iontorrent

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

USER GUIDE

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IVD

For In Vitro Diagnostic Use.

Thermo Fisher SCIENTIFIC

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Products manufactured in Singapore:

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

Life Technologies Corporation |
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Products manufactured in Frederick:

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

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A.0	26 August 2021	New Oncomine™ Dx Target Test user guide for FDA submission.

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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.

Product information

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 non-small cell lung cancer (NSCLC) and DNA isolated from cholangiocarcinoma (CC), in formalin-fixed, paraffin-embedded (FFPE) tumor 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 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) and deletions in 23 genes from DNA and fusions in ROS1 and RET from RNA isolated from formalin-fixed, paraffin-embedded (FFPE) tumor tissue samples from patients with non-small cell lung cancer (NSCLC), and IDH1 SNVs from FFPE tumor tissue samples from patients with cholangiocarcinoma (CC) using the Ion PGM™ Dx System.

The test is indicated as a companion diagnostic to aid in selecting NSCLC and CC 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
Non-small cell lung cancer (NSCLC)	BRAF	BRAF V600E mutations	TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib)
	EGFR	EGFR L858R mutation, EGFR Exon 19 deletions	IRESSA® (gefitinib)
	RET	RET fusions	GAVRETO™ (pralsetinib)
	ROS1	ROS1 fusions	XALKORI® (crizotinib)
Cholangiocarcinoma (CC)	IDH1	IDH1 R132C, IDH1 R132G, IDH1 R132H, IDH1 R132L, and IDH1 R132S	TIBSOVO® (ivosidenib)

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

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

Table 2 List of variants with established analytical performance only

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

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

Theory of operation

Overview

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

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

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

- 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 have not been directly demonstrated.

Software compatibility and requirements

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

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

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

Materials provided

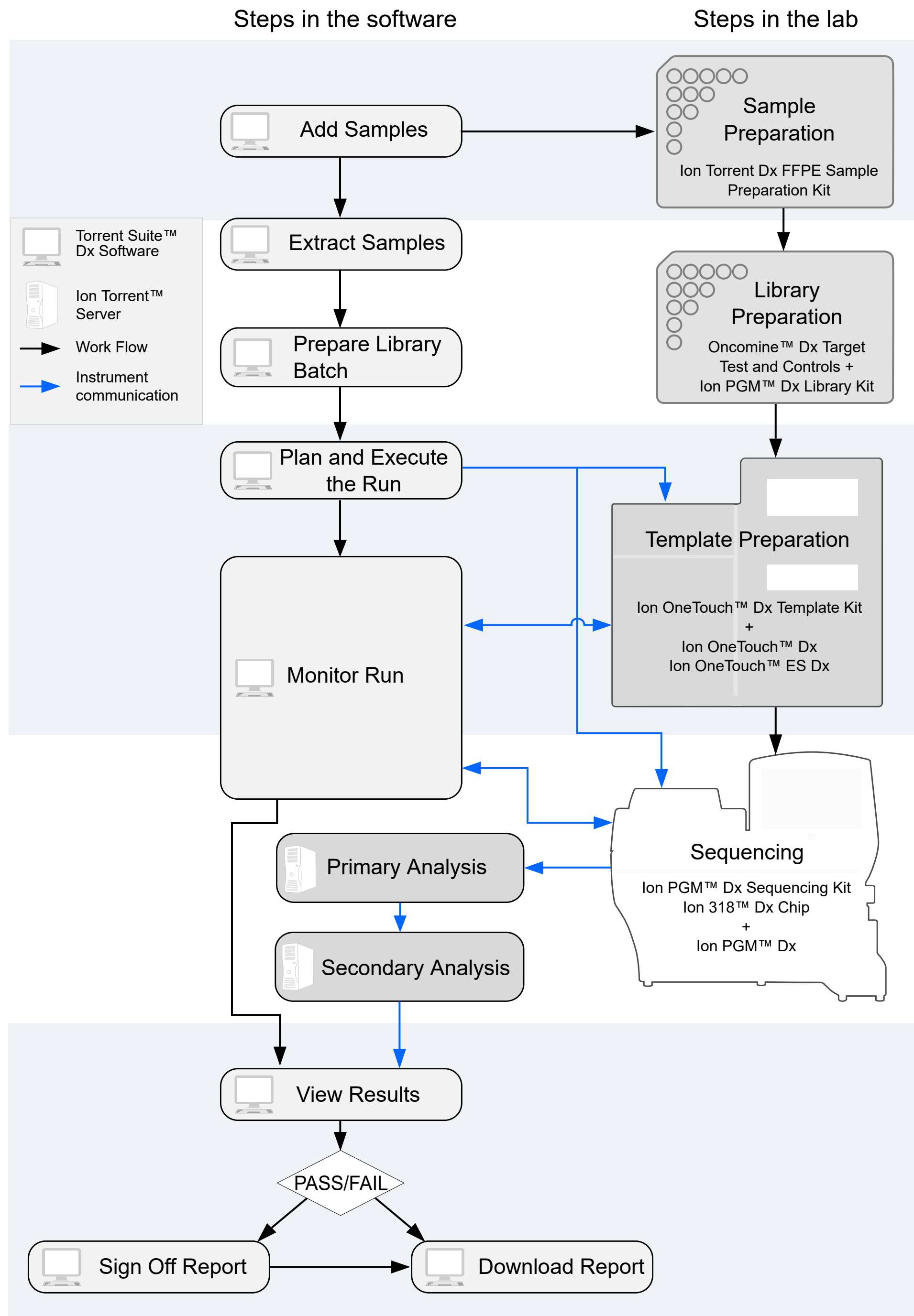
Oncomine™ Dx Target Test Kit

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

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

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

Oncomine™ Dx Target Test system diagram



2 Pass/fail criteria and repeat strategy

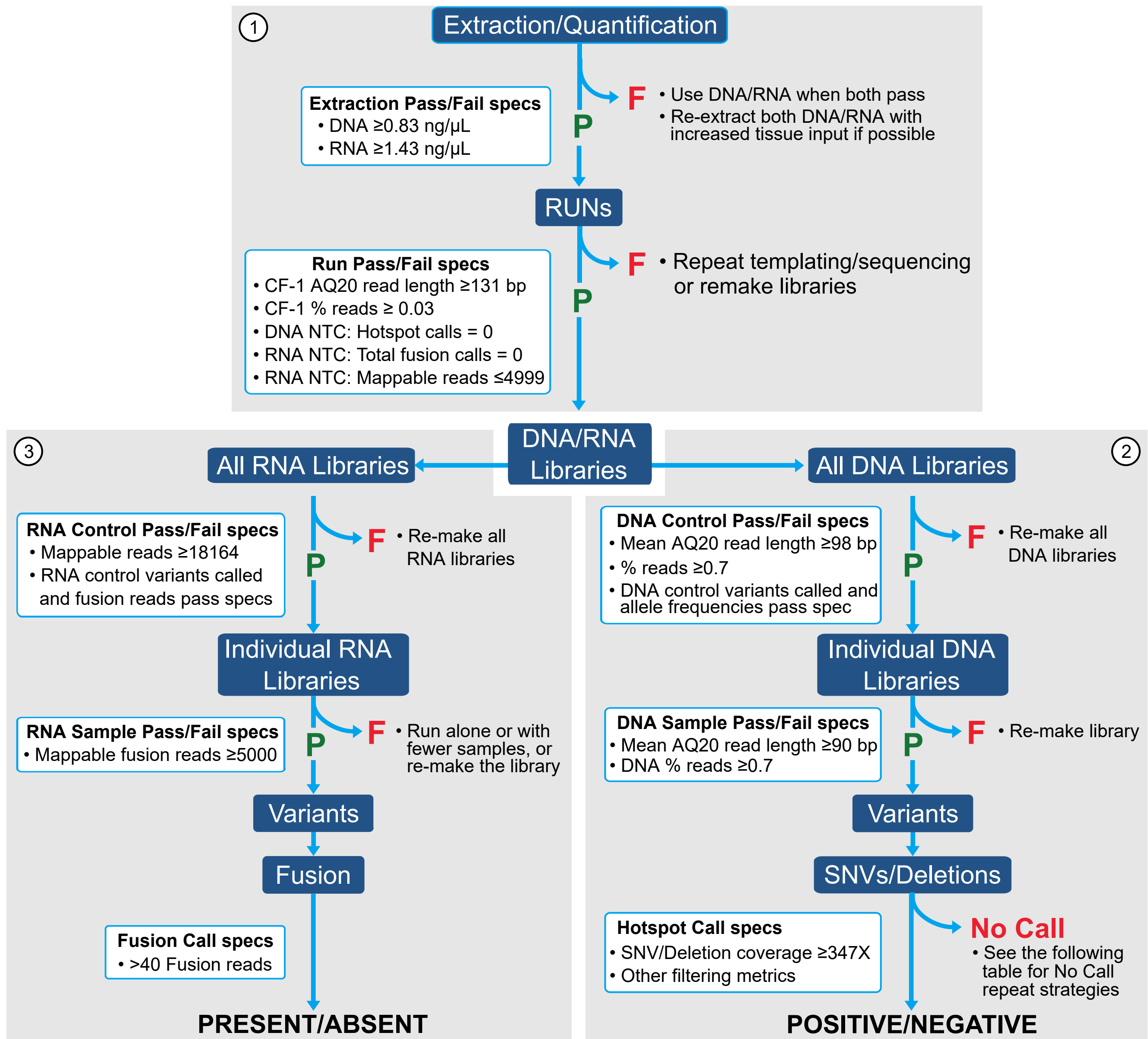
Quality control pass/fail criteria

Metric	Criteria
Run QC	
CF-1 Mean AQ20 Read Length (bp)	≥131
CF-1 Percent Reads (%)	≥0.03
DNA NTC	Hotspot calls = 0
RNA NTC	Total fusion calls = 0
RNA NTC Mappable Reads	≤4999
DNA Library	
Mean AQ20 Read Length (bp)	≥90
Percent Reads (%)	≥0.7
RNA Library	
Mappable Fusion Reads	≥5000
DNA Control	
COSM476_AF	Variant called and AF ≥0.05
COSM521_AF	Variant called and AF ≥0.05
COSM6223_AF	Variant called and AF ≥0.05
COSM6224_AF	Variant called and AF ≥0.05
COSM760_AF	Variant called and AF ≥0.05
COSM28747_AF	Variant called and AF ≥0.05
Mean AQ20 Read Length (bp)	≥98
Percent Reads (%)	≥0.7
RNA Control	
Mappable Reads	≥18164
ROS1 Fusion Reads	Variant called and fusion reads ≥349

Pass/fail specifications and repeat strategy

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

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



Point of failure		Tissue type	Recommended repeat strategy
1	RUNs	NSCLC and CC	Repeat templating and sequencing or remake the libraries.
2	DNA Library	NSCLC and CC	Remake all DNA libraries.
3	RNA library	NSCLC	Remake all RNA libraries.
		CC	Unnecessary to remake cholangiocarcinoma 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 and Deletion Variant Specifications	All Single Nucleotide Variants (SNVs) and Deletions (Dels) must have coverage ≥347 reads and pass all Variant Caller filtering metrics in order to have a reportable result for the variant.	Any SNVs and deletions that do not meet the coverage criteria will result in a "No Call" for the variant. The operator may run the sample alone or with fewer samples to obtain reportable results for the variant. If the repeat run fails to meet the minimum coverage requirement, the operator may remake the library to obtain reportable results for the variant.

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 non-small cell lung cancer and cholangiocarcinoma 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 13.

For example, if the RNA Control fails in a mixed run, it is not necessary to remake cholangiocarcinoma 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 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 cholangiocarcinoma (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 4.5–5.7% AF.

Table 7 LoD of clinical IDH1 variants

Variant	ID	Sample type	Estimated LoD (AF)
R132C	COSM28747	Clinical sample	4.5%
R132G	COSM28749	Clinical sample	5.7%
R132H	COSM28746	Cell line	4.9%
R132L	COSM28750	Cell line	5.1%
R132S	COSM28748	Cell line	5.3%

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 (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.

Tissue input study

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

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

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

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

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

DNA and RNA input—Study 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.

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—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	COSM17242 3	ERBB3 V104M	32	32	0	0	100% (89.1%, 100%)	100% (89.1%, 100%)	0% (0%, 10.9%)	0% (0%, 10.9%)	100% (79.4%, 100%)	100% (79.4%, 100%)
K	COSM775	PIK3 H1047R	30[1]	29	0	1	96.7% (82.8%, 99.9%)	100% (88.1%, 100%)	0% (0%, 11.6%)	0% (0%, 11.9%)	93.3% (68.1%, 99.8%)	100% (76.8%, 100%)
M	COSM715	FGR3 S249C	32	32	0	0	100% (89.1%, 100%)	100% (89.1%, 100%)	0% (0%, 10.9%)	0% (0%, 10.9%)	100% (79.4%, 100%)	100% (79.4%, 100%)

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

Interfering substances studies

Interfering substances—Study I

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

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

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

Table 12 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 12) 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 12) on assay performance, and the results were compared to the control (no interferents) condition. For the 6 interferents tested, both the positive concordance and the overall concordance for all samples was 100%. These data support the claim that paraffin, xylene, ethanol, hemoglobin, protease, or wash buffer do not affect assay performance at the level tested in detection of the RET fusions.

Limit of Detection (LoD) studies

Limit of Detection (LoD)—Study I

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

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

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

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

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

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

Limit of Detection (LoD)—Study II

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

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

Limit of Detection (LoD)—Study III

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

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

Tumor content studies

Tumor content—Study I

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

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

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

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

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

Table 13 Tumor content range in ROS1-positive samples used in clinical studies

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

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

Tumor content—Study II

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

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

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

Table 14 Tumor content range in ROS1-positive samples used in clinical studies

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

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

Assay reproducibility studies

Assay reproducibility—Study I

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

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

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

Table 15 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 16 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 17 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

Table 17 Representative variant approach—Study II *(continued)*

Variant category	No. of clinical specimens used	No. of levels tested[1]	No. of samples tested
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 18 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 19 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 20 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 21 Reproducibility results (RET fusion)—Study III

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

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

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

Panel accuracy study

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

The following reference detection methods were used:

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

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

Accuracy data was analyzed by the following:

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

The results are shown in the following tables.

Table 22 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 23 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 24 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 25 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 26 Exon 19 deletion—Concordance

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

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

Table 27 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 28 Overall concordance

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

RET clinical study

RET study—concordance evaluation

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

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

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

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

For testing with the Archer assay, of the 135 RET fusion-positive samples by the LLTs, in addition to the 54 cancelled for the Oncomine™ Dx Target Test, 11 more samples were cancelled due to insufficient RNA concentration, and 4 samples were not tested on the Archer Assay. Of the remaining 66 samples, 43 were positive by the Archer assay, 17 samples were called negative, and 6 samples yielded an invalid result. Of the 103 RET fusion-negative samples (by LLT), 1 sample had insufficient RNA volume for testing, 93 were negative, 1 was positive, and 8 were invalid by the Archer assay.

The PPA was defined as the proportion of RET fusion-positive specimens as called by the Archer assay that were also RET fusion-positive as called by the Oncomine™ Dx Target Test, and the NPA was defined as the proportion of RET fusion-negative specimens as called by the Archer assay that were also RET fusion-negative as called by the Oncomine™ Dx Target Test. Concordance between the Oncomine™ Dx Target Test and the Archer assay is shown in Table 29.

Table 29 Concordance between the Archer assay and the Oncomine™ Dx Target Test

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

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

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

RET study—clinical effectiveness

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

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

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

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

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

ROS1 clinical 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 30 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 31 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 32 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.

Variants detected by the Oncomine™ Dx Target Test

Variants detected in non-small cell lung cancer (NSCLC)

DNA variants detected by the Oncomine™ Dx Target Test

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

(continued)

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

(continued)

Gene	Exon	Amino Acid Change	Nucleotide Change	Variant ID
CDK4	2	p.Arg24His	c.71G>A	COSM1989836
CDK4	2	p.Arg24Cys	c.70C>T	COSM1677139
CDK4	2	p.Arg24Ser	c.70C>A	COSM3463914
CDK4	2	p.Lys22Met	c.65A>T	COSM3463915
CDK4	2	p.Lys22Arg	c.65A>G	COSM232013
CDK4	2	p.Lys22Gln	c.64A>C	OM3153
DDR2	5	p.Arg124Trp	c.370C>T	COSM4024594
DDR2	5	p.Arg124Leu	c.371G>T	COSM400880
EGFR	21	p.Leu858Met	c.2572C>A	COSM12366
EGFR	21	p.Leu861Gln	c.2582T>A	COSM6213
EGFR	21	p.Leu861Arg	c.2582T>G	COSM12374
EGFR	21	p.Leu858Arg	c.2573T>G	COSM6224
EGFR	20	p.Ser768Ile	c.2303G>T	COSM6241
EGFR	20	p.Cys797Ser	c.2389T>A	COSM6493937
EGFR	20	p.Cys797Ser	c.2390G>C	COSM5945664
EGFR	19	p.Lys745_Glu749del	c.2233_2247delAAGGAATTAAGAGAA	COSM26038
EGFR	19	p.Lys745_Ala750delinsThr	c.2234_2248delAGGAATTAAGAGAAG	COSM1190791
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_Thr751del	c.2236_2253delGAATTAAGAGAAGCAACA	COSM12728

(continued)

Gene	Exon	Amino Acid Change	Nucleotide Change	Variant ID
EGFR	19	p.Glu746_Ala750del	c.2236_2250delGAATTAAGAG AAGCA	COSM6225
EGFR	19	p.Glu746_Arg748del	c.2239_2247delTTAAGAGAA	COSM6218
EGFR	19	p.Glu746_Thr751delinsAla	c.2237_2251delAATTAAGAGAA GCAA	COSM12678 **Note:** A false negative call was observed for this variant when tested with plasmid targets for 1 out of 4 of the replicates tested.
EGFR	19	p.Leu747_Thr751delinsPro	c.2239_2251delTTAAGAGAAG CAAinsC	COSM12383
EGFR	19	p.Leu747_Ala750delinsPro	c.2239_2248delTTAAGAGAAG insC	COSM12382
EGFR	19	p.Glu746_Ser752delinsAsp	c.2238_2255delATTAAGAGAA GCAACATC	COSM6220
EGFR	19	p.Leu747_Thr751del	c.2240_2254delTAAGAGAAGC AACAT	COSM12369
EGFR	19	p.Glu746_Ser752delinsVal	c.2237_2255delAATTAAGAGAA GCAACATCinsT	COSM12384
EGFR	19	p.Glu746_Thr751delinsValAla	c.2237_2253delAATTAAGAGAA GCAACAinsTTGCT	COSM12416
EGFR	19	p.Leu747_Ser752del	c.2239_2256delTTAAGAGAAG CAACATCT	COSM6255
EGFR	19	p.Leu747_Thr751delinsGln	c.2238_2252delATTAAGAGAA GCAACinsGCA	COSM12419
EGFR	19	p.Leu747_Ala750delinsPro	c.2238_2248delATTAAGAGAAG insGC	COSM12422
EGFR	19	p.Leu747_Pro753delinsGln	c.2239_2258delTTAAGAGAAG CAACATCTCCinsCA	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_Pro753delinsSer	c.2240_2257delTAAGAGAAGC AACATCTC	COSM12370

(continued)

Gene	Exon	Amino Acid Change	Nucleotide Change	Variant ID
EGFR	19	p.Leu747_Thr751delinsSer	c.2240_2251delTAAGAGAAGCAA	COSM6210
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	15	p.Gly598Ala	c.1793G>C	COSM3412196
EGFR	15	p.Gly598Val	c.1793G>T	COSM21690
EGFR	12	p.Ser492Arg	c.1474A>C	COSM236671
EGFR	12	p.Ser492Arg	c.1476C>A	COSM236670
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	3	p.Arg108Gly	c.322A>G	COSM1451536
EGFR	3	p.Arg108Lys	c.323G>A	COSM21683
ERBB2	22	p.Arg896Cys	c.2686C>T	COSM14066
ERBB2	22	p.Arg896His	c.2687G>A	COSM119971
ERBB2	21	p.Val842Ile	c.2524G>A	COSM14065
ERBB2	20	p.Gly776Val	c.2327G>T	COSM18609
ERBB2	20	p.Val777Leu	c.2329G>T	COSM14062
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	18	p.Thr733Ile	c.2198C>T	COSM14059

(continued)

Gene	Exon	Amino Acid Change	Nucleotide Change	Variant ID
ERBB2	17	p.Arg678Gln	c.2033G>A	COSM436498
ERBB2	8	p.Ser310Tyr	c.929C>A	COSM94225
ERBB2	8	p.Ser310Phe	c.929C>T	COSM48358
ERBB3	9	p.Glu332Lys	c.994G>A	COSM254677
ERBB3	8	p.Asp297Tyr	c.889G>T	COSM160822
ERBB3	8	p.Asp297Val	c.890A>T	COSM941490
ERBB3	6	p.Ala232Thr	c.694G>A	COSM4043440
ERBB3	6	p.Ala232Val	c.695C>T	COSM1242239
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	2	p.Met60Leu	c.178A>T	COSM1606366
ERBB3	2	p.Met60Lys	c.179T>A	COSM254678
ERBB3	2	p.Met60Arg	c.179T>G	COSM941484
FGFR2	14	p.Lys659Asn	c.1977G>T	COSM49173
FGFR2	14	p.Lys659Asn	c.1977G>C	COSM683054
FGFR2	14	p.Lys659Met	c.1976A>T	COSM49175
FGFR2	14	p.Lys659Glu	c.1975A>G	COSM36909
FGFR2	12	p.Asn549Lys	c.1647T>G	COSM36902
FGFR2	12	p.Asn549Lys	c.1647T>A	COSM36912
FGFR2	12	p.Asn549Ser	c.1646A>G	COSM3665553
FGFR2	12	p.Asn549His	c.1645A>C	COSM250083
FGFR2	9	p.Cys382Tyr	c.1145G>A	COSM915493
FGFR2	9	p.Cys382Arg	c.1144T>C	COSM36906
FGFR2	9	p.Tyr375Cys	c.1124A>G	COSM36904
FGFR2	9	p.Tyr375His	c.1123T>C	COSM1560916

(continued)

Gene	Exon	Amino Acid Change	Nucleotide Change	Variant ID
FGFR2	8	p.Ala314Asp	c.941C>A	COSM49171
FGFR2	7	p.Pro253Leu	c.758C>T	COSM537801
FGFR2	7	p.Pro253Arg	c.758C>G	COSM49170
FGFR2	7	p.Ser252Trp	c.755C>G	COSM36903
FGFR3	16	p.Gly697Cys	c.2089G>T	COSM24802
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	7	p.Arg248Cys	c.742C>T	COSM714
FGFR3	7	p.Ser249Cys	c.746C>G	COSM715
HRAS	3	p.Gln61His	c.183G>T	COSM502
HRAS	3	p.Gln61His	c.183G>C	COSM503
HRAS	3	p.Gln61Leu	c.182A>T	COSM498
HRAS	3	p.Gln61Arg	c.182A>G	COSM499
HRAS	3	p.Gln61Pro	c.182A>C	COSM500
HRAS	3	p.Gln61Lys	c.181C>A	COSM496
HRAS	2	p.Gly13Val	c.38G>T	COSM489
HRAS	2	p.Gly13Asp	c.38G>A	COSM490
HRAS	2	p.Gly13Cys	c.37G>T	COSM488
HRAS	2	p.Gly13Arg	c.37G>C	COSM486
HRAS	2	p.Gly13Ser	c.37G>A	COSM487
HRAS	2	p.Gly12Val	c.35G>T	COSM483
HRAS	2	p.Gly12Ala	c.35G>C	COSM485
HRAS	2	p.Gly12Asp	c.35G>A	COSM484
HRAS	2	p.Gly12Cys	c.34G>T	COSM481
HRAS	2	p.Gly12Arg	c.34G>C	COSM482
HRAS	2	p.Gly12Ser	c.34G>A	COSM480
IDH1	4	p.Arg132Leu	c.395G>T	COSM28750

(continued)

Gene	Exon	Amino Acid Change	Nucleotide Change	Variant ID
IDH1	4	p.Arg132His	c.395G>A	COSM28746
IDH1	4	p.Arg132Cys	c.394C>T	COSM28747
IDH1	4	p.Arg132Gly	c.394C>G	COSM28749
IDH1	4	p.Arg132Ser	c.394C>A	COSM28748
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
KIT	13	p.Lys642Glu	c.1924A>G	COSM1304
KIT	13	p.Val654Ala	c.1961T>C	COSM12706
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	8	p.Asp419del	c.1255_1257delGAC	COSM29014
KIT	8	p.Asp419_Arg420del	c.1255_1260delGACAGG	COSM1578132
KRAS	4	p.Ala146Val	c.437C>T	COSM19900

(continued)

Gene	Exon	Amino Acid Change	Nucleotide Change	Variant ID
KRAS	4	p.Ala146Pro	c.436G>C	COSM19905
KRAS	4	p.Ala146Thr	c.436G>A	COSM19404
KRAS	4	p.Lys117Asn	c.351A>T	COSM28519
KRAS	4	p.Lys117Asn	c.351A>C	COSM19940
KRAS	3	p.Gln61His	c.183A>T	COSM555
KRAS	3	p.Gln61His	c.183A>C	COSM554
KRAS	3	p.Gln61Leu	c.182A>T	COSM553
KRAS	3	p.Gln61Arg	c.182A>G	COSM552
KRAS	3	p.Gln61Pro	c.182A>C	COSM551
KRAS	3	p.Gln61Glu	c.181C>G	COSM550
KRAS	3	p.Gln61Lys	c.181C>A	COSM549
KRAS	3	p.Gln61Lys	c.180_181delTCinsAA	COSM87298
KRAS	3	p.Ala59Gly	c.176C>G	COSM28518
KRAS	3	p.Ala59Glu	c.176C>A	COSM547
KRAS	3	p.Ala59Thr	c.175G>A	COSM546
KRAS	2	p.Gly13Asp	c.38_39delGCinsAT	COSM531 **Note:** The nucleotide change of COSM531 overlaps that of COSM532, so a positive COSM531 sample will also result in a positive call for COSM532.
KRAS	2	p.Gly13Val	c.38G>T	COSM534
KRAS	2	p.Gly13Ala	c.38G>C	COSM533
KRAS	2	p.Gly13Asp	c.38G>A	COSM532
KRAS	2	p.Gly13Cys	c.37G>T	COSM527
KRAS	2	p.Gly13Arg	c.37G>C	COSM529
KRAS	2	p.Gly13Ser	c.37G>A	COSM528

(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.Gly12Cys	c.34G>T	COSM516
KRAS	2	p.Gly12Arg	c.34G>C	COSM518
KRAS	2	p.Gly12Ser	c.34G>A	COSM517
MAP2K1	6	p.Glu203Lys	c.607G>A	COSM232755
MAP2K1	6	p.Glu203Val	c.608A>T	COSM3386991
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	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

(continued)

Gene	Exon	Amino Acid Change	Nucleotide Change	Variant ID
MAP2K1	2	p.Lys57Met	c.170A>T	MAN124
MAP2K1	2	p.Lys57Asn	c.171G>C	COSM5520914
MAP2K1	2	p.Lys57Asn	c.171G>T	COSM1235478
MAP2K2	2	p.Gln60Pro	c.179A>C	COSM145610
MAP2K2	2	p.Phe57Leu	c.171T>G	OM3158
MAP2K2	2	p.Phe57Leu	c.171T>A	COSM3389034
MAP2K2	2	p.Phe57Val	c.169T>G	COSM3534171
MAP2K2	2	p.Phe57Leu	c.169T>C	COSM1235618
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
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	14	p.Thr1010Ile	c.3029C>T	COSM707
MET	14	p.Tyr1021Asn	c.3061T>A	COSM48564
MET	14	p.Tyr1021Phe	c.3062A>T	COSM339515
MET	Intronic	NA	c.3082+1G>A	COSM29633[1]
MET	Intronic	NA	c.3082+1G>T	COSM24687[1]
MET	14	NA	c3082+2T>C	COSM35468[1]
MTOR	53	p.Leu2427Arg	c.7280T>G	COSM2119114
MTOR	53	p.Leu2427Gln	c.7280T>A	COSM1185313
MTOR	47	p.Ser2215Phe	c.6644C>T	COSM1686998
MTOR	47	p.Ser2215Tyr	c.6644C>A	COSM20417
MTOR	47	p.Ser2215Pro	c.6643T>C	COSM1560108

(continued)

Gene	Exon	Amino Acid Change	Nucleotide Change	Variant ID
MTOR	43	p.Val2006Phe	c.6016G>T	COSM249481
MTOR	43	p.Val2006Leu	c.6016G>C	COSM1134662
MTOR	43	p.Val2006Ile	c.6016G>A	COSM893804
MTOR	43	p.Thr1977Arg	c.5930C>G	COSM462602 **Note:** Some "no calls" were observed for this analytical variant due to strand bias with plasmid targets. This does not impact clinical test results.
MTOR	43	p.Thr1977Lys	c.5930C>A	COSM462601
MTOR	43	p.Thr1977Ser	c.5929A>T	COSM1289945 **Note:** Some "no calls" were observed for this analytical variant due to strand bias with plasmid targets. This does not impact clinical test results.
MTOR	40	p.Phe1888Leu	c.5664C>G	COSM462604
MTOR	40	p.Phe1888Leu	c.5664C>A	COSM893813
MTOR	40	p.Phe1888Val	c.5662T>G	COSM893814
MTOR	40	p.Phe1888Leu	c.5662T>C	COSM3358967
MTOR	40	p.Phe1888Ile	c.5662T>A	COSM3358968
MTOR	39	p.Glu1799Lys	c.5395G>A	COSM180789
MTOR	30	p.Cys1483Trp	c.4449C>G	OM3149
MTOR	30	p.Cys1483Phe	c.4448G>T	COSM462616
MTOR	30	p.Cys1483Tyr	c.4448G>A	COSM462615
MTOR	30	p.Cys1483Arg	c.4447T>C	COSM3747775
NRAS	4	p.Ala146Val	c.437C>T	COSM4170228
NRAS	4	p.Ala146Thr	c.436G>A	COSM27174
NRAS	4	p.Lys117Asn	c.351G>T	MAN13
NRAS	3	p.Gln61His	c.183A>T	COSM585

(continued)

Gene	Exon	Amino Acid Change	Nucleotide Change	Variant ID
NRAS	3	p.Gln61His	c.183A>C	COSM586
NRAS	3	p.Gln61Leu	c.182A>T	COSM583
NRAS	3	p.Gln61Arg	c.182A>G	COSM584
NRAS	3	p.Gln61Pro	c.182A>C	COSM582
NRAS	3	p.Gln61Glu	c.181C>G	COSM581
NRAS	3	p.Gln61Lys	c.181C>A	COSM580
NRAS	3	p.Ala59Thr	c.175G>A	COSM578
NRAS	2	p.Gly13Val	c.38G>T	COSM574
NRAS	2	p.Gly13Ala	c.38G>C	COSM575
NRAS	2	p.Gly13Asp	c.38G>A	COSM573
NRAS	2	p.Gly13Cys	c.37G>T	COSM570
NRAS	2	p.Gly13Arg	c.37G>C	COSM569
NRAS	2	p.Gly13Ser	c.37G>A	COSM571
NRAS	2	p.Gly12Val	c.35G>T	COSM566
NRAS	2	p.Gly12Ala	c.35G>C	COSM565
NRAS	2	p.Gly12Asp	c.35G>A	COSM564
NRAS	2	p.Gly12Cys	c.34G>T	COSM562
NRAS	2	p.Gly12Arg	c.34G>C	COSM561
NRAS	2	p.Gly12Ser	c.34G>A	COSM563
PDGFRA	18	p.Asp842_His845del	c.2526_2537delCATCATGCAT GA	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_2538delATCATGCATGA T	COSM12400
PDGFRA	18	p.Ile843_Ser847delinsThr	c.2528_2539delTCATGCATGAT T	COSM12407
PDGFRA	14	p.Asn659Tyr	c.1975A>T	COSM22416

(continued)

Gene	Exon	Amino Acid Change	Nucleotide Change	Variant ID
PDGFRA	14	p.Asn659Lys	c.1977C>A	COSM22415
PDGFRA	14	p.Asn659Lys	c.1977C>G	COSM22414
PDGFRA	12	p.Val561Asp	c.1682T>A	COSM739
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
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	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	10	p.Pro539Arg	c.1616C>G	COSM759
PIK3CA	10	p.Glu542Lys	c.1624G>A	COSM760
PIK3CA	10	p.Glu542Val	c.1625A>T	COSM762
PIK3CA	10	p.Glu545Lys	c.1633G>A	COSM763
PIK3CA	10	p.Glu545Gln	c.1633G>C	COSM27133
PIK3CA	10	p.Glu545Ala	c.1634A>C	COSM12458
PIK3CA	10	p.Glu545Gly	c.1634A>G	COSM764

(continued)

Gene	Exon	Amino Acid Change	Nucleotide Change	Variant ID
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	8	p.Cys420Arg	c.1258T>C	COSM757
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	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	2	p.Arg38Ser	c.112C>A	COSM87310
PIK3CA	2	p.Arg38Gly	c.112C>G	COSM40945
PIK3CA	2	p.Arg38Cys	c.112C>T	COSM744
PIK3CA	2	p.Arg38His	c.113G>A	COSM745
PIK3CA	2	p.Glu39Lys	c.115G>A	COSM30625
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

(continued)

Gene	Exon	Amino Acid Change	Nucleotide Change	Variant ID
PIK3CA	2	p.Arg108His	c.323G>A	COSM27497
PIK3CA	2	p.Lys111Glu	c.331A>G	COSM13570
RAF1	12	p.Thr421Met	c.1262_1263delCCinsTG	MAN9
RAF1	7	p.Ser257Leu	c.770C>T	COSM181063
RAF1	7	p.Ser257Trp	c.770C>G	COSM581519
RET	16	p.Met918Thr	c.2753T>C	COSM965
RET	15	p.Ala883Phe	c.2646_2648delAGCinsTTT	COSM981 **Note:** The nucleotide change of COSM981 overlaps that of COSM133167, so a positive COSM981 sample will also result in a positive call for COSM133167.
RET	15	p.Ala883Ser	c.2647G>T	COSM133167
RET	15	p.Asp898_Glu901del	c.2694_2705delTGTTTATGAAG A	COSM962
RET	13	p.Glu768Gly	c.2303A>G	COSM1347811
RET	13	p.Glu768Asp	c.2304G>C	COSM21338
RET	11	p.Cys634Arg	c.1900T>C	COSM966
RET	10	p.Cys618Arg	c.1852T>C	COSM29803
RET	10	p.Cys618Tyr	c.1853G>A	COSM980
RET	10	p.Cys620Arg	c.1858T>C	COSM29804
ROS1	38	p.Gly2032Arg	c.6094G>C	MAN11
ROS1	38	p.Gly2032Arg	c.6094G>A	MAN10
ROS1	36	p.Leu1951Met	c.5851C>A	COSM1072521

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

Fusion isoforms detected by the Oncomine™ Dx Target Test

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

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

Variants detected in cholangiocarcinoma (CC)

DNA variants detected by the Oncomine™ Dx Target Test

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

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