4392192649	chr10	73544551	73544730	179	61%	A/G	Het	SNP	1	REF	Homo	NA	NA
AMPL4393619016	chr10	73553085	73553267	182	61%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL2455591517	chr10	73558803	73558973	170	59%	G	Homo	SNP	1	A/G	Het	SNP	1
AMPL2455591517	chr10	73558803	73558973	170	59%	A	Homo	SNP	1	G/A	Het	SNP	1
AMPL2455591517	chr10	73558803	73558973	170	59%	T	Homo	SNP	1	C/T	Het	SNP	1
AMPL4325322943	chr10	88441423	88441548	125	63%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4170634321	chr10	88683068	88683184	116	41%	T/C	Het	SNP	1	C	Homo	SNP	1
AMPL652088025	chr10	104157695	104157806	111	50%	REF	Homo	NA	NA	T/G	Het	SNP	1
AMPL652088025	chr10	104157695	104157806	111	50%	T	Homo	SNP	1	C/T	Het	SNP	1
AMPL4392182082	chr10	121429694	121429875	181	66%	G	Homo	SNP	1	G	Homo	SNP	1
AMPL1519565349	chr10	123243120	123243289	169	49%	A	Homo	SNP	1	REF	Homo	NA	NA
AMPL408214700	chr10	123310818	123310945	127	59%	REF	Homo	NA	NA	G	Homo	SNP	1
AMPL4395267587	chr11	2592435	2592618	183	64%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL4392872919	chr11	4123233	4123402	169	36%	C/G	Het	SNP	1	G	Homo	SNP	1
AMPL402546844	chr11	4141069	4141161	92	42%	C/A	Het	SNP	1	REF	Homo	NA	NA
AMPL404067077	chr11	4159389	4159562	173	39%	A/G	Het	SNP	1	A/G	Het	SNP	1
AMPL404067077	chr11	4159389	4159562	173	39%	G/A	Het	SNP	1	A	Homo	SNP	1
AMPL659232630	chr11	17522560	17522717	157	59%	G/A	Het	SNP	1	REF	Homo	NA	NA
AMPL4395306426	chr11	17537601	17537783	182	49%	REF	Homo	NA	NA	G/C	Het	SNP	1
AMPL4392187570	chr11	36595534	36595682	148	47%	A/G	Het	SNP	1	G	Homo	SNP	1
AMPL4395394628	chr11	44151504	44151656	152	41%	T/A	Het	SNP	1	REF	Homo	NA	NA
AMPL4392903847	chr11	47260304	47260405	101	50%	REF	Homo	NA	NA	A/G	Het	SNP	1
AMPL4394224699	chr11	47364670	47364855	185	63%	G	Homo	SNP	1	G	Homo	SNP	1
AMPL4320019211	chr11	47371415	47371599	184	69%	A	Homo	DEL	1	AG/A	Het	DEL	1
AMPL4392351914	chr11	47463411	47463574	163	66%	A	Homo	DEL	1	A	Homo	DEL	1
AMPL3198712213	chr11	69462780	69462959	179	63%	G/A	Het	SNP	1	REF	Homo	NA	NA

Amplicon Information						NA12878				NA19240			
Amplicon Name	Chromosome	Start	End	Length	% GC	Genotype Expected	Zygosity	Variation Type	Variation Length	Genotype Expected	Zygosity	Variation Type	Variation Length
AMPL4394465691	chr11	71728837	71728991	154	58%	A	Homo	DEL	6	AGTCAAC/A	Het	DEL	6
AMPL4393628011	chr11	76867830	76867993	163	60%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL4394223117	chr11	76894174	76894343	169	64%	C	Homo	SNP	1	G/C	Het	SNP	1
AMPL4394360574	chr11	76895653	76895809	156	64%	REF	Homo	NA	NA	GGGAGGCGGGGACACCAGGGCCT/G	Het	DEL	22
AMPL4394214041	chr11	76901159	76901343	184	58%	T	Homo	SNP	1	T	Homo	SNP	1
AMPL4394678126	chr11	76901513	76901697	184	65%	TGCTGGGGCCTGGAGC	Homo	INS	15	REF	Homo	NA	NA
AMPL4394220527	chr11	76910689	76910823	134	60%	T	Homo	SNP	1	C/T	Het	SNP	1
AMPL3674673921	chr11	76917145	76917312	167	62%	A/G	Het	SNP	1	G	Homo	SNP	1
AMPL583119206	chr11	76919386	76919553	167	51%	A/T	Het	SNP	1	A/T	Het	SNP	1
AMPL583119206	chr11	76919386	76919553	167	51%	C/A	Het	SNP	1	A	Homo	SNP	1
AMPL3735262350	chr11	89017929	89017999	70	49%	A	Homo	SNP	1	REF	Homo	NA	NA
AMPL623995564	chr11	108121359	108121498	139	29%	REF	Homo	NA	NA	A/G	Het	SNP	1
AMPL1486637447	chr11	108139082	108139185	103	35%	REF	Homo	NA	NA	CTTAGTG/C	Het	DEL	6
AMPL3777975057	chr11	108150156	108150275	119	35%	CT/C	Het	DEL	1	REF	Homo	NA	NA
AMPL3778824349	chr11	108151661	108151772	111	23%	T/TA	Het	INS	1	T/TA	Het	INS	1
AMPL3378946700	chr11	119148489	119148660	171	38%	T	Homo	SNP	1	T	Homo	SNP	1
AMPL4394922626	chr11	125497377	125497553	176	34%	G/T	Het	SNP	1	T	Homo	SNP	1
AMPL588811071	chr11	125525141	125525249	108	48%	G	Homo	SNP	1	G	Homo	SNP	1
AMPL3817158157	chr11	128675206	128675375	169	40%	T/A	Het	SNP	1	T/A	Het	SNP	1
AMPL4394216319	chr12	2694583	2694746	163	63%	C/T	Het	SNP	1	REF	Homo	NA	NA
AMPL4326420418	chr12	2760874	2761041	167	67%	GA/G	Het	DEL	1	G	Homo	DEL	1
AMPL3829029013	chr12	2794750	2794920	170	57%	T/G	Het	SNP	1	G	Homo	SNP	1
AMPL4395303483	chr12	7362126	7362282	156	47%	REF	Homo	NA	NA	G/T	Het	SNP	1
AMPL4324007004	chr12	22063699	22063792	93	41%	T	Homo	SNP	1	T	Homo	SNP	1
AMPL4324007004	chr12	22063699	22063792	93	41%	C	Homo	SNP	1	C	Homo	SNP	1
AMPL4320523169	chr12	25358617	25358699	82	43%	CTT/C	Het	DEL	2	CTT/C	Het	DEL	2
AMPL395328621	chr12	25362710	25362877	167	27%	REF	Homo	NA	NA	A/G	Het	SNP	1
AMPL4395288076	chr12	33021844	33022022	178	51%	REF	Homo	NA	NA	A/G	Het	SNP	1
AMPL538987753	chr12	40645164	40645334	170	36%	C/A	Het	SNP	1	C/A	Het	SNP	1
AMPL4394633624	chr12	40681030	40681208	178	33%	REF	Homo	NA	NA	C/CT	Het	INS	1
AMPL4392357297	chr12	40757029	40757196	167	24%	ACATG	Homo	INS	4	ACATG	Homo	INS	4
AMPL4393606166	chr12	48374502	48374685	183	57%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL4393136614	chr12	49424591	49424735	144	64%	REF	Homo	NA	NA	T/TG	Het	INS	1
AMPL4394209036	chr12	56435842	56436002	160	61%	C/G	Het	SNP	1	REF	Homo	NA	NA
AMPL4394553261	chr12	56437683	56437851	168	46%	REF	Homo	NA	NA	T/TAG	Het	INS	2
AMPL1496946897	chr12	103234160	103234340	180	46%	REF	Homo	NA	NA	A/G	Het	SNP	1
AMPL1496946897	chr12	103234160	103234340	180	46%	T/C	Het	SNP	1	REF	Homo	NA	NA
AMPL3519304014	chr12	103306534	103306608	74	41%	REF	Homo	NA	NA	A/G	Het	SNP	1
AMPL4392413266	chr12	111350946	111351129	183	57%	TC/T	Het	DEL	1	REF	Homo	NA	NA
AMPL4395265123	chr12	111962825	111962995	170	33%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL3295318277	chr12	112919769	112919953	184	36%	REF	Homo	NA	NA	C/A	Het	SNP	1
AMPL4317912554	chr12	121431245	121431363	118	61%	T/A	Het	SNP	1	REF	Homo	NA	NA
AMPL4393793084	chr13	28585576	28585760	184	58%	CA/C	Het	DEL	1	CA/C	Het	DEL	1
AMPL4395343628	chr13	28597348	28597520	172	34%	ATC/A	Het	DEL	2	REF	Homo	NA	NA
AMPL1517948259	chr13	28608377	28608547	170	38%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL4393860661	chr13	28609966	28610122	156	35%	T/C	Het	SNP	1	REF	Homo	NA	NA
AMPL4373761294	chr13	28611325	28611457	132	34%	T/G	Het	SNP	1	REF	Homo	NA	NA
AMPL4393823209	chr13	28623609	28623787	178	34%	G/T	Het	SNP	1	REF	Homo	NA	NA
AMPL4393823209	chr13	28623609	28623787	178	34%	C/T	Het	SNP	1	REF	Homo	NA	NA
AMPL420799557	chr13	48947421	48947496	75	33%	T	Homo	SNP	1	T	Homo	SNP	1
AMPL416087660	chr13	49033706	49033825	119	26%	A	Homo	SNP	1	A	Homo	SNP	1
AMPL4374301969	chr13	103517942	103518118	176	39%	REF	Homo	NA	NA	A/G	Het	SNP	1
AMPL4392815412	chr13	103527760	103527936	176	40%	C	Homo	SNP	1	C	Homo	SNP	1
AMPL4392815412	chr13	103527760	103527936	176	40%	C	Homo	SNP	1	C	Homo	SNP	1
AMPL4393570611	chr13	110833626	110833799	173	51%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL4395233644	chr13	110858855	110859032	177	46%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4392696459	chr13	110864027	110864190	163	22%	REF	Homo	NA	NA	AG/A	Het	DEL	1

Amplicon Information						NA12878				NA19240			
Amplicon Name	Chromosome	Start	End	Length	% GC	Genotype Expected	Zygosity	Variation Type	Variation Length	Genotype Expected	Zygosity	Variation Type	Variation Length
AMPL1608248833	chr14	21778581	21778755	174	26%	AAAT/A	Het	DEL	3	REF	Homo	NA	NA
AMPL4393590705	chr14	21792740	21792894	154	47%	REF	Homo	NA	NA	A	Homo	SNP	1
AMPL4395233955	chr14	23855348	23855515	167	64%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL4393557392	chr14	23865796	23865973	177	54%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4395246850	chr14	23866622	23866800	178	55%	REF	Homo	NA	NA	G	Homo	SNP	1
AMPL4395355849	chr14	23888241	23888381	140	52%	T/TG	Het	INS	1	TG	Homo	INS	1
AMPL4392670346	chr14	51062126	51062305	179	32%	T	Homo	DEL	2	T	Homo	DEL	2
AMPL4393550446	chr14	51094599	51094782	183	37%	REF	Homo	NA	NA	A/C	Het	SNP	1
AMPL3498752779	chr14	51204934	51205092	158	39%	T	Homo	SNP	1	T	Homo	SNP	1
AMPL4393769156	chr14	56139260	56139365	105	34%	GT/G	Het	DEL	1	REF	Homo	NA	NA
AMPL557689580	chr14	62164530	62164685	155	32%	REF	Homo	NA	NA	CT/C	Het	DEL	1
AMPL4392824175	chr14	62194172	62194304	132	43%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4392796221	chr14	81534645	81534757	112	45%	REF	Homo	NA	NA	A/T	Het	SNP	1
AMPL3637426447	chr14	81574766	81574913	147	33%	REF	Homo	NA	NA	G/C	Het	SNP	1
AMPL3199428974	chr14	81610497	81610667	170	49%	C	Homo	SNP	1	G/C	Het	SNP	1
AMPL777571747	chr14	88852164	88852343	179	72%	T	Homo	DEL	1	T	Homo	DEL	1
AMPL4393547526	chr14	88894012	88894175	163	31%	REF	Homo	NA	NA	A/T	Het	SNP	1
AMPL4394883639	chr14	95572033	95572197	164	38%	REF	Homo	NA	NA	A/C	Het	SNP	1
AMPL391855502	chr15	28235684	28235864	180	56%	T	Homo	SNP	1	REF	Homo	NA	NA
AMPL4392820663	chr15	39880713	39880891	178	52%	A/G	Het	SNP	1	G	Homo	SNP	1
AMPL4392803596	chr15	39886341	39886519	178	42%	REF	Homo	NA	NA	A	Homo	SNP	1
AMPL4374504539	chr15	40457155	40457311	156	37%	REF	Homo	NA	NA	G/GT	Het	INS	1
AMPL4395008361	chr15	40476060	40476215	155	36%	REF	Homo	NA	NA	T/TA	Het	INS	1
AMPL4374372220	chr15	40494801	40494975	174	40%	A/T	Het	SNP	1	T	Homo	SNP	1
AMPL4374372220	chr15	40494801	40494975	174	40%	A/C	Het	SNP	1	REF	Homo	NA	NA
AMPL4392783695	chr15	40498416	40498584	168	43%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL4392776958	chr15	40898606	40898724	118	35%	G/C	Het	SNP	1	G/C	Het	SNP	1
AMPL4394859257	chr15	40914671	40914844	173	39%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL4392795183	chr15	40915154	40915305	151	38%	A/G	Het	SNP	1	G	Homo	SNP	1
AMPL3763697326	chr15	40915785	40915961	176	41%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL4392776634	chr15	40949499	40949581	82	33%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL4394847665	chr15	40954280	40954452	172	41%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4393146133	chr15	41797467	41797647	180	61%	REF	Homo	NA	NA	T/TG	Het	INS	1
AMPL4392805233	chr15	43571313	43571466	153	54%	G	Homo	SNP	1	G	Homo	SNP	1
AMPL4393330449	chr15	48703039	48703216	177	29%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL4393549181	chr15	48719613	48719791	178	37%	REF	Homo	NA	NA	G/T	Het	SNP	1
AMPL4393560571	chr15	48722794	48722972	178	49%	REF	Homo	NA	NA	A/G	Het	SNP	1
AMPL4394644825	chr15	48759924	48760067	143	44%	REF	Homo	NA	NA	AT/A	Het	DEL	1
AMPL562172230	chr15	48787737	48787910	173	39%	REF	Homo	NA	NA	TA/T	Het	DEL	1
AMPL562219796	chr15	48797186	48797347	161	51%	REF	Homo	NA	NA	G	Homo	SNP	1
AMPL4393539439	chr15	48805866	48806027	161	27%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL4392146647	chr15	67457242	67457419	177	59%	G	Homo	SNP	1	G	Homo	SNP	1
AMPL769894769	chr15	72105889	72105999	110	58%	A	Homo	DEL	1	A	Homo	DEL	1
AMPL3374963808	chr15	72638919	72639093	174	51%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL3720788183	chr15	73614695	73614876	181	45%	GGT/G	Het	DEL	2	REF	Homo	NA	NA
AMPL3720788183	chr15	73614695	73614876	181	45%	T/C	Het	SNP	1	REF	Homo	NA	NA
AMPL4395377014	chr15	74336548	74336725	177	61%	C	Homo	SNP	1	REF	Homo	NA	NA
AMPL4392352458	chr15	89859827	89860005	178	53%	A/AC	Het	INS	1	REF	Homo	NA	NA
AMPL4392678393	chr15	89864234	89864394	160	57%	G/GCTAC	Het	INS	4	GCTAC	Homo	INS	4
AMPL4393760723	chr15	89869687	89869838	151	53%	A/AC	Het	INS	1	REF	Homo	NA	NA
AMPL3681904054	chr15	91337435	91337551	116	33%	REF	Homo	NA	NA	C/A	Het	SNP	1
AMPL4394458394	chr15	91358164	91358333	169	41%	G/GGAA	Het	INS	3	GGAA	Homo	INS	3
AMPL4393140419	chr15	99482289	99482470	181	39%	REF	Homo	NA	NA	G/GA	Het	INS	1
AMPL4373740005	chr16	14025882	14026059	177	42%	A	Homo	SNP	1	A	Homo	SNP	1
AMPL3691760208	chr16	15810982	15811144	162	65%	T	Homo	SNP	1	C/T	Het	SNP	1
AMPL3691760208	chr16	15810982	15811144	162	65%	T	Homo	SNP	1	C/T	Het	SNP	1
AMPL4392126885	chr16	15818055	15818233	178	55%	C	Homo	SNP	1	A/C	Het	SNP	1

Amplicon Information						NA12878				NA19240			
Amplicon Name	Chromosome	Start	End	Length	% GC	Genotype Expected	Zygosity	Variation Type	Variation Length	Genotype Expected	Zygosity	Variation Type	Variation Length
AMPL4392372494	chr16	15818379	15818561	182	62%	CG/C	Het	DEL	1	REF	Homo	NA	NA
AMPL4393856361	chr16	15865383	15865559	176	54%	G/A	Het	SNP	1	REF	Homo	NA	NA
AMPL4373672931	chr16	27456502	27456666	164	57%	REF	Homo	NA	NA	G/C	Het	SNP	1
AMPL4392120439	chr16	57946844	57947023	179	63%	A/T	Het	SNP	1	A/T	Het	SNP	1
AMPL4392120439	chr16	57946844	57947023	179	63%	A/G	Het	SNP	1	A/G	Het	SNP	1
AMPL4300890407	chr16	89806276	89806419	143	46%	A/T	Het	SNP	1	T	Homo	SNP	1
AMPL4301194040	chr16	89831404	89831586	182	53%	C/A	Het	SNP	1	REF	Homo	NA	NA
AMPL544389827	chr16	89836261	89836432	171	66%	C/T	Het	SNP	1	T	Homo	SNP	1
AMPL4216700451	chr16	89849352	89849532	180	57%	T	Homo	SNP	1	T	Homo	SNP	1
AMPL1156353735	chr16	89865962	89866103	141	38%	T/C	Het	SNP	1	C	Homo	SNP	1
AMPL4300833980	chr16	89869666	89869844	178	43%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL4300833980	chr16	89869666	89869844	178	43%	C	Homo	SNP	1	C	Homo	SNP	1
AMPL4393523704	chr16	89874777	89874951	174	32%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4395213682	chr16	89880764	89880934	170	34%	REF	Homo	NA	NA	C/G	Het	SNP	1
AMPL789566107	chr17	1554535	1554713	178	63%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL4393540427	chr17	1578547	1578719	172	50%	REF	Homo	NA	NA	A/G	Het	SNP	1
AMPL789212884	chr17	1580725	1580901	176	49%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4393492671	chr17	1585039	1585218	179	55%	REF	Homo	NA	NA	A/G	Het	SNP	1
AMPL4393748172	chr17	5288544	5288670	126	44%	GC/G	Het	DEL	1	REF	Homo	NA	NA
AMPL4394161757	chr17	6331703	6331883	180	65%	T/C	Het	SNP	1	REF	Homo	NA	NA
AMPL4394161757	chr17	6331703	6331883	180	65%	T/G	Het	SNP	1	REF	Homo	NA	NA
AMPL4393462046	chr17	7348707	7348867	160	70%	REF	Homo	NA	NA	G/T	Het	SNP	1
AMPL4392661656	chr17	7579577	7579754	177	59%	CCCCCAGCCCTCCAGGT/C	Het	DEL	16	CCCCCAGCCCTCCAGGT/C	Het	DEL	16
AMPL3889188256	chr17	11650979	11651145	166	53%	A/G	Het	SNP	1	A/G	Het	SNP	1
AMPL4395202607	chr17	11687442	11687600	158	41%	REF	Homo	NA	NA	G/T	Het	SNP	1
AMPL4392398765	chr17	11696967	11697134	167	32%	C/CA	Het	INS	1	CA	Homo	INS	1
AMPL4392113442	chr17	11778129	11778308	179	50%	G/A	Het	SNP	1	G/A	Het	SNP	1
AMPL4395337088	chr17	15133617	15133792	175	53%	CAG/C	Het	DEL	2	CAG/C	Het	DEL	2
AMPL4393477737	chr17	29528505	29528684	179	24%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4305062531	chr17	29559839	29560015	176	38%	A	Homo	SNP	1	C/A	Het	SNP	1
AMPL4392565249	chr17	29562937	29563112	175	35%	TTG	Homo	INS	2	REF	Homo	NA	NA
AMPL4395050447	chr17	37657457	37657532	75	27%	REF	Homo	NA	NA	C/CT	Het	INS	1
AMPL4393470475	chr17	37816372	37816557	185	58%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL4392777432	chr17	37830819	37830993	174	63%	A/G	Het	SNP	1	G	Homo	SNP	1
AMPL4395183962	chr17	39742431	39742560	129	53%	REF	Homo	NA	NA	C	Homo	SNP	1
AMPL2949788169	chr17	39923513	39923694	181	60%	G	Homo	SNP	1	G	Homo	SNP	1
AMPL4392758534	chr17	45360799	45360978	179	63%	REF	Homo	NA	NA	A/G	Het	SNP	1
AMPL4393492728	chr17	48263815	48263994	179	60%	REF	Homo	NA	NA	A/G	Het	SNP	1
AMPL4392107402	chr17	48265381	48265517	136	64%	G	Homo	SNP	1	A/G	Het	SNP	1
AMPL4392107402	chr17	48265381	48265517	136	64%	C	Homo	SNP	1	C	Homo	SNP	1
AMPL4392128611	chr17	48268147	48268297	150	63%	G	Homo	SNP	1	G	Homo	SNP	1
AMPL549199875	chr17	48269250	48269402	152	62%	A	Homo	SNP	1	A	Homo	SNP	1
AMPL549072782	chr17	48275081	48275258	177	50%	REF	Homo	NA	NA	TA/T	Het	DEL	1
AMPL4392509220	chr17	48276939	48277127	188	73%	G/C	Het	SNP	1	G/C	Het	SNP	1
AMPL4392510383	chr17	53342737	53342872	135	45%	G/GTTTC	Het	INS	4	REF	Homo	NA	NA
AMPL4395349481	chr17	56688438	56688616	178	53%	GT/G	Het	DEL	1	REF	Homo	NA	NA
AMPL1104437054	chr17	59760934	59761106	172	33%	G	Homo	SNP	1	G	Homo	SNP	1
AMPL4104053523	chr17	59763278	59763431	153	37%	G	Homo	SNP	1	G	Homo	SNP	1
AMPL4393041536	chr17	66519805	66519940	135	36%	REF	Homo	NA	NA	C/CT	Het	INS	1
AMPL4394409931	chr17	72306014	72306194	180	58%	CCT/C	Het	DEL	2	REF	Homo	NA	NA
AMPL4392109226	chr17	73720786	73720965	179	65%	G	Homo	SNP	1	G	Homo	SNP	1
AMPL573434660	chr17	78013946	78014117	171	63%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4392117695	chr17	78055681	78055837	156	58%	C/T	Het	SNP	1	REF	Homo	NA	NA
AMPL4392117695	chr17	78055681	78055837	156	58%	T/G	Het	SNP	1	G	Homo	SNP	1
AMPL4395175643	chr17	78059986	78060167	181	57%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4373650991	chr17	78070956	78071139	183	58%	C	Homo	SNP	1	REF	Homo	NA	NA
AMPL4392110866	chr17	78073465	78073637	172	62%	A/G	Het	SNP	1	REF	Homo	NA	NA

Amplicon Information						NA12878				NA19240			
Amplicon Name	Chromosome	Start	End	Length	% GC	Genotype Expected	Zygosity	Variation Type	Variation Length	Genotype Expected	Zygosity	Variation Type	Variation Length
AMPL4392110866	chr17	78073465	78073637	172	62%	T/C	Het	SNP	1	REF	Homo	NA	NA
AMPL4394139126	chr17	78078672	78078790	118	67%	C	Homo	SNP	1	REF	Homo	NA	NA
AMPL555450618	chr17	78081618	78081797	179	70%	A	Homo	SNP	1	REF	Homo	NA	NA
AMPL555283876	chr17	78084718	78084889	171	60%	G/A	Het	SNP	1	REF	Homo	NA	NA
AMPL4392759751	chr17	78302071	78302241	170	41%	REF	Homo	NA	NA	A	Homo	SNP	1
AMPL4392767076	chr17	78319054	78319218	164	55%	REF	Homo	NA	NA	A	Homo	SNP	1
AMPL4394809934	chr17	78327284	78327435	151	60%	REF	Homo	NA	NA	A	Homo	SNP	1
AMPL4394147071	chr18	21119201	21119377	176	39%	G	Homo	SNP	1	G	Homo	SNP	1
AMPL3289654030	chr18	28666393	28666570	177	30%	TTAA	Homo	INS	3	TTAA	Homo	INS	3
AMPL4392726050	chr18	28669555	28669731	176	23%	CTTAA/C	Het	DEL	4	C	Homo	DEL	4
AMPL3961593542	chr18	29104474	29104652	178	25%	C/A	Het	SNP	1	REF	Homo	NA	NA
AMPL4394756215	chr18	50831835	50832014	179	37%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL4394758212	chr18	59166465	59166636	171	56%	C/T	Het	SNP	1	C/T	Het	SNP	1
AMPL4392098735	chr18	77477404	77477589	185	69%	C/T	Het	SNP	1	REF	Homo	NA	NA
AMPL4392422488	chr19	1619669	1619838	169	60%	A/AGGGTGGGGTG	Het	INS	10	A/AGGGTG	Het	INS	5
AMPL4393028379	chr19	3113443	3113629	186	76%	REF	Homo	NA	NA	C	Homo	DEL	15
AMPL700490202	chr19	16593266	16593419	153	45%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL3764819988	chr19	17937608	17937762	154	66%	C/T	Het	SNP	1	REF	Homo	NA	NA
AMPL4393070345	chr19	18876284	18876438	154	65%	REF	Homo	NA	NA	GA/G	Het	DEL	1
AMPL4374033508	chr19	30303473	30303659	186	78%	C/CGGACG	Het	INS	5	REF	Homo	NA	NA
AMPL597978528	chr19	35523495	35523664	169	63%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL3569591894	chr19	38939302	38939435	133	65%	C	Homo	SNP	1	C	Homo	SNP	1
AMPL4393411927	chr19	38968440	38968616	176	66%	REF	Homo	NA	NA	G/C	Het	SNP	1
AMPL4393455182	chr19	38973498	38973678	180	58%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL4393432724	chr19	38995283	38995457	174	60%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL3695208132	chr19	38996902	38997076	174	61%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL4393407496	chr19	38997355	38997526	171	60%	REF	Homo	NA	NA	T/TTCA	Het	INS	3
AMPL4393407496	chr19	38997355	38997526	171	60%	REF	Homo	NA	NA	G/C	Het	SNP	1
AMPL4392668212	chr19	39034509	39034663	154	59%	REF	Homo	NA	NA	GC/G	Het	DEL	1
AMPL397538170	chr19	39057443	39057625	182	60%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4394405741	chr19	41928787	41928952	165	64%	GC	Homo	INS	1	G/GC	Het	INS	1
AMPL4392096706	chr19	41930301	41930484	183	66%	G	Homo	SNP	1	A/G	Het	SNP	1
AMPL4393123724	chr19	45864902	45865063	161	66%	REF	Homo	NA	NA	C/CG	Het	INS	1
AMPL4395386501	chr19	45922245	45922420	175	58%	G/T	Het	SNP	1	REF	Homo	NA	NA
AMPL4392102936	chr19	55644240	55644416	176	62%	C	Homo	SNP	1	C	Homo	SNP	1
AMPL4297998702	chr19	55653230	55653332	102	63%	A/C	Het	SNP	1	REF	Homo	NA	NA
AMPL532298001	chr19	55667556	55667736	180	71%	C/A	Het	SNP	1	REF	Homo	NA	NA
AMPL4392537380	chr20	31019281	31019438	157	49%	AT/A	Het	DEL	1	REF	Homo	NA	NA
AMPL4394751711	chr20	31022692	31022838	146	59%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4395148488	chr20	32000402	32000582	180	61%	REF	Homo	NA	NA	A/G	Het	SNP	1
AMPL4394736429	chr20	41306521	41306680	159	61%	REF	Homo	NA	NA	A/G	Het	SNP	1
AMPL551935865	chr20	43252834	43253007	173	61%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL4392073003	chr20	50407090	50407255	165	52%	T/C	Het	SNP	1	REF	Homo	NA	NA
AMPL4394129588	chr20	50408398	50408557	159	60%	G	Homo	SNP	1	G	Homo	SNP	1
AMPL595971724	chr21	35821740	35821910	170	63%	C	Homo	SNP	1	C	Homo	SNP	1
AMPL4394610057	chr21	45707413	45707593	180	71%	REF	Homo	NA	NA	CA/C	Het	DEL	1
AMPL4392084924	chr21	45717457	45717636	179	65%	C	Homo	SNP	1	C	Homo	SNP	1
AMPL4392744809	chr21	46313347	46313530	183	61%	REF	Homo	NA	NA	G/T	Het	SNP	1
AMPL4392755421	chr22	24158805	24158987	182	55%	C/A	Het	SNP	1	C/A	Het	SNP	1
AMPL4395355273	chr22	36688108	36688247	139	64%	G/A	Het	SNP	1	REF	Homo	NA	NA
AMPL4373723911	chr22	36710103	36710277	174	60%	T/C	Het	SNP	1	REF	Homo	NA	NA
AMPL4392449135	chr22	39636854	39637033	179	53%	A	Homo	DEL	4	REF	Homo	NA	NA
AMPL4392752461	chr22	41573992	41574172	180	63%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL3920741189	chr22	42526477	42526643	166	61%	T	Homo	SNP	1	C/T	Het	SNP	1
AMPL3289833537	chrX	32305727	32305905	178	30%	A/AAT	Het	INS	2	REF	Homo	NA	NA
AMPL4395147724	chrX	32583655	32583821	166	34%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4392928879	chrX	33357203	33357360	157	40%	REF	Homo	NA	NA	G/A	Het	SNP	1

Amplicon Information						NA12878				NA19240			
Amplicon Name	Chromosome	Start	End	Length	% GC	Genotype Expected	Zygosity	Variation Type	Variation Length	Genotype Expected	Zygosity	Variation Type	Variation Length
AMPL4394730094	chrX	44833754	44833923	169	37%	C/A	Het	SNP	1	A	Homo	SNP	1
AMPL1676134309	chrX	44966687	44966825	138	39%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL1676134309	chrX	44966687	44966825	138	39%	REF	Homo	NA	NA	GT/G	Het	DEL	1
AMPL749578720	chrX	70595055	70595210	155	46%	REF	Homo	NA	NA	T/C	Het	SNP	1
AMPL4373417951	chrX	70613049	70613140	91	38%	REF	Homo	NA	NA	T/A	Het	SNP	1
AMPL4392707708	chrX	70627405	70627547	142	46%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL4394086736	chrX	77298747	77298920	173	32%	A	Homo	SNP	1	A	Homo	SNP	1
AMPL3700904683	chrX	79283457	79283544	87	47%	REF	Homo	NA	NA	C	Homo	SNP	1
AMPL553632290	chrX	107829816	107829986	170	44%	REF	Homo	NA	NA	G/C	Het	SNP	1
AMPL4393378805	chrX	107929262	107929420	158	56%	REF	Homo	NA	NA	C/T	Het	SNP	1
AMPL569668692	chrX	153760051	153760229	178	73%	REF	Homo	NA	NA	GTCC/G	Het	DEL	3
AMPL4314163957	chrX	154065760	154065933	173	61%	REF	Homo	NA	NA	G/A	Het	SNP	1
AMPL529493999	chrX	154158197	154158369	172	36%	REF	Homo	NA	NA	C	Homo	SNP	1
AMPL4392743687	chr1	114940545	114940708	163	34%	No variants*				No variants*			
AMPL3840038528	chr1	144873863	144873997	134	54%	No variants*				No variants*			
AMPL4393344413	chr1	144909911	144910000	89	36%	No variants*				No variants*			
AMPL4394508046	chr1	144915520	144915680	160	49%	No variants*				No variants*			
AMPL4393316443	chr1	144994811	144994992	181	62%	No variants*				No variants*			
AMPL4392299366	chr1	237850588	237850742	154	33%	No variants*				No variants*			
AMPL912086672	chr2	141707684	141707848	164	28%	No variants*				No variants*			
AMPL4218950823	chr3	10088170	10088344	174	34%	No variants*				No variants*			
AMPL3256513166	chr3	38627039	38627214	175	46%	No variants*				No variants*			
AMPL4392692126	chr3	48602446	48602601	155	61%	No variants*				No variants*			
AMPL4374007010	chr3	187442590	187442772	182	59%	No variants*				No variants*			
AMPL4395311255	chr4	1809082	1809218	136	60%	No variants*				No variants*			
AMPL4395318837	chr4	106191761	106191898	137	32%	No variants*				No variants*			
AMPL3676543350	chr6	51930797	51930972	175	30%	No variants*				No variants*			
AMPL3435076154	chr6	117631334	117631510	176	36%	No variants*				No variants*			
AMPL3498792122	chr7	6036957	6037130	173	38%	No variants*				No variants*			
AMPL4392491672	chr7	140434509	140434683	174	31%	No variants*				No variants*			
AMPL4322896544	chr7	151970960	151971126	166	27%	No variants*				No variants*			
AMPL668652008	chr8	145738681	145738858	177	70%	No variants*				No variants*			
AMPL3797942774	chr9	135772895	135773048	153	35%	No variants*				No variants*			
AMPL4393636823	chr9	137676710	137676883	173	62%	No variants*				No variants*			
AMPL4394566481	chr10	73572319	73572470	151	64%	No variants*				No variants*			
AMPL4313036002	chr10	75874121	75874273	152	52%	No variants*				No variants*			
AMPL2675981375	chr10	76781817	76781993	176	46%	No variants*				No variants*			
AMPL644684281	chr10	88458921	88459093	172	49%	No variants*				No variants*			
AMPL528631298	chr11	32456688	32456864	176	74%	No variants*				No variants*			
AMPL4298148304	chr11	61725541	61725702	161	60%	No variants*				No variants*			
AMPL4394624154	chr12	40703006	40703181	175	32%	No variants*				No variants*			
AMPL4235578244	chr14	21796697	21796872	175	38%	No variants*				No variants*			
AMPL3733781601	chr14	21802697	21802871	174	43%	No variants*				No variants*			
AMPL4393119864	chr14	95579309	95579428	119	26%	No variants*				No variants*			
AMPL4085226812	chr16	2142906	2142992	86	63%	No variants*				No variants*			
AMPL4392701696	chr17	7124675	7124850	175	59%	No variants*				No variants*			
AMPL4392708195	chr18	32711821	32711997	176	44%	No variants*				No variants*			
AMPL1118439227	chr20	40898964	40899142	178	65%	No variants*				No variants*			
AMPL4373586961	chr21	27348309	27348481	172	30%	No variants*				No variants*			
AMPL4394432367	chr22	30051616	30051777	161	43%	No variants*				No variants*			
AMPL3680965544	chr22	42524148	42524322	174	63%	No variants*				No variants*			
AMPL4392728909	chrX	53221980	53222116	136	61%	No variants*				No variants*			

* The SVA panel contains 39 amplicons which do not have a variant in our hotspot file.

Table 26 Sequencing Characteristics by Genome

Sequencing characteristics for each of the 632 amplicons generated by the SVA panel are presented for both genomes used in the study (NA12878 and NA12940). Data are from a representative sequencing run (four samples per run) of each genome selected prior to the start of the study.

		NA12878		NA19240		Variant
Amplicon Name	Length	Coverage Depth (Avg)[1]	# Aligned Reads[2]	Coverage Depth (Avg)[1]	# Aligned Reads[2]	Type[3]
AMPL4394358129	178	1383	1418	1417	1465	SNP
AMPL4394343000	168	568	584	557	589	SNP
AMPL4393046521	185	711	1108	831	1100	Indel
AMPL4392504456	178	167	267	136	236	Indel
AMPL4393385216	180	2684	2723	2880	2916	SNP
AMPL4393372520	173	4133	4162	4614	4662	SNP
AMPL4392440990	179	2146	2366	2170	2206	Indel Indel
AMPL4392338993	171	1807	1830	2181	2242	SNP
AMPL3200084096	176	1981	2119	1730	1918	SNP
AMPL959019077	173	105	112	301	327	Indel
AMPL4297944685	177	1703	1742	1814	1855	SNP
AMPL4298066535	154	2213	2231	2668	2696	SNP
AMPL4392347320	181	2908	2998	2996	3073	SNP
AMPL4392659170	162	3660	3694	3722	3761	SNP
AMPL4392650892	180	1266	1279	1520	1536	SNP
AMPL4373731419	171	1683	1710	1805	1831	SNP
AMPL4392328096	154	1293	1309	1270	1289	SNP
AMPL4392651712	167	726	731	1815	1836	SNP
AMPL4392320712	126	1044	1048	979	987	SNP
AMPL4392327972	175	1637	1656	1468	1484	SNP
AMPL4392743687	163	1693	1818	1949	2145	NA
AMPL4322363520	154	2807	2853	2959	3011	SNP
AMPL3289619107	178	1600	1615	1887	1922	SNP SNP
AMPL4392073064	177	1247	2191	1375	2496	SNP
AMPL4301817011	166	2559	2588	2621	2653	SNP
AMPL413175205	154	1408	1422	1529	1561	SNP
AMPL1871943647	159	8229	8244	8233	8279	SNP
AMPL3840258113	176	4607	4785	5082	5302	SNP
AMPL1090715363	170	2329	2365	2517	2552	SNP
AMPL4392034654	144	8496	8682	8779	8969	SNP
AMPL3840038528	134	5205	5365	5259	5437	NA
AMPL4392047471	169	266	274	338	343	SNP
AMPL4392040940	178	3155	3232	3306	3457	SNP
AMPL4392033882	172	6221	6271	7158	7213	SNP
AMPL4392030295	177	455	491	520	550	SNP
AMPL4392028734	174	5255	5377	4796	4895	SNP
AMPL4393344413	89	11861	11914	13811	13904	NA
AMPL4394508046	160	3163	3204	3799	3853	NA
AMPL2858925985	171	7088	7153	7325	7408	SNP
AMPL4392024816	172	7985	8047	8826	8895	SNP SNP
AMPL4392025061	170	4758	4842	5557	5671	SNP
AMPL4394524216	181	6127	6252	6249	6390	Indel
AMPL4394090071	175	2670	2718	2704	2748	SNP SNP
AMPL4393326327	175	7531	7704	7885	8148	SNP SNP
AMPL4393283848	175	3149	3197	4031	4098	SNP
AMPL4394093339	113	20381	20427	18576	18704	SNP
AMPL4393316443	181	988	1009	1092	1123	NA
AMPL4373875155	166	6271	6424	5484	5670	SNP
AMPL4392008110	115	16919	17510	17397	18115	SNP SNP
AMPL3819598533	184	2871	2952	2570	2656	SNP
AMPL398678636	182	2196	2235	2751	2785	SNP

Amplicon Name	Length	NA12878		NA19240		Variant Type[3]
		Coverage Depth (Avg)[1]	# Aligned Reads[2]	Coverage Depth (Avg)[1]	# Aligned Reads[2]	
AMPL4392693991	171	378	400	440	475	Indel
AMPL3731499965	146	2802	2883	2891	2958	SNP
AMPL4393296666	167	2859	2898	3237	3293	SNP
AMPL526439206	187	1826	1939	1025	1093	Indel
AMPL4393093192	177	2017	2072	2017	2092	Indel
AMPL4392317144	116	3515	3615	3548	3639	SNP
AMPL4304199970	171	2922	2979	3391	3454	Indel
AMPL4103839312	156	4273	4308	4393	4436	SNP
AMPL4394463576	174	3377	3496	3075	3290	Indel
AMPL4394318742	172	1407	1428	1472	1491	SNP
AMPL4392325406	175	1353	1387	1478	1506	SNP
AMPL4324701736	113	1593	1603	1870	1885	SNP
AMPL3257535118	175	2720	3546	3231	3907	Indel
AMPL4392598603	177	1906	2568	1950	2660	Indel
AMPL4394085835	167	2803	2826	3012	3042	SNP
AMPL4394068372	153	2418	2431	2571	2601	SNP
AMPL4039838351	156	1367	1383	1437	1451	SNP
AMPL4392315810	185	1567	1607	1456	1491	SNP
AMPL4392611784	171	998	1138	1201	1358	SNP SNP
AMPL4304947808	159	2602	2630	2672	2717	SNP
AMPL4392311692	173	1637	1660	1756	1789	SNP
AMPL3198880818	168	2456	2478	2666	2695	SNP
AMPL4395108860	160	3224	3350	2990	3506	SNP
AMPL4318413913	179	1696	1744	1831	1873	SNP
AMPL4392298562	173	2453	2470	2640	2673	SNP
AMPL461585857	178	1051	1073	1245	1279	SNP
AMPL4394997727	159	3265	3299	3108	3159	Indel
AMPL4393286038	124	3361	3427	3360	3416	SNP
AMPL4392312449	131	1115	1121	953	963	SNP
AMPL4373352228	167	127	127	207	211	SNP
AMPL4392299366	154	273	433	336	522	NA
AMPL598421930	179	3406	3729	3317	3637	SNP
AMPL4394316952	173	2349	2398	2509	2568	SNP
AMPL3497995644	144	824	832	937	947	SNP
AMPL4391994088	157	2983	3050	3908	3977	SNP
AMPL4393073176	172	2556	2595	2869	2933	Indel
AMPL3666903404	160	295	315	321	354	SNP
AMPL735306822	83	2720	2741	3481	3524	SNP
AMPL4374432645	160	2122	2163	2312	2361	SNP
AMPL4394446055	153	3503	3723	3799	4262	Indel
AMPL4394058159	174	3421	3453	3616	3666	SNP
AMPL3770343132	169	2678	2734	2540	2601	SNP
AMPL4394312266	172	2833	2860	2986	3023	SNP
AMPL4373941627	179	823	975	1172	1291	Indel
AMPL3434544210	164	1399	1749	1790	2181	Indel
AMPL4392293614	177	2537	2565	2744	2772	SNP
AMPL4391994985	165	1929	1980	1786	1865	SNP
AMPL4394057009	174	2334	2447	2457	2695	SNP
AMPL912086672	164	1498	1508	1656	1679	NA
AMPL905336021	117	4351	4374	4512	4572	SNP
AMPL4394077238	169	2884	2896	3042	3052	SNP
AMPL1705717513	83	2460	2481	2360	2387	SNP
AMPL4392286218	173	1539	1547	1649	1666	SNP
AMPL4394302264	175	1110	1130	1257	1277	SNP
AMPL4394548445	164	3916	3965	3873	3921	SNP
AMPL4392296241	76	1546	1708	1416	1608	SNP
AMPL4309761177	70	1904	1915	2083	2083	Indel

Amplicon Name	Length	NA12878		NA19240		Variant Type[3]
		Coverage Depth (Avg)[1]	# Aligned Reads[2]	Coverage Depth (Avg)[1]	# Aligned Reads[2]	
AMPL556873511	168	1890	1928	2607	2671	SNP SNP
AMPL4392283526	178	2151	2184	2592	2641	SNP
AMPL4392722819	175	1676	1702	1884	1920	Indel
AMPL4392277638	133	2368	2375	2437	2453	SNP
AMPL4373957362	152	3391	3423	3618	3659	Indel
AMPL4392651475	175	2436	2463	3006	3036	SNP
AMPL3379180838	131	3736	3783	4345	4424	SNP
AMPL3454598707	156	2599	2634	2819	2863	Indel
AMPL3498234957	171	1106	1132	1348	1383	Indel
AMPL3073489807	162	1068	1072	1203	1207	SNP
AMPL4392487356	143	2353	2415	2707	2773	Indel
AMPL4392495150	159	2714	2778	3050	3113	Indel
AMPL4394453234	174	2252	2717	2713	3285	Indel
AMPL581765267	176	2840	2986	3653	3852	Indel
AMPL4391973685	167	2871	2909	3126	3168	SNP
AMPL4392278772	169	2812	2871	2751	2816	SNP
AMPL4392278508	146	1432	1445	1723	1783	SNP
AMPL4374039730	185	3007	3039	3441	3483	Indel
AMPL4394049619	152	5207	5315	5628	5726	SNP
AMPL4218950823	174	2045	2063	2345	2382	NA
AMPL4219350794	168	2740	2817	2773	2860	SNP
AMPL969040897	173	101	107	227	252	Indel
AMPL4392714050	159	1586	1596	1787	1804	Indel
AMPL4395093302	176	2840	2903	3129	3213	SNP
AMPL3255695397	170	2763	2845	2611	2703	SNP
AMPL3256513166	175	1409	2732	1765	3196	NA
AMPL4394436054	173	1640	1681	1781	1969	Indel
AMPL4391970287	181	3524	3633	4344	4489	SNP
AMPL4393295145	176	1603	1643	1753	1791	SNP
AMPL4392692126	155	2007	2816	1992	2813	NA
AMPL4393058046	89	3465	3462	3322	3343	Indel
AMPL4395087540	177	3240	3359	3596	3795	SNP
AMPL4393144102	82	3619	3641	5245	5289	SNP
AMPL4392648148	173	3728	3758	3790	3816	SNP
AMPL4392262531	177	1533	2058	1298	1805	SNP
AMPL4392638186	178	1983	2121	1974	2148	SNP
AMPL4392477090	151	2802	3244	2607	3161	Indel
AMPL4394514215	108	4485	4497	4132	4164	SNP
AMPL4392619457	156	1234	1297	1025	1073	SNP
AMPL748437993	178	1196	2013	1245	2033	SNP
AMPL4393315786	138	1142	1142	1076	1079	SNP
AMPL736692473	112	1413	1441	1495	1543	SNP
AMPL4305635694	168	72	78	109	126	SNP
AMPL4395062735	118	3161	3207	4109	4178	SNP
AMPL4219407492	125	1608	1616	1486	1505	SNP
AMPL4222168082	169	3088	3141	3528	3616	SNP
AMPL4301889960	154	3175	3183	3475	3501	SNP SNP
AMPL4392603343	145	6585	6628	4812	4873	SNP
AMPL4374007010	182	1472	1546	1494	1590	NA
AMPL4393227469	172	1210	1247	1385	1445	SNP
AMPL4392596733	163	706	718	709	717	SNP
AMPL4395311255	136	672	796	691	808	NA
AMPL4392251505	141	2203	2237	2273	2316	SNP
AMPL4227407944	153	2069	2087	1955	1972	SNP
AMPL395434724	175	1958	1977	1732	1755	Indel
AMPL4395087555	179	1216	1249	1135	1171	SNP
AMPL4322933975	148	2413	2455	2482	2526	Indel

Amplicon Name	Length	NA12878		NA19240		Variant Type[3]
		Coverage Depth (Avg)[1]	# Aligned Reads[2]	Coverage Depth (Avg)[1]	# Aligned Reads[2]	
AMPL4394545846	132	2763	2802	2924	2972	SNP
AMPL4394601334	152	2595	2605	2376	2381	Indel
AMPL4373689558	181	2780	2810	3184	3247	SNP
AMPL4391975825	181	2766	2798	3332	3366	SNP
AMPL4393733107	183	2807	3043	2826	3296	Indel
AMPL3498362734	140	1606	1618	1519	1526	SNP
AMPL413738456	115	1241	1254	1719	1753	SNP
AMPL4395318837	137	2226	3228	3998	4989	NA
AMPL4392997426	173	2508	2550	2448	2511	Indel
AMPL4392261532	180	2667	2714	2550	2616	SNP
AMPL2742861328	167	1653	1673	1555	1587	SNP
AMPL4392257952	172	1932	1963	2513	2573	SNP
AMPL4308774096	151	5132	5156	5205	5242	SNP SNP
AMPL4392228266	149	3555	3570	3592	3610	SNP
AMPL3467347768	164	1874	1900	1905	1936	SNP
AMPL552238544	172	1279	1297	1371	1390	SNP
AMPL551297934	170	1714	1753	1907	1944	SNP
AMPL3498059646	175	2292	2302	2384	2402	SNP
AMPL4393190958	180	3925	3961	4142	4172	SNP
AMPL4392317694	175	1716	2294	2226	2556	Indel
AMPL4393183760	140	682	694	796	809	SNP
AMPL1629455589	170	2076	2101	2291	2339	SNP
AMPL393611061	119	3289	3276	3788	3788	SNP
AMPL3653082204	176	1729	1751	1668	1690	SNP
AMPL4391964653	171	2482	2515	3197	3249	SNP
AMPL1211083733	185	2118	2214	1977	2279	SNP
AMPL4392743054	177	576	904	429	717	Indel
AMPL407783594	168	2592	2633	2822	2865	SNP
AMPL1663497431	178	1781	1857	1933	1977	SNP
AMPL863277015	182	1244	1345	1255	1357	SNP
AMPL4394285134	155	499	509	608	615	SNP
AMPL4393684226	180	3217	3310	3058	3136	Indel
AMPL3433472167	173	562	578	600	616	SNP
AMPL4394027906	176	2388	2430	2805	2866	SNP
AMPL416374362	113	3671	3684	3750	3767	SNP
AMPL4393199548	170	2383	2421	2532	2594	SNP
AMPL4392452727	133	2827	2922	3175	3265	Indel
AMPL4394422924	183	554	583	541	574	Indel
AMPL4392438280	177	1001	1074	880	979	Indel
AMPL3438454613	114	2098	2100	1837	1846	SNP
AMPL4394498383	180	1190	1196	1445	1457	SNP
AMPL4392232288	185	394	582	1344	1921	SNP SNP SNP
AMPL4392247152	159	1832	1876	1979	2033	SNP
AMPL4392197049	185	348	579	323	601	SNP
AMPL4393991123	182	2939	3033	3220	3345	SNP SNP
AMPL4393996246	182	1351	1387	1505	1547	SNP
AMPL4392574004	134	32	33	14	14	SNP
AMPL4392530564	187	996	1045	1291	1377	SNP
AMPL4392171571	153	1224	1775	1297	1832	SNP
AMPL4392174571	152	2152	2225	2442	2552	SNP
AMPL3682590436	166	2555	2662	2813	2953	SNP
AMPL4392240361	176	1377	2306	1490	2577	SNP
AMPL4395049446	185	840	882	860	911	SNP
AMPL3518568818	172	1951	1968	2330	2366	SNP
AMPL4394999207	171	1843	1888	2059	2109	SNP
AMPL4393163023	174	4042	4113	4147	4226	SNP
AMPL4393980045	176	1886	1918	2036	2067	SNP

Amplicon Name	Length	NA12878		NA19240		Variant Type[3]
		Coverage Depth (Avg)[1]	# Aligned Reads[2]	Coverage Depth (Avg)[1]	# Aligned Reads[2]	
AMPL4393983602	176	3400	3437	3749	3785	SNP
AMPL4393695146	174	2030	2661	1936	2570	SNP
AMPL3676543350	175	735	992	753	962	NA
AMPL4343482687	73	531	528	1569	1558	SNP
AMPL4393984337	154	1016	1043	1129	1164	SNP
AMPL4059164024	158	1511	1541	1723	1763	SNP
AMPL4392391519	161	2088	2881	2016	2840	Indel
AMPL4394279241	172	589	601	715	728	SNP
AMPL4393723902	176	2706	2741	2729	2757	SNP
AMPL4392346671	167	892	895	981	986	Indel
AMPL4373578084	177	2775	2811	2850	2873	SNP SNP
AMPL4393984326	152	906	926	904	933	SNP
AMPL4393961771	176	677	688	685	695	SNP
AMPL4373985988	161	714	1330	616	1138	Indel
AMPL4392235190	178	3020	3079	3306	3363	SNP
AMPL4392253940	170	1046	1086	1191	1245	SNP
AMPL4392992251	119	3677	3694	3520	3552	SNP
AMPL4374147776	174	1237	1267	1389	1421	Indel
AMPL4373707639	93	2218	2233	2155	2170	SNP
AMPL4392220658	157	4473	4498	4385	4416	SNP
AMPL4394603296	152	1717	1790	2090	2167	Indel
AMPL3435076154	176	1399	1410	2638	2663	NA
AMPL4393051580	169	972	986	1016	1034	Indel
AMPL4393964460	150	2363	2389	2630	2662	SNP
AMPL3434408361	175	1974	2002	2443	2461	SNP
AMPL4392985517	163	3845	3884	4576	4651	SNP
AMPL4395015331	180	2827	2858	3227	3267	SNP
AMPL4392990319	166	1861	1871	2119	2135	SNP
AMPL4392441515	177	4369	4528	4955	5096	Indel
AMPL4392216992	147	3943	3950	4047	4061	SNP SNP
AMPL4393954745	173	2988	3016	3289	3337	SNP
AMPL4393010759	162	2463	2479	2277	2328	Indel
AMPL711877918	180	2243	2518	2594	2875	SNP
AMPL4392573539	172	569	839	656	1036	Indel
AMPL4373702125	150	4681	4699	4731	4738	SNP
AMPL4393942602	172	2073	2096	1855	1890	SNP
AMPL4393950751	171	2241	2339	2460	2545	SNP
AMPL4392987625	166	3152	3178	3654	3691	SNP
AMPL4316030239	153	3992	4077	4606	4720	Indel
AMPL4394982619	168	4157	4374	4046	4253	SNP
AMPL1158164934	169	1919	1938	2355	2384	SNP SNP
AMPL3683333814	125	4522	4556	4951	4999	SNP
AMPL755591047	125	2491	2588	3225	3236	SNP
AMPL3498792122	173	1046	1451	1381	1759	NA
AMPL575431534	185	120	125	128	139	SNP SNP SNP
AMPL4394281382	164	3687	3700	3603	3616	SNP
AMPL575510303	173	298	305	453	466	Indel
AMPL4393743713	175	1966	2034	2348	2401	SNP
AMPL4392200573	149	2665	2675	2816	2833	SNP
AMPL4393737942	175	1925	1959	1706	1740	SNP
AMPL4393656617	184	59	68	55	63	SNP
AMPL4394594066	167	2711	2810	2999	3089	Indel
AMPL571636108	162	1657	1681	2299	2341	SNP
AMPL868949058	169	919	947	934	969	SNP
AMPL4231728591	152	1737	1796	1620	1691	SNP
AMPL692547671	173	183	232	233	279	SNP
AMPL4323577210	141	3004	3047	3482	3606	SNP

Amplicon Name	Length	NA12878		NA19240		Variant Type[3]
		Coverage Depth (Avg)[1]	# Aligned Reads[2]	Coverage Depth (Avg)[1]	# Aligned Reads[2]	
AMPL413836218	170	1516	1555	1689	1743	Indel
AMPL4392936584	172	2656	2712	2821	2904	SNP
AMPL4393915769	168	2761	2789	3519	3574	SNP
AMPL4022901967	125	2118	2138	2246	2279	SNP
AMPL415167643	172	1778	1788	1914	1931	SNP
AMPL4323505451	144	3244	3297	3266	3355	SNP
AMPL4393686515	172	1261	1308	1660	1722	SNP
AMPL4374016890	180	5047	5174	6135	6264	Indel
AMPL4374230397	179	2036	2079	2229	2272	SNP
AMPL4374521900	174	2849	2895	3077	3118	Indel
AMPL4392992071	82	5602	5610	5843	5879	SNP
AMPL719291358	173	1030	1038	972	988	SNP SNP
AMPL4393683810	172	2297	2429	2661	2848	SNP
AMPL4392932301	164	1243	1254	1331	1346	SNP
AMPL403750326	118	2401	2421	2667	2708	SNP
AMPL4374399269	169	4301	4412	4529	4672	SNP
AMPL4374398779	166	30	30	36	37	SNP SNP
AMPL4392203039	139	1690	1727	1705	1734	SNP
AMPL4392491672	174	561	817	964	1299	NA
AMPL4395001028	163	821	899	790	953	Indel
AMPL4395342492	175	6151	6256	6929	7060	SNP
AMPL4392223494	174	1160	1192	1461	1499	SNP
AMPL4392215902	169	1922	2374	2171	2765	SNP
AMPL4393114509	183	4050	4189	4605	4817	Indel
AMPL4394953784	173	1741	1770	1812	1837	SNP
AMPL596607752	171	318	357	350	425	SNP
AMPL596173137	171	996	1003	1192	1204	SNP
AMPL4320903836	178	472	493	521	541	Indel
AMPL417791888	170	3430	3476	3404	3459	SNP
AMPL4307576268	154	4193	4238	4494	4541	SNP
AMPL413267348	172	1039	1049	1366	1386	Indel
AMPL3498610579	175	1851	1879	1905	1933	SNP
AMPL4322896544	166	4324	4422	4030	4156	NA
AMPL4392211943	160	244	253	284	294	SNP
AMPL4393613220	175	1603	1826	1686	1933	SNP
AMPL2692711234	131	3873	3959	4187	4349	SNP
AMPL4392926980	168	4144	4211	4441	4518	SNP
AMPL3676783348	168	1948	1959	2054	2064	SNP
AMPL2692395510	176	2377	2502	3021	3254	Indel
AMPL3936418821	174	1644	1680	1786	1868	Indel
AMPL4374406487	158	2831	2900	3293	3403	SNP
AMPL4393927397	126	2181	2192	2491	2507	SNP SNP
AMPL4373781431	170	1538	1570	1741	1782	SNP
AMPL4393043045	168	3624	3670	4464	4560	Indel
AMPL4038401239	169	1538	1615	1819	1953	SNP
AMPL4392423431	171	2515	2622	2432	2624	Indel
AMPL4392201407	178	289	298	329	332	SNP SNP
AMPL4302499643	177	829	839	786	800	SNP
AMPL704947325	188	567	582	572	591	SNP
AMPL4202336805	188	340	499	314	478	SNP SNP
AMPL4203136737	185	265	274	357	364	SNP
AMPL4302482618	93	1199	1199	1298	1294	Indel
AMPL4392191781	182	313	332	348	364	SNP
AMPL668652008	177	556	577	657	678	NA
AMPL3659553353	157	779	792	844	859	SNP
AMPL4392890404	145	2190	2198	2379	2504	SNP
AMPL4374509052	127	737	749	774	791	Indel

Amplicon Name	Length	NA12878		NA19240		Variant Type[3]
		Coverage Depth (Avg)[1]	# Aligned Reads[2]	Coverage Depth (Avg)[1]	# Aligned Reads[2]	
AMPL4394429615	178	1235	1652	1100	1514	Indel
AMPL4392895580	169	1961	1980	1902	1928	SNP
AMPL4373372000	170	1425	1472	1313	1362	Indel
AMPL1153902623	161	3011	3106	3733	3805	SNP
AMPL4393659645	176	1249	1287	1175	1217	SNP
AMPL3679236852	121	797	807	844	865	SNP
AMPL3498104622	178	2614	2689	2541	2612	SNP
AMPL708625773	172	512	844	471	806	SNP
AMPL4302673454	171	3284	3408	3376	3553	SNP
AMPL1705657360	173	957	974	893	909	SNP
AMPL1702865108	168	2524	2565	3286	3325	Indel
AMPL4392692973	171	1245	1263	1244	1263	Indel
AMPL4195002255	93	1205	1496	1424	1807	Indel
AMPL3797942774	153	1053	1598	1336	1898	NA
AMPL3498144888	149	3037	3096	2805	2857	SNP
AMPL3299722549	178	1565	1671	1834	2052	SNP
AMPL4393636823	173	985	1091	1066	1157	NA
AMPL4392675093	181	1955	2245	2021	2356	Indel
AMPL4393781331	180	2642	2745	2763	2851	Indel
AMPL4395333937	94	672	679	1169	1194	Indel
AMPL4393786655	170	2190	2271	3032	3130	Indel
AMPL4393896650	109	3369	3390	3597	3615	SNP
AMPL4392914397	160	2208	2227	2513	2545	SNP
AMPL4395281946	171	2002	2041	1948	2000	SNP
AMPL4395290340	143	2744	2752	2586	2593	SNP
AMPL4393619887	117	118	120	303	311	SNP
AMPL4394604242	174	4203	4414	4021	4254	Indel
AMPL3710790718	98	1468	1481	1595	1623	SNP
AMPL4395266793	81	2759	2772	3108	3126	SNP
AMPL4392189306	114	2825	2823	2439	2444	SNP
AMPL3671540502	161	1257	1272	1403	1423	SNP
AMPL4392192649	179	3438	3602	3886	4182	SNP
AMPL4393619016	182	1767	1806	1934	1982	SNP
AMPL2455591517	170	3451	3483	3601	3644	SNP SNP SNP
AMPL4394566481	151	1279	1468	1587	1806	NA
AMPL4313036002	152	1695	3244	1886	3453	NA
AMPL2675981375	176	2303	2709	2406	2861	NA
AMPL4325322943	125	1335	1358	1355	1381	SNP
AMPL644684281	172	849	1261	788	1225	NA
AMPL4170634321	116	1710	1796	1718	1788	SNP
AMPL652088025	111	4130	4152	3781	3806	SNP SNP
AMPL4392182082	181	545	563	596	618	SNP
AMPL1519565349	169	2000	2098	1975	2092	SNP
AMPL408214700	127	1861	1883	1824	1850	SNP
AMPL4395267587	183	1206	1310	1283	1456	SNP
AMPL4392872919	169	1733	1775	1855	1918	SNP
AMPL402546844	92	4536	4550	4568	4610	SNP
AMPL404067077	173	2309	2420	2834	2968	SNP SNP
AMPL659232630	157	1857	1985	1892	2077	SNP
AMPL4395306426	182	2077	2104	2118	2142	SNP
AMPL528631298	176	4	4	8	9	NA
AMPL4392187570	148	2575	2603	2454	2487	SNP
AMPL4395394628	152	2619	2634	2908	2924	SNP
AMPL4392903847	101	2022	2028	2151	2173	SNP
AMPL4394224699	185	1133	1160	1108	1141	SNP
AMPL4320019211	184	343	471	345	457	Indel
AMPL4392351914	163	1433	1478	1621	1692	Indel

Amplicon Name	Length	NA12878		NA19240		Variant Type[3]
		Coverage Depth (Avg)[1]	# Aligned Reads[2]	Coverage Depth (Avg)[1]	# Aligned Reads[2]	
AMPL4298148304	161	1719	2219	1678	2257	NA
AMPL3198712213	179	1941	1957	2185	2212	SNP
AMPL4394465691	154	2604	2833	2697	3010	Indel
AMPL4393628011	163	53	57	58	60	SNP
AMPL4394223117	169	2324	2463	2442	2587	SNP
AMPL4394360574	156	1144	1480	1094	1278	Indel
AMPL4394214041	184	1190	1263	1087	1183	SNP
AMPL4394678126	184	5	6	9	11	Indel
AMPL4394220527	134	3639	3650	3746	3781	SNP
AMPL3674673921	167	787	794	827	833	SNP
AMPL583119206	167	1916	1941	1755	1787	SNP SNP
AMPL3735262350	70	2394	2387	2206	2209	SNP
AMPL623995564	139	499	623	649	856	SNP
AMPL1486637447	103	1355	1387	1471	1563	Indel
AMPL3777975057	119	1158	1163	1325	1335	Indel
AMPL3778824349	111	22	22	83	85	Indel
AMPL3378946700	171	1209	1226	1427	1458	SNP
AMPL4394922626	176	1832	1846	2069	2098	SNP
AMPL588811071	108	4331	4360	4495	4548	SNP
AMPL3817158157	169	1233	1241	1524	1539	SNP
AMPL4394216319	163	2670	2700	2820	2860	SNP
AMPL4326420418	167	1428	1466	1290	1338	Indel
AMPL3829029013	170	2109	2126	1280	1337	SNP
AMPL4395303483	156	2189	2721	3091	3445	SNP
AMPL4324007004	93	1406	1427	1391	1410	SNP SNP
AMPL4320523169	82	1009	1213	1107	1380	Indel
AMPL395328621	167	723	737	1236	1268	SNP
AMPL4395288076	178	3563	3597	3634	3682	SNP
AMPL538987753	170	1988	2016	2187	2226	SNP
AMPL4394633624	178	2988	3026	3028	3087	Indel
AMPL4394624154	175	2502	2763	2762	3123	NA
AMPL4392357297	167	765	790	1154	1229	Indel
AMPL4393606166	183	3194	3380	3315	3519	SNP
AMPL4393136614	144	2264	2288	2644	2693	Indel
AMPL4394209036	160	1382	1742	1125	1207	SNP
AMPL4394553261	168	900	924	1037	1069	Indel
AMPL1496946897	180	1737	1765	1991	2030	SNP SNP
AMPL3519304014	74	2099	2089	2167	2170	SNP
AMPL4392413266	183	2287	2708	2660	2924	Indel
AMPL4395265123	170	1873	1890	2355	2377	SNP
AMPL3295318277	184	4269	4334	4868	4949	SNP
AMPL4317912554	118	3635	3669	3652	3690	SNP
AMPL4393793084	184	2726	2904	2730	2869	Indel
AMPL4395343628	172	549	602	745	796	Indel
AMPL1517948259	170	1983	2006	1993	2025	SNP
AMPL4393860661	156	2910	2928	3422	3452	SNP
AMPL4373761294	132	1722	1734	2172	2199	SNP
AMPL4393823209	178	3489	3527	3780	3835	SNP SNP
AMPL420799557	75	1814	1809	2330	2327	SNP
AMPL416087660	119	1784	1809	2790	2902	SNP
AMPL4374301969	176	4344	4381	4832	4885	SNP
AMPL4392815412	176	2918	3018	3183	3283	SNP SNP
AMPL4393570611	173	1525	1590	1413	1470	SNP
AMPL4395233644	177	2824	2961	2675	2876	SNP
AMPL4392696459	163	276	312	865	964	Indel
AMPL1608248833	174	1130	1256	1153	1365	Indel
AMPL4393590705	154	4309	4330	4999	5042	SNP

Amplicon Name	Length	NA12878		NA19240		Variant Type[3]
		Coverage Depth (Avg)[1]	# Aligned Reads[2]	Coverage Depth (Avg)[1]	# Aligned Reads[2]	
AMPL4235578244	175	3572	3598	3910	3955	NA
AMPL3733781601	174	3547	3560	3867	3880	NA
AMPL4395233955	167	801	811	844	859	SNP
AMPL4393557392	177	2487	2533	2505	2601	SNP
AMPL4395246850	178	2214	2252	2555	2593	SNP
AMPL4395355849	140	2244	2247	2187	2189	Indel
AMPL4392670346	179	1144	1369	1639	1968	Indel
AMPL4393550446	183	2388	2419	2794	2845	SNP
AMPL3498752779	158	2320	2358	2359	2407	SNP
AMPL4393769156	105	3968	4039	3857	3904	Indel
AMPL557689580	155	1215	1277	1304	1365	Indel
AMPL4392824175	132	2135	2150	2549	2587	SNP
AMPL4392796221	112	2751	2750	2931	2952	SNP
AMPL3637426447	147	2191	2334	2487	2875	SNP
AMPL3199428974	170	1414	1456	1439	1473	SNP
AMPL777571747	179	543	567	534	567	Indel
AMPL4393547526	163	2616	2813	2726	2903	SNP
AMPL4394883639	164	2462	2516	2546	2845	SNP
AMPL4393119864	119	1248	1313	1820	1912	NA
AMPL391855502	180	2269	2366	2045	2303	SNP
AMPL4392820663	178	2633	2666	2529	2589	SNP
AMPL4392803596	178	3559	3620	4255	4333	SNP
AMPL4374504539	156	4947	4967	5184	5216	Indel
AMPL4395008361	155	3939	3979	4577	4640	Indel
AMPL4374372220	174	1610	1650	1760	1781	SNP SNP
AMPL4392783695	168	3149	3194	2893	2938	SNP
AMPL4392776958	118	5001	5021	5008	5049	SNP
AMPL4394859257	173	1193	1222	1217	1243	SNP
AMPL4392795183	151	1790	1806	1993	2018	SNP
AMPL3763697326	176	3908	3910	4061	4071	SNP
AMPL4392776634	82	1726	1736	1682	1704	SNP
AMPL4394847665	172	1011	1035	1068	1088	SNP
AMPL4393146133	180	653	779	535	722	Indel
AMPL4392805233	153	2240	2254	2544	2586	SNP
AMPL4393330449	177	2972	3283	3068	3518	SNP
AMPL4393549181	178	4429	4617	4909	5280	SNP
AMPL4393560571	178	3118	3160	3308	3341	SNP
AMPL4394644825	143	3155	3237	3153	3263	Indel
AMPL562172230	173	3526	3574	3955	4035	Indel
AMPL562219796	161	2040	2074	2172	2209	SNP
AMPL4393539439	161	2073	2106	2446	2512	SNP
AMPL4392146647	177	2724	2736	3027	3051	SNP
AMPL769894769	110	4322	4439	4226	4369	Indel
AMPL3374963808	174	2840	2885	3495	3549	SNP
AMPL3720788183	181	927	1019	912	1056	Indel SNP
AMPL4395377014	177	2213	2540	2422	2806	SNP
AMPL4392352458	178	1093	1127	1381	1462	Indel
AMPL4392678393	160	2437	2474	2483	2521	Indel
AMPL4393760723	151	1066	1087	874	895	Indel
AMPL3681904054	116	1683	1706	1975	2029	SNP
AMPL4394458394	169	3391	3459	3355	3475	Indel
AMPL4393140419	181	2998	3148	3166	3389	Indel
AMPL4085226812	86	4225	4204	4092	4077	NA
AMPL4373740005	177	1667	1724	1742	1791	SNP
AMPL3691760208	162	1607	1651	1447	1486	SNP SNP
AMPL4392126885	178	1687	1723	1765	1816	SNP
AMPL4392372494	182	1627	1700	1556	1627	Indel

Amplicon Name	Length	NA12878		NA19240		Variant Type[3]
		Coverage Depth (Avg)[1]	# Aligned Reads[2]	Coverage Depth (Avg)[1]	# Aligned Reads[2]	
AMPL4393856361	176	1475	1526	1417	1461	SNP
AMPL4373672931	164	1382	1404	1574	1594	SNP
AMPL4392120439	179	2040	2135	2275	2402	SNP SNP
AMPL4300890407	143	5882	5910	6315	6365	SNP
AMPL4301194040	182	1690	1714	1968	1989	SNP
AMPL544389827	171	746	766	898	928	SNP
AMPL4216700451	180	1140	1513	1230	1666	SNP
AMPL1156353735	141	1127	1134	1402	1419	SNP
AMPL4300833980	178	1901	1947	2212	2265	SNP SNP
AMPL4393523704	174	3408	3475	3861	3925	SNP
AMPL4395213682	170	1445	1475	1777	1811	SNP
AMPL789566107	178	1800	1982	2176	2376	SNP
AMPL4393540427	172	1410	1440	1662	1698	SNP
AMPL789212884	176	3740	3819	4956	5068	SNP
AMPL4393492671	179	3155	3181	3799	3834	SNP
AMPL4393748172	126	2754	2782	3254	3299	Indel
AMPL4394161757	180	1182	1210	1397	1425	SNP SNP
AMPL4392701696	175	1190	1662	1118	1674	NA
AMPL4393462046	160	711	1009	838	1226	SNP
AMPL4392661656	177	924	1496	919	1491	Indel
AMPL3889188256	166	3074	3232	3097	3242	SNP
AMPL4395202607	158	2416	2442	2706	2743	SNP
AMPL4392398765	167	2339	2363	2647	2684	Indel
AMPL4392113442	179	3107	3153	2820	2883	SNP
AMPL4395337088	175	2881	2944	2875	2980	Indel
AMPL4393477737	179	773	818	984	1048	SNP
AMPL4305062531	176	1244	1265	1317	1335	SNP
AMPL4392565249	175	2210	2336	2719	2850	Indel
AMPL4395050447	75	401	406	916	926	Indel
AMPL4393470475	185	750	769	822	844	SNP
AMPL4392777432	174	488	501	468	486	SNP
AMPL4395183962	129	2694	2703	2713	2737	SNP
AMPL2949788169	181	1151	1759	1631	2424	SNP
AMPL4392758534	179	2243	2350	2157	2307	SNP
AMPL4393492728	179	1149	1386	1071	1296	SNP
AMPL4392107402	136	1876	1917	2275	2318	SNP SNP
AMPL4392128611	150	1923	1944	2164	2196	SNP
AMPL549199875	152	3002	3076	3344	3433	SNP
AMPL549072782	177	1649	2356	1896	3076	Indel
AMPL4392509220	188	93	147	107	157	SNP
AMPL4392510383	135	3108	3258	3103	3243	Indel
AMPL4395349481	178	4107	4186	4549	4639	Indel
AMPL1104437054	172	2595	2642	2822	2894	SNP
AMPL4104053523	153	3892	3928	4089	4172	SNP
AMPL4393041536	135	2611	2639	2537	2578	Indel
AMPL4394409931	180	2264	2336	2229	2327	Indel
AMPL4392109226	179	642	651	829	848	SNP
AMPL573434660	171	1166	1178	1318	1337	SNP
AMPL4392117695	156	2581	2608	2662	2687	SNP SNP
AMPL4395175643	181	1516	1537	1800	1825	SNP
AMPL4373650991	183	2217	2256	2444	2511	SNP
AMPL4392110866	172	1039	1066	1224	1265	SNP SNP
AMPL4394139126	118	2038	2226	2551	2703	SNP
AMPL555450618	179	361	367	482	497	SNP
AMPL555283876	171	2868	2903	3293	3334	SNP
AMPL4392759751	170	2989	3033	2951	3032	SNP
AMPL4392767076	164	4465	4491	4956	5013	SNP

Amplicon Name	Length	NA12878		NA19240		Variant Type[3]
		Coverage Depth (Avg)[1]	# Aligned Reads[2]	Coverage Depth (Avg)[1]	# Aligned Reads[2]	
AMPL4394809934	151	1079	1110	1202	1226	SNP
AMPL4394147071	176	1981	2000	1992	2015	SNP
AMPL3289654030	177	1944	2012	2020	2122	Indel
AMPL4392726050	176	153	168	566	620	Indel
AMPL3961593542	178	1874	1958	2826	2935	SNP
AMPL4392708195	176	1196	1254	1112	1353	NA
AMPL4394756215	179	3789	3911	4149	4277	SNP
AMPL4394758212	171	2270	2327	2415	2501	SNP
AMPL4392098735	185	916	949	901	942	SNP
AMPL4392422488	169	238	331	445	608	Indel
AMPL4393028379	186	125	146	207	252	Indel
AMPL700490202	153	2851	2861	1903	1914	SNP
AMPL3764819988	154	981	992	1059	1076	SNP
AMPL4393070345	154	936	1036	880	973	Indel
AMPL4374033508	186	95	127	92	123	Indel
AMPL597978528	169	935	1293	1005	1408	SNP
AMPL3569591894	133	1208	1216	1074	1078	SNP
AMPL4393411927	176	468	727	528	832	SNP
AMPL4393455182	180	1269	1598	1350	1673	SNP
AMPL4393432724	174	978	1015	1063	1148	SNP
AMPL3695208132	174	383	401	383	429	SNP
AMPL4393407496	171	3093	3197	3386	3696	Indel SNP
AMPL4392668212	154	3591	3614	4245	4293	Indel
AMPL397538170	182	3289	3476	3545	3820	SNP
AMPL4394405741	165	921	1441	1174	1735	Indel
AMPL4392096706	183	743	762	850	876	SNP
AMPL4393123724	161	1032	1082	1016	1061	Indel
AMPL4395386501	175	2925	2961	2801	2854	SNP
AMPL4392102936	176	1386	1420	1343	1378	SNP
AMPL4297998702	102	4865	4874	4734	4745	SNP
AMPL532298001	180	560	568	504	513	SNP
AMPL4392537380	157	2332	2390	2728	2789	Indel
AMPL4394751711	146	1894	1932	2038	2102	SNP
AMPL4395148488	180	2863	2894	3222	3265	SNP
AMPL1118439227	178	83	158	86	183	NA
AMPL4394736429	159	1158	1170	1294	1308	SNP
AMPL551935865	173	1846	1878	2099	2150	SNP
AMPL4392073003	165	4480	4508	4132	4168	SNP
AMPL4394129588	159	1428	1978	1245	1805	SNP
AMPL4373586961	172	1590	1644	1727	1805	NA
AMPL595971724	170	1068	1082	1121	1143	SNP
AMPL4394610057	180	423	561	387	550	Indel
AMPL4392084924	179	456	482	519	556	SNP
AMPL4392744809	183	1551	1737	1737	1962	SNP
AMPL4392755421	182	3019	3053	3455	3502	SNP
AMPL4394432367	161	2104	2480	2099	2518	NA
AMPL4395355273	139	1442	1454	1462	1480	SNP
AMPL4373723911	174	1632	1669	1800	1841	SNP
AMPL4392449135	179	1510	3006	1665	3188	Indel
AMPL4392752461	180	1792	1874	2008	2086	SNP
AMPL3680965544	174	1835	1884	2043	2106	NA
AMPL3920741189	166	4783	4864	3419	3468	SNP
AMPL3289833537	178	1451	1558	1685	1804	Indel
AMPL4395147724	166	2555	2597	2479	2521	SNP
AMPL4392928879	157	3029	3062	2877	2936	SNP
AMPL4394730094	169	922	1290	930	1317	SNP
AMPL1676134309	138	1777	1783	1992	2009	SNP Indel

Amplicon Name	Length	NA12878		NA19240		Variant Type[3]
		Coverage Depth (Avg)[1]	# Aligned Reads[2]	Coverage Depth (Avg)[1]	# Aligned Reads[2]	
AMPL4392728909	136	1155	1235	1226	1309	NA
AMPL749578720	155	2666	2695	3039	3073	SNP
AMPL4373417951	91	2183	2616	1788	2188	SNP
AMPL4392707708	142	2225	2255	2526	2568	SNP
AMPL4394086736	173	2016	2067	2149	2230	SNP
AMPL3700904683	87	1381	1412	1366	1401	SNP
AMPL553632290	170	872	908	1036	1082	SNP
AMPL4393378805	158	1592	1612	1623	1640	SNP
AMPL569668692	178	137	168	119	144	Indel
AMPL4314163957	173	803	827	879	896	SNP
AMPL529493999	172	2321	3022	2709	3568	SNP

[1] Coverage Depth (Average) = sum of coverage depth of all bases in the amplicon / length of the amplicon.

[2] # Aligned Reads = number of reads aligned to the amplicon listed.

[3] Where an amplicon spans two variant locations the variant type for both locations is listed e.g., SNP SNP. NA = not applicable, amplicon does not contain a variant in our hotspot file.

Table 27a Summary results for Sensitivity and Reproducibility by Operator and test site.

The following table demonstrates that the sensitivity and reproducibility of the Ion PGM™ Dx System to make correct SNV and Indel variant calls was independent of operator and test site.

DNA #	Site	Op	# runs	SNVs						Indels					
				# SNVs	# Correct SNV Calls	# No Calls	# Incorrect Calls	Mean SNV Sensitivity[1]	% with ≥95% Reproducibility[3]	# Indels	# Correct Indel Calls	# No Calls	# Incorrect Calls	Mean Indel Sensitivity[2]	% with ≥95% Reproducibility[3]
NA12878	1	1	52	302	15565	133	6	99.96%	293/302 = 97.02%	97	3816	890	338	91.86%	58/97 = 59.79%
		2	52	302	15550	146	8	99.95%	292/302 = 96.69%	97	3807	874	363	91.29%	61/97 = 62.89%
	2	1	51	302	15203	183	16	99.89%	287/302 = 95.03%	97	3673	868	406	90.05%	58/97 = 59.79%
		2	52	302	15556	143	5	99.97%	294/302 = 97.35%	97	3846	852	346	91.75%	62/97 = 63.92%
	3	1	52	302	15538	156	10	99.94%	290/302 = 96.03%	97	3788	889	367	91.17%	59/97 = 60.82%
		2	52	302	15522	156	26	99.83%	292/302 = 96.69%	97	3811	893	340	91.81%	60/97 = 61.86%
NA19240	1	1	51	373	18567	353	103	99.45%	347/373 = 93.03%	100	4005	876	219	94.82%	68/100 = 68.00%
		2	51	373	18589	340	94	99.50%	351/373 = 94.10%	100	4006	881	213	94.95%	70/100 = 70.00%
	2	1	52	373	18890	403	103	99.46%	344/373 = 92.23%	100	4020	948	232	94.54%	66/100 = 66.00%
		2	52	373	18974	309	113	99.41%	353/373 = 94.64%	100	4061	923	216	94.95%	66/100 = 66.00%
	3	1	52	373	18955	332	109	99.43%	353/373 = 94.64%	100	4006	971	223	94.73%	65/100 = 65.00%
		2	52	373	18942	327	127	99.33%	351/373 = 94.10%	100	4044	948	208	95.11%	66/100 = 66.00%

[1] Mean SNV sensitivity, excluding No Calls, over all valid runs for a given DNA sample will be calculated using the formula Sensitivity = TP/(TP+FN), where TP = total number of SNV positions called correctly (including zygosity determination) when compared to the variant reference database sequence, and FN = total number of SNVs present in the variant reference database sequence but not called correctly by the Ion PGM™ Dx System.

[2] Mean Indel sensitivity, excluding No Calls, over all valid runs for a given DNA sample will be calculated using the formula Sensitivity = TP/(TP+FN), where TP = total number of Indel positions called correctly (including zygosity determination) when compared to the variant reference database sequence, and FN = total number of Indels present in the variant reference database sequence but not called correctly by the Ion PGM™ Dx System.

[3] Reproducibility for SNVs and Indels is defined as the number of variant locations called with a minimum of 95% reproducibility across all sample runs. Reproducibility calculations were performed including no calls where a no-call result is counted as not being reproducible.

Table 27b Summary results for Specificity by Operator and test site.

The following table demonstrates the specificity of the Ion PGM™ Dx System in correctly identifying reference base positions when compared to hg19 was independent of operator and test site.

DNA #	Site	Op	# runs	Reference Locations				
				# Reference Locations	# Correct Reference Calls	# No Calls	# Incorrect Calls	Mean Specificity[1]
NA12878	1	1	52	237	11103	1082	139	98.76%
		2	52	237	11235	963	126	98.89%
	2	1	51	237	10840	1117	130	98.81%
		2	52	237	11212	989	123	98.91%
	3	1	52	237	11139	1048	137	98.79%
		2	52	237	11188	993	143	98.74%
NA19240	1	1	51	163	7188	857	268	96.41%
		2	51	163	7234	810	269	96.41%
	2	1	52	163	7285	929	262	96.53%
		2	52	163	7372	819	285	96.28%
	3	1	52	163	7350	852	274	96.41%
		2	52	163	7362	835	279	96.35%

[1] Specificity, excluding No Calls, over all valid runs for a given DNA sample was calculated using the formula: Specificity = TN/(TN + FP), where TN = total number of wild type hotspot positions present in the reference database sequence called correctly by the Ion PGM™ Dx System, and FP = total number of wild type hotspot positions present in the reference database sequence that are not correctly called as WT by the Ion PGM™ Dx System.

Table 28a **Reproducibility Results: Operator-to-Operator: NA12878**

The following table shows operator-to-operator reproducibility results for the Ion PGM™ Dx System (per variant). Results are presented for NA12878 and are inclusive of all the sequencing runs per operator.

		Operator 1					Operator 2					Operator 3					Operator 4					Operator 5					Operator 6				
Location	Variant Type	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]
chr1_103343469_103343470_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_103355221_103355222_REF	REF	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_103461507_103461508_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100
chr1_103467614_103467615_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_11194590_11194591_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_11303152_11303153_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_116243876_116243877_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_116247789_116247790_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_116247825_116247826_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_120480393_120480394_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_120509048_120509049_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_120512302_120512303_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144852389_144852390_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144854179_144854180_REF	REF	52	0	0	52	0	52	0	0	52	0	51	0	0	51	0	52	0	0	52	0	52	0	0	52	0	52	0	0	52	0
chr1_144866642_144866643_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144871888_144871889_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144874640_144874641_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	50	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144879263_144879264_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144882580_144882581_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144882822_144882823_SNP	SNP	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144886196_144886197_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144918956_144918957_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144921923_144921924_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144921949_144921950_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144922170_144922171_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144923728_144923729_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144931329_144931330_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144931391_144931392_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144931580_144931581_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144931606_144931607_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144946700_144946701_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144994657_144994658_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_145016019_145016020_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_145021149_145021150_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_145021182_145021183_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_145075682_145075683_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_156106184_156106185_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_156107612_156107613_REF	REF	52	29	23	0	100	52	35	17	0	100	51	36	15	0	100	52	46	6	0	100	52	22	30	0	100	52	39	13	0	100
chr1_156128219_156128220_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_156834186_156834187_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_156848909_156848910_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_164761715_164761715_REF	REF	52	51	1	0	100	52	52	0	0	100	51	50	1	0	100	52	49	3	0	100	52	50	2	0	100	52	52	0	0	100
chr1_171076965_171076966_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_171085314_171085314_REF	REF	52	43	9	0	100	52	45	7	0	100	51	41	10	0	100	52	37	15	0	100	52	40	12	0	100	52	31	21	0	100
chr1_182554556_182554557_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_185069314_185069314_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	51	1	0	100
chr1_197325907_197325908_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_197404769_197404770_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_201335898_201335899_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_201341175_201341180_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_204433511_204433511_Indel	Indel	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_204501382_204501383_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_204515862_204515863_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_206650064_206650065_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_20972304_20972318_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_20972373_20972374_Indel	Indel	52	50	0	2	96.15	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	50	0	2	96.15	52	51	0	1	98.08
chr1_209804094_209804095_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_215821938_215821939_REF	REF	52	52	0	0	100	52	52	0	0	100	51	47	4	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_215821970_215821971_REF	REF	52	52	0	0	100	52	52	0	0	100	51	50	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_216052344_216052345_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_216258250_216258251_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_226555301_226555302_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_226580020_226580021_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_227071524_227071525_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_2337276_2337277_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_2340072_2340073_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_236883420_236883421_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_236925843_236925844_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_236978992_236978992_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_237060849_237060850_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_237711796_237711797_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_237814782_237814783_SNP	SNP	52	50	2	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_237863717_237863718_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_237969637_237969638_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_41296827_41296828_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_45797504_45797505_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_6529182_6529188_REF	REF	52	11	41	0	100	52	18	34	0	100	51	7	44	0	100	52	12	40	0	100	52	12	40	0	100	52	16	36	0	100
chr1_6530965_6530965_Indel	Indel	52	10	31	11	47.62	52	4	18	30	11.76	51	8	30	13	38.10	52	4	28	20	16.67	52	0	35	17	0	52	8	34	10	44.44
chr1_68894730_68894730_Indel	Indel	52	47	5	0	100	52	49	2	1	98.00	51	48	3	0	100	52	52	0	0	100	52	50	2	0	100	52	47	5	0	100
chr1_94466658_94466659_SNP	SNP	52	50	2	0	100	52	50	2	0	100	51	50	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_94471074_94471075_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_94474327_94474328_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_94481504_94481505_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_94564482_94564483_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_98015290_98015291_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_112725684_112725684_Indel	Indel	52	0	1	51	0	52	0	0	52	0	51	0	0	51	0	52	0	0	52	0	52	0	2	50	0	52	0	1	51	0
chr2_112786048_112786049_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_113999533_113999534_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_141130547_141130548_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100

		Operator 1					Operator 2					Operator 3					Operator 4					Operator 5					Operator 6				
Location	Variant Type	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]
chr2_141819587_141819588_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_141945948_141945949_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_148657116_148657117_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_167163042_167163043_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_167168092_167168093_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_175613272_175613273_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_175622219_175622220_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_178098087_178098088_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	51	1	0	100
chr2_182413237_182413238_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_182413258_182413259_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_182413601_182413602_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_189866342_189866343_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_189907936_189907937_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_189953285_189953285_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_191109532_191109533_REF	REF	52	52	0	0	100	52	51	1	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_198257794_198257795_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_198267770_198267770_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_209101905_209101906_Indel	Indel	52	0	52	0	0	52	0	52	0	0	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0
chr2_212812096_212812097_REF	REF	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_216240126_216240128_Indel	Indel	52	48	0	4	92.31	52	46	0	6	88.46	51	46	0	5	90.20	52	51	0	1	98.08	52	45	0	7	86.54	52	47	0	5	90.38
chr2_216248707_216248709_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_216274853_216274855_Indel	Indel	52	50	0	2	96.15	52	51	0	1	98.08	51	49	0	2	96.08	52	52	0	0	100	52	52	0	0	100	52	50	0	2	96.15
chr2_216299571_216299571_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_219545308_219545309_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_220284875_220284876_SNP	SNP	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100
chr2_223161623_223161624_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_24905926_24905927_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_24974944_24974945_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_25471169_25471170_REF	REF	52	51	1	0	100	52	50	2	0	100	51	50	1	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100
chr2_29416571_29416572_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_42510017_42510018_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	50	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_48030837_48030838_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_48032874_48032878_Indel	Indel	52	52	0	0	100	52	51	1	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_61760892_61760893_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_71795151_71795152_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_71829820_71829821_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_71838499_71838500_REF	REF	52	2	50	0	100	52	3	49	0	100	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	11	41	0	100
chr3_10085129_10085130_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_10133948_10133949_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_123419732_123419733_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_123453060_123453061_REF	REF	52	16	36	0	100	52	16	36	0	100	51	13	38	0	100	52	20	32	0	100	52	9	43	0	100	52	14	38	0	100
chr3_134644635_134644636_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_138456725_138456726_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100
chr3_138665063_138665064_REF	REF	52	31	20	1	96.88	52	40	12	0	100	51	22	27	2	91.67	52	42	10	0	100	52	41	6	5	89.13	52	30	14	8	78.95
chr3_142222283_142222284_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_142277535_142277536_REF	REF	52	52	0	0	100	52	52	0	0	100	51	50	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_142281611_142281612_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_178922272_178922273_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_178922273_178922274_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_180366066_180366067_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_188326954_188326955_REF	REF	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_30733401_30733403_Indel	Indel	52	45	6	1	97.83	52	48	4	0	100	51	50	1	0	100	52	52	0	0	100	52	48	3	1	97.96	52	49	1	2	96.08
chr3_3192524_3192524_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_32188248_32188249_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_37695191_37695192_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_38592405_38592406_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_46501284_46501284_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_47125384_47125385_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_47163342_47163343_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_52584431_52584431_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_52584714_52584715_REF	REF	52	52	0	0	100	52	52	0	0	100	51	50	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_52662849_52662850_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_63973739_63973740_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_63981634_63981635_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_63982219_63982220_REF	REF	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100
chr3_65361369_65361373_Indel	Indel	52	32	0	20	61.54	52	22	0	30	42.31	51	21	0	30	41.18	52	36	0	16	69.23	52	31	0	21	59.62	52	37	0	15	71.15
chr3_81627174_81627175_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr4_103533630_103533631_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr4_103534559_103534560_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr4_106154990_106154998_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr4_106155750_106155751_REF	REF	52	52	0	0	100	52	52	0	0	100	51	50	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr4_106190731_106190732_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr4_114117474_114117474_REF	REF	52	50	2	0	100	52	49	3	0	100	51	45	6	0	100	52	46	6	0	100	52	47	5	0	100	52	51	1	0	100
chr4_146576417_146576418_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr4_16037351_16037352_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr4_2834813_2834814_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr4_55151958_55151958_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr4_87967234_87967235_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr4_88056719_88056720_Indel	Indel	52	24	28	0	100	52	22	30	0	100	51	24	27	0	100	52	21	31	0	100	52	23	29	0	100	52	22	30	0	100
chr4_88959380_88959381_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr4_90743317_90743317_REF	REF	52	49	3	0	100	52	50	2	0	100	51	49	2	0	100	52	51	1	0	100	52	48	4	0	100	52	50	2	0	100
chr4_995304_995305_REF	REF	52	52	0	0	100	52	52	0	0	100	51	50	1	0	100	52	51	1	0	100	52	51	1	0	100	52	52	0	0	100
chr5_112162853_112162854_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_112175769_112175770_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_131915212_131915213_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_13811774_13811775_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_13829607_13829608_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_13829610_13829611_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_13864727_13864728_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_13871053_13871054_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_149435607_149435608_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	50	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_149441016_149441017_Indel	Indel	52	0	52	0	0	52	0	52	0	0	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0

		Operator 1					Operator 2					Operator 3					Operator 4					Operator 5					Operator 6				
Location	Variant Type	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]
chr5_149456994_149456995_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_149460552_149460553_SNP	SNP	52	51	1	0	100	52	51	1	0	100	51	44	7	0	100	52	50	2	0	100	52	49	3	0	100	52	52	0	0	100
chr5_149749125_149749126_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_149776231_149776232_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_156190172_156190172_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_176517460_176517461_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_176517796_176517797_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_176522727_176522728_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_176636881_176636882_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_180045700_180045700_Indel	Indel	52	50	0	2	96.15	52	50	0	2	96.15	51	47	0	4	92.16	52	48	0	4	92.31	52	50	0	2	96.15	52	51	0	1	98.08
chr5_180046492_180046494_REF	REF	52	0	52	0	0	52	0	52	0	0	51	0	49	2	0	52	0	52	0	0	52	0	52	0	0	52	0	51	1	0
chr5_180047739_180047739_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	50	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_180052945_180052946_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_35873604_35873605_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_35874574_35874575_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_35876273_35876274_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_38499763_38499764_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_38528951_38528953_Indel	Indel	52	0	0	52	0	52	2	1	49	3.92	51	0	0	51	0	52	0	0	52	0	52	0	2	50	0	52	2	0	50	3.85
chr5_55250726_55250727_REF	REF	52	52	0	0	100	52	52	0	0	100	51	50	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_55251930_55251931_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_7870972_7870973_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_116950733_116950734_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_116951505_116951507_REF	REF	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	51	1	0	100	52	52	0	0	100	52	52	0	0	100
chr6_117686699_117686701_REF	REF	52	52	0	0	100	52	51	1	0	100	51	51	0	0	100	52	52	0	0	100	52	51	1	0	100	52	51	1	0	100
chr6_117708970_117708971_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_117730818_117730819_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_135515447_135515448_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_135518107_135518108_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_135521488_135521489_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_138195961_138195964_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_143792605_143792606_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_143792674_143792675_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	47	4	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_152453290_152453291_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_152461048_152461052_REF	REF	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_152469330_152469331_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_152539572_152539573_Indel	Indel	52	0	52	0	0	52	0	52	0	0	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0
chr6_152557345_152557346_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_152660309_152660310_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_152671474_152671475_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_152711394_152711395_REF	REF	52	52	0	0	100	52	51	1	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_152793375_152793376_Indel	Indel	52	30	22	0	100	52	37	15	0	100	51	46	5	0	100	52	49	3	0	100	52	41	11	0	100	52	37	15	0	100
chr6_152826296_152826297_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_16327329_16327330_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_167271710_167271711_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_167271715_167271716_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_31978686_31978687_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_31978733_31978734_REF	REF	52	0	52	0	0	52	0	52	0	0	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0
chr6_31978745_31978746_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_31978686_31978687_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_31978733_31978734_REF	REF	52	0	52	0	0	52	0	52	0	0	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0
chr6_31978745_31978746_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_32032742_32032743_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_32064725_32064726_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100
chr6_32164868_32164869_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_32164873_32164874_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_32188296_32188297_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_33132313_33132314_REF	REF	52	7	43	2	77.78	52	10	41	1	90.91	51	3	46	2	60.00	52	10	41	1	90.91	52	16	36	0	100.00	52	14	38	0	100
chr6_33134799_33134800_REF	REF	52	52	0	0	100	52	52	0	0	100	51	50	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_33136637_33136638_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	51	1	0	100	52	52	0	0	100	52	52	0	0	100
chr6_33139327_33139328_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_33141160_33141161_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_33145368_33145369_SNP	SNP	52	44	8	0	100	52	42	10	0	100	51	43	8	0	100	52	45	7	0	100	52	38	14	0	100	52	42	10	0	100
chr6_41565471_41565472_REF	REF	52	52	0	0	100	52	52	0	0	100	51	50	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_49425520_49425521_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_51618169_51618170_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_51776534_51776535_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_51875249_51875250_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_51891010_51891011_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_51921613_51921614_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_51935746_51935747_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_56373535_56373536_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_56463409_56463410_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_65016977_65016979_Indel	Indel	52	18	34	0	100	52	15	37	0	100	51	11	40	0	100	52	2	50	0	100	52	9	42	1	90.00	52	6	46	0	100
chr6_65098767_65098768_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_65301503_65301504_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_65611952_65611952_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_66005856_66005857_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_66005887_66005888_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_69666683_69666684_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_70071172_70071173_SNP	SNP	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_70950281_70950283_Indel	Indel	52	15	34	3	83.33	52	12	40	0	100	51	17	33	1	94.44	52	12	35	5	70.59	52	2	46	4	33.33	52	4	44	4	50.00
chr6_70965124_70965125_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	48	3	0	100	52	52	0	0	100	52	50	2	0	100	52	50	2	0	100
chr6_70990669_70990670_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_91226285_91226286_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_91266349_91266349_REF	REF	52	0	52	0	0	52	0	52	0	0	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0
chr6_93964318_93964319_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_100411277_100411278_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_106508977_106508978_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_106508986_106508987_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_107353127_107353128_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_124486979_124486980_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_124492037_124492038_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	51	1	0	100
chr7_126079143_126079144_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_126410121_126410122_REF	REF	52	12	39	1	92.31	52	9	42	1	90.00	51	11	40	0	100	52	15	37	0	100	52	20	31	1	95.24	52	16	36	0	100
chr7_126410170_126410171_SNP	SNP	52	33	18	1	97.06	52	34	17	1	97.14	51	37	13	1	97.37	52	45	7	0	100	52	40	11	1	97.56	52	40	12	0	100

Location	Variant Type	Operator 1					Operator 2					Operator 3					Operator 4					Operator 5					Operator 6				
		Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]
chr7_128034628_128034629_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_142566684_142566684_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_143016927_143016928_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_143036547_143036548_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_143043239_143043240_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	49	2	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_148504716_148504717_REF	REF	52	0	52	0	0	52	0	52	0	0	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0
chr7_148543524_148543525_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_150644427_150644428_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_150648671_150648672_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_150654622_150654622_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_151873852_151873853_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_151874497_151874498_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_151896573_151896573_REF	REF	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_151949067_151949068_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_21582916_21582917_SNP	SNP	52	43	9	0	100	52	35	15	2	94.59	51	44	6	1	97.78	52	31	21	0	100	52	30	22	0	100	52	37	15	0	100
chr7_21582962_21582963_SNP	SNP	52	51	1	0	100	52	52	0	0	100	51	49	2	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_21582963_21582964_SNP	SNP	52	51	1	0	100	52	52	0	0	100	51	49	2	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_21640617_21640618_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_21659554_21659554_Indel	Indel	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	50	0	2	96.15	52	52	0	0	100
chr7_21723572_21723573_REF	REF	52	51	1	0	100	52	52	0	0	100	51	48	3	0	100	52	50	2	0	100	52	50	2	0	100	52	49	3	0	100
chr7_21778448_21778449_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_21784431_21784432_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_2962292_2962293_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_2962752_2962753_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_33148711_33148712_SNP	SNP	52	42	10	0	100	52	49	3	0	100	51	35	14	2	94.59	52	50	2	0	100	52	43	3	6	87.76	52	32	9	11	74.42
chr7_37904065_37904068_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_37916454_37916455_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_55228052_55228053_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_55266416_55266417_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_73472049_73472050_SNP	SNP	52	48	4	0	100	52	50	2	0	100	51	51	0	0	100	52	50	2	0	100	52	51	1	0	100	52	46	6	0	100
chr7_91632305_91632306_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_91652178_91652178_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_91695716_91695717_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_91712697_91712698_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_91713971_91713972_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_91714910_91714911_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_91726926_91726927_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_92146686_92146687_REF	REF	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_92244630_92244635_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_94047160_94047161_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_98534946_98534948_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_113364695_113364696_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_114327081_114327084_REF	REF	52	52	0	0	100	52	51	1	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_11614574_11614575_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_118819577_118819578_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_126056215_126056215_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_144992075_144992076_REF	REF	52	52	0	0	100	52	52	0	0	100	51	48	3	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_144992102_144992103_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_144995493_144995494_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_144996407_144996408_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_144998168_144998169_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_144998189_144998190_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_145000055_145000056_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	51	1	0	100
chr8_145005688_145005688_Indel	Indel	52	25	27	0	100	52	16	36	0	100	51	16	35	0	100	52	23	29	0	100	52	21	31	0	100	52	21	31	0	100
chr8_145008341_145008342_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	49	1	1	98	52	52	0	0	100	52	49	3	0	100	52	52	0	0	100
chr8_21979102_21979103_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_30925750_30925751_SNP	SNP	52	45	4	3	93.75	52	43	7	2	95.56	51	29	19	3	90.63	52	37	15	0	100	52	48	4	0	100	52	33	8	11	75.00
chr8_30977716_30977717_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_30999122_30999123_REF	REF	52	52	0	0	100	52	52	0	0	100	51	50	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_31005017_31005017_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_48805816_48805816_Indel	Indel	52	18	29	5	78.26	52	13	39	0	100	51	18	33	0	100	52	21	30	1	95.45	52	18	33	1	94.74	52	17	35	0	100
chr8_90967710_90967711_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_90970861_90970862_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_90970934_90970935_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr9_111685078_111685078_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr9_130587052_130587052_Indel	Indel	52	45	7	0	100	52	45	7	0	100	51	46	5	0	100	52	48	4	0	100	52	44	8	0	100	52	43	9	0	100
chr9_135786903_135786904_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr9_137659235_137659236_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr9_137688780_137688800_Indel	Indel	52	52	0	0	100	52	51	0	1	98.08	51	43	2	6	87.76	52	52	0	0	100	52	49	0	3	94.23	52	52	0	0	100
chr9_15479634_15479635_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr9_34485236_34485237_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr9_5081779_5081780_REF	REF	52	52	0	0	100	52	52	0	0	100	51	50	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr9_5090640_5090641_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr9_71650751_71650752_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	48	4	0	100	52	52	0	0	100
chr9_8331574_8331574_Indel	Indel	52	1	51	0	100	52	0	52	0	0	51	0	51	0	0	52	0	52	0	0	52	1	51	0	100	52	0	52	0	0
chr9_8338847_8338849_Indel	Indel	52	1	0	51	1.92	52	0	0	52	0	51	0	0	51	0	52	0	0	52	0	52	0	0	52	0	52	0	0	52	0
chr9_8460590_8460591_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr9_8484397_8484398_Indel	Indel	52	0	52	0	0	52	0	52	0	0	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0
chr9_93639848_93639849_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr9_94486320_94486321_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr9_97934366_97934367_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr9_98229388_98229389_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr9_98232223_98232224_Indel	Indel	52	23	29	0	100	52	34	18	0	100	51	32	19	0	100	52	36	15	1	97.30	52	44	8	0	100	52	28	24	0	100
chr10_104157710_104157711_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_104157726_104157727_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_121429761_121429762_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	48	0	3	94.12	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_123243196_123243197_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_123310870_123310871_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_27294449_27294451_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_3818449_3818449_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_3820113_3820113_Indel	Indel	52	0	0	52	0	52	0	0	52	0	51	0	0	51	0	52	0	0	52	0	52	0	3	49	0	52	0	0	52	0
chr10_51568377_51568378_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100

		Operator 1					Operator 2					Operator 3					Operator 4					Operator 5					Operator 6				
Location	Variant Type	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]
chr10_51582893_51582894_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_55591312_55591313_SNP	SNP	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_55755490_55755491_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_55826469_55826470_REF	REF	52	36	16	0	100	52	37	15	0	100	51	36	15	0	100	52	43	9	0	100	52	31	21	0	100	52	37	15	0	100
chr10_55892600_55892602_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_55943183_55943184_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_55955484_55955485_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_56423967_56423968_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_70405236_70405237_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_73544637_73544638_SNP	SNP	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	50	2	0	100
chr10_73553176_73553177_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_73558851_73558852_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_73558885_73558886_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_73558951_73558952_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_88441479_88441480_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_88683121_88683122_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_108121445_108121446_REF	REF	52	50	2	0	100	52	52	0	0	100	51	51	0	0	100	52	51	1	0	100	52	51	1	0	100	52	52	0	0	100
chr11_108139106_108139112_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_108150207_108150208_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_108151707_108151707_Indel	Indel	52	28	21	3	90.32	52	36	14	2	94.74	51	32	15	4	88.89	52	39	12	1	97.50	52	32	16	4	88.89	52	37	12	3	92.50
chr11_119148572_119148573_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_125497465_125497466_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_125525194_125525195_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_128675291_128675292_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_17522637_17522638_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_17537704_17537705_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_2592530_2592531_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_36595599_36595600_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_4123344_4123345_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_4141131_4141132_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_4159456_4159457_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_4159465_4159466_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_44151576_44151577_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_47260318_47260319_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_47364761_47364762_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_47371484_47371485_Indel	Indel	52	45	7	0	100	52	51	1	0	100	51	50	1	0	100	52	44	8	0	100	52	46	6	0	100	52	51	1	0	100
chr11_47463483_47463484_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_69462909_69462910_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_71728908_71728914_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_76867902_76867903_REF	REF	52	22	30	0	100	52	19	33	0	100	51	16	35	0	100	52	24	28	0	100	52	23	29	0	100	52	26	26	0	100
chr11_76894234_76894235_SNP	SNP	52	52	0	0	100	52	46	6	0	100	51	40	10	1	97.56	52	50	2	0	100	52	50	2	0	100	52	52	0	0	100
chr11_76895770_76895792_REF	REF	52	0	7	45	0	52	0	4	48	0	51	0	7	44	0	52	0	6	46	0	52	0	8	44	0	52	0	6	46	0
chr11_76901272_76901273_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_76901623_76901623_Indel	Indel	52	4	45	3	57.14	52	5	41	6	45.45	51	2	37	12	14.29	52	7	41	4	63.64	52	17	32	3	85.00	52	16	31	5	76.19
chr11_76910765_76910766_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_76917219_76917220_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_76919467_76919468_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_76919477_76919478_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_89017960_89017961_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_103234214_103234215_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_103234251_103234252_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_103306549_103306550_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_111351029_111351030_Indel	Indel	52	32	20	0	100	52	33	19	0	100	51	28	23	0	100	52	30	22	0	100	52	27	25	0	100	52	34	18	0	100
chr12_111962923_111962924_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_112919868_112919869_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_121431271_121431272_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_22063736_22063737_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_22063748_22063749_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_25358662_25358664_Indel	Indel	52	12	38	2	85.71	52	13	37	2	86.67	51	7	43	1	87.50	52	6	44	2	75.00	52	3	48	1	75.00	52	5	44	3	62.50
chr12_25362776_25362777_REF	REF	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_2694637_2694638_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_2760965_2760966_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_2794790_2794791_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_33021933_33021934_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_40645256_40645257_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_40681142_40681142_REF	REF	52	43	9	0	100	52	46	6	0	100	51	44	7	0	100	52	41	11	0	100	52	48	4	0	100	52	44	8	0	100
chr12_40757114_40757114_Indel	Indel	52	50	0	2	96.15	52	50	2	0	100	51	36	0	15	70.59	52	44	0	8	84.62	52	40	6	6	86.96	52	36	6	10	78.26
chr12_48374615_48374616_REF	REF	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_49424665_49424665_REF	REF	52	34	18	0	100	52	44	8	0	100	51	9	42	0	100	52	17	35	0	100	52	15	37	0	100	52	29	23	0	100
chr12_56435928_56435929_SNP	SNP	52	51	1	0	100	52	51	1	0	100	51	49	2	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_56437777_56437777_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100
chr12_7362203_7362204_REF	REF	52	0	14	38	0	52	0	28	24	0	51	0	22	29	0	52	0	28	24	0	52	0	18	34	0	52	0	17	35	0
chr13_103518035_103518036_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr13_103527848_103527849_SNP	SNP	52	51	1	0	100	52	50	2	0	100	51	47	4	0	100	52	46	6	0	100	52	49	2	1	98	52	50	2	0	100
chr13_103527929_103527930_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr13_110833701_110833702_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr13_110858991_110858992_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr13_110864181_110864182_REF	REF	52	49	3	0	100	52	50	2	0	100	51	44	7	0	100	52	52	0	0	100	52	50	2	0	100	52	50	2	0	100
chr13_28585649_28585650_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100
chr13_28597447_28597449_Indel	Indel	52	49	1	2	96.08	52	49	0	3	94.23	51	38	0	13	74.51	52	51	0	1	98.08	52	52	0	0	100	52	50	0	2	96.15
chr13_28608458_28608459_REF	REF	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr13_28610044_28610045_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr13_28611381_28611382_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr13_28623698_28623699_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr13_28623758_28623759_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr13_48947468_48947469_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr13_49033746_49033747_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_21778717_21778720_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_21792810_21792811_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_23855425_23855426_REF	REF	52	52	0	0	100	52	52	0	0	100	51	49	2	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_23865884_23865885_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_23866712_23866713_REF	REF	52	52	0	0	100	52	52	0	0	100	51	50	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100

		Operator 1					Operator 2					Operator 3					Operator 4					Operator 5					Operator 6				
Location	Variant Type	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]
chr14_23888323_23888323_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_51062236_51062238_Indel	Indel	52	42	0	10	80.77	52	41	0	11	78.85	51	40	0	11	78.43	52	50	0	2	96.15	52	44	0	8	84.62	52	47	0	5	90.38
chr14_51094701_51094702_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_51204995_51204996_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_56139313_56139314_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_62164607_62164608_REF	REF	52	42	10	0	100	52	45	7	0	100	51	41	10	0	100	52	42	10	0	100	52	48	4	0	100	52	49	3	0	100
chr14_62194238_62194239_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_81534708_81534709_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_81574815_81574816_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_81610582_81610583_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_88852279_88852280_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100
chr14_88894094_88894095_REF	REF	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_95572110_95572111_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_28235772_28235773_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_39880821_39880822_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_39886429_39886430_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_40457238_40457238_REF	REF	52	42	10	0	100	52	45	7	0	100	51	46	5	0	100	52	41	11	0	100	52	46	6	0	100	52	37	15	0	100
chr15_40476131_40476131_REF	REF	52	50	2	0	100	52	49	3	0	100	51	42	9	0	100	52	51	1	0	100	52	46	6	0	100	52	49	3	0	100
chr15_40494951_40494952_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_40494959_40494960_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_40498502_40498503_REF	REF	52	52	0	0	100	52	51	1	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_40898642_40898643_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_40914771_40914772_REF	REF	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_40915189_40915190_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_40915893_40915894_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_40949525_40949526_REF	REF	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_40954369_40954370_REF	REF	52	52	0	0	100	52	52	0	0	100	51	50	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_41797573_41797573_REF	REF	52	0	52	0	0	52	0	52	0	0	51	0	51	0	0	52	0	52	0	0	52	0	51	1	0	52	0	52	0	0
chr15_43571389_43571390_SNP	SNP	52	3	49	0	100	52	4	48	0	100	51	2	49	0	100	52	1	51	0	100	52	0	51	1	0	52	1	51	0	100
chr15_48703129_48703130_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_48719700_48719701_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_48722883_48722884_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_48759993_48759994_REF	REF	52	49	3	0	100	52	52	0	0	100	51	47	4	0	100	52	48	4	0	100	52	49	3	0	100	52	52	0	0	100
chr15_48787842_48787843_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_48797306_48797307_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_48805937_48805938_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_67457334_67457335_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_72105928_72105929_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	50	1	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100
chr15_72639002_72639003_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_73614702_73614704_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_73614833_73614834_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_74336632_74336633_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_89859932_89859932_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_89864316_89864316_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_89869768_89869768_Indel	Indel	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_91337504_91337505_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_91358271_91358271_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_99482380_99482380_REF	REF	52	0	52	0	0	52	0	52	0	0	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0
chr16_14026006_14026007_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_15811022_15811023_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_15811061_15811062_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_15818140_15818141_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_15818492_15818493_Indel	Indel	52	0	50	2	0	52	0	52	0	0	51	0	49	2	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0
chr16_15865471_15865472_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_27456612_27456613_REF	REF	52	52	0	0	100	52	52	0	0	100	51	47	4	0	100	52	49	3	0	100	52	52	0	0	100	52	51	1	0	100
chr16_57946936_57946937_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_57946972_57946973_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_89806346_89806347_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_89831509_89831510_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_89836322_89836323_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_89849479_89849480_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_89866042_89866043_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_89869703_89869704_REF	REF	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_89869760_89869761_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_89874873_89874874_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_89880821_89880822_REF	REF	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_11651056_11651057_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_11687520_11687521_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_11697049_11697049_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_11778239_11778240_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_15133705_15133707_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_1554635_1554636_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100
chr17_1578645_1578646_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_1580830_1580831_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_1585129_1585130_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_29528561_29528562_REF	REF	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_29559931_29559932_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_29563073_29563073_Indel	Indel	52	10	12	30	25.00	52	10	21	21	32.26	51	6	19	26	18.75	52	11	10	31	26.19	52	3	7	42	6.67	52	8	19	25	24.24
chr17_37657460_37657460_REF	REF	52	0	52	0	0	52	0	52	0	0	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0
chr17_37816460_37816461_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_37830899_37830900_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_39742491_39742492_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_39923613_39923614_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_45360888_45360889_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_48263902_48263903_REF	REF	52	52	0	0	100	52	52	0	0	100	51	50	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_48265425_48265426_SNP	SNP	52	51	1	0	100	52	45	7	0	100	51	35	16	0	100	52	50	2	0	100	52	50	2	0	100	52	45	7	0	100
chr17_48265494_48265495_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_48268222_48268223_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_48269301_48269302_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_48275181_48275182_REF	REF	52	20	32	0	100	52	14	38	0	100	51	10	41	0	100	52	7	45	0	100	52	9	43	0	100	52	5	47	0	100
chr17_48276970_48276971_SNP	SNP	52	44	6	2	95.65	52	37	12	3	92.50	51	38	9	4	90.48	52	36	11	5	87.80	52	33	18	1	97.06	52	27	21	4	87.10
chr17_5288607_5288608_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100

		Operator 1					Operator 2					Operator 3					Operator 4					Operator 5					Operator 6				
Location	Variant Type	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]
chr17_53342796_53342796_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	51	1	0	100	52	52	0	0	100	52	52	0	0	100
chr17_56688511_56688512_Indel	Indel	52	49	3	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	51	1	0	100
chr17_59760995_59760996_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_59763346_59763347_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_6331802_6331803_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_6331835_6331836_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_66519857_66519857_REF	REF	52	45	7	0	100	52	48	4	0	100	51	45	6	0	100	52	48	4	0	100	52	46	6	0	100	52	49	3	0	100
chr17_72306116_72306118_Indel	Indel	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_7348763_7348764_REF	REF	52	18	34	0	100	52	37	15	0	100	51	27	24	0	100	52	40	12	0	100	52	32	20	0	100	52	37	15	0	100
chr17_73720899_73720900_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_7579643_7579659_Indel	Indel	52	16	15	21	43.24	52	11	2	39	22.00	51	5	11	35	12.50	52	20	4	28	41.67	52	16	12	24	40.00	52	23	2	27	46.00
chr17_78014020_78014021_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_78055756_78055757_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_78055757_78055758_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_78060108_78060109_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_78071051_78071052_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_78073561_78073562_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_78073588_78073589_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_78078708_78078709_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_78081706_78081707_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_78084768_78084769_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_78302156_78302157_REF	REF	52	52	0	0	100	52	52	0	0	100	51	50	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_78319135_78319136_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_78327357_78327358_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr18_21119290_21119291_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr18_28666526_28666526_Indel	Indel	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr18_28669664_28669668_Indel	Indel	52	51	1	0	100	52	51	1	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr18_29104563_29104564_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr18_50831932_50831933_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr18_59166540_59166541_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr18_77477514_77477515_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_1619749_1619749_Indel	Indel	52	7	45	0	100	52	3	49	0	100	51	1	50	0	100	52	0	52	0	0	52	0	52	0	0	52	1	51	0	100
chr19_16593330_16593331_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_17937757_17937758_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_18876367_18876368_REF	REF	52	42	10	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_30303508_30303508_Indel	Indel	52	23	28	1	95.83	52	29	23	0	100	51	21	29	1	95.45	52	26	26	0	100	52	20	26	6	76.92	52	13	32	7	65.00
chr19_3113516_3113531_REF	REF	52	37	15	0	100	52	50	2	0	100	51	36	15	0	100	52	52	0	0	100	52	44	8	0	100	52	36	15	1	97.30
chr19_35523611_35523612_REF	REF	52	37	15	0	100	52	48	4	0	100	51	37	14	0	100	52	48	4	0	100	52	40	12	0	100	52	45	7	0	100
chr19_38939407_38939408_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_38968558_38968559_REF	REF	52	19	33	0	100	52	24	28	0	100	51	19	32	0	100	52	28	24	0	100	52	22	30	0	100	52	24	28	0	100
chr19_38973588_38973589_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_38995437_38995438_REF	REF	52	52	0	0	100	52	52	0	0	100	51	50	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_38996989_38996990_REF	REF	52	52	0	0	100	52	52	0	0	100	51	49	2	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_38997364_38997364_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_38997458_38997459_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_39034584_39034585_REF	REF	52	19	33	0	100	52	15	37	0	100	51	13	38	0	100	52	15	37	0	100	52	13	39	0	100	52	15	37	0	100
chr19_39057615_39057616_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	51	1	0	100
chr19_41928867_41928867_Indel	Indel	52	3	49	0	100	52	0	52	0	0	51	1	50	0	100	52	0	52	0	0	52	0	52	0	0	52	1	51	0	100
chr19_41930395_41930396_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_45864924_45864924_REF	REF	52	0	52	0	0	52	0	52	0	0	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0
chr19_45922322_45922323_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_55644324_55644325_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_55653296_55653297_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_55667646_55667647_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr20_31019360_31019361_Indel	Indel	52	22	29	1	95.65	52	10	42	0	100	51	7	42	2	77.78	52	15	35	2	88.24	52	21	30	1	95.45	52	22	30	0	100
chr20_31022765_31022766_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr20_32000482_32000483_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr20_41306599_41306600_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr20_43252914_43252915_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr20_50407161_50407162_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr20_50408481_50408482_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr21_35821820_35821821_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr21_45707515_45707516_REF	REF	52	52	0	0	100	52	52	0	0	100	51	46	5	0	100	52	52	0	0	100	52	50	2	0	100	52	52	0	0	100
chr21_45717549_45717550_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr21_46313441_46313442_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr22_24158898_24158899_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr22_36688177_36688178_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr22_36710182_36710183_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr22_39636944_39636948_Indel	Indel	52	42	9	1	97.67	52	41	10	1	97.62	51	40	9	2	95.24	52	44	8	0	100	52	44	8	0	100	52	46	6	0	100
chr22_41574086_41574087_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr22_42526548_42526549_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chrX_107829906_107829907_REF	REF	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chrX_107929336_107929337_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chrX_153760143_153760146_REF	REF	52	0	52	0	0	52	0	52	0	0	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0
chrX_154065842_154065843_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chrX_154158284_154158285_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chrX_32305879_32305879_Indel	Indel	52	47	1	4	92.16	52	50	0	2	96.15	51	34	0	17	66.67	52	41	0	11	78.85	52	42	0	10	80.77	52	44	0	8	84.62
chrX_32583739_32583740_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chrX_33357271_33357272_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chrX_44833840_44833841_SNP	SNP	52	42	10	0	100	52	39	13	0	100	51	43	8	0	100	52	38	14	0	100	52	41	11	0	100	52	42	10	0	100
chrX_44966794_44966795_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chrX_44966805_44966806_REF	REF	52	48	4	0	100	52	49	3	0	100	51	48	3	0	100	52	52	0	0	100	52	50	2	0	100	52	50	2	0	100
chrX_70595131_70595132_REF	REF	52	51	1	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chrX_70613072_70613073_REF	REF	52	33	19	0	100	52	51	1	0	100	51	46	5	0	100	52	51	1	0	100	52	42	10	0	100	52	45	7	0	100
chrX_70627476_70627477_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chrX_77298856_77298857_SNP	SNP	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chrX_79283508_79283509_REF	REF	52	52	0	0	100	52	52	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100

[1] % Correct Calls = Correct calls/(Correct calls + Incorrect calls)

Operator 1 & 2 are from Site 1
Operator 3 & 4 are from Site 2
Operator 5 & 6 are from Site 3

Table 28b **Reproducibility Results: Operator-to-Operator: NA19240**

The following table shows operator-to-operator reproducibility results for the Ion PGM™ Dx System (per variant). Results are presented for NA19240 and are inclusive of all the sequencing runs per operator.

		Operator 1					Operator 2					Operator 3					Operator 4					Operator 5					Operator 6				
Location	Variant Type	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]
chr1_103343469_103343470_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_103355221_103355222_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_103461507_103461508_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_103467614_103467615_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_11194590_11194591_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_11303152_11303153_SNP	SNP	51	47	4	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_116243876_116243877_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_116247789_116247790_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_116247825_116247826_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_120480393_120480394_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	51	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_120509048_120509049_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_120512302_120512303_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144852389_144852390_SNP	SNP	51	0	0	51	0	51	0	0	51	0	52	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0	52	0
chr1_144854179_144854180_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144866642_144866643_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144871888_144871889_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144874640_144874641_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	51	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144879263_144879264_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144882580_144882581_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144882822_144882823_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144886196_144886197_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144918956_144918957_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144921923_144921924_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144921949_144921950_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144922170_144922171_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144923728_144923729_REF	REF	51	41	10	0	100	51	47	4	0	100	52	42	10	0	100	52	49	3	0	100	52	41	11	0	100	52	49	3	0	100
chr1_144931329_144931330_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144931391_144931392_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144931580_144931581_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	49	3	0	100	52	51	1	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144931606_144931607_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144946700_144946701_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_144994657_144994658_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_145016019_145016020_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_145021149_145021150_SNP	SNP	51	41	10	0	100	51	45	6	0	100	52	42	10	0	100	52	42	10	0	100	52	45	7	0	100	52	46	6	0	100
chr1_145021182_145021183_SNP	SNP	51	44	7	0	100	51	44	7	0	100	52	37	15	0	100	52	42	10	0	100	52	43	9	0	100	52	39	13	0	100
chr1_145075682_145075683_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_156106184_156106185_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_156107612_156107613_Indel	Indel	51	21	30	0	100	51	25	26	0	100	52	18	34	0	100	52	19	33	0	100	52	23	29	0	100	52	33	19	0	100
chr1_156128219_156128220_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_156834186_156834187_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_156848909_156848910_Indel	Indel	51	0	0	51	0	51	0	0	51	0	52	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0	52	0
chr1_164761715_164761715_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_171076965_171076966_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100
chr1_171085314_171085314_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_182554556_182554557_REF	REF	51	51	0	0	100	51	51	0	0	100	52	50	2	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100
chr1_185069314_185069314_REF	REF	51	24	27	0	100	51	29	22	0	100	52	27	25	0	100	52	31	21	0	100	52	27	25	0	100	52	20	32	0	100
chr1_197325907_197325908_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_197404769_197404770_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_201335898_201335899_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_201341175_201341180_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_204433511_204433511_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_204501382_204501383_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_204515862_204515863_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_206650064_206650065_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_20972304_20972318_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_20972373_20972374_REF	REF	51	41	10	0	100	51	39	12	0	100	52	28	24	0	100	52	34	18	0	100	52	34	18	0	100	52	34	18	0	100
chr1_209804094_209804095_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	50	2	0	100	52	52	0	0	100
chr1_215821938_215821939_SNP	SNP	51	49	2	0	100	51	51	0	0	100	52	49	3	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_215821970_215821971_SNP	SNP	51	50	1	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	50	2	0	100	52	52	0	0	100
chr1_216052344_216052345_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_216258250_216258251_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_226555301_226555302_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_226580020_226580021_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_227071524_227071525_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_2337276_2337277_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_2340072_2340073_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_236883420_236883421_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_236925843_236925844_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_236978992_236978992_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_237060849_237060850_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_237711796_237711797_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_237814782_237814783_SNP	SNP	51	51	0	0	100	51	50	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_237863717_237863718_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_237969637_237969638_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	51	1	0	100
chr1_41296827_41296828_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_45797504_45797505_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_6529182_6529188_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	50	2	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_6530965_6530965_REF	REF	51	0	33	18	0	51	0	24	27	0	52	0	31	21	0	52	0	22	30	0	52	0	34	18	0	52	0	26	26	0
chr1_68894730_68894730_REF	REF	51	42	9	0	100	51	37	14	0	100	52	48	4	0	100	52	44	8	0	100	52	44	8	0	100	52	40	12	0	100
chr1_94466658_94466659_SNP	SNP	51	49	2	0	100	51	46	5	0	100	52	50	2	0	100	52	50	2	0	100	52	52	0	0	100	52	52	0	0	100
chr1_94471074_94471075_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_94474327_94474328_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_94481504_94481505_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_94564482_94564483_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr1_98015290_98015291_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_112725684_112725684_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	48	4	0	100	52	51	1	0	100
chr2_112786048_112786049_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_113999533_113999534_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_141130547_141130548_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100

		Operator 1					Operator 2					Operator 3					Operator 4					Operator 5					Operator 6				
Location	Variant Type	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]
chr2_141819587_141819588_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_141945948_141945949_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_148657116_148657117_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_167163042_167163043_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_167168092_167168093_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_175613272_175613273_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_175622219_175622220_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_178098087_178098088_REF	REF	51	42	9	0	100	51	44	7	0	100	52	44	8	0	100	52	43	9	0	100	52	48	4	0	100	52	49	3	0	100
chr2_182413237_182413238_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_182413258_182413259_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_182413601_182413602_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_189866342_189866343_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_189907936_189907937_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_189953285_189953285_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_191109532_191109533_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_198257794_198257795_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_198267770_198267770_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_209101905_209101906_Indel	Indel	51	0	51	0	0	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0
chr2_212812096_212812097_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_216240126_216240128_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_216248707_216248709_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_216274853_216274855_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_216299571_216299571_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_219545308_219545309_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_220284875_220284876_REF	REF	51	50	1	0	100	51	50	1	0	100	52	52	0	0	100	52	51	1	0	100	52	51	1	0	100	52	50	2	0	100
chr2_223161623_223161624_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_24905926_24905927_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_24974944_24974945_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_25471169_25471170_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_29416571_29416572_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100
chr2_42510017_42510018_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100
chr2_48030837_48030838_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_48032874_48032878_Indel	Indel	51	50	1	0	100	51	49	2	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100	52	52	0	0	100
chr2_61760892_61760893_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_71795151_71795152_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_71829820_71829821_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr2_71838499_71838500_Indel	Indel	51	24	27	0	100	51	20	31	0	100	52	13	39	0	100	52	27	25	0	100	52	15	37	0	100	52	29	23	0	100
chr3_10085129_10085130_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_10133948_10133949_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_123419732_123419733_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_123453060_123453061_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_134644635_134644636_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_138456725_138456726_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_138665063_138665064_SNP	SNP	51	45	6	0	100	51	50	1	0	100	52	42	8	2	95.45	52	52	0	0	100	52	42	6	4	91.30	52	35	9	8	81.40
chr3_142222283_142222284_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_142277535_142277536_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_142281611_142281612_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_178922272_178922273_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_178922273_178922274_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_180366066_180366067_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_188326954_188326955_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_30733401_30733403_REF	REF	51	0	29	22	0	51	0	32	19	0	52	0	20	32	0	52	1	22	29	3.33	52	0	24	28	0	52	0	28	24	0
chr3_3192524_3192524_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_32188248_32188249_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_37695191_37695192_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_38592405_38592406_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_46501284_46501284_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_47125384_47125385_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_47163342_47163343_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_52584431_52584431_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_52584714_52584715_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_52662849_52662850_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_63973739_63973740_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_63981634_63981635_REF	REF	51	51	0	0	100	51	50	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_63982219_63982220_SNP	SNP	51	23	28	0	100	51	25	26	0	100	52	43	9	0	100	52	44	8	0	100	52	47	5	0	100	52	41	11	0	100
chr3_65361369_65361373_REF	REF	51	50	1	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr3_81627174_81627175_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr4_103533630_103533631_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr4_103534559_103534560_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr4_106154990_106154998_REF	REF	51	5	0	46	9.80	51	3	0	48	5.88	52	17	0	35	32.69	52	5	0	47	9.62	52	18	0	34	34.62	52	3	0	49	5.77
chr4_106155750_106155751_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr4_106190731_106190732_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr4_114117474_114117474_Indel	Indel	51	49	2	0	100	51	33	18	0	100	52	27	25	0	100	52	31	21	0	100	52	22	30	0	100	52	48	4	0	100
chr4_146576417_146576418_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr4_16037351_16037352_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr4_2834813_2834814_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr4_55151958_55151958_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr4_87967234_87967235_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr4_88056719_88056720_Indel	Indel	51	28	23	0	100	51	22	29	0	100	52	25	27	0	100	52	22	30	0	100	52	25	27	0	100	52	21	31	0	100
chr4_88959380_88959381_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr4_90743317_90743317_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr4_995304_995305_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_112162853_112162854_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_112175769_112175770_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_131915212_131915213_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_13811774_13811775_REF	REF	51	49	2	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	51	1	0	100
chr5_13829607_13829608_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_13829610_13829611_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_13864727_13864728_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_13871053_13871054_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_149435607_149435608_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_149441016_149441017_REF	REF	51	0	51	0	0	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0

Location	Variant Type	Operator 1					Operator 2					Operator 3					Operator 4					Operator 5					Operator 6				
		Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]
chr5_149456994_149456995_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_149460552_149460553_REF	REF	51	9	42	0	100	51	1	50	0	100	52	0	52	0	0	52	1	51	0	100	52	2	50	0	100	52	2	50	0	100
chr5_149749125_149749126_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_149776231_149776232_REF	REF	51	51	0	0	100	51	49	2	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100	52	52	0	0	100
chr5_156190172_156190172_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_176517460_176517461_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_176517796_176517797_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_176522727_176522728_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_176636881_176636882_SNP	SNP	51	46	5	0	100	51	39	12	0	100	52	25	27	0	100	52	37	15	0	100	52	31	21	0	100	52	49	3	0	100
chr5_180045700_180045700_Indel	Indel	51	43	8	0	100	51	28	23	0	100	52	40	12	0	100	52	35	17	0	100	52	40	12	0	100	52	45	7	0	100
chr5_180046492_180046494_Indel	Indel	51	44	0	7	86.27	51	51	0	0	100	52	51	0	1	98.08	52	51	0	1	98.08	52	51	0	1	98.08	52	47	0	5	90.38
chr5_180047739_180047739_REF	REF	51	47	0	4	92.16	51	51	0	0	100	52	50	0	2	96.15	52	51	0	1	98.08	52	52	0	0	100	52	52	0	0	100
chr5_180052945_180052946_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_35873604_35873605_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_35874574_35874575_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_35876273_35876274_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_38499763_38499764_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_38528951_38528953_Indel	Indel	51	0	0	51	0	51	0	0	51	0	52	0	0	52	0	52	0	0	52	0	52	0	6	46	0	52	0	0	52	0
chr5_55250726_55250727_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_55251930_55251931_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr5_7870972_7870973_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_116950733_116950734_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_116951505_116951507_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_117686699_117686701_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_117708970_117708971_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_117730818_117730819_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_135515447_135515448_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_135518107_135518108_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_135521488_135521489_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_138195961_138195964_REF	REF	51	50	1	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_143792605_143792606_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_143792674_143792675_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_152453290_152453291_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_152461048_152461052_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_152469330_152469331_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100	52	52	0	0	100
chr6_152539572_152539573_Indel	Indel	51	0	51	0	0	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0
chr6_152557345_152557346_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_152660309_152660310_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_152671474_152671475_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_152711394_152711395_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_152793375_152793376_Indel	Indel	51	29	22	0	100	51	36	15	0	100	52	48	4	0	100	52	48	4	0	100	52	43	9	0	100	52	36	16	0	100
chr6_152826296_152826297_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_16327329_16327330_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_167271710_167271711_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	49	3	0	100	52	50	2	0	100	52	52	0	0	100	52	52	0	0	100
chr6_167271715_167271716_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_31978686_31978687_REF	REF	51	0	51	0	0	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0
chr6_31978733_31978734_SNP	SNP	51	47	4	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_31978745_31978746_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_32032742_32032743_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_32064725_32064726_REF	REF	51	49	2	0	100	51	51	0	0	100	52	47	5	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100
chr6_32164868_32164869_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_32164873_32164874_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_32188296_32188297_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_33132313_33132314_SNP	SNP	51	32	18	1	96.97	51	31	19	1	96.88	52	23	26	3	88.46	52	32	18	2	94.12	52	29	21	2	93.55	52	38	14	0	100
chr6_33134799_33134800_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_33136637_33136638_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_33139327_33139328_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_33141160_33141161_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_33145368_33145369_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_41565471_41565472_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_49425520_49425521_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_51618169_51618170_Indel	Indel	51	7	0	44	13.73	51	17	0	34	33.33	52	6	0	46	11.54	52	17	0	35	32.69	52	13	0	39	25.00	52	17	0	35	32.69
chr6_51776534_51776535_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_51875249_51875250_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_51891010_51891011_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_51921613_51921614_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_51935746_51935747_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_56373535_56373536_REF	REF	51	51	0	0	100	51	51	0	0	100	52	51	1	0	100	52	51	1	0	100	52	52	0	0	100	52	52	0	0	100
chr6_56463409_56463410_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_65016977_65016979_Indel	Indel	51	14	34	3	82.35	51	12	39	0	100	52	7	45	0	100	52	3	49	0	100	52	8	43	1	88.89	52	2	49	1	66.67
chr6_65098767_65098768_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_65301503_65301504_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_65611952_65611952_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_66005856_66005857_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_66005887_66005888_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_69666683_69666684_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_70071172_70071173_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_70950281_70950283_REF	REF	51	13	38	0	100	51	15	36	0	100	52	16	36	0	100	52	11	41	0	100	52	19	33	0	100	52	20	32	0	100
chr6_70965124_70965125_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	51	1	0	100	52	52	0	0	100	52	52	0	0	100	52	50	2	0	100
chr6_70990669_70990670_REF	REF	51	49	2	0	100	51	51	0	0	100	52	49	3	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_91226285_91226286_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr6_91266349_91266349_Indel	Indel	51	0	51	0	0	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0
chr6_93964318_93964319_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_100411277_100411278_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100	52	52	0	0	100
chr7_106508977_106508978_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_106508986_106508987_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_107353127_107353128_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_124486979_124486980_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_124492037_124492038_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_126079143_126079144_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_126410121_126410122_SNP	SNP	51	32	18	1	96.97	51	28	23	0	100	52	29	22	1	96.67	52	31	19	2	93.94	52	37	15	0	100	52	32	19	1	96.97
chr7_126410170_126410171_SNP	SNP	51	31	19	1	96.88	51	28	23	0	100	52	29	22	1	96.67	52	32	19	1	96.97	52	39	13	0	100	52	37	14	1	97.37

		Operator 1					Operator 2					Operator 3					Operator 4					Operator 5					Operator 6				
Location	Variant Type	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]
chr7_128034628_128034629_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_142566684_142566684_Indel	Indel	51	49	0	2	96.08	51	51	0	0	100	52	51	0	1	98.08	52	49	0	3	94.23	52	51	0	1	98.08	52	51	0	1	98.08
chr7_143016927_143016928_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_143036547_143036548_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_143043239_143043240_SNP	SNP	51	18	31	2	90.00	51	28	23	0	100	52	31	19	2	93.94	52	34	18	0	100	52	36	16	0	100	52	33	19	0	100
chr7_148504716_148504717_Indel	Indel	51	0	51	0	0	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0
chr7_148543524_148543525_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_150644427_150644428_REF	REF	51	51	0	0	100	51	51	0	0	100	52	45	7	0	100	52	52	0	0	100	52	45	7	0	100	52	52	0	0	100
chr7_150648671_150648672_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_150654622_150654622_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_151873852_151873853_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_151874497_151874498_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_151896573_151896573_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100	52	52	0	0	100
chr7_151949067_151949068_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_21582916_21582917_REF	REF	51	6	45	0	100	51	7	44	0	100	52	2	50	0	100	52	5	47	0	100	52	7	45	0	100	52	5	47	0	100
chr7_21582962_21582963_REF	REF	51	43	8	0	100	51	50	1	0	100	52	39	13	0	100	52	48	4	0	100	52	43	9	0	100	52	40	12	0	100
chr7_21582963_21582964_REF	REF	51	43	8	0	100	51	50	1	0	100	52	38	14	0	100	52	49	3	0	100	52	43	9	0	100	52	40	12	0	100
chr7_21640617_21640618_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_21659554_21659554_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100
chr7_21723572_21723573_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_21778448_21778449_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_21784431_21784432_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_2962292_2962293_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_2962752_2962753_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_33148711_33148712_SNP	SNP	51	39	12	0	100	51	51	0	0	100	52	42	8	2	95.45	52	50	2	0	100	52	42	4	6	87.50	52	31	12	9	77.50
chr7_37904065_37904068_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_37916454_37916455_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_55228052_55228053_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_55266416_55266417_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_73472049_73472050_SNP	SNP	51	49	1	1	98.00	51	51	0	0	100	52	47	4	1	97.92	52	50	2	0	100	52	51	1	0	100	52	47	5	0	100
chr7_91632305_91632306_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_91652178_91652178_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_91695716_91695717_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_91712697_91712698_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_91713971_91713972_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100	52	52	0	0	100
chr7_91714910_91714911_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_91726926_91726927_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_92146686_92146687_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_92244630_92244635_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_94047160_94047161_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr7_98534946_98534948_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_113364695_113364696_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_114327081_114327084_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	51	0	1	98.08	52	52	0	0	100
chr8_11614574_11614575_REF	REF	51	49	2	0	100	51	50	1	0	100	52	48	4	0	100	52	52	0	0	100	52	50	2	0	100	52	52	0	0	100
chr8_118819577_118819578_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_126056215_126056215_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_144992075_144992076_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_144992102_144992103_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_144995493_144995494_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	51	1	0	100
chr8_144996407_144996408_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_144998168_144998169_REF	REF	51	51	0	0	100	51	50	1	0	100	52	50	2	0	100	52	52	0	0	100	52	47	5	0	100	52	46	6	0	100
chr8_144998189_144998190_REF	REF	51	51	0	0	100	51	50	1	0	100	52	50	2	0	100	52	52	0	0	100	52	47	5	0	100	52	46	6	0	100
chr8_145000055_145000056_REF	REF	51	51	0	0	100	51	51	0	0	100	52	47	5	0	100	52	52	0	0	100	52	49	3	0	100	52	47	5	0	100
chr8_145005688_145005688_REF	REF	51	3	19	29	9.38	51	0	26	25	0	52	0	33	19	0	52	0	29	23	0	52	0	27	25	0	52	0	32	20	0
chr8_145008341_145008342_REF	REF	51	42	9	0	100	51	51	0	0	100	52	50	2	0	100	52	52	0	0	100	52	47	5	0	100	52	50	2	0	100
chr8_21979102_21979103_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_30925750_30925751_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100	52	52	0	0	100
chr8_30977716_30977717_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_30999122_30999123_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_31005017_31005017_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_48805816_48805816_Indel	Indel	51	16	28	7	69.57	51	13	38	0	100	52	21	31	0	100	52	22	30	0	100	52	17	31	4	80.95	52	15	37	0	100
chr8_90967710_90967711_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_90970861_90970862_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr8_90970934_90970935_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr9_111685078_111685078_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr9_130587052_130587052_REF	REF	51	1	50	0	100	51	2	49	0	100	52	1	51	0	100	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0
chr9_135786903_135786904_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr9_137659235_137659236_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr9_137688780_137688800_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	44	4	4	91.67	52	50	0	2	96.15	52	50	0	2	96.15	52	52	0	0	100
chr9_15479634_15479635_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr9_34485236_34485237_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr9_5081779_5081780_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr9_5090640_5090641_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr9_71650751_71650752_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	51	1	0	100
chr9_8331574_8331574_REF	REF	51	49	1	1	98	51	50	0	1	98.04	52	51	1	0	100	52	52	0	0	100	52	51	0	1	98.08	52	52	0	0	100
chr9_8338847_8338849_REF	REF	51	0	51	0	0	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0
chr9_8460590_8460591_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr9_8484397_8484398_REF	REF	51	0	51	0	0	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0
chr9_93639848_93639849_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr9_94486320_94486321_SNP	SNP	51	45	6	0	100	51	36	15	0	100	52	45	7	0	100	52	41	11	0	100	52	41	11	0	100	52	46	6	0	100
chr9_97934366_97934367_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr9_98229388_98229389_SNP	SNP	51	42	9	0	100	51	48	3	0	100	52	39	13	0	100	52	44	8	0	100	52	44	8	0	100	52	45	7	0	100
chr9_98232223_98232224_REF	REF	51	21	30	0	100	51	33	18	0	100	52	29	23	0	100	52	34	18	0	100	52	34	18	0	100	52	28	24	0	100
chr10_104157710_104157711_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_104157726_104157727_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_121429761_121429762_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_123243196_123243197_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_123310870_123310871_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_27294449_27294451_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_3818449_3818449_REF	REF	51	47	4	0	100	51	46	5	0	100	52	48	4	0	100	52	49	3	0	100	52	48	4	0	100	52	49	3	0	100
chr10_3820113_3820113_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_51568377_51568378_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100

		Operator 1					Operator 2					Operator 3					Operator 4					Operator 5					Operator 6				
Location	Variant Type	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]
chr10_51582893_51582894_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_55591312_55591313_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_55755490_55755491_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_55826469_55826470_SNP	SNP	51	50	1	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	51	0	1	98.08	52	52	0	0	100
chr10_55892600_55892602_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_55943183_55943184_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_55955484_55955485_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_56423967_56423968_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_70405236_70405237_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_73544637_73544638_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_73553176_73553177_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_73558851_73558852_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_73558885_73558886_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_73558951_73558952_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_88441479_88441480_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr10_88683121_88683122_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_108121445_108121446_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_108139106_108139112_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_108150207_108150208_REF	REF	51	44	7	0	100	51	46	5	0	100	52	44	8	0	100	52	43	9	0	100	52	46	6	0	100	52	46	6	0	100
chr11_108151707_108151707_Indel	Indel	51	28	19	4	87.50	51	31	17	3	91.18	52	35	13	4	89.74	52	29	18	5	85.29	52	32	17	3	91.43	52	29	19	4	87.88
chr11_119148572_119148573_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_125497465_125497466_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_125525194_125525195_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_128675291_128675292_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_17522637_17522638_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_17537704_17537705_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_2592530_2592531_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_36595599_36595600_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_4123344_4123345_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_4141131_4141132_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_4159456_4159457_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_4159465_4159466_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_44151576_44151577_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_47260318_47260319_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_47364761_47364762_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_47371484_47371485_Indel	Indel	51	47	4	0	100	51	50	1	0	100	52	51	1	0	100	52	49	3	0	100	52	48	4	0	100	52	52	0	0	100
chr11_47463483_47463484_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_69462909_69462910_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_71728908_71728914_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_76867902_76867903_SNP	SNP	51	47	4	0	100	51	46	5	0	100	52	39	13	0	100	52	47	5	0	100	52	50	2	0	100	52	47	5	0	100
chr11_76894234_76894235_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_76895770_76895792_Indel	Indel	51	4	41	6	40.00	51	6	33	12	33.33	52	10	37	5	66.67	52	3	36	13	18.75	52	12	34	6	66.67	52	1	43	8	11.11
chr11_76901272_76901273_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_76901623_76901623_REF	REF	51	0	36	15	0	51	0	39	12	0	52	0	39	13	0	52	0	37	15	0	52	0	33	19	0	52	0	38	14	0
chr11_76910765_76910766_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_76917219_76917220_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_76919467_76919468_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_76919477_76919478_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr11_89017960_89017961_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_103234214_103234215_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_103234251_103234252_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_103306549_103306550_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_111351029_111351030_REF	REF	51	22	29	0	100	51	18	33	0	100	52	13	39	0	100	52	20	32	0	100	52	23	29	0	100	52	33	19	0	100
chr12_111962923_111962924_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_112919868_112919869_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_121431271_121431272_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_22063736_22063737_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_22063748_22063749_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_25358662_25358664_Indel	Indel	51	13	36	2	86.67	51	16	33	2	88.89	52	9	42	1	90.00	52	11	39	2	84.62	52	4	48	0	100	52	6	45	1	85.71
chr12_25362776_25362777_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_2694637_2694638_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_2760965_2760966_Indel	Indel	51	50	0	1	98.04	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_2794790_2794791_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_33021933_33021934_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_40645256_40645257_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_40681142_40681142_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_40757114_40757114_Indel	Indel	51	51	0	0	100	51	49	2	0	100	52	35	3	14	71.43	52	42	1	9	82.35	52	38	3	11	77.55	52	35	5	12	74.47
chr12_48374615_48374616_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_49424665_49424665_Indel	Indel	51	18	33	0	100	51	33	18	0	100	52	5	45	2	71.43	52	2	50	0	100	52	2	49	1	66.67	52	9	43	0	100
chr12_56435928_56435929_REF	REF	51	45	6	0	100	51	50	1	0	100	52	47	5	0	100	52	52	0	0	100	52	50	2	0	100	52	51	1	0	100
chr12_56437777_56437777_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr12_7362203_7362204_SNP	SNP	51	0	16	35	0	51	0	21	30	0	52	0	24	28	0	52	0	17	35	0	52	0	22	30	0	52	0	14	38	0
chr13_103518035_103518036_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr13_103527848_103527849_SNP	SNP	51	51	0	0	100	51	48	3	0	100	52	48	4	0	100	52	49	3	0	100	52	50	2	0	100	52	52	0	0	100
chr13_103527929_103527930_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr13_110833701_110833702_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr13_110858991_110858992_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr13_110864181_110864182_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	51	1	0	100	52	52	0	0	100	52	52	0	0	100	52	51	1	0	100
chr13_28585649_28585650_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr13_28597447_28597449_REF	REF	51	51	0	0	100	51	51	0	0	100	52	51	1	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100
chr13_28608458_28608459_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr13_28610044_28610045_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr13_28611381_28611382_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr13_28623698_28623699_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr13_28623758_28623759_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr13_48947468_48947469_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr13_49033746_49033747_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_21778717_21778720_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_21792810_21792811_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_23855425_23855426_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_23865884_23865885_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_23866712_23866713_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100

		Operator 1					Operator 2					Operator 3					Operator 4					Operator 5					Operator 6				
Location	Variant Type	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]
chr14_23888323_23888323_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_51062236_51062238_Indel	Indel	51	44	0	7	86.27	51	42	0	9	82.35	52	45	0	7	86.54	52	49	0	3	94.23	52	39	0	13	75.00	52	46	0	6	88.46
chr14_51094701_51094702_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_51204995_51204996_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_56139313_56139314_REF	REF	51	46	5	0	100	51	45	6	0	100	52	45	7	0	100	52	47	5	0	100	52	48	4	0	100	52	46	6	0	100
chr14_62164607_62164608_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	51	1	0	100	52	50	2	0	100
chr14_62194238_62194239_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_81534708_81534709_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_81574815_81574816_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_81610582_81610583_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_88852279_88852280_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_88894094_88894095_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr14_95572110_95572111_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_28235772_28235773_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_39880821_39880822_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_39886429_39886430_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	51	1	0	100
chr15_40457238_40457238_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_40476131_40476131_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	38	14	0	100	52	49	3	0	100	52	47	5	0	100	52	45	7	0	100
chr15_40494951_40494952_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_40494959_40494960_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_40498502_40498503_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_40898642_40898643_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_40914771_40914772_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_40915189_40915190_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_40915893_40915894_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_40949525_40949526_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_40954369_40954370_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_41797573_41797573_Indel	Indel	51	0	51	0	0	51	0	51	0	0	52	0	51	1	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0
chr15_43571389_43571390_SNP	SNP	51	3	48	0	100	51	2	49	0	100	52	3	49	0	100	52	3	49	0	100	52	0	52	0	0	52	0	52	0	0
chr15_48703129_48703130_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_48719700_48719701_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_48722883_48722884_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_48759993_48759994_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_48787842_48787843_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_48797306_48797307_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_48805937_48805938_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_67457334_67457335_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_72105928_72105929_Indel	Indel	51	50	1	0	100	51	50	1	0	100	52	50	2	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_72639002_72639003_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_73614702_73614704_REF	REF	51	51	0	0	100	51	50	1	0	100	52	45	7	0	100	52	52	0	0	100	52	52	0	0	100	52	51	1	0	100
chr15_73614833_73614834_REF	REF	51	51	0	0	100	51	50	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_74336632_74336633_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_89859932_89859932_REF	REF	51	42	9	0	100	51	45	6	0	100	52	45	7	0	100	52	38	14	0	100	52	46	6	0	100	52	49	3	0	100
chr15_89864316_89864316_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_89869768_89869768_REF	REF	51	47	4	0	100	51	48	3	0	100	52	45	7	0	100	52	52	0	0	100	52	49	3	0	100	52	51	1	0	100
chr15_91337504_91337505_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_91358271_91358271_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr15_99482380_99482380_Indel	Indel	51	0	51	0	0	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0
chr16_14026006_14026007_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_15811022_15811023_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_15811061_15811062_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_15818140_15818141_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_15818492_15818493_REF	REF	51	10	41	0	100	51	4	47	0	100	52	5	47	0	100	52	8	44	0	100	52	8	44	0	100	52	20	32	0	100
chr16_15865471_15865472_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_27456612_27456613_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_57946936_57946937_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_57946972_57946973_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_89806346_89806347_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_89831509_89831510_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_89836322_89836323_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_89849479_89849480_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_89866042_89866043_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_89869703_89869704_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_89869760_89869761_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_89874873_89874874_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr16_89880821_89880822_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_11651056_11651057_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_11687520_11687521_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_11697049_11697049_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_11778239_11778240_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_15133705_15133707_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_1554635_1554636_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_1578645_1578646_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_1580830_1580831_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_1585129_1585130_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_29528561_29528562_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_29559931_29559932_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_29563073_29563073_REF	REF	51	0	0	51	0	51	0	1	50	0	52	0	2	50	0	52	0	2	50	0	52	0	0	52	0	52	0	3	49	0
chr17_37657460_37657460_Indel	Indel	51	0	51	0	0	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0
chr17_37816460_37816461_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_37830899_37830900_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_39742491_39742492_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100
chr17_39923613_39923614_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_45360888_45360889_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_48263902_48263903_SNP	SNP	51	41	10	0	100	51	48	3	0	100	52	49	3	0	100	52	52	0	0	100	52	50	2	0	100	52	50	2	0	100
chr17_48265425_48265426_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	49	3	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100
chr17_48265494_48265495_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_48268222_48268223_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_48269301_48269302_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_48275181_48275182_Indel	Indel	51	16	20	15	51.61	51	8	29	14	36.36	52	25	22	5	83.33	52	20	27	5	80.00	52	24	22	6	80.00	52	4	46	2	66.67
chr17_48276970_48276971_SNP	SNP	51	39	1	11	78.00	51	32	7	12	72.73	52	32	9	11	74.42	52	28	3	21	57.14	52	26	12	14	65.00	52	19	15	18	51.35
chr17_5288607_5288608_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100

Location	Variant Type	Operator 1					Operator 2					Operator 3					Operator 4					Operator 5					Operator 6				
		Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]	Total Calls	Correct Calls	No Calls	Miscalls	% Correct Calls[1]
chr17_53342796_53342796_REF	REF	51	51	0	0	100	51	51	0	0	100	52	51	1	0	100	52	52	0	0	100	52	51	1	0	100	52	50	2	0	100
chr17_56688511_56688512_REF	REF	51	47	4	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	50	2	0	100
chr17_59760995_59760996_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_59763346_59763347_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_6331802_6331803_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_6331835_6331836_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_66519857_66519857_Indel	Indel	51	38	13	0	100	51	48	3	0	100	52	45	7	0	100	52	50	2	0	100	52	42	10	0	100	52	51	1	0	100
chr17_72306116_72306118_REF	REF	51	46	5	0	100	51	50	1	0	100	52	49	3	0	100	52	52	0	0	100	52	48	4	0	100	52	47	5	0	100
chr17_7348763_7348764_SNP	SNP	51	40	11	0	100	51	40	11	0	100	52	41	11	0	100	52	42	10	0	100	52	41	11	0	100	52	35	17	0	100
chr17_73720899_73720900_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_7579643_7579659_Indel	Indel	51	16	16	19	45.71	51	8	6	37	17.78	52	12	4	36	25.00	52	11	7	34	24.44	52	9	7	36	20.00	52	17	6	29	36.96
chr17_78014020_78014021_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_78055756_78055757_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_78055757_78055758_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_78060108_78060109_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_78071051_78071052_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_78073561_78073562_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_78073588_78073589_REF	REF	51	51	0	0	100	51	51	0	0	100	52	51	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_78078708_78078709_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_78081706_78081707_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_78084768_78084769_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_78302156_78302157_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_78319135_78319136_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr17_78327357_78327358_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr18_21119290_21119291_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr18_28666526_28666526_Indel	Indel	51	50	1	0	100	51	50	1	0	100	52	51	0	1	98.08	52	52	0	0	100	52	52	0	0	100	52	51	1	0	100
chr18_28669664_28669668_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	51	1	0	100
chr18_29104563_29104564_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr18_50831932_50831933_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr18_59166540_59166541_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr18_77477514_77477515_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_1619749_1619749_Indel	Indel	51	47	4	0	100	51	44	7	0	100	52	50	2	0	100	52	46	6	0	100	52	47	5	0	100	52	40	12	0	100
chr19_16593330_16593331_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_17937757_17937758_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_18876367_18876368_Indel	Indel	51	45	6	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	51	1	0	100	52	50	2	0	100
chr19_30303508_30303508_REF	REF	51	0	51	0	0	51	0	51	0	0	52	1	48	3	25.00	52	0	52	0	0	52	0	47	5	0	52	0	45	7	0
chr19_3113516_3113531_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	47	5	0	100	52	52	0	0	100	52	50	2	0	100	52	48	4	0	100
chr19_35523611_35523612_SNP	SNP	51	48	3	0	100	51	51	0	0	100	52	48	4	0	100	52	52	0	0	100	52	51	1	0	100	52	52	0	0	100
chr19_38939407_38939408_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_38968558_38968559_SNP	SNP	51	45	6	0	100	51	43	8	0	100	52	44	8	0	100	52	46	6	0	100	52	42	10	0	100	52	47	5	0	100
chr19_38973588_38973589_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_38995437_38995438_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_38996989_38996990_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	50	2	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_38997364_38997364_Indel	Indel	51	1	50	0	100	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	1	51	0	100	52	0	52	0	0
chr19_38997458_38997459_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_39034584_39034585_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_39057615_39057616_SNP	SNP	51	51	0	0	100	51	49	2	0	100	52	50	2	0	100	52	52	0	0	100	52	52	0	0	100	52	51	1	0	100
chr19_41928867_41928867_Indel	Indel	51	3	48	0	100	51	2	49	0	100	52	3	49	0	100	52	3	49	0	100	52	0	52	0	0	52	2	50	0	100
chr19_41930395_41930396_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_45864924_45864924_Indel	Indel	51	0	51	0	0	51	0	51	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0
chr19_45922322_45922323_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_55644324_55644325_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_55653296_55653297_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr19_55667646_55667647_REF	REF	51	50	1	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	49	3	0	100	52	50	2	0	100
chr20_31019360_31019361_REF	REF	51	8	43	0	100	51	7	44	0	100	52	2	50	0	100	52	6	46	0	100	52	4	48	0	100	52	15	37	0	100
chr20_31022765_31022766_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr20_32000482_32000483_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr20_41306599_41306600_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr20_43252914_43252915_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr20_50407161_50407162_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr20_50408481_50408482_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr21_35821820_35821821_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr21_45707515_45707516_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	51	1	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr21_45717549_45717550_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr21_46313441_46313442_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr22_24158898_24158899_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr22_36688177_36688178_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr22_36710182_36710183_REF	REF	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr22_39636944_39636948_REF	REF	51	0	20	31	0	51	0	15	36	0	52	0	17	35	0	52	0	14	38	0	52	0	12	40	0	52	0	14	38	0
chr22_41574086_41574087_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chr22_42526548_42526549_SNP	SNP	51	5	46	0	100	51	1	50	0	100	52	1	51	0	100	52	2	50	0	100	52	4	48	0	100	52	5	47	0	100
chrX_107829906_107829907_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chrX_107929336_107929337_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chrX_153760143_153760146_Indel	Indel	51	50	1	0	100	51	50	1	0	100	52	42	10	0	100	52	49	3	0	100	52	37	15	0	100	52	47	5	0	100
chrX_154065842_154065843_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chrX_154158284_154158285_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chrX_32305879_32305879_REF	REF	51	0	0	51	0	51	0	0	51	0	52	0	0	52	0	52	0	0	52	0	52	0	0	52	0	52	0	0	52	0
chrX_32583739_32583740_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chrX_33357271_33357272_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chrX_44833840_44833841_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chrX_44966794_44966795_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chrX_44966805_44966806_Indel	Indel	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chrX_70595131_70595132_SNP	SNP	51	47	4	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	51	1	0	100
chrX_70613072_70613073_SNP	SNP	51	31	20	0	100	51	34	17	0	100	52	36	16	0	100	52	33	19	0	100	52	28	24	0	100	52	26	26	0	100
chrX_70627476_70627477_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chrX_77298856_77298857_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100
chrX_79283508_79283509_SNP	SNP	51	51	0	0	100	51	51	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100	52	52	0	0	100

[1] % Correct Calls = Correct calls/(Correct calls + Incorrect calls)

Operator 1 & 2 are from Site 1
Operator 3 & 4 are from Site 2
Operator 5 & 6 are from Site 3

Table 29a Summary results for Sensitivity and Reproducibility by test site and instrument system.

The following table demonstrates that the sensitivity and reproducibility of the Ion PGM™ Dx System to make correct SNV and Indel variant calls was independent of test site and instrument system.

DNA #	Site	Instrument	# runs	SNVs						Indels					
				# SNVs	# Correct SNV Calls	# No Calls	# Incorrect Calls	Mean SNV Sensitivity[1]	% with ≥95% Reproducibility[3]	# Indels	# Correct Indel Calls	# No Calls	# Incorrect Calls	Mean Indel Sensitivity[2]	% with ≥95% Reproducibility[3]
NA12878	1	1	52	302	15547	149	8	99.95%	291/302 = 96.36%	97	3805	872	367	91.20%	58/97 = 59.79%
		2	52	302	15568	130	6	99.96%	293/302 = 97.02%	97	3818	892	334	91.96%	59/97 = 60.82%
	2	1	52	302	15486	205	13	99.92%	289/302 = 95.70%	97	3726	914	404	90.22%	60/97 = 61.86%
		2	51	302	15273	121	8	99.95%	294/302 = 97.35%	97	3793	806	348	91.60%	61/97 = 62.89%
	3	1	52	302	15516	164	24	99.85%	290/302 = 96.03%	97	3795	893	356	91.42%	59/97 = 60.82%
		2	52	302	15544	148	12	99.92%	294/302 = 97.35%	97	3804	889	351	91.55%	60/97 = 61.86%
NA19240	1	1	50	373	18211	341	98	99.46%	346/373 = 92.76%	100	3909	875	216	94.76%	68/100 = 68.00%
		2	52	373	18945	352	99	99.48%	351/373 = 94.10%	100	4102	882	216	95.00%	68/100 = 68.00%
	2	1	52	373	18908	383	105	99.45%	347/373 = 93.03%	100	3963	979	258	93.89%	66/100 = 66.00%
		2	52	373	18956	329	111	99.42%	352/373 = 94.37%	100	4118	892	190	95.59%	68/100 = 68.00%
	3	1	52	373	18905	359	132	99.31%	351/373 = 94.10%	100	4026	962	212	95.00%	65/100 = 65.00%
		2	52	373	18992	300	104	99.46%	353/373 = 94.64%	100	4024	957	219	94.84%	65/100 = 65.00%

[1] Mean SNV sensitivity, excluding No Calls, over all valid runs for a given DNA sample will be calculated using the formula Sensitivity = TP/(TP+FN), where TP = total number of SNV positions called correctly (including zygosity determination) when compared to the variant reference database sequence, and FN = total number of SNVs present in the variant reference database sequence but not called correctly by the Ion PGM™ Dx System.

[2] Mean Indel sensitivity, excluding No Calls, over all valid runs for a given DNA sample will be calculated using the formula Sensitivity = TP/(TP+FN), where TP = total number of Indel positions called correctly (including zygosity determination) when compared to the variant reference database sequence, and FN = total number of Indels present in the variant reference database sequence but not called correctly by the Ion PGM™ Dx System.

[3] Reproducibility for SNVs and Indels is defined as the number of variant locations called with a minimum of 95% reproducibility across all sample runs. Reproducibility calculations were performed including no calls where a no-call result is counted as not being reproducible.

Table 29b Summary results for Specificity by test site and instrument system.

The following table demonstrates the specificity of the Ion PGM™ Dx System in correctly identifying reference base positions when compared to hg19 was independent of test site and instrument system.

DNA #	Site	Op	# runs	Reference Locations				
				# Reference Locations	# Correct Reference Calls	# No Calls	# Incorrect Calls	Mean Specificity[1]
NA12878	1	1	52	237	11167	1016	141	98.75%
		2	52	237	11171	1029	124	98.90%
	2	1	52	237	11123	1072	129	98.85%
		2	51	237	10929	1034	124	98.88%
	3	1	52	237	11106	1065	153	98.64%
		2	52	237	11221	976	127	98.88%
NA19240	1	1	50	163	7073	830	247	96.63%
		2	52	163	7349	837	290	96.20%
	2	1	52	163	7287	951	238	96.84%
		2	52	163	7370	797	309	95.98%
	3	1	52	163	7312	878	286	96.24%
		2	52	163	7400	809	267	96.52%

[1] Specificity, excluding No Calls, over all valid runs for a given DNA sample was calculated using the formula: Specificity = TN/(TN + FP), where TN = total number of wild type hotspot positions present in the reference database sequence called correctly by the Ion PGM™ Dx System, and FP = total number of wild type hotspot positions present in the reference database sequence that are not correctly called as WT by the Ion PGM™ Dx System.

Table 30a Reproducibility Results: Variant calling at different levels of pooling: NA12878

The following table confirms that the number of sample libraries pooled into a sequencing run (4 vs. 16 sample libraries) did not affect the Ion PGM™ Dx System's ability to reproducibly make the correct variant call.

Location	Variant Type	NA12878 (4 samples/run)[1]					NA12878 (16 samples/run)[2]				
		Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]	Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]
chr1_103343469_103343470_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_103355221_103355222_REF	REF	119	119	0	0	100	192	191	1	0	100
chr1_103461507_103461508_SNP	SNP	119	118	1	0	100	192	192	0	0	100
chr1_103467614_103467615_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_11194590_11194591_REF	REF	119	119	0	0	100	192	192	0	0	100
chr1_11303152_11303153_REF	REF	119	119	0	0	100	192	192	0	0	100
chr1_116243876_116243877_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_116247789_116247790_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_116247825_116247826_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_120480393_120480394_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_120509048_120509049_REF	REF	119	119	0	0	100	192	192	0	0	100
chr1_120512302_120512303_REF	REF	119	119	0	0	100	192	192	0	0	100
chr1_144852389_144852390_REF	REF	119	119	0	0	100	192	192	0	0	100
chr1_144854179_144854180_REF	REF	119	0	0	119	0	192	0	0	192	0
chr1_144866642_144866643_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_144871888_144871889_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_144874640_144874641_SNP	SNP	119	119	0	0	100	192	191	1	0	100
chr1_144879263_144879264_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_144882580_144882581_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_144882822_144882823_SNP	SNP	119	119	0	0	100	192	191	1	0	100
chr1_144886196_144886197_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_144918956_144918957_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_144921923_144921924_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_144921949_144921950_REF	REF	119	119	0	0	100	192	192	0	0	100
chr1_144922170_144922171_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_144923728_144923729_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr1_144931329_144931330_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_144931391_144931392_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_144931580_144931581_REF	REF	119	119	0	0	100	192	192	0	0	100
chr1_144931606_144931607_REF	REF	119	119	0	0	100	192	192	0	0	100
chr1_144946700_144946701_REF	REF	119	119	0	0	100	192	192	0	0	100
chr1_144994657_144994658_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_145016019_145016020_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_145021149_145021150_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_145021182_145021183_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_145075682_145075683_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_156106184_156106185_REF	REF	119	119	0	0	100	192	192	0	0	100
chr1_156107612_156107613_REF	REF	119	76	43	0	100	192	131	61	0	100
chr1_156128219_156128220_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_156834186_156834187_REF	REF	119	119	0	0	100	192	192	0	0	100
chr1_156848909_156848910_REF	REF	119	119	0	0	100	192	192	0	0	100
chr1_164761715_164761715_REF	REF	119	115	4	0	100	192	189	3	0	100
chr1_171076965_171076966_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_171085314_171085314_REF	REF	119	91	28	0	100	192	146	46	0	100
chr1_182554556_182554557_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_185069314_185069314_Indel	Indel	119	118	1	0	100	192	192	0	0	100
chr1_197325907_197325908_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_197404769_197404770_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_201335898_201335899_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_201341175_201341180_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr1_204433511_204433511_Indel	Indel	119	118	1	0	100	192	192	0	0	100
chr1_204501382_204501383_SNP	SNP	119	119	0	0	100	192	192	0	0	100

Location	Variant Type	NA12878 (4 samples/run)[1]					NA12878 (16 samples/run)[2]				
		Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]	Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]
chr1_204515862_204515863_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_206650064_206650065_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_20972304_20972318_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr1_20972373_20972374_Indel	Indel	119	116	0	3	97.48	192	190	0	2	98.96
chr1_209804094_209804095_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_215821938_215821939_REF	REF	119	119	0	0	100	192	188	4	0	100
chr1_215821970_215821971_REF	REF	119	119	0	0	100	192	191	1	0	100
chr1_216052344_216052345_REF	REF	119	119	0	0	100	192	192	0	0	100
chr1_216258250_216258251_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_226555301_226555302_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_226580020_226580021_REF	REF	119	119	0	0	100	192	192	0	0	100
chr1_227071524_227071525_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_2337276_2337277_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_2340072_2340073_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_236883420_236883421_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_236925843_236925844_REF	REF	119	119	0	0	100	192	192	0	0	100
chr1_236978992_236978992_REF	REF	119	119	0	0	100	192	192	0	0	100
chr1_237060849_237060850_REF	REF	119	119	0	0	100	192	192	0	0	100
chr1_237711796_237711797_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_237814782_237814783_SNP	SNP	119	119	0	0	100	192	190	2	0	100
chr1_237863717_237863718_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_237969637_237969638_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_41296827_41296828_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_45797504_45797505_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_6529182_6529188_REF	REF	119	61	58	0	100	192	15	177	0	100
chr1_6530965_6530965_Indel	Indel	119	9	72	38	19.15	192	25	104	63	28.41
chr1_68894730_68894730_Indel	Indel	119	116	3	0	100	192	177	14	1	99.44
chr1_94466658_94466659_SNP	SNP	119	115	4	0	100	192	191	1	0	100
chr1_94471074_94471075_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_94474327_94474328_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr1_94481504_94481505_REF	REF	119	119	0	0	100	192	192	0	0	100
chr1_94564482_94564483_REF	REF	119	119	0	0	100	192	192	0	0	100
chr1_98015290_98015291_REF	REF	119	119	0	0	100	192	192	0	0	100
chr2_112725684_112725684_Indel	Indel	119	0	1	118	0	192	0	3	189	0
chr2_112786048_112786049_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr2_113999533_113999534_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr2_141130547_141130548_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr2_141819587_141819588_REF	REF	119	119	0	0	100	192	192	0	0	100
chr2_141945948_141945949_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr2_148657116_148657117_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr2_167163042_167163043_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr2_167168092_167168093_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr2_175613272_175613273_REF	REF	119	119	0	0	100	192	192	0	0	100
chr2_175622219_175622220_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr2_178098087_178098088_Indel	Indel	119	119	0	0	100	192	191	1	0	100
chr2_182413237_182413238_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr2_182413258_182413259_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr2_182413601_182413602_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr2_189866342_189866343_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr2_189907936_189907937_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr2_189953285_189953285_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr2_191109532_191109533_REF	REF	119	119	0	0	100	192	191	1	0	100
chr2_198257794_198257795_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr2_198267770_198267770_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr2_209101905_209101906_Indel	Indel	119	0	119	0	0	192	0	192	0	0
chr2_212812096_212812097_REF	REF	119	119	0	0	100	192	191	1	0	100
chr2_216240126_216240128_Indel	Indel	119	104	0	15	87.39	192	179	0	13	93.23

Location	Variant Type	NA12878 (4 samples/run)[1]					NA12878 (16 samples/run)[2]				
		Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]	Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]
chr2_216248707_216248709_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr2_216274853_216274855_Indel	Indel	119	117	0	2	98.32	192	187	0	5	97.40
chr2_216299571_216299571_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr2_219545308_219545309_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr2_220284875_220284876_SNP	SNP	119	118	1	0	100	192	191	1	0	100
chr2_223161623_223161624_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr2_24905926_24905927_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr2_24974944_24974945_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr2_25471169_25471170_REF	REF	119	117	2	0	100	192	189	3	0	100
chr2_29416571_29416572_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr2_42510017_42510018_SNP	SNP	119	118	1	0	100	192	192	0	0	100
chr2_48030837_48030838_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr2_48032874_48032878_Indel	Indel	119	118	1	0	100	192	192	0	0	100
chr2_61760892_61760893_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr2_71795151_71795152_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr2_71829820_71829821_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr2_71838499_71838500_REF	REF	119	5	114	0	100	192	11	181	0	100
chr3_10085129_10085130_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr3_10133948_10133949_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr3_123419732_123419733_REF	REF	119	119	0	0	100	192	192	0	0	100
chr3_123453060_123453061_REF	REF	119	58	61	0	100	192	30	162	0	100
chr3_134644635_134644636_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr3_138456725_138456726_REF	REF	119	119	0	0	100	192	191	1	0	100
chr3_138665063_138665064_REF	REF	119	100	17	2	98.04	192	106	72	14	88.33
chr3_142222283_142222284_REF	REF	119	119	0	0	100	192	192	0	0	100
chr3_142277535_142277536_REF	REF	119	119	0	0	100	192	191	1	0	100
chr3_142281611_142281612_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr3_178922272_178922273_REF	REF	119	119	0	0	100	192	192	0	0	100
chr3_178922273_178922274_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr3_180366066_180366067_REF	REF	119	119	0	0	100	192	192	0	0	100
chr3_188326954_188326955_REF	REF	119	119	0	0	100	192	191	1	0	100
chr3_30733401_30733403_Indel	Indel	119	119	0	0	100	192	173	15	4	97.74
chr3_3192524_3192524_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr3_32188248_32188249_REF	REF	119	119	0	0	100	192	192	0	0	100
chr3_37695191_37695192_REF	REF	119	119	0	0	100	192	192	0	0	100
chr3_38592405_38592406_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr3_46501284_46501284_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr3_47125384_47125385_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr3_47163342_47163343_REF	REF	119	119	0	0	100	192	192	0	0	100
chr3_52584431_52584431_REF	REF	119	119	0	0	100	192	192	0	0	100
chr3_52584714_52584715_REF	REF	119	119	0	0	100	192	191	1	0	100
chr3_52662849_52662850_REF	REF	119	119	0	0	100	192	192	0	0	100
chr3_63973739_63973740_REF	REF	119	119	0	0	100	192	192	0	0	100
chr3_63981634_63981635_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr3_63982219_63982220_REF	REF	119	119	0	0	100	192	190	2	0	100
chr3_65361369_65361373_Indel	Indel	119	69	0	50	58	192	110	0	82	57.29
chr3_81627174_81627175_REF	REF	119	119	0	0	100	192	192	0	0	100
chr4_103533630_103533631_REF	REF	119	119	0	0	100	192	192	0	0	100
chr4_103534559_103534560_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr4_106154990_106154998_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr4_106155750_106155751_REF	REF	119	119	0	0	100	192	191	1	0	100
chr4_106190731_106190732_REF	REF	119	119	0	0	100	192	192	0	0	100
chr4_114117474_114117474_REF	REF	119	109	10	0	100	192	179	13	0	100
chr4_146576417_146576418_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr4_16037351_16037352_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr4_2834813_2834814_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr4_55151958_55151958_Indel	Indel	119	119	0	0	100	192	192	0	0	100

Location	Variant Type	NA12878 (4 samples/run)[1]					NA12878 (16 samples/run)[2]				
		Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]	Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]
chr4_87967234_87967235_REF	REF	119	119	0	0	100	192	192	0	0	100
chr4_88056719_88056720_Indel	Indel	119	53	66	0	100	192	83	109	0	100
chr4_88959380_88959381_REF	REF	119	119	0	0	100	192	192	0	0	100
chr4_90743317_90743317_REF	REF	119	113	6	0	100	192	184	8	0	100
chr4_995304_995305_REF	REF	119	119	0	0	100	192	189	3	0	100
chr5_112162853_112162854_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr5_112175769_112175770_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr5_131915212_131915213_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr5_13811774_13811775_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr5_13829607_13829608_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr5_13829610_13829611_REF	REF	119	119	0	0	100	192	192	0	0	100
chr5_13864727_13864728_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr5_13871053_13871054_REF	REF	119	119	0	0	100	192	192	0	0	100
chr5_149435607_149435608_SNP	SNP	119	119	0	0	100	192	191	1	0	100
chr5_149441016_149441017_Indel	Indel	119	0	119	0	0	192	0	192	0	0
chr5_149456994_149456995_REF	REF	119	119	0	0	100	192	192	0	0	100
chr5_149460552_149460553_SNP	SNP	119	111	8	0	100	192	186	6	0	100
chr5_149749125_149749126_REF	REF	119	119	0	0	100	192	192	0	0	100
chr5_149776231_149776232_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr5_156190172_156190172_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr5_176517460_176517461_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr5_176517796_176517797_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr5_176522727_176522728_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr5_176636881_176636882_REF	REF	119	119	0	0	100	192	192	0	0	100
chr5_180045700_180045700_Indel	Indel	119	115	0	4	97	192	181	0	11	94.27
chr5_180046492_180046494_REF	REF	119	0	119	0	0	192	0	189	3	0
chr5_180047739_180047739_Indel	Indel	119	119	0	0	100	192	191	1	0	100
chr5_180052945_180052946_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr5_35873604_35873605_REF	REF	119	119	0	0	100	192	192	0	0	100
chr5_35874574_35874575_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr5_35876273_35876274_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr5_38499763_38499764_REF	REF	119	119	0	0	100	192	192	0	0	100
chr5_38528951_38528953_Indel	Indel	119	4	1	114	3	192	0	2	190	0
chr5_55250726_55250727_REF	REF	119	119	0	0	100	192	191	1	0	100
chr5_55251930_55251931_REF	REF	119	119	0	0	100	192	192	0	0	100
chr5_7870972_7870973_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_116950733_116950734_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_116951505_116951507_REF	REF	119	119	0	0	100	192	190	2	0	100
chr6_117686699_117686701_REF	REF	119	119	0	0	100	192	189	3	0	100
chr6_117708970_117708971_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_117730818_117730819_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_135515447_135515448_REF	REF	119	119	0	0	100	192	192	0	0	100
chr6_135518107_135518108_REF	REF	119	119	0	0	100	192	192	0	0	100
chr6_135521488_135521489_REF	REF	119	119	0	0	100	192	192	0	0	100
chr6_138195961_138195964_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr6_143792605_143792606_REF	REF	119	119	0	0	100	192	192	0	0	100
chr6_143792674_143792675_SNP	SNP	119	119	0	0	100	192	188	4	0	100
chr6_152453290_152453291_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_152461048_152461052_REF	REF	119	118	1	0	100	192	192	0	0	100
chr6_152469330_152469331_REF	REF	119	119	0	0	100	192	192	0	0	100
chr6_152539572_152539573_Indel	Indel	119	0	119	0	0	192	0	192	0	0
chr6_152557345_152557346_REF	REF	119	119	0	0	100	192	192	0	0	100
chr6_152660309_152660310_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_152671474_152671475_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_152711394_152711395_REF	REF	119	119	0	0	100	192	191	1	0	100
chr6_152793375_152793376_Indel	Indel	119	93	26	0	100	192	147	45	0	100
chr6_152826296_152826297_REF	REF	119	119	0	0	100	192	192	0	0	100

Location	Variant Type	NA12878 (4 samples/run)[1]					NA12878 (16 samples/run)[2]				
		Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]	Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]
chr6_16327329_16327330_REF	REF	119	119	0	0	100	192	192	0	0	100
chr6_167271710_167271711_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_167271715_167271716_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_31978686_31978687_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_31978733_31978734_REF	REF	119	0	119	0	0	192	0	192	0	0
chr6_31978745_31978746_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_32032742_32032743_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_32064725_32064726_SNP	SNP	119	119	0	0	100	192	191	1	0	100
chr6_32164868_32164869_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_32164873_32164874_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_32188296_32188297_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_33132313_33132314_REF	REF	119	60	59	0	100	192	0	186	6	0
chr6_33134799_33134800_REF	REF	119	119	0	0	100	192	191	1	0	100
chr6_33136637_33136638_SNP	SNP	119	119	0	0	100	192	191	1	0	100
chr6_33139327_33139328_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_33141160_33141161_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_33145368_33145369_SNP	SNP	119	99	20	0	100	192	155	37	0	100
chr6_41565471_41565472_REF	REF	119	119	0	0	100	192	191	1	0	100
chr6_49425520_49425521_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_51618169_51618170_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_51776534_51776535_REF	REF	119	119	0	0	100	192	192	0	0	100
chr6_51875249_51875250_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_51891010_51891011_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_51921613_51921614_REF	REF	119	119	0	0	100	192	192	0	0	100
chr6_51935746_51935747_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_56373535_56373536_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_56463409_56463410_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_65016977_65016979_Indel	Indel	119	19	100	0	100	192	42	149	1	97.67
chr6_65098767_65098768_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_65301503_65301504_REF	REF	119	119	0	0	100	192	192	0	0	100
chr6_65611952_65611952_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr6_66005856_66005857_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_66005887_66005888_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_69666683_69666684_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_70071172_70071173_SNP	SNP	119	119	0	0	100	192	191	1	0	100
chr6_70950281_70950283_Indel	Indel	119	19	99	1	95	192	43	133	16	72.88
chr6_70965124_70965125_SNP	SNP	119	112	7	0	100	192	192	0	0	100
chr6_70990669_70990670_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr6_91226285_91226286_REF	REF	119	119	0	0	100	192	192	0	0	100
chr6_91266349_91266349_REF	REF	119	0	119	0	0	192	0	192	0	0
chr6_93964318_93964319_REF	REF	119	119	0	0	100	192	192	0	0	100
chr7_100411277_100411278_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr7_106508977_106508978_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr7_106508986_106508987_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr7_107353127_107353128_REF	REF	119	119	0	0	100	192	192	0	0	100
chr7_124486979_124486980_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr7_124492037_124492038_REF	REF	119	119	0	0	100	192	191	1	0	100
chr7_126079143_126079144_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr7_126410121_126410122_REF	REF	119	81	38	0	100	192	2	187	3	40.00
chr7_126410170_126410171_SNP	SNP	119	115	4	0	100	192	114	74	4	96.61
chr7_128034628_128034629_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr7_142566684_142566684_REF	REF	119	119	0	0	100	192	192	0	0	100
chr7_143016927_143016928_REF	REF	119	119	0	0	100	192	192	0	0	100
chr7_143036547_143036548_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr7_143043239_143043240_SNP	SNP	119	119	0	0	100	192	190	2	0	100
chr7_148504716_148504717_REF	REF	119	0	119	0	0	192	0	192	0	0
chr7_148543524_148543525_SNP	SNP	119	119	0	0	100	192	192	0	0	100

Location	Variant Type	NA12878 (4 samples/run)[1]					NA12878 (16 samples/run)[2]				
		Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]	Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]
chr7_150644427_150644428_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr7_150648671_150648672_REF	REF	119	119	0	0	100	192	192	0	0	100
chr7_150654622_150654622_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr7_151873852_151873853_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr7_151874497_151874498_REF	REF	119	119	0	0	100	192	192	0	0	100
chr7_151896573_151896573_REF	REF	119	119	0	0	100	192	191	1	0	100
chr7_151949067_151949068_REF	REF	119	119	0	0	100	192	192	0	0	100
chr7_21582916_21582917_SNP	SNP	119	45	74	0	100	192	175	14	3	98.31
chr7_21582962_21582963_SNP	SNP	119	119	0	0	100	192	189	3	0	100
chr7_21582963_21582964_SNP	SNP	119	119	0	0	100	192	189	3	0	100
chr7_21640617_21640618_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr7_21659554_21659554_Indel	Indel	119	119	0	0	100	192	189	1	2	98.95
chr7_21723572_21723573_REF	REF	119	112	7	0	100	192	188	4	0	100
chr7_21778448_21778449_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr7_21784431_21784432_REF	REF	119	119	0	0	100	192	192	0	0	100
chr7_2962292_2962293_REF	REF	119	119	0	0	100	192	192	0	0	100
chr7_2962752_2962753_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr7_33148711_33148712_SNP	SNP	119	109	9	1	99	192	142	32	18	88.75
chr7_37904065_37904068_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr7_37916454_37916455_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr7_55228052_55228053_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr7_55266416_55266417_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr7_73472049_73472050_SNP	SNP	119	114	5	0	100	192	182	10	0	100
chr7_91632305_91632306_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr7_91652178_91652178_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr7_91695716_91695717_REF	REF	119	119	0	0	100	192	192	0	0	100
chr7_91712697_91712698_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr7_91713971_91713972_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr7_91714910_91714911_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr7_91726926_91726927_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr7_92146686_92146687_REF	REF	119	119	0	0	100	192	191	1	0	100
chr7_92244630_92244635_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr7_94047160_94047161_REF	REF	119	119	0	0	100	192	192	0	0	100
chr7_98534946_98534948_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr8_113364695_113364696_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr8_114327081_114327084_REF	REF	119	119	0	0	100	192	191	1	0	100
chr8_11614574_11614575_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr8_118819577_118819578_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr8_126056215_126056215_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr8_144992075_144992076_REF	REF	119	119	0	0	100	192	189	3	0	100
chr8_144992102_144992103_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr8_144995493_144995494_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr8_144996407_144996408_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr8_144998168_144998169_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr8_144998189_144998190_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr8_145000055_145000056_SNP	SNP	119	119	0	0	100	192	191	1	0	100
chr8_145005688_145005688_Indel	Indel	119	54	65	0	100	192	68	124	0	100
chr8_145008341_145008342_SNP	SNP	119	117	2	0	100	192	189	2	1	99.47
chr8_21979102_21979103_REF	REF	119	119	0	0	100	192	192	0	0	100
chr8_30925750_30925751_SNP	SNP	119	85	24	10	89.47	192	150	33	9	94.34
chr8_30977716_30977717_REF	REF	119	119	0	0	100	192	192	0	0	100
chr8_30999122_30999123_REF	REF	119	119	0	0	100	192	191	1	0	100
chr8_31005017_31005017_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr8_48805816_48805816_Indel	Indel	119	31	85	3	91.18	192	74	114	4	94.87
chr8_90967710_90967711_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr8_90970861_90970862_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr8_90970934_90970935_SNP	SNP	119	119	0	0	100	192	192	0	0	100

Location	Variant Type	NA12878 (4 samples/run)[1]					NA12878 (16 samples/run)[2]				
		Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]	Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]
chr9_111685078_111685078_REF	REF	119	119	0	0	100	192	192	0	0	100
chr9_130587052_130587052_Indel	Indel	119	95	24	0	100	192	176	16	0	100
chr9_135786903_135786904_REF	REF	119	119	0	0	100	192	192	0	0	100
chr9_137659235_137659236_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr9_137688780_137688800_Indel	Indel	119	116	0	3	97.48	192	183	2	7	96.32
chr9_15479634_15479635_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr9_34485236_34485237_REF	REF	119	119	0	0	100	192	192	0	0	100
chr9_5081779_5081780_REF	REF	119	119	0	0	100	192	191	1	0	100
chr9_5090640_5090641_REF	REF	119	119	0	0	100	192	192	0	0	100
chr9_71650751_71650752_SNP	SNP	119	119	0	0	100	192	188	4	0	100
chr9_8331574_8331574_Indel	Indel	119	0	119	0	0	192	2	190	0	100
chr9_8338847_8338849_Indel	Indel	119	0	0	119	0	192	1	0	191	0.52
chr9_8460590_8460591_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr9_8484397_8484398_Indel	Indel	119	0	119	0	0	192	0	192	0	0
chr9_93639848_93639849_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr9_94486320_94486321_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr9_97934366_97934367_REF	REF	119	119	0	0	100	192	192	0	0	100
chr9_98229388_98229389_REF	REF	119	119	0	0	100	192	192	0	0	100
chr9_98232223_98232224_Indel	Indel	119	61	57	1	98	192	136	56	0	100
chr10_104157710_104157711_REF	REF	119	119	0	0	100	192	192	0	0	100
chr10_104157726_104157727_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr10_121429761_121429762_SNP	SNP	119	118	0	1	99	192	190	0	2	98.96
chr10_123243196_123243197_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr10_123310870_123310871_REF	REF	119	119	0	0	100	192	192	0	0	100
chr10_27294449_27294451_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr10_3818449_3818449_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr10_3820113_3820113_Indel	Indel	119	0	2	117	0	192	0	1	191	0
chr10_51568377_51568378_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr10_51582893_51582894_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr10_55591312_55591313_SNP	SNP	119	119	0	0	100	192	191	1	0	100
chr10_55755490_55755491_REF	REF	119	119	0	0	100	192	192	0	0	100
chr10_55826469_55826470_REF	REF	119	118	1	0	100	192	102	90	0	100
chr10_55892600_55892602_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr10_55943183_55943184_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr10_55955484_55955485_REF	REF	119	119	0	0	100	192	192	0	0	100
chr10_56423967_56423968_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr10_70405236_70405237_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr10_73544637_73544638_SNP	SNP	119	117	2	0	100	192	191	1	0	100
chr10_73553176_73553177_REF	REF	119	119	0	0	100	192	192	0	0	100
chr10_73558851_73558852_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr10_73558885_73558886_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr10_73558951_73558952_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr10_88441479_88441480_REF	REF	119	119	0	0	100	192	192	0	0	100
chr10_88683121_88683122_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr11_108121445_108121446_REF	REF	119	119	0	0	100	192	188	4	0	100
chr11_108139106_108139112_REF	REF	119	119	0	0	100	192	192	0	0	100
chr11_108150207_108150208_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr11_108151707_108151707_Indel	Indel	119	105	11	3	97.22	192	99	79	14	87.61
chr11_119148572_119148573_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr11_125497465_125497466_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr11_125525194_125525195_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr11_128675291_128675292_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr11_17522637_17522638_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr11_17537704_17537705_REF	REF	119	119	0	0	100	192	192	0	0	100
chr11_2592530_2592531_REF	REF	119	119	0	0	100	192	192	0	0	100
chr11_36595599_36595600_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr11_4123344_4123345_SNP	SNP	119	119	0	0	100	192	192	0	0	100

Location	Variant Type	NA12878 (4 samples/run)[1]					NA12878 (16 samples/run)[2]				
		Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]	Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]
chr11_4141131_4141132_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr11_4159456_4159457_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr11_4159465_4159466_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr11_44151576_44151577_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr11_47260318_47260319_REF	REF	119	119	0	0	100	192	192	0	0	100
chr11_47364761_47364762_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr11_47371484_47371485_Indel	Indel	119	107	12	0	100	192	180	12	0	100
chr11_47463483_47463484_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr11_69462909_69462910_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr11_71728908_71728914_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr11_76867902_76867903_REF	REF	119	111	8	0	100	192	19	173	0	100
chr11_76894234_76894235_SNP	SNP	119	107	12	0	100	192	183	8	1	99.46
chr11_76895770_76895792_REF	REF	119	0	17	102	0	192	0	21	171	0
chr11_76901272_76901273_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr11_76901623_76901623_Indel	Indel	119	47	70	2	96	192	4	157	31	11.43
chr11_76910765_76910766_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr11_76917219_76917220_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr11_76919467_76919468_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr11_76919477_76919478_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr11_89017960_89017961_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr12_103234214_103234215_REF	REF	119	119	0	0	100	192	192	0	0	100
chr12_103234251_103234252_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr12_103306549_103306550_REF	REF	119	119	0	0	100	192	192	0	0	100
chr12_111351029_111351030_Indel	Indel	119	73	46	0	100	192	111	81	0	100
chr12_111962923_111962924_REF	REF	119	119	0	0	100	192	192	0	0	100
chr12_112919868_112919869_REF	REF	119	119	0	0	100	192	192	0	0	100
chr12_121431271_121431272_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr12_22063736_22063737_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr12_22063748_22063749_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr12_25358662_25358664_Indel	Indel	119	13	103	3	81	192	33	151	8	80.49
chr12_25362776_25362777_REF	REF	119	119	0	0	100	192	191	1	0	100
chr12_2694637_2694638_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr12_2760965_2760966_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr12_2794790_2794791_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr12_33021933_33021934_REF	REF	119	119	0	0	100	192	192	0	0	100
chr12_40645256_40645257_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr12_40681142_40681142_REF	REF	119	97	22	0	100	192	169	23	0	100
chr12_40757114_40757114_Indel	Indel	119	94	13	12	88.68	192	162	1	29	84.82
chr12_48374615_48374616_REF	REF	119	119	0	0	100	192	191	1	0	100
chr12_49424665_49424665_REF	REF	119	57	62	0	100	192	91	101	0	100
chr12_56435928_56435929_SNP	SNP	119	117	2	0	100	192	190	2	0	100
chr12_56437777_56437777_REF	REF	119	119	0	0	100	192	191	1	0	100
chr12_7362203_7362204_REF	REF	119	0	22	97	0	192	0	105	87	0
chr13_103518035_103518036_REF	REF	119	119	0	0	100	192	192	0	0	100
chr13_103527848_103527849_SNP	SNP	119	111	8	0	100	192	182	9	1	99.45
chr13_103527929_103527930_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr13_110833701_110833702_REF	REF	119	119	0	0	100	192	192	0	0	100
chr13_110858991_110858992_REF	REF	119	119	0	0	100	192	192	0	0	100
chr13_110864181_110864182_REF	REF	119	119	0	0	100	192	176	16	0	100
chr13_28585649_28585650_Indel	Indel	119	118	1	0	100	192	192	0	0	100
chr13_28597447_28597449_Indel	Indel	119	112	0	7	94.12	192	177	1	14	92.67
chr13_28608458_28608459_REF	REF	119	119	0	0	100	192	191	1	0	100
chr13_28610044_28610045_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr13_28611381_28611382_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr13_28623698_28623699_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr13_28623758_28623759_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr13_48947468_48947469_SNP	SNP	119	119	0	0	100	192	192	0	0	100

Location	Variant Type	NA12878 (4 samples/run)[1]					NA12878 (16 samples/run)[2]				
		Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]	Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]
chr13_49033746_49033747_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr14_21778717_21778720_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr14_21792810_21792811_REF	REF	119	119	0	0	100	192	192	0	0	100
chr14_23855425_23855426_REF	REF	119	119	0	0	100	192	190	2	0	100
chr14_23865884_23865885_REF	REF	119	119	0	0	100	192	192	0	0	100
chr14_23866712_23866713_REF	REF	119	119	0	0	100	192	191	1	0	100
chr14_23888323_23888323_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr14_51062236_51062238_Indel	Indel	119	107	0	12	89.92	192	157	0	35	81.77
chr14_51094701_51094702_REF	REF	119	119	0	0	100	192	192	0	0	100
chr14_51204995_51204996_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr14_56139313_56139314_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr14_62164607_62164608_REF	REF	119	101	18	0	100	192	166	26	0	100
chr14_62194238_62194239_REF	REF	119	119	0	0	100	192	192	0	0	100
chr14_81534708_81534709_REF	REF	119	119	0	0	100	192	192	0	0	100
chr14_81574815_81574816_REF	REF	119	119	0	0	100	192	192	0	0	100
chr14_81610582_81610583_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr14_88852279_88852280_Indel	Indel	119	119	0	0	100	192	191	1	0	100
chr14_88894094_88894095_REF	REF	119	119	0	0	100	192	191	1	0	100
chr14_95572110_95572111_REF	REF	119	119	0	0	100	192	192	0	0	100
chr15_28235772_28235773_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr15_39880821_39880822_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr15_39886429_39886430_REF	REF	119	119	0	0	100	192	192	0	0	100
chr15_40457238_40457238_REF	REF	119	103	16	0	100	192	154	38	0	100
chr15_40476131_40476131_REF	REF	119	113	6	0	100	192	174	18	0	100
chr15_40494951_40494952_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr15_40494959_40494960_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr15_40498502_40498503_REF	REF	119	119	0	0	100	192	191	1	0	100
chr15_40898642_40898643_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr15_40914771_40914772_REF	REF	119	119	0	0	100	192	191	1	0	100
chr15_40915189_40915190_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr15_40915893_40915894_REF	REF	119	119	0	0	100	192	192	0	0	100
chr15_40949525_40949526_REF	REF	119	119	0	0	100	192	191	1	0	100
chr15_40954369_40954370_REF	REF	119	119	0	0	100	192	191	1	0	100
chr15_41797573_41797573_REF	REF	119	0	118	1	0	192	0	192	0	0
chr15_43571389_43571390_SNP	SNP	119	7	112	0	100	192	4	187	1	80.00
chr15_48703129_48703130_REF	REF	119	119	0	0	100	192	192	0	0	100
chr15_48719700_48719701_REF	REF	119	119	0	0	100	192	192	0	0	100
chr15_48722883_48722884_REF	REF	119	119	0	0	100	192	192	0	0	100
chr15_48759993_48759994_REF	REF	119	114	5	0	100	192	183	9	0	100
chr15_48787842_48787843_REF	REF	119	119	0	0	100	192	192	0	0	100
chr15_48797306_48797307_REF	REF	119	119	0	0	100	192	192	0	0	100
chr15_48805937_48805938_REF	REF	119	119	0	0	100	192	192	0	0	100
chr15_67457334_67457335_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr15_72105928_72105929_Indel	Indel	119	118	1	0	100	192	191	1	0	100
chr15_72639002_72639003_REF	REF	119	119	0	0	100	192	192	0	0	100
chr15_73614702_73614704_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr15_73614833_73614834_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr15_74336632_74336633_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr15_89859932_89859932_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr15_89864316_89864316_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr15_89869768_89869768_Indel	Indel	119	118	1	0	100	192	192	0	0	100
chr15_91337504_91337505_REF	REF	119	119	0	0	100	192	192	0	0	100
chr15_91358271_91358271_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr15_99482380_99482380_REF	REF	119	0	119	0	0	192	0	192	0	0
chr16_14026006_14026007_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr16_15811022_15811023_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr16_15811061_15811062_SNP	SNP	119	119	0	0	100	192	192	0	0	100

Location	Variant Type	NA12878 (4 samples/run)[1]					NA12878 (16 samples/run)[2]				
		Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]	Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]
chr16_15818140_15818141_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr16_15818492_15818493_Indel	Indel	119	0	118	1	0	192	0	189	3	0
chr16_15865471_15865472_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr16_27456612_27456613_REF	REF	119	116	3	0	100	192	187	5	0	100
chr16_57946936_57946937_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr16_57946972_57946973_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr16_89806346_89806347_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr16_89831509_89831510_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr16_89836322_89836323_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr16_89849479_89849480_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr16_89866042_89866043_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr16_89869703_89869704_REF	REF	119	119	0	0	100	192	191	1	0	100
chr16_89869760_89869761_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr16_89874873_89874874_REF	REF	119	119	0	0	100	192	192	0	0	100
chr16_89880821_89880822_REF	REF	119	119	0	0	100	192	191	1	0	100
chr17_11651056_11651057_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr17_11687520_11687521_REF	REF	119	119	0	0	100	192	192	0	0	100
chr17_11697049_11697049_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr17_11778239_11778240_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr17_15133705_15133707_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr17_1554635_1554636_REF	REF	119	118	1	0	100	192	192	0	0	100
chr17_1578645_1578646_REF	REF	119	119	0	0	100	192	192	0	0	100
chr17_1580830_1580831_REF	REF	119	119	0	0	100	192	192	0	0	100
chr17_1585129_1585130_REF	REF	119	119	0	0	100	192	192	0	0	100
chr17_29528561_29528562_REF	REF	119	119	0	0	100	192	191	1	0	100
chr17_29559931_29559932_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr17_29563073_29563073_Indel	Indel	119	22	37	60	26.83	192	26	51	115	18.44
chr17_37657460_37657460_REF	REF	119	0	119	0	0	192	0	192	0	0
chr17_37816460_37816461_REF	REF	119	119	0	0	100	192	192	0	0	100
chr17_37830899_37830900_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr17_39742491_39742492_REF	REF	119	119	0	0	100	192	192	0	0	100
chr17_39923613_39923614_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr17_45360888_45360889_REF	REF	119	119	0	0	100	192	192	0	0	100
chr17_48263902_48263903_REF	REF	119	119	0	0	100	192	191	1	0	100
chr17_48265425_48265426_SNP	SNP	119	111	8	0	100	192	165	27	0	100
chr17_48265494_48265495_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr17_48268222_48268223_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr17_48269301_48269302_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr17_48275181_48275182_REF	REF	119	21	98	0	100	192	44	148	0	100
chr17_48276970_48276971_SNP	SNP	119	61	53	5	92.42	192	154	24	14	91.67
chr17_5288607_5288608_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr17_53342796_53342796_Indel	Indel	119	118	1	0	100	192	192	0	0	100
chr17_56688511_56688512_Indel	Indel	119	117	2	0	100	192	190	2	0	100
chr17_59760995_59760996_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr17_59763346_59763347_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr17_6331802_6331803_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr17_6331835_6331836_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr17_66519857_66519857_REF	REF	119	103	16	0	100	192	178	14	0	100
chr17_72306116_72306118_Indel	Indel	119	119	0	0	100	192	192	0	0	100
chr17_7348763_7348764_REF	REF	119	108	11	0	100	192	83	109	0	100
chr17_73720899_73720900_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr17_7579643_7579659_Indel	Indel	119	33	20	66	33.33	192	58	26	108	34.94
chr17_78014020_78014021_REF	REF	119	119	0	0	100	192	192	0	0	100
chr17_78055756_78055757_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr17_78055757_78055758_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr17_78060108_78060109_REF	REF	119	119	0	0	100	192	192	0	0	100
chr17_78071051_78071052_SNP	SNP	119	119	0	0	100	192	192	0	0	100

Location	Variant Type	NA12878 (4 samples/run)[1]					NA12878 (16 samples/run)[2]				
		Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]	Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]
chr17_78073561_78073562_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr17_78073588_78073589_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr17_78078708_78078709_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr17_78081706_78081707_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr17_78084768_78084769_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr17_78302156_78302157_REF	REF	119	118	1	0	100	192	192	0	0	100
chr17_78319135_78319136_REF	REF	119	119	0	0	100	192	192	0	0	100
chr17_78327357_78327358_REF	REF	119	119	0	0	100	192	192	0	0	100
chr18_21119290_21119291_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr18_28666526_28666526_Indel	Indel	119	118	1	0	100	192	192	0	0	100
chr18_28669664_28669668_Indel	Indel	119	119	0	0	100	192	190	2	0	100
chr18_29104563_29104564_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr18_50831932_50831933_REF	REF	119	119	0	0	100	192	192	0	0	100
chr18_59166540_59166541_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr18_77477514_77477515_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr19_1619749_1619749_Indel	Indel	119	4	115	0	100	192	8	184	0	100
chr19_16593330_16593331_REF	REF	119	119	0	0	100	192	192	0	0	100
chr19_17937757_17937758_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr19_18876367_18876368_REF	REF	119	117	2	0	100	192	184	8	0	100
chr19_30303508_30303508_Indel	Indel	119	36	81	2	95	192	96	83	13	88.07
chr19_3113516_3113531_REF	REF	119	116	3	0	100	192	139	52	1	99.29
chr19_35523611_35523612_REF	REF	119	114	5	0	100	192	141	51	0	100
chr19_38939407_38939408_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr19_38968558_38968559_REF	REF	119	100	19	0	100	192	36	156	0	100
chr19_38973588_38973589_REF	REF	119	119	0	0	100	192	192	0	0	100
chr19_38995437_38995438_REF	REF	119	119	0	0	100	192	191	1	0	100
chr19_38996989_38996990_REF	REF	119	119	0	0	100	192	190	2	0	100
chr19_38997364_38997364_REF	REF	119	119	0	0	100	192	192	0	0	100
chr19_38997458_38997459_REF	REF	119	119	0	0	100	192	192	0	0	100
chr19_39034584_39034585_REF	REF	119	39	80	0	100	192	51	141	0	100
chr19_39057615_39057616_REF	REF	119	118	1	0	100	192	192	0	0	100
chr19_41928867_41928867_Indel	Indel	119	4	115	0	100	192	1	191	0	100
chr19_41930395_41930396_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr19_45864924_45864924_REF	REF	119	0	119	0	0	192	0	192	0	0
chr19_45922322_45922323_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr19_55644324_55644325_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr19_55653296_55653297_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr19_55667646_55667647_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr20_31019360_31019361_Indel	Indel	119	35	80	4	89.74	192	62	128	2	96.88
chr20_31022765_31022766_REF	REF	119	119	0	0	100	192	192	0	0	100
chr20_32000482_32000483_REF	REF	119	119	0	0	100	192	192	0	0	100
chr20_41306599_41306600_REF	REF	119	119	0	0	100	192	192	0	0	100
chr20_43252914_43252915_REF	REF	119	119	0	0	100	192	192	0	0	100
chr20_50407161_50407162_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr20_50408481_50408482_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr21_35821820_35821821_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr21_45707515_45707516_REF	REF	119	119	0	0	100	192	185	7	0	100
chr21_45717549_45717550_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr21_46313441_46313442_REF	REF	119	119	0	0	100	192	192	0	0	100
chr22_24158898_24158899_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr22_36688177_36688178_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr22_36710182_36710183_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chr22_39636944_39636948_Indel	Indel	119	98	21	0	100	192	159	29	4	97.55
chr22_41574086_41574087_REF	REF	119	119	0	0	100	192	192	0	0	100
chr22_42526548_42526549_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chrX_107829906_107829907_REF	REF	119	119	0	0	100	192	191	1	0	100
chrX_107929336_107929337_REF	REF	119	119	0	0	100	192	192	0	0	100

Location	Variant Type	NA12878 (4 samples/run)[1]					NA12878 (16 samples/run)[2]				
		Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]	Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]
chrX_153760143_153760146_REF	REF	119	0	119	0	0	192	0	192	0	0
chrX_154065842_154065843_REF	REF	119	119	0	0	100	192	192	0	0	100
chrX_154158284_154158285_REF	REF	119	119	0	0	100	192	192	0	0	100
chrX_32305879_32305879_Indel	Indel	119	103	0	16	86.55	192	155	1	36	81.15
chrX_32583739_32583740_REF	REF	119	119	0	0	100	192	192	0	0	100
chrX_33357271_33357272_REF	REF	119	119	0	0	100	192	192	0	0	100
chrX_44833840_44833841_SNP	SNP	119	99	20	0	100	192	146	46	0	100
chrX_44966794_44966795_REF	REF	119	119	0	0	100	192	192	0	0	100
chrX_44966805_44966806_REF	REF	119	113	6	0	100	192	184	8	0	100
chrX_70595131_70595132_REF	REF	119	119	0	0	100	192	191	1	0	100
chrX_70613072_70613073_REF	REF	119	114	5	0	100	192	154	38	0	100
chrX_70627476_70627477_REF	REF	119	119	0	0	100	192	192	0	0	100
chrX_77298856_77298857_SNP	SNP	119	119	0	0	100	192	192	0	0	100
chrX_79283508_79283509_REF	REF	119	119	0	0	100	192	192	0	0	100

[1] 60 sequencing runs in total

[2] 24 sequencing runs in total

[3] % positive agreement = (correct calls/total calls [excluding no calls]) × 100

Table 30b Reproducibility Results: Variant calling at different levels of pooling: NA19240

The following table confirms that the number of sample libraries pooled into a sequencing run (4 vs. 16 sample libraries) did not affect the Ion PGM™ Dx System's ability to reproducibly make the correct variant call.

Location	Variant Type	NA19240 (4 samples/run)[1]					NA19240 (16 samples/run)[2]				
		Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]	Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]
chr1_103343469_103343470_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_103355221_103355222_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_103461507_103461508_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_103467614_103467615_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_11194590_11194591_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_11303152_11303153_SNP	SNP	119	118	1	0	100	191	188	3	0	100
chr1_116243876_116243877_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_116247789_116247790_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_116247825_116247826_REF	REF	119	119	0	0	100	191	191	0	0	100
chr1_120480393_120480394_SNP	SNP	119	119	0	0	100	191	190	1	0	100
chr1_120509048_120509049_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_120512302_120512303_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_144852389_144852390_SNP	SNP	119	0	0	119	0	191	0	0	191	0
chr1_144854179_144854180_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_144866642_144866643_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_144871888_144871889_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_144874640_144874641_SNP	SNP	119	119	0	0	100	191	190	1	0	100
chr1_144879263_144879264_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_144882580_144882581_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_144882822_144882823_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_144886196_144886197_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_144918956_144918957_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_144921923_144921924_REF	REF	119	119	0	0	100	191	191	0	0	100
chr1_144921949_144921950_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_144922170_144922171_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_144923728_144923729_REF	REF	119	107	12	0	100	191	162	29	0	100
chr1_144931329_144931330_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_144931391_144931392_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_144931580_144931581_SNP	SNP	119	118	1	0	100	191	188	3	0	100
chr1_144931606_144931607_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_144946700_144946701_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_144994657_144994658_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_145016019_145016020_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_145021149_145021150_SNP	SNP	119	93	26	0	100	191	168	23	0	100
chr1_145021182_145021183_SNP	SNP	119	97	22	0	100	191	152	39	0	100
chr1_145075682_145075683_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_156106184_156106185_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_156107612_156107613_Indel	Indel	119	38	81	0	100	191	101	90	0	100
chr1_156128219_156128220_REF	REF	119	119	0	0	100	191	191	0	0	100
chr1_156834186_156834187_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_156848909_156848910_Indel	Indel	119	0	0	119	0	191	0	0	191	0
chr1_164761715_164761715_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr1_171076965_171076966_SNP	SNP	119	119	0	0	100	191	190	1	0	100
chr1_171085314_171085314_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr1_182554556_182554557_REF	REF	119	117	2	0	100	191	190	1	0	100
chr1_185069314_185069314_REF	REF	119	65	54	0	100	191	93	98	0	100
chr1_197325907_197325908_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_197404769_197404770_REF	REF	119	119	0	0	100	191	191	0	0	100
chr1_201335898_201335899_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_201341175_201341180_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr1_204433511_204433511_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr1_204501382_204501383_REF	REF	119	119	0	0	100	191	191	0	0	100

Location	Variant Type	NA19240 (4 samples/run)[1]					NA19240 (16 samples/run)[2]				
		Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]	Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]
chr1_204515862_204515863_REF	REF	119	119	0	0	100	191	191	0	0	100
chr1_206650064_206650065_REF	REF	119	119	0	0	100	191	191	0	0	100
chr1_20972304_20972318_REF	REF	119	119	0	0	100	191	191	0	0	100
chr1_20972373_20972374_REF	REF	119	87	32	0	100	191	123	68	0	100
chr1_209804094_209804095_SNP	SNP	119	119	0	0	100	191	189	2	0	100
chr1_215821938_215821939_SNP	SNP	119	115	4	0	100	191	190	1	0	100
chr1_215821970_215821971_SNP	SNP	119	116	3	0	100	191	191	0	0	100
chr1_216052344_216052345_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_216258250_216258251_REF	REF	119	119	0	0	100	191	191	0	0	100
chr1_226555301_226555302_REF	REF	119	119	0	0	100	191	191	0	0	100
chr1_226580020_226580021_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_227071524_227071525_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_2337276_2337277_REF	REF	119	119	0	0	100	191	191	0	0	100
chr1_2340072_2340073_REF	REF	119	119	0	0	100	191	191	0	0	100
chr1_236883420_236883421_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_236925843_236925844_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_236978992_236978992_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr1_237060849_237060850_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_237711796_237711797_REF	REF	119	119	0	0	100	191	191	0	0	100
chr1_237814782_237814783_SNP	SNP	119	119	0	0	100	191	190	1	0	100
chr1_237863717_237863718_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_237969637_237969638_REF	REF	119	119	0	0	100	191	190	1	0	100
chr1_41296827_41296828_REF	REF	119	119	0	0	100	191	191	0	0	100
chr1_45797504_45797505_REF	REF	119	119	0	0	100	191	191	0	0	100
chr1_6529182_6529188_Indel	Indel	119	117	2	0	100	191	191	0	0	100
chr1_6530965_6530965_REF	REF	119	0	63	56	0	191	0	107	84	0
chr1_68894730_68894730_REF	REF	119	116	3	0	100	191	139	52	0	100
chr1_94466658_94466659_SNP	SNP	119	111	8	0	100	191	188	3	0	100
chr1_94471074_94471075_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_94474327_94474328_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_94481504_94481505_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_94564482_94564483_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr1_98015290_98015291_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr2_112725684_112725684_Indel	Indel	119	118	1	0	100	191	187	4	0	100
chr2_112786048_112786049_REF	REF	119	119	0	0	100	191	191	0	0	100
chr2_113999533_113999534_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr2_141130547_141130548_REF	REF	119	119	0	0	100	191	191	0	0	100
chr2_141819587_141819588_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr2_141945948_141945949_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr2_148657116_148657117_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr2_167163042_167163043_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr2_167168092_167168093_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr2_175613272_175613273_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr2_175622219_175622220_REF	REF	119	119	0	0	100	191	191	0	0	100
chr2_178098087_178098088_REF	REF	119	104	15	0	100	191	166	25	0	100
chr2_182413237_182413238_REF	REF	119	119	0	0	100	191	191	0	0	100
chr2_182413258_182413259_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr2_182413601_182413602_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr2_189866342_189866343_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr2_189907936_189907937_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr2_189953285_189953285_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr2_191109532_191109533_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr2_198257794_198257795_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr2_198267770_198267770_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr2_209101905_209101906_Indel	Indel	119	0	119	0	0	191	0	191	0	0
chr2_212812096_212812097_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr2_216240126_216240128_REF	REF	119	119	0	0	100	191	191	0	0	100

Location	Variant Type	NA19240 (4 samples/run)[1]					NA19240 (16 samples/run)[2]				
		Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]	Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]
chr2_216248707_216248709_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr2_216274853_216274855_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr2_216299571_216299571_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr2_219545308_219545309_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr2_220284875_220284876_REF	REF	119	118	1	0	100	191	186	5	0	100
chr2_223161623_223161624_REF	REF	119	119	0	0	100	191	191	0	0	100
chr2_24905926_24905927_REF	REF	119	119	0	0	100	191	191	0	0	100
chr2_24974944_24974945_REF	REF	119	119	0	0	100	191	191	0	0	100
chr2_25471169_25471170_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr2_29416571_29416572_SNP	SNP	119	118	1	0	100	191	191	0	0	100
chr2_42510017_42510018_SNP	SNP	119	118	1	0	100	191	191	0	0	100
chr2_48030837_48030838_REF	REF	119	119	0	0	100	191	191	0	0	100
chr2_48032874_48032878_Indel	Indel	119	118	1	0	100	191	188	3	0	100
chr2_61760892_61760893_REF	REF	119	119	0	0	100	191	191	0	0	100
chr2_71795151_71795152_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr2_71829820_71829821_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr2_71838499_71838500_Indel	Indel	119	54	65	0	100	191	74	117	0	100
chr3_10085129_10085130_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr3_10133948_10133949_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr3_123419732_123419733_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr3_123453060_123453061_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr3_134644635_134644636_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr3_138456725_138456726_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr3_138665063_138665064_SNP	SNP	119	112	4	3	97.39	191	154	26	11	93.33
chr3_142222283_142222284_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr3_142277535_142277536_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr3_142281611_142281612_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr3_178922272_178922273_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr3_178922273_178922274_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr3_180366066_180366067_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr3_188326954_188326955_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr3_30733401_30733403_REF	REF	119	1	29	89	1.11	191	0	126	65	0
chr3_3192524_3192524_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr3_32188248_32188249_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr3_37695191_37695192_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr3_38592405_38592406_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr3_46501284_46501284_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr3_47125384_47125385_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr3_47163342_47163343_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr3_52584431_52584431_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr3_52584714_52584715_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr3_52662849_52662850_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr3_63973739_63973740_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr3_63981634_63981635_REF	REF	119	119	0	0	100	191	190	1	0	100
chr3_63982219_63982220_SNP	SNP	119	76	43	0	100	191	147	44	0	100
chr3_65361369_65361373_REF	REF	119	118	1	0	100	191	191	0	0	100
chr3_81627174_81627175_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr4_103533630_103533631_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr4_103534559_103534560_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr4_106154990_106154998_REF	REF	119	25	0	94	21.01	191	26	0	165	13.61
chr4_106155750_106155751_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr4_106190731_106190732_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr4_114117474_114117474_Indel	Indel	119	80	39	0	100	191	130	61	0	100
chr4_146576417_146576418_REF	REF	119	119	0	0	100	191	191	0	0	100
chr4_16037351_16037352_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr4_2834813_2834814_REF	REF	119	119	0	0	100	191	191	0	0	100
chr4_55151958_55151958_Indel	Indel	119	119	0	0	100	191	191	0	0	100

Location	Variant Type	NA19240 (4 samples/run)[1]					NA19240 (16 samples/run)[2]				
		Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]	Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]
chr4_87967234_87967235_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr4_88056719_88056720_Indel	Indel	119	58	61	0	100	191	85	106	0	100
chr4_88959380_88959381_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr4_90743317_90743317_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr4_995304_995305_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr5_112162853_112162854_REF	REF	119	119	0	0	100	191	191	0	0	100
chr5_112175769_112175770_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr5_131915212_131915213_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr5_13811774_13811775_REF	REF	119	118	1	0	100	191	189	2	0	100
chr5_13829607_13829608_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr5_13829610_13829611_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr5_13864727_13864728_REF	REF	119	119	0	0	100	191	191	0	0	100
chr5_13871053_13871054_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr5_149435607_149435608_REF	REF	119	119	0	0	100	191	191	0	0	100
chr5_149441016_149441017_REF	REF	119	0	119	0	0	191	0	191	0	0
chr5_149456994_149456995_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr5_149460552_149460553_REF	REF	119	4	115	0	100	191	11	180	0	100
chr5_149749125_149749126_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr5_149776231_149776232_REF	REF	119	119	0	0	100	191	188	3	0	100
chr5_156190172_156190172_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr5_176517460_176517461_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr5_176517796_176517797_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr5_176522727_176522728_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr5_176636881_176636882_SNP	SNP	119	77	42	0	100	191	150	41	0	100
chr5_180045700_180045700_Indel	Indel	119	93	26	0	100	191	138	53	0	100
chr5_180046492_180046494_Indel	Indel	119	117	0	2	98.32	191	178	0	13	93.19
chr5_180047739_180047739_REF	REF	119	119	0	0	100	191	184	0	7	96.34
chr5_180052945_180052946_REF	REF	119	119	0	0	100	191	191	0	0	100
chr5_35873604_35873605_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr5_35874574_35874575_REF	REF	119	119	0	0	100	191	191	0	0	100
chr5_35876273_35876274_REF	REF	119	119	0	0	100	191	191	0	0	100
chr5_38499763_38499764_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr5_38528951_38528953_Indel	Indel	119	0	0	119	0	191	0	6	185	0
chr5_55250726_55250727_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr5_55251930_55251931_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr5_7870972_7870973_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_116950733_116950734_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_116951505_116951507_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr6_117686699_117686701_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr6_117708970_117708971_REF	REF	119	119	0	0	100	191	191	0	0	100
chr6_117730818_117730819_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_135515447_135515448_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_135518107_135518108_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_135521488_135521489_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_138195961_138195964_REF	REF	119	118	1	0	100	191	191	0	0	100
chr6_143792605_143792606_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_143792674_143792675_REF	REF	119	119	0	0	100	191	191	0	0	100
chr6_152453290_152453291_REF	REF	119	119	0	0	100	191	191	0	0	100
chr6_152461048_152461052_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr6_152469330_152469331_SNP	SNP	119	118	1	0	100	191	191	0	0	100
chr6_152539572_152539573_Indel	Indel	119	0	119	0	0	191	0	191	0	0
chr6_152557345_152557346_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_152660309_152660310_REF	REF	119	119	0	0	100	191	191	0	0	100
chr6_152671474_152671475_REF	REF	119	119	0	0	100	191	191	0	0	100
chr6_152711394_152711395_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_152793375_152793376_Indel	Indel	119	95	24	0	100	191	145	46	0	100
chr6_152826296_152826297_SNP	SNP	119	119	0	0	100	191	191	0	0	100

Location	Variant Type	NA19240 (4 samples/run)[1]					NA19240 (16 samples/run)[2]				
		Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]	Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]
chr6_16327329_16327330_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_167271710_167271711_SNP	SNP	119	117	2	0	100	191	188	3	0	100
chr6_167271715_167271716_REF	REF	119	119	0	0	100	191	191	0	0	100
chr6_31978686_31978687_REF	REF	119	0	119	0	0	191	0	191	0	0
chr6_31978733_31978734_SNP	SNP	119	116	3	0	100	191	190	1	0	100
chr6_31978745_31978746_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_32032742_32032743_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_32064725_32064726_REF	REF	119	118	1	0	100	191	184	7	0	100
chr6_32164868_32164869_REF	REF	119	119	0	0	100	191	191	0	0	100
chr6_32164873_32164874_REF	REF	119	119	0	0	100	191	191	0	0	100
chr6_32188296_32188297_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_33132313_33132314_SNP	SNP	119	110	7	2	98.21	191	75	109	7	91.46
chr6_33134799_33134800_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_33136637_33136638_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_33139327_33139328_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_33141160_33141161_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_33145368_33145369_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_41565471_41565472_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_49425520_49425521_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_51618169_51618170_Indel	Indel	119	18	0	101	15.13	191	59	0	132	30.89
chr6_51776534_51776535_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_51875249_51875250_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_51891010_51891011_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_51921613_51921614_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_51935746_51935747_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_56373535_56373536_REF	REF	119	118	1	0	100	191	190	1	0	100
chr6_56463409_56463410_REF	REF	119	119	0	0	100	191	191	0	0	100
chr6_65016977_65016979_Indel	Indel	119	18	100	1	94.74	191	28	159	4	87.5
chr6_65098767_65098768_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_65301503_65301504_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_65611952_65611952_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr6_66005856_66005857_REF	REF	119	119	0	0	100	191	191	0	0	100
chr6_66005887_66005888_REF	REF	119	119	0	0	100	191	191	0	0	100
chr6_69666683_69666684_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_70071172_70071173_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_70950281_70950283_REF	REF	119	25	94	0	100	191	69	122	0	100
chr6_70965124_70965125_SNP	SNP	119	116	3	0	100	191	191	0	0	100
chr6_70990669_70990670_REF	REF	119	117	2	0	100	191	188	3	0	100
chr6_91226285_91226286_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr6_91266349_91266349_Indel	Indel	119	0	119	0	0	191	0	191	0	0
chr6_93964318_93964319_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr7_100411277_100411278_SNP	SNP	119	118	1	0	100	191	191	0	0	100
chr7_106508977_106508978_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr7_106508986_106508987_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr7_107353127_107353128_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr7_124486979_124486980_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr7_124492037_124492038_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr7_126079143_126079144_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr7_126410121_126410122_SNP	SNP	119	113	5	1	99.12	191	76	111	4	95.00
chr7_126410170_126410171_SNP	SNP	119	114	4	1	99.13	191	82	106	3	96.47
chr7_128034628_128034629_REF	REF	119	119	0	0	100	191	191	0	0	100
chr7_142566684_142566684_Indel	Indel	119	116	0	3	97.48	191	186	0	5	97.38
chr7_143016927_143016928_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr7_143036547_143036548_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr7_143043239_143043240_SNP	SNP	119	67	52	0	100	191	113	74	4	96.58
chr7_148504716_148504717_Indel	Indel	119	0	119	0	0	191	0	191	0	0
chr7_148543524_148543525_SNP	SNP	119	119	0	0	100	191	191	0	0	100

Location	Variant Type	NA19240 (4 samples/run)[1]					NA19240 (16 samples/run)[2]				
		Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]	Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]
chr7_150644427_150644428_REF	REF	119	117	2	0	100	191	179	12	0	100
chr7_150648671_150648672_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr7_150654622_150654622_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr7_151873852_151873853_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr7_151874497_151874498_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr7_151896573_151896573_Indel	Indel	119	118	1	0	100	191	191	0	0	100
chr7_151949067_151949068_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr7_21582916_21582917_REF	REF	119	22	97	0	100	191	10	181	0	100
chr7_21582962_21582963_REF	REF	119	116	3	0	100	191	147	44	0	100
chr7_21582963_21582964_REF	REF	119	115	4	0	100	191	148	43	0	100
chr7_21640617_21640618_REF	REF	119	119	0	0	100	191	191	0	0	100
chr7_21659554_21659554_Indel	Indel	119	119	0	0	100	191	190	1	0	100
chr7_21723572_21723573_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr7_21778448_21778449_REF	REF	119	119	0	0	100	191	191	0	0	100
chr7_21784431_21784432_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr7_2962292_2962293_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr7_2962752_2962753_REF	REF	119	119	0	0	100	191	191	0	0	100
chr7_33148711_33148712_SNP	SNP	119	106	12	1	99.07	191	149	26	16	90.30
chr7_37904065_37904068_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr7_37916454_37916455_REF	REF	119	119	0	0	100	191	191	0	0	100
chr7_55228052_55228053_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr7_55266416_55266417_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr7_73472049_73472050_SNP	SNP	119	112	7	0	100	191	183	6	2	98.92
chr7_91632305_91632306_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr7_91652178_91652178_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr7_91695716_91695717_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr7_91712697_91712698_REF	REF	119	119	0	0	100	191	191	0	0	100
chr7_91713971_91713972_REF	REF	119	119	0	0	100	191	190	1	0	100
chr7_91714910_91714911_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr7_91726926_91726927_REF	REF	119	119	0	0	100	191	191	0	0	100
chr7_92146686_92146687_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr7_92244630_92244635_REF	REF	119	119	0	0	100	191	191	0	0	100
chr7_94047160_94047161_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr7_98534946_98534948_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr8_113364695_113364696_REF	REF	119	119	0	0	100	191	191	0	0	100
chr8_114327081_114327084_Indel	Indel	119	119	0	0	100	191	190	0	1	99.48
chr8_11614574_11614575_REF	REF	119	118	1	0	100	191	183	8	0	100
chr8_118819577_118819578_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr8_126056215_126056215_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr8_144992075_144992076_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr8_144992102_144992103_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr8_144995493_144995494_REF	REF	119	119	0	0	100	191	190	1	0	100
chr8_144996407_144996408_REF	REF	119	119	0	0	100	191	191	0	0	100
chr8_144998168_144998169_REF	REF	119	118	1	0	100	191	178	13	0	100
chr8_144998189_144998190_REF	REF	119	118	1	0	100	191	178	13	0	100
chr8_145000055_145000056_REF	REF	119	118	1	0	100	191	179	12	0	100
chr8_145005688_145005688_REF	REF	119	0	56	63	0	191	3	110	78	3.70
chr8_145008341_145008342_REF	REF	119	114	5	0	100	191	178	13	0	100
chr8_21979102_21979103_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr8_30925750_30925751_REF	REF	119	119	0	0	100	191	190	1	0	100
chr8_30977716_30977717_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr8_30999122_30999123_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr8_31005017_31005017_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr8_48805816_48805816_Indel	Indel	119	27	89	3	90.00	191	77	106	8	90.59
chr8_90967710_90967711_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr8_90970861_90970862_REF	REF	119	119	0	0	100	191	191	0	0	100
chr8_90970934_90970935_REF	REF	119	119	0	0	100	191	191	0	0	100

Location	Variant Type	NA19240 (4 samples/run)[1]					NA19240 (16 samples/run)[2]				
		Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]	Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]
chr9_111685078_111685078_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr9_130587052_130587052_REF	REF	119	4	115	0	100	191	0	191	0	0
chr9_135786903_135786904_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr9_137659235_137659236_REF	REF	119	119	0	0	100	191	191	0	0	100
chr9_137688780_137688800_Indel	Indel	119	115	0	4	96.64	191	183	4	4	97.86
chr9_15479634_15479635_REF	REF	119	119	0	0	100	191	191	0	0	100
chr9_34485236_34485237_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr9_5081779_5081780_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr9_5090640_5090641_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr9_71650751_71650752_SNP	SNP	119	119	0	0	100	191	190	1	0	100
chr9_8331574_8331574_REF	REF	119	119	0	0	100	191	186	2	3	98.41
chr9_8338847_8338849_REF	REF	119	0	119	0	0	191	0	191	0	0
chr9_8460590_8460591_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr9_8484397_8484398_REF	REF	119	0	119	0	0	191	0	191	0	0
chr9_93639848_93639849_REF	REF	119	119	0	0	100	191	191	0	0	100
chr9_94486320_94486321_SNP	SNP	119	85	34	0	100	191	169	22	0	100
chr9_97934366_97934367_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr9_98229388_98229389_SNP	SNP	119	98	21	0	100	191	164	27	0	100
chr9_98232223_98232224_REF	REF	119	54	65	0	100	191	125	66	0	100
chr10_104157710_104157711_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr10_104157726_104157727_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr10_121429761_121429762_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr10_123243196_123243197_REF	REF	119	119	0	0	100	191	191	0	0	100
chr10_123310870_123310871_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr10_27294449_27294451_REF	REF	119	119	0	0	100	191	191	0	0	100
chr10_3818449_3818449_REF	REF	119	110	9	0	100	191	177	14	0	100
chr10_3820113_3820113_REF	REF	119	119	0	0	100	191	191	0	0	100
chr10_51568377_51568378_REF	REF	119	119	0	0	100	191	191	0	0	100
chr10_51582893_51582894_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr10_55591312_55591313_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr10_55755490_55755491_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr10_55826469_55826470_SNP	SNP	119	119	0	0	100	191	189	1	1	99.47
chr10_55892600_55892602_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr10_55943183_55943184_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr10_55955484_55955485_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr10_56423967_56423968_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr10_70405236_70405237_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr10_73544637_73544638_REF	REF	119	119	0	0	100	191	191	0	0	100
chr10_73553176_73553177_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr10_73558851_73558852_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr10_73558885_73558886_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr10_73558951_73558952_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr10_88441479_88441480_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr10_88683121_88683122_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr11_108121445_108121446_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr11_108139106_108139112_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr11_108150207_108150208_REF	REF	119	101	18	0	100	191	168	23	0	100
chr11_108151707_108151707_Indel	Indel	119	97	14	8	92.38	191	87	89	15	85.29
chr11_119148572_119148573_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr11_125497465_125497466_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr11_125525194_125525195_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr11_128675291_128675292_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr11_17522637_17522638_REF	REF	119	119	0	0	100	191	191	0	0	100
chr11_17537704_17537705_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr11_2592530_2592531_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr11_36595599_36595600_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr11_4123344_4123345_SNP	SNP	119	119	0	0	100	191	191	0	0	100

Location	Variant Type	NA19240 (4 samples/run)[1]					NA19240 (16 samples/run)[2]				
		Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]	Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]
chr11_4141131_4141132_REF	REF	119	119	0	0	100	191	191	0	0	100
chr11_4159456_4159457_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr11_4159465_4159466_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr11_44151576_44151577_REF	REF	119	119	0	0	100	191	191	0	0	100
chr11_47260318_47260319_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr11_47364761_47364762_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr11_47371484_47371485_Indel	Indel	119	114	5	0	100	191	183	8	0	100
chr11_47463483_47463484_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr11_69462909_69462910_REF	REF	119	119	0	0	100	191	191	0	0	100
chr11_71728908_71728914_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr11_76867902_76867903_SNP	SNP	119	116	3	0	100	191	160	31	0	100
chr11_76894234_76894235_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr11_76895770_76895792_Indel	Indel	119	24	79	16	60.00	191	12	145	34	26.09
chr11_76901272_76901273_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr11_76901623_76901623_REF	REF	119	0	79	40	0	191	0	143	48	0
chr11_76910765_76910766_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr11_76917219_76917220_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr11_76919467_76919468_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr11_76919477_76919478_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr11_89017960_89017961_REF	REF	119	119	0	0	100	191	191	0	0	100
chr12_103234214_103234215_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr12_103234251_103234252_REF	REF	119	119	0	0	100	191	191	0	0	100
chr12_103306549_103306550_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr12_111351029_111351030_REF	REF	119	57	62	0	100	191	72	119	0	100
chr12_111962923_111962924_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr12_112919868_112919869_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr12_121431271_121431272_REF	REF	119	119	0	0	100	191	191	0	0	100
chr12_22063736_22063737_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr12_22063748_22063749_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr12_25358662_25358664_Indel	Indel	119	9	108	2	82	191	50	135	6	89.29
chr12_25362776_25362777_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr12_2694637_2694638_REF	REF	119	119	0	0	100	191	191	0	0	100
chr12_2760965_2760966_Indel	Indel	119	118	0	1	99	191	191	0	0	100
chr12_2794790_2794791_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr12_33021933_33021934_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr12_40645256_40645257_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr12_40681142_40681142_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr12_40757114_40757114_Indel	Indel	119	93	13	13	87.74	191	157	1	33	82.63
chr12_48374615_48374616_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr12_49424665_49424665_Indel	Indel	119	36	80	3	92.31	191	33	158	0	100
chr12_56435928_56435929_REF	REF	119	116	3	0	100	191	179	12	0	100
chr12_56437777_56437777_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr12_7362203_7362204_SNP	SNP	119	0	36	83	0	191	0	78	113	0
chr13_103518035_103518036_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr13_103527848_103527849_SNP	SNP	119	115	4	0	100	191	183	8	0	100
chr13_103527929_103527930_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr13_110833701_110833702_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr13_110858991_110858992_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr13_110864181_110864182_Indel	Indel	119	119	0	0	100	191	189	2	0	100
chr13_28585649_28585650_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr13_28597447_28597449_REF	REF	119	117	2	0	100	191	191	0	0	100
chr13_28608458_28608459_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr13_28610044_28610045_REF	REF	119	119	0	0	100	191	191	0	0	100
chr13_28611381_28611382_REF	REF	119	119	0	0	100	191	191	0	0	100
chr13_28623698_28623699_REF	REF	119	119	0	0	100	191	191	0	0	100
chr13_28623758_28623759_REF	REF	119	119	0	0	100	191	191	0	0	100
chr13_48947468_48947469_SNP	SNP	119	119	0	0	100	191	191	0	0	100

Location	Variant Type	NA19240 (4 samples/run)[1]					NA19240 (16 samples/run)[2]				
		Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]	Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]
chr13_49033746_49033747_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr14_21778717_21778720_REF	REF	119	119	0	0	100	191	191	0	0	100
chr14_21792810_21792811_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr14_23855425_23855426_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr14_23865884_23865885_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr14_23866712_23866713_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr14_23888323_23888323_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr14_51062236_51062238_Indel	Indel	119	107	0	12	89.92	191	158	0	33	82.72
chr14_51094701_51094702_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr14_51204995_51204996_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr14_56139313_56139314_REF	REF	119	106	13	0	100	191	171	20	0	100
chr14_62164607_62164608_Indel	Indel	119	116	3	0	100	191	191	0	0	100
chr14_62194238_62194239_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr14_81534708_81534709_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr14_81574815_81574816_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr14_81610582_81610583_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr14_88852279_88852280_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr14_88894094_88894095_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr14_95572110_95572111_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr15_28235772_28235773_REF	REF	119	119	0	0	100	191	191	0	0	100
chr15_39880821_39880822_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr15_39886429_39886430_SNP	SNP	119	119	0	0	100	191	190	1	0	100
chr15_40457238_40457238_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr15_40476131_40476131_Indel	Indel	119	106	13	0	100	191	175	16	0	100
chr15_40494951_40494952_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr15_40494959_40494960_REF	REF	119	119	0	0	100	191	191	0	0	100
chr15_40498502_40498503_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr15_40898642_40898643_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr15_40914771_40914772_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr15_40915189_40915190_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr15_40915893_40915894_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr15_40949525_40949526_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr15_40954369_40954370_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr15_41797573_41797573_Indel	Indel	119	0	119	0	0	191	0	190	1	0
chr15_43571389_43571390_SNP	SNP	119	6	113	0	100	191	5	186	0	100
chr15_48703129_48703130_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr15_48719700_48719701_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr15_48722883_48722884_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr15_48759993_48759994_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr15_48787842_48787843_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr15_48797306_48797307_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr15_48805937_48805938_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr15_67457334_67457335_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr15_72105928_72105929_Indel	Indel	119	118	1	0	100	191	188	3	0	100
chr15_72639002_72639003_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr15_73614702_73614704_REF	REF	119	119	0	0	100	191	182	9	0	100
chr15_73614833_73614834_REF	REF	119	119	0	0	100	191	190	1	0	100
chr15_74336632_74336633_REF	REF	119	119	0	0	100	191	191	0	0	100
chr15_89859932_89859932_REF	REF	119	106	13	0	100	191	159	32	0	100
chr15_89864316_89864316_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr15_89869768_89869768_REF	REF	119	112	7	0	100	191	180	11	0	100
chr15_91337504_91337505_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr15_91358271_91358271_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr15_99482380_99482380_Indel	Indel	119	0	119	0	0	191	0	191	0	0
chr16_14026006_14026007_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr16_15811022_15811023_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr16_15811061_15811062_SNP	SNP	119	119	0	0	100	191	191	0	0	100

Location	Variant Type	NA19240 (4 samples/run)[1]					NA19240 (16 samples/run)[2]				
		Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]	Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]
chr16_15818140_15818141_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr16_15818492_15818493_REF	REF	119	15	104	0	100	191	40	151	0	100
chr16_15865471_15865472_REF	REF	119	119	0	0	100	191	191	0	0	100
chr16_27456612_27456613_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr16_57946936_57946937_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr16_57946972_57946973_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr16_89806346_89806347_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr16_89831509_89831510_REF	REF	119	119	0	0	100	191	191	0	0	100
chr16_89836322_89836323_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr16_89849479_89849480_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr16_89866042_89866043_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr16_89869703_89869704_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr16_89869760_89869761_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr16_89874873_89874874_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr16_89880821_89880822_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_11651056_11651057_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_11687520_11687521_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_11697049_11697049_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr17_11778239_11778240_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_15133705_15133707_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr17_1554635_1554636_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_1578645_1578646_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_1580830_1580831_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_1585129_1585130_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_29528561_29528562_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_29559931_29559932_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_29563073_29563073_REF	REF	119	0	4	115	0	191	0	4	187	0
chr17_37657460_37657460_Indel	Indel	119	0	119	0	0	191	0	191	0	0
chr17_37816460_37816461_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_37830899_37830900_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_39742491_39742492_SNP	SNP	119	119	0	0	100	191	190	1	0	100
chr17_39923613_39923614_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_45360888_45360889_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_48263902_48263903_SNP	SNP	119	107	12	0	100	191	183	8	0	100
chr17_48265425_48265426_SNP	SNP	119	118	1	0	100	191	188	3	0	100
chr17_48265494_48265495_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_48268222_48268223_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_48269301_48269302_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_48275181_48275182_Indel	Indel	119	33	65	21	61.11	191	64	101	26	71.11
chr17_48276970_48276971_SNP	SNP	119	61	23	35	63.54	191	115	24	52	68.86
chr17_5288607_5288608_REF	REF	119	119	0	0	100	191	191	0	0	100
chr17_53342796_53342796_REF	REF	119	117	2	0	100	191	189	2	0	100
chr17_56688511_56688512_REF	REF	119	117	2	0	100	191	187	4	0	100
chr17_59760995_59760996_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_59763346_59763347_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_6331802_6331803_REF	REF	119	119	0	0	100	191	191	0	0	100
chr17_6331835_6331836_REF	REF	119	119	0	0	100	191	191	0	0	100
chr17_66519857_66519857_Indel	Indel	119	103	16	0	100	191	171	20	0	100
chr17_72306116_72306118_REF	REF	119	113	6	0	100	191	179	12	0	100
chr17_7348763_7348764_SNP	SNP	119	108	11	0	100	191	131	60	0	100
chr17_73720899_73720900_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_7579643_7579659_Indel	Indel	119	24	23	72	25.00	191	49	23	119	29.17
chr17_78014020_78014021_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_78055756_78055757_REF	REF	119	119	0	0	100	191	191	0	0	100
chr17_78055757_78055758_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_78060108_78060109_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_78071051_78071052_REF	REF	119	119	0	0	100	191	191	0	0	100

Location	Variant Type	NA19240 (4 samples/run)[1]					NA19240 (16 samples/run)[2]				
		Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]	Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]
chr17_78073561_78073562_REF	REF	119	119	0	0	100	191	191	0	0	100
chr17_78073588_78073589_REF	REF	119	119	0	0	100	191	190	1	0	100
chr17_78078708_78078709_REF	REF	119	119	0	0	100	191	191	0	0	100
chr17_78081706_78081707_REF	REF	119	119	0	0	100	191	191	0	0	100
chr17_78084768_78084769_REF	REF	119	119	0	0	100	191	191	0	0	100
chr17_78302156_78302157_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_78319135_78319136_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr17_78327357_78327358_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr18_21119290_21119291_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr18_28666526_28666526_Indel	Indel	119	117	2	0	100	191	189	1	1	99.47
chr18_28669664_28669668_Indel	Indel	119	119	0	0	100	191	190	1	0	100
chr18_29104563_29104564_REF	REF	119	119	0	0	100	191	191	0	0	100
chr18_50831932_50831933_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr18_59166540_59166541_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr18_77477514_77477515_REF	REF	119	119	0	0	100	191	191	0	0	100
chr19_1619749_1619749_Indel	Indel	119	93	26	0	100	191	181	10	0	100
chr19_16593330_16593331_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr19_17937757_17937758_REF	REF	119	119	0	0	100	191	191	0	0	100
chr19_18876367_18876368_Indel	Indel	119	115	4	0	100	191	186	5	0	100
chr19_30303508_30303508_REF	REF	119	1	116	2	33.33	191	0	178	13	0
chr19_3113516_3113531_Indel	Indel	119	118	1	0	100	191	181	10	0	100
chr19_35523611_35523612_SNP	SNP	119	117	2	0	100	191	185	6	0	100
chr19_38939407_38939408_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr19_38968558_38968559_SNP	SNP	119	116	3	0	100	191	151	40	0	100
chr19_38973588_38973589_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr19_38995437_38995438_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr19_38996989_38996990_SNP	SNP	119	118	1	0	100	191	190	1	0	100
chr19_38997364_38997364_Indel	Indel	119	1	118	0	100	191	1	190	0	100
chr19_38997458_38997459_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr19_39034584_39034585_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chr19_39057615_39057616_SNP	SNP	119	117	2	0	100	191	188	3	0	100
chr19_41928867_41928867_Indel	Indel	119	5	114	0	100	191	8	183	0	100
chr19_41930395_41930396_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr19_45864924_45864924_Indel	Indel	119	0	119	0	0	191	0	191	0	0
chr19_45922322_45922323_REF	REF	119	119	0	0	100	191	191	0	0	100
chr19_55644324_55644325_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr19_55653296_55653297_REF	REF	119	119	0	0	100	191	191	0	0	100
chr19_55667646_55667647_REF	REF	119	117	2	0	100	191	187	4	0	100
chr20_31019360_31019361_REF	REF	119	20	99	0	100	191	22	169	0	100
chr20_31022765_31022766_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr20_32000482_32000483_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr20_41306599_41306600_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr20_43252914_43252915_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr20_50407161_50407162_REF	REF	119	119	0	0	100	191	191	0	0	100
chr20_50408481_50408482_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr21_35821820_35821821_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr21_45707515_45707516_Indel	Indel	119	119	0	0	100	191	190	1	0	100
chr21_45717549_45717550_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr21_46313441_46313442_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr22_24158898_24158899_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr22_36688177_36688178_REF	REF	119	119	0	0	100	191	191	0	0	100
chr22_36710182_36710183_REF	REF	119	119	0	0	100	191	191	0	0	100
chr22_39636944_39636948_REF	REF	119	0	31	88	0	191	0	61	130	0
chr22_41574086_41574087_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chr22_42526548_42526549_SNP	SNP	119	11	108	0	100	191	7	184	0	100
chrX_107829906_107829907_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chrX_107929336_107929337_SNP	SNP	119	119	0	0	100	191	191	0	0	100

Location	Variant Type	NA19240 (4 samples/run)[1]					NA19240 (16 samples/run)[2]				
		Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]	Total Calls	Correct Calls	No Calls	Miscalls	% Positive Agreement[3]
chrX_153760143_153760146_Indel	Indel	119	110	9	0	100	191	165	26	0	100
chrX_154065842_154065843_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chrX_154158284_154158285_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chrX_32305879_32305879_REF	REF	119	0	0	119	0	191	0	0	191	0
chrX_32583739_32583740_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chrX_33357271_33357272_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chrX_44833840_44833841_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chrX_44966794_44966795_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chrX_44966805_44966806_Indel	Indel	119	119	0	0	100	191	191	0	0	100
chrX_70595131_70595132_SNP	SNP	119	116	3	0	100	191	189	2	0	100
chrX_70613072_70613073_SNP	SNP	119	77	42	0	100	191	111	80	0	100
chrX_70627476_70627477_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chrX_77298856_77298857_SNP	SNP	119	119	0	0	100	191	191	0	0	100
chrX_79283508_79283509_SNP	SNP	119	119	0	0	100	191	191	0	0	100

[1] 60 sequencing runs in total

[2] 24 sequencing runs in total

[3] % positive agreement = (correct calls/total calls [excluding no calls]) × 100

Table 31 Accuracy results: Per base analysis

Sequencing accuracy for each of the 632 amplicons generated by the SVA panel are presented for both genomes used in the study (NA12878 and NA19240). Data is from a representative sequencing run of each genome selected prior to the start of the study. Correct calls include both variant positions correctly called as variant (compared to the sponsor-generated reference database sequence), as well as reference positions correctly called reference (compared to the human genome assembly sequence hg19).

			NA12878							NA19240						
Amplicon Name	Amplicon Genomic Content (GC; Other Features)[1]	Amplicon Length	Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]	Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]
AMPL4394358129	65% GC	178	178	178	0	178	0	100	100	178	178	0	177	1	99.44	99.44
AMPL4394343000	74% GC	168	168	168	0	168	0	100	100	168	166	2	166	0	98.81	100
AMPL4393046521	64% GC;8TCC	185	185	178	7	178	0	99.44	100	185	177	8	177	0	98.88	100
AMPL4392504456	76% GC	178	178	171	7	170	1	96.05	99.42	178	163	15	162	1	91.53	99.39
AMPL4393385216	54% GC	180	180	180	0	180	0	100	100	180	180	0	180	0	100	100
AMPL4393372520	50% GC;6A	173	173	173	0	173	0	100	100	173	173	0	173	0	100	100
AMPL4392440990	49% GC;9T	179	179	164	15	162	2	98.78	98.78	179	163	16	162	1	98.78	99.39
AMPL4392338993	66% GC	171	171	170	1	170	0	99.42	100	171	171	0	171	0	100	100
AMPL3200084096	65% GC;6G	176	176	173	3	173	0	98.30	100	176	171	5	171	0	97.16	100
AMPL959019077	23% GC;6A;7T	173	173	171	2	170	1	98.84	99.42	173	172	1	172	0	100	100
AMPL4297944685	64% GC;6G	177	177	177	0	176	1	99.44	99.44	177	177	0	176	1	99.44	99.44
AMPL4298066535	42% GC;7A	154	154	153	1	153	0	99.35	100	154	154	0	154	0	100	100
AMPL4392347320	39% GC	181	181	179	2	179	0	98.90	100	181	179	2	179	0	98.90	100
AMPL4392659170	49% GC	162	162	162	0	162	0	100	100	162	161	1	161	0	99.38	100
AMPL4392650892	62% GC	180	180	179	1	179	0	99.44	100	180	180	0	180	0	100	100
AMPL4373731419	40% GC;6A	171	171	171	0	171	0	100	100	171	171	0	171	0	100	100
AMPL4392328096	40% GC	154	154	153	1	153	0	99.35	100	154	153	1	153	0	99.35	100
AMPL4392651712	24% GC;8A	167	167	167	0	167	0	100	100	167	167	0	167	0	100	100
AMPL4392320712	39% GC	126	126	126	0	126	0	100	100	126	126	0	126	0	100	100
AMPL4392327972	30% GC	175	175	175	0	175	0	100	100	175	175	0	175	0	100	100
AMPL4392743687	34% GC;12A	163	163	162	1	162	0	99.39	100	163	163	0	163	0	100	100
AMPL4322363520	38% GC;6ATC	154	154	153	1	153	0	99.35	100	154	154	0	154	0	100	100
AMPL3289619107	56% GC	178	178	178	0	178	0	100	100	178	176	2	176	0	98.88	100
AMPL4392073064	50% GC;7A	177	177	147	30	147	0	83.05	100	177	59	118	59	0	33.33	100
AMPL4301817011	45% GC	166	166	166	0	166	0	100	100	166	166	0	166	0	100	100
AMPL413175205	54% GC	154	154	154	0	154	0	100	100	154	154	0	154	0	100	100
AMPL1871943647	57% GC	159	159	159	0	159	0	100	100	159	159	0	158	1	99.37	99.37
AMPL3840258113	43% GC	176	176	176	0	173	3	98.30	98.30	176	176	0	174	2	98.86	98.86
AMPL1090715363	61% GC	170	170	170	0	169	1	99.41	99.41	170	170	0	169	1	99.41	99.41
AMPL4392034654	43% GC	144	144	144	0	143	1	99.31	99.31	144	144	0	144	0	100	100
AMPL3840038528	54% GC	134	134	132	2	131	1	97.76	99.24	134	133	1	132	1	98.51	99.25
AMPL4392047471	51% GC	169	169	168	1	167	1	98.82	99.40	169	168	1	168	0	99.41	100
AMPL4392040940	52% GC	178	178	178	0	177	1	99.44	99.44	178	178	0	177	1	99.44	99.44
AMPL4392033882	51% GC	172	172	172	0	172	0	100	100	172	172	0	172	0	100	100
AMPL4392030295	51% GC	177	177	177	0	177	0	100	100	177	177	0	176	1	99.44	99.44
AMPL4392028734	51% GC	174	174	174	0	174	0	100	100	174	174	0	174	0	100	100
AMPL4393344413	36% GC	89	89	89	0	89	0	100	100	89	89	0	88	1	98.88	98.88
AMPL4394508046	49% GC	160	160	160	0	158	2	98.75	98.75	160	160	0	158	2	98.75	98.75
AMPL2858925985	39% GC	171	171	171	0	171	0	100	100	171	171	0	169	2	98.83	98.83
AMPL4392024816	49% GC	172	172	172	0	172	0	100	100	172	172	0	171	1	99.42	99.42
AMPL4392025061	46% GC	170	170	170	0	169	1	99.41	99.41	170	170	0	169	1	99.41	99.41
AMPL4394524216	44% GC;6T	181	181	180	1	180	0	100	100	181	180	1	180	0	100	100
AMPL4394090071	56% GC	175	175	174	1	174	0	99.43	100	175	174	1	174	0	99.43	100
AMPL4393326327	57% GC	175	175	174	1	174	0	99.43	100	175	175	0	175	0	100	100
AMPL4393283848	43% GC	175	175	174	1	173	1	98.86	99.43	175	174	1	173	1	98.86	99.43
AMPL4394093339	56% GC	113	113	113	0	113	0	100	100	113	112	1	112	0	99.12	100
AMPL4393316443	62% GC;6T	181	181	181	0	180	1	99.45	99.45	181	180	1	179	1	98.90	99.44
AMPL4373875155	40% GC;9A	166	166	166	0	166	0	100	100	166	166	0	166	0	100	100
AMPL4392008110	48% GC	115	115	114	1	114	0	99.13	100	115	113	2	113	0	98.26	100
AMPL3819598533	73% GC	184	184	183	1	183	0	99.46	100	184	184	0	184	0	100	100

Amplicon Name	Amplicon Genomic Content (GC; Other Features)[1]	Amplicon Length	NA12878							NA19240						
			Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]	Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]
AMPL398678636	62% GC	182	182	181	1	181	0	99.45	100	182	182	0	182	0	100	100
AMPL4392693991	59% GC	171	171	170	1	169	1	99.41	99.41	171	169	2	169	0	99.41	100
AMPL3731499965	54% GC	146	146	146	0	146	0	100	100	146	146	0	146	0	100	100
AMPL4393296666	63% GC	167	167	167	0	167	0	100	100	167	167	0	167	0	100	100
AMPL526439206	65% GC	187	187	186	1	185	1	99.46	99.46	187	186	1	185	1	99.46	99.46
AMPL4393093192	46% GC;6T	177	177	176	1	176	0	100	100	177	176	1	176	0	100	100
AMPL4392317144	42% GC;6A	116	116	116	0	116	0	100	100	116	116	0	115	1	99.14	99.14
AMPL4304199970	33% GC	171	171	170	1	170	0	100	100	171	170	1	170	0	100	100
AMPL4103839312	47% GC	156	156	156	0	156	0	100	100	156	156	0	156	0	100	100
AMPL4394463576	28% GC;9A;6T;6T	174	174	171	3	171	0	98.84	100	174	171	3	171	0	98.84	100
AMPL4394318742	45% GC	172	172	172	0	172	0	100	100	172	172	0	172	0	100	100
AMPL4392325406	39% GC	175	175	175	0	175	0	100	100	175	175	0	175	0	100	100
AMPL4324701736	63% GC	113	113	113	0	113	0	100	100	113	110	3	110	0	97.35	100
AMPL3257535118	54% GC	175	175	169	6	169	0	99.41	100	175	167	8	167	0	98.24	100
AMPL4392598603	55% GC;6C	177	177	174	3	174	0	98.86	100	177	174	3	174	0	98.86	100
AMPL4394085835	37% GC;6T	167	167	167	0	167	0	100	100	167	167	0	167	0	100	100
AMPL4394068372	35% GC;6T	153	153	153	0	153	0	100	100	153	153	0	153	0	100	100
AMPL4039838351	61% GC	156	156	156	0	156	0	100	100	156	156	0	156	0	100	100
AMPL4392315810	60% GC	185	185	185	0	185	0	100	100	185	185	0	185	0	100	100
AMPL4392611784	60% GC;6G	171	171	168	3	168	0	98.25	100	171	171	0	171	0	100	100
AMPL4304947808	43% GC	159	159	159	0	159	0	100	100	159	159	0	159	0	100	100
AMPL4392311692	30% GC	173	173	173	0	173	0	100	100	173	173	0	173	0	100	100
AMPL3198880818	54% GC	168	168	168	0	168	0	100	100	168	167	1	167	0	99.40	100
AMPL4395108860	54% GC	160	160	158	2	158	0	98.75	100	160	159	1	159	0	99.38	100
AMPL4318413913	60% GC	179	179	179	0	179	0	100	100	179	179	0	179	0	100	100
AMPL4392298562	35% GC;6A	173	173	173	0	173	0	100	100	173	173	0	173	0	100	100
AMPL461585857	62% GC;6T	178	178	178	0	178	0	100	100	178	178	0	178	0	100	100
AMPL4394997727	35% GC	159	159	157	2	157	0	99.37	100	159	158	1	158	0	100	100
AMPL4393286038	37% GC	124	124	124	0	124	0	100	100	124	124	0	124	0	100	100
AMPL4392312449	53% GC	131	131	131	0	131	0	100	100	131	131	0	131	0	100	100
AMPL4373352228	32% GC	167	167	167	0	166	1	99.40	99.40	167	167	0	166	1	99.40	99.40
AMPL4392299366	33% GC;6T;9T;6T;18T	154	154	98	56	98	0	63.64	100	154	101	53	100	1	64.94	99.01
AMPL598421930	37% GC;13T	179	179	176	3	176	0	98.32	100	179	178	1	178	0	99.44	100
AMPL4394316952	34% GC;6A;7T	173	173	173	0	173	0	100	100	173	173	0	173	0	100	100
AMPL3497995644	40% GC	144	144	144	0	144	0	100	100	144	143	1	143	0	99.31	100
AMPL4391994088	56% GC	157	157	157	0	157	0	100	100	157	157	0	157	0	100	100
AMPL4393073176	51% GC;6A	172	172	170	2	170	0	99.42	100	172	170	2	170	0	99.42	100
AMPL3666903404	61% GC	160	160	160	0	160	0	100	100	160	160	0	160	0	100	100
AMPL735306822	35% GC	83	83	83	0	83	0	100	100	83	83	0	83	0	100	100
AMPL4374432645	39% GC;6G	160	160	160	0	160	0	100	100	160	159	1	159	0	99.38	100
AMPL4394446055	29% GC	153	153	147	6	146	1	97.99	99.32	153	147	6	146	1	97.99	99.32
AMPL4394058159	29% GC;6T	174	174	174	0	174	0	100	100	174	174	0	173	1	99.43	99.43
AMPL3770343132	59% GC	169	169	169	0	169	0	100	100	169	169	0	169	0	100	100
AMPL4394312266	57% GC	172	172	172	0	172	0	100	100	172	172	0	172	0	100	100
AMPL4373941627	68% GC	179	179	177	2	176	1	98.88	99.44	179	178	1	176	2	98.88	98.88
AMPL3434544210	43% GC;7TG	164	164	161	3	160	1	98.16	99.38	164	160	4	160	0	98.16	100
AMPL4392293614	53% GC	177	177	176	1	176	0	99.44	100	177	177	0	177	0	100	100
AMPL4391994985	56% GC	165	165	163	2	163	0	98.79	100	165	164	1	164	0	99.39	100
AMPL4394057009	32% GC	174	174	174	0	174	0	100	100	174	172	2	172	0	98.85	100
AMPL912086672	28% GC	164	164	164	0	163	1	99.39	99.39	164	164	0	161	3	98.17	98.17
AMPL905336021	32% GC	117	117	117	0	117	0	100	100	117	116	1	116	0	99.15	100
AMPL4394077238	34% GC;6T	169	169	169	0	169	0	100	100	169	169	0	169	0	100	100
AMPL1705717513	40% GC	83	83	83	0	83	0	100	100	83	83	0	83	0	100	100
AMPL4392286218	36% GC	173	173	173	0	173	0	100	100	173	173	0	173	0	100	100
AMPL4394302264	49% GC	175	175	175	0	175	0	100	100	175	175	0	175	0	100	100

Accuracy results: Per base analysis

Amplicon Name	Amplicon Genomic Content (GC; Other Features)[1]	Amplicon Length	NA12878							NA19240						
			Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]	Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]
AMPL4394548445	46% GC	164	164	164	0	164	0	100	100	164	164	0	164	0	100	100
AMPL4392296241	62% GC	76	76	76	0	76	0	100	100	76	76	0	76	0	100	100
AMPL4309761177	34% GC	70	70	69	1	69	0	100	100	70	69	1	69	0	100	100
AMPL556873511	29% GC	168	168	167	1	167	0	99.40	100	168	168	0	168	0	100	100
AMPL4392283526	41% GC	178	178	178	0	178	0	100	100	178	178	0	178	0	100	100
AMPL4392722819	45% GC	175	175	174	1	174	0	100	100	175	173	2	173	0	99.43	100
AMPL4392277638	50% GC	133	133	133	0	133	0	100	100	133	133	0	133	0	100	100
AMPL4373957362	27% GC;7T	152	152	151	1	151	0	100	100	152	151	1	151	0	100	100
AMPL4392651475	45% GC;6A	175	175	175	0	175	0	100	100	175	175	0	174	1	99.43	99.43
AMPL3379180838	44% GC	131	131	130	1	130	0	99.24	100	131	130	1	130	0	99.24	100
AMPL3454598707	34% GC	156	156	155	1	155	0	100	100	156	155	1	155	0	100	100
AMPL3498234957	33% GC;10A	171	171	169	2	169	0	99.41	100	171	169	2	169	0	99.41	100
AMPL3073489807	29% GC	162	162	162	0	162	0	100	100	162	162	0	162	0	100	100
AMPL4392487356	42% GC	143	143	141	2	141	0	100	100	143	141	2	141	0	100	100
AMPL4392495150	41% GC	159	159	157	2	157	0	100	100	159	157	2	157	0	100	100
AMPL4394453234	39% GC;11AC	174	174	171	3	171	0	99.42	100	174	168	6	168	0	97.67	100
AMPL581765267	33% GC;7T	176	176	174	2	171	3	97.71	98.28	176	174	2	171	3	97.71	98.28
AMPL4391973685	55% GC	167	167	167	0	167	0	100	100	167	167	0	167	0	100	100
AMPL4392278772	60% GC	169	169	169	0	169	0	100	100	169	169	0	169	0	100	100
AMPL4392278508	64% GC;6C	146	146	146	0	146	0	100	100	146	146	0	146	0	100	100
AMPL4374039730	32% GC	185	185	184	1	184	0	100	100	185	184	1	184	0	100	100
AMPL4394049619	41% GC;7T	152	152	150	2	148	2	97.37	98.67	152	150	2	148	2	97.37	98.67
AMPL4218950823	34% GC	174	174	173	1	173	0	99.43	100	174	174	0	174	0	100	100
AMPL4219350794	46% GC	168	168	168	0	168	0	100	100	168	168	0	168	0	100	100
AMPL969040897	25% GC;9AT	173	173	171	2	171	0	100	100	173	171	2	170	1	99.42	99.42
AMPL4392714050	47% GC	159	159	158	1	158	0	100	100	159	158	1	158	0	100	100
AMPL4395093302	52% GC	176	176	176	0	176	0	100	100	176	176	0	176	0	100	100
AMPL3255695397	52% GC	170	170	170	0	170	0	100	100	170	170	0	170	0	100	100
AMPL3256513166	46% GC;5GT	175	175	60	115	60	0	34.29	100	175	60	115	60	0	34.29	100
AMPL4394436054	58% GC	173	173	172	1	172	0	100	100	173	171	2	169	2	98.26	98.83
AMPL4391970287	44% GC	181	181	180	1	180	0	99.45	100	181	181	0	181	0	100	100
AMPL4393295145	41% GC;7T	176	176	176	0	176	0	100	100	176	175	1	174	1	98.86	99.43
AMPL4392692126	61% GC;6C	155	155	121	34	119	2	76.77	98.35	155	102	53	101	1	65.16	99.02
AMPL4393058046	45% GC	89	89	88	1	88	0	100	100	89	87	2	87	0	98.86	100
AMPL4395087540	52% GC	177	177	177	0	177	0	100	100	177	177	0	177	0	100	100
AMPL4393144102	29% GC	82	82	82	0	82	0	100	100	82	82	0	82	0	100	100
AMPL4392648148	39% GC;7T	173	173	173	0	173	0	100	100	173	173	0	173	0	100	100
AMPL4392262531	50% GC	177	177	177	0	177	0	100	100	177	174	3	174	0	98.31	100
AMPL4392638186	39% GC;24A	178	178	178	0	178	0	100	100	178	175	3	175	0	98.31	100
AMPL4392477090	38% GC;5AG;9AG	151	151	146	5	146	0	99.32	100	151	145	6	145	0	98.64	100
AMPL4394514215	40% GC	108	108	108	0	108	0	100	100	108	108	0	108	0	100	100
AMPL4392619457	66% GC	156	156	156	0	156	0	100	100	156	156	0	156	0	100	100
AMPL748437993	48% GC	178	178	104	74	104	0	58.43	100	178	65	113	65	0	36.52	100
AMPL4393315786	46% GC;6T	138	138	138	0	138	0	100	100	138	138	0	138	0	100	100
AMPL736692473	38% GC;6A	112	112	112	0	112	0	100	100	112	112	0	112	0	100	100
AMPL4305635694	79% GC;6G	168	168	168	0	168	0	100	100	168	168	0	167	1	99.40	99.40
AMPL4395062735	31% GC	118	118	118	0	118	0	100	100	118	118	0	118	0	100	100
AMPL4219407492	38% GC	125	125	125	0	124	1	99.20	99.20	125	125	0	124	1	99.20	99.20
AMPL4222168082	32% GC;6A	169	169	167	2	167	0	98.82	100	169	164	5	164	0	97.04	100
AMPL4301889960	35% GC;6T	154	154	154	0	153	1	99.35	99.35	154	154	0	153	1	99.35	99.35
AMPL4392603343	37% GC	145	145	145	0	145	0	100	100	145	145	0	145	0	100	100
AMPL4374007010	59% GC	182	182	182	0	182	0	100	100	182	182	0	181	1	99.45	99.45
AMPL4393227469	32% GC;8T	172	172	172	0	172	0	100	100	172	172	0	172	0	100	100
AMPL4392596733	60% GC;6G	163	163	162	1	162	0	99.39	100	163	162	1	162	0	99.39	100
AMPL4395311255	60% GC;8TG;9GT	136	136	136	0	134	2	98.53	98.53	136	135	1	133	2	97.79	98.52

Amplicon Name	Amplicon Genomic Content (GC; Other Features)[1]	Amplicon Length	NA12878							NA19240						
			Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]	Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]
AMPL4392251505	59% GC	141	141	141	0	141	0	100	100	141	141	0	141	0	100	100
AMPL4227407944	41% GC	153	153	153	0	153	0	100	100	153	153	0	153	0	100	100
AMPL395434724	53% GC	175	175	174	1	173	1	99.43	99.43	175	174	1	173	1	99.43	99.43
AMPL4395087555	43% GC	179	179	177	2	177	0	98.88	100	179	179	0	179	0	100	100
AMPL4322933975	38% GC;8T	148	148	144	4	144	0	97.96	100	148	143	5	143	0	97.28	100
AMPL4394545846	49% GC	132	132	132	0	132	0	100	100	132	132	0	132	0	100	100
AMPL4394601334	46% GC	152	152	150	2	150	0	99.34	100	152	151	1	150	1	99.34	99.34
AMPL4373689558	44% GC	181	181	181	0	181	0	100	100	181	181	0	181	0	100	100
AMPL4391975825	57% GC	181	181	179	2	179	0	98.90	100	181	179	2	179	0	98.90	100
AMPL4393733107	29% GC	183	183	175	8	175	0	100	100	183	175	8	174	1	99.43	99.43
AMPL3498362734	41% GC;6A	140	140	140	0	140	0	100	100	140	140	0	140	0	100	100
AMPL413738456	26% GC;7T	115	115	115	0	115	0	100	100	115	115	0	115	0	100	100
AMPL4395318837	32% GC;12TA	137	137	137	0	133	4	97.08	97.08	137	131	6	129	2	94.16	98.47
AMPL4392997426	35% GC;8A	173	173	172	1	172	0	100	100	173	172	1	171	1	99.42	99.42
AMPL4392261532	48% GC	180	180	180	0	180	0	100	100	180	180	0	180	0	100	100
AMPL2742861328	44% GC	167	167	167	0	167	0	100	100	167	167	0	167	0	100	100
AMPL4392257952	40% GC	172	172	172	0	172	0	100	100	172	172	0	172	0	100	100
AMPL4308774096	41% GC	151	151	151	0	151	0	100	100	151	151	0	151	0	100	100
AMPL4392228266	49% GC;6A	149	149	149	0	149	0	100	100	149	149	0	149	0	100	100
AMPL3467347768	40% GC;6T	164	164	164	0	164	0	100	100	164	164	0	164	0	100	100
AMPL552238544	43% GC	172	172	172	0	172	0	100	100	172	170	2	170	0	98.84	100
AMPL551297934	45% GC;6T;7A	170	170	170	0	170	0	100	100	170	170	0	170	0	100	100
AMPL3498059646	51% GC	175	175	175	0	175	0	100	100	175	174	1	174	0	99.43	100
AMPL4393190958	36% GC;8T	180	180	180	0	180	0	100	100	180	180	0	180	0	100	100
AMPL4392317694	43% GC;14CA	175	175	171	4	170	1	98.27	99.42	175	170	5	169	1	97.69	99.41
AMPL4393183760	42% GC	140	140	140	0	140	0	100	100	140	140	0	140	0	100	100
AMPL1629455589	37% GC	170	170	170	0	170	0	100	100	170	170	0	170	0	100	100
AMPL393611061	40% GC	119	119	119	0	119	0	100	100	119	119	0	119	0	100	100
AMPL3653082204	45% GC	176	176	176	0	176	0	100	100	176	176	0	176	0	100	100
AMPL4391964653	31% GC;6T;6A	171	171	170	1	170	0	99.42	100	171	171	0	171	0	100	100
AMPL1211083733	61% GC	185	185	185	0	185	0	100	100	185	185	0	185	0	100	100
AMPL4392743054	59% GC;6C	177	177	111	66	111	0	63.07	100	177	73	104	73	0	41.48	100
AMPL407783594	52% GC	168	168	167	1	167	0	99.40	100	168	166	2	166	0	98.81	100
AMPL1663497431	62% GC;6G	178	178	177	1	177	0	99.44	100	178	175	3	175	0	98.31	100
AMPL863277015	65% GC	182	182	176	6	176	0	96.70	100	182	180	2	180	0	98.90	100
AMPL4394285134	58% GC;5AAG	155	155	155	0	155	0	100	100	155	155	0	155	0	100	100
AMPL4393684226	41% GC	180	180	179	1	178	1	99.44	99.44	180	179	1	179	0	100	100
AMPL3433472167	70% GC	173	173	173	0	173	0	100	100	173	173	0	173	0	100	100
AMPL4394027906	56% GC	176	176	176	0	176	0	100	100	176	175	1	175	0	99.43	100
AMPL416374362	65% GC	113	113	113	0	113	0	100	100	113	113	0	113	0	100	100
AMPL4393199548	41% GC	170	170	170	0	170	0	100	100	170	169	1	169	0	99.41	100
AMPL4392452727	62% GC	133	133	133	0	133	0	100	100	133	133	0	133	0	100	100
AMPL4394422924	74% GC	183	183	181	2	180	1	99.45	99.45	183	181	2	179	2	98.90	98.90
AMPL4392438280	62% GC;9GT	177	177	176	1	175	1	99.43	99.43	177	175	2	175	0	99.43	100
AMPL3438454613	71% GC	114	114	114	0	114	0	100	100	114	114	0	114	0	100	100
AMPL4394498383	62% GC	180	180	180	0	180	0	100	100	180	180	0	180	0	100	100
AMPL4392232288	64% GC	185	185	2	183	2	0	1.08	100	185	3	182	2	1	1.08	66.67
AMPL4392247152	63% GC	159	159	159	0	159	0	100	100	159	159	0	159	0	100	100
AMPL4392197049	69% GC	185	185	184	1	184	0	99.46	100	185	183	2	183	0	98.92	100
AMPL4393991123	60% GC	182	182	182	0	182	0	100	100	182	181	1	181	0	99.45	100
AMPL4393996246	62% GC	182	182	182	0	182	0	100	100	182	182	0	182	0	100	100
AMPL4392574004	63% GC	134	134	133	1	132	1	98.51	99.25	134	2	132	1	1	0.75	50.00
AMPL4392530564	66% GC	187	187	185	2	185	0	98.93	100	187	186	1	186	0	99.47	100
AMPL4392171571	63% GC;7C	153	153	54	99	53	1	34.64	98.15	153	54	99	54	0	35.29	100
AMPL4392174571	66% GC	152	152	152	0	152	0	100	100	152	152	0	152	0	100	100

Amplicon Name	Amplicon Genomic Content (GC; Other Features)[1]	Amplicon Length	NA12878							NA19240						
			Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]	Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]
AMPL3682590436	60% GC;6G	166	166	164	2	164	0	98.80	100	166	166	0	166	0	100	100
AMPL4392240361	58% GC	176	176	116	60	116	0	65.91	100	176	105	71	105	0	59.66	100
AMPL4395049446	68% GC	185	185	184	1	184	0	99.46	100	185	185	0	185	0	100	100
AMPL3518568818	33% GC;6T	172	172	172	0	172	0	100	100	172	172	0	172	0	100	100
AMPL4394999207	46% GC;9A	171	171	171	0	170	1	99	99	171	170	1	168	2	98.82	98.82
AMPL4393163023	38% GC;6T	174	174	174	0	174	0	100	100	174	174	0	174	0	100	100
AMPL4393980045	39% GC	176	176	176	0	176	0	100	100	176	176	0	176	0	100	100
AMPL4393983602	45% GC	176	176	176	0	176	0	100	100	176	176	0	176	0	100	100
AMPL4393695146	44% GC;7A;6T	174	174	102	72	102	0	58.62	100	174	121	53	121.00	0	69.54	100
AMPL3676543350	30% GC;6A;14A	175	175	174	1	174	0	99.43	100	175	175	0	175	0	100	100
AMPL4343482687	33% GC	73	73	73	0	73	0	100	100	73	73	0	73	0	100	100
AMPL4393984337	44% GC	154	154	154	0	154	0	100	100	154	154	0	154	0	100	100
AMPL4059164024	36% GC	158	158	156	2	156	0	98.73	100	158	156	2	156	0	98.73	100
AMPL4392391519	34% GC;11GA	161	161	157	4	157	0	98.74	100	161	157	4	157	0	98.74	100
AMPL4394279241	26% GC	172	172	171	1	171	0	99.42	100	172	170	2	170	0	98.84	100
AMPL4393723902	39% GC	176	176	176	0	176	0	100	100	176	176	0	176	0	100	100
AMPL4392346671	37% GC	167	167	166	1	166	0	100	100	167	166	1	166	0	100	100
AMPL4373578084	43% GC	177	177	177	0	177	0	100	100	177	177	0	177	0	100	100
AMPL4393984326	41% GC;10A	152	152	152	0	152	0	100	100	152	152	0	152	0	100	100
AMPL4393961771	39% GC	176	176	176	0	176	0	100	100	176	176	0	176	0	100	100
AMPL4373985988	29% GC;10A;6C;8AT;6TA	161	161	34	127	34	0	21.38	100	161	36	125	36	0	22.64	100
AMPL4392235190	36% GC	178	178	178	0	178	0	100	100	178	178	0	178	0	100	100
AMPL4392253940	60% GC;6G;6G	170	170	169	1	169	0	99.41	100	170	169	1	169	0	99.41	100
AMPL4392992251	41% GC;6T	119	119	119	0	119	0	100	100	119	119	0	119	0	100	100
AMPL4374147776	37% GC;6C;9A;6A	174	174	169	5	169	0	97.69	100	174	172	2	172	0	99.42	100
AMPL4373707639	33% GC	93	93	93	0	93	0	100	100	93	93	0	93	0	100	100
AMPL4392220658	35% GC;7A	157	157	157	0	157	0	100	100	157	157	0	157	0	100	100
AMPL4394603296	34% GC;6T;5TG;7TG	152	152	150	2	150	0	100	100	152	150	2	150	0	100	100
AMPL3435076154	36% GC;6TAA	176	176	176	0	175	1	99.43	99.43	176	176	0	175	1	99.43	99.43
AMPL4393051580	46% GC	169	169	167	2	167	0	100	100	169	167	2	167	0	100	100
AMPL4393964460	51% GC	150	150	150	0	150	0	100	100	150	150	0	150	0	100	100
AMPL3434408361	45% GC	175	175	175	0	175	0	100	100	175	175	0	175	0	100	100
AMPL4392985517	42% GC	163	163	163	0	163	0	100	100	163	163	0	163	0	100	100
AMPL4395015331	44% GC;5TC	180	180	180	0	180	0	100	100	180	180	0	180	0	100	100
AMPL4392990319	42% GC	166	166	166	0	166	0	100	100	166	166	0	166	0	100	100
AMPL4392441515	46% GC	177	177	174	3	174	0	100	100	177	173	4	173	0	99.43	100
AMPL4392216992	27% GC;6T	147	147	147	0	147	0	100	100	147	147	0	147	0	100	100
AMPL4393954745	49% GC	173	173	173	0	173	0	100	100	173	173	0	173	0	100	100
AMPL4393010759	53% GC	162	162	158	4	158	0	100	100	162	158	4	157	1	99.37	99.37
AMPL711877918	62% GC	180	180	180	0	180	0	100	100	180	180	0	180	0	100	100
AMPL4392573539	30% GC;7T;13A	172	172	167	5	167	0	97.66	100	172	170	2	170	0	99.42	100
AMPL4373702125	37% GC	150	150	150	0	150	0	100	100	150	150	0	150	0	100	100
AMPL4393942602	29% GC	172	172	172	0	172	0	100	100	172	172	0	172	0	100	100
AMPL4393950751	43% GC	171	171	168	3	167	1	97.66	99.40	171	170	1	170	0	99.42	100
AMPL4392987625	45% GC	166	166	166	0	166	0	100	100	166	166	0	166	0	100	100
AMPL4316030239	37% GC;9A;6A	153	153	152	1	151	1	99.34	99.34	153	152	1	151	1	99.34	99.34
AMPL4394982619	42% GC	168	168	168	0	167	1	99.40	99.40	168	168	0	168	0	100	100
AMPL1158164934	45% GC	169	169	169	0	169	0	100	100	169	169	0	169	0	100	100
AMPL3683333814	62% GC	125	125	125	0	125	0	100	100	125	125	0	125	0	100	100
AMPL755591047	64% GC	125	125	125	0	125	0	100	100	125	125	0	125	0	100	100
AMPL3498792122	38% GC;6A;17A	173	173	173	0	172	1	99.42	99.42	173	171	2	170	1	98.27	99.42
AMPL575431534	72% GC;5GAG	185	185	183	2	183	0	98.92	100	185	182	3	182	0	98.38	100
AMPL4394281382	33% GC	164	164	164	0	164	0	100	100	164	164	0	164	0	100	100
AMPL575510303	28% GC;6A;7A	173	173	170	3	170	0	98.84	100	173	171	2	171	0	99.42	100
AMPL4393743713	40% GC;6A	175	175	175	0	175	0	100	100	175	175	0	175	0	100	100

Amplicon Name	Amplicon Genomic Content (GC; Other Features)[1]	Amplicon Length	NA12878							NA19240						
			Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]	Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]
AMPL4392200573	41% GC;6A	149	149	149	0	149	0	100	100	149	149	0	149	0	100	100
AMPL4393737942	50% GC	175	175	174	1	174	0	99.43	100	175	174	1	174	0	99.43	100
AMPL4393656617	78% GC;6C	184	184	184	0	184	0	100	100	184	183	1	183	0	99.46	100
AMPL4394594066	31% GC;6A	167	167	164	3	163	1	99.39	99.39	167	164	3	163	1	99.39	99.39
AMPL571636108	31% GC;7A	162	162	162	0	162	0	100	100	162	162	0	162	0	100	100
AMPL868949058	30% GC;6T	169	169	169	0	168	1	99.41	99.41	169	169	0	168	1	99.41	99.41
AMPL4231728591	51% GC	152	152	152	0	152	0	100	100	152	151	1	151	0	99.34	100
AMPL692547671	63% GC	173	173	173	0	173	0	100	100	173	173	0	173	0	100	100
AMPL4323577210	34% GC	141	141	141	0	141	0	100	100	141	141	0	141	0	100	100
AMPL413836218	30% GC;6T;6A	170	170	168	2	168	0	99.41	100	170	167	3	167	0	98.82	100
AMPL4392936584	33% GC	172	172	172	0	172	0	100	100	172	172	0	172	0	100	100
AMPL4393915769	36% GC	168	168	168	0	168	0	100	100	168	168	0	168	0	100	100
AMPL4022901967	32% GC;6A	125	125	125	0	125	0	100	100	125	125	0	125	0	100	100
AMPL415167643	41% GC	172	172	171	1	171	0	99.42	100	172	172	0	172	0	100	100
AMPL4323505451	34% GC	144	144	144	0	144	0	100	100	144	144	0	144	0	100	100
AMPL4393686515	35% GC;9T	172	172	171	1	171	0	99.42	100	172	172	0	172	0	100	100
AMPL4374016890	35% GC;7A	180	180	175	5	175	0	100	100	180	175	5	175	0	100	100
AMPL4374230397	53% GC	179	179	179	0	179	0	100	100	179	179	0	179	0	100	100
AMPL4374521900	47% GC	174	174	172	2	172	0	100	100	174	172	2	171	1	99.42	99.42
AMPL4392992071	50% GC	82	82	82	0	82	0	100	100	82	82	0	82	0	100	100
AMPL719291358	61% GC	173	173	173	0	173	0	100	100	173	173	0	173	0	100	100
AMPL4393683810	31% GC	172	172	171	1	171	0	99.42	100	172	171	1	171	0	99.42	100
AMPL4392932301	34% GC	164	164	164	0	164	0	100	100	164	164	0	164	0	100	100
AMPL403750326	31% GC	118	118	118	0	118	0	100	100	118	118	0	118	0	100	100
AMPL4374399269	37% GC	169	169	169	0	169	0	100	100	169	169	0	169	0	100	100
AMPL4374398779	36% GC;6A	166	166	164	2	164	0	98.80	100	166	166	0	166	0	100	100
AMPL4392203039	60% GC	139	139	139	0	139	0	100	100	139	139	0	139	0	100	100
AMPL4392491672	31% GC;11A;10A;9A;6A;9A;6A	174	174	141	33	141	0	81.03	100	174	150	24	146	4	83.91	97.33
AMPL4395001028	65% GC	163	163	162	1	162	0	100	100	163	162	1	162	0	100	100
AMPL4395342492	46% GC	175	175	175	0	175	0	100	100	175	175	0	174	1	99.43	99.43
AMPL4392223494	45% GC	174	174	174	0	174	0	100	100	174	174	0	174	0	100	100
AMPL4392215902	58% GC	169	169	163	6	163	0	96.45	100	169	163	6	163	0	96.45	100
AMPL4393114509	38% GC;6G	183	183	181	2	181	0	99.45	100	183	181	2	181	0	99.45	100
AMPL4394953784	38% GC	173	173	173	0	173	0	100	100	173	173	0	173	0	100	100
AMPL596607752	74% GC;7C	171	171	171	0	171	0	100	100	171	168	3	167	1	97.66	99.40
AMPL596173137	61% GC	171	171	171	0	171	0	100	100	171	171	0	171	0	100	100
AMPL4320903836	68% GC	178	178	176	2	176	0	99.44	100	178	176	2	176	0	99.44	100
AMPL417791888	41% GC	170	170	170	0	170	0	100	100	170	170	0	170	0	100	100
AMPL4307576268	42% GC	154	154	154	0	154	0	100	100	154	154	0	154	0	100	100
AMPL413267348	30% GC	172	172	171	1	171	0	100	100	172	171	1	171	0	100	100
AMPL3498610579	37% GC;6A;9A	175	175	175	0	175	0	100	100	175	175	0	175	0	100	100
AMPL4322896544	27% GC;6A	166	166	166	0	162	4	97.59	97.59	166	164	2	162	2	97.59	98.78
AMPL4392211943	68% GC	160	160	159	1	159	0	99.38	100	160	159	1	159	0	99.38	100
AMPL4393613220	63% GC;7G	175	175	172	3	172	0	98.29	100	175	172	3	172	0	98.29	100
AMPL2692711234	27% GC;7T	131	131	130	1	130	0	99.24	100	131	130	1	130	0	99.24	100
AMPL4392926980	32% GC	168	168	168	0	168	0	100	100	168	168	0	167	1	99.40	99.40
AMPL3676783348	38% GC	168	168	168	0	168	0	100	100	168	168	0	168	0	100	100
AMPL2692395510	35% GC	176	176	175	1	175	0	100	100	176	175	1	175	0	100	100
AMPL3936418821	62% GC;6C	174	174	173	1	173	0	100	100	174	172	2	171	1	98.84	99.42
AMPL4374406487	35% GC	158	158	158	0	158	0	100	100	158	158	0	158	0	100	100
AMPL4393927397	33% GC	126	126	126	0	126	0	100	100	126	126	0	126	0	100	100
AMPL4373781431	35% GC	170	170	168	2	168	0	98.82	100	170	170	0	170	0	100	100
AMPL4393043045	26% GC	168	168	165	3	165	0	100	100	168	163	5	163	0	98.79	100
AMPL4038401239	53% GC	169	169	168	1	168	0	99.41	100	169	168	1	168	0	99.41	100
AMPL4392423431	37% GC	171	171	170	1	170	0	100	100	171	170	1	170	0	100	100

Amplicon Name	Amplicon Genomic Content (GC; Other Features)[1]	Amplicon Length	NA12878							NA19240						
			Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]	Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]
AMPL4392201407	65% GC	178	178	178	0	178	0	100	100	178	178	0	178	0	100	100
AMPL4302499643	71% GC	177	177	177	0	177	0	100	100	177	177	0	177	0	100	100
AMPL704947325	66% GC	188	188	188	0	188	0	100	100	188	188	0	188	0	100	100
AMPL4202336805	75% GC	188	188	187	1	187	0	99.47	100	188	188	0	188	0	100	100
AMPL4203136737	70% GC	185	185	185	0	185	0	100	100	185	185	0	185	0	100	100
AMPL4302482618	73% GC	93	93	91	2	91	0	98.91	100	93	91	2	91	0	98.91	100
AMPL4392191781	74% GC;6G	182	182	182	0	182	0	100	100	182	182	0	182	0	100	100
AMPL668652008	70% GC	177	177	177	0	176	1	99.44	99.44	177	176	1	175	1	98.87	99.43
AMPL3659553353	36% GC	157	157	157	0	157	0	100	100	157	157	0	157	0	100	100
AMPL4392890404	28% GC	145	145	145	0	145	0	100	100	145	145	0	145	0	100	100
AMPL4374509052	31% GC	127	127	125	2	125	0	99.21	100	127	126	1	126	0	100	100
AMPL4394429615	39% GC;9A;9AG	178	178	174	4	173	1	98.30	99.43	178	172	6	172	0	97.73	100
AMPL4392895580	39% GC;6A	169	169	169	0	169	0	100	100	169	169	0	169	0	100	100
AMPL4373372000	34% GC;10A	170	170	168	2	168	0	99.41	100	170	168	2	168	0	99.41	100
AMPL1153902623	35% GC	161	161	159	2	159	0	98.76	100	161	161	0	161	0	100	100
AMPL4393659645	52% GC	176	176	176	0	176	0	100	100	176	176	0	176	0	100	100
AMPL3679236852	79% GC	121	121	121	0	121	0	100	100	121	120	1	120	0	99.17	100
AMPL3498104622	41% GC	178	178	177	1	176	1	98.88	99.44	178	177	1	175	2	98.31	98.87
AMPL708625773	69% GC	172	172	31	141	31	0	18.02	100	172	32	140	32	0	18.60	100
AMPL4302673454	32% GC	171	171	171	0	171	0	100	100	171	171	0	171	0	100	100
AMPL1705657360	46% GC	173	173	173	0	173	0	100	100	173	173	0	172	1	99.42	99.42
AMPL1702865108	41% GC;8A	168	168	167	1	167	0	100	100	168	167	1	167	0	100	100
AMPL4392692973	44% GC	171	171	170	1	170	0	100	100	171	169	2	168	1	98.82	99.41
AMPL4195002255	65% GC	93	93	62	31	62	0	67.39	100	93	62	31	62	0	67	100
AMPL3797942774	35% GC;7T;18A;6A	153	153	150	3	150	0	98.04	100	153	150	3	150	0	98	100
AMPL3498144888	50% GC	149	149	148	1	148	0	99.33	100	149	149	0	149	0	100	100
AMPL3299722549	62% GC	178	178	175	3	175	0	98.31	100	178	176	2	176	0	98.88	100
AMPL4393636823	62% GC;6G	173	173	173	0	170	3	98.27	98.27	173	173	0	171	2	98.84	98.84
AMPL4392675093	62% GC;6C	181	181	159	22	158	1	98.14	99.37	181	160	21	159	1	98.76	99.38
AMPL4393781331	55% GC;6G	180	180	179	1	178	1	99.44	99.44	180	179	1	179	0	100	100
AMPL4395333937	27% GC;6T	94	94	94	0	92	2	97.87	97.87	94	93	1	93	0	100	100
AMPL4393786655	24% GC	170	170	167	3	167	0	99.40	100	170	168	2	168	0	100	100
AMPL4393896650	48% GC	109	109	109	0	109	0	100	100	109	109	0	109	0	100	100
AMPL4392914397	50% GC	160	160	160	0	160	0	100	100	160	160	0	160	0	100	100
AMPL4395281946	29% GC	171	171	171	0	171	0	100	100	171	171	0	171	0	100	100
AMPL4395290340	40% GC	143	143	143	0	143	0	100	100	143	143	0	143	0	100	100
AMPL4393619887	25% GC	117	117	117	0	117	0	100	100	117	117	0	117	0	100	100
AMPL4394604242	40% GC;10CA	174	174	171	3	170	1	98.84	99.42	174	172	2	172	0	100	100
AMPL3710790718	36% GC	98	98	98	0	98	0	100	100	98	98	0	98	0	100	100
AMPL4395266793	44% GC	81	81	81	0	81	0	100	100	81	81	0	81	0	100	100
AMPL4392189306	43% GC	114	114	114	0	114	0	100	100	114	114	0	114	0	100	100
AMPL3671540502	47% GC	161	161	161	0	161	0	100	100	161	161	0	161	0	100	100
AMPL4392192649	61% GC	179	179	177	2	176	1	98.32	99.44	179	177	2	177	0	98.88	100
AMPL4393619016	61% GC	182	182	182	0	182	0	100	100	182	182	0	182	0	100	100
AMPL2455591517	59% GC	170	170	170	0	170	0	100	100	170	170	0	170	0	100	100
AMPL4394566481	64% GC	151	151	150	1	150	0	99.34	100	151	151	0	150	1	99.34	99.34
AMPL4313036002	52% GC;14GT;7GT	152	152	57	95	56	1	36.84	98.25	152	57	95	56	1	36.84	98.25
AMPL2675981375	46% GC;8GAA	176	176	157	19	157	0	89.20	100	176	154	22	153	1	86.93	99.35
AMPL4325322943	63% GC	125	125	124	1	124	0	99.20	100	125	125	0	125	0	100	100
AMPL644684281	49% GC;14TC	172	172	130	42	129	1	75.00	99.23	172	122	50	119	3	69.19	97.54
AMPL4170634321	41% GC	116	116	116	0	116	0	100	100	116	116	0	116	0	100	100
AMPL652088025	50% GC	111	111	111	0	111	0	100	100	111	111	0	111	0	100	100
AMPL4392182082	66% GC	181	181	180	1	179	1	98.90	99.44	181	180	1	179	1	98.90	99.44
AMPL1519565349	49% GC;5GT	169	169	168	1	168	0	99.41	100	169	169	0	169	0	100	100
AMPL408214700	59% GC;6C	127	127	126	1	126	0	99.21	100	127	126	1	126	0	99.21	100

Amplicon Name	Amplicon Genomic Content (GC; Other Features)[1]	Amplicon Length	NA12878							NA19240						
			Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]	Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]
AMPL4395267587	64% GC	183	183	182	1	182	0	99.45	100	183	183	0	183	0	100	100
AMPL4392872919	36% GC	169	169	169	0	169	0	100	100	169	169	0	169	0	100	100
AMPL402546844	42% GC	92	92	92	0	92	0	100	100	92	91	1	91	0	98.91	100
AMPL404067077	39% GC	173	173	173	0	173	0	100	100	173	172	1	172	0	99.42	100
AMPL659232630	59% GC	157	157	155	2	155	0	98.73	100	157	153	4	153	0	97.45	100
AMPL4395306426	49% GC	182	182	182	0	182	0	100	100	182	182	0	182	0	100	100
AMPL528631298	74% GC	176	176	0	176	0	0	0	0	176	1	175	0	1	0	0
AMPL4392187570	47% GC;6A	148	148	148	0	148	0	100	100	148	147	1	147	0	99.32	100
AMPL4395394628	41% GC	152	152	152	0	152	0	100	100	152	152	0	152	0	100	100
AMPL4392903847	50% GC	101	101	101	0	101	0	100	100	101	101	0	101	0	100	100
AMPL4394224699	63% GC;6C	185	185	184	1	184	0	99.46	100	185	184	1	184	0	99.46	100
AMPL4320019211	69% GC;7C	184	184	107	77	107	0	58.47	100	184	105	79	105	0	57.38	100
AMPL4392351914	66% GC	163	163	162	1	162	0	100	100	163	162	1	162	0	100	100
AMPL4298148304	60% GC;5TCC	161	161	161	0	160	1	99.38	99.38	161	161	0	160	1	99.38	99.38
AMPL3198712213	63% GC	179	179	179	0	179	0	100	100	179	179	0	179	0	100	100
AMPL4394465691	58% GC	154	154	148	6	148	0	100	100	154	148	6	148	0	100	100
AMPL4393628011	60% GC	163	163	161	2	161	0	98.77	100	163	161	2	161	0	98.77	100
AMPL4394223117	64% GC	169	169	168	1	168	0	99.41	100	169	167	2	167	0	98.82	100
AMPL4394360574	64% GC	156	156	122	34	116	6	82.86	95.08	156	134	22	134	0	100	100
AMPL4394214041	58% GC;7GT	184	184	183	1	183	0	99.46	100	184	182	2	182	0	98.91	100
AMPL4394678126	65% GC	184	184	0	184	0	0	0	0	184	2	182	0	2	0	0
AMPL4394220527	60% GC	134	134	134	0	134	0	100	100	134	134	0	134	0	100	100
AMPL3674673921	62% GC	167	167	167	0	167	0	100	100	167	166	1	166	0	99.40	100
AMPL583119206	51% GC	167	167	167	0	167	0	100	100	167	167	0	167	0	100	100
AMPL3735262350	49% GC	70	70	70	0	70	0	100	100	70	70	0	70	0	100	100
AMPL623995564	29% GC;15T	139	139	134	5	134	0	96.40	100	139	136	3	136	0	97.84	100
AMPL1486637447	35% GC	103	103	96	7	96	0	98.97	100	103	97	6	97	0	100	100
AMPL3777975057	35% GC	119	119	118	1	118	0	100	100	119	118	1	118	0	100	100
AMPL3778824349	23% GC;10T	111	111	108	3	108	0	98.18	100	111	110	1	110	0	100	100
AMPL3378946700	38% GC	171	171	171	0	171	0	100	100	171	171	0	171	0	100	100
AMPL4394922626	34% GC	176	176	176	0	176	0	100	100	176	176	0	176	0	100	100
AMPL588811071	48% GC	108	108	108	0	108	0	100	100	108	108	0	108	0	100	100
AMPL3817158157	40% GC	169	169	169	0	169	0	100	100	169	169	0	169	0	100	100
AMPL4394216319	63% GC	163	163	162	1	162	0	99.39	100	163	161	2	161	0	98.77	100
AMPL4326420418	67% GC	167	167	166	1	165	1	99.40	99.40	167	166	1	166	0	100	100
AMPL3829029013	57% GC	170	170	170	0	170	0	100	100	170	169	1	169	0	99.41	100
AMPL4395303483	47% GC	156	156	155	1	154	1	98.72	99.35	156	155	1	155	0	99.36	100
AMPL4324007004	41% GC	93	93	93	0	93	0	100	100	93	93	0	93	0	100	100
AMPL4320523169	43% GC;6G;8T	82	82	78	4	78	0	97.50	100	82	72	10	72	0	90.00	100
AMPL395328621	27% GC	167	167	167	0	167	0	100	100	167	167	0	167	0	100	100
AMPL4395288076	51% GC	178	178	178	0	178	0	100	100	178	178	0	178	0	100	100
AMPL538987753	36% GC;6T	170	170	170	0	170	0	100	100	170	170	0	170	0	100	100
AMPL4394633624	33% GC	178	178	176	2	175	1	98.87	99.43	178	176	2	175	1	98.87	99.43
AMPL4394624154	32% GC;9GT	175	175	171	4	170	1	97.14	99.42	175	170	5	169	1	96.57	99.41
AMPL4392357297	24% GC;9T	167	167	165	2	165	0	99.40	100	167	165	2	165	0	99.40	100
AMPL4393606166	57% GC	183	183	182	1	181	1	98.91	99.45	183	183	0	182	1	99.45	99.45
AMPL4393136614	64% GC;6G	144	144	143	1	143	0	100	100	144	142	2	142	0	99.30	100
AMPL4394209036	61% GC	160	160	159	1	159	0	99.38	100	160	159	1	159	0	99.38	100
AMPL4394553261	46% GC	168	168	167	1	167	0	100	100	168	167	1	167	0	100	100
AMPL1496946897	46% GC	180	180	180	0	180	0	100	100	180	180	0	180	0	100	100
AMPL3519304014	41% GC	74	74	74	0	74	0	100	100	74	74	0	74	0	100	100
AMPL4392413266	57% GC;6G;5TG	183	183	182	1	181	1	99.45	99.45	183	182	1	182	0	100	100
AMPL4395265123	33% GC	170	170	169	1	169	0	99.41	100	170	170	0	170	0	100	100
AMPL3295318277	36% GC;7T	184	184	184	0	184	0	100	100	184	184	0	184	0	100	100
AMPL4317912554	61% GC	118	118	117	1	117	0	99.15	100	118	118	0	118	0	100	100

Amplicon Name	Amplicon Genomic Content (GC; Other Features)[1]	Amplicon Length	NA12878							NA19240						
			Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]	Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]
AMPL4393793084	58% GC	184	184	180	4	180	0	98.36	100	184	180	4	180	0	98.36	100
AMPL4395343628	34% GC;8T;6T	172	172	170	2	170	0	100	100	172	170	2	169	1	99.41	99.41
AMPL1517948259	38% GC	170	170	170	0	170	0	100	100	170	170	0	170	0	100	100
AMPL4393860661	35% GC	156	156	156	0	154	2	98.72	98.72	156	155	1	155	0	99.36	100
AMPL4373761294	34% GC	132	132	132	0	132	0	100	100	132	132	0	132	0	100	100
AMPL4393823209	34% GC	178	178	178	0	178	0	100	100	178	178	0	178	0	100	100
AMPL420799557	33% GC;6T;7T	75	75	75	0	75	0	100	100	75	74	1	74	0	98.67	100
AMPL416087660	26% GC;7A	119	119	119	0	119	0	100	100	119	119	0	119	0	100	100
AMPL4374301969	39% GC	176	176	176	0	176	0	100	100	176	176	0	176	0	100	100
AMPL4392815412	40% GC	176	176	176	0	176	0	100	100	176	176	0	176	0	100	100
AMPL4393570611	51% GC;12A	173	173	172	1	172	0	99.42	100	173	171	2	171	0	98.84	100
AMPL4395233644	46% GC	177	177	176	1	176	0	99.44	100	177	175	2	174	1	98.31	99.43
AMPL4392696459	22% GC	163	163	162	1	162	0	100	100	163	160	3	159	1	98.15	99.38
AMPL1608248833	26% GC	174	174	171	3	170	1	99.42	99.42	174	170	4	169	1	98.83	99.41
AMPL4393590705	47% GC	154	154	154	0	154	0	100	100	154	154	0	154	0	100	100
AMPL4235578244	38% GC	175	175	175	0	175	0	100	100	175	174	1	174	0	99.43	100
AMPL3733781601	43% GC	174	174	174	0	174	0	100	100	174	174	0	174	0	100	100
AMPL4395233955	64% GC	167	167	167	0	167	0	100	100	167	167	0	166	1	99.40	99.40
AMPL4393557392	54% GC	177	177	177	0	177	0	100	100	177	177	0	177	0	100	100
AMPL4395246850	55% GC;6A	178	178	177	1	177	0	99.44	100	178	175	3	175	0	98.31	100
AMPL4395355849	52% GC	140	140	139	1	139	0	100	100	140	139	1	139	0	100	100
AMPL4392670346	32% GC;8T;12TC;6TG	179	179	176	3	175	1	98.87	99.43	179	175	4	174	1	98.31	99.43
AMPL4393550446	37% GC	183	183	183	0	183	0	100	100	183	183	0	183	0	100	100
AMPL3498752779	39% GC;6A	158	158	157	1	157	0	99.37	100	158	158	0	158	0	100	100
AMPL4393769156	34% GC	105	105	104	1	104	0	100	100	105	104	1	104	0	100	100
AMPL557689580	32% GC;10T	155	155	154	1	154	0	100	100	155	154	1	154	0	100	100
AMPL4392824175	43% GC	132	132	132	0	132	0	100	100	132	132	0	132	0	100	100
AMPL4392796221	45% GC	112	112	112	0	112	0	100	100	112	112	0	112	0	100	100
AMPL3637426447	33% GC;9T;13T	147	147	145	2	145	0	98.64	100	147	143	4	142	1	96.60	99.30
AMPL3199428974	49% GC	170	170	170	0	170	0	100	100	170	170	0	170	0	100	100
AMPL777571747	72% GC	179	179	178	1	177	1	99.44	99.44	179	178	1	178	0	100	100
AMPL4393547526	31% GC	163	163	162	1	162	0	99.39	100	163	162	1	162	0	99.39	100
AMPL4394883639	38% GC	164	164	164	0	164	0	100	100	164	164	0	163	1	99.39	99.39
AMPL4393119864	26% GC;6A	119	119	119	0	119	0	100	100	119	118	1	117	1	98.32	99.15
AMPL391855502	56% GC	180	180	180	0	180	0	100	100	180	180	0	180	0	100	100
AMPL4392820663	52% GC	178	178	178	0	178	0	100	100	178	177	1	177	0	99.44	100
AMPL4392803596	42% GC;8T	178	178	177	1	177	0	99.44	100	178	177	1	177	0	99.44	100
AMPL4374504539	37% GC	156	156	155	1	155	0	100	100	156	154	2	154	0	99.35	100
AMPL4395008361	36% GC	155	155	153	2	153	0	99.35	100	155	154	1	154	0	100	100
AMPL4374372220	40% GC	174	174	174	0	174	0	100	100	174	174	0	174	0	100	100
AMPL4392783695	43% GC	168	168	168	0	168	0	100	100	168	168	0	168	0	100	100
AMPL4392776958	35% GC	118	118	118	0	118	0	100	100	118	118	0	118	0	100	100
AMPL4394859257	39% GC	173	173	172	1	172	0	99.42	100	173	172	1	172	0	99.42	100
AMPL4392795183	38% GC	151	151	151	0	151	0	100	100	151	150	1	150	0	99.34	100
AMPL3763697326	41% GC	176	176	175	1	175	0	99.43	100	176	176	0	175	1	99.43	99.43
AMPL4392776634	33% GC;8T	82	82	82	0	82	0	100	100	82	82	0	82	0	100	100
AMPL4394847665	41% GC	172	172	171	1	171	0	99.42	100	172	172	0	172	0	100	100
AMPL4393146133	61% GC;7G	180	180	177	3	177	0	98.88	100	180	178	2	178	0	99.44	100
AMPL4392805233	54% GC	153	153	152	1	152	0	99.35	100	153	152	1	151	1	98.69	99.34
AMPL4393330449	29% GC	177	177	176	1	176	0	99.44	100	177	176	1	176	0	99.44	100
AMPL4393549181	37% GC;6A	178	178	178	0	178	0	100	100	178	177	1	177	0	99.44	100
AMPL4393560571	49% GC	178	178	178	0	178	0	100	100	178	178	0	178	0	100	100
AMPL4394644825	44% GC	143	143	142	1	142	0	100	100	143	142	1	142	0	100	100
AMPL562172230	39% GC	173	173	172	1	172	0	100	100	173	172	1	172	0	100	100
AMPL562219796	51% GC;5AC	161	161	161	0	161	0	100	100	161	161	0	160	1	99.38	99.38

Amplicon Name	Amplicon Genomic Content (GC; Other Features)[1]	Amplicon Length	NA12878							NA19240						
			Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]	Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]
AMPL4393539439	27% GC;6T	161	161	160	1	160	0	99.38	100	161	160	1	159	1	98.76	99.38
AMPL4392146647	59% GC	177	177	177	0	177	0	100	100	177	177	0	177	0	100	100
AMPL769894769	58% GC	110	110	108	2	108	0	99.08	100	110	105	5	105	0	96.33	100
AMPL3374963808	51% GC	174	174	174	0	174	0	100	100	174	174	0	174	0	100	100
AMPL3720788183	45% GC;6T	181	181	179	2	179	0	100	100	181	178	3	178	0	99.44	100
AMPL4395377014	61% GC	177	177	176	1	176	0	99.44	100	177	177	0	177	0	100	100
AMPL4392352458	53% GC	178	178	177	1	177	0	100	100	178	176	2	176	0	99.44	100
AMPL4392678393	57% GC	160	160	159	1	159	0	100	100	160	159	1	159	0	100	100
AMPL4393760723	53% GC	151	151	150	1	150	0	100	100	151	150	1	149	1	99.33	99.33
AMPL3681904054	33% GC	116	116	115	1	115	0	99.14	100	116	115	1	115	0	99.14	100
AMPL4394458394	41% GC;7T	169	169	168	1	168	0	100	100	169	168	1	167	1	99.40	99.40
AMPL4393140419	39% GC;10A;7T	181	181	178	3	178	0	98.89	100	181	178	3	178	0	98.89	100
AMPL4085226812	63% GC	86	86	86	0	86	0	100	100	86	86	0	86	0	100	100
AMPL4373740005	42% GC	177	177	177	0	177	0	100	100	177	177	0	177	0	100	100
AMPL3691760208	65% GC	162	162	162	0	162	0	100	100	162	162	0	161	1	99.38	99.38
AMPL4392126885	55% GC	178	178	178	0	178	0	100	100	178	178	0	178	0	100	100
AMPL4392372494	62% GC;6G	182	182	180	2	180	0	99.45	100	182	180	2	180	0	99.45	100
AMPL4393856361	54% GC	176	176	175	1	175	0	99.43	100	176	175	1	175	0	99.43	100
AMPL4373672931	57% GC	164	164	164	0	164	0	100	100	164	164	0	164	0	100	100
AMPL4392120439	63% GC	179	179	179	0	179	0	100	100	179	179	0	179	0	100	100
AMPL4300890407	46% GC	143	143	143	0	143	0	100	100	143	143	0	143	0	100	100
AMPL4301194040	53% GC;5CA	182	182	182	0	182	0	100	100	182	182	0	182	0	100	100
AMPL544389827	66% GC	171	171	171	0	171	0	100	100	171	171	0	171	0	100	100
AMPL4216700451	57% GC	180	180	178	2	178	0	98.89	100	180	178	2	178	0	98.89	100
AMPL1156353735	38% GC	141	141	141	0	141	0	100	100	141	141	0	141	0	100	100
AMPL4300833980	43% GC;6T	178	178	178	0	178	0	100	100	178	178	0	178	0	100	100
AMPL4393523704	32% GC;7A;7T	174	174	173	1	173	0	99.43	100	174	174	0	174	0	100	100
AMPL4395213682	34% GC	170	170	170	0	170	0	100	100	170	170	0	170	0	100	100
AMPL789566107	63% GC	178	178	178	0	178	0	100	100	178	178	0	178	0	100	100
AMPL4393540427	50% GC	172	172	172	0	172	0	100	100	172	172	0	172	0	100	100
AMPL789212884	49% GC	176	176	176	0	176	0	100	100	176	176	0	176	0	100	100
AMPL4393492671	55% GC	179	179	179	0	179	0	100	100	179	179	0	179	0	100	100
AMPL4393748172	44% GC	126	126	125	1	125	0	100	100	126	125	1	125	0	100	100
AMPL4394161757	65% GC	180	180	180	0	180	0	100	100	180	178	2	178	0	98.89	100
AMPL4392701696	59% GC	175	175	174	1	170	4	97.14	97.70	175	173	2	170	3	97.14	98.27
AMPL4393462046	70% GC;6G	160	160	129	31	129	0	80.63	100	160	159	1	159	0	99.38	100
AMPL4392661656	59% GC;6C;6C	177	177	133	44	133	0	82.61	100	177	61	116	61	0	37.89	100
AMPL3889188256	53% GC	166	166	164	2	163	1	98.19	99.39	166	166	0	166	0	100	100
AMPL4395202607	41% GC	158	158	158	0	158	0	100	100	158	158	0	158	0	100	100
AMPL4392398765	32% GC;11A	167	167	166	1	166	0	100	100	167	166	1	166	0	100	100
AMPL4392113442	50% GC	179	179	178	1	177	1	98.88	99.44	179	177	2	176	1	98.32	99.44
AMPL4395337088	53% GC	175	175	173	2	172	1	99.42	99.42	175	173	2	173	0	100	100
AMPL4393477737	24% GC;7A;7T	179	179	176	3	176	0	98.32	100	179	177	2	177	0	98.88	100
AMPL4305062531	38% GC	176	176	176	0	176	0	100	100	176	176	0	176	0	100	100
AMPL4392565249	35% GC;6T;13T	175	175	174	1	172	2	98.85	98.85	175	173	2	171	2	98.28	98.84
AMPL4395050447	27% GC;10T;8T	75	75	73	2	73	0	98.65	100	75	73	2	73	0	98.65	100
AMPL4393470475	58% GC	185	185	184	1	184	0	99.46	100	185	184	1	184	0	99.46	100
AMPL4392777432	63% GC;6C	174	174	173	1	173	0	99.43	100	174	173	1	173	0	99.43	100
AMPL4395183962	53% GC	129	129	129	0	129	0	100	100	129	129	0	129	0	100	100
AMPL2949788169	60% GC	181	181	79	102	79	0	43.65	100	181	74	107	74	0	40.88	100
AMPL4392758534	63% GC	179	179	179	0	179	0	100	100	179	179	0	179	0	100	100
AMPL4393492728	60% GC	179	179	179	0	179	0	100	100	179	179	0	179	0	100	100
AMPL4392107402	64% GC	136	136	136	0	136	0	100	100	136	136	0	136	0	100	100
AMPL4392128611	63% GC	150	150	150	0	150	0	100	100	150	150	0	150	0	100	100
AMPL549199875	62% GC	152	152	152	0	152	0	100	100	152	151	1	151	0	99.34	100

Amplicon Name	Amplicon Genomic Content (GC; Other Features)[1]	Amplicon Length	NA12878							NA19240						
			Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]	Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]
AMPL549072782	50% GC;7A	177	177	175	2	175	0	99.43	100	177	175	2	174	1	98.86	99.43
AMPL4392509220	73% GC	188	188	111	77	110	1	58.51	99.10	188	112	76	111	1	59.04	99.11
AMPL4392510383	45% GC;7T;6A	135	135	134	1	134	0	100	100	135	134	1	134	0	100	100
AMPL4395349481	53% GC	178	178	177	1	177	0	100	100	178	177	1	177	0	100	100
AMPL1104437054	33% GC;7T	172	172	172	0	172	0	100	100	172	172	0	172	0	100	100
AMPL4104053523	37% GC	153	153	152	1	152	0	99.35	100	153	151	2	151	0	98.69	100
AMPL4393041536	36% GC	135	135	133	2	133	0	99.25	100	135	133	2	133	0	99.25	100
AMPL4394409931	58% GC	180	180	178	2	178	0	100	100	180	177	3	177	0	99.44	100
AMPL4392109226	65% GC	179	179	179	0	179	0	100	100	179	178	1	178	0	99.44	100
AMPL573434660	63% GC	171	171	171	0	171	0	100	100	171	171	0	171	0	100	100
AMPL4392117695	58% GC	156	156	156	0	156	0	100	100	156	156	0	156	0	100	100
AMPL4395175643	57% GC	181	181	181	0	181	0	100	100	181	181	0	181	0	100	100
AMPL4373650991	58% GC	183	183	183	0	182	1	99.45	99.45	183	183	0	183	0	100	100
AMPL4392110866	62% GC	172	172	172	0	172	0	100	100	172	172	0	172	0	100	100
AMPL4394139126	67% GC	118	118	116	2	116	0	98.31	100	118	115	3	115	0	97.46	100
AMPL555450618	70% GC	179	179	179	0	179	0	100	100	179	179	0	179	0	100	100
AMPL555283876	60% GC;6C	171	171	169	2	169	0	98.83	100	171	169	2	169	0	98.83	100
AMPL4392759751	41% GC;6A	170	170	170	0	170	0	100	100	170	170	0	170	0	100	100
AMPL4392767076	55% GC	164	164	164	0	164	0	100	100	164	164	0	164	0	100	100
AMPL4394809934	60% GC	151	151	149	2	149	0	98.68	100	151	149	2	149	0	98.68	100
AMPL4394147071	39% GC	176	176	175	1	175	0	99.43	100	176	176	0	176	0	100	100
AMPL3289654030	30% GC	177	177	176	1	176	0	100	100	177	176	1	176	0	100	100
AMPL4392726050	23% GC	176	176	172	4	172	0	100	100	176	172	4	172	0	100	100
AMPL3961593542	25% GC	178	178	177	1	177	0	99.44	100	178	177	1	177	0	99.44	100
AMPL4392708195	44% GC;8T;8TC	176	176	174	2	174	0	98.86	100	176	172	4	172	0	97.73	100
AMPL4394756215	37% GC	179	179	179	0	179	0	100	100	179	179	0	179	0	100	100
AMPL4394758212	56% GC	171	171	171	0	171	0	100	100	171	171	0	171	0	100	100
AMPL4392098735	69% GC	185	185	185	0	185	0	100	100	185	184	1	184	0	99.46	100
AMPL4392422488	60% GC	169	169	120	49	120	0	71.43	100	169	65	104	64	1	38.10	98.46
AMPL4393028379	76% GC	186	186	170	16	170	0	99.42	100	186	170	16	170	0	99.42	100
AMPL700490202	45% GC;7A	153	153	153	0	152	1	99.35	99.35	153	153	0	153	0	100	100
AMPL3764819988	66% GC	154	154	153	1	153	0	99.35	100	154	152	2	152	0	98.70	100
AMPL4393070345	65% GC;6G	154	154	153	1	153	0	100	100	154	153	1	153	0	100	100
AMPL4374033508	78% GC	186	186	184	2	184	0	99.46	100	186	111	75	111	0	60.00	100
AMPL597978528	63% GC	169	169	168	1	168	0	99.41	100	169	166	3	166	0	98.22	100
AMPL3569591894	65% GC;6C	133	133	133	0	133	0	100	100	133	133	0	133	0	100	100
AMPL4393411927	66% GC;6G	176	176	69	107	69	0	39.20	100	176	70	106	70	0	39.77	100
AMPL4393455182	58% GC	180	180	138	42	138	0	76.67	100	180	122	58	122	0	67.78	100
AMPL4393432724	60% GC;6C	174	174	173	1	173	0	99.43	100	174	173	1	172	1	98.85	99.42
AMPL3695208132	61% GC;6C;6G;7G	174	174	172	2	172	0	98.85	100	174	173	1	173	0	99.43	100
AMPL4393407496	60% GC	171	171	170	1	170	0	100	100	171	169	2	169	0	99.41	100
AMPL4392668212	59% GC	154	154	153	1	153	0	100	100	154	153	1	153	0	100	100
AMPL397538170	60% GC	182	182	179	3	179	0	98.35	100	182	180	2	180	0	98.90	100
AMPL4394405741	64% GC	165	165	82	83	82	0	50.00	100	165	117	48	117	0	71.34	100
AMPL4392096706	66% GC	183	183	181	2	181	0	98.91	100	183	183	0	183	0	100	100
AMPL4393123724	66% GC;6G	161	161	159	2	158	1	98.75	99.37	161	159	2	157	2	98.13	98.74
AMPL4395386501	58% GC	175	175	175	0	175	0	100	100	175	175	0	175	0	100	100
AMPL4392102936	62% GC	176	176	176	0	176	0	100	100	176	176	0	176	0	100	100
AMPL4297998702	63% GC	102	102	102	0	102	0	100	100	102	102	0	102	0	100	100
AMPL532298001	71% GC	180	180	180	0	180	0	100	100	180	180	0	180	0	100	100
AMPL4392537380	49% GC;7T	157	157	155	2	155	0	99.36	100	157	155	2	155	0	99.36	100
AMPL4394751711	59% GC	146	146	146	0	146	0	100	100	146	146	0	146	0	100	100
AMPL4395148488	61% GC	180	180	179	1	179	0	99.44	100	180	179	1	179	0	99.44	100
AMPL1118439227	65% GC	178	178	153	25	153	0	85.96	100	178	157	21	156	1	87.64	99.36
AMPL4394736429	61% GC;6C	159	159	158	1	158	0	99.37	100	159	158	1	158	0	99.37	100

Amplicon Name	Amplicon Genomic Content (GC; Other Features)[1]	Amplicon Length	NA12878							NA19240						
			Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]	Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]
AMPL551935865	61% GC	173	173	173	0	173	0	100	100	173	173	0	173	0	100	100
AMPL4392073003	52% GC	165	165	165	0	165	0	100	100	165	165	0	165	0	100	100
AMPL4394129588	60% GC;6G	159	159	154	5	154	0	96.86	100	159	155	4	155	0	97.48	100
AMPL4373586961	30% GC;6A	172	172	171	1	171	0	99.42	100	172	171	1	170	1	98.84	99.42
AMPL595971724	63% GC;6G	170	170	170	0	170	0	100	100	170	170	0	170	0	100	100
AMPL4394610057	71% GC;6C	180	180	178	2	178	0	99.44	100	180	178	2	178	0	99.44	100
AMPL4392084924	65% GC	179	179	179	0	179	0	100	100	179	179	0	179	0	100	100
AMPL4392744809	61% GC	183	183	182	1	182	0	99.45	100	183	183	0	183	0	100	100
AMPL4392755421	55% GC	182	182	182	0	182	0	100	100	182	182	0	182	0	100	100
AMPL4394432367	43% GC;16T	161	161	161	0	161	0	100	100	161	160	1	160	0	99.38	100
AMPL4395355273	64% GC	139	139	139	0	139	0	100	100	139	139	0	139	0	100	100
AMPL4373723911	60% GC	174	174	174	0	174	0	100	100	174	174	0	174	0	100	100
AMPL4392449135	53% GC;10TC;10AC;8TC	179	179	144	35	142	2	81.14	98.61	179	149	30	147	2	84.00	98.66
AMPL4392752461	63% GC	180	180	180	0	180	0	100	100	180	180	0	180	0	100	100
AMPL3680965544	63% GC;6C;7G	174	174	174	0	172	2	98.85	98.85	174	174	0	174	0	100	100
AMPL3920741189	61% GC	166	166	165	1	158	7	95.18	95.76	166	164	2	157	7	94.58	95.73
AMPL3289833537	30% GC;7A;7AT	178	178	177	1	177	0	100	100	178	177	1	176	1	99.44	99.44
AMPL4395147724	34% GC;6A	166	166	166	0	166	0	100	100	166	166	0	166	0	100	100
AMPL4392928879	40% GC	157	157	157	0	157	0	100	100	157	157	0	157	0	100	100
AMPL4394730094	37% GC;6G	169	169	169	0	169	0	100	100	169	154	15	154	0	91.12	100
AMPL1676134309	39% GC	138	138	137	1	137	0	100	100	138	137	1	137	0	100	100
AMPL4392728909	61% GC;6C;6C;6G	136	136	132	4	132	0	97.06	100	136	132	4	132	0	97.06	100
AMPL749578720	46% GC	155	155	155	0	155	0	100	100	155	155	0	155	0	100	100
AMPL4373417951	38% GC;20TG	91	91	90	1	90	0	98.90	100	91	87	4	87	0	95.60	100
AMPL4392707708	46% GC	142	142	142	0	142	0	100	100	142	142	0	142	0	100	100
AMPL4394086736	32% GC	173	173	173	0	173	0	100	100	173	172	1	172	0	99.42	100
AMPL3700904683	47% GC;6T	87	87	87	0	87	0	100	100	87	86	1	86	0	98.85	100
AMPL553632290	44% GC;10T	170	170	169	1	169	0	99.41	100	170	170	0	170	0	100	100
AMPL4393378805	56% GC	158	158	158	0	158	0	100	100	158	158	0	158	0	100	100
AMPL569668692	73% GC	178	178	14	164	14	0	8.00	100	178	53	125	52	1	29.71	98.11
AMPL4314163957	61% GC	173	173	173	0	173	0	100	100	173	173	0	173	0	100	100
AMPL529493999	36% GC	172	172	129	43	129	0	75.00	100	172	147	25	147	0	85.47	100

[1] other features include homopolymers, di-nucleotide repeats, tri-nucleotide repeats (e.g., 6A indicates a homopolymer six adenine residues in length, 8TCC indicates the 8 repeats of the tri-nucleotide 'TCC'.

[2] the covered regions of the amplicon fragment.

[3] total number of calls made (correct calls + incorrect calls) for the pre-selected sequencing run.

[4] number of incorrect calls for the pre-selected sequencing run.

[5] % correct calls including no calls = # correct calls / (# calls + # no calls)

[6] % correct calls excluding no calls = # correct calls / # calls

Table 32 Accuracy results: NA12878 vs NIST per base analysis

The following table shows sequencing accuracy results for NA12878 when compared to the highly confident NA12878 reference sequence defined by the National Institutes of Standards and Technology (NIST). Sequencing accuracy results for all amplicons generated by the SVA panel for which ≥50% of the amplicon is covered by NA12878 NIST highly confident reference sequence are presented. Data is from a representative sequencing run selected prior to the start of the study. Correct calls include both variant positions correctly called as variant, as well as reference positions correctly called reference.

Amplicon Name	Amplicon Genomic Content (GC; Other Features)[1]	Amplicon Length	NA12878 vs NIST						
			Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]
AMPL4394358129	65% GC	178	178	178	0	178	0	100	100
AMPL4394343000	74% GC	168	168	168	0	168	0	100	100
AMPL4393046521	64% GC;8TCC	185	109	109	0	109	0	100	100
AMPL4392504456	76% GC	178	138	135	3	134	1	97.81	99.26
AMPL4393385216	54% GC	180	180	180	0	180	0	100	100
AMPL4393372520	50% GC;6A	173	146	146	0	146	0	100	100
AMPL4392440990	49% GC;9T	179	175	160	15	159	1	99.38	99.38
AMPL4392338993	66% GC	171	171	170	1	170	0	99.42	100
AMPL3200084096	65% GC;6G	176	142	139	3	139	0	97.89	100
AMPL4297944685	64% GC;6G	177	177	177	0	176	1	99.44	99.44
AMPL4298066535	42% GC;7A	154	136	136	0	136	0	100	100
AMPL4392347320	39% GC	181	155	153	2	153	0	98.71	100
AMPL4392659170	49% GC	162	162	162	0	162	0	100	100
AMPL4392650892	62% GC	180	180	179	1	179	0	99.44	100
AMPL4373731419	40% GC;6A	171	171	171	0	171	0	100	100
AMPL4392328096	40% GC	154	152	151	1	151	0	99.34	100
AMPL4392651712	24% GC;8A	167	167	167	0	167	0	100	100
AMPL4392320712	39% GC	126	126	126	0	126	0	100	100
AMPL4392327972	30% GC	175	175	175	0	175	0	100	100
AMPL4392743687	34% GC;12A	163	132	132	0	132	0	100	100
AMPL3289619107	56% GC	178	178	178	0	178	0	100	100
AMPL4392073064	50% GC;7A	177	149	119	30	119	0	79.87	100
AMPL4301817011	45% GC	166	109	109	0	109	0	100	100
AMPL413175205	54% GC	154	154	154	0	154	0	100	100
AMPL398678636	62% GC	182	109	108	1	108	0	99.08	100
AMPL4392693991	59% GC	171	105	104	1	104	0	100	100
AMPL3731499965	54% GC	146	146	146	0	146	0	100	100
AMPL4393296666	63% GC	167	167	167	0	167	0	100	100
AMPL526439206	65% GC	187	187	186	1	186	0	100	100
AMPL4393093192	46% GC;6T	177	118	117	1	117	0	100	100
AMPL4392317144	42% GC;6A	116	116	116	0	116	0	100	100
AMPL4304199970	33% GC	171	154	153	1	153	0	100	100
AMPL4103839312	47% GC	156	153	153	0	153	0	100	100
AMPL4394318742	45% GC	172	172	172	0	172	0	100	100
AMPL4392325406	39% GC	175	144	144	0	144	0	100	100
AMPL4324701736	63% GC	113	84	84	0	84	0	100	100
AMPL3257535118	54% GC	175	145	139	6	139	0	99.29	100
AMPL4392598603	55% GC;6C	177	145	142	3	142	0	98.61	100

Amplicon Name	Amplicon Genomic Content (GC; Other Features)[1]	Amplicon Length	NA12878 vs NIST						
			Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]
AMPL4394085835	37% GC;6T	167	136	136	0	136	0	100	100
AMPL4394068372	35% GC;6T	153	153	153	0	153	0	100	100
AMPL4039838351	61% GC	156	156	156	0	156	0	100	100
AMPL4392611784	60% GC;6G	171	169	166	3	166	0	98.22	100
AMPL4304947808	43% GC	159	159	159	0	159	0	100	100
AMPL4392311692	30% GC	173	144	144	0	144	0	100	100
AMPL3198880818	54% GC	168	142	142	0	142	0	100	100
AMPL4395108860	54% GC	160	158	157	1	157	0	99.37	100
AMPL4318413913	60% GC	179	132	132	0	132	0	100	100
AMPL461585857	62% GC;6T	178	176	176	0	176	0	100	100
AMPL4394997727	35% GC	159	157	155	2	155	0	99.36	100
AMPL4393286038	37% GC	124	124	124	0	124	0	100	100
AMPL4392312449	53% GC	131	131	131	0	131	0	100	100
AMPL4373352228	32% GC	167	167	167	0	167	0	100	100
AMPL598421930	37% GC;13T	179	121	121	0	121	0	100	100
AMPL4394316952	34% GC;6A;7T	173	147	147	0	147	0	100	100
AMPL3497995644	40% GC	144	144	144	0	144	0	100	100
AMPL4391994088	56% GC	157	128	128	0	128	0	100	100
AMPL4393073176	51% GC;6A	172	137	136	1	136	0	100	100
AMPL3666903404	61% GC	160	110	110	0	110	0	100	100
AMPL735306822	35% GC	83	54	54	0	54	0	100	100
AMPL4374432645	39% GC;6G	160	160	160	0	160	0	100	100
AMPL4394446055	29% GC	153	101	99	2	99	0	98.02	100
AMPL3770343132	59% GC	169	140	140	0	140	0	100	100
AMPL4394312266	57% GC	172	172	172	0	172	0	100	100
AMPL4373941627	68% GC	179	179	177	2	177	0	99.44	100
AMPL4391994985	56% GC	165	165	163	2	163	0	98.79	100
AMPL4394057009	32% GC	174	174	174	0	174	0	100	100
AMPL912086672	28% GC	164	136	136	0	136	0	100	100
AMPL905336021	32% GC	117	117	117	0	117	0	100	100
AMPL4394077238	34% GC;6T	169	115	115	0	115	0	100	100
AMPL4392286218	36% GC	173	173	173	0	173	0	100	100
AMPL4394548445	46% GC	164	162	162	0	162	0	100	100
AMPL4392296241	62% GC	76	76	76	0	76	0	100	100
AMPL556873511	29% GC	168	168	167	1	167	0	99.40	100
AMPL4392283526	41% GC	178	178	178	0	178	0	100	100
AMPL4392722819	45% GC	175	175	174	1	174	0	100	100
AMPL4392277638	50% GC	133	133	133	0	133	0	100	100
AMPL4373957362	27% GC;7T	152	87	87	0	87	0	100	100
AMPL4392651475	45% GC;6A	175	175	175	0	175	0	100	100
AMPL3379180838	44% GC	131	131	130	1	130	0	99.24	100
AMPL3498234957	33% GC;10A	171	137	137	0	137	0	100	100
AMPL3073489807	29% GC	162	162	162	0	162	0	100	100

Amplicon Name	Amplicon Genomic Content (GC; Other Features)[1]	Amplicon Length	NA12878 vs NIST						
			Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]
AMPL4392487356	42% GC	143	143	141	2	141	0	100	100
AMPL4392495150	41% GC	159	159	157	2	157	0	100	100
AMPL4394453234	39% GC;11AC	174	133	132	1	132	0	99.25	100
AMPL581765267	33% GC;7T	176	150	149	1	149	0	100	100
AMPL4391973685	55% GC	167	167	167	0	167	0	100	100
AMPL4392278772	60% GC	169	132	132	0	132	0	100	100
AMPL4392278508	64% GC;6C	146	117	117	0	117	0	100	100
AMPL4219350794	46% GC	168	168	168	0	168	0	100	100
AMPL4395093302	52% GC	176	176	176	0	176	0	100	100
AMPL3255695397	52% GC	170	170	170	0	170	0	100	100
AMPL4394436054	58% GC	173	173	172	1	172	0	100	100
AMPL4391970287	44% GC	181	181	180	1	180	0	99.45	100
AMPL4393295145	41% GC;7T	176	123	123	0	123	0	100	100
AMPL4392692126	61% GC;6C	155	126	93	33	92	1	73.02	98.92
AMPL4393058046	45% GC	89	89	88	1	88	0	100	100
AMPL4395087540	52% GC	177	151	151	0	151	0	100	100
AMPL4393144102	29% GC	82	82	82	0	82	0	100	100
AMPL4392648148	39% GC;7T	173	147	147	0	147	0	100	100
AMPL4392262531	50% GC	177	128	128	0	128	0	100	100
AMPL4394514215	40% GC	108	108	108	0	108	0	100	100
AMPL748437993	48% GC	178	147	99	48	99	0	67.35	100
AMPL4393315786	46% GC;6T	138	107	107	0	107	0	100	100
AMPL736692473	38% GC;6A	112	112	112	0	112	0	100	100
AMPL4305635694	79% GC;6G	168	107	107	0	107	0	100	100
AMPL4395062735	31% GC	118	118	118	0	118	0	100	100
AMPL4219407492	38% GC	125	125	125	0	125	0	100	100
AMPL4222168082	32% GC;6A	169	169	167	2	167	0	98.82	100
AMPL4301889960	35% GC;6T	154	154	154	0	154	0	100	100
AMPL4374007010	59% GC	182	182	182	0	182	0	100	100
AMPL4393227469	32% GC;8T	172	142	142	0	142	0	100	100
AMPL4392596733	60% GC;6G	163	163	162	1	162	0	99.39	100
AMPL4395311255	60% GC;8TG;9GT	136	81	81	0	81	0	100	100
AMPL4392251505	59% GC	141	111	111	0	111	0	100	100
AMPL4227407944	41% GC	153	153	153	0	153	0	100	100
AMPL4395087555	43% GC	179	179	177	2	177	0	98.88	100
AMPL4322933975	38% GC;8T	148	114	110	4	110	0	97.35	100
AMPL4394545846	49% GC	132	132	132	0	132	0	100	100
AMPL4394601334	46% GC	152	152	150	2	150	0	99.34	100
AMPL4373689558	44% GC	181	181	181	0	181	0	100	100
AMPL4391975825	57% GC	181	121	119	2	119	0	98.35	100
AMPL3498362734	41% GC;6A	140	112	112	0	112	0	100	100
AMPL4392997426	35% GC;8A	173	115	114	1	114	0	100	100
AMPL4392261532	48% GC	180	180	180	0	180	0	100	100

Amplicon Name	Amplicon Genomic Content (GC; Other Features)[1]	Amplicon Length	NA12878 vs NIST						
			Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]
AMPL552238544	43% GC	172	172	172	0	172	0	100	100
AMPL551297934	45% GC;6T;7A	170	138	138	0	138	0	100	100
AMPL3498059646	51% GC	175	175	175	0	175	0	100	100
AMPL4393190958	36% GC;8T	180	153	153	0	153	0	100	100
AMPL4392317694	43% GC;14CA	175	111	111	0	111	0	100	100
AMPL4393183760	42% GC	140	98	98	0	98	0	100	100
AMPL1629455589	37% GC	170	170	170	0	170	0	100	100
AMPL3653082204	45% GC	176	146	146	0	146	0	100	100
AMPL4391964653	31% GC;6T;6A	171	171	170	1	170	0	99.42	100
AMPL1211083733	61% GC	185	185	185	0	185	0	100	100
AMPL407783594	52% GC	168	168	167	1	167	0	99.40	100
AMPL1663497431	62% GC;6G	178	146	145	1	145	0	99.32	100
AMPL863277015	65% GC	182	182	176	6	176	0	96.70	100
AMPL4394285134	58% GC;5AAG	155	121	121	0	121	0	100	100
AMPL3433472167	70% GC	173	173	173	0	173	0	100	100
AMPL4394027906	56% GC	176	148	148	0	148	0	100	100
AMPL416374362	65% GC	113	113	113	0	113	0	100	100
AMPL4393199548	41% GC	170	170	170	0	170	0	100	100
AMPL3438454613	71% GC	114	114	114	0	114	0	100	100
AMPL4394498383	62% GC	180	172	172	0	172	0	100	100
AMPL4392247152	63% GC	159	157	157	0	157	0	100	100
AMPL4392197049	69% GC	185	185	184	1	184	0	99.46	100
AMPL4393991123	60% GC	182	182	182	0	182	0	100	100
AMPL4393996246	62% GC	182	150	150	0	150	0	100	100
AMPL4392574004	63% GC	134	84	84	0	84	0	100	100
AMPL4392530564	66% GC	187	187	185	2	185	0	98.93	100
AMPL4392174571	66% GC	152	152	152	0	152	0	100	100
AMPL3682590436	60% GC;6G	166	166	164	2	164	0	98.80	100
AMPL4392240361	58% GC	176	176	116	60	116	0	65.91	100
AMPL4395049446	68% GC	185	185	184	1	184	0	99.46	100
AMPL3518568818	33% GC;6T	172	156	156	0	156	0	100	100
AMPL4394999207	46% GC;9A	171	143	143	0	143	0	100	100
AMPL4393163023	38% GC;6T	174	174	174	0	174	0	100	100
AMPL4393980045	39% GC	176	176	176	0	176	0	100	100
AMPL4393983602	45% GC	176	176	176	0	176	0	100	100
AMPL4393695146	44% GC;7A;6T	174	148	77	71	77	0	52.03	100
AMPL4343482687	33% GC	73	73	73	0	73	0	100	100
AMPL4393984337	44% GC	154	123	123	0	123	0	100	100
AMPL4059164024	36% GC	158	158	156	2	156	0	98.73	100
AMPL4394279241	26% GC	172	114	113	1	113	0	99.12	100
AMPL4393723902	39% GC	176	176	176	0	176	0	100	100
AMPL4392346671	37% GC	167	167	166	1	166	0	100	100
AMPL4393984326	41% GC;10A	152	79	79	0	79	0	100	100

Amplicon Name	Amplicon Genomic Content (GC; Other Features)[1]	Amplicon Length	NA12878 vs NIST						
			Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]
AMPL4393961771	39% GC	176	176	176	0	176	0	100	100
AMPL4392235190	36% GC	178	178	178	0	178	0	100	100
AMPL4392253940	60% GC;6G;6G	170	90	89	1	89	0	98.89	100
AMPL4392992251	41% GC;6T	119	91	91	0	91	0	100	100
AMPL4373707639	33% GC	93	93	93	0	93	0	100	100
AMPL4392220658	35% GC;7A	157	95	95	0	95	0	100	100
AMPL4394603296	34% GC;6T;5TG;7TG	152	92	92	0	92	0	100	100
AMPL4393051580	46% GC	169	169	167	2	167	0	100	100
AMPL4393964460	51% GC	150	150	150	0	150	0	100	100
AMPL3434408361	45% GC	175	175	175	0	175	0	100	100
AMPL4392985517	42% GC	163	163	163	0	163	0	100	100
AMPL4395015331	44% GC;5TC	180	157	157	0	157	0	100	100
AMPL4392990319	42% GC	166	166	166	0	166	0	100	100
AMPL4392216992	27% GC;6T	147	147	147	0	147	0	100	100
AMPL4393954745	49% GC	173	144	144	0	144	0	100	100
AMPL4393010759	53% GC	162	162	158	4	158	0	100	100
AMPL711877918	62% GC	180	150	150	0	150	0	100	100
AMPL4392573539	30% GC;7T;13A	172	114	113	1	113	0	99.12	100
AMPL4373702125	37% GC	150	150	150	0	150	0	100	100
AMPL4393942602	29% GC	172	142	142	0	142	0	100	100
AMPL4393950751	43% GC	171	168	167	1	167	0	99.40	100
AMPL4392987625	45% GC	166	166	166	0	166	0	100	100
AMPL4316030239	37% GC;9A;6A	153	97	97	0	97	0	100	100
AMPL4394982619	42% GC	168	168	168	0	168	0	100	100
AMPL1158164934	45% GC	169	141	141	0	141	0	100	100
AMPL3683333814	62% GC	125	125	125	0	125	0	100	100
AMPL755591047	64% GC	125	96	96	0	96	0	100	100
AMPL575431534	72% GC;5GAG	185	182	181	1	181	0	99.45	100
AMPL4394281382	33% GC	164	164	164	0	164	0	100	100
AMPL575510303	28% GC;6A;7A	173	147	144	3	144	0	98.63	100
AMPL4393743713	40% GC;6A	175	147	147	0	147	0	100	100
AMPL4392200573	41% GC;6A	149	149	149	0	149	0	100	100
AMPL4393737942	50% GC	175	175	174	1	174	0	99.43	100
AMPL4393656617	78% GC;6C	184	184	184	0	184	0	100	100
AMPL4394594066	31% GC;6A	167	138	135	3	135	0	100	100
AMPL571636108	31% GC;7A	162	136	136	0	136	0	100	100
AMPL868949058	30% GC;6T	169	169	169	0	169	0	100	100
AMPL4231728591	51% GC	152	150	150	0	150	0	100	100
AMPL4323577210	34% GC	141	141	141	0	141	0	100	100
AMPL4392936584	33% GC	172	145	145	0	145	0	100	100
AMPL4393915769	36% GC	168	168	168	0	168	0	100	100
AMPL4022901967	32% GC;6A	125	118	118	0	118	0	100	100
AMPL415167643	41% GC	172	143	142	1	142	0	99.30	100

Amplicon Name	Amplicon Genomic Content (GC; Other Features)[1]	Amplicon Length	NA12878 vs NIST						
			Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]
AMPL4323505451	34% GC	144	144	144	0	144	0	100	100
AMPL4393686515	35% GC;9T	172	119	119	0	119	0	100	100
AMPL4374230397	53% GC	179	179	179	0	179	0	100	100
AMPL4392992071	50% GC	82	82	82	0	82	0	100	100
AMPL719291358	61% GC	173	144	144	0	144	0	100	100
AMPL4393683810	31% GC	172	165	164	1	164	0	99.39	100
AMPL4374399269	37% GC	169	169	169	0	169	0	100	100
AMPL4392203039	60% GC	139	121	121	0	121	0	100	100
AMPL4395001028	65% GC	163	163	162	1	162	0	100	100
AMPL4395342492	46% GC	175	163	163	0	163	0	100	100
AMPL4392223494	45% GC	174	143	143	0	143	0	100	100
AMPL4392215902	58% GC	169	169	163	6	163	0	96.45	100
AMPL596607752	74% GC;7C	171	171	171	0	171	0	100	100
AMPL596173137	61% GC	171	171	171	0	171	0	100	100
AMPL4320903836	68% GC	178	122	121	1	121	0	99.18	100
AMPL417791888	41% GC	170	170	170	0	170	0	100	100
AMPL4307576268	42% GC	154	154	154	0	154	0	100	100
AMPL413267348	30% GC	172	170	169	1	169	0	100	100
AMPL4393613220	63% GC;7G	175	143	142	1	142	0	99.30	100
AMPL2692711234	27% GC;7T	131	78	78	0	78	0	100	100
AMPL4392926980	32% GC	168	168	168	0	168	0	100	100
AMPL3676783348	38% GC	168	168	168	0	168	0	100	100
AMPL2692395510	35% GC	176	176	175	1	175	0	100	100
AMPL3936418821	62% GC;6C	174	174	173	1	173	0	100	100
AMPL4374406487	35% GC	158	126	126	0	126	0	100	100
AMPL4393927397	33% GC	126	101	101	0	101	0	100	100
AMPL4373781431	35% GC	170	170	168	2	168	0	98.82	100
AMPL4393043045	26% GC	168	139	136	3	136	0	100	100
AMPL4038401239	53% GC	169	169	168	1	168	0	99.41	100
AMPL4392423431	37% GC	171	171	170	1	170	0	100	100
AMPL4392201407	65% GC	178	152	152	0	152	0	100	100
AMPL4302499643	71% GC	177	173	173	0	173	0	100	100
AMPL704947325	66% GC	188	134	134	0	134	0	100	100
AMPL4202336805	75% GC	188	103	103	0	103	0	100	100
AMPL4203136737	70% GC	185	112	112	0	112	0	100	100
AMPL4392191781	74% GC;6G	182	148	148	0	148	0	100	100
AMPL668652008	70% GC	177	119	119	0	119	0	100	100
AMPL3659553353	36% GC	157	157	157	0	157	0	100	100
AMPL4392890404	28% GC	145	145	145	0	145	0	100	100
AMPL4392895580	39% GC;6A	169	169	169	0	169	0	100	100
AMPL1153902623	35% GC	161	161	159	2	159	0	98.76	100
AMPL3679236852	79% GC	121	77	77	0	77	0	100	100
AMPL3498104622	41% GC	178	178	177	1	177	0	99.44	100

Amplicon Name	Amplicon Genomic Content (GC; Other Features)[1]	Amplicon Length	NA12878 vs NIST						
			Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]
AMPL708625773	69% GC	172	172	31	141	31	0	18.02	100
AMPL4302673454	32% GC	171	171	171	0	171	0	100	100
AMPL1705657360	46% GC	173	173	173	0	173	0	100	100
AMPL4392692973	44% GC	171	171	170	1	170	0	100	100
AMPL3797942774	35% GC;7T;18A;6A	153	90	88	2	88	0	97.78	100
AMPL3498144888	50% GC	149	149	148	1	148	0	99.33	100
AMPL3299722549	62% GC	178	178	175	3	175	0	98.31	100
AMPL4392675093	62% GC;6C	181	156	134	22	134	0	98.53	100
AMPL4393781331	55% GC;6G	180	180	179	1	179	0	100	100
AMPL4393786655	24% GC	170	92	92	0	92	0	100	100
AMPL4393896650	48% GC	109	109	109	0	109	0	100	100
AMPL4392914397	50% GC	160	155	155	0	155	0	100	100
AMPL4395281946	29% GC	171	171	171	0	171	0	100	100
AMPL4395290340	40% GC	143	143	143	0	143	0	100	100
AMPL4393619887	25% GC	117	117	117	0	117	0	100	100
AMPL3710790718	36% GC	98	98	98	0	98	0	100	100
AMPL4395266793	44% GC	81	81	81	0	81	0	100	100
AMPL4392189306	43% GC	114	59	59	0	59	0	100	100
AMPL4392192649	61% GC	179	150	148	2	148	0	98.67	100
AMPL4393619016	61% GC	182	182	182	0	182	0	100	100
AMPL2455591517	59% GC	170	170	170	0	170	0	100	100
AMPL4394566481	64% GC	151	149	148	1	148	0	99.33	100
AMPL4325322943	63% GC	125	125	124	1	124	0	99.20	100
AMPL652088025	50% GC	111	111	111	0	111	0	100	100
AMPL4392182082	66% GC	181	181	180	1	180	0	99.45	100
AMPL1519565349	49% GC;5GT	169	140	139	1	139	0	99.29	100
AMPL408214700	59% GC;6C	127	127	126	1	126	0	99.21	100
AMPL4395267587	64% GC	183	183	182	1	182	0	99.45	100
AMPL4392872919	36% GC	169	167	167	0	167	0	100	100
AMPL402546844	42% GC	92	92	92	0	92	0	100	100
AMPL659232630	59% GC	157	155	154	1	154	0	99.35	100
AMPL4395306426	49% GC	182	144	144	0	144	0	100	100
AMPL528631298	74% GC	176	113	0	113	0	0	0	0
AMPL4392187570	47% GC;6A	148	121	121	0	121	0	100	100
AMPL4395394628	41% GC	152	152	152	0	152	0	100	100
AMPL4392903847	50% GC	101	101	101	0	101	0	100	100
AMPL4394224699	63% GC;6C	185	185	184	1	184	0	99.46	100
AMPL4320019211	69% GC;7C	184	158	93	65	93	0	59.24	100
AMPL4392351914	66% GC	163	134	133	1	133	0	100	100
AMPL4298148304	60% GC;5TCC	161	118	118	0	118	0	100	100
AMPL3198712213	63% GC	179	153	153	0	153	0	100	100
AMPL4394465691	58% GC	154	154	148	6	148	0	100	100
AMPL4393628011	60% GC	163	163	161	2	161	0	98.77	100

Amplicon Name	Amplicon Genomic Content (GC; Other Features)[1]	Amplicon Length	NA12878 vs NIST						
			Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]
AMPL4394223117	64% GC	169	148	147	1	147	0	99.32	100
AMPL4394360574	64% GC	156	156	122	34	118	4	84.29	96.72
AMPL4394214041	58% GC;7GT	184	98	97	1	97	0	98.98	100
AMPL4394678126	65% GC	184	134	0	134	0	0	0	0
AMPL4394220527	60% GC	134	134	134	0	134	0	100	100
AMPL3674673921	62% GC	167	167	167	0	167	0	100	100
AMPL583119206	51% GC	167	139	139	0	139	0	100	100
AMPL1486637447	35% GC	103	73	67	6	67	0	100	100
AMPL3378946700	38% GC	171	171	171	0	171	0	100	100
AMPL4394922626	34% GC	176	176	176	0	176	0	100	100
AMPL588811071	48% GC	108	108	108	0	108	0	100	100
AMPL3817158157	40% GC	169	140	140	0	140	0	100	100
AMPL4394216319	63% GC	163	163	162	1	162	0	99.39	100
AMPL4395303483	47% GC	156	156	155	1	154	1	98.72	99.35
AMPL4324007004	41% GC	93	72	72	0	72	0	100	100
AMPL4395288076	51% GC	178	135	135	0	135	0	100	100
AMPL538987753	36% GC;6T	170	170	170	0	170	0	100	100
AMPL4394633624	33% GC	178	178	176	2	176	0	99.44	100
AMPL4392357297	24% GC;9T	167	167	165	2	165	0	99.40	100
AMPL4393606166	57% GC	183	183	182	1	182	0	99.45	100
AMPL4393136614	64% GC;6G	144	144	143	1	143	0	100	100
AMPL4394209036	61% GC	160	160	159	1	159	0	99.38	100
AMPL4394553261	46% GC	168	168	167	1	167	0	100	100
AMPL1496946897	46% GC	180	160	160	0	160	0	100	100
AMPL3519304014	41% GC	74	74	74	0	74	0	100	100
AMPL4392413266	57% GC;6G;5TG	183	183	182	1	182	0	100	100
AMPL4395265123	33% GC	170	170	169	1	169	0	99.41	100
AMPL3295318277	36% GC;7T	184	151	151	0	151	0	100	100
AMPL4317912554	61% GC	118	118	117	1	117	0	99.15	100
AMPL4395343628	34% GC;8T;6T	172	170	168	2	168	0	100	100
AMPL1517948259	38% GC	170	170	170	0	170	0	100	100
AMPL4393860661	35% GC	156	156	156	0	156	0	100	100
AMPL4373761294	34% GC	132	132	132	0	132	0	100	100
AMPL4393823209	34% GC	178	178	178	0	178	0	100	100
AMPL420799557	33% GC;6T;7T	75	75	75	0	75	0	100	100
AMPL416087660	26% GC;7A	119	119	119	0	119	0	100	100
AMPL4374301969	39% GC	176	176	176	0	176	0	100	100
AMPL4392815412	40% GC	176	176	176	0	176	0	100	100
AMPL4393570611	51% GC;12A	173	173	172	1	172	0	99.42	100
AMPL4395233644	46% GC	177	177	176	1	176	0	99.44	100
AMPL4392696459	22% GC	163	163	162	1	162	0	100	100
AMPL4392670346	32% GC;8T;12TC;6TG	179	143	142	1	142	0	99.30	100
AMPL4393550446	37% GC	183	183	183	0	183	0	100	100

Amplicon Name	Amplicon Genomic Content (GC; Other Features)[1]	Amplicon Length	NA12878 vs NIST						
			Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]
AMPL3498752779	39% GC;6A	158	156	155	1	155	0	99.36	100
AMPL557689580	32% GC;10T	155	155	154	1	154	0	100	100
AMPL4392824175	43% GC	132	132	132	0	132	0	100	100
AMPL4392796221	45% GC	112	83	83	0	83	0	100	100
AMPL3637426447	33% GC;9T;13T	147	101	100	1	100	0	99.01	100
AMPL3199428974	49% GC	170	143	143	0	143	0	100	100
AMPL4393547526	31% GC	163	127	126	1	126	0	99.21	100
AMPL4394883639	38% GC	164	164	164	0	164	0	100	100
AMPL4393119864	26% GC;6A	119	117	117	0	117	0	100	100
AMPL391855502	56% GC	180	180	180	0	180	0	100	100
AMPL4392820663	52% GC	178	178	178	0	178	0	100	100
AMPL4392803596	42% GC;8T	178	178	177	1	177	0	99.44	100
AMPL4374504539	37% GC	156	156	155	1	155	0	100	100
AMPL4395008361	36% GC	155	155	153	2	153	0	99.35	100
AMPL4374372220	40% GC	174	174	174	0	174	0	100	100
AMPL4392783695	43% GC	168	168	168	0	168	0	100	100
AMPL4392776634	33% GC;8T	82	82	82	0	82	0	100	100
AMPL4394847665	41% GC	172	172	171	1	171	0	99.42	100
AMPL4393146133	61% GC;7G	180	180	177	3	177	0	98.88	100
AMPL4392805233	54% GC	153	153	152	1	152	0	99.35	100
AMPL4393330449	29% GC	177	177	176	1	176	0	99.44	100
AMPL4393549181	37% GC;6A	178	178	178	0	178	0	100	100
AMPL4393560571	49% GC	178	178	178	0	178	0	100	100
AMPL4394644825	44% GC	143	143	142	1	142	0	100	100
AMPL562172230	39% GC	173	173	172	1	172	0	100	100
AMPL562219796	51% GC;5AC	161	161	161	0	161	0	100	100
AMPL4393539439	27% GC;6T	161	161	160	1	160	0	99.38	100
AMPL4392146647	59% GC	177	177	177	0	177	0	100	100
AMPL769894769	58% GC	110	110	108	2	108	0	99.08	100
AMPL3374963808	51% GC	174	174	174	0	174	0	100	100
AMPL3720788183	45% GC;6T	181	122	122	0	122	0	100	100
AMPL4395377014	61% GC	177	177	176	1	176	0	99.44	100
AMPL3681904054	33% GC	116	116	115	1	115	0	99.14	100
AMPL4393140419	39% GC;10A;7T	181	178	176	2	176	0	99.44	100
AMPL4085226812	63% GC	86	86	86	0	86	0	100	100
AMPL4373740005	42% GC	177	177	177	0	177	0	100	100
AMPL4393856361	54% GC	176	176	175	1	175	0	99.43	100
AMPL4373672931	57% GC	164	164	164	0	164	0	100	100
AMPL4392120439	63% GC	179	142	142	0	142	0	100	100
AMPL4300890407	46% GC	143	143	143	0	143	0	100	100
AMPL4301194040	53% GC;5CA	182	182	182	0	182	0	100	100
AMPL544389827	66% GC	171	171	171	0	171	0	100	100
AMPL4216700451	57% GC	180	180	178	2	178	0	98.89	100

Amplicon Name	Amplicon Genomic Content (GC; Other Features)[1]	Amplicon Length	NA12878 vs NIST						
			Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]
AMPL1156353735	38% GC	141	141	141	0	141	0	100	100
AMPL4300833980	43% GC;6T	178	178	178	0	178	0	100	100
AMPL4393523704	32% GC;7A;7T	174	138	137	1	137	0	99.28	100
AMPL4395213682	34% GC	170	170	170	0	170	0	100	100
AMPL789566107	63% GC	178	178	178	0	178	0	100	100
AMPL4393540427	50% GC	172	172	172	0	172	0	100	100
AMPL789212884	49% GC	176	176	176	0	176	0	100	100
AMPL4393492671	55% GC	179	179	179	0	179	0	100	100
AMPL4394161757	65% GC	180	180	180	0	180	0	100	100
AMPL4392701696	59% GC	175	173	172	1	170	2	98.27	98.84
AMPL4393462046	70% GC;6G	160	160	129	31	129	0	80.63	100
AMPL3889188256	53% GC	166	166	164	2	164	0	98.80	100
AMPL4395202607	41% GC	158	158	158	0	158	0	100	100
AMPL4392113442	50% GC	179	179	178	1	178	0	99.44	100
AMPL4395050447	27% GC;10T;8T	75	75	73	2	73	0	98.65	100
AMPL4393470475	58% GC	185	185	184	1	184	0	99.46	100
AMPL4392777432	63% GC;6C	174	174	173	1	173	0	99.43	100
AMPL2949788169	60% GC	181	181	79	102	79	0	43.65	100
AMPL4392758534	63% GC	179	179	179	0	179	0	100	100
AMPL4393492728	60% GC	179	179	179	0	179	0	100	100
AMPL4392107402	64% GC	136	136	136	0	136	0	100	100
AMPL4392128611	63% GC	150	150	150	0	150	0	100	100
AMPL549199875	62% GC	152	152	152	0	152	0	100	100
AMPL549072782	50% GC;7A	177	177	175	2	175	0	99.43	100
AMPL4393041536	36% GC	135	135	133	2	133	0	99.25	100
AMPL4392109226	65% GC	179	177	177	0	177	0	100	100
AMPL573434660	63% GC	171	169	169	0	169	0	100	100
AMPL4392117695	58% GC	156	156	156	0	156	0	100	100
AMPL4395175643	57% GC	181	181	181	0	181	0	100	100
AMPL4373650991	58% GC	183	183	183	0	183	0	100	100
AMPL4392110866	62% GC	172	172	172	0	172	0	100	100
AMPL4394139126	67% GC	118	118	116	2	116	0	98.31	100
AMPL555450618	70% GC	179	179	179	0	179	0	100	100
AMPL555283876	60% GC;6C	171	171	169	2	169	0	98.83	100
AMPL4392759751	41% GC;6A	170	170	170	0	170	0	100	100
AMPL4392767076	55% GC	164	164	164	0	164	0	100	100
AMPL4394809934	60% GC	151	151	149	2	149	0	98.68	100
AMPL4394147071	39% GC	176	122	121	1	121	0	99.18	100
AMPL3289654030	30% GC	177	177	176	1	176	0	100	100
AMPL3961593542	25% GC	178	178	177	1	177	0	99.44	100
AMPL4392708195	44% GC;8T;8TC	176	93	91	2	91	0	97.85	100
AMPL4394756215	37% GC	179	152	152	0	152	0	100	100
AMPL4394758212	56% GC	171	171	171	0	171	0	100	100

Amplicon Name	Amplicon Genomic Content (GC; Other Features)[1]	Amplicon Length	NA12878 vs NIST						
			Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]
AMPL4392098735	69% GC	185	185	185	0	185	0	100	100
AMPL4393028379	76% GC	186	186	170	16	170	0	99.42	100
AMPL700490202	45% GC;7A	153	153	153	0	153	0	100	100
AMPL3764819988	66% GC	154	154	153	1	153	0	99.35	100
AMPL4393070345	65% GC;6G	154	154	153	1	153	0	100	100
AMPL597978528	63% GC	169	169	168	1	168	0	99.41	100
AMPL3569591894	65% GC;6C	133	133	133	0	133	0	100	100
AMPL4393411927	66% GC;6G	176	176	69	107	69	0	39.20	100
AMPL4393455182	58% GC	180	180	138	42	138	0	76.67	100
AMPL4393432724	60% GC;6C	174	174	173	1	173	0	99.43	100
AMPL3695208132	61% GC;6C;6G;7G	174	174	172	2	172	0	98.85	100
AMPL4393407496	60% GC	171	171	170	1	170	0	100	100
AMPL4392668212	59% GC	154	154	153	1	153	0	100	100
AMPL4394405741	64% GC	165	165	82	83	82	0	50	100
AMPL4392096706	66% GC	183	183	181	2	181	0	98.91	100
AMPL4395386501	58% GC	175	175	175	0	175	0	100	100
AMPL4392102936	62% GC	176	176	176	0	176	0	100	100
AMPL4297998702	63% GC	102	102	102	0	102	0	100	100
AMPL532298001	71% GC	180	180	180	0	180	0	100	100
AMPL4394751711	59% GC	146	146	146	0	146	0	100	100
AMPL4395148488	61% GC	180	180	179	1	179	0	99.44	100
AMPL1118439227	65% GC	178	178	153	25	153	0	85.96	100
AMPL4394736429	61% GC;6C	159	142	141	1	141	0	99.30	100
AMPL551935865	61% GC	173	173	173	0	173	0	100	100
AMPL4392073003	52% GC	165	165	165	0	165	0	100	100
AMPL4394129588	60% GC;6G	159	159	154	5	154	0	96.86	100
AMPL4373586961	30% GC;6A	172	172	171	1	171	0	99.42	100
AMPL595971724	63% GC;6G	170	170	170	0	170	0	100	100
AMPL4394610057	71% GC;6C	180	180	178	2	178	0	99.44	100
AMPL4392084924	65% GC	179	179	179	0	179	0	100	100
AMPL4392744809	61% GC	183	183	182	1	182	0	99.45	100
AMPL4395355273	64% GC	139	139	139	0	139	0	100	100
AMPL4373723911	60% GC	174	174	174	0	174	0	100	100
AMPL4392449135	53% GC;10TC;10AC;8TC	179	97	94	3	94	0	96.91	100
AMPL4392752461	63% GC	180	170	170	0	170	0	100	100
AMPL4395147724	34% GC;6A	166	141	141	0	141	0	100	100
AMPL4392928879	40% GC	157	157	157	0	157	0	100	100
AMPL4394730094	37% GC;6G	169	169	169	0	169	0	100	100
AMPL1676134309	39% GC	138	107	106	1	106	0	100	100
AMPL749578720	46% GC	155	136	136	0	136	0	100	100
AMPL4392707708	46% GC	142	140	140	0	140	0	100	100
AMPL553632290	44% GC;10T	170	142	142	0	142	0	100	100
AMPL4393378805	56% GC	158	158	158	0	158	0	100	100

Amplicon Name	Amplicon Genomic Content (GC; Other Features)[1]	Amplicon Length	NA12878 vs NIST						
			Covered Fragment Size[2]	Calls[3]	No Calls	Correct Calls	Miscalls[4]	% Correct Calls including No Calls[5]	% Correct Calls excluding No Calls[6]
AMPL569668692	73% GC	178	148	14	134	14	0	9.66	100
AMPL4314163957	61% GC	173	173	173	0	173	0	100	100

[1] other features include homopolymers, di-nucleotide repeats, tri-nucleotide repeats (e.g., 6A indicates a homopolymer six adenine residues in length, 8TCC indicates the 8 repeats of the tri-nucleotide 'TCC'.

[2] length of the amplicon fragment covered by the NIST highly confident sequence for NA12878.

[3] total number of calls made (correct calls + miscalls) for the pre-selected sequencing run.

[4] number of incorrect calls for the pre-selected sequencing run.

[5] % correct calls including no calls = 100 × (# correct calls / [# calls + # no calls])

[6] % correct calls excluding no calls = 100 × (# correct calls / # calls)

Table 33 Summary of Overall Accuracy of Ion PGM™ Dx Sequencing Results at Variant and Non-Variant Locations

Sample	# Amplicons	% Amplicon Coverage	Variant Expected	Variants Correctly Called	Variants Missed	Non-variant Bases Called Correctly	PPA[1]	PPA[2]	NPA[3]	NPA[4]	Overall Agreement (Accuracy)[5]	Overall Agreement (Accuracy)[6]
NA12878	632	96.98[a]	399	379	6	98189	98.43	94.24	99.89	96.88	96.87	99.88
NA19240	632	96.53[a]	473	449	4	97631	99.12	94.93	99.86	96.4	96.39	99.86
NA12878_NIST	470	67.40[b]	299	281	7	68286	97.57	93.98	99.99	97.77	97.76	99.98

[a] Percentage of the amplicon bases that were analyzed by Variant Caller after filtering.

[b] Percentage of the 470 amplicons for which NIST generated high confident region sequence listed a call. The remaining amplicon bases did not fall into the NIST high confident region sequence.

[1] Positive percent agreement (PPA) = 100 × TP/(TP + FN). TP = variants called correctly when compared to the sponsor-generated variant reference sequence. FN = variants not called correctly. No-calls were not used in the calculation.

[2] Positive percent agreement (PPA) = 100 × TP/(TP + FN + NC). TP = variants called correctly when compared to the sponsor-generated variant reference sequence. FN = variants not called correctly.

[3] Negative percent agreement (NPA) = 100 × TN/(FP + TN). TN = Wild Type or Reference positions correctly identified as reference. FP = Wild Type positions not correctly called as WT. No-calls were not used in the calculation.

[4] Negative percent agreement (NPA) = 100 × TN/(FP + TN + NC). TN = Wild Type or Reference positions correctly identified as reference. FP = Wild Type positions not correctly called as WT.

[5] Percent Overall agreement including no calls.

[6] Percent Overall agreement excluding no calls. No calls were not used in the calculation.

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support
- Order and web support
- Safety Data Sheets (SDSs; also known as MSDSs)

Additional product documentation, including user guides and Certificates of Analysis, are available by contacting Customer Support.

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are printed and shipped with the product.

Obtaining Certificates of Conformance

The Certificate of Conformance provides information on conformance testing of each instrument provided with the system. Certificates of Conformance are shipped with the instrument, and are also available by contacting Customer Support at **thermofisher.com/support**.

Ion PGM™ Dx System

Publication Number MAN0016696 Revision D.0

This guide contains the information needed to prepare your site for installation of the Ion PGM™ Dx Sequencer, Ion Torrent™ Server, Ion OneTouch™ Dx Instrument, Ion OneTouch™ ES Dx Instrument, and Ion PGM™ Dx Chip Minifuge.

Site preparation workflow

A service representative will contact you to schedule the installation. When the installation is scheduled:

1. Receive and inspect the shipment (see "Receive and inspect the shipment" on page 20).
2. Move the crated instrument to the installation site (see "Move the crated instrument to the installation site" on page 20).
3. Complete the site preparation activities (see "Site preparation activities" on page 2).
4. Ensure the purchase order is complete.

Installation timeline and training

After the Ion PGM™ Dx System is uncrated, installation and testing takes 8 hours (qualification and training requires an additional 32 hours):

During and/or after installation, the service representative reviews data and provides some basic operator training. For additional training and reference information, see the user documents provided with the Ion PGM™ Dx System.

CE IVD

For In Vitro Diagnostic Use.

Site preparation activities

IMPORTANT! Complete all activities below before the scheduled installation date. If the activities are not complete when the service representative arrives, the scheduled installation may be postponed.

- Review the customer responsibilities and assign personnel (see "Customer responsibilities" on page 3).
- Identify an installation site that meets all requirements in this guide:
  - Space and clearance (see "Instrument clearances" on page 5)
  - Environmental (see "Environmental requirements" on page 11)
  - Gas (see "Gas requirements" on page 12)
  - Ventilation and waste collection (see "Ventilation and waste collection requirements" on page 13)
  - Electrical (see "Electrical requirements" on page 14)
  - Network (see "Network requirements" on page 17)
  - Safety (see "Safety requirements" on page 18)
- Confirm that all materials needed for installation and operation are available (see "Materials for installation and operation" on page 19).
- Confirm that the product was received and inspected (see "Receive and inspect the shipment" on page 20):
  - All items on the shipping list are the same items ordered at the time of purchase.
  - Any damage to shipping containers was reported to field service.
  - Any damage or mishandling was recorded on the shipping documents.
  - The reagents box was unpacked and stored as specified.
- Confirm the installation site is cleared and ready for instrument installation (see "Move the crated instrument to the installation site" on page 20).
- Confirm the crated instrument and other shipping containers have been moved to the installation site (see "Move the crated instrument to the installation site" on page 20).

Customer responsibilities

Personnel	Responsibilities
Site preparation/ installation coordinator	• Reviews the site preparation guide for safety information and instrument requirements. • Coordinates personnel and tasks. • Chooses the site. • Reviews checklists with applicable personnel, then with the service representative to verify that the site is properly prepared. • Receives and inspects the Ion PGM™ Dx System. • Stores the reagents box according to the specifications indicated in the product inserts. • Schedules the installation and informs personnel of the installation day. • Ensures that the site is clear of unnecessary material on the installation day. • Is available to assist the service representative throughout installation.
Laboratory safety representative	• Reviews the site preparation guide for safety information. • Ensures that the required safety practices and equipment are in place. • Is in the vicinity and available to the service representative at all times while the service representative is at the customer's facility.
Laboratory personnel/ primary users	• Review safety information. • Ensures that all customer-provided materials for installation are present at the site. • Ensures that primary users (responsible for training other users) are available during the installation, so that they can be trained on the instrument.
Facilities personnel	• Ensures that the installation requirements are met for: – Space at the installation site – Building clearances – Temperature and humidity – Waste collection – Electrical supply – Computer – Safety and installation materials • If possible, moves the crated Ion PGM™ Dx System to the site before the installation date. • Is available to assist service representative and laboratory personnel throughout installation. • If applicable, ensures that at least two people are available to help the service representative move and position the instrument.

(continued)

Personnel	Responsibilities
Network or IT specialist (if the instrument will be connected to a network)	• Ensures that active, tested local area network (LAN) connections are in place before the scheduled installation date. • Ensures that network hardware is compatible with an RJ45-type connector. • If necessary, supplies additional cables. • Is available during installation to connect the Ion PGM™ Dx System to the network. • If applicable, provides and installs a network or dedicated printer. ⚠ **CAUTION!** Do not attempt to connect the Ion PGM™ Dx System components to the network before the service representative arrives.

Site requirements

Dimensions and weights

To prepare for installation, provide space for receipt and configuration of the components listed in this section. This section provides dimensions and weights for the packages you will receive, and it describes the dimensions of the components after installation and configuration.

Crate dimensions and weights

Crate	Height	Length (depth)	Width	Weight
Ion PGM™ Dx Sequencer	67.3 cm (26.5 in)	66.0 cm (26.0 in)	74.9 cm (29.5 in)	39.1 kg (95.0 lbs)
Ion Torrent™ Server	71.9 cm (28.3 in)	70.6 cm (27.8 in)	34.3 cm (13.5 in)	29.9 kg (66.0 lbs)
Ion OneTouch™ Dx Instrument	45.7 cm (18.0 in)	45.7 cm (18.0 in)	53.3 cm (21.0 in)	20.0 kg (44.0 lbs)
Ion OneTouch™ ES Dx Instrument	36.8 cm (14.5 in)	36.8 cm (14.5 in)	44.4 cm (17.5 in)	5.9 kg (~13.0 lbs)
Ion PGM™ Dx Chip Minifuge	16.3 cm (6.4 in)	18.2 cm (7.2 in)	23.5 cm (9.2 in)	0.9 kg (2.0 lbs)

Instrument dimensions and weights

Component	Height	Length (depth)	Width	Weight
Ion PGM™ Dx Sequencer	53.3 cm (21.0 in)	50.8 cm (20.0 in)	61.0 cm (24.0 in)	29.5 kg (65.0 lbs)
Ion Torrent™ Server	56.6 cm (22.3 in)	53.8 cm (21.2 in)	21.6 cm (8.5 in)	24.9 kg (55.0 lbs)
Ion OneTouch™ Dx Instrument	30.5 cm (12.0 in)	40.6 cm (16.0 in)	35.6 cm (14.0 in)	17.0 kg (37.5 lbs)
Ion OneTouch™ ES Dx Instrument	24.1 cm (9.5 in)	40.6 cm (16.0 in)	27.9 cm (11.0 in)	5.4 kg (12.0 lbs)
Ion PGM™ Dx Chip Minifuge	11.2 cm (4.4 in)	15.3 cm (6.0 in)	15.3 cm (6.0 in)	0.8 kg (1.8 lbs)

Instrument clearances

During instrument setup and maintenance, it is necessary to access the back and sides of the instruments. If the back of an instrument faces a wall, it will be necessary to have enough space to rotate it on the bench for access.

IMPORTANT! For safety, the power outlet used for powering the instrument components must be accessible at all times.

Component	Top	Front	Left	Right	Back
Ion PGM™ Dx Sequencer	30.5 cm (12.0 in)	30.5 cm (12.0 in) [1]	10.0 cm (4.0 in)	20.0 cm (8.0 in)	10.0 cm (4.0 in)
Ion Torrent™ Server	5.0 cm (2.0 in)	30.5 cm (12.0 in)	5.0 cm (2.0 in)		61.0 cm (24.0 in)
Ion OneTouch™ Dx Instrument	30.5 cm (12.0 in)	30.5 cm (12.0 in)	10.0 cm (4.0 in)		10.0 cm (4.0 in)
Ion OneTouch™ ES Dx Instrument	30.5 cm (12.0 in)	30.5 cm (12.0 in)	30.5 cm (12.0 in)		30.5 cm (4.0 in)
Ion PGM™ Dx Chip Minifuge	30.5 cm (12.0 in)	10.0 cm (4.0 in)	10.0 cm (4.0 in)		10.0 cm (4.0 in)

[1] The instrument requires: 30.5 cm (12.0 in) from front edge of bench to sequencer bezel, 20.3 cm (8.0 in) from front edge of bench to the conical tubes, and 90.0 cm (36.0 in) aisle in front of bench for operator access.

Placement of the instruments and server

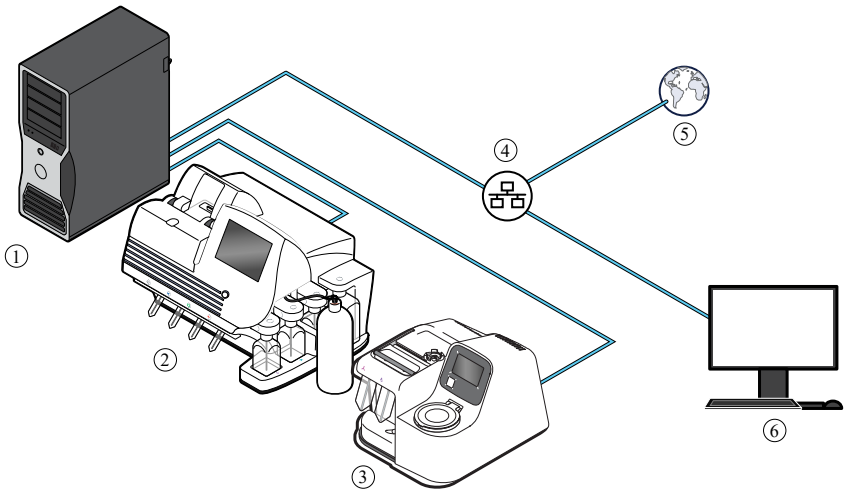
The installation room size must accommodate both the Ion PGM™ Dx Sequencer and the Ion Torrent™ Server. If the Ion PGM™ Dx Sequencer, the Ion Torrent™ Server, and the Ion OneTouch™ Dx Instrument are placed on a stationary or mobile bench, verify that the bench meets the requirements in "Dimensions and weights" on page 4.

Note: We are not responsible for any damage caused by using a laboratory bench that does not meet the minimum weight capacities requirements.

System layout

We support the layout in which the Ion Torrent™ Server is directly connected to the Ion PGM™ Dx Sequencer, rather than through the local area network from a remote location such as a server room. Data are most robustly transferred from the Ion PGM™ Dx Sequencer to the Ion Torrent™ Server when they are directly connected by a standard Category 6 Ethernet cable provided with the installation materials.

IMPORTANT! The Ion PGM™ Dx Sequencer must be connected to the Ion Torrent™ Server by a standard Category 6 Ethernet cable. We do not troubleshoot data transfer issues associated with an indirect connection between the Ion PGM™ Dx Sequencer and the Ion Torrent™ Server.



1. Ion Torrent™ Server
2. Ion PGM™ Dx Sequencer
3. Ion OneTouch™ Dx Instrument
4. Local area network
5. Internet
6. Torrent Suite™ Dx Software

Planning the installation

In preparation for the Ion PGM™ Dx System installation, you must plan the layout of your laboratory to accommodate library and template preparation activities, in addition to those related to chip preparation and sequencing. This section describes the stations involved in the sequencer workflow and the basic laboratory layouts.

Laboratory layout

When designing your laboratory layout, follow good laboratory practices to ensure reliable and contamination-free PCR results. Pay particular attention to the need to separate the areas for pre- and post-PCR activities. Isolating the amplicon source, separating pre-PCR from post-PCR activities, and dedicating laboratory supplies and/or equipment to each space can significantly reduce the potential for contamination.

As shown in the illustrations below, the Ion PGM™ Dx System can be deployed in both one- and two-room laboratory configurations. The two-room layout is highly recommenced due to the protection that it affords against contamination; however, the one-room layout will produce acceptable results if proper precautions are observed.

If you choose to deploy the Ion PGM™ Dx System in a one-room layout:

- Establish clearly-labeled, separate sets of pipettes for the library preparation, emulsion PCR (emPCR) setup, and emulsion breaking stations.
- Always move from "clean" to "dirty" (for example, from pre- to post-PCR). We do not recommend moving from "dirty" to "clean" (for example, do not handle post-amplification samples and then make libraries).

Note: The positions of the stations in the pre-and post- PCR rooms are not important.

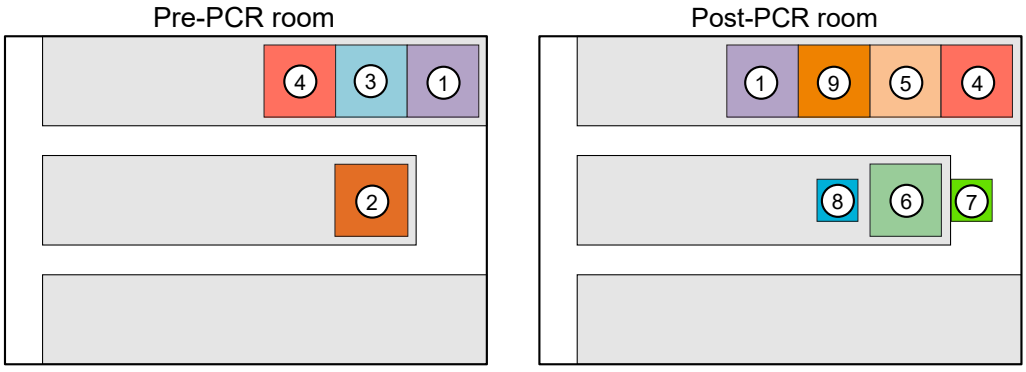


Figure 1 Two-room layout

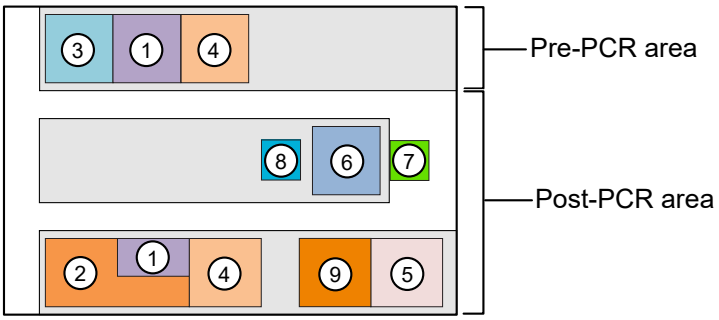


Figure 2 One-room layout

1. Centrifuge
2. Amplification mixture setup area
3. Library setup area
4. Pipettes
5. Ion OneTouch™ Dx Instrument, Ion OneTouch™ ES Dx Instrument, and Veriti™ Dx Thermal Cycler
6. Ion PGM™ Dx Sequencer and Ion Torrent™ Server
7. Gas cylinder
8. Ion PGM™ Dx Chip Minifuge

Laboratory workstations

The following table describes workstations associated with the generic laboratory layout for the Ion PGM™ Dx System. The stations are categorized in terms of their involvement pre- and post-PCR activities.

Note: Some stations (pipette and centrifuge) are present in both environments, but the equipment is not necessarily shared between the pre- and post-PCR stations.

#	Station	Location	Description/requirements
1	Centrifuges	Pre- and post-PCR areas	Centrifuges are used during library preparation and chip loading, so access is required in both the pre- and post-PCR areas. When planning centrifuge placement: • Consider dedicating separate centrifuges for the pre- and post-PCR operations to minimize contamination. • If only one centrifuge is available, place it in a central location, which is accessible from both areas. • Most importantly, place the centrifuges in convenient locations.

(continued)

#	Station	Location	Description/requirements
2	Amplification mixture setup area	Pre-PCR area	Whenever possible, amplification mixture preparation should be performed within a dedicated hood. When selecting a location for amplification mixture preparation: • The amplification mixture setup station requires a set of dedicated pipettes. • If only one hood is available within the pre-PCR area, dedicate the hood for amplification mixture preparation and perform library preparation elsewhere. • If a hood is unavailable within the pre-PCR area, select a bench that is sterilized regularly and preferably isolated from the neighboring stations. **IMPORTANT!** Separation of the amplification mixture preparation area from the other stations is critical to preventing contamination. **IMPORTANT!** If possible, physically separate the library and amplification mixture setup areas.
3	Library setup area	Pre-PCR area	The use of a dedicated hood allows for UV treatment of the library preparation area and minimizes contamination from post-PCR material containing adaptors. When selecting a location for library preparation: • In a one-room laboratory layout, the use of a dedicated hood for library preparation is highly recommended, but not required. • The library area setup station requires a set of dedicated pipettes. • If a dedicated hood is unavailable, select a bench that is preferably isolated from the neighboring stations.
4	Pipettes	Pre- and Post-PCR areas	Both the pre- and post-PCR areas require a complete set of dedicated pipettes. If necessary, the pipettes can be shared between neighboring stations, except for the amplification mixture and library setup stations, both of which require dedicated sets of pipettes.

(continued)

#	Station	Location	Description/requirements
5	Ion OneTouch™ Dx Instrument and primer-annealing thermal cycler	Post-PCR area	Ion OneTouch™ Dx Instrument and Primer-Annealing Thermal Cycler The Ion OneTouch™ Dx Instrument performs automated preparation of templated Ion Sphere™ Particles. The Veriti™ Dx Thermal Cycler is used to perform the primer-annealing step during template preparation. When selecting a location, confirm that the location meets all clearance and environmental requirements for the Ion OneTouch™ Dx Instrument and thermal cycler.
6	Ion PGM™ Dx Sequencer and Ion Torrent™ Server	Post-PCR area	When planning the placement of the Ion PGM™ Dx Sequencer and Ion Torrent™ Server, confirm that the location meets all clearance and environmental requirements described in this document. **IMPORTANT!** The Ion PGM™ Dx Sequencer is sensitive to both electrical noise and temperature changes. **Note:** Because the Ion Torrent™ Server requires a direct connection to the Ion PGM™ Dx Sequencer via a standard Category 6 Ethernet cable, the server is typically installed to the bench directly beneath the instrument.
7	Gas cylinder	Post-PCR area	The Ion PGM™ Dx Sequencer requires a constant supply of nitrogen gas, typically provided by a gas cylinder located within 3m (10ft) of the instrument. **IMPORTANT!** The gas cylinder must be chained to a wall or bench.
8	Ion PGM™ Dx Chip Minifuge	Post-PCR area	The Ion PGM™ Dx Chip Minifuge is designed for loading chips, and should be located near the Ion PGM™ Dx Sequencer. It comes in two configurations, depending on your power supply: 120 VAC and 220–240 VAC. ⚠ **CAUTION!** Never plug a 120 VAC minifuge into an 220–240 VAC outlet, or vice versa. Operating the minifuge with a supply voltage outside the range specified on the label may cause a fire or electric shock.

Environmental requirements

Ensure that the room where the instruments have been installed is maintained under the correct environmental conditions. Avoid placing the instruments next to heaters, cooling ducts, or in direct sunlight. Place the sequencer at least a meter away from major sources of electronic noise, such as refrigerators or microwaves.

CAUTION! Use of the instruments in an unspecified manner may result in the protection provided by the instruments to be impaired.

Component	Acceptable range
Altitude	Between sea level and 2,000 meters (6,500 feet) above sea level
Humidity: operating	10–90% relative humidity
Humidity: transportation and storage	20–80% relative humidity
Temperature: operating	15–30°C (59–86°F) At or above 1,800 meters (5,906 feet), the system must not be used if the temperature is above 29.5°C.
Temperature: transportation and storage	–30°C to 60°C (–22°F to 140°F)
Vibration	Ensure that benches where instruments are to be installed are free of vibration and have no contact with equipment that causes vibration (freezers, pumps, and similar equipment). Vibration can reduce the quality of sequencing measurements.
Pollution	The system has a Pollution Degree rating of II (2). The system may only be installed in an environment that has nonconductive pollutants, such as dust particles or wood chips. Typical environments with a Pollution Degree II (2) rating are laboratories, sales, and commercial areas.
Overvoltage category	The instruments have an installation (overvoltage) category of II (2).
Other conditions	For indoor use only. Keep away from any vents that could expel particulate material on the system components.

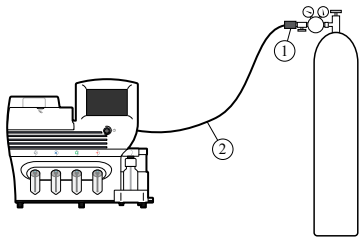
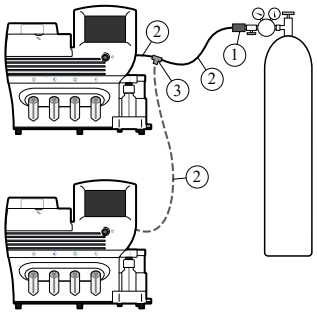
Gas requirements

CAUTION! Thermo Fisher Scientific recommends the use of nitrogen gas with the Ion PGM™ Dx System. The use of alternative gasses is currently not supported and may adversely affect system performance.

An nitrogen gas cylinder must be:

- Connected to the Ion PGM™ Dx Sequencer. See the figure below for a schematic and list of tubing and fittings.
- Able to supply 30 psi for up to three sets of installations of the Ion PGM™ Dx Sequencer.

Note: In the following figure, dashed lines indicate an optional configuration for multipoint tie-in.

Installation	Example (see the following table for annotated parts)
• Single instrument • Single Room	
• Multiple instruments • Single or Multiple Room	

Items in the following table ship with the Ion PGM™ Dx System:

	Description
1	Nylon and Nickel- Plated Brass Tube Fitting Adapter for 1/4-in Tube OD × 1/4-in NPT Female Pipe
2	Clear Tygothane® C-210-A Polyurethane Tubing 1/8-in ID, 1/4 in OD
3	*(Optional)* Nylon Tee for 1/4-in Tube OD
	Regulator, relieving-type, 7.25-58 psi, 28 scfm, no gauge, 1/4-in NPT
	(Not shown) Syringe, 10CC, Female Luer-Lok™ fitting
	(Not shown) Cleaning Tray

Note: If the gas cylinders will be placed more than 10 ft from the Ion PGM™ Dx Sequencer, use the same type of low-permeability tubing supplied with the Ion PGM™ Dx System (Tygothane® C-210-A).

Gas cylinders

You must supply the required nitrogen gas cylinder and accessories for the installation. This instrument requires a pressurized house line or one size 1-A nitrogen gas cylinder that holds approximately 7.2 m^3 (257 ft^3) of gas when full. Use only prepurified nitrogen of 99.998% (grade 4.8) or greater purity.

CAUTION! Damage to the instrument and its products can result from using impure gas, gases other than nitrogen, or an inadequate amount of gas.

WARNING! EXPLOSION HAZARD. Pressurized gas cylinders are potentially explosive. Always cap the gas cylinder when it is not in use, and attach it firmly to the wall or gas cylinder cart with approved brackets or chains.

WARNING! Gas cylinders are heavy and may topple over, potentially causing personal injury and tank damage. Cylinders should be firmly secured to a wall or work surface. Please contact your Environmental Health and Safety Coordinator for guidance on the proper installation of a gas cylinder.

Pressure regulator

You must supply a two-gauge regulator with a Compressed Gas Association (CGA) 580-cylinder adapter on the inlet side and a Swagelok® (or equivalent) end-fitting that accepts 6.35-mm (0.25-in.) outer diameter tubing. The primary gauge (0-3000 psi; 0-25,000 kPa recommended) measures tank pressure, and the secondary gauge (0-50 psi; 0-350 kPa recommended) measures regulated pressure. The secondary gauge must allow regulation between 25 and 45 psi via a Compressed Gas Association (CGA) 580-cylinder adapter with a needle-type shutoff valve on the exit side. The needle valves should have Swagelok® (or equivalent) end-fittings ready for connection to 6.35-mm (0.25-in.) outer diameter tubing.

Attaching the cylinder

Attach the pressurized gas cylinder firmly to a wall or gas cylinder cart by means of approved straps or chains.

Ventilation and waste collection requirements

WARNING! The instrumentation must be installed and operated in a well-ventilated environment as defined as having a minimum airflow of 6–10 air changes per hour. Contact your environmental health and safety coordinator to confirm that all instruments are installed and operated in an environment with sufficient ventilation.

Ventilation requirements

Allow at least 50 cm (20 in.) of clearance around the Ion OneTouch™ Dx Instrument for ventilation.

Disposing of waste

WARNING! CHEMICAL HAZARD. Refer to Safety Data Sheets (SDSs) and local regulations for handling and disposing of plastic consumables. Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of plastic consumables.

WARNING! CHEMICAL HAZARD. Before handling chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING! CHEMICAL HAZARD. Waste produced by instruments can be hazardous and can cause injury, illness, or death.

Cleaning or decontamination

See the user documentation for your Ion PGM™ Dx System for information on how to clean or decontaminate the instrument.

Wear appropriate protection, including gloves, laboratory goggles, and coat whenever you work with the fluids that are used on this instrument, or parts that may come into contact with these fluids.

Use only the cleaning agents as described in the user documentation for your Ion PGM™ Dx System. Use of cleaning agents that are not described in user documentation can impair the instrument. Contact Technical Support if you have questions.

Wipe off any liquid on or near the instrument using a lint-free tissue.

Electrical requirements

CAUTION! Do not unpack or plug in any components until a field service representative has configured them for the proper operating voltage.

WARNING! For safety, the power outlet used for powering the instrument must be accessible at all times. In case of emergency, you must be able to immediately disconnect the main power supply to all the equipment. Allow adequate space between the wall and the equipment so that the power cords can be disconnected in case of emergency.

- Electric receptacle required: 2-prong with ground pin
- Main AC line voltage tolerances must be at most ±10% percent of nominal voltage.

- Power cords are provided with the instruments. If not suitable for installation in your region, ensure any power cord you do use is:
  - Maximum 10 feet (3 meters) in length
  - Grounding type
  - Compatible with the power supply receptacles used to connect to main power
  - Suitable for the rating of the instrument and main power supply
  - Compliant with local safety requirements (for example, UL Listed for North America, JIS approved for Japan, HAR or agency certified for Europe)
- (Ion OneTouch™ Dx Instrument only) Fuse Rating: 6 A, 250 VAC, Type M. Replace only with the same fuse type and rating.

WARNING! FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

Device	Rated voltage [1,2]	Rated frequency	Rated current [3]
Ion PGM™ Dx Sequencer	110/120VAC 220/240VAC	50/60 Hz	9 A
Ion Torrent™ Server [4]	110/120VAC 220/240VAC	50/60 Hz	11 A
Ion OneTouch™ Dx Instrument with power supply	110/120VAC 220/240VAC	50/60 Hz	5.5 A
Ion OneTouch™ ES Dx Instrument	110/120VAC 220/240VAC	50/60 Hz	375 mA 160 mA
Ion PGM™ Dx Chip Minifuge	120 VAC 220–240 VAC	50/60 Hz	130 mA 65 mA

[1] In Japan, rated voltages of 100 VAC and 200 VAC are acceptable.

[2] If the supplied power fluctuates beyond the rated voltage, a power line regulator may be required. High or low voltages can adversely affect the electronic components of the instrument.

[3] Based on rated current at minimum input voltage.

[4] Minimum Efficiency: 65% (Energy Star Qualified); 85% Efficient Power Supply.

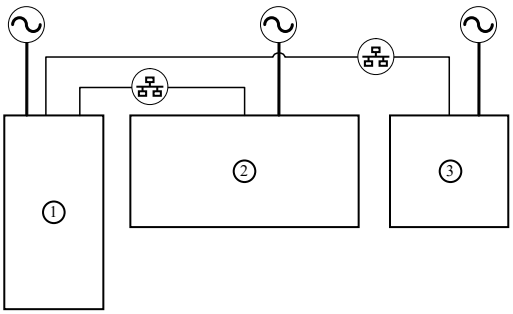
Electrical protective devices

We recommend several protective devices to protect the system in environments with large voltage and power fluctuations.

Device	Description
Power line regulator	We recommend the use of a 1.5-kVA power line regulator in areas where the supplied power fluctuates in excess of ± 10% of the normal voltage. Power fluctuations can adversely affect the function of the instrument and computer. **Note:** A power line regulator monitors the input current and adjusts the power supplied to the instrument or computer. It does not protect against a power surge or failure.
Uninterruptible power supply (UPS)	We recommend the use of a 1.5-kVA uninterruptible power supply (UPS), especially in areas prone to power failure. Power failures and other events that abruptly terminate the function of the instrument and computer can corrupt data and possibly damage the system. ⚠ **WARNING! PHYSICAL INJURY HAZARD.** Do not attempt to lift the UPS unit without assistance of at least two people. Improper lifting can cause painful and permanent back injury. Refer to the UPS manufacturer user guide for more information. **IMPORTANT!** UPSs provide power for a limited time. They are meant to delay the effects of a power outage, not to serve as replacement power sources. In the event of a power loss, power off the instrument and computer unless you expect to regain power within the battery life of the UPS.
Surge protector	We recommend the use of a 10-kVA surge protector (line conditioner) in areas with frequent electrical storms or near devices that are electrically noisy, such as refrigerators, air conditioners, or centrifuges. Short-duration, high-voltage power fluctuations can abruptly terminate the function of, and thereby damage the components of, the computer and the instrument. **Note:** A dedicated line and ground between the instrument, computer, and the building's main electrical service can also prevent problems caused by power fluctuations.

Electrical requirements for the Ion PGM™ Dx Sequencer and Ion Torrent™ Server

Use an approved UL Listed detachable power supply cord to connect the sequencer to the wall. Route power cords away from the workspace to avoid accidental disconnection.



1. Ion Torrent™ Server
2. Ion PGM™ Dx System
3. Ion OneTouch™ Dx Instrument

Power cords are provided with the instrument. If not suitable for installation in your region, ensure any power cord you do use is:

- Maximum 10 ft (3 m) in length
- Grounding type
- Compatible with the power supply receptacles used to connect to main power
- Suitable for the rating of the instrument and mains power supply
- Compliant with local safety requirements (for example, UL Listed for North America, JIS approved for Japan, HAR or agency certified for Europe)

Network requirements

The Ion PGM™ Dx Sequencer, Ion Torrent™ Server, and Ion OneTouch™ Dx Instrument are factory-configured for the TCP/IP protocol and include fast Ethernet adapters (10/100Mbps) for use with RJ45-type connectors. The product includes one 3-m (9.8-ft) 1GB Ethernet cable used to connect the Ion Torrent™ Server to the Ion PGM™ Dx Sequencer, and one 3-m (9.8-ft) 10/100Mbps Ethernet cable used to connect the Ion Torrent™ Server to the Ion OneTouch™ Dx Instrument. You must supply a standard Category 6 Ethernet cable of the required length to connect the Ion Torrent™ Server to your LAN.

If the Ion OneTouch™ Dx Instrument will be connected to a local area network (LAN), an active, tested LAN connection must be in place before the scheduled installation date. Due to differences in network connections, the service representative cannot configure the system to access a specific network.

Network configuration

The following requirements must be met before installation of the Ion Torrent™ Server. Discuss any discrepancies in the checklist with your field service representative prior to the visit.

- A static (or dynamic) IP address must be reserved for the Ion Torrent™ Server.

  Note: If necessary, field service can provide the server's MAC address prior to the installation.

- The room where the Ion Torrent™ Server will be deployed must contain at least one active network jack.
- The site DNS Server must be configured for the Ion Torrent™ Server so that users can access the server URL from their workstations.
- An information technologies resource must be available to assist with the network connection on the date that the Ion Torrent™ Server will be installed.
- If the Ion Torrent™ Server will be more than 10 feet from the Ion PGM™ Dx System , you must provide a standard Category 6 Ethernet Cable of sufficient length at the time of installation.

  Note: The supported configuration is a direct connection between the instrument and the server.

- Use a shielded Ethernet cable when connecting to the Ion OneTouch™ Dx Instrument.

Antivirus software recommendation

Thermo Fisher Scientific has tested ClamAV antivirus software from Ubuntu™ on the Ion Torrent™ Server and shown that it does not interfere with the assay or Torrent Suite™ Dx Software. For more information, visit **help.ubuntu.com/community/ClamAV**.

Safety requirements

Safety practices

A safety representative from your facility must ensure that:

- Personnel establish and follow all applicable safety practices and policies to protect laboratory personnel from potential hazards.
- All applicable safety devices and equipment are available at all times.

Required safety equipment

Your laboratory has specific safety practices and policies designed to protect laboratory personnel from potential hazards that are present. Follow all applicable safety-related procedures at all times.

The following safety equipment and protection from hazards must be available at the installation site:

- Protection from any sources of hazardous chemicals, radiation (for example, lasers, radioisotopes, radioactive wastes, and contaminated equipment), and potentially infectious biological material that may be present in the area where the service representative will work.
- Appropriate fire extinguisher:
  - You are responsible for providing an appropriate fire extinguisher for use on or near the equipment.
  - The types and sizes of fire extinguishers shall be suitable for use on electrical and chemical fires as specified in current codes, regulations, and/or standards, and with approval of the Fire Marshall or other authority having jurisdiction.
  - The installation of appropriate fire extinguishers shall be in addition to other fire-protection systems and not as a substitute or alternative to them.
- Eyewash
- Safety shower
- Eye and hand protection
- Adequate ventilation, including vent line/fume hood, if applicable
- Biohazard waste container, if applicable
- First-aid equipment
- Spill cleanup equipment
- Applicable Safety Data Sheets (SDSs)

Materials for installation and operation

Template and library preparation

For a complete list of materials and equipment required for template and library preparation, see the *Ion PGM™ Dx System User Guide* (Pub. No. MAN0016694).

Installation checklist

See the *Ion PGM™ Dx System Pre-Installation Checklist* for the materials required for instrument installation. The checklist specifies the materials that must be present onsite before installation and subsequent training can take place.

Receive and inspect the shipment

1. Verify that the items shown on the shipping list are the items that were ordered at the time of purchase.
2. Carefully inspect the shipping containers. Report any damage to the shipping company and to your service representative. Record any damage or mishandling on the shipping documents.
3. Immediately unpack the reagents or installation kit box (boxed separately from the instrument components). Store the reagents at the temperatures specified on the product packaging or labels.

IMPORTANT! Other than reagents or plates that require storage at specific conditions, do not unpack shipping containers at this time. To protect yourself from liability for damage that occurred during shipping, inspect the shipping containers and report damage as described above.

IMPORTANT! Do not unpack shipping containers at this time. To protect yourself from liability for damage that occurred during shipping, inspect the shipping containers and report damage as described above.

Move the crated instrument to the installation site

1. Clear the installation site of all unnecessary materials.
2. If possible, move the crated instrument and other shipping containers to the installation site. Do not uncrate.

CAUTION! PHYSICAL INJURY HAZARD. Do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more people.

CAUTION! Do not tip the crated instrument on end. Tipping may damage the instrument hardware and electronics.

Note: After installation, retain the crate and instrument packaging in case you need to relocate the instrument.

Instrument warranty

For new Ion Torrent™ instruments, Life Technologies warrants to and only to buyer for twelve (12) months from the date of shipping, that the Ion Torrent™ software and Ion Torrent™ instruments are free from defects in material and workmanship and conform to Life Technologies' published specifications in all material respects. Where a valid and timely claim in respect of breach of Ion Torrent™ warranty is submitted to Life Technologies, Life Technologies may, at its discretion, replace, repair or modify the Ion Torrent™ instrument. Any agreed replacement shall be at 1:1, like-kind basis, at no cost to the buyer. For Ion Torrent™ chips or reagents reasonably determined by Life Technologies to be defective, independent of user error, shall be replaced by Life Technologies on a 1:1, like-kind basis at no cost to buyer, provided that such defective Ion Torrent™ chips or reagents were used by buyer prior to their expiration date, or if there is no expiration date, the Ion Torrent™ chips or reagents were used within six (6) months of receipt, and the defect was promptly reported with appropriate detail to Life Technologies' technical support.

NO OTHER WARRANTIES SHALL BE APPLICABLE TO ION TORRENT PRODUCTS (WHETHER OR NOT ANY FURTHER WARRANTY DOCUMENTATION MAY BE INCLUDED IN THE SHIPMENT), WITH THE EXCEPTION OF THIRD PARTY WARRANTIES WITH RESPECT TO THIRD PARTY PRODUCT. ANY THIRD PARTY PRODUCTS ARE NOT COVERED BY THIS SECTION AND ANY WARRANTIES FOR THIRD PARTY PRODUCTS ARE PROVIDED BY THE ORIGINAL MANUFACTURER OF THE THIRD PARTY PRODUCT. Warranties are made only to buyer purchasing the Ion Torrent™ Product directly from Life Technologies, are not transferable and do not extend to the benefit of any other person or entity, unless otherwise expressly stated in writing by Life Technologies. ANY PRODUCT NOT COVERED BY AN EXPRESS WRITTEN WARRANTY IS SOLD AND PROVIDED "AS IS," WITHOUT WARRANTY OF ANY KIND, STATUTORY, EXPRESS OR IMPLIED. Any description of Ion Torrent™ Product recited in Life Technologies' quotation is for the sole purpose of identifying Ion Torrent™ Product, and any such description is not part of any contract between Life Technologies and buyer and does not constitute a warranty that Ion Torrent™ Product shall conform to that description. Any sample or model used in connection with Life Technologies' quotation is for illustrative purposes only, and is not part of any contract between Life Technologies and buyer and does not constitute a warranty that Ion Torrent™ Product will conform to the sample or model. No affirmation of fact or promise made by Life Technologies, whether or not in Life Technologies' quotation, shall constitute a warranty that Ion Torrent™ Product will conform to the affirmation or promise. Unless otherwise specified in writing in documentation shipped with Ion Torrent™ Product or otherwise agreed by Life Technologies in writing. Life Technologies does not provide service or support for custom products or other products made to buyer's specifications. THE WARRANTIES IDENTIFIED IN THIS CLAUSE ARE LIFE TECHNOLOGIES' SOLE AND EXCLUSIVE WARRANTIES WITH RESPECT TO Ion Torrent™ PRODUCT AND ARE IN LIEU OF ALL OTHER WARRANTIES, STATUTORY, EXPRESS OR IMPLIED, ALL OF WHICH OTHER WARRANTIES ARE EXPRESSLY DISCLAIMED, INCLUDING WITHOUT LIMITATION ANY IMPLIED WARRANTY OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, NON-INFRINGEMENT, OR REGARDING RESULTS OBTAINED THROUGH THE USE OF ANY PRODUCT (INCLUDING, WITHOUT LIMITATION, ANY CLAIM OF INACCURATE, INVALID OR INCOMPLETE RESULTS), WHETHER ARISING FROM A STATUTE OR OTHERWISE IN LAW OR FROM A COURSE OF PERFORMANCE, DEALING OR USAGE OF TRADE.

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support
- Order and web support
- Safety Data Sheets (SDSs; also known as MSDSs)

Additional product documentation, including user guides and Certificates of Analysis, are available by contacting Customer Support.

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are printed and shipped with the product.

Obtaining Certificates of Conformance

The Certificate of Conformance provides information on conformance testing of each instrument provided with the system. Certificates of Conformance are shipped with the instrument, and are also available by contacting Customer Support at **thermofisher.com/support**.

Life Technologies Holdings Pte Ltd |
Block 33 |
Marsiling Industrial Estate Road 3 |
#07-06, Singapore 739256

Products manufactured in Singapore:

Ion PGM™ Dx Instrument System
Ion PGM™ Dx Sequencer
Ion OneTouch™ Dx Instrument
Ion OneTouch™ ES Dx Instrument
Ion PGM™ Dx Chip Minifuge (120V or 230V)
Ion PGM™ Wireless Scanner
Ion Torrent™ Server
Torrent Suite™ Dx Software
Torrent Suite™ Assay Development Software
Veriti™ Dx 96-well Thermal Cycler, 0.2 mL

Life Technologies Corporation |
7335 Executive Way |
Frederick, MD 21704 | USA

Products manufactured in Frederick:

Ion Torrent Dx FFPE Sample Preparation Kit
Ion PGM™ Dx Library Kit
Ion OneTouch™ Dx Template Kit
Ion PGM™ Dx Sequencing Kit
Ion 318™ Dx Chip
Ion OneTouch™ Rack Kit
DynaMag™ Dx 96-Well Plate Magnet
DynaMag™ Dx 16 2-mL Magnet

EC REP Life Technologies Europe B.V.
Kwartsweg 2, 2665 NN Bleiswijk
The Netherlands

The information in this guide is subject to change without notice.

DISCLAIMER

TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0016696

Revision	Date	Description of change
D.0	4 August 2020	Information about a recommended antivirus software program was added. See "Antivirus software recommendation" on page 18.
C.0	10 December 2019	• The EC-REP address was updated. • The following manufactured products were added to the list on page 22: Torrent Suite™ Assay Development Software, Ion Torrent Dx FFPE Sample Preparation Kit, DynaMag™ Dx 16 2-mL Magnet, and Veriti™ Dx 96-well Thermal Cycler, 0.2 mL.
B.0	26 May 2017	Updates to operating temperature at or above 1,800 meters, operating humidity, syringe size shipped with the instrument, and a note about damage from shipping.
A.0	23 January 2017	Revision to Pub. No. 4474570 Rev. D for FDA submission.