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

USER GUIDE

Publication Number MAN0019390

Revision A.0

IVD

For In Vitro Diagnostic Use. | Rx Only

ThermoFisher SCIENTIFIC

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Products manufactured at this site:

- 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, Maryland 21704 USA

Products manufactured at this site:

- 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

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Revision	Date	Description
A.0	23 September 2022	New Oncomine™ Dx Target Test user guide for FDA submission

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

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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), cholangiocarcinoma (CC), and thyroid cancer (TC) 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 and TC, and one related to CC.

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), deletions, and insertions 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), IDH1 SNVs from FFPE tumor tissue samples from patients with cholangiocarcinoma (CC), RET SNVs, MNVs, and deletions from DNA isolated from FFPE tumor samples from patients with medullary thyroid cancer (MTC), and RET fusions from RNA isolated from FFPE tumor tissue samples from patients with thyroid cancer (TC) using the Ion PGM™ Dx System.

The test is indicated as a companion diagnostic to aid in selecting NSCLC, CC, MTC, and TC patients for treatment with the targeted therapies listed in Table 1 in accordance with the approved therapeutic product labeling.

Table 1 List of variants for therapeutic use

Tissue type	Gene	Variant	Targeted therapy
NSCLC	BRAF	BRAF V600E mutations	TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib)
	EGFR	EGFR L858R mutation, EGFR exon 19 deletions	IRESSA® (gefitinib)
	EGFR	EGFR exon 20 insertions	EXKIVITY™ (mobocertinib)
			RYBREVANT™ (amivantamab-vmjw)
	ERBB2/HER2	ERBB2/HER2 activating mutations (SNVs and exon 20 insertions)	ENHERTU® (fam-trastuzumab deruxtecan-nxki)

Table 1 List of variants for therapeutic use *(continued)*

Tissue type	Gene	Variant	Targeted therapy
NSCLC	RET	RET fusions	GAVRETO™ (pralsetinib) RETEVMO® (selpercatinib)
	ROS1	ROS1 fusions	XALKORI® (crizotinib)
CC	IDH1	IDH1 R132C, IDH1 R132G, IDH1 R132H, IDH1 R132L, and IDH1 R132S	TIBSOVO® (ivosidenib)
MTC	RET	RET mutations (SNVs, MNVs, and deletions)	RETEVMO® (selpercatinib)
TC	RET	RET fusions	RETEVMO® (selpercatinib)

Safe and effective use has not been established for selecting therapies using this device for the variants listed in tissue types other than those 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 in NSCLC

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 a 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 a 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 use of fine needle aspirates for thyroid cancer (TC) specimens has not been validated.
- The Oncomine™ Dx Target Test has been validated to detect the following somatic mutations: single-nucleotide variations (SNVs), multi-nucleotide variations (MNVs), deletions of 3, 6, 9, 12, 15, and 18 base pairs, and insertions of 3, 6, 9, and 12 base pairs in DNA, and fusions in RNA.
- 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 has not been validated for the detection of RET insertions.
- Users are cautioned that DNA variant-positive calls in the RET genomic region have been observed to produce multiple variant calls, even when only one variant is present. These RET variants are all activating and do not change the patient's clinical appropriateness for selpercatinib.
- High variation in fusion reads can be observed with fusion-positive samples. A decrease in fusion reads over time has been observed when testing slides from TC tissue under storage.
- For NSCLC, the Oncomine™ Dx Target Test assay definition file includes prevalent but not all rare or newly identified RET isoforms, ROS1 isoforms, EGFR exon 20 insertions, and ERBB2/HER2 activating mutations. The Oncomine™ Dx Target Test may miss rare or newly identified:
  - RET isoforms carried by a subset of patients who may derive benefit from pralsetinib or selpercatinib
  - ROS1 isoforms carried by a subset of patients who may derive benefit from crizotinib
  - EGFR exon 20 insertions carried by a subset of patients who may derive benefit from mobocertinib or amivantamab-vmjw
  - ERBB2/HER2 activating mutations carried by a subset of patients who may derive benefit from fam-trastuzumab deruxtecan-nxki
- The safe and effective use of the variants reported in Table 2 has not been established for selecting therapy using this device. The variants for KRAS (COSM512/p.Gly12Phe/c.34_35delGGinsTT and COSM516/p.Gly12Cys/c.34G>T), MET (COSM707/p.Thr1010Ile/c.3029C>T) and PIK3CA (COSM754/p.Asn345Lys/c.1035T>A) have been analytically validated. Performance of all other variants identified by the test, other than the clinically validated therapeutic variants and analytically validated variants, has not been directly demonstrated.

CAUTION! U.S. Federal law restricts this device to sale by or on the order of a physician.

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

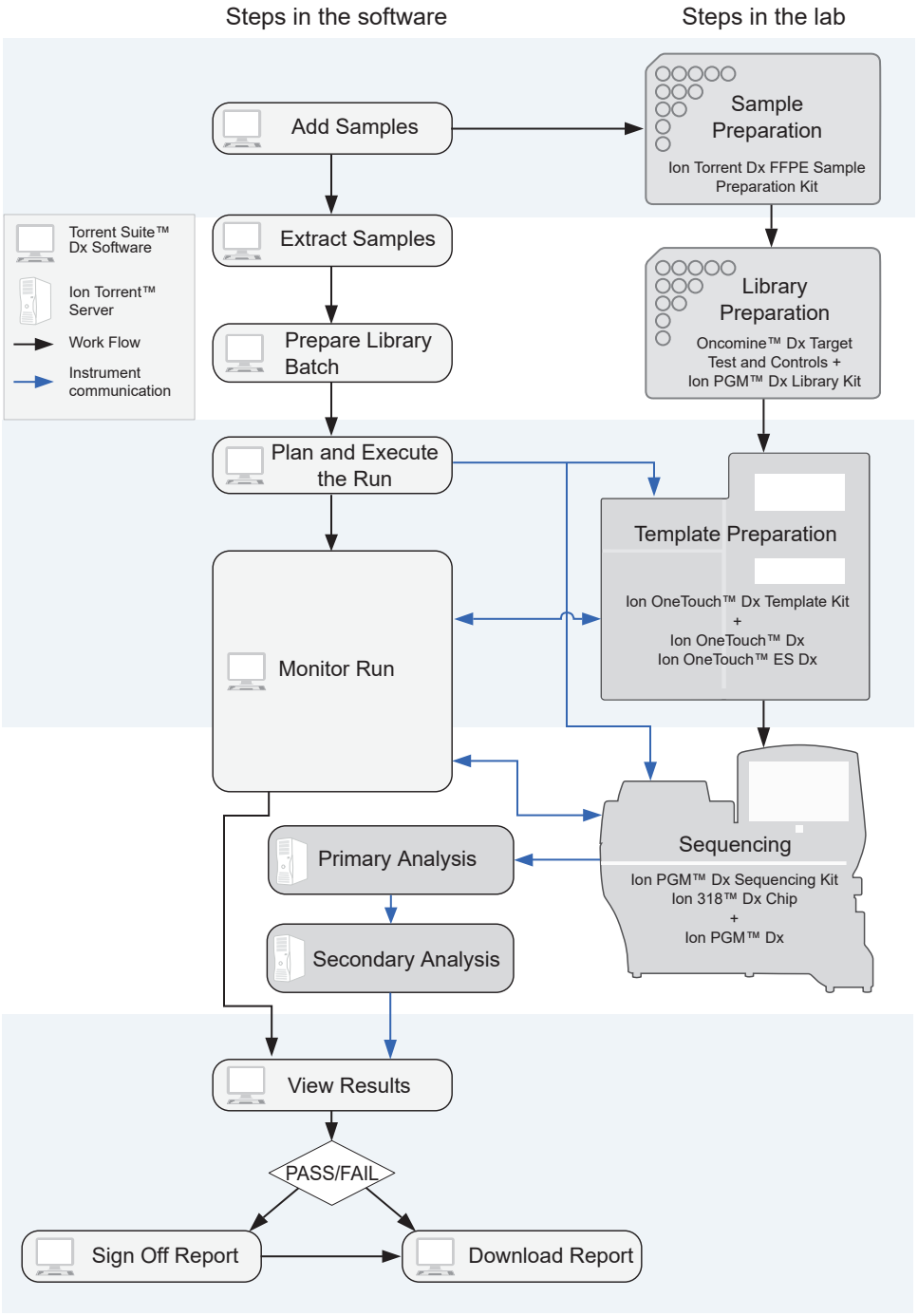
Oncomine™ Dx Target Test Kit

The Oncomine™ Dx Target Test Kit (Cat. No. A51695) 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	A52639

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
COSM28747_AF	Variant called and AF ≥0.05
COSM12558_AF	Variant called and AF ≥0.05
Mean AQ20 Read Length (bp)	≥98
Percent Reads (%)	≥0.7

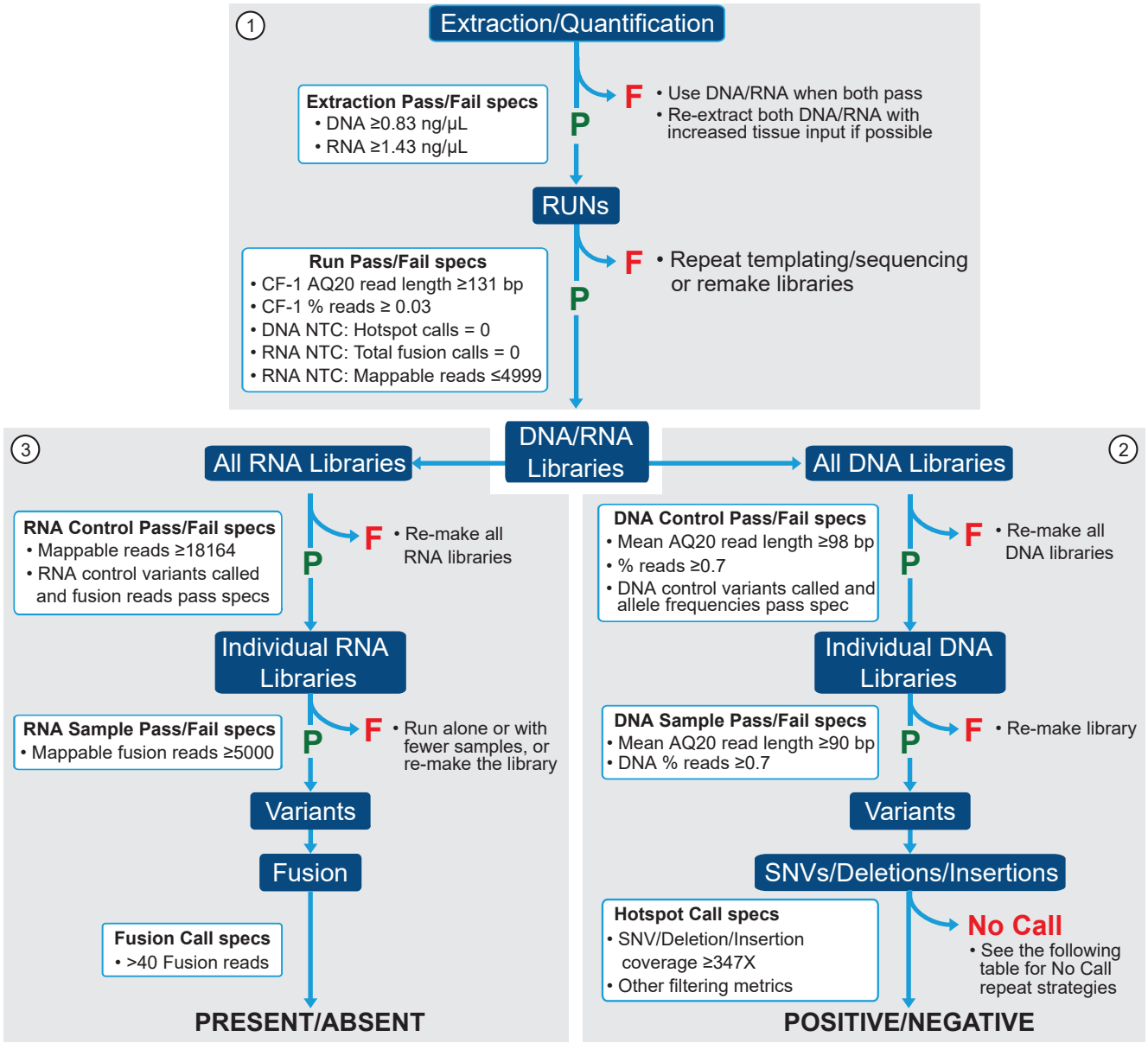
(continued)

Metric	Criteria
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 and table to determine the best course of action.



Point of failure		Tissue type	Recommended repeat strategy
1	RUNs	NSCLC, CC, MTC, and TC	Repeat templating and sequencing or remake the libraries.
2	DNA Library	NSCLC, CC, MTC, and TC	Remake all DNA libraries.
3	RNA library	NSCLC and TC	Remake all RNA libraries.
		CC and MTC	Unnecessary to remake cholangiocarcinoma and medullary thyroid cancer sample libraries, because RNA fusion variants are not reported for these samples.

Point of failure	Specification type	Passing criteria	Repeat strategy
1	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.
1	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.
1	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.

(continued)

Point of failure	Specification type	Passing criteria	Repeat strategy
2	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.
3	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.
2	Library DNA Sample	Any individual DNA sample library must meet the following specifications to have reportable results for the DNA sample library: • Mean AQ20 Read Length (bp) ≥90. • Percent Reads ≥0.7.	Run the DNA sample library alone, or with fewer DNA sample libraries. If the DNA sample library 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)

Point of failure	Specification type	Passing criteria	Repeat strategy
3	Library RNA Sample	Any individual RNA sample library must have ≥5000 Mappable Fusion Reads to have reportable results for the RNA sample library.	Run the RNA sample library alone, or with fewer RNA sample libraries. If the RNA sample library 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.
2	SNV, Deletion, and Insertion Variant Specifications	All Single Nucleotide Variants (SNVs), Deletions (Dels), and Insertions (Ins) must have coverage ≥347 reads and pass all Variant Caller filtering metrics in order to have a reportable result for the variant.	Any SNVs, deletions, and insertions 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.

No Call repeat strategies

Point of failure	Observation (Example FR tag)[1]	Reason[2]	Repeat strategy
2	*MINCOV<347, PosCov<2, NegCov<2**	Coverage	Repeat the run with fewer samples per chip may improve coverage.
2	*NODATA*	No data	Repeating the run with fewer samples per chip may improve coverage.
2	*QualityScore<8*	Quality score	Remake the DNA and/or RNA libraries starting from nucleic acid sample to improve the quality score if the quality score is due to low coverage.
2	*STDBIAS0.99034>0.96, STDBIASPVAL 0.299<=1*	Strand bias	Remake the DNA and/or RNA libraries starting from nucleic acid sample to improve strand coverage.

[1] The reason for a No Call can be determined by examining the value of the FR tag for a given variant, listed in the output VCF file.

[2] While presence of ALL of the reasons for No Call is not necessary for a No Call assignment, more than one condition may occur simultaneously. In cases where multiple reasons are observed, such that one recommends repeat while the other does not, repeating sample from library preparation is recommended.

Pass/fail specifications and repeat strategy—mixed runs

If you combine different tissue types (for example, NSCLC and CC samples) in a single Oncomine™ Dx Target Test run and experience a run or sample failure, follow the repeat strategy recommendations according to the tissue type of the samples that require retesting that are listed in “Pass/fail specifications and repeat strategy” on page 16.

For example, if the RNA Control fails in a mixed run, it is not necessary to remake CC sample libraries, because RNA fusion variants are not reported for these samples.

Performance characteristics

Cholangiocarcinoma (CC)—Analytical studies

Tissue input study

Fifteen (15) 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 that are 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.

Five (5) resection samples with ≥20% tumor content were prepared without macrodissection, 5 resection samples with <20% to ≥10% tumor cell content were macrodissected, and 5 samples were collected by core needle biopsy (CNB). For the resection samples with ≥100 mm^2 surface area, 1 x 5 µm section was used per extraction. For resection samples with <100 mm^2, 4 x 5 µm sections were used per extraction. For CNBs, all of which had a surface area <30 mm^2, 9 x 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 15 samples tested, 93.3% (14/15) 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 0.72 ng/µL and 0.81 ng/µL respectively. The low concentrations were likely caused by insufficient tissue input as the sample only had a single core biopsy per slide.

Guard band testing study

Guard band testing was performed to evaluate the tolerance levels of the Proteinase K digestion and inactivation steps during FFPE sample preparation when using the Oncomine™ Dx Target Test with CC samples.

The tolerance level for each test condition (volume, temperature, and time for digestion and inactivation) was evaluated by comparing DNA and RNA concentrations across 3 test levels: Low, Standard Operating Protocol (SOP)/Nominal, and High. For each test condition and level, DNA and RNA were extracted from 1 IDH1 variant-positive FFPE CC sample and an FFPE BRAF V600E cell-line (in triplicate) and sequenced using the Oncomine™ Dx Target Test.

No statistically significant difference was observed between the levels for all 3 test conditions for the samples tested.

FFPE block stability study

Stability of CC FFPE tissue blocks at room temperature was established when tested with the Oncomine™ Dx Target Test.

Three (3) IDH1 (2 R132G and 1 R132C) variant-positive clinical sample blocks were tested in duplicate at baseline, 3 months + 2 weeks, 6 months + 2 weeks, and 12 months + 2 weeks' time points.

Linear regression analyses and stability estimates from each sample across the timepoints demonstrated that CC FFPE tissue blocks were stable for up to 12 months.

FFPE slide stability study

Stability of paraffin dipped and un-dipped FFPE CC tissue sections mounted on slides was established when using the Oncomine™ Dx Target Test.

Three (3) IDH1 (2 R132C and 1 R132G) variant-positive clinical samples from dipped and un-dipped FFPE CC tissue sections were tested at baseline, 3 months + 1 week, 6 months + 1 week, 9 months + 1 week, and 12 months + 1 week time points.

Linear regression analyses and stability estimates from each sample across the timepoints demonstrated that CC FFPE tissue sections mounted on slides were stable for up to 12 months.

Sample processing reproducibility study

The reproducibility and repeatability of IDH1 R132 variant detection using the Oncomine™ Dx Target Test were evaluated with 2 IDH1 WT samples and 4 IDH1 R132 (2 R132C and 2 R132G) variant-positive samples at a single test site. The site had 2 Ion PGM™ Dx instrument systems and 2 operators.

Each sample was tested 6 times by each operator, for a total of 12 replicates per sample. After repeat testing, there was a single invalid reaction (1/72 or 1.39%).

The negative call rate, positive call rate, and within-run repeatability were calculated for each IDH1 R132 variant-positive sample at the expected IDH1 R132 variant location. The results are shown in Table 5.

Including no calls the negative call rate for the IDH1 WT sample was 100% at all IDH1 R132 variant locations. Including no calls the positive call rate from the expected IDH1 R132 positive variants was 100%.

Table 5 Reproducibility call rates

Sample	Variant Identification	Variant (amino acid change)	# of valid sample results (N)	# of positive calls (A)	# of negative calls (B)	# of no calls (C)	Positive call rate + 95% C.I.		Negative call rate + 95% C.I.		Within-run repeatability + 95% C.I.	
							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
A	COSM28747	R132C	11	11	0	0	100% (71.5%, 100%)	100% (71.5%, 100%)	0% (0%, 28.5%)	0% (0%, 28.5%)	100% (47.8%, 100%)	100% (47.8%, 100%)
B	COSM28749	R132G	12	12	0	0	100% (73.5%, 100%)	100% (73.5%, 100%)	0% (0%, 26.5%)	0% (0%, 26.5%)	100% (54.1%, 100%)	100% (54.1%, 100%)
D	COSM28747	R132C	12	12	0	0	100% (73.5%, 100%)	100% (73.5%, 100%)	0% (0%, 26.5%)	0% (0%, 26.5%)	100% (54.1%, 100%)	100% (54.1%, 100%)
E	COSM28749	R132G	12	12	0	0	100% (73.5%, 100%)	100% (73.5%, 100%)	0% (0%, 26.5%)	0% (0%, 26.5%)	100% (54.1%, 100%)	100% (54.1%, 100%)

Interfering substances study

Two (2) potentially interfering substances that may be found in CC FFPE tissue samples, hemoglobin and bile acids, were evaluated using the Oncomine™ Dx Target Test on the Ion PGM™ Dx System.

The guidelines for 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.

Table 6 Interfering substances and amounts

Potential interfering substance	Step	Amount of substance
Hemoglobin	After deparaffinization, hemoglobin was added to the Digestion Buffer used to pre-wet the tissue section.	4 mg/mL
Bile acids	After deparaffinization, bile acids were added to the Digestion Buffer used to pre-wet the tissue section.	30 nmol/mL

Three (3) IDH1 R132 (1 R132G and 2 R132C) variant-positive and 1 WT FFPE CC clinical samples (2 replicates each) were extracted in the presence and absence of the excess endogenous substance and processed through the entire assay workflow. The concordance between variant calls in samples with and without interfering substances was calculated for each substance under investigation.

With no calls excluded, the results of testing with hemoglobin and bile acids showed 100% concordance with the control condition for both the IDH1 R132 variant-positive and WT FFPE CC samples. This data supports the claim that hemoglobin, and bile acids do not impact Oncomine™ Dx Target Test assay performance at the level tested.

Limit of Detection (LoD) study

The LoD was evaluated for all 5 IDH1 R132 variants that are detected by the Oncomine™ Dx Target Test in clinical samples. The LoD is the lowest allelic frequency (AF) of the IDH1 R132 variants that can be detected at least 95% of the time. DNA from variant-containing samples or cell lines were blended with DNA from WT FFPE CC samples at multiple levels and used as input DNA for the test. A minimum of 120 data points was generated for each IDH1 R132 variant by testing 6 titration levels, 2 reagent lots, and 10 replicates (per level per lot).

The LoD of the 5 IDH1 R132 variants ranged from 3.7–5.5% AF.

Table 7 LoD of clinical IDH1 variants

Variant	ID	Sample type	Estimated LoD (AF)
R132C	COSM28747	Clinical sample	4.9%
R132G	COSM28749	Clinical sample	5.5%
R132H	COSM28746	Cell line	4.4%
R132L	COSM28750	Cell line	3.7%
R132S	COSM28748	Cell line	3.9%

Assay reproducibility study

The reproducibility and repeatability of IDH1 R132 variant detection using the Oncomine™ Dx Target Test were assessed with 1 IDH1 WT sample and 3 IDH1 R132 variant-positive samples at 2 allelic frequency (AF) levels. Testing was performed at 4 testing sites, each site had 2 Ion PGM™ Dx instrument systems, 2 operators, and completed testing using 4 lots of reagents.

Thirty-six (36) replicates per sample were tested across all sites. Overall, there were 72 sequencing events per variant and samples were run in duplicate for repeatability analysis. After repeat testing, there was a single invalid reaction (1/252 or 0.4%).

The negative call rate, positive call rate, and within-run repeatability were calculated for each IDH1 R132 variant-positive sample at the expected IDH1 R132 variant location. The results are shown in Table 8.

The overall positive call rate for the IDH1 R132 variants was 92.6% when including no calls and 97.1% when excluding no calls. The negative call rate for the IDH1 WT sample was 100% at all IDH1 R132 variant locations.

Table 8 Reproducibility results

Sample	Variant Identification	Variant (amino acid change)	# of valid sample results (N)	# of positive calls (A)	# of negative calls (B)	# of no calls (C)	Positive call rate + 95% CI		Relative LoD
							Including no calls (A/N)	Excluding no calls (A/(A+B))	
D1	COSM28747	R132C	36	36	0	0	100% (90.3%, 100%)	100% (90.3%, 100%)	2.1–2.7X
D2	COSM28747	R132C	36	35	0	1	97.2% (85.5%, 99.9%)	100% (90.0%, 100%)	0.98–1.4X
D3	COSM28749	R132G	36	36	0	0	100% (90.3%, 100%)	100% (90.3%, 100%)	1.9–2.5X
D4	COSM28749	R132G	36	36	0	0	100% (90.3%, 100%)	100% (90.3%, 100%)	0.9–1.3X
D5	COSM28750	R132L	36	36	0	0	100% (90.3%, 100%)	100% (90.3%, 100%)	1.4–1.8X
D6	COSM28750	R132L	35	20	6	9[1]	57.1% (39.4%, 73.7%)	76.9% (56.4%, 91.0%)	0.65–0.94X
D7	Wild-type (WT)	N/A	36	0	0	0	0% (0%, 9.17%)	0% (90.3%, 100%)	N/A

[1] A number of no calls were seen because the LoD for this variant is close to the assay AF cutoff of 2.5%.

Cholangiocarcinoma (CC)—Clinical studies

IDH1 clinical study

IDH1 study—concordance evaluation

A total of 383 samples were obtained for this study. Both slides cut from FFPE blocks and extracted DNA were used. Of these, 187 were identified by the enrolling clinical trial assay (CTA) as IDH1 variant-positive, 187 were identified as IDH1 variant-negative, and 9 samples were invalid based on the enrolling CTA.

The 187 IDH1 variant-positive samples set were previously enrolled into the AG-120-C-005 clinical study. The IDH1 variant-negative samples were randomly selected from the CTA patient population that had yielded a valid negative result on the CTA assay. The IDH1 invalid samples were randomly selected from the CTA patient population that yielded invalid results.

Of the 187 IDH1 variant-positive samples from the CTA testing, 174 were positive on Oncomine™ Dx Target Test. Of the 174 samples IDH1 positive by Oncomine™ Dx Target Test, 172 were IDH1 positive from the NDA population as 2 samples tested by the CTA were not part of the efficacy population. Of the 187 IDH1 CTA positive samples in the bridging population, 1 sample was called negative, 6 samples were invalid, 6 samples were cancelled due to failure to meet test input requirements due to low or insufficient sample availability.

Of the 187 IDH1 variant-negative samples tested, 0 were identified as IDH1 variant-positive by the Oncomine™ Dx Target Test, 14 were invalid, 5 yielded no calls, and 2 were not tested due to insufficient sample availability, leaving 166 confirmed IDH1 variant-negative samples by the Oncomine™ Dx Target Test.

Of the 9 CTA invalid samples tested, 3 yielded Oncomine™ Dx Target Test positive results, 3 yielded negative results, 2 were confirmed invalid, and 1 was not tested due to insufficient sample availability.

In summary, 9 samples were cancelled (not tested), 22 had invalid Oncomine™ Dx Target Test results, 5 samples were no calls, 177 were IDH1 variant-positive, and 170 were IDH1 variant-negative by the Oncomine™ Dx Target Test.

The PPA was defined as the proportion of IDH1 variant-positive specimens as called by the CTA assay that were also IDH1 variant-positive by the Oncomine™ Dx Target Test. The NPA was defined as the proportion of IDH1 variant-negative specimens as called by the CTA assay that were also IDH1 variant-negative by the Oncomine™ Dx Target Test. The unadjusted concordances by variant and overall concordance (OPA) are shown in Table 9.

Table 9 IDH1—Bridging concordance results (unadjusted)

Parameter	Agreed	Total	Agreement	Exact 95% CIs
PPA exclude UNK	174	175	99.4%	96.9%, 100.0%
NPA exclude UNK	166	166	100.0%	97.8%, 100.0%
OPA exclude UNK	340	341	99.7%	98.4%, 100.0%
PPA include UNK	174	181	96.1%	92.2%, 98.4%

Table 9 IDH1—Bridging concordance results (unadjusted) *(continued)*

Parameter	Agreed	Total	Agreement	Exact 95% CIs
NPA include UNK	166	185	89.7%	84.4%, 93.7%
OPA include UNK	340	366	92.9%	89.8%, 95.3%

Of the enrolled samples, 349 samples were analyzed using the Oncomine™ Dx Target Test to demonstrate positive percent agreement (PPA) and negative percent agreement (NPA) concordance with a validated reference detection method (Sanger assay).

One hundred and sixty-eight (168) specimens from patients that tested positive using the Sanger assay were analyzed using the Oncomine™ Dx Target Test. In addition, 181 specimens that tested negative using the Sanger assay were analyzed using the Oncomine™ Dx Target Test.

Of the IDH1 variant-positive samples, 164 generated valid results from the Oncomine™ Dx Target Test. Three samples had invalid results due to failed QC metrics for the sequencing runs, and one generated a no call due to insufficient coverage.

Of the IDH1 variant-negative samples, 170 generated valid results from the Oncomine™ Dx Target Test. Ten samples had invalid results due to failed QC metrics for the sequencing runs, and one sample generated a no call due to insufficient coverage.

The PPA was defined as the proportion of IDH1 variant-positive specimens as called by the Sanger assay that were also IDH1 variant-positive as called by the Oncomine™ Dx Target Test, and the NPA was defined as the proportion of IDH1 variant-negative specimens as called by the Sanger assay that were also IDH1 variant-negative as called by the Oncomine™ Dx Target Test. The concordances and overall concordance (OPA; overall percent agreement) are shown in Table 10.

Table 10 IDH1—Accuracy concordance results

Agreement measure	Excluding invalids and no calls		Including invalids and no calls	
	Percent agreement	95% CI	Percent agreement	95% CI
PPA	99.4% (163/164)	(96.7%, 100.0%)	97.0% (163/168)	(93.2%, 99.0%)
NPA	96.5% (164/170)	(92.5%, 98.7%)	90.6% (164/181)	(85.4%, 94.4%)
OPA	97.9% (327/334) [1]	(95.7%, 99.2%)	93.7% (327/349)	(90.6%, 96.0%)

[1] Seven samples were found to be discordant in this analysis, where one was called a false negative and six were called false positives with the Oncomine™ Dx Target Test.

IDH1 study—clinical effectiveness

The clinical effectiveness of the Oncomine™ Dx Target Test was evaluated by measuring progression-free survival (PFS) for patients with CC who tested positive for IDH1 R132 variants (R132C, R132G, R132H, R132L, and R132S) by both the Clinical Trial Assay (CTA) and the Oncomine™ Dx Target Test. Progression-free survival and hazard ratio were calculated for patients who were selected for treatment with ivosidenib.

The primary efficacy outcome measurements between treatment and control arms (including PFS, hazard ratio, and overall survival) were examined in the AG120-C-005 study, based on the evaluable Oncomine™ Dx Target Test results (confirmed positive by the Oncomine™ Dx Target Test; 172 total

patients) and the study population (185 total patients) that included samples not confirmed with the Oncomine™ Dx Target Test.

The clinical efficacy (represented by PFS) determined in the Oncomine™ Dx Target Test positive population (N=115 treatment vs. 57 placebo) showed a HR=0.37 with 95% CI of (0.25, 0.55), and is similar to the Oncomine™ Dx Target Test positive *plus unevaluable* population (N=123 treatment vs. 61 placebo; HR=0.38; 95% CI: 0.26, 0.55) and the overall *CTA*+ population (primary endpoint of the AG120-C-005 study) (N=124 treatment vs. 61 placebo; HR = 0.37; 95% CI: 0.25, 0.54). These results suggest that no efficacy bias was introduced into the Oncomine™ Dx Target Test positive population.

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

Non-small cell lung cancer (NSCLC)—Analytical studies

Limit of blank studies

Limit of Blank (LoB)—Study I

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.

Limit of Blank (LoB)—Study II

A second study was performed to test the frequency of false positive calls for EGFR exon 20 insertion variants detected by the Oncomine™ Dx Target Test in wild-type samples. In this study, nucleic acid was extracted from 4 wild-type (WT) FFPE NSCLC clinical samples and carried through from library preparation to sequencing. Samples that are WT at all EGFR 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 the 4 WT samples prepared as FFPE sections on slides. The study was conducted using two different lots of the Oncomine™ Dx Target Test Kit. Operators made 18 library replicates for each sample and kit lot, giving a total of 4 samples × 18 replicates × 2 lots = 144 libraries sequenced.

The result at each variant location for each sample was tabulated. For all 4 samples, there were no positive calls at any of the EGFR exon 20 insertion variant locations analyzed by the test. The false positive rate was therefore zero.

Limit of Blank (LoB)—Study III

A further study was performed to test the frequency of false positive calls for EGFR exon 20 insertions detected by the Oncomine™ Dx Target Test in wild-type samples. In this study, operators used pre extracted DNA from 4 WT FFPE NSCLC clinical samples and carried it through from library preparation to sequencing. Samples that are WT at all EGFR 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.

The study was conducted using two different lots of the Oncomine™ Dx Target Test Kit. Operators made 18 library replicates for each sample and kit lot, giving a total of 4 samples × 18 replicates × 2 lots = 144 libraries sequenced.

The result at each variant location for each sample was tabulated. For all 4 samples, there were no positive calls at any of the variant locations analyzed by the test. The false positive rate was therefore zero.

Limit of Blank (LoB)—Study IV

A study was performed to test the frequency of false positive calls for ERBB2/HER2 exon 20 insertions detected by the Oncomine™ Dx Target Test in wild-type samples. In this study, operators used pre extracted DNA from 4 WT FFPE NSCLC clinical samples and carried it through from library preparation to sequencing. Samples that are WT at all ERBB2/HER2 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.

The study was conducted using two different lots of the Oncomine™ Dx Target Test Kit. Operators made 18 library replicates for each sample and kit lot, giving a total of 4 samples × 18 replicates × 2 lots = 144 libraries sequenced.

The result at each variant location for each sample was tabulated. For all 4 samples, there were no positive calls at any of the variant locations analyzed by the test. The false positive rate was therefore zero.

Limit of Blank (LoB)—Study V

A study was performed to test the frequency of false positive calls for ERBB2/HER2 SNVs detected by the Oncomine™ Dx Target Test in wild-type samples. In this study, operators used DNA extracted from 4 WT FFPE NSCLC clinical samples and carried it through from library preparation to sequencing. Samples that are WT at all ERBB2/HER2 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.

The study was conducted using two different lots of the Oncomine™ Dx Target Test Kit. Operators made 18 library replicates for each sample and kit lot, giving a total of 4 samples × 18 replicates × 2 lots = 144 libraries sequenced.

The result at each variant location for each sample was tabulated. For all 4 samples, there were no positive calls at any of the variant locations analyzed by the test. The false positive rate was therefore zero.

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.

Guard band testing studies

Guard band testing—initial studies

The tolerances encompassing the workflow steps in library preparation, template preparation, and sequencing were assessed in 20 separate studies corresponding to the most critical workflow steps of the test which could lead to assay failure. Each study included 3 test points, which included testing in low condition, nominal condition as defined by the user guide, and high condition. The guard band testing range for each experiment was designed such that the maximum and minimum test points challenged the system, while still being within operational error range. Each study was conducted across multiple runs, utilizing multiple operators and instrument systems. For each study, 6 independent libraries were prepared per condition. Libraries were pooled into 3 sets of pools with one pool tested at the low condition, one at the high condition, and the remaining samples tested at the nominal condition. Each pool was tested in a single system run, resulting in a total of 3 pools tested in 3 runs, with 3 or 6 replicates per condition.

Of the 20 studies, one study, Thermal Cycling Temperature Offset, showed a significant difference in performance when the temperature deviation in the PCR thermocycler during all thermocycling steps was increased by either +0.8°C or +1°C, which resulted in the samples failing the test QC parameters. The acceptable tolerance was therefore defined as –1°C to +0.5°C of the specified temperature. Of the remaining studies, 8 showed no significant difference in results, while 11 showed a statistically significant difference. However, it is recommended that each step in assay preparation and sequencing be followed according to the user guide.

Guard band testing—Study II

Guard band testing was performed to define Oncomine™ Dx Target Test tolerances by testing critical assay steps at key points of the NSCLC workflow, from library preparation through sequencing, using a blend of variants with EGFR exon 20 insertions.

Tolerances were tested across 11 test conditions across 3 test levels: Low, Standard Operating Protocol (SOP)/Nominal, and High. A DNA blend of nucleic acids isolated from clinical FFPE samples with the EFGR insertion was used in 10 test conditions related to volumes, temperature, and time, while a DNA control was used in a test condition related to DNA control volume. For a test condition to be considered acceptable, 6 library replicates must pass sample validity metrics (Sample AQ20 mean read length and % reads), and the EGFR variant within the clinical blended sample must be called present using the Oncomine™ Dx Target Test. Following initial testing, narrower acceptable ranges were established for EtOH (1–1.75 µL) and thermal cycling temperature offset (–1.0°C to +0.5°C).

The study demonstrated acceptable performance of the test at tolerance ranges that are significant deviations from the SOP-defined condition.

Guard band testing—Study III

Guard band testing was performed to define Oncomine™ Dx Target Test tolerances by testing critical assay steps at key points of the NSCLC workflow, from library preparation through sequencing, using one ERBB2/HER2 exon 20 insertion variant blend.

Tolerances were tested across 11 test conditions across 3 test levels: Low, Standard Operating Protocol (SOP)/Nominal, and High. A DNA blend of nucleic acids isolated from clinical FFPE samples with the ERBB2/HER2 exon 20 insertion was used in 10 test conditions related to volumes, temperature, and time, while a DNA control was used in a test condition related to DNA control volume. For a test condition to be considered acceptable, 6 library replicates must pass sample validity metrics (Sample

AQ20 mean read length and % reads), and the ERBB2/HER2 exon 20 insertion variant within the clinical blended sample must be called present using the Oncomine™ Dx Target Test. Following initial testing, a narrower acceptable range was established for EtOH (1.0–1.75 µL) and thermal cycling temperature offset (–1.0°C to +0.5°C).

The study demonstrated acceptable performance of the test at tolerance ranges that are significant deviations from the SOP-defined condition.

Guard band testing—Study IV

Guard band testing was performed to define Oncomine™ Dx Target Test tolerances by testing critical assay steps at key points of the NSCLC workflow, from library preparation through sequencing, using two ERBB2/HER2 SNV variant blends.

Tolerances were tested across 4 test conditions across 3 test levels: Low, Standard Operating Protocol (SOP)/Nominal, and High. DNA blends of nucleic acids isolated from clinical FFPE samples with the ERBB2/HER2 SNV were used in 4 test conditions: the volume of DNA panel, the volume of LIB HiFi Mix, the residual volume of ethanol and the temperature offset for the thermal cycler. For a test condition to be considered acceptable, 6 library replicates must meet assay performance requirements (MAPD and allelic frequency of ERBB2/HER2 SNVs), and the ERBB2/HER2 variant within the clinical blended sample must be called present using the Oncomine™ Dx Target Test. Following initial testing, a narrower acceptable range was established for EtOH (0–1.0 µL).

The study demonstrated acceptable performance of the test at tolerance ranges that are significant deviations from the SOP-defined condition.

Stability of assay intermediates studies

Stability of assay intermediates—Study I

The workflow for the Oncomine™ Dx Target Test incorporates several optional stopping points to hold assay intermediates. The stability of the intermediate products was evaluated by incorporating all of the 13 optional extended hold times specified in the user guide. A total of 3 samples (2 FFPE clinical samples and 1 FFPE cell line sample) were included in this study. The SNV, deletions, and fusion variant types were represented by samples which contained EGFR L858R, EGFR exon 19 deletion, BRAF V600E, a ROS1 fusion, and other representative variants. Each sample was tested under 3 different test conditions.

- Nominal (no-hold)
- Library hold—30-day hold of eluted libraries at –30°C to –10°C
- Combo hold—remaining stopping points in library preparation, template preparation, and sequencing, tested at the maximum hold time specified in the user guide

For DNA, allelic frequency and the log-transformed median absolute pairwise difference [log(MAPD)] were used as metrics to evaluate stability. For RNA, the log-transformed fusion reads and the log-transformed normalized read ratio [for example, log(fusion reads/total mapped reads)] were used as metrics to evaluate stability. In all of the evaluations, the results of the test conditions with the incorporated hold times were compared to the samples tested without the hold times. The study results support the conclusion that the 30-day library hold and combo hold conditions did not result in a decrease in Oncomine™ Dx Target Test performance relative to the nominal test condition.

Stability of assay intermediates—Study II

The stability of assay intermediates study was performed to test whether hold times in stopping points specified in the Oncomine™ Dx Target Test user guides affect test performance. The study was conducted in two separate parts that had the same study design and acceptance criteria. DNA corresponding to one of two EGFR exon 20 insertion variants, COSM1238030 (3 bp insertion), and COSM26720 (12 bp insertion), at mean allelic frequency (AF) of 2.5X LoD (1.9X to 3.5X LoD, 10% to 18% AF, respectively) was used in each part of the study to test assay performance at baseline, then compare it to performance after the stopping point holds that are specified in the Oncomine™ Dx Target Test user guides are included in the workflow. The Oncomine™ Dx Target Test library preparation workflow allows a total of 9 stopping points, each with a maximum hold time. The no-hold/hold performance was tested in three conditions:

- Nominal (no-hold)
- Library hold—30-day hold of eluted libraries at –30°C to –10°C
- Combo hold—8 remaining stopping points in library preparation, tested at the hold time specified in the user guide

The study demonstrated that the assay intermediates are stable after pre-defined hold times and Oncomine™ Dx Target Test performance was not affected by the hold times. Both EGFR exon 20 insertion variants (3 and 12 bp) were called correctly 100% of the time in the clinical sample blends across both hold conditions and the control condition. A T-Test was also performed to compare the mean AFs observed across the sample blends tested in each test hold condition compared with the no-hold condition. The p value for each test was >0.05, and together the p values showed no statistically significant differences in the mean variant AF between the hold and the no-hold conditions.

Stability of assay intermediates—Study III

The stability of assay intermediates study was performed to test whether hold times in stopping points specified in the Oncomine™ Dx Target Test user guides affect test performance. The study was conducted in two separate parts that had the same study design and acceptance criteria. One clinical ERBB2/HER2 exon 20 insertion-positive (COSM20959, 12-bp insertion) DNA blend was prepared at target allele frequency levels of 2–3X the limit of detection for testing was used in each part of the study to test assay performance at baseline, then compare it to performance after the stopping point holds that are specified in the Oncomine™ Dx Target Test user guides are included in the workflow. The Oncomine™ Dx Target Test library preparation workflow allows a total of 9 stopping points, each with a maximum hold time. The no-hold/hold performance was tested in three conditions:

- Nominal (no-hold)
- Library hold—30-day hold of eluted libraries at –30°C to –10°C
- Combo hold—8 remaining stopping points in library preparation, tested at the hold time specified in the user guide

The study demonstrated that the assay intermediates are stable after pre-defined hold times and Oncomine™ Dx Target Test performance was not affected by the hold times. The ERBB2/HER2 exon 20 insertion-positive variant was called correctly 100% of the time in the clinical sample blend across both hold conditions and the control condition.

Stability of assay intermediates—Study IV

The stability of assay intermediates study was performed to test whether hold times in stopping points specified in the Oncomine™ Dx Target Test user guides affect test performance. Two dual variant ERBB2/HER2 SNV-positive DNA blends were prepared at target allele frequency levels of 1.5–3X the limit of detection for testing. Both DNA blends were used in each part of the study to test assay performance at baseline, then compare it to performance after the stopping point holds that are specified in the Oncomine™ Dx Target Test user guides are included in the workflow. The Oncomine™ Dx Target Test library preparation workflow allows a total of 9 stopping points, each with a maximum hold time. The no-hold/hold performance was tested in three conditions:

- Nominal (no-hold)
- Library hold—30-day hold of eluted libraries at –30°C to –10°C
- Combo hold—8 remaining stopping points in library preparation, tested at the hold time specified in the user guide

The study demonstrated that the assay intermediates were stable after pre-defined hold times and Oncomine™ Dx Target Test performance was not affected by the hold times. All ERBB2/HER2 SNV-positive variants were called correctly 100% of the time in the clinical sample blends across both hold conditions and the control condition.

DNA and RNA input studies

DNA and RNA input—Study I

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.

DNA and RNA input—Study II

Two EGFR exon 20 insertion-positive variant DNA blends at allele frequency levels 2–3X the limit of detection were prepared for testing. A total of 96 libraries at various DNA input-level combinations within the range of 5–15 ng were tested, including positive controls and NTC controls, with 6 replicate libraries each 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 for the Oncomine™ Dx Target Test.

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

DNA input—Study III

Two ERBB2/HER2 exon 20 insertion-positive DNA blends were prepared at target allele frequency levels of 2–3X the limit of detection for testing. A total of 96 libraries at various DNA input-levels (5–15 ng) were tested, including positive controls and NTC controls, with 6 replicate libraries for each test condition.

The study demonstrated a 100% positive variant call rate within the input range tested. The results support the DNA 10-ng input requirement for the Oncomine™ Dx Target Test.

DNA input—Study IV

Two dual variant ERBB2/HER2 SNV-positive DNA blends were prepared at target allele frequency levels of 1.5–3X the limit of detection for testing. A total of 88 libraries at various DNA input-levels (5–15 ng) were tested, including positive controls and NTC controls, with 6 replicate libraries for each test condition.

The study demonstrated a 100% positive variant call rate within the input range tested. The results support the DNA 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 832 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 studies

Sample processing reproducibility—Study I

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 11. 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 11 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	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%)
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 11 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.

Sample processing reproducibility—Study II

The reproducibility and repeatability of variant detection using the Oncomine™ Dx Target Test were assessed with 2 WT samples and 2 EGFR exon 20 insertion variant-positive samples at 3 testing sites. Each sample was extracted two times and tested using 3 lots of reagents at each site, for a total of 18 replicates per sample. Each site had 2 Ion PGM™ Dx instrument systems and 2 operators.

The call rate, no call rate, positive call rate, negative call rate, and within-run repeatability were computed for each EGFR exon 20 insertion variant. 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 12. Including no calls, all positive call rates from positive variant locations were 100%.

Excluding no calls and combining data across all study samples, the estimate of repeatability was 100% for both EGFR exon 20 insertion variants. The lower limit of the 95% two-sided confidence interval (CI) for repeatability exceeded 100% at all variant locations.

Including no calls from the data, the estimate of repeatability was 100% at all EGFR exon 20 insertion variant locations. Including no calls, the lower limit of the 95% two-sided confidence interval for repeatability exceeded 81.5% at all variant locations.

Table 12 Call rates at positive variant locations

Sample	Variant identification	# of valid sample results (N)	# of positive calls (A)	# of negative calls (B)	# of no calls (C)	Positive call rate + 95% C.I.		Negative call rate + 95% C.I.		Within-run repeatability + 95% C.I.	
						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
D1	COSM12376	18	18	0	0	100% (81.5%, 100%)	100% (81.5%, 100%)	0% (0%, 18.5%)	0% (0%, 18.5%)	100% (66.4%, 100%)	100% (66.4%, 100%)
D2	COSM12380	18	18	0	0	100% (81.5%, 100%)	100% (81.5%, 100%)	0% (0%, 18.5%)	0% (0%, 18.5%)	100% (66.4%, 100%)	100% (66.4%, 100%)

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.

Table 13 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 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 13) 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 13) 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.

Interfering substances—Study IV

A study was performed to demonstrate that the performance of the Oncomine™ Dx Target Test in detecting EGFR exon 20 insertion-positive variants is not affected by the presence of potentially interfering substances.

A total of 3 FFPE samples (2 EGFR exon 20 insertion-positive, and 1 WT) with 2 replicates each were used to evaluate the impact of the listed interferents (Table 13) 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 EGFR exon 20 insertion-positive variants.

Interfering substances—Study V

A study was performed to demonstrate that the performance of the Oncomine™ Dx Target Test in detecting ERBB2/HER2 exon 20 insertion-positive variants is not affected by the presence of potentially interfering substances.

One ERBB2/HER2 exon 20 insertion-positive and one variant negative (WT) FFPE samples with 2 replicates each were used to evaluate the impact of the listed interferents (Table 13) on assay performance, and the results were compared to the control (no interferents) condition. For the 6 interferents tested, the positive, negative, 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 ERBB2/HER2 exon 20 insertion-positive variants.

Interfering substances—Study VI

A study was performed to demonstrate that the performance of the Oncomine™ Dx Target Test in detecting ERBB2/HER2 SNV-positive variants is not affected by the presence of potentially interfering substances.

Three ERBB2/HER2 SNV-positive and one variant negative (WT) FFPE samples with 2 replicates each were used to evaluate the impact of 6 interferents (Table 13) on assay performance, and the results were compared to the control (no interferents) condition. For the interferents tested, the positive, negative, and the overall concordance for all samples was 100%. These data support the claim that paraffin, xylene, ethanol, protease, or wash buffer do not affect assay performance at the level tested in detection of ERBB2/HER2 SNVs.

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).

Limit of Detection (LoD)—Study IV

The LoD of the Oncomine™ Dx Target Test was calculated by testing 2 clinical EGFR exon 20 insertion-positive specimens using the Oncomine™ Dx Target Test DNA workflow.

DNA extracted from EGFR exon 20 insertion-positive clinical FFPE specimens was blended with DNA extracted from WT clinical FFPE specimens to achieve 6 dilution levels. For each EGFR exon 20 insertion variant, 10 replicates per dilution level were tested with 2 reagent lots, giving a total of 120 data points. The LoD of the assay for EGFR exon 20 insertion detection was determined to be 4.8–5.2% allelic frequency.

Limit of Detection (LoD)—Study V

The LoD of the Oncomine™ Dx Target Test was calculated by testing 2 clinical ERBB2/HER2 exon 20 insertion-positive specimens using the Oncomine™ Dx Target Test DNA workflow.

DNA extracted from ERBB2/HER2 exon 20 insertion-positive clinical FFPE specimens was blended with DNA extracted from WT clinical FFPE specimens to achieve 6 dilution levels. For each ERBB2/HER2 exon 20 insertion variant, 10 replicates per dilution level were tested with 2 reagent lots, giving a total of 120 data points. The LoD of the assay for ERBB2/HER2 exon 20 insertion detection was determined to be 4.8–5.0% allelic frequency.

Limit of Detection (LoD)—Study VI

The LoD of the Oncomine™ Dx Target Test was calculated by testing 3 clinical ERBB2/HER2 SNV-positive specimens containing 4 different ERBB2/HER2 SNVs across 4 different exons using the Oncomine™ Dx Target Test DNA workflow.

DNA extracted from ERBB2/HER2 SNV-positive clinical FFPE specimens was blended with DNA extracted from WT clinical FFPE specimens to achieve 6 dilution levels. For each ERBB2/HER2 SNV variant, 20 replicates per dilution level were tested with 2 reagent lots, giving a total of 120 data points. The LoD of the assay for the 4 clinical ERBB2/HER2 SNVs was determined to be 4.5–5.8% allelic frequency.

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 14 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 20–29%
- 8 samples with tumor content 30–39%
- 10 samples with tumor content 40–49%
- 14 samples with tumor content 50–69%
- 21 samples with tumor content 70–90%

Table 15 Tumor content range in ROS1-positive samples used in clinical studies

ROS1-positive samples	Tumor-content range
0	<20%
1	20–29%
1	30–39%
0	40–49%
4	50–69%
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.

Tumor content—Study III

In a further study, the tumor cell content in FFPE samples used as input material was calculated for 149 FFPE clinical samples. 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 54 EGFR exon 20 insertion-positive and 95 EGFR exon 20 insertion-negative samples were included in the study analysis. All samples gave valid results for both the Oncomine™ Dx Target Test (passing Run, DNA Control, and DNA 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:

- 21 samples with tumor content <20%
- 41 samples with tumor content 20–29%
- 26 samples with tumor content 30–39%
- 27 samples with tumor content 40–59%
- 34 samples with tumor content 60–100%

Table 16 Tumor content range in EGFR exon 20 insertion-positive samples used in clinical studies

EGFR exon 20 insertion-positive samples	Tumor-content range
10	<20%
20	20–29%
6	30–39%
9	40–59%
9	60–100%

The corresponding 95% Clopper Pearson Exact CIs of the PPA, NPA, and OPA overlapped between tumor content levels. This shows that the EGFR exon 20 insertion detection performance of the Oncomine™ Dx Target Test was equivalent 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.

Tumor content—Study IV

To determine the minimum tumor cell content required in FFPE samples used as input material, 127 pre-characterized clinical samples were analyzed using the Oncomine™ Dx Target Test. The tumor cell content of each specimen and region of interest was estimated before the study by an external pathology lab.

A total of 61 EGFR exon 20 insertion-positive and 66 EGFR exon 20 insertion-negative samples were included in the study analysis. All samples gave valid results for both the Oncomine™ Dx Target Test and the reference method test. These valid samples were used for the tumor content study analysis. The observed tumor content had the following distribution:

- 33 samples with tumor content >0–30%
- 10 samples with tumor content ≥30–40%
- 32 samples with tumor content ≥40–60%
- 52 samples with tumor content ≥60–100%

Table 17 Tumor content range in EGFR exon 20 insertion-positive samples used in clinical studies

22	>0–30%
5	≥30–40%
10	≥40–60%
24	≥60–100%

The corresponding two-sided 95% CIs of the PPA, NPA, and OPA overlapped between tumor content levels. This shows that the EGFR exon 20 insertion 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, all samples were accurately called 100% of the time.

Tumor content—Study V

In a further study, the tumor cell content in FFPE samples used as input material was calculated for 147 FFPE clinical samples. 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 38 ERBB2/HER2 exon 20 insertion-positive or ERBB2/HER2 SNV-positive samples and 109 ERBB2/HER2 variant-negative samples were included in the study analysis. All samples gave valid results for both the Oncomine™ Dx Target Test (passing Run, DNA Control, and DNA 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:

- 2 samples with tumor content <20%
- 9 samples with tumor content 20–29%
- 15 samples with tumor content 30–39%
- 44 samples with tumor content 40–59%
- 77 samples with tumor content 60–100%

Table 18 Tumor content range in ERBB2/HER2 exon 20 insertion-positive and SNV-positive samples used in clinical studies

ERBB2/HER2 exon 20 insertion-positive or SNV-positive samples	Tumor-content range
0	<20%
5	20–29%
4	30–39%
9	40–59%
20	60–100%

The corresponding 95% Clopper Pearson Exact CIs of the PPA, NPA, and OPA overlapped between tumor content levels. This shows that the ERBB2/HER2 SNV and exon 20 insertion detection performance of the Oncomine™ Dx Target Test was equivalent 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.

Tumor content—Study VI

In a further study, the tumor cell content in FFPE samples used as input material was calculated for 216 FFPE clinical samples. The tumor cell content of each specimen and region of interest was estimated before the study by an external pathology lab.

A total of 91 RET fusion-positive and 125 RET fusion-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:

- 78 samples with tumor content <30%
- 47 samples with tumor content 30–39%
- 39 samples with tumor content 40–59%
- 52 samples with tumor content 60–100%

Table 19 Tumor content range in RET fusion-positive samples used in clinical studies

RET fusion-positive samples	Tumor-content range
34	<30%
14	30–39%
18	40–59%
25	60–100%

The corresponding 95% Clopper Pearson Exact CIs of the PPA, NPA, and OPA overlapped between tumor content levels. This shows that the RET fusion detection performance of the Oncomine™ Dx Target Test was equivalent 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 20 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 21 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 22 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 23 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 24 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 25 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
CCDC6-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 26 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%.

Assay reproducibility—Study IV

A study was performed to evaluate the reproducibility and repeatability of the Oncomine™ Dx Target Test for detection of EGFR exon 20 insertion variants using FFPE DNA from 2 EGFR variant-positive samples (blended with WT clinical samples) and 2 EGFR variant-negative (WT) samples.

Table 27 Sample description—Study IV

EGFR exon 20 insertion	No. of clinical specimens used	No. of levels tested	No. of samples tested
COSM1238028	1	2	2
COSM12376	1	2	2
EGFR-negative/WT DNA	2	N/A	2

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

Six pre-extracted DNA sample blends (4 insertion positive blends and 2 WT specimens) and placeholder RNA 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 DNA sample blends. Samples were run in duplicate using 2 different reagent lots at all study sites. The study design resulted in a total of 24 test determinations per sample blend tested.

The reproducibility results are summarized in the following table.

Table 28 Reproducibility results (EGFR exon 20 insertions)—Study IV

Description	Variants evaluated across the samples	Call rate excluding no calls[1]		Call rate including no calls[1]	
		Mean	Median	Mean	Median
EGFR exon 20 insertion-positive DNA (positive calls)	2	100%	100%	99.7%	100%
WT DNA (negative calls)	2	100%	100%	100%	100%

[1] Analysis includes invalid results.

Estimates of the repeatability were reported to be 100% for both COSM1238028 and COSM12376 (excluding no calls). The CV across both DNA locations ranged from 11.3% to 16.7%.

Assay reproducibility—Study V

A study was performed to evaluate the reproducibility and repeatability of the Oncomine™ Dx Target Test for detection of ERBB2/HER2 exon 20 insertion variants using FFPE DNA from 2 ERBB2/HER2 variant-positive samples (blended with WT clinical samples) and 2 ERBB2/HER2 variant-negative (WT) samples.

Table 29 Sample description—Study V

ERBB2/HER2 exon 20 insertion	No. of clinical specimens used	No. of samples tested
COSM20959	1	2
COSM12552	1	2
ERBB2/HER2-negative/WT DNA	2	2

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

Six pre-extracted DNA sample blends (4 insertion positive blends and 2 WT specimens) and placeholder RNA 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 DNA sample blends. Samples were run in duplicate using 2 different reagent lots at all study sites. The study design resulted in a total of 72 test determinations per sample blend tested.

The reproducibility results are summarized in the following table.

Table 30 Reproducibility results (ERBB2/HER2 exon 20 insertions)—Study V

Description	Variants evaluated across the samples	Call rate excluding no calls[1]		Call rate including no calls[1]	
		Mean	Median	Mean	Median
ERBB2/HER2 exon 20 insertion-positive DNA (positive calls)	2	100%	100%	98.6%	100%
WT DNA (negative calls)	2	100%	100%	100%	100%

[1] Analysis includes invalid results.

Estimates of the repeatability were reported to be 100% for both COSM20959 and COSM12552 (excluding no calls). The CV across both DNA locations ranged from 9.8% to 19.2%.

Assay reproducibility—Study VI

A study was performed to evaluate the reproducibility and repeatability of the Oncomine™ Dx Target Test for detection of ERBB2/HER2 SNVs using FFPE DNA from 3 ERBB2/HER2 SNV-positive samples (blended with WT clinical samples) and 4 ERBB2/HER2 variant-negative (WT) samples.

Table 31 Sample description—Study VI

ERBB2/HER2 SNV	No. of clinical specimens used	No. of samples tested
COSM14060 and COSM48358	1	2
COSM18609	1	2
COSM436498	1	2
ERBB2/HER2-negative/WT DNA	4	4

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

Seven pre-extracted DNA sample blends (3 SNV positive specimens and 4 WT specimens) and placeholder RNA 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 DNA sample blends. Samples were run in duplicate using 2 different reagent lots at all study sites. The study design resulted in a total of 72 test determinations per sample blend tested.

The reproducibility results are summarized in the following table.

Table 32 Reproducibility results (ERBB2/HER2 SNVs)—Study VI

Description	Variants evaluated across the samples	Call rate excluding no calls[1]		Call rate including no calls[1]	
		Mean	Median	Mean	Median
ERBB2/HER2 SNV-positive DNA (positive calls)	4	100%	100%	100%	100%
WT DNA (negative calls)	4	100%	100%	98.3%	98.4%

[1] Analysis includes invalid results.

Estimates of the repeatability were reported to be 100% for all 4 ERBB2/HER2 SNVs. The CV across all DNA locations ranged from 9.3% to 18.6%.

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 33 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 34 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 35 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%)

Non-small cell lung cancer (NSCLC)—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 positive percent agreement (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 negative percent agreement (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 overall percent agreement (OPA), and the results are shown in the following table:

Table 36 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 L858R and exon 19 deletions 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 37 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%)	74.5% (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 38 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 39 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%)

EGFR exon 20 insertions clinical study

EGFR exon 20 insertions concordance evaluation—Study I

To evaluate the ability of the Oncomine™ Dx Target Test to identify the EGFR exon 20 insertion biomarker in FFPE NSCLC tumor specimens, 87 specimens from patients that tested positive using local laboratory tests (LLT) were analyzed using the Oncomine™ Dx Target Test and a validated reference NGS assay (henceforth referred to as the reference assay). In addition, 110 samples screened as EGFR exon 20 insertion-negative were analyzed with the Oncomine™ Dx Target Test and the reference assay.

Of the 87 EGFR exon 20 insertion variant-positive samples, 63 were positive by the Oncomine™ Dx Target Test, 12 samples were negative, 6 samples yielded an invalid result, and 6 samples generated no calls. For the reference assay, 55 out the 87 samples were positive, 13 samples were negative, and 19 samples yielded an invalid result.

Of the 110 CTA-screened expected negative samples, 89 yielded negative calls with the Oncomine™ Dx Target Test, 19 samples yielded an invalid result, and 2 samples generated no calls. For the reference assay, 92 out of the 110 samples were negative, and 18 yielded invalid results.

In all, 160 samples were used to evaluate concordance between the Oncomine™ Dx Target Test as an investigational method and the reference assay. A total of 37 samples were excluded due insufficient material and invalid results between the two tests.

The PPA was defined as the proportion of EGFR-positive specimens as called by the reference 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 reference assay that were also EGFR-negative as called by the Oncomine™ Dx Target Test. The concordance between the Oncomine™ Dx Target Test and the reference assay is shown in the following table.

Table 40 EGFR exon 20 insertions—Concordance

Agreement measure	Excluding unknowns[1]		Including unknowns[1]	
	Percent agreement	95% CI	Percent agreement	95% CI
PPA	100% (54/54)	(93.4%, 100%)	98.2% (54/55)	(90.3%, 100%)
NPA	100% (95/95)	(96.2%, 100%)	90.5% (95/105)	(83.2.0%, 95.3%)
OPA	100% (149/149)	(97.6%, 100%)	93.1% (149/160)	(88.0%, 96.5%)

[1] Unknowns are defined as values due to insufficient sample, or sample QC sequencing failure resulting in an invalid result or No Call for the variant.

EGFR exon 20 insertions clinical effectiveness—Study I

The clinical effectiveness of the Oncomine™ Dx Target Test was evaluated by measuring the overall response rate (ORR; complete response or partial response) and duration of response (DOR) for patients with NSCLC that tested positive for EGFR exon 20 insertion variants by the Oncomine™ Dx Target Test. The ORR and DOR were calculated for 102 patients with prior platinum treatment selected for treatment with mobocertinib in the AP32788-15-101 trial, 27 of whom were responders.

The ORR was 26.7% (95% CI: 16.1, 39.7) in the patient population testing positive for EGFR exon 20 insertions with the Oncomine™ Dx Target Test (16/60) in comparison to 18.2% (95% CI: 2.3, 51.8) in the patient population testing negative for EGFR exon 20 insertions (2/11). The 2 samples from responding

patients that tested negative with the Oncomine™ Dx Target Test also tested negative with the reference assay, suggesting that the samples harbored novel variants that are not currently reported by either test.

The 26.7% ORR was similar to the ORR of 26.5% (95% CI: 18.2, 36.1) for the entire CDx or Oncomine™ Dx Target Test population (27/102) due to a large number of samples (n=10) that were unavailable for testing, of which 4 were from patients that showed partial response. The 26.7% ORR was consistent with the response rate of the LLT-positive population.

The median DOR by the Kaplan-Meier (KM) method was 11.07 months (95% CI: 9.13, 16.59) in the patient population testing positive for EGFR exon 20 insertions with the Oncomine™ Dx Target Test (n = 16), in comparison to 13.83 months (95% CI: 11.07, 16.59) for samples testing negative for EGFR exon 20 insertions (n = 2).

In total, 60 Oncomine™ Dx Target Test EGFR exon 20 insertion-positive, prior platinum-treated patients were included in the drug efficacy evaluation, 52 of which were concordant with the reference assay and LLT results, and 8 with only the LLT results available (reference assay unknown).

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

EGFR exon 20 insertions concordance evaluation—Study II

To evaluate the ability of the Oncomine™ Dx Target Test to identify the EGFR exon 20 insertion biomarker in FFPE NSCLC tumor specimens, 55 specimens from patients that tested positive using the clinical trial assay (CTA) were analyzed using the Oncomine™ Dx Target Test and a validated reference NGS assay. In addition, 103 commercially sourced samples screened as EGFR exon 20 insertion-negative were analyzed with the Oncomine™ Dx Target Test and the reference NGS assay.

Of the EGFR exon 20 insertion-positive samples, 46 generated valid results from both the reference NGS assay and the Oncomine™ Dx Target Test. One sample had invalid results due to sample QC failure with both assays.

Of the CTA screened expected negative samples, 60 generated valid results from both the Oncomine™ Dx Target Test and the reference NGS assay. The Oncomine™ Dx Target Test had 6 invalid results and the reference NGS assay had 2 invalid results.

An additional cohort of 23 samples that tested positive for EGFR exon 20 insertions with the CTA was included in the analysis, from which 5 samples were excluded because of insufficient material or invalid results. In both the Oncomine™ Dx Target Test and the reference NGS assay, 15 samples were positive, and 3 samples were EGFR exon 20 insertion-negative. Two of the 3 samples were found to be positive for a novel EGFR exon 20 insertion variant.

In all, 135 samples were used to evaluate concordance between the Oncomine™ Dx Target Test as an investigational method and the reference NGS assay. A total of 83 samples were excluded due to insufficient material, invalid results, or no calls by both assays.

The PPA was defined as the proportion of EGFR exon 20 insertion-positive specimens as called by the reference NGS assay that were also EGFR exon 20 insertion-positive as called by the Oncomine™ Dx Target Test. The NPA was defined as the proportion of EGFR exon 20 insertion-negative specimens as called by the reference NGS assay that were also EGFR exon 20 insertion-negative as called by the

Oncomine™ Dx Target Test. The concordances by variant and overall concordance are shown in the following table:

Table 41 EGFR exon 20 insertion—Concordance

Agreement measure	Excluding unknowns[1]		Including unknowns[1]	
	Percent agreement	95% CI	Percent agreement	95% CI
PPA	100% (61/61)	(94.1%, 100%)	98.4% (61/62)	(91.3%, 100%)
NPA	100% (67/67)	(94.6%, 100%)	91.8% (67/73)	(83.0%, 96.9%)
OPA	100% (128/128)	(97.2%, 100%)	94.8% (128/135)	(89.6%, 97.9%)

[1] Unknowns are defined as values due to insufficient sample, or sample QC sequencing failure resulting in an invalid result or No Call for the variant.

EGFR exon 20 insertion clinical effectiveness—Study II

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 EGFR exon 20 insertion variant by both the CTA and the Oncomine™ Dx Target Test. The ORR was calculated for patients selected for treatment with RYBREVANT™ (amivantamab-vmjw), with prior platinum treatment, and with no prior systemic treatment.

In the efficacy population, the ORR for the NSCLC patients with prior platinum treatment was 51.6% (95% CI: 38.6, 64.5).

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

ERBB2/HER2 clinical study

ERBB2/HER2 concordance evaluation—Study I

To evaluate the ability of the Oncomine™ Dx Target Test to identify activating ERBB2/HER2 SNV and exon 20 insertion biomarkers (ERBB2/HER2 activating mutation) in FFPE NSCLC tumor specimens, 101 specimens that tested positive using local laboratory tests (LLT) were analyzed using the Oncomine™ Dx Target Test and a validated reference NGS assay (henceforth referred to as the reference assay). In addition, 119 samples screened as ERBB2/HER2-negative were analyzed with the Oncomine™ Dx Target Test and the reference assay.

Of the 101 samples from the ERBB2/HER2 activating mutation-positive cohort that were tested, 38 samples were positive by both the Oncomine™ Dx Target Test and reference assay. Three samples were ERBB2/HER2-negative by both the Oncomine™ Dx Target Test and reference assay. One sample was discordant, giving a ERBB2/HER2-negative result on the reference assay, and a ERBB2/HER2-positive result on the Oncomine™ Dx Target Test (false positive). One ERBB2/HER2 activating mutation-positive and 1 ERBB2/HER2-negative sample by the reference assay were called unknown by the Oncomine™ Dx Target Test. One sample called ERBB2/HER2-positive by the Oncomine™ Dx Target Test was unknown by the reference assay.

Of the 119 samples from the ERBB2/HER2-negative cohort that were tested, 108 samples were negative by both the Oncomine™ Dx Target Test and reference assay. One sample was ERBB2/HER2-negative by the Oncomine™ Dx Target Test but produced unknown results by the reference assay.

Seven samples that were unknown by the Oncomine™ Dx Target Test were ERBB2/HER2-negative by the reference assay.

In all, 159 samples were used to evaluate concordance between the Oncomine™ Dx Target Test as an investigational method and the reference assay. A total of 61 samples were excluded due insufficient material between the two tests.

The PPA was defined as the proportion of ERBB2/HER2 activating mutation-positive specimens as called by the reference assay that were also ERBB2/HER2-positive as called by the Oncomine™ Dx Target Test, and the NPA was defined as the proportion of ERBB2/HER2-negative specimens as called by the reference assay that were also ERBB2/HER2-negative as called by the Oncomine™ Dx Target Test. The concordances by variant and overall concordance are shown in the following table linearly.

Table 42 ERBB2/HER2 SNV and exon 20 insertions—concordance

Agreement measure	Excluding unknowns[1]		Including unknowns[1]	
	Percent agreement	95% CI	Percent agreement	95% CI
PPA	100% (38/38)	(90.8%, 100%)	97.4% (38/39)	(86.5%, 99.9%)
NPA	99.1% (108/109)	(95.0%, 100%)	92.3% (108/117)	(85.9%, 96.4%)
OPA	99.3% (146/147)	(96.3%, 100%)	93.6% (146/156)	(88.5%, 96.9%)

[1] Unknowns are defined as values due to insufficient sample, or sample QC sequencing failure resulting in an invalid result or No Call for the variant.

ERBB2/HER2 clinical effectiveness—Study I

The efficacy of fam-trastuzumab deruxtecan-nxki(ENHERTU®) was evaluated in Daiichi Sankyo DS8201-A-U204 (DESTINY Lung 01, n=91) and DS8201-A-U206 (DESTINY Lung 02, n=52) studies. Demographic and baseline disease characteristics were similar for patients in both the DESTINY-Lung 01 and DESTINY-Lung 02 studies. Also, the response rates were consistent across the evaluated dose levels (5.4 mg/kg and 6.4 mg/kg). The efficacy of ENHERTU® (fam-trastuzumab deruxtecan-nxki) in both study populations (DESTINY Lung 01 and DESTINY Lung 02) and in those subjects positive for ERBB2 activating mutations (SNVs and exon 20 insertions) by the Oncomine™ Dx Target Test was comparable.

The safety and effectiveness of the Oncomine™ Dx Target Test for selecting NSCLC subjects who may benefit from treatment with ENHERTU® (fam-trastuzumab deruxtecan-nxki) was demonstrated through testing of DNA in tissue specimens from patients enrolled into one of two Daiichi Sankyo Studies DS8201-A-U204 (DESTINY Lung 01; NCT03505710) used to support the efficacy of ENHERTU® (fam-trastuzumab deruxtecan-nxki). The clinical effectiveness of the Oncomine™ Dx Target Test was evaluated by measuring the objective response rate (ORR; complete response or partial response) and duration of response (DOR) for patients with NSCLC that tested positive for ERBB2/HER2 activating mutations (SNVs or exon 20 insertions) by the Oncomine™ Dx Target Test. The ORR and DOR were calculated for 91 patients selected for treatment with ENHERTU® in the DS8201-A-U204 trial. The ORR was 58.3% (95% CI: 43.2, 72.4) in the patient population testing positive for ERBB2/HER2 SNVs or exon 20 insertions with the Oncomine™ Dx Target Test (28/48) in comparison to 52.4% (95% CI: 36.4, 68.0) in the patient population unevaluable by the Oncomine™ Dx Target Test (22/42). One sample from responding patients that tested negative with the Oncomine™ Dx Target Test also tested negative with the reference assay.

The efficacy in the Oncomine™ Dx Target Test CDx cohort (ORR 58.3%, 95% CI: 43.2, 72.4), was clinically meaningful, given the patient population, and supported the efficacy observed as reported in the drug label (ORR 57.7%, 95% CI 43.2, 71.3) trial (DS8201-A-U206).

The median DOR in subjects that were Oncomine™ Dx Target Test+/CTA+ was 12 months (95% CI 5.5, 18.2), compared to 9.3 months (95% CI 5.7, 14.7) for the CTA+ Cohort 2 population. The DOR for DESTINY Lung 02 was 8.7 months (95% CI 7.1, NE).

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

RET clinical studies

RET concordance evaluation—Study I

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 43.

Table 43 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 clinical effectiveness—Study I

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.

RET concordance evaluation—Study II

To evaluate the ability of the Oncomine™ Dx Target Test to identify the RET fusion biomarker in FFPE NSCLC tumor specimens, 203 specimens that tested positive using local laboratory tests (LLT) were analyzed using the Oncomine™ Dx Target Test. A subset of these samples (123) were also tested with a validated next generation sequencing (NGS) assay, henceforth referred to as the reference assay. In addition, 124 samples screened by a representative LLT as RET fusion-negative were analyzed with the Oncomine™ Dx Target Test and the reference assay.

Of the 203 RET fusion-positive samples, 161 were positive by the Oncomine™ Dx Target Test, 30 samples were negative, and 12 samples yielded an invalid result. For the reference assay, 92 samples were positive, 12 samples were negative, and 19 samples yielded an invalid result. Eighty

samples were not part of the analytical accuracy sample set and/or were not tested by the reference method.

Of the 124 LLT-negative samples, 118 yielded negative calls with the Oncomine™ Dx Target Test, 5 samples yielded an invalid result, and 1 sample was not tested due to insufficient RNA quantity. For the reference assay, 114 out of the 124 samples were negative, 9 yielded invalid results, and 1 sample was not tested due to insufficient material.

In all, 217 samples were used to evaluate concordance between the Oncomine™ Dx Target Test as an investigational method and the reference assay.

The PPA was defined as the proportion of RET fusion-positive specimens as called by the reference assay that were also RET-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 reference assay that were also RET fusion-negative as called by the Oncomine™ Dx Target Test. Concordance between the Oncomine™ Dx Target Test and the reference assay is shown in Table 44.

Table 44 Concordance between the Oncomine™ Dx Target Test and the reference assay

Agreement measure	Excluding unknowns[1]		Including unknowns[1]	
	Percent agreement	95% CI	Percent agreement	95% CI
PPA	92.3% (84/91)	(84.8%, 96.9%)	92.3% (84/91)	(84.8%, 96.9%)
NPA	96.8% (121/125)	(92.0%, 99.1%)	96.0% (121/126)	(91.0%, 98.7%)
OPA	94.9% (205/216)	(91.1%, 97.4%)	94.5% (205/217)	(90.5%, 97.1%)

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

RET clinical effectiveness—Study II

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 LLTs and the Oncomine™ Dx Target Test. The ORR was calculated for patients selected for treatment with the RET inhibitor selpercatinib, with prior platinum treatment, and with no prior systemic treatment. The disease control rate (DCR; proportion of patients who have a complete response, partial response, or stable disease) and clinical benefit rate (CBR; proportion of patients who have a complete response, partial response, or stable disease for ≥16 weeks) were also calculated for these patients. In total, 77 Oncomine™ Dx Target Test RET fusion-positive patients from the LIBRETTO-001 clinical study (58 from the prior platinum treatment cohort, 19 from a treatment-naive cohort) were included in the evaluation. The ORR for patients testing positive for RET fusions with the Oncomine™ Dx Target Test was compared with the ORR for patients in the full drug efficacy population (LLT-positive; n = 144).

- ORR for the Oncomine™ Dx Target Test RET fusion-positive NSCLC patients with prior platinum treatment (n = 58) was 67.2% (95% CI: 53.7%, 79.0%) with 39 patients having achieved best confirmed response of CR or PR.

  For the same population,
  - DCR was 91.4% (95% CI: 81.0%, 97.1%)
  - CBR was 84.5% (95% CI: 72.6%, 92.7%)
- ORR for the Oncomine™ Dx Target Test RET fusion-positive NSCLC patients in the treatment-naive cohort (n = 19) was 78.9% (95% CI: 54.4%, 93.95%) with 15 patients having achieved best confirmed response of CR or PR.

For the same population,

- DCR was 89.5% (95% CI: 66.9%, 98.7%)
- CBR was 89.5% (95% CI: 66.9%, 98.7%)

- ORR for Oncomine™ Dx Target Test RET fusion-positive NSCLC patients combined across prior treatment and treatment-naive cohorts (n = 77) was 70.1% (95% CI: 58.6%, 80.0%) with 54 patients having achieved best confirmed response of CR or PR.

  For the same population,

  - DCR was 90.9% (95% CI: 82.2%, 96.3%)
  - CBR was 85.7% (95% CI: 75.9%, 92.7%)

For comparison, the results for the primary efficacy endpoint of ORR observed in the corresponding drug efficacy set (LLT RET fusion-positive) of the prior platinum treatment cohort was 63.8% (95% CI: 53.9%, 73.0%), and for the treatment-naive cohort was 84.6% (95% CI: 69.5%, 94.1%). Across both cohorts, the ORR observed in the combined drug efficacy analysis sets was 69.4% (95% CI: 61.2%, 76.8%), with 100 patients having achieved best confirmed response of CR or PR.

For the same population,

- DCR was 93.1% (95% CI: 87.6%, 96.6%)
- CBR was 87.5% (95% CI: 81.0%, 92.4%)

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

ROS1 clinical studies

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 45 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 46 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 47 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.

Medullary thyroid cancer and thyroid cancer—Analytical studies

Limit of Blank (LoB) study

A study was performed to test the frequency of false positive calls for RET DNA variants and RET RNA fusions detected by the Oncomine™ Dx Target Test in wild-type (WT) clinical samples. For DNA, a previously tested set of negative FFPE clinical non-small cell lung cancer (NSCLC) samples known to be WT for RET DNA variant locations was reanalyzed to evaluate the false positive rate and verify that the LoB = 0. For RNA, a set of negative FFPE clinical thyroid cancer (TC) samples known to be WT for RET fusion isoforms was tested to evaluate the false positive rate and similarly verify that the LoB = 0. Samples that are WT at all RET locations are expected to produce a negative or absent call at each location. By definition (EP17-A2 guidelines), the 95th percentile of test results on blank samples equals zero.

The study was conducted using two different lots of the Oncomine™ Dx Target Test Kit. Operators made 18 library replicates for each of 8 unique samples (4 NSCLC and 4 TC) and kit lot, giving a total of 8 samples × 18 replicates × 2 lots = 288 libraries sequenced. The updated Oncomine™ Dx Target Test Kit RNA workflow was used for RNA library preparation.

The result at each variant location for each sample was tabulated. For all 8 samples, there were no positive calls at any of the variant locations analyzed by the test. The false positive rate was therefore zero.

Tissue input study

A study was performed to determine if thyroid cancer samples extracted using the Ion Torrent Dx Total Nucleic Acid Isolation Kit yield DNA and RNA at the concentrations that are 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. A total of 25 FFPE thyroid samples were analyzed, including 15 resection, 5 core needle biopsy (CNB), and 5 fine needle aspirate (FNA) samples.

Fourteen resection samples with ≥20% tumor content were prepared without macrodissection, 1 resection sample with <20% to ≥10% tumor cell content was macrodissected, and the 5 CNB and 5 FNA samples were prepared without macrodissection. For resection samples with ≥20% tumor cell content, 1–2 × 5 µm sections were used per extraction. For the resection sample with <20% tumor cell content and ≥10% tumor cell content that was macrodissected, 2 × 5 µm sections were used in the extraction. For CNBs, 9 × 5 µm sections were used per extraction. For FNAs, 7 × 5 µm sections were used per extraction. DNA and RNA concentrations were determined using the Ion Torrent Dx DNA Quantification Kit and Ion Torrent Dx RNA Quantification Kit, respectively.

Of the samples tested, 100% (25/25) yielded a DNA concentration of ≥0.83 ng/µL and an RNA concentration of ≥1.43 ng/µL, meeting the minimum concentration requirements.

Guard band testing study

Guard band testing was performed to define Oncomine™ Dx Target Test tolerances by testing critical assay steps at key points of the TC sample preparation and cDNA preparation and target amplification workflows. One clinical sample that was positive for a RET DNA variant (COSM965), and one clinical sample that was positive for a RET RNA fusion (CCDC6-RET.C1R12.COSF1271) were used in the study.

For DNA, the study included one condition to test the tolerance of Proteinase K enzyme volume in the digestion step in FFPE sample preparation using the RET DNA variant-positive clinical sample.

For RNA, the study included 7 conditions to test the tolerance of critical components and steps during FFPE sample preparation through cDNA target amplification using the RET RNA fusion-positive clinical sample. Conditions included Proteinase K volume, DNase volume, DNase incubation time, cDNA synthesis 10X Enzyme Mix volume, cDNA synthesis 5X Reaction Mix volume, Oncomine™ Dx Target Test—RNA panel volume, and LIB HiFi Mix enzyme volume.

For each test condition, 3 levels were tested to determine the tolerance range for each condition: Low, Standard Operating Protocol (SOP-Nominal), and High. The Low and High levels were set at 25% below and 25% above the SOP-Nominal volume or time, respectively. A total of 17 runs were performed to generate 9 DNA data points and 63 RNA data points for analysis (3 replicates per condition and level).

All conditions tested (9/9 RET DNA variant-positive samples and 63/63 RET RNA fusion-positive samples) yielded positive calls. All DNA and RNA yields were within a maximum mean difference of less than 50%.

The study showed acceptable performance of the test at tolerance ranges that are significant deviations from the SOP-defined condition.

RNA input study

A study was performed to compare RET fusion reads over a range of RNA:DNA input ratios to determine the sensitivity of fusion reads to input ratio. RNA was prepared from RET fusion-positive and wild-type RET fusion-negative thyroid cancer FFPE clinical samples and blended to fusion read levels of approximately 1–1.5X LoD. A DNA blend composed of two common RET variants was used to prepare a DNA library to function as a filler library in Oncomine™ Dx Target Test runs.

Sample RNA and DNA libraries were prepared with input ratios corresponding to the range of levels shown in Table 48. Six replicates of each input ratio were run, and the RET fusion reads were tabulated. The results showed a 100% call rate for RET fusions across the RNA:DNA input ratios tested, and further showed that mapped reads and log of fusion reads were not impacted by varying the input ratio from the standard RNA:DNA ratio of 10 ng:10 ng.

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

Table 48 RNA:DNA input ratio

RNA:DNA input (ng)	Average log fusion reads
10:10 (SOP)	2.39
5:15	2.63
6.5:15	2.62
8.5:15	2.60
10:15	2.39
15:15	2.53

FFPE tissue slide stability study

A study was performed to evaluate the stability of FFPE slide sections as a tissue source for the detection of RET variants in medullary thyroid cancer (MTC) and thyroid cancer (TC) with the Oncomine™ Dx Target Test. FFPE sections from 4 clinical samples, each containing a unique RET DNA variant or RNA fusion, were tested with the Oncomine™ Dx Target Test workflow at baseline (T0) and 4 time points after slide preparation: 3 months, 6 months, 9 months, and 12 months. The RET variant samples tested in the study, which represent prevalent DNA variants and RNA fusions found in MTC and TC, are listed in Table 49. Slide-mounted 5-µM tissue sections (non-paraffin dipped) from each sample were prepared from FFPE tissue blocks at the start of the study and stored at room temperature (15°C to 30°C) during the study.

Table 49 Samples tested

Tissue type	Variant type	RET variant ID	Variant amino acid change
MTC	SNV	COSM965	p.M918T
MTC	Deletion	COSM962	p.D898_E901del
TC	Fusion	CCDC6-RET.C1R12.COSF1271	N/A
TC	Fusion	NCOA4-RET.N7R12	N/A

At each time point, 2 replicate nucleic acid extractions were performed for all clinical samples using the Ion Torrent Dx Total Nucleic Acid Isolation Kit. Each extraction used 1–2 slides per sample. Samples were quantified, carried through the library and template preparation workflow steps, then sequenced using Oncomine™ Dx Target Test kit components.

The mean allelic frequency (AF) of the SNV and deletion in the DNA samples, and the mean log10-fusion reads in the RNA samples were determined by results analysis with Torrent Suite™ Dx Software. Study results are shown in Table 50 and Table 51.

Table 50 DNA variants

RET variant ID	Mean allelic frequency					
	T0 baseline	3 mo.	6 mo.	9 mo.	12 mo.	Lower threshold (0.7 × T0 baseline)
COSM965	0.467	0.463	0.435	0.440	0.449	0.327
COSM962	0.713	0.652	0.710	0.656	0.680	0.499

Table 51 RNA fusions

RET variant ID	Mean log10 fusion reads					
	T0 baseline	3 mo.	6 mo.	9 mo.	12 mo.	Lower threshold (0.6 × T0 baseline)
CCDC6-RET.C1R12.COSF1271	3.298	2.903	2.488	2.873	2.865	1.979
NCOA4-RET.N7R12	3.664	3.348	3.149	3.258	3.309	2.198

Overall, 100% of both DNA and RNA samples yielded positive calls. For RET DNA mutations, the AF was not significantly different for every time point up to and including 12 months, and no statistically significant difference in percent AF was observed in any resulting RET DNA mutation data. For RET RNA fusions, while the percent positive calls are 100% across all timepoints, a significant decrease in actual fusion reads (>50%) for samples with both CCDC6-RET and NCOA4 -RET variants were observed after three months, and the trend is maintained for all the later timepoints. Since the RNA Control QC metrics displayed a similar trend in both total mappable read and control variant fusion reads as seen with the clinical samples, the decrease in fusion reads can be traced to amplifiability differences and higher performing replicates in the run conducted at baseline (T0) relative to each subsequent timepoint through 12 months. Potential factors that may have contributed to the higher baseline performance include but are not limited to the quality of library preparation and recovery, variance in library pooling, templating and sequencing efficiency disparity, and variance in chip loading. These data indicated that the observed difference between baseline and subsequent timepoints is not correlated with RET fusions, TC tissue samples, or TC FFPE slide samples stored for up to 12 months.

Extracted RNA stability study

A study was performed to test the storage and freeze-thaw stability of RNA extracted from medullary thyroid cancer (MTC) FFPE samples. Testing was conducted to establish stability data for up to 12 months when extracted RNA is prepared using the Ion Torrent Dx FFPE Sample Preparation Kit for use with the Oncomine™ Dx Target Test.

Two RNA sample blends were prepared and tested with the Oncomine™ Dx Target Test, each including a RET fusion at fusion read levels 1.0– 1.5× above the limit of detection (LoD) of the test. Clinical specimens used to create the RNA sample blends included the 2 RET RNA fusion isoforms with the highest clinical prevalence in TC (CCDC6-RET.C1R12.COSF1271 and NCOA4-RET.N7R12). Each RNA sample blend was taken through the Oncomine™ Dx Target Test workflow at baseline (T0), and then at 3 months, 6 months, 9 months, and 12 months to demonstrate the stability of extracted RNA when stored at –90°C to –60°C. Testing up to 2 weeks beyond the required stability timepoint (date) was allowed to provide sufficient time to take the RNA sample blends through the entire workflow.

Two aliquots of each RNA sample blend were tested at each timepoint to evaluate the effect of freeze-thaw cycles on the ability to obtain valid sequencing results using the Oncomine™ Dx Target Test. One aliquot (Aliquot R1) was tested after going through a single freeze-thaw cycle where the frozen sample was allowed to thaw at room temperature until no ice crystals were present, then kept on ice until use. A second aliquot (Aliquot R2) was tested after going through 3 freeze-thaw cycles. In this case, a freeze-thaw cycle was defined as the frozen sample being thawed at room temperature until no ice crystals were present, kept on ice for one hour, then returned to the freezer for a minimum of

24 hours before beginning another freeze-thaw cycle. At each time point, 3 replicates of Aliquot R1 and 3 replicates of Aliquot R2 were tested in a single run for each RNA sample blend (2 total runs per time point). Sequencing results from each timepoint were compared to baseline results for each RNA sample blend.

As shown in Table 52, the percent positive calls are 100% across all timepoints for both RNA sample blends, and no statistically significant difference in percent fusion reads was observed in any resulting RET RNA fusion data.

Table 52 Extracted RNA stability

Sample blend	RET variant ID	Freeze-thaw cycles	Mean log10 fusion reads					
			T0 baseline	3 mo.	6 mo.	9 mo.	12 mo.	Lower threshold (0.6 × T0 baseline)
Smpl1	CCDC6-RET.C1R12.COSF1271	1	2.305	2.232	2.325	2.155	1.987	1.383
		3	2.262	2.399	2.289	2.213	2.454	1.357
Smpl2	NCOA4-RET.N7R12	1	2.451	2.354	2.121	2.202	2.147	1.471
		3	2.437	2.521	2.065	2.431	2.394	1.462

Sample processing reproducibility

The reproducibility and repeatability of variant detection using the Oncomine™ Dx Target Test were assessed with 2 RET DNA variant-positive medullary thyroid cancer (MTC) FFPE samples, and 2 RET fusion-positive thyroid cancer (TC) FFPE samples at 1 testing site. In addition, 2 WT TC FFPE samples were included in the study. Each sample was extracted 12 times (3 FFPE extraction kit lots × 4 replicates per kit lot) at one internal test site with 2 operators, for a total of 12 replicates per sample. The testing site used 2 Ion PGM™ Dx instrument systems.

The call rate, no call rate, positive call rate, negative call rate, and within-run repeatability were computed for each RET variant and WT sample. Including no calls and excluding known positive variant locations, the negative call rate at each clinical variant location for the 4 RET variant samples was 100%. Including no calls, the negative call rate at each clinical variant location for the two WT samples was 100% and 95.8%.

The results at positive variant locations are shown in Table 53. Including no calls, all positive call rates from positive variant locations were 100%.

When combining data across all study samples, excluding or including no calls, the estimate of repeatability was 100% for the tested RET DNA variant locations and RET RNA fusions. The lower limit of the 95% two-sided confidence interval (CI) for repeatability exceeded 54% at all variant locations.

Table 53 Call rates at positive variant locations

Sample	Variant identification (Variant Type)	# of valid sample results (N)	# of positive calls (A)	# of negative calls (B)	# of no calls (C)	Positive call rate + 95% C.I.		Negative call rate + 95% C.I.		Within-run repeatability + 95% C.I.	
						Including no calls (A/N)[1]	Excluding no calls (A/(A+B))	Including no calls (B/N)[1]	Excluding no calls (B/(A+B))	Including no calls[1]	Excluding no calls
1	COSM965 p.Met918Thr (SNV)	12	12	0	0	100% (73.5%, 100%)	100% (73.5%, 100%)	0% (0%, 26.5%)	0% (0%, 26.5%)	100% (54.1%, 100%)	100% (54.1%, 100%)
2	COSM962 p.Asp898_Glu901del (Deletion)	12	12	0	0	100% (73.5%, 100%)	100% (73.5%, 100%)	0% (0%, 26.5%)	0% (0%, 26.5%)	100% (54.1%, 100%)	100% (54.1%, 100%)
3	CCDC6-RET.C1R12.COSF1271 (Fusion)	12	12	0	N/A	N/A	100% (73.5%, 100%)	N/A	0% (0%, 26.5%)	N/A	100% (54.1%, 100%)
4	NCOA4-RET.N7R12 (Fusion)	12	12	0	N/A	N/A	100% (73.5%, 100%)	N/A	0% (0%, 26.5%)	N/A	100% (54.1%, 100%)
5	N/A (WT)	48	0	48	0	0% (0%, 7.4%)	0% (0%, 7.4%)	100% (92.6%, 100%)	100% (92.6%, 100%)	100% (85.8%, 100%)	100% (85.8%, 100%)
6	N/A (WT)	48	0	46	2	0% (0%, 7.4%)	0% (0%, 7.7%)	95.8% (85.7%, 99.5%)	100% (92.3%, 100%)	95.8% (78.9%, 99.9%)	100% (85.2%, 100%

[1] No calls are a possible result for only DNA variants and are not applicable to RNA fusion targets.

Interfering substances study

A study was performed to evaluate the performance of the Oncomine™ Dx Target Test in detecting RET DNA variants and RET RNA fusions in thyroid cancer (TC) FFPE samples in the presence of two potentially interfering substances that are known to be high in TC specimens: hemoglobin and colloid.

A total of 4 TC FFPE clinical samples (2 RET DNA variant-positive and 2 RET RNA fusion-positive) were used to evaluate the impact of hemoglobin and colloid on assay performance, and the results were compared to the control (no interferents) condition. Two wild-type TC samples with high colloid content were used for blending with RET DNA variant and RET RNA fusion samples to achieve a higher colloid content. In total, 72 data points were generated using 4 TC FFPE samples with the 2 interferent conditions (hemoglobin at 4 mg/ml, colloid at >40%), and control to evaluate the impact on Oncomine™ Dx Target Test performance.

For the 2 interferents tested, both the positive concordance and the overall concordance with control for all samples was 100%. These data support the claim that hemoglobin and colloid do not affect assay performance at the level tested in detection of the RET DNA variants and RET RNA fusions.

Limit of Detection (LoD) study

The LoD was evaluated for 4 representative RET DNA variants and 2 RET RNA fusion isoforms detected by the Oncomine™ Dx Target Test in clinical TC samples. For RET DNA variants, the LoD is the lowest allelic frequency of SNV, MNV, or deletion variants that can be detected at least 95% of the time. For RET RNA fusions, the LoD is the lowest fusion reads that can be detected at least 95% of the time. Variant-positive samples were blended with WT samples and used as the input DNA and RNA for the test. LoD was established using a representative variant approach. RET variants were selected in the following categories:

Table 54 LoD study variants

RET variant category	No. of variants tested
Simple SNV	2
MNV	1
12-bp deletion	1
RNA fusion	2

Two DNA sample blends (each with 2 RET DNA variants) were created for the study by blending RET variant-positive DNA with RET WT DNA. Two RNA sample blends (each with one RET RNA fusion) were created for the study by blending RET fusion-positive RNA with RET WT RNA. At least 120 data points were generated for each representative variant by testing the sample blends at 6 dilution levels, with 2 reagent lots, and 10 replicates per level per lot for a total of 720 data points.

Based on 4 representative RET DNA variants assessed in clinical samples, the LoDs for RET DNA variants tested in clinical samples (supported by the results from the assay reproducibility study) were determined to have allelic frequencies ranging from 4.9% to 5.5%.

Based on 2 representative RET fusion isoforms assessed in clinical samples, the LoD for RET RNA fusions tested in clinical samples was 236 fusion reads (higher of the LoD observed for the 2 isoforms tested).

Tumor content study

The tumor cell content in FFPE samples used as input material was calculated for clinical thyroid cancer samples to determine whether tumor content affected the performance of the Oncomine™ Dx Target Test. The tumor cell content of each specimen and region of interest was estimated before the study by an external pathology lab. In total, 133 specimens were included in the study analysis as follows:

- Sixty-eight (68) FFPE medullary thyroid cancer (MTC) samples, including 15 RET mutation-positive and 53 RET mutation-negative samples, were included in the study analysis.
- Sixty-five (65) FFPE thyroid cancer (TC) samples, including 9 RET fusion-positive and 56 RET fusion-negative samples, were included in the study analysis.

All samples gave valid results for both the Oncomine™ Dx Target Test (Passing Run, Control, and 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:

- 8 samples with tumor content <30%
- 4 samples with tumor content ≥30–40%
- 17 samples with tumor content >40–60%
- 104 samples with tumor content >60–100%

Table 55 Tumor content range in RET mutation-positive samples used in clinical studies

RET mutation-positive samples	Tumor-content range
0	<30%
1	≥30–40%
3	>40–60%
11	>60–100%

Table 56 Tumor content range in RET fusion-positive samples used in clinical studies

RET fusion-positive samples	Tumor-content range
2	<30%
0	≥30–40%
2	>40–60%
5	>60–100%

The PPA, NPA, and OPA agreement between the Oncomine™ Dx Target Test and the reference method test was 100% across all tumor content ranges. The corresponding 95% Clopper Pearson Exact CIs of the PPA, NPA, and OPA overlapped between tumor content levels. This result shows that the RET mutation and RET fusion detection performance of the Oncomine™ Dx Target Test was equivalent 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 study

A study was performed to evaluate the reproducibility and repeatability of the Oncomine™ Dx Target Test, independent of sample processing steps, for detection of RET DNA variants and RET RNA fusions. For DNA, FFPE DNA from 4 RET DNA variant-positive thyroid cancer (TC) samples (blended with WT samples) and WT clinical samples were used. For RNA, 2 RET RNA fusion-positive thyroid cancer (TC) samples (blended with WT samples) and fusion-negative (WT) samples were used.

Table 57 Sample description

RET variant category	RET variant ID	No. of clinical specimens used	No. of levels tested[1]	No. of sample blends tested
SNV	COSM965	1	2	2
MNV	COSM977	1	2	2
Deletion	COSM962	1	2	2
SNV	COSM1738369	1	2	2
Fusion	CCDC6-RET.C1R12.COSF1271	1	2	2
Fusion	NCOA4-RET.N7R12	1	2	2
WT DNA	N/A	7	N/A	NA
WT RNA	N/A	6	N/A	NA

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

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

In initial studies, 6 pre-extracted DNA sample blends (4 variant-positive blends and 2 WT blends) and 6 pre-extracted RNA sample blends (4 fusion-positive blends and 2 WT blends) were used for library preparation. Sample libraries were pooled and sequenced at 3 sites by a total of 6 operators. At each site, 2 operators were assigned to 2 instrument systems and were responsible for testing the sample blends. Across the 3 sites, 72 sample library sequencing results (2 libraries × 12 sequencing runs × 3 sites) were generated for each DNA and RNA sample blend and 144 data points (72 replicates per sample blend × 2 variant target levels; 0.9X–1.5X and 2X–3X LoD) were generated for each unique RET variant.

Three additional DNA sample blends (2 RET DNA variant-positive and 1 RET DNA WT sample blends), and 3 additional RNA sample blends (2 RET RNA fusion-positive and 1 RET RNA WT sample blends) were prepared to more closely approach the 2 LoD levels targeted in the study (0.9X–1.5X and 2X–3X LoD). Thirty-six additional runs (12 runs/site) were performed by the 6 operators at 3 sites to sequence these blends.

The reproducibility results are summarized in Table 58 and Table 59.

Table 58 Reproducibility results (RET DNA variants)

Description	Variants evaluated across the samples	Call rate excluding no calls		Call rate including no calls	
		Mean	Median	Mean	Median
RET variant-positive DNA (positive calls)	4	100%	100%	100%	100%
WT DNA (negative calls)	4	100%	100%	99.3%	100%

Table 59 Reproducibility results (RET RNA fusions)

Description	Isoforms evaluated across the samples	Call rate	
		Mean	Median
RET fusion-positive RNA (positive calls)	2	97.4%	97.9%
WT RNA (negative calls)	2	100%	100%

Estimates of within-run repeatability were 100% for the RET DNA variants tested, with one WT blend showing a 97.9% repeatability with no calls included. Repeatability estimates for the RET RNA fusion blends tested ranged from 88.9% to 100%.

Medullary thyroid cancer and thyroid cancer—Clinical studies

RET clinical study

RET study—concordance evaluation for medullary thyroid cancer (MTC) samples

To evaluate the ability of the Oncomine™ Dx Target Test to identify RET DNA variants in FFPE MTC tumor specimens, 46 RET DNA variant-positive specimens from patients enrolled in the LIBRETTO-001 clinical trial were analyzed with the Oncomine™ Dx Target Test and a validated reference next generation sequencing (NGS) method, henceforth referred to as the reference assay. In addition, 81 commercially procured TC samples were screened by a representative local laboratory test (LLT) for RET DNA variant-negative samples.

Of the 46 RET DNA variant-positive samples, 36 were positive by the Oncomine™ Dx Target Test, 6 samples were negative, 3 samples yielded an invalid result, and 1 sample was excluded due to insufficient DNA quantity. For the reference assay, 35 samples were positive, 7 samples were negative, and 1 sample yielded an invalid result.

Of the 81 LLT-negative samples, 54 were negative with the Oncomine™ Dx Target Test, 1 sample was positive, 25 samples yielded an invalid result, 1 sample was not tested due to insufficient DNA quantity. For the reference assay, 59 samples were negative, 1 sample was positive, and 18 yielded invalid results.

In all, 102 samples were used to evaluate concordance between the Oncomine™ Dx Target Test as an investigational method and the reference assay.

The PPA was defined as the proportion of RET DNA variant-positive specimens as called by the reference assay that were also RET DNA variant-positive as called by the Oncomine™ Dx Target Test, and the NPA was defined as the proportion of RET DNA variant-negative specimens as called by the reference assay that were also RET DNA variant-negative as called by the Oncomine™ Dx Target Test. Concordance between the Oncomine™ Dx Target Test and the reference assay is shown in Table 60.

Table 60 Concordance between Oncomine™ Dx Target Test and the reference assay—RET DNA variants (MTC)

Agreement measure	Excluding unknowns[1]		Including unknowns[1]	
	Percent agreement	95% CI	Percent agreement	95% CI
PPA	100.0% (36/36)	(90.3%, 100.0%)	100.0% (36/36)	(90.3%, 100.0%)
NPA	98.3% (57/58)	(90.8%, 100.0%)	86.4% (57/66)	(75.7%, 93.6%)
OPA	98.9% (93/94)	(94.2%, 100.0%)	91.2% (93/102)	(83.9%, 95.9%)

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

RET study—clinical effectiveness in medullary thyroid cancer (MTC)

The clinical effectiveness of the Oncomine™ Dx Target Test was evaluated by measuring the overall response rate (ORR) for patients with medullary thyroid cancer (MTC) that tested positive for a RET DNA variant by both LLTs and the Oncomine™ Dx Target Test. The ORR was calculated for patients selected for treatment with the RET inhibitor selpercatinib, with prior vandetinib/cabozantinib treatment, and vandetinib/cabozantinib naive. The disease control rate (DCR; proportion of patients who have a complete response (CR), partial response (PR), or stable disease) and clinical benefit rate (CBR; proportion of patients who have a complete response, partial response, or stable disease for ≥16 weeks) were also calculated for these patients. In total, 97 Oncomine™ Dx Target Test RET DNA variant-positive patients from the LIBRETTO-001 clinical study (38 from the prior treatment cohort, 59 from the treatment-naive cohort) were included in the evaluation. The ORR for patients testing positive for RET DNA variants with the Oncomine™ Dx Target Test was compared with the ORR for patients in the full drug efficacy population (LLT-positive; n = 142).

- ORR for the Oncomine™ Dx Target Test RET DNA variant-positive MTC patients with prior cabozantinib and/or vandetanib treatment (n = 38) was 68.4% (95% CI: 51.4%, 82.5%) with 26 patients having achieved best confirmed response of CR or PR.

  For the same population,
  - DCR was 94.7% (95% CI: 82.3%, 99.4%)
  - CBR was 92.1% (95% CI: 78.6%, 98.3%)
- ORR for the Oncomine™ Dx Target Test RET DNA variant-positive MTC patients in the treatment-naive cohort (n = 59) was 78.0% (95% CI: 65.3%, 87.7%) with 46 patients having achieved best confirmed response of CR or PR.

  For the same population,
  - DCR was 94.9% (95% CI: 85.9%, 98.9%)
  - CBR was 93.2% (95% CI: 83.5%, 98.1%)
- ORR for Oncomine™ Dx Target Test RET DNA variant-positive MTC patients combined across prior treatment and treatment-naive cohorts (n = 97) was 74.2% (95% CI: 64.35%, 82.6%) with 72 patients having achieved best confirmed response of CR or PR.

  For the same population,
  - DCR was 94.9% (95% CI: 88.4%, 98.3%)
  - CBR was 92.8% (95% CI: 85.7%, 97.1%)

For comparison, the results for the primary efficacy endpoint of ORR observed in the corresponding drug efficacy set (LLT RET DNA variant-positive) of the prior treatment cohort was 69.1% (95% CI: 55.2%, 80.9%), and for the treatment-naive cohort was 73.6% (95% CI: 63.0%, 82.45%). Across both cohorts, the ORR observed in the combined drug efficacy analysis sets was 71.8% (95% CI: 63.7%, 79.05%), with 102 patients having achieved best confirmed response of CR or PR.

For the same population,

- DCR was 95.1% (95% CI: 90.1%, 98.0%)
- CBR was 93.0% (95% CI: 87.4%, 96.6%)

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

RET study—concordance evaluation for thyroid cancer (TC) samples

To evaluate the ability of the Oncomine™ Dx Target Test to identify RET fusions in FFPE TC tumor specimens, 31 RET fusion-positive specimens from patients enrolled in the LIBRETTO-001 clinical trial, were analyzed with the Oncomine™ Dx Target Test and a validated reference next generation sequencing (NGS) method, henceforth referred to as the reference assay. In addition, 68 commercially procured TC samples were screened by a representative local laboratory test (LLT) for RET fusion-negative samples.

Of the 31 RET fusion-positive samples, 25 were positive by the Oncomine™ Dx Target Test, 2 samples were negative, 2 samples yielded an invalid result, and 2 samples were not tested due to insufficient RNA quantity. For the reference assay, 25 samples were positive, 2 samples were negative, and 2 samples yielded an invalid result.

Of the 68 LLT-negative samples, 58 were negative with the Oncomine™ Dx Target Test, and 10 samples yielded an invalid result. For the reference assay, 60 samples were negative, and 7 yielded invalid results.

In all, 87 samples were used to evaluate concordance between the Oncomine™ Dx Target Test as an investigational method and the reference assay.

The PPA was defined as the proportion of RET fusion-positive specimens as called by the reference 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 reference assay that were also RET fusion-negative as called by the Oncomine™ Dx Target Test. Concordance between the Oncomine™ Dx Target Test and the reference assay is shown in Table 61.

Table 61 Concordance between Oncomine™ Dx Target Test and the reference assay—RET fusions (TC)

Agreement measure	Excluding unknowns[1]		Including unknowns[1]	
	Percent agreement	95% CI	Percent agreement	95% CI
PPA	100.0% (25/25)	(86.3%, 100.0%)	100.0% (25/25)	(86.3%, 100.0%)
NPA	100.0% (57/57)	(93.7%, 100.0%)	91.9% (57/62)	(82.2%, 97.3%)
OPA	100.0% (82/82)	(95.6%, 100.0%)	94.3% (82/87)	(87.1%, 98.1%)

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

RET study—clinical effectiveness in thyroid cancer (TC)

The clinical effectiveness of the Oncomine™ Dx Target Test was evaluated by measuring the overall response rate (ORR) for patients with thyroid cancer (TC) that tested positive for a RET fusion by both LLTs and the Oncomine™ Dx Target Test. The ORR was calculated for patients selected for treatment with the RET inhibitor selpercatinib, with prior systemic treatment, and with no prior systemic treatment. The disease control rate (DCR; the proportion of patients who have a complete response (CR), partial response (PR), or stable disease) and clinical benefit rate (CBR; the proportion of patients who have a complete response, partial response, or stable disease for ≥16 weeks) were also calculated for these patients. In total, 23 Oncomine™ Dx Target Test RET fusion-positive patients from the LIBRETTO-001 clinical study (13 from the prior treatment cohort, 10 from the treatment-naive cohort) were included in the evaluation. The ORR for patients testing positive for RET fusions with the Oncomine™ Dx Target Test was compared with the ORR for patients in the full drug efficacy population (LLT-positive; n = 36).

- ORR for the Oncomine™ Dx Target Test RET fusion-positive TC patients with prior treatment (n = 13) was 69.2% (95% CI: 38.6%, 90.9%) with 9 patients having achieved best confirmed response of CR or PR.

  For the same population,
  - DCR was 100.0% (95% CI: 75.3%, 100.0%)
  - CBR was 92.3% (95% CI: 64.0%, 99.8%)
- ORR for the Oncomine™ Dx Target Test RET fusion-positive TC patients in the treatment-naive cohort (n = 10) was 100.0% (95% CI: 69.15%, 100.0%) with all 10 patients having achieved best confirmed response of CR or PR.

  For the same population,
  - DCR was 100.0% (95% CI: 69.2%, 100.0%)
  - CBR was 100.0% (95% CI: 69.2%, 100.0%)
- ORR for Oncomine™ Dx Target Test RET fusion-positive TC patients combined across prior treatment and treatment-naive cohorts (n = 23) was 82.6% (95% CI: 61.2%, 95.05%) with 19 patients having achieved best confirmed response of CR or PR.

  For the same population,
  - DCR was 100.0% (95% CI: 85.2%, 100.0%)
  - CBR was 95.7% (95% CI: 78.1%, 99.9%)

For comparison, the ORR for RET fusion-positive TC patients observed in the corresponding drug efficacy set (LLT RET fusion-positive), combined across prior treatment and treatment-naive cohorts (n = 36), was 83.3% (95% CI: 67.2%, 93.6%) with 30 patients having achieved best confirmed response of CR or PR.

For the same population,

- DCR was 100.0% (95% CI: 90.3%, 100.0%)
- CBR was 97.2% (95% CI: 85.5%, 99.9%)

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 in non-small cell lung cancer (NSCLC)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
AKT1	3	p.Glu17Lys	c.49G>A	COSM33765
ALK	21	p.Gly1128Ala	c.3383G>C	COSM98475
ALK	22	p.Leu1152Arg	c.3455T>G	COSM97185
ALK	22	p.Leu1152Pro	c.3455T>C	COSM1407659
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.Ile1171Asn	c.3512T>A	COSM28498
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	23	p.Phe1174Val	c.3520T>G	COSM28054
ALK	23	p.Phe1174Leu	c.3520T>C	COSM28057
ALK	23	p.Phe1174Ile	c.3520T>A	COSM28491
ALK	23	p.Phe1174Cys	c.3521T>G	COSM28059
ALK	23	p.Phe1174Ser	c.3521T>C	COSM53063
ALK	23	p.Phe1174Leu	c.3522C>G	COSM28061

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
ALK	23	p.Phe1174Leu	c.3522C>A	COSM28055
ALK	23	p.Val1180Leu	c.3538G>C	COSM4381101
ALK	23	p.Leu1196Met	c.3586C>A	COSM99137
ALK	23	p.Leu1196Gln	c.3587T>A	COSM1169447
ALK	23	p.Gly1202Arg	c.3604G>A	COSM144250
ALK	23	p.Ser1206Tyr	c.3617C>A	COSM144251
ALK	24	p.Phe1245Val	c.3733T>G	COSM28499
ALK	24	p.Phe1245Ile	c.3733T>A	COSM28492
ALK	24	p.Phe1245Cys	c.3734T>G	COSM28500
ALK	24	p.Phe1245Leu	c.3735C>G	COSM28062
ALK	24	p.Phe1245Leu	c.3735C>A	COSM28493
ALK	25	p.Arg1275Leu	c.3824G>T	COSM28060
ALK	25	p.Arg1275Gln	c.3824G>A	COSM28056
BRAF	11	p.Gly466Val	c.1397G>T	COSM451
BRAF	11	p.Gly466Glu	c.1397G>A	COSM453
BRAF	11	p.Gly469Arg	c.1405G>A	COSM457
BRAF	11	p.Gly469Val	c.1406G>T	COSM459
BRAF	11	p.Gly469Ala	c.1406G>C	COSM460
BRAF	15	p.Asp594Asn	c.1780G>A	COSM27639
BRAF	15	p.Asp594Gly	c.1781A>G	COSM467
BRAF	15	p.Val600Arg	c.1798_1799delGTinsAG	COSM474
BRAF	15	p.Val600Lys	c.1798_1799delGTinsAA	COSM473
BRAF	15	p.Val600Glu	c.1799T>A	COSM476
BRAF	15	p.Val600Glu	c.1799_1800delTGinsAA	COSM475
BRAF	15	p.Val600_Lys601delinsGlu	c.1799_1801delTGA	COSM1133
BRAF	15	p.Lys601Glu	c.1801A>G	COSM478
CDK4	2	p.Lys22Gln	c.64A>C	OM3153
CDK4	2	p.Lys22Arg	c.65A>G	COSM232013

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
CDK4	2	p.Lys22Met	c.65A>T	COSM3463915
CDK4	2	p.Arg24Cys	c.70C>T	COSM1677139
CDK4	2	p.Arg24Ser	c.70C>A	COSM3463914
CDK4	2	p.Arg24Leu	c.71G>T	COSM363684
CDK4	2	p.Arg24His	c.71G>A	COSM1989836
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_Thr751del	c.2236_2253delGAATTAAGAGAAGCAACA	COSM12728
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.Glu746_Arg748del	c.2239_2247delTTAAGAGAA	COSM6218
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_Ala750delinsPro	c.2239_2248delTTAAGAGAAGinsC	COSM12382
EGFR	19	p.Leu747_Thr751delinsPro	c.2239_2251delTTAAGAGAAGCAAinsC	COSM12383
EGFR	19	p.Leu747_Thr751del	c.2240_2254delTAAGAGAAGCAACAT	COSM12369
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	NA	NA	COSM26720[1]
EGFR	20	p.Met766_Ala767insAlaThrLeu	c.2302_2303insCGCTGGCCA	COSM12425
EGFR	20	p.Met766_Ala767insAlaSerVal	c.2308_2309insCCAGCGTGG	COSM12376
EGFR	20	p.Met766_Ala767insAlaIle	c.2302_2303insTAGCCA	COSM13559
EGFR	20	p.Ala767_Ser768insSerValAsp	c.2311_2312insGCGTGGACA	COSM13428
EGFR	20	p.Ala767_Ser768insSerValGly	c.2308_2309insGCAGCGTGG	COSM18429
EGFR	20	p.Ala767_Ser768insSerValGly	c.2308_2309insGGAGCGTGG	COSM1235344
EGFR	20	p.Ala767_Ser768insTyrValMet	c.2301_2302insTACGTGATG	COSM1651740
EGFR	20	p.Ser768_Val769insValCys	c.2307_2308insTGCGTG	COSM12379
EGFR	20	p.Ser768_Val769insValAspAsn	c.2313_2314insGTGGACAAC	COSM20885
EGFR	20	p.Ser768_Val769insValGlyVal	c.2308_2309insGGGTCGTGG	COSM18430
EGFR	20	p.Ser768_Val769insValAlaAsn	c.2303_2304insTGTGGCCAA	COSM1651741
EGFR	20	p.Ser768Ile	c.2303G>T	COSM6241
EGFR	20	p.Val769dup	c.2308_2309insTGG	COSM6506514
EGFR	20	p.Val769_Asp770insAspGly	c.2310_2311insGGGGAC	COSM85795
EGFR	20	p.Val769_Asp770insAspAsnPro	c.2316_2317insGACAACCCC	COSM1651745
EGFR	20	p.Val769_Asp770insAspGly	c.2310_2311insGGCGAC	COSM22955

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
EGFR	20	p.Val769_Asp770insGluArgGly	c.2309_2310insGCGTGGAGA	COSM1651742
EGFR	20	p.Asp769insAlaSerVal	c.2309_2310delACinsCCAGCGTGGAT	COSM13558
EGFR	20	p.Asp770_Asn771insAsnProHis	c.2319_2320insAACCCCCAC	COSM12381
EGFR	20	p.Asp770_Asn771insTyr	c.2310_2311insTAC	COSM1238030
EGFR	20	p.Asp770delinsGlyTyr	c.2308_2309insGTT	COSM12427
EGFR	20	p.Asp770_Asn771insAsnPro	c.2316_2317insAACCCC	MAN123
EGFR	20	p.Asp770_Asn771insAsnProHisGly	c.2320_2321insGCAACCCCCACG	COSM51544
EGFR	20	p.Asp770_Asn771insThr	c.2311_2312insCCA	COSM5023008
EGFR	20	p.Asp770_Asn771insMetAlaThrPro	c.2311_2312insTGGCCACCCCCA	COSM26719
EGFR	20	p.Asp770_Asn771insGlnArgGly	c.2310_2311insCAGCGTGGC	COSM4970107
EGFR	20	p.Asp770_Asn771insGly	c.2310_2311insGGC	COSM13004
EGFR	20	p.Asp770_Asn771delinsAlaGlyGly	c.2309_2312delACAAinsCTGGTGG	COSM12737
EGFR	20	p.Asp770_Asn771insSerValGlu	c.2311_2312insGCGTCGAAA	COSM1651743
EGFR	20	p.Asp770_Asn771insAlaProTrp	c.2310_2311insGCACCGTGG	COSM20886
EGFR	20	p.Asp770_Asn771insGlyThr	c.2310_2311insGGCACA	COSM1238029
EGFR	20	p.Asp770_Asn771insGlyLeu	c.2310_2311insGGGTTA	COSM48921
EGFR	20	p.Asp770_Asn771insGlyPhe	c.2310_2311insGGGTTT	COSM655155
EGFR	20	p.Asp770_Asn771insGly	c.2310_2311insGGT	COSM12378
EGFR	20	p.Asn771dup	c.2313_2314insAAC	COSM13003
EGFR	20	p.Asn771_Pro772insHis	c.2314_2315insACC	COSM1238031
EGFR	20	p.Asn771_Pro772insProHis	c.2319_2320insCCCCAC	COSM12380
EGFR	20	p.Asn771_Pro772insProThrHis	c.2315_2316insGACACACCC	COSM48923
EGFR	20	p.Asn771_Pro772insArgHis	c.2314_2315insGGCACC	COSM166390
EGFR	20	p.Asn771delinsThrHis	c.2311_2312insCAC	COSM22946
EGFR	20	p.Asn771delinsSerGlyHis	c.2311_2312insGTGGCC	COSM1651744
EGFR	20	p.Asn771delinsValHis	c.2311_2311delAinsGTCC	COSM5023007

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
EGFR	20	p.Asn771delinsLysLeu	c.2312_2313insACT	COSM6438147
EGFR	20	p.Pro772_His773insHisAla	c.2320_2321insCCCACG	COSM1238028
EGFR	20	p.Pro772_His773insHisAsn	c.2319_2320insAACCAC	COSM5023006
EGFR	20	p.Pro772_His773insHisVal	c.2321_2322insCCACGT	COSM18432
EGFR	20	p.Pro772_His773insThrPro	c.2316_2316delCinsAACCCCT	COSM12388
EGFR	20	p.Pro772_His773insThrPro	c.2322_2323insCACGTG	COSM22948
EGFR	20	p.His773dup	c.2319_2320insCAC	COSM12377
EGFR	20	p.Pro772_His773insVal	c.2316_2317insGTT	COSM255205
EGFR	20	p.His773delinsProAsnProTyr	c.2317_2318insCTAACCCCT	COSM1735761
EGFR	20	p.His773_Val774insThrGlnProPro	c.2319_2320insACACAACCCCCC	COSM3727813
EGFR	20	p.His773_Val774insGln	c.2319_2320insCAG	COSM131552
EGFR	20	p.Val774_Cys775insProArg	c.2322_2323insCCACGT	COSM4170223
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	7	p.Ser252Trp	c.755C>G	COSM36903
FGFR2	7	p.Pro253Leu	c.758C>T	COSM537801
FGFR2	7	p.Pro253Arg	c.758C>G	COSM49170
FGFR2	8	p.Ala314Asp	c.941C>A	COSM49171
FGFR2	9	p.Tyr375His	c.1123T>C	COSM1560916
FGFR2	9	p.Tyr375Cys	c.1124A>G	COSM36904
FGFR2	9	p.Cys382Arg	c.1144T>C	COSM36906
FGFR2	9	p.Cys382Tyr	c.1145G>A	COSM915493
FGFR2	12	p.Asn549His	c.1645A>C	COSM250083
FGFR2	12	p.Asn549Ser	c.1646A>G	COSM3665553
FGFR2	12	p.Asn549Lys	c.1647T>G	COSM36902
FGFR2	12	p.Asn549Lys	c.1647T>A	COSM36912

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
FGFR2	14	p.Lys659Glu	c.1975A>G	COSM36909
FGFR2	14	p.Lys659Met	c.1976A>T	COSM49175
FGFR2	14	p.Lys659Asn	c.1977G>T	COSM49173
FGFR2	14	p.Lys659Asn	c.1977G>C	COSM683054
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	2	p.Gly12Ser	c.34G>A	COSM480
HRAS	2	p.Gly12Cys	c.34G>T	COSM481
HRAS	2	p.Gly12Arg	c.34G>C	COSM482
HRAS	2	p.Gly12Val	c.35G>T	COSM483
HRAS	2	p.Gly12Asp	c.35G>A	COSM484
HRAS	2	p.Gly12Ala	c.35G>C	COSM485
HRAS	2	p.Gly13Arg	c.37G>C	COSM486
HRAS	2	p.Gly13Ser	c.37G>A	COSM487
HRAS	2	p.Gly13Cys	c.37G>T	COSM488
HRAS	2	p.Gly13Val	c.38G>T	COSM489
HRAS	2	p.Gly13Asp	c.38G>A	COSM490
HRAS	3	p.Gln61Lys	c.181C>A	COSM496
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.Gln61His	c.183G>T	COSM502
HRAS	3	p.Gln61His	c.183G>C	COSM503
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	2	p.Gly12Ser	c.34G>A	COSM517
KRAS	2	p.Gly12Arg	c.34G>C	COSM518

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
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
KRAS	2	p.Gly12Asp	c.35G>A	COSM521
KRAS	2	p.Gly13Cys	c.37G>T	COSM527
KRAS	2	p.Gly13Ser	c.37G>A	COSM528
KRAS	2	p.Gly13Arg	c.37G>C	COSM529
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	3	p.Ala59Thr	c.175G>A	COSM546
KRAS	3	p.Ala59Gly	c.176C>G	COSM28518
KRAS	3	p.Ala59Glu	c.176C>A	COSM547
KRAS	3	p.Gln61Lys	c.180_181delTCinsAA	COSM87298
KRAS	3	p.Gln61Lys	c.181C>A	COSM549
KRAS	3	p.Gln61Glu	c.181C>G	COSM550
KRAS	3	p.Gln61Pro	c.182A>C	COSM551
KRAS	3	p.Gln61Arg	c.182A>G	COSM552
KRAS	3	p.Gln61Leu	c.182A>T	COSM553

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
KRAS	3	p.Gln61His	c.183A>C	COSM554
KRAS	3	p.Gln61His	c.183A>T	COSM555
KRAS	4	p.Lys117Asn	c.351A>T	COSM28519
KRAS	4	p.Lys117Asn	c.351A>C	COSM19940
KRAS	4	p.Ala146Thr	c.436G>A	COSM19404
KRAS	4	p.Ala146Pro	c.436G>C	COSM19905
KRAS	4	p.Ala146Val	c.437C>T	COSM19900
KRAS	2	p.Gly12Cys	c.34G>T	COSM516
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

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
MAP2K2	2	p.Phe57Val	c.169T>G	COSM3534171
MAP2K2	2	p.Phe57Leu	c.169T>C	COSM1235618
MAP2K2	2	p.Phe57Leu	c.171T>G	OM3158
MAP2K2	2	p.Phe57Leu	c.171T>A	COSM3389034
MAP2K2	2	p.Gln60Pro	c.179A>C	COSM145610
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	30	p.Cys1483Arg	c.4447T>C	COSM3747775
MTOR	30	p.Cys1483Phe	c.4448G>T	COSM462616
MTOR	30	p.Cys1483Tyr	c.4448G>A	COSM462615
MTOR	30	p.Cys1483Trp	c.4449C>G	OM3149
MTOR	39	p.Glu1799Lys	c.5395G>A	COSM180789
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	40	p.Phe1888Leu	c.5664C>G	COSM462604

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
MTOR	40	p.Phe1888Leu	c.5664C>A	COSM893813
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	43	p.Thr1977Lys	c.5930C>A	COSM462601
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.
MTOR	43	p.Val2006Leu	c.6016G>C	COSM1134662
MTOR	43	p.Val2006Ile	c.6016G>A	COSM893804
MTOR	43	p.Val2006Phe	c.6016G>T	COSM249481
MTOR	47	p.Ser2215Pro	c.6643T>C	COSM1560108
MTOR	47	p.Ser2215Tyr	c.6644C>A	COSM20417
MTOR	47	p.Ser2215Phe	c.6644C>T	COSM1686998
MTOR	53	p.Leu2427Gln	c.7280T>A	COSM1185313
MTOR	53	p.Leu2427Arg	c.7280T>G	COSM2119114
NRAS	2	p.Gly12Cys	c.34G>T	COSM562
NRAS	2	p.Gly12Arg	c.34G>C	COSM561
NRAS	2	p.Gly12Ser	c.34G>A	COSM563
NRAS	2	p.Gly12Ala	c.35G>C	COSM565
NRAS	2	p.Gly12Asp	c.35G>A	COSM564
NRAS	2	p.Gly12Val	c.35G>T	COSM566
NRAS	2	p.Gly13Arg	c.37G>C	COSM569
NRAS	2	p.Gly13Ser	c.37G>A	COSM571

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
NRAS	2	p.Gly13Cys	c.37G>T	COSM570
NRAS	2	p.Gly13Ala	c.38G>C	COSM575
NRAS	2	p.Gly13Asp	c.38G>A	COSM573
NRAS	2	p.Gly13Val	c.38G>T	COSM574
NRAS	3	p.Ala59Thr	c.175G>A	COSM578
NRAS	3	p.Gln61Lys	c.181C>A	COSM580
NRAS	3	p.Gln61Glu	c.181C>G	COSM581
NRAS	3	p.Gln61Arg	c.182A>G	COSM584
NRAS	3	p.Gln61Pro	c.182A>C	COSM582
NRAS	3	p.Gln61Leu	c.182A>T	COSM583
NRAS	3	p.Gln61His	c.183A>C	COSM586
NRAS	3	p.Gln61His	c.183A>T	COSM585
NRAS	4	p.Lys117Asn	c.351G>T	MAN13
NRAS	4	p.Ala146Thr	c.436G>A	COSM27174
NRAS	4	p.Ala146Val	c.437C>T	COSM4170228
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

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
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
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

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
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
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	7	p.Ser257Leu	c.770C>T	COSM181063
RAF1	7	p.Ser257Trp	c.770C>G	COSM581519

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
RAF1	12	p.Thr421Met	c.1262_1263delCCinsTG	MAN9
RET	10	p.Cys618Arg	c.1852T>C	COSM29803
RET	10	p.Cys618Tyr	c.1853G>A	COSM980
RET	10	p.Cys620Arg	c.1858T>C	COSM29804
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 COSM4594154, so a positive COSM981 sample will also result in a positive call for COSM4594154.
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	36	p.Leu1951Met	c.5851C>A	COSM1072521
ROS1	38	p.Gly2032Arg	c.6094G>C	MAN11
ROS1	38	p.Gly2032Arg	c.6094G>A	MAN10

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

Fusion isoforms detected in non-small cell lung cancer (NSCLC)

RET fusion isoforms (N=44)	
ACBD5-RET.A11R12	KIF5B-RET.K16R12.COSF1230
AFAP1-RET.A3R12	KIF5B-RET.K18R12
AKAP13-RET.A35R12	KIF5B-RET.K22R12.COSF1253
AKAP13-RET.A36R12	KIF5B-RET.K23R11
CCDC6-RET.C1R11	KIF5B-RET.K23R11mid
CCDC6-RET.C1R11.1	KIF5B-RET.K23R12.COSF1234
CCDC6-RET.C1R12	KIF5B-RET.K24R8.COSF1236
CCDC6-RET.C1R12.COSF1271	KIF5B-RET.K24R10
CCDC6-RET.C2R12	KIF5B-RET.K24R11.COSF1262
CCDC6-RET.C8R11	KTN1-RET.K29R12.COSF1513
CCDC6-RET.C8R12full	NCOA4-RET.N7R12
CUX1-RET.C10R12	NCOA4_ELE1-RET.E6R12
ERC1-RET.E12R12	PCM1-RET.P29R12
ERC1-RET.E17R12	PRKAR1A-RET.P7R12
ERC1-RET.E7R12	RUFY2-RET.R9R12
ERC1_ELKS-RET.E11R12.COSF1507	SPECC1L-RET.S10R11.NGS.1
FKBP15-RET.F25R12	SPECC1L-RET.S10R12
GOLGA5-RET.G7R12	TBL1XR1-RET.T9R11.NGS.1
HOOK3-RET.H11R12	TBL1XR1-RET.T9R12
KIAA1468-RET.K10R12	TRIM24-RET.T9R12
KIF5B-RET.K15R11.COSF1255.1	TRIM27-RET.T3R12
KIF5B-RET.K15R12.COSF1232	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

IDH1 DNA variants detected in cholangiocarcinoma (CC)

Gene	Amino Acid Change	Nucleotide Change	Variant ID
IDH1	p.Arg132Cys	c.394C>T	COSM28747
IDH1	p.Arg132Ser	c.394C>A	COSM28748
IDH1	p.Arg132Gly	c.394C>G	COSM28749
IDH1	p.Arg132Leu	c.395G>T	COSM28750
IDH1	p.Arg132His	c.395G>A	COSM28746

RET DNA variants detected in medullary thyroid cancer (MTC)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
RET	10	p.Cys609Ser	c.1825T>A	RETC609S2
RET	10	p.Cys609Arg	c.1825T>C	RETC609R
RET	10	p.Cys609Gly	c.1825T>G	RETC609G
RET	10	p.Cys609Tyr	c.1826G>A	COSM967
RET	10	p.Cys609Ser	c.1826G>C	RETC609S
RET	10	p.Cys609Phe	c.1826G>T	RETC609F
RET	10	p.Cys609Trp	c.1827C>G	RETC609W
RET	10	p.Cys611Ser	c.1831T>A	COSM6984745
RET	10	p.Cys611Arg	c.1831T>C	COSM87269
RET	10	p.Cys611Gly	c.1831T>G	RETC611G
RET	10	p.Cys611Tyr	c.1832G>A	COSM4440700
RET	10	p.Cys611Tyr	c.1832_1833delGCinsAT	RETC611Y
RET	10	p.Cys611Ser	c.1832G>C	RETC611S
RET	10	p.Cys611Phe	c.1832G>T	RETC611F
RET	10	p.Cys611Phe	c.1832_1833delGCinsTT	RETC611F2
RET	10	p.Cys618Thr	c.1852_1853delTGinsAC	RETC618T **Note:** The nucleotide change of RETC618T overlaps that of RETC618S, so a positive RETC618T sample will also result in a positive call for RETC618S.
RET	10	p.Cys618Ser	c.1852T>A	COSM87267
RET	10	p.Cys618Gly	c.1852T>G	RETC618G
RET	10	p.Cys618Arg	c.1852T>C	COSM29803
RET	10	p.Cys618Tyr	c.1853G>A	COSM980
RET	10	p.Cys618Ser	c.1853G>C	RETC618S
RET	10	p.Cys618Phe	c.1853G>T	RETC618F
RET	10	p.Cys618Trp	c.1854C>G	RETC618W

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
RET	10	p.Cys620Arg	c.1858T>C	COSM29804
RET	10	p.Cys620Ser	c.1858T>A	COSM5946160
RET	10	p.Cys620Gly	c.1858T>G	RETC620G
RET	10	p.Cys620Tyr	c.1859G>A	COSM7403807
RET	10	p.Cys620Ser	c.1859G>C	COSM29805
RET	10	p.Cys620Phe	c.1859G>T	RETC620F
RET	10	p.Cys620Trp	c.1860C>G	COSM249815
RET	—	NA	NA	RETD627_L633delinsAH[1]
RET	11	p.Pro628_Leu633del	c.1882_1899delCCACTGTGCGACGAGCTG	RETP628_L633del
RET	11	p.Leu629_Glu632del	c.1891_1902delGACGAGCTGTGC	RETD631_C634del
RET	11	p.Leu629_Ile638delinsCysAsp	c.1884_1913delACTGTGCGACGAGCTGTGCCGCACGGTGATinsGTGCGA	RETL629_I638delinsCD
RET	11	p.Cys630Ser	c.1888T>A	RETC630S
RET	11	p.Cys630Arg	c.1888T>C	COSM964
RET	11	p.Cys630Gly	c.1888T>G	COSM29806
RET	11	p.Cys630Tyr	c.1889G>A	RETC630Y
RET	11	p.Cys630Phe	c.1889G>T	RETC630F
RET	11	p.Asp631del	c.1893_1895delCGA	RETD631del
RET	11	p.Asp631_Leu633delinsVal	c.1892_1897delACGAGC	RETD631_L633delinsV
RET	11	p.Asp631Tyr	c.1891G>T	RETD631Y
RET	11	p.Asp631_Leu633delinsGlu	c.1893_1898delCGAGCT	COSM983
RET	11	p.Glu632_Ala639delinsHisArg	c.1894_1917delGAGCTGTGCCGCACGGTGATCGCAinsCACCGT	COSM5945861
RET	11	p.Glu632_Leu633del	c.1894_1899delGAGCTG	COSM968
RET	11	p.Glu632_Thr636delinsSerSer	c.1894_1906delGAGCTGTGCCGCAinsAGCT	COSM1048
RET	11	p.Glu632_Cys634delinsGly	c.1895_1900delAGCTGT	COSM973

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
RET	11	p.Glu632_Leu633delinsVal	c.1895_1897delAGC	COSM982
RET	11	p.Glu632_Ala640delinsValArgPro	c.1895_1918delAGCTGTGCCGCACGGTGATCGCAGinsTGCGGC	COSM1049
RET	11	p.Glu632_Cys634delinsAspValArg	c.1896_1900delGCTGTinsCGTGC	RETE632_C634insDVR **Note:** The nucleotide change of RETE632_C634insDVR overlaps that of COSM966, so a positive RETE632_C634insDVR sample will also result in a positive call for COSM966.
RET	11	p.Cys634Ser	c.1900T>A	COSM1237918
RET	11	p.Cys634Gly	c.1900T>G	COSM1738369
RET	11	p.Cys634Arg	c.1900T>C	COSM966
RET	11	p.Cys634Tyr	c.1901G>A	COSM974
RET	11	p.Cys634Ser	c.1901G>C	COSM1666664
RET	11	p.Cys634Phe	c.1901G>T	COSM1237919
RET	11	p.Cys634Leu	c.1901_1902delGCinsTG	RETC634L **Note:** The nucleotide change of RETC634L overlaps that of COSM975, so a positive RETC634L sample will also result in a positive call for COSM975.
RET	11	p.Cys634Trp	c.1902C>G	COSM975
RET	11	p.Ala640Gly	c.1919C>G	RETA640G
RET	11	p.Val642Ile	c.1924G>A	COSM6005497
RET	11	p.Ser649Leu	c.1946C>T	COSM4170226
RET	13	p.Glu768Gln	c.2302G>C	COSM1716312
RET	13	p.Glu768Gly	c.2303A>G	COSM1347811
RET	13	p.Glu768Asp	c.2304G>T	RETE768D
RET	13	p.Glu768Asp	c.2304G>C	COSM21338

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
RET	13	p.Arg770Gln	c.2309G>A	RETR770Q
RET	13	p.Asn777Ser	c.2330A>G	RETN777S
RET	13	p.Val778Ile	c.2332G>A	COSM3807173
RET	13	p.Gln781Arg	c.2342A>G	COSM87265
RET	15	p.Leu881Val	c.2641C>G	RETL881V
RET	15	p.Ala883Phe	c.2646_2648delAGCinsTTT	COSM981 **Note:** The nucleotide change of COSM981 overlaps that of COSM4594154, so a positive COSM981 sample will also result in a positive call for COSM4594154.
RET	15	p.Ala883Ser	c.2647G>T	COSM133167
RET	15	p.Ala883Thr	c.2647G>A	COSM100081
RET	15	p.Ala883Phe	c.2647_2648delGCinsTT	COSM977 **Note:** The nucleotide change of COSM977 overlaps that of COSM4594154, so a positive COSM977 sample will also result in a positive call for COSM4594154.
RET	15	p.Ala883Tyr	c.2647_2648delGCinsTA	RETA883Y
RET	15	p.Ala883Val	c.2648C>T	COSM4594154
RET	15	p.Glu884Val	c.2651A>T	COSM1570338
RET	15	p.Arg886Trp	c.2656C>T	COSM6942691
RET	15	p.Ser891Ala	c.2671T>G	COSM5945860
RET	15	p.Asp898_Glu901del	c.2694_2705delTGTTTATGAAGA	COSM962
RET	15	p.Asp898_Glu902del	c.2695_2709delGTTTATGAAGAGGAT	COSM5991595
RET	15	p.Asp903_Ser904delinsGluPro	c.2709_2710delTTinsAC	RETD903_S904EP1
RET	15	p.Asp903_Ser904delinsGluPro	c.2709_2710delTTinsGC	RETD903_S904EP2

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
RET	15	p.Ser904Cys	c.2711C>G	RETS904C
RET	15	p.Ser904Phe	c.2711C>T	COSM6438204
RET	16	p.Gly911Asp	c.2732G>A	COSM20888
RET	16	p.Arg912Trp	c.2734C>T	COSM3415038
RET	16	p.Arg912Pro	c.2735G>C	RETR912P
RET	16	p.Arg912Leu	c.2735G>T	COSM188545
RET	16	p.Met918Val	c.2752A>G	RETM918V
RET	16	p.Met918Thr	c.2753T>C	COSM965
RET	16	p.Ser922Pro	c.2764T>C	COSM26636

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

RET fusion isoforms detected in thyroid cancer (TC)

RET fusion isoforms (N=44)	
ACBD5-RET.A11R12	KIF5B-RET.K16R12.COSF1230
AFAP1-RET.A3R12	KIF5B-RET.K18R12
AKAP13-RET.A35R12	KIF5B-RET.K22R12.COSF1253
AKAP13-RET.A36R12	KIF5B-RET.K23R11
CCDC6-RET.C1R11	KIF5B-RET.K23R11mid
CCDC6-RET.C1R11.1	KIF5B-RET.K23R12.COSF1234
CCDC6-RET.C1R12	KIF5B-RET.K24R8.COSF1236
CCDC6-RET.C1R12.COSF1271	KIF5B-RET.K24R10
CCDC6-RET.C2R12	KIF5B-RET.K24R11.COSF1262
CCDC6-RET.C8R11	KTN1-RET.K29R12.COSF1513
CCDC6-RET.C8R12full	NCOA4-RET.N7R12
CUX1-RET.C10R12	NCOA4_ELE1-RET.E6R12
ERC1-RET.E12R12	PCM1-RET.P29R12
ERC1-RET.E17R12	PRKAR1A-RET.P7R12
ERC1-RET.E7R12	RUFY2-RET.R9R12
ERC1_ELKS-RET.E11R12.COSF1507	SPECC1L-RET.S10R11.NGS.1
FKBP15-RET.F25R12	SPECC1L-RET.S10R12
GOLGA5-RET.G7R12	TBL1XR1-RET.T9R11.NGS.1
HOOK3-RET.H11R12	TBL1XR1-RET.T9R12
KIAA1468-RET.K10R12	TRIM24-RET.T9R12
KIF5B-RET.K15R11.COSF1255.1	TRIM27-RET.T3R12
KIF5B-RET.K15R12.COSF1232	TRIM33-RET.T16R12

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ion torrent

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

USER GUIDE

for use with Torrent Suite™ Dx Software 5.12.5

Publication Number MAN0019391

Revision A.0

IVD

For In Vitro Diagnostic Use. | Rx Only

ThermoFisher SCIENTIFIC

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

Products manufactured at this site:

- 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, Maryland 21704 USA

Products manufactured at this site:

- 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

Revision history: Pub. No. MAN0019391

Revision	Date	Description
A.0	23 September 2022	New Oncomine™ Dx Target Test user guide for FDA submission

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

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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*.

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*.

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*.

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*.

Materials provided

Oncomine™ Dx Target Test Kit

The Oncomine™ Dx Target Test Kit (Cat. No. A51695) 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	A52639

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
Non-small cell lung cancer (NSCLC)	
Resection or surgical biopsies	2 × 5-micron sections
Core needle biopsies	9 × 5-micron sections
Fine needle aspirates	7 × 5-micron sections
Cholangiocarcinoma (CC)	
Resection or surgical biopsies	4 × 5-micron sections
Core needle biopsies	9 × 5-micron sections
Thyroid cancer (TC) and medullary thyroid cancer (MTC)	
Resection or surgical biopsies	2 × 5-micron sections
Core needle biopsies	9 × 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 12 months at 15–30°C. Stability studies for blocks and slides with DNA insertions are ongoing.

Extracted DNA can be stored at –30°C to –10°C for up to 12 months, including 3 freeze-thaw cycles. Stability studies for extracted DNA (insertions) are ongoing, but a minimum stability of 5 months, including one freeze-thaw cycle, has been established.

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 (59–86°F).
- 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: 10–20% 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. If an initial extraction leads 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.

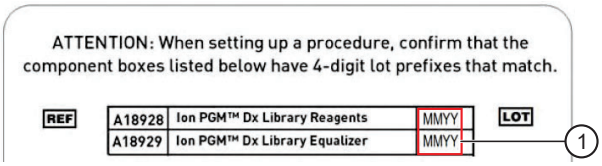
Kit interchangeability and component box lot matching

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.

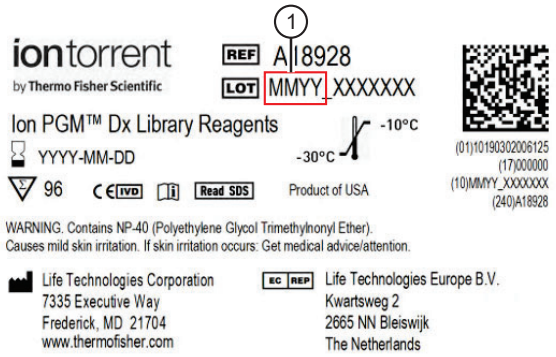
Note: The Oncomine™ Dx Target RNA Control and Oncomine™ Dx Target RNA Control Diluent in the Oncomine™ Dx Target Test, Controls, and Diluent Kit must be lot-matched. However, the controls do not require lot-matching with the Oncomine™ Dx Target Test DNA and RNA Panel.

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:

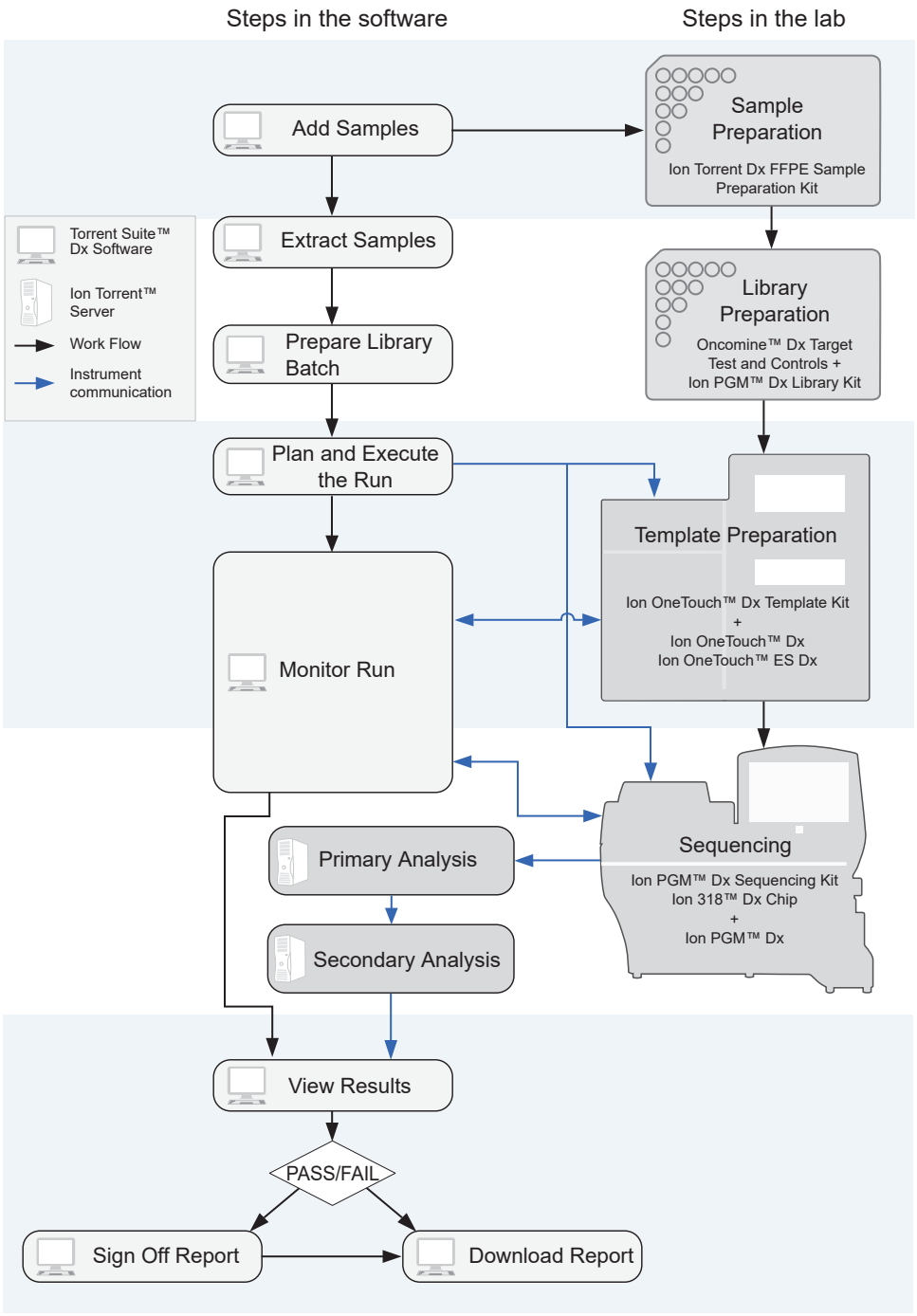


An example box label with lot information is shown below:



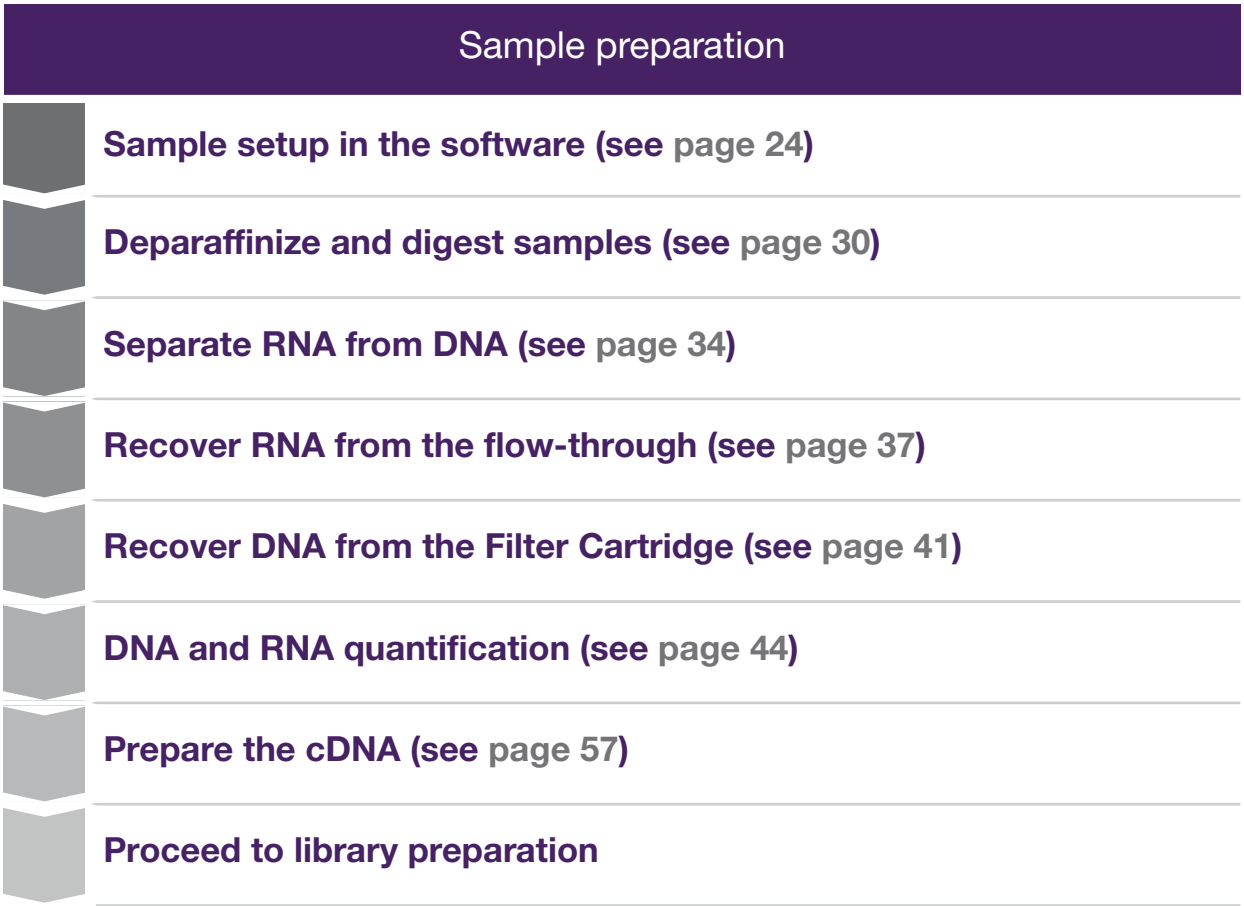
1 Lot prefix

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.



Library preparation workflow

Library preparation
Prepare a library batch in the software (see page 62)
Prepare reagents and equipment (see page 65)
Amplify the cDNA (see page 66)
Amplify the DNA (see page 69)
Transfer the cDNA amplicons (see page 72)
Partially digest amplicons (see page 73)
Ligate barcode adapters (see page 75)
Prepare the LIB HiFi Mix plus LIB Primers mix (see page 76)
Purify the barcode-adapted library (see page 77)
Amplify the barcode-adapted library (see page 78)
Prepare the LIB Beads (see page 78)
Add LIB Capture to the amplified sample library (see page 79)
Add the LIB Beads and wash (see page 79)
Elute the library (see page 80)

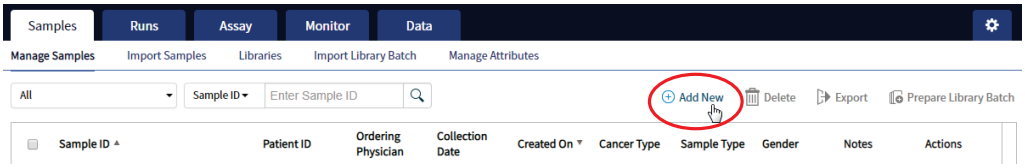
Library preparation

Proceed to *Oncomine™ Dx Target Test Part III: Template Preparation User Guide*

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.

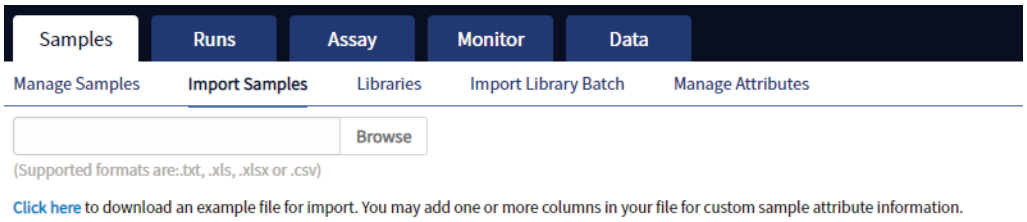
(continued)

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 26 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 83.

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.

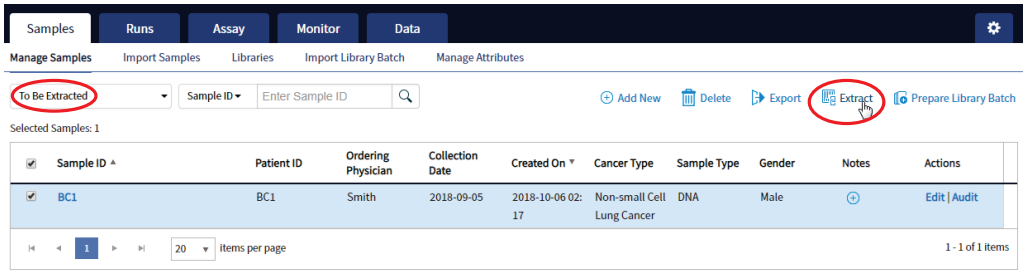
Note: LIMS users must create custom attributes before importing sample and Planned Run information from LIMS for the attributes to be propagated through to output files. The software ignores all input file content that is not a recognized attribute.

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.



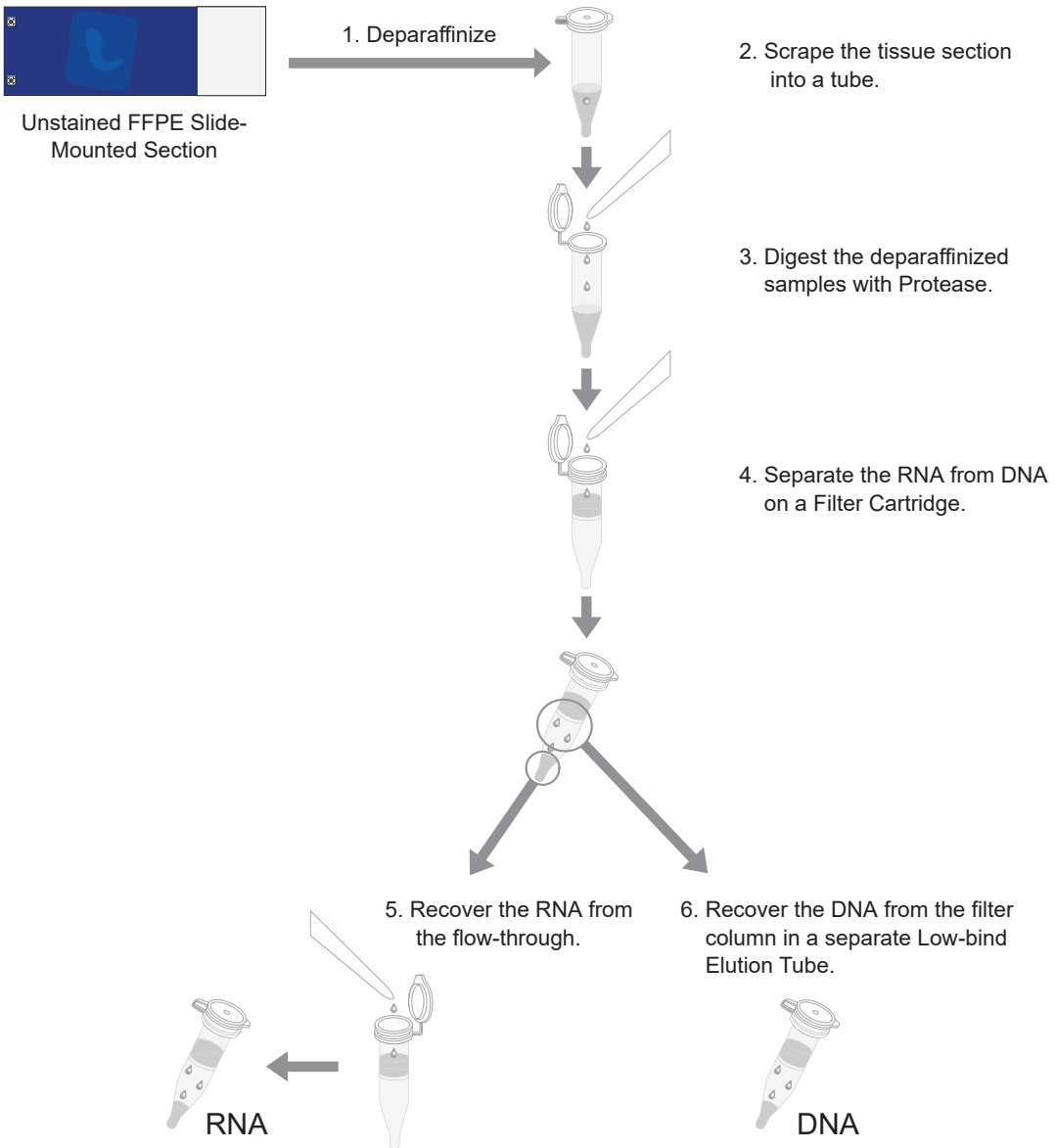
3. Click **Extract**. In the dialog box, scan the barcode that is printed on the Ion Torrent Dx Total Nucleic Acid Isolation Kit (box 1 of 2, Part No. A32434).

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 32.

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 33.

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 31.

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.

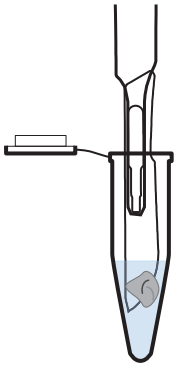
In this procedure, scrape each tissue section into the appropriate labeled 1.5-mL low-retention microcentrifuge tube containing 1X Digestion Buffer (prepared in "Prepare 1X Digestion Buffer" on page 30).

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 36.

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 41.
6. Proceed to “Recover the RNA from the flow-through” on page 37.

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 36.
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 30) 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 30) 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.

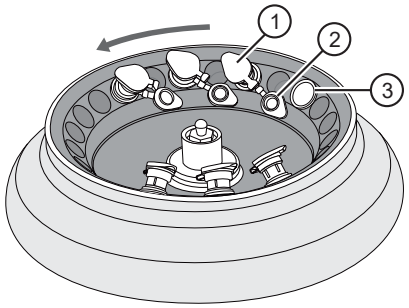
2. Wet the pipette tip by slowly pipetting up and down 3 times in the pre-heated Elution Solution.

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 5 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 36).
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.

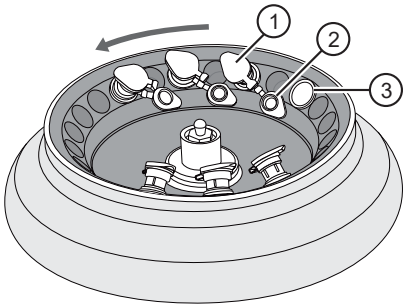
2. Wet the pipette tip by slowly pipetting up and down 3 times in the pre-heated Elution Solution.

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. Stability studies for extracted DNA (insertions) are ongoing, but a minimum stability of 5 months, including one freeze-thaw cycle, has been established.

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 5 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 (for example, 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 (for up to 12 months) and the quantified RNA sample at –90°C to –60°C (for up to 12 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 50 and page 54.
- 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 54. 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 12 months. Stability studies for extracted DNA (insertions) are ongoing, but a minimum stability of 5 months, including one freeze-thaw cycle, has been established.

(continued)

DNA concentration	Dilution procedure
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 54. 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 12 months. Stability studies for extracted DNA (insertions) are ongoing, but a minimum stability of 5 months, including one freeze-thaw cycle, has been established.

(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 54. 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 12 months. Stability studies for extracted DNA (insertions) are ongoing, but a minimum stability of 5 months, including one freeze-thaw cycle, has been established.

(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 54. 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 12 months. Stability studies for extracted DNA (insertions) are ongoing, but a minimum stability of 5 months, including one freeze-thaw cycle, has been established.

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 58. Do not store the diluted RNA samples for longer than necessary. **Note:** Store the remaining undiluted RNA sample at –90°C to –60°C for up to 5 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 58. Do not store the diluted RNA samples for longer than necessary. **Note:** Store the remaining undiluted RNA sample at –90°C to –60°C for up to 5 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 58. 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 –90°C to –60°C for up to 5 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 58. 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 –90°C to –60°C for up to 5 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 RNA Control	A32443	–90°C to –60°C
Oncomine™ Dx Target RNA Control Diluent	A38872	–90°C to –60°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 48 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.

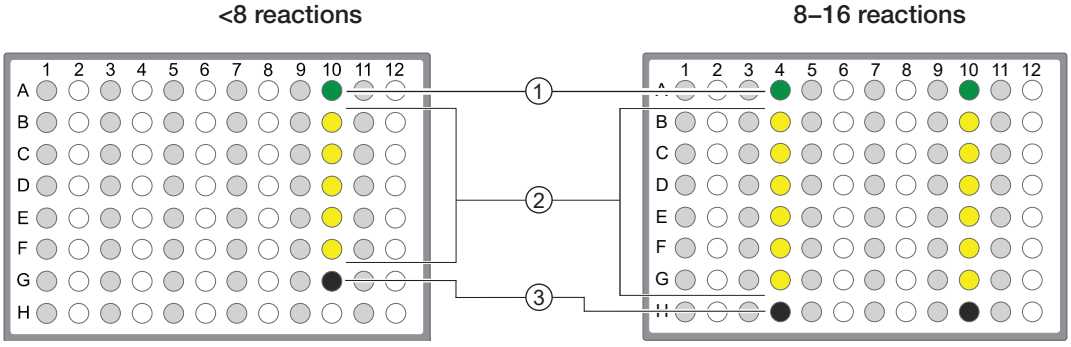
Prepare a master mix for up to 16 cDNA synthesis reactions.

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.

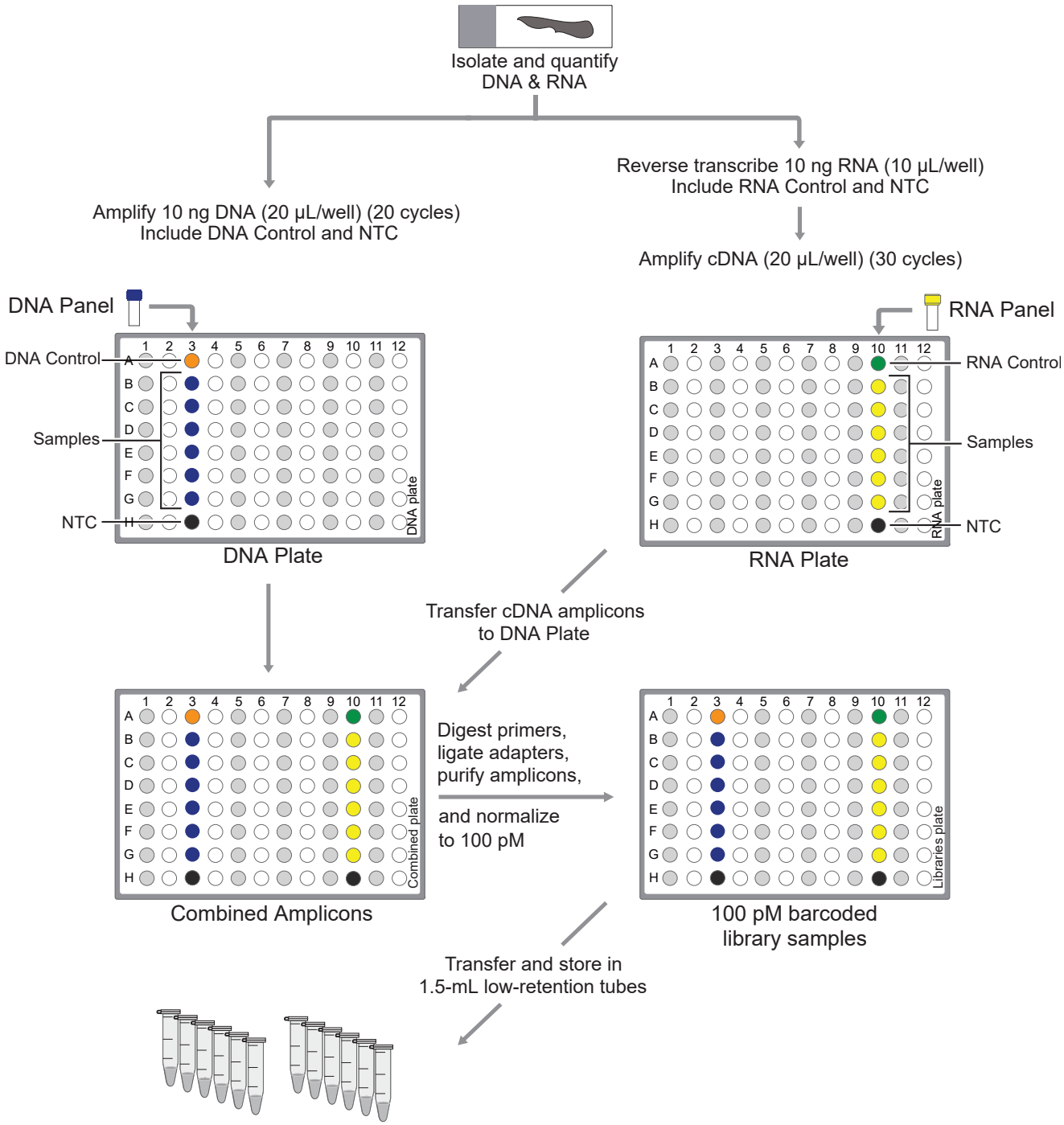
	Temperature	Time
☐	42°C	30 minutes
☐	85°C	5 minutes
☐	10°C	Hold (up to 1 hour)

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

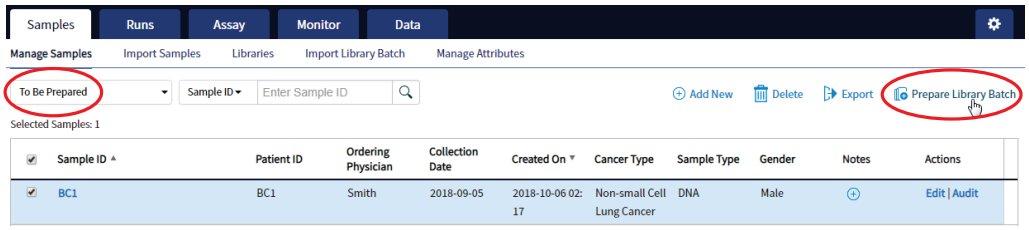
In Torrent Suite™ Dx Software, samples that are entered into the software are placed in library batches for processing and tracking. A library batch consists of a group of libraries that are prepared at the same time.

Note:

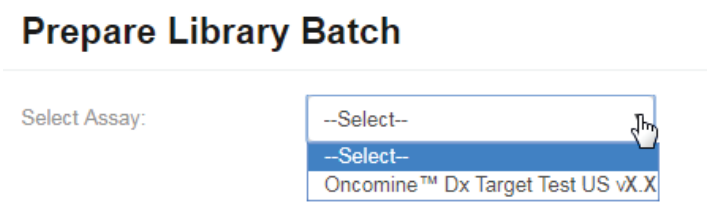
- Each library in a library batch must have a unique library name. When combining libraries in the same run, each must also have a unique barcode.
- Control libraries must be included in the same library batch as the sample library they control for.
- Fields identified with a red asterisk (*) are required.

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.



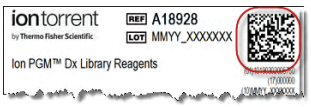
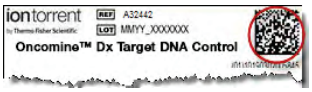
3. Select up to 6 samples in the list, then click **Prepare Library Batch**.
   The **Prepare Library Batch** dialog opens. Required fields are indicated with a red asterisk(*).
4. In the **Select Assay** dropdown list, select **Oncomine™ Dx Target Test**. The assay determines specific parameters of 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 74.

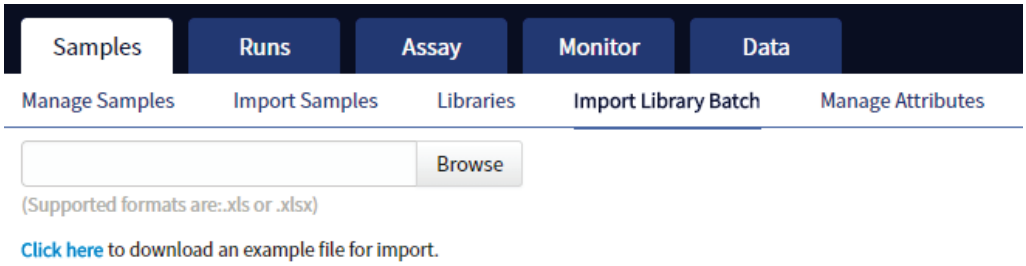
IMPORTANT! Be careful to ensure that the actual barcodes used to create the libraries match the barcodes that are entered in the **Prepare Library Batch** dialog.

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

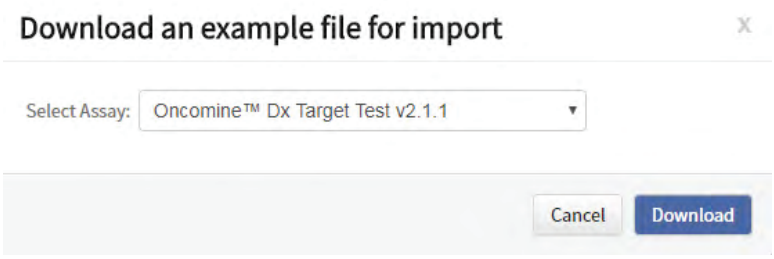
10. Click **Save** to save your selections and close the dialog.
The **Libraries** screen opens, listing the libraries that you created. Libraries that are prepared in the same batch have the same **Library Batch ID**.

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 83.

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.

Oncomine™ Dx Target RNA Control	A32443	–90°C to –60°C
Oncomine™ Dx Target RNA Control Diluent	A38872	–90°C to –60°C

Amplify the cDNA

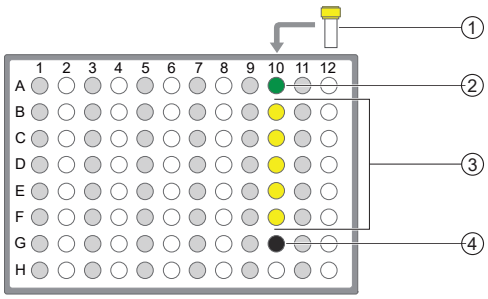
Kit components used in this procedure

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 67. The number of reactions depends on the configuration of your "RNA/cDNA" plate (prepared as described in “Reverse transcribe the RNA” on page 58).

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.

☐	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 68.

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 66. The number of reactions depends on the configuration of your "RNA/cDNA" plate (prepared as described in "Reverse transcribe the RNA" on page 58).

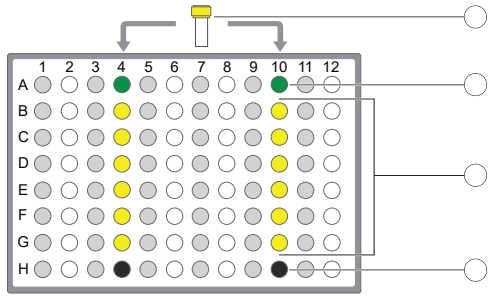
For 8–16 amplification reactions (including controls), make a master mix for n+1 reactions, where "n" is the number of reactions you are preparing.

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

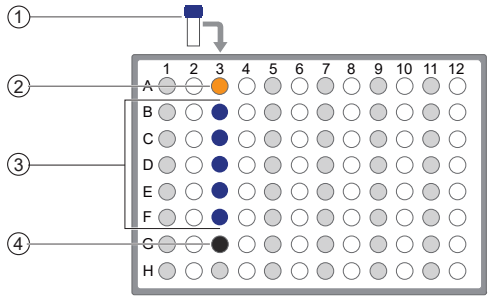
Kit component	Box
No Template Control (purple cap)	Ion Torrent Dx No Template Control Kit (Part No. A32444, stored at 15°C to 30°C)
Oncomine™ Dx Target Test—DNA panel (blue cap)	Oncomine™ Dx Target Test DNA and RNA Panel (Part No. A32441, stored at –30°C to –10°C)
LIB HiFi Mix (red cap)	Ion PGM™ Dx Library 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 71.

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.

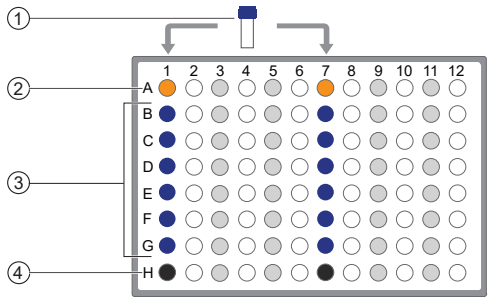
9. Proceed to “Amplify the DNA targets” on page 72.

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 69.

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

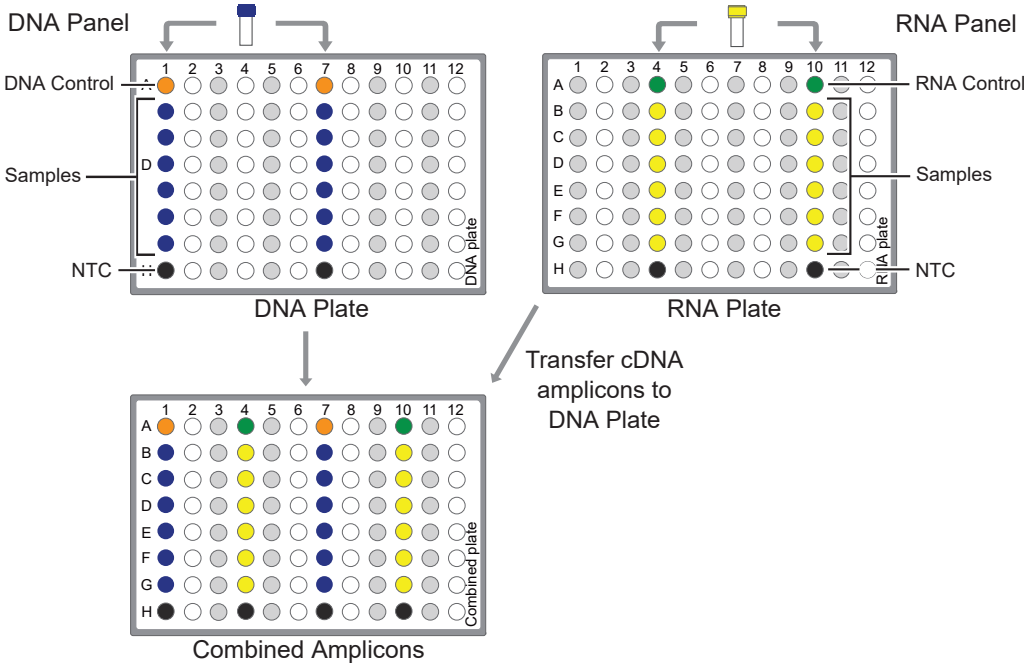
1. After thermal cycling, transfer the 96-well plate to a designated post-PCR preparation area.

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 73.

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 76) 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	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.

Observation	Possible cause	Recommended action
Library batch import fails *(continued)*	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.

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*.

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, visit **thermofisher.com/support**.

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-2020-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/publications/i/item/9789240011311

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 15223-1:2021 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
	UNIQUE DEVICE IDENTIFIER		
	IN VITRO DIAGNOSTIC MEDICAL DEVICE		
	INDICATES CONFORMITY WITH UNITED KINGDOM REQUIREMENTS		

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 48).

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 90.

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 90.

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 58.

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 for up to 12 months, and the remaining undiluted RNA sample at –90°C to –60°C for up to 5 months. Stability studies for extracted DNA with insertions are ongoing, but a minimum stability of 5 months has been established.

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

for use with Torrent Suite™ Dx Software 5.12.5

Publication Number MAN0019392

Revision A.0

IVD

For In Vitro Diagnostic Use. | Rx Only

ThermoFisher SCIENTIFIC

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

Products manufactured at this site:

- 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, Maryland 21704 USA

Products manufactured at this site:

- 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

Revision history: Pub. No. MAN0019392

Revision	Date	Description
A.0	23 September 2022	New Oncomine™ Dx Target Test user guide for FDA submission

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

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*.

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*.

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*.

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*.

Materials provided

Oncomine™ Dx Target Test Kit

The Oncomine™ Dx Target Test Kit (Cat. No. A51695) 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	A52639

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.

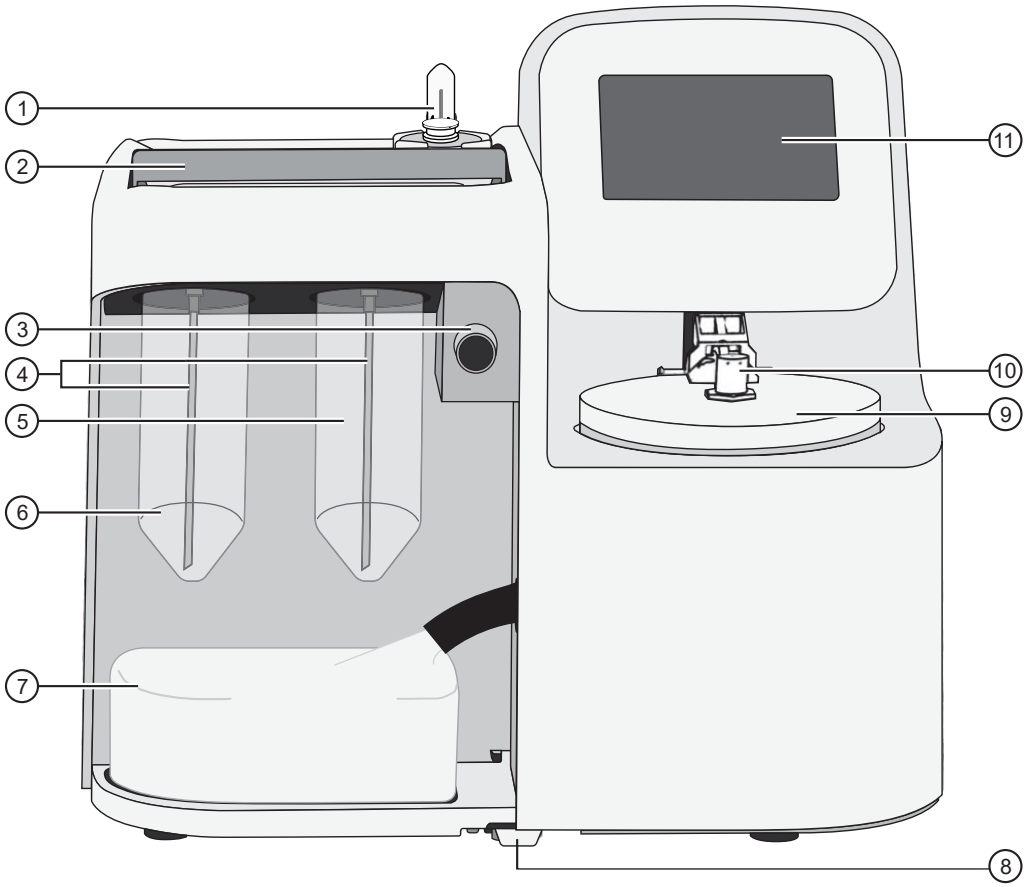
The DynaMag™ Dx 16 2-mL Magnet holds 16 standard 1.5-mL or 2-mL microcentrifuge tubes, and collects bead-bound biomolecules in suspension at the sides of the tubes, allowing removal of fluid without disturbing the bead pellets.

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 3 meters (10 feet) 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 (59–86°F).
- 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.

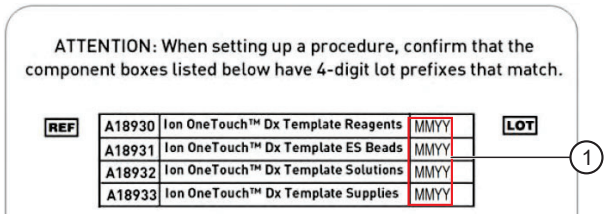
Kit interchangeability and component box lot matching

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.

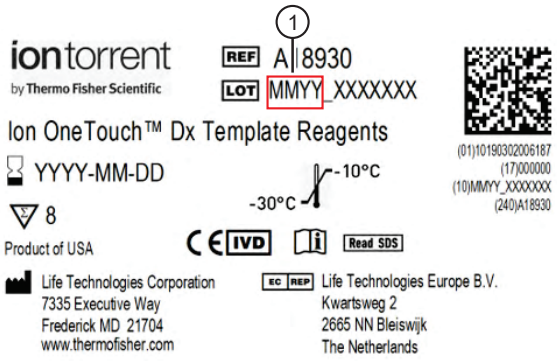
Note: The Oncomine™ Dx Target RNA Control and Oncomine™ Dx Target RNA Control Diluent in the Oncomine™ Dx Target Test, Controls, and Diluent Kit must be lot-matched. However, the controls do not require lot-matching with the Oncomine™ Dx Target Test DNA and RNA Panel.

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:

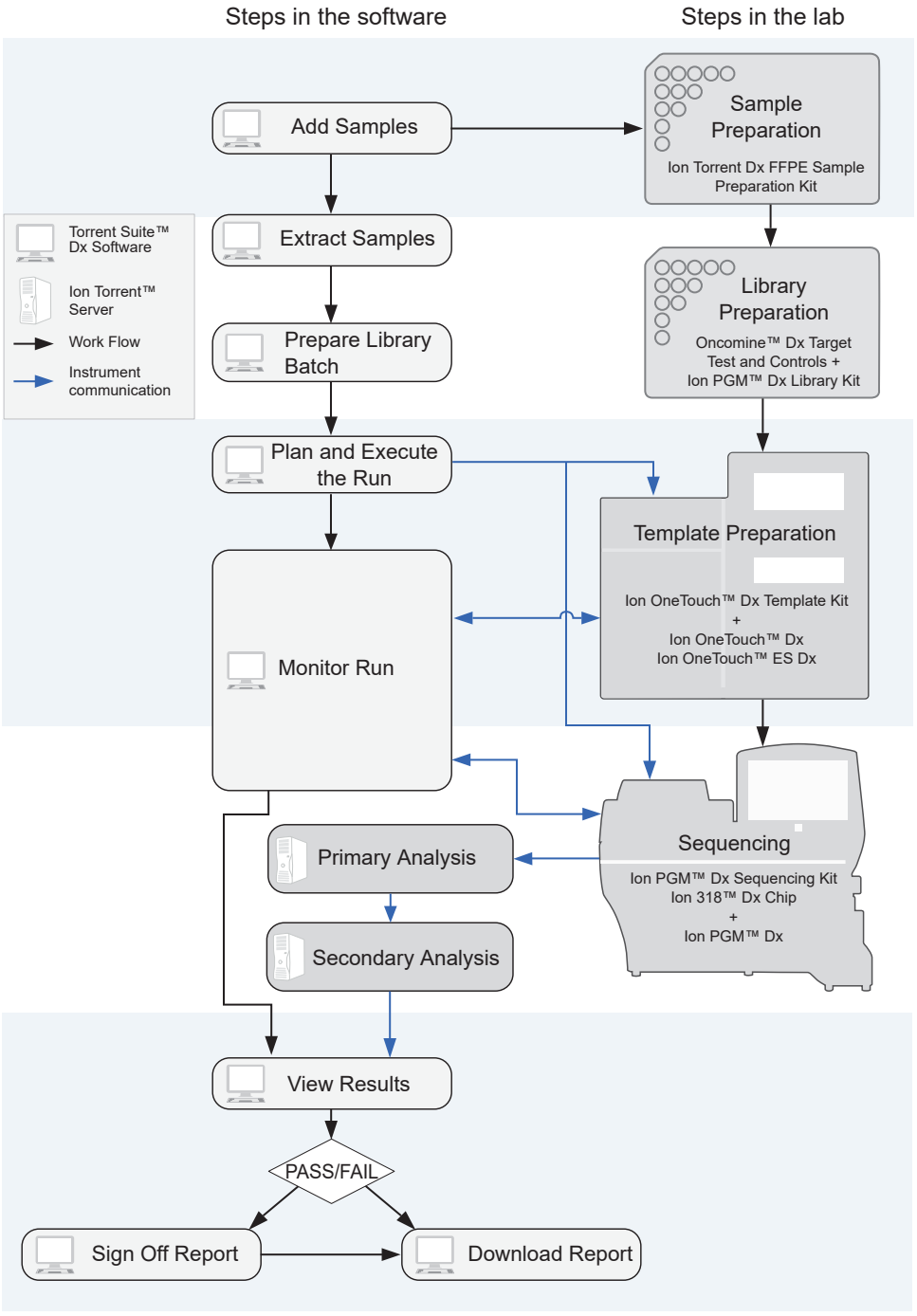


① Lot prefix

Library preparation

Libraries must be prepared as described in the *Oncomine™ Dx Target Test Part II: Sample and Library Preparation User Guide*.

Oncomine™ Dx Target Test system diagram



Template preparation workflow

The total workflow time is 7 hours, including 1 hour of hands-on time.

Template preparation
Previous guide: *Oncomine™ Dx Target Test Part II: Sample and Library Preparation User Guide*
Plan and execute a run with the software (see page 23)
Pool sample and control libraries (see page 27)
Clean the Ion OneTouch™ Dx Instrument before a run (see page 30)
Run the Ion OneTouch™ Dx Instrument (see page 43)
Prepare the Ion OneTouch™ ES Dx Instrument and perform the run (see page 49)
Clean the Ion OneTouch™ Dx Instrument after the run (see page 51)
Proceed to: *Oncomine™ Dx Target Test Part IV: Sequencing and Results Reports User Guide*

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*.

Create a Planned Run

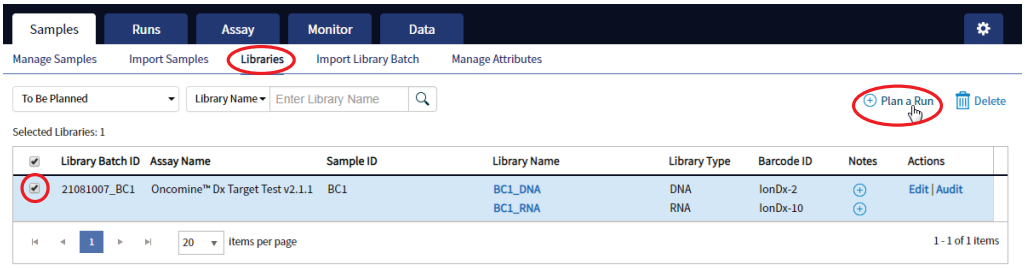
Libraries that are ready to be entered into a Planned Run are listed under the **Samples** tab in the **Libraries** screen.

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.

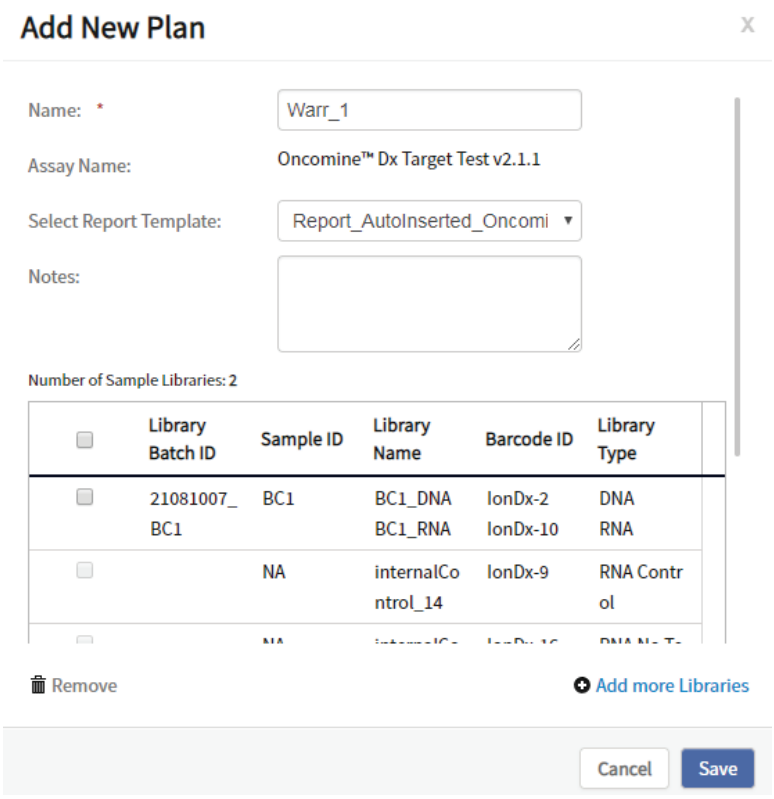
   Note:
   - Libraries prepared with the same assay in the same library batch can be combined and run together, as long as they have unique library names and Barcode IDs.
   - To plan a run with the Oncomine™ Dx Target Test assay, a report template must be created and associated with the assay by an Administrator 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.

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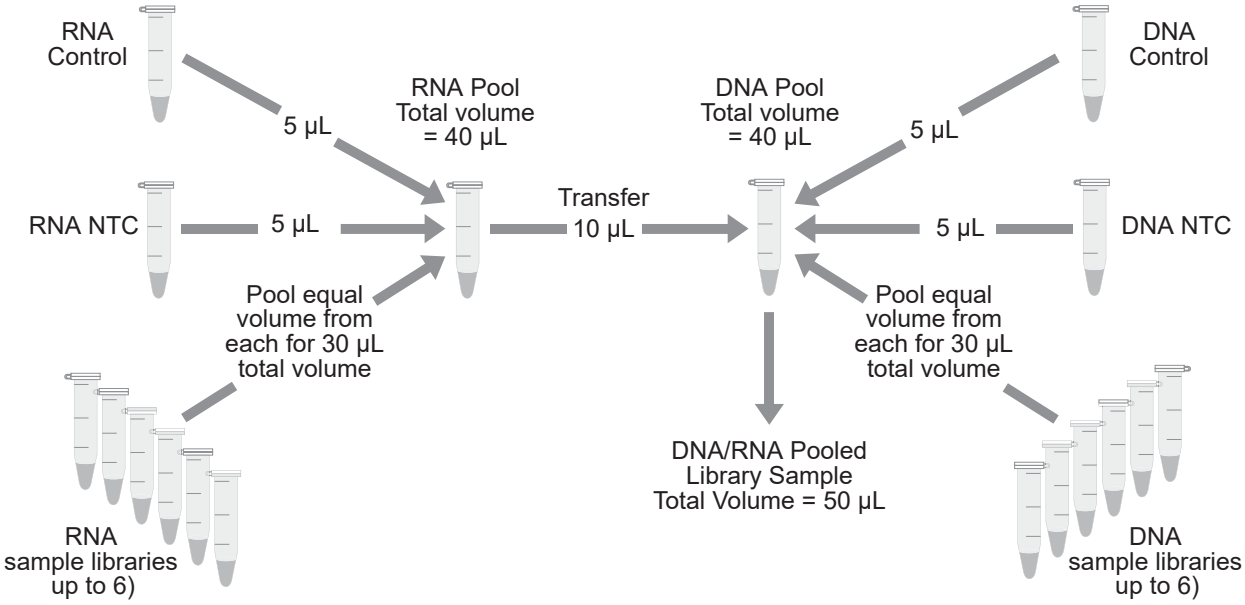
4 Pool sample and control libraries

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

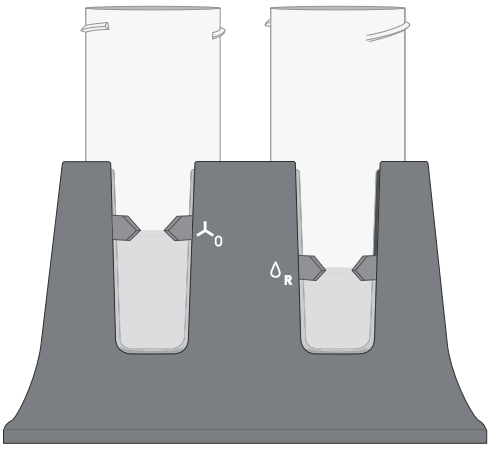
Before you perform a new run on the Ion OneTouch™ Dx Instrument, you must clean the instrument. Until you clean the instrument, the **Run** button on the instrument remains disabled.

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.

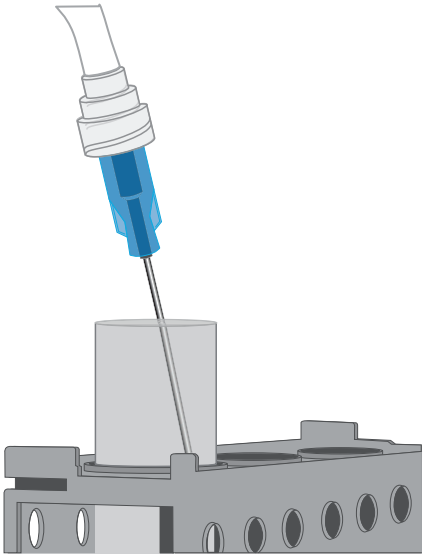
   IMPORTANT! When removing reagent tubes, do not touch the reagent sippers or allow the reagent sippers or reagent tubes to come into contact with any surfaces.
8. Place the TMPL Reagent Tube in the position marked with an "R" on the Ion OneTouch™ Solutions Rack.

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**.

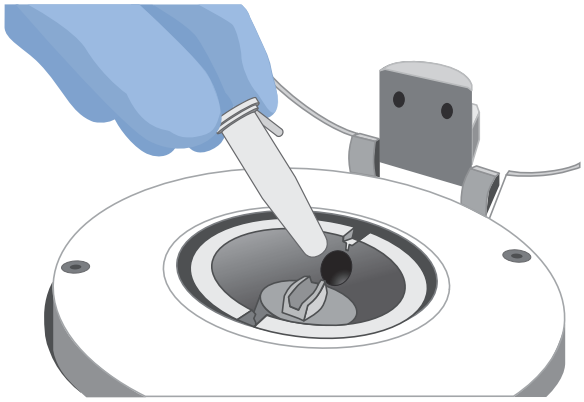
3. Enter the Tube Label text that you entered into the software when you executed the run, then press **Next**.

   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**.

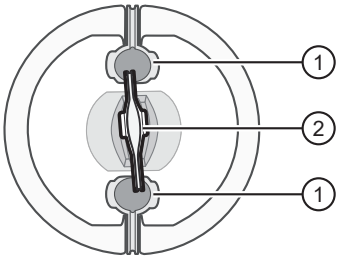
Install the TMPL Recovery Tubes, TMPL Recovery Router, and TMPL Amplification Plate

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.

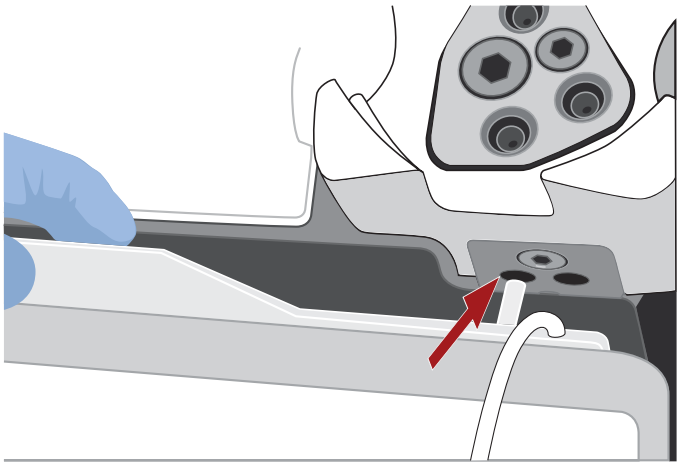


① TMPL Recovery Tube
② TMPL Recovery Router

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.



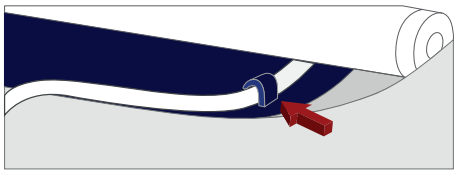
CAUTION! Hot Surface. Use care when working near the heat block to avoid burns from the hot components.

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**.

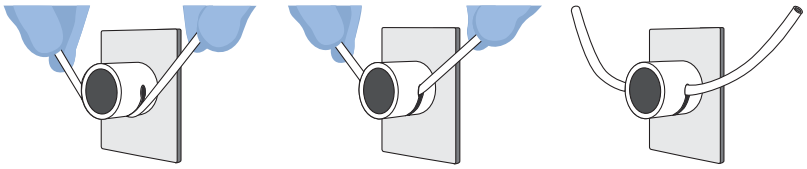
Note: In the following steps, ensure that the tubing is not kinked or twisted at any point along its length.

7. Thread the tubing through the tubing holder.



Note: Examine the tubing to ensure there are no kinks in the tubing before proceeding.

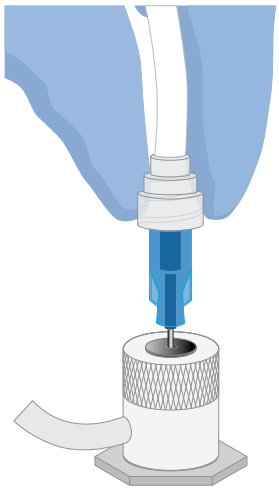
8. Align the tubing with the slot that runs along the bottom of the pinch valve. Gently pull the tubing up into the slot until it is secure in the notch.



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**.

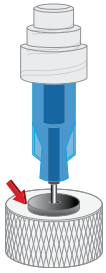
10. Ensure that the injector needle is screwed tightly onto the tubing. Hold the centrifuge lid down with one hand, and with your other hand insert the injector needle straight down into the injector hub. Push down until the injector touches the hub.

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.

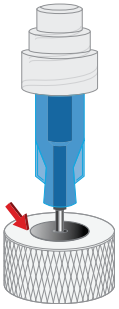


11. Gently push the injector down again and release. You should hear a click from the hub. Then press **Next**.

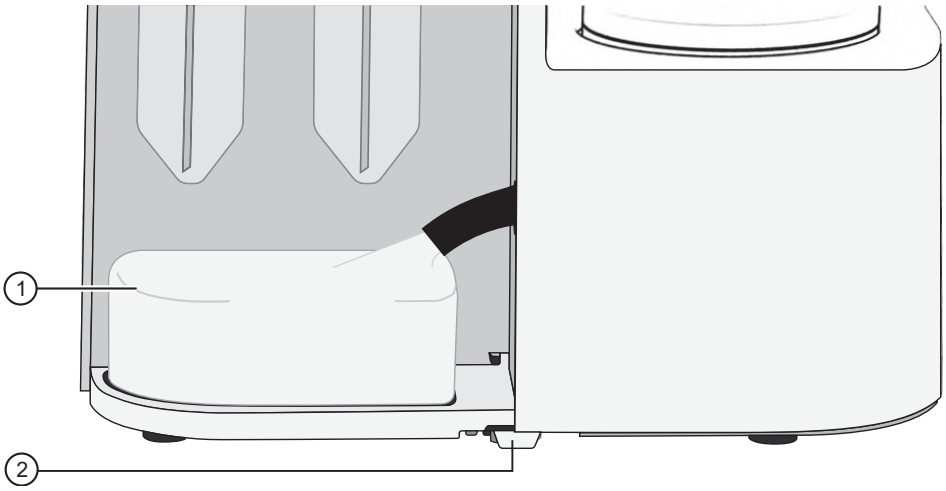
Up position



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

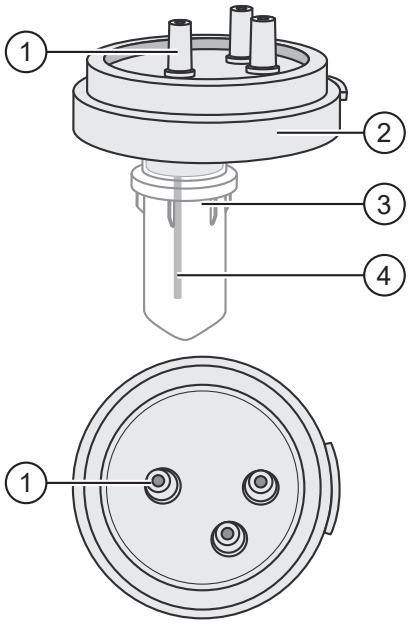
Proceed immediately to "Fill the TMPL Emulsion Cartridge" on page 40.

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.

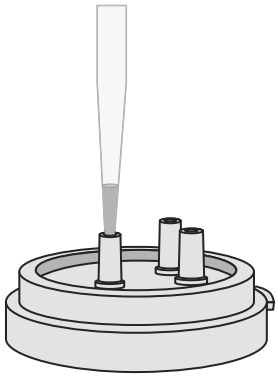


① Sample port (above the Reaction Tube)
② TMPL Emulsion Cartridge
③ Reaction Tube
④ Short tube from the sample port into the Reaction Tube

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.

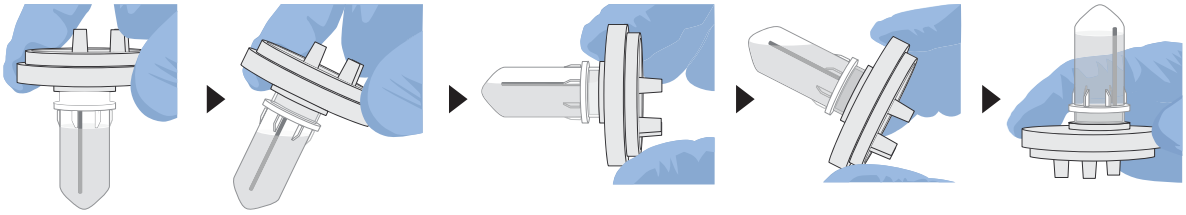
Install the filled TMPL Emulsion Cartridge

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*.

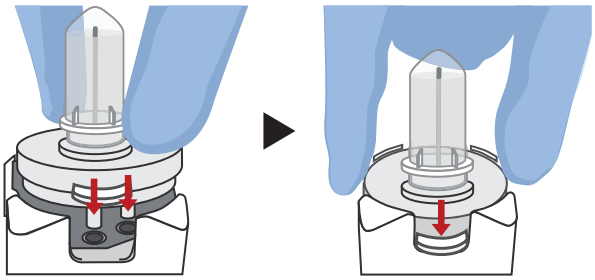
2. Lift the TMPL Emulsion Cartridge straight out of the tube rack.

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.



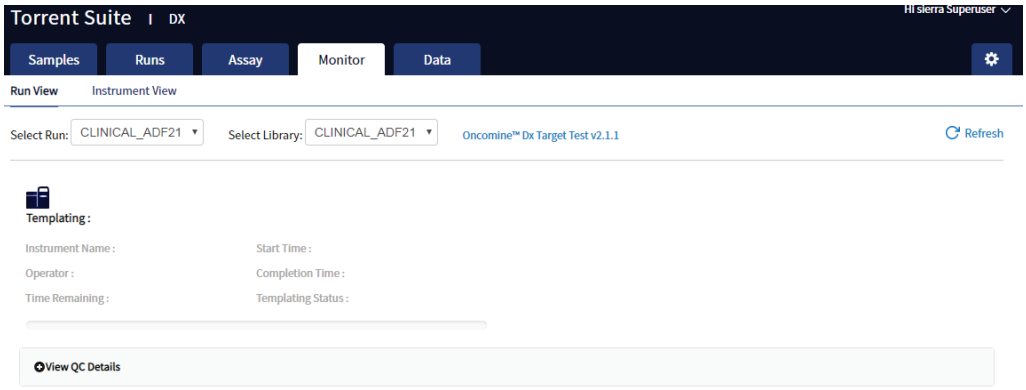
Note: After inserting the TMPL Emulsion Cartridge, bubbles may shoot up into the Reaction Tube. This is normal.

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.



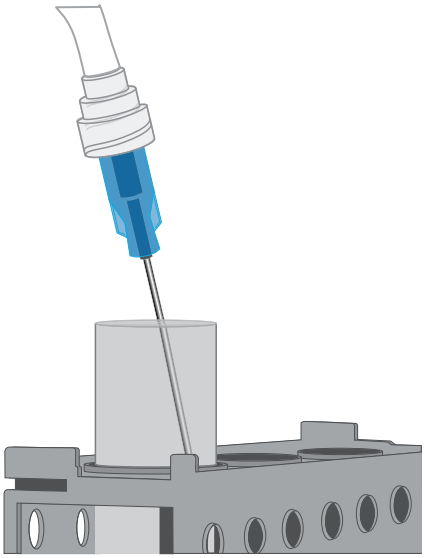
   - To cancel a run, press **Abort**, then press **Yes** to confirm. If there is a high-pressure event on the instrument, the instrument aborts the run automatically.
   - If a run is aborted, you must power-cycle the Ion OneTouch™ Dx Instrument before repeating the run preparation and run. Not doing so 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.

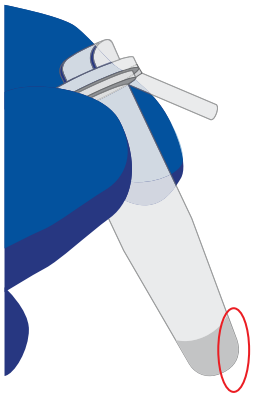
Note: When using multiple instruments, use a new disposable wipe for every instrument.

3. Remove and discard the TMPL Recovery Router.

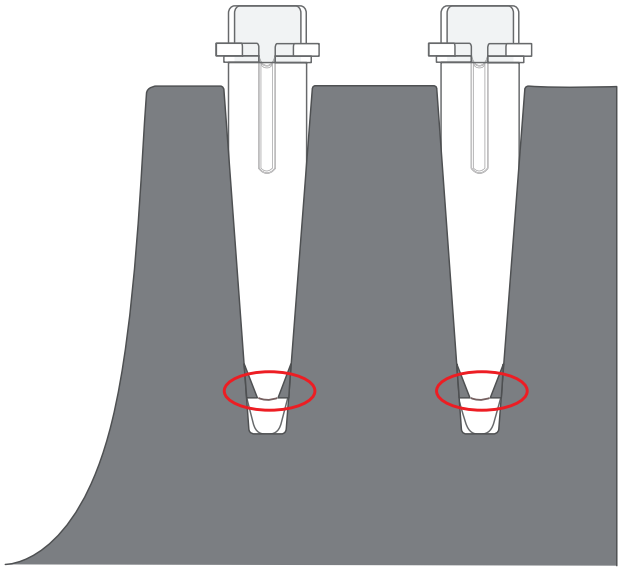
4. Carefully remove the two TMPL Recovery Tubes from the instrument and insert them in the Ion OneTouch™ Sample Rack, with each tube arm inserted into the slot on the back of the rack. Close the instrument lid.

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.

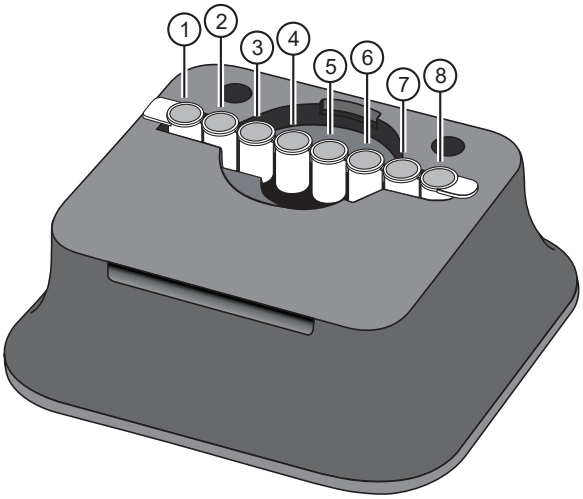
   When facing the rack, make sure that the square tab of the TMPL ES Strip Tube is on the left and the round tab is on the right.

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.

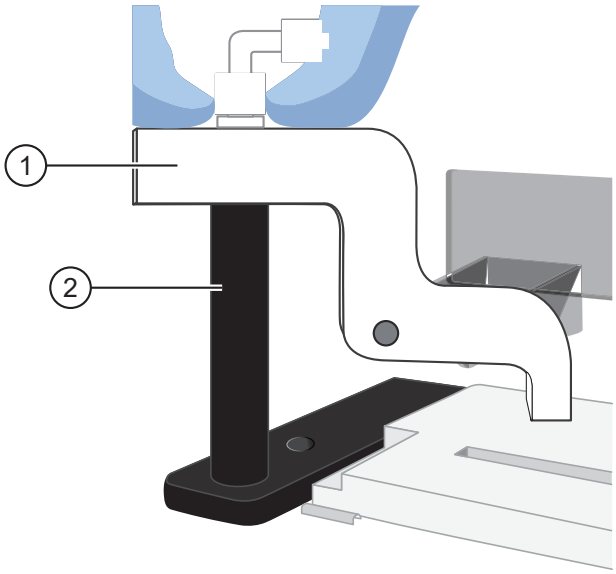
IMPORTANT! The volume of TMPL Neutral Soln added to the TMPL Sample Collection Tube is critical and must be accurate.

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.

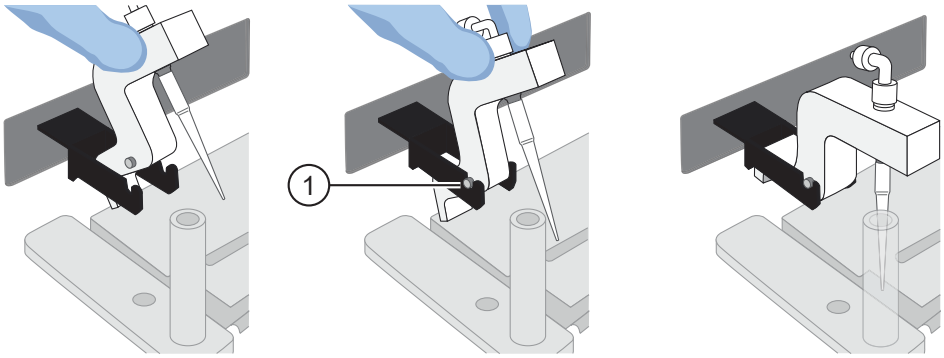


① Tip Arm
② Tip Loader

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.

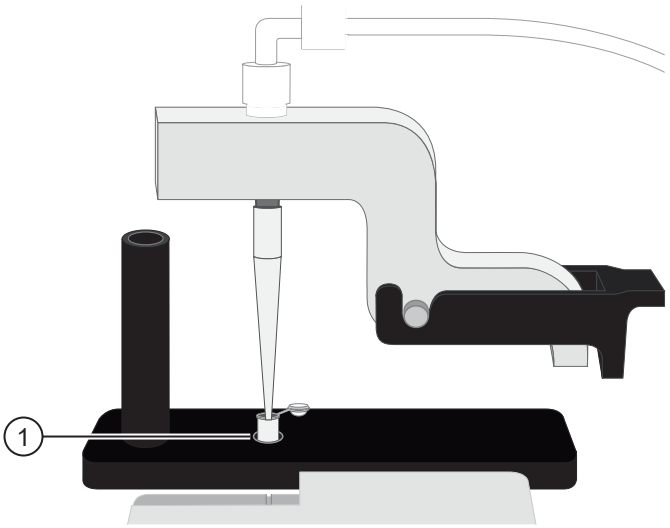


① Tip Arm pins resting in the notches in the cradle

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.

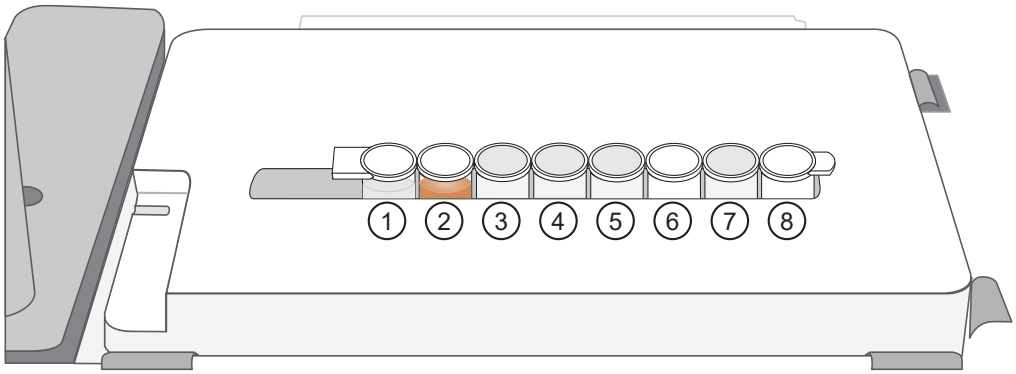
IMPORTANT! Be sure that the Tip Loader is aligned properly in its trough. If the Tip Loader is too far forward or back, the tip will miss the tube and eject sample onto the tip loader.



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

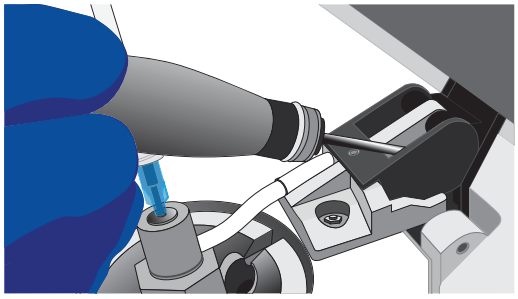
Ion OneTouch™ Dx Instrument

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	• 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").

Observation	Possible cause	Recommended action
Centrifuge does not spin *(continued)*	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 65). 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 65). 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.

Observation	Possible cause	Recommended action
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 65).
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 65).
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 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

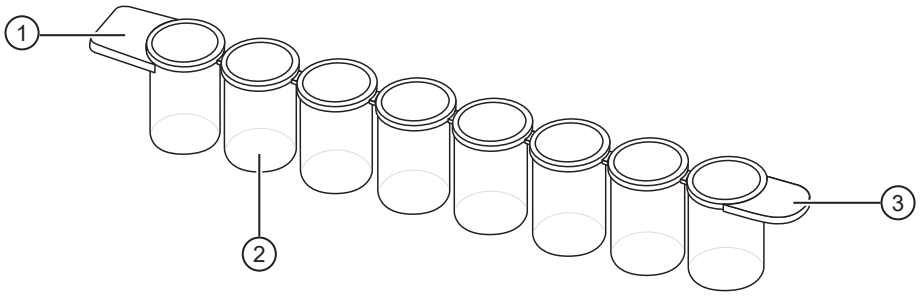
WARNING! ELECTRICAL SHOCK HAZARD. Severe electrical shock, which could cause physical injury or death, can result from working on an instrument when the high voltage power supply is operating. To avoid electrical shock, disconnect the power supply to the instrument, unplug the power cord, and wait at 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**.

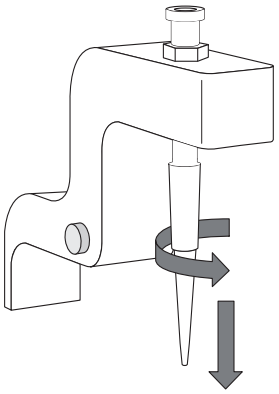
7. Wait for the instrument to aspirate the solution from Well 2 and completely remove the tip from Well 2, then *manually* push the 8-well strip to the left so that Well 4 is positioned directly under the Tip Arm.

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.

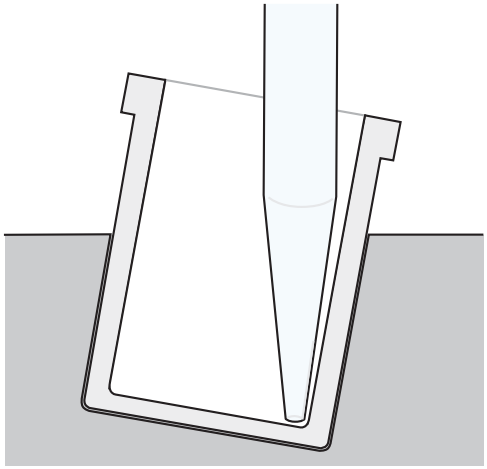
12. Remove and discard the used 8-well strip.

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 65).
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 65).

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

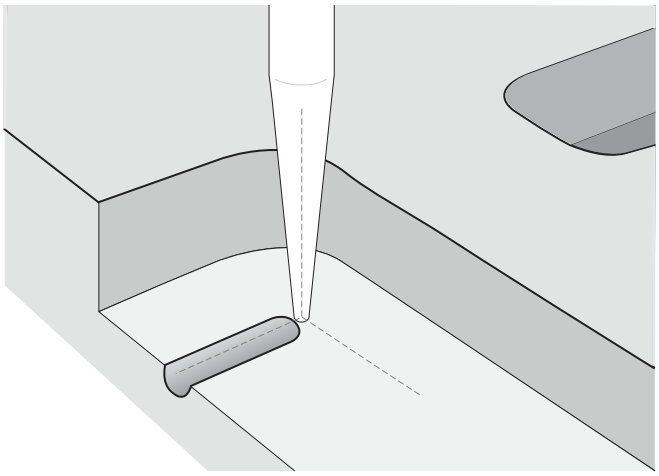
1. Install a new tip.
2. Restore the factory default settings:
   a. Power OFF the instrument, then wait 3 seconds.
   b. While holding down **Vert. Adjust**, power ON the instrument.
3. Put the instrument into calibration mode:
   a. Power OFF the instrument.
   b. While holding down **Select/Calibrate**, 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.

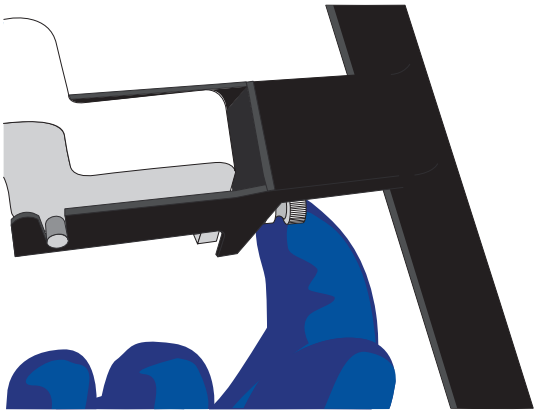
4. Press **Vert. Adjust**. The instrument displays "ASP" (Aspirate or z-bottom position).

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.

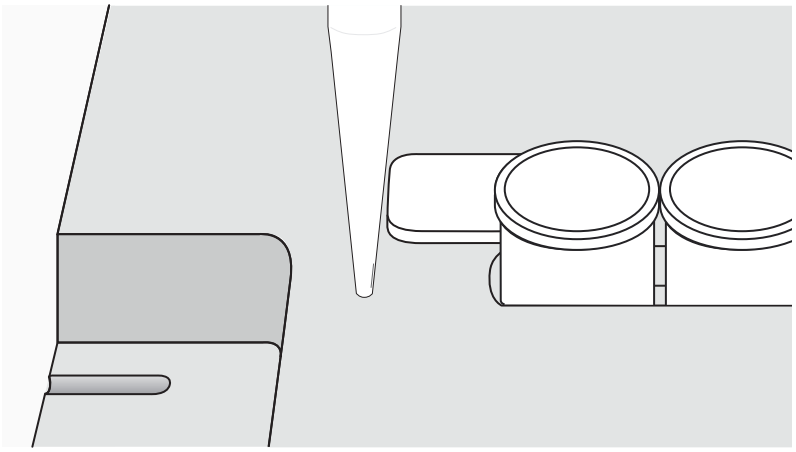
   Note: While you press the button, the instrument cycles through several values before “CAL” is displayed.

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 62).

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, visit **thermofisher.com/support**.

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, 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)

(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 15223-1:2021 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
	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
	UNIQUE DEVICE IDENTIFIER		
	IN VITRO DIAGNOSTIC MEDICAL DEVICE		
	INDICATES CONFORMITY WITH UNITED KINGDOM REQUIREMENTS		

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.

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*.

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.

F 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**.