

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

USER GUIDE

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Revision D.02

IVD

For In Vitro Diagnostic Use. | Rx Only

ThermoFisher
S C I E N T I F I C



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Products manufactured at this site:

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



Life Technologies Corporation |
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Products manufactured at this site:

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

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: **MAN0018948 D.02 (English)**

Revision	Date	Description
D.02	16 October 2024	<ul style="list-style-type: none"> • Updated “Assay warnings and limitations” on page 11. • Updated with claims and supporting material for IDH1 and IDH2 mutations in astrocytoma and oligodendroglioma.
D.01	25 June 2024	Draft for FDA review updated with post-market commitment study summaries.
D.0	18 October 2023	<ul style="list-style-type: none"> • Updated “Assay warnings and limitations” on page 11. • Updated with an additional analytical study for cholangiocarcinoma—“Interfering substances—Study II” on page 26.
C.0	18 April 2023	<ul style="list-style-type: none"> • Updated with claims and supporting material for RET fusions in NSCLC and thyroid cancer, and RET mutations in medullary thyroid cancer. • Updated for Torrent Suite™ Dx Software 5.14.
B.0	19 January 2023	<ul style="list-style-type: none"> • Updated with claims and supporting material for ERBB2 SNVs and insertions. • Updated labeling.
A.0	10 December 2021	New Oncomine™ Dx Target Test user guide for commercial release—updated with claims and supporting material for IDH1 SNVs and EGFR exon 20 insertion variants.

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

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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 OncoPrint™ 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.

OncoPrint™ Dx Target Test user guides

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

- *OncoPrint™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide*
- *OncoPrint™ Dx Target Test Part II: Sample and Library Preparation User Guide*
- *OncoPrint™ Dx Target Test Part III: Template Preparation User Guide*
- *OncoPrint™ Dx Target Test Part IV: Sequencing and Results Reports User Guide*
- *OncoPrint™ Dx Target Test Part V: Torrent Suite™ Dx Software 5.14 Reference User Guide*

All five guides are required to complete the entire OncoPrint™ 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 OncoPrint™ 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 changes in human DNA and RNA isolated from non-small cell lung cancer (NSCLC), cholangiocarcinoma (CC), astrocytoma (AC), oligodendroglioma (OG), anaplastic thyroid cancer (ATC), medullary thyroid cancer (MTC), and thyroid cancer (TC), tumor specimens in formalin-fixed, paraffin-embedded (FFPE) tissue samples. Detection of these variants is performed using the Ion PGM™ Dx System.

The Oncomine™ Dx Target Test (Cat. No. A49755) provides a set of primers in two panels that target key regions of 23 genes related to NSCLC, one gene related to CC, two genes related to AC and OG, one gene related to ATC, and one gene related to TC and MTC.

Intended use

The Oncomine™ Dx Target Test is a qualitative *in vitro* diagnostic test that uses targeted high-throughput, parallel-sequencing technology to detect single nucleotide variants (SNVs), deletions, and insertions in 23 genes from DNA and fusions in ROS1 and RET from RNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor tissue samples from patients with non-small cell lung cancer (NSCLC), IDH1 SNVs from FFPE tumor tissue samples from patients with cholangiocarcinoma (CC), BRAF V600E mutations from FFPE tumor tissue samples from patients with anaplastic thyroid cancer (ATC), IDH1 and IDH2 SNVs from FFPE tumor tissue samples from patients with astrocytoma (AC) or oligodendroglioma (OG), RET SNVs, multi-nucleotide variants (MNVs), and deletions from DNA isolated from FFPE tumor tissue samples from patients with medullary thyroid cancer (MTC), and RET fusions from RNA isolated from FFPE tumor tissue samples from patients with thyroid cancer (TC) using the Ion PGM™ Dx System.

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

Table 1 List of variants for therapeutic use

Tissue type	Gene	Variant	Targeted therapy
NSCLC	BRAF	BRAF V600E mutations	TAFINLAR [®] (dabrafenib) in combination with MEKINIST [®] (trametinib)
	EGFR	EGFR L858R mutation, EGFR exon 19 deletions	IRESSA [®] (gefitinib)
	EGFR	EGFR exon 20 insertions	RYBREVANT [™] (amivantamab-vmjw)
	ERBB2/HER2	ERBB2/HER2 activating mutations (SNVs and exon 20 insertions)	ENHERTU [®] (fam-trastuzumab deruxtecan-nxki)
	RET	RET fusions	GAVRETO [™] (pralsetinib) RETEVMO [®] (selpercatinib)
	ROS1	ROS1 fusions	XALKORI [®] (crizotinib)
CC	IDH1	IDH1 R132C, IDH1 R132G, IDH1 R132H, IDH1 R132L, and IDH1 R132S mutations	TIBSOVO [®] (ivosidenib)
ATC	BRAF	BRAF V600E mutations	TAFINLAR [®] (dabrafenib) in combination with MEKINIST [®] (trametinib)
MTC	RET	RET mutations (SNVs, MNVs, and deletions)	RETEVMO [®] (selpercatinib)
TC	RET	RET fusions	RETEVMO [®] (selpercatinib)
AC and OG	IDH1, IDH2	IDH1 R132C, IDH1 R132G, IDH1 R132H, IDH1 R132L, IDH1 R132S, IDH2 R172M, IDH2 R172K, IDH2 R172W, IDH2 R172S, and IDH2 R172G mutations	VORANIGO [®] (vorasidenib)

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

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

Table 2 List of variants with established analytical performance only in NSCLC

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

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

Theory of operation

Overview

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

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

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

Sample and library preparation

The system has been validated with DNA and RNA isolated from FFPE tissue samples using the Ion Torrent Dx FFPE Sample Preparation Kit. Samples are prepared as slide-mounted 5-micron FFPE sections, which are deparaffinized before use. The samples must be macrodissected and enriched for tumor content if the tumor content is less than 20% and the tumor content in the region of interest is greater than or equal to 10%, or if the tissue is highly necrotic. The samples are digested, then the DNA and RNA are isolated and quantified. The minimum concentration and R² values that are required for library preparation are shown in Table 3.

Table 3 Required sample concentrations and R² 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 OncoPrint™ Dx Target Test, Controls, and Diluent Kit and Ion PGM™ Dx Library Kit. Libraries created using these kits have a distinguishing nucleic acid sequence barcode that is incorporated into each amplicon. Information about each sample and its resulting libraries are entered into Torrent Suite™ Dx Software, which tracks the progress of the sample from library preparation through analysis. The specific Assay Definition File for a particular set of targets and therapies defines the sample and library information required and tracked by the software.

Template preparation and sequencing

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

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

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

Data analysis

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

Results

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

Table 4 Electronic results and reports generated by the software

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

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

Assay warnings and limitations

- Use of this product must be limited to personnel trained in the techniques of PCR, NGS, and the use of the Oncomine™ Dx Target Test and the Ion PGM™ Dx System.
- The Oncomine™ Dx Target Test has only been validated for use with FFPE tumor slide specimens. The use of fine needle aspirates for thyroid cancer (TC) specimens has not been validated.
- The Oncomine™ Dx Target Test has been validated to detect the following somatic mutations: single-nucleotide variations (SNVs), multinucleotide variations (MNVs), deletions of 3, 6, 9, 12, 15, and 18 base pairs, and insertions of 3, 6, 9, and 12 base pairs in DNA, and fusions in RNA.
- The Oncomine™ Dx Target Test is only validated for use with the Ion PGM™ Dx System and the Veriti™ Dx 96-well Thermal Cycler, 0.2 mL.
- The Oncomine™ Dx Target Test is only validated for use with 10 ng each of DNA and RNA per sample. Input amounts less than or greater 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.

- Interference in variant calling can be observed at higher concentrations of chenodeoxycholic acid (≥ 30 nmol/mL bile acid) in cholangiocarcinoma (CC) clinical FFPE samples with IDH1 variants present at an allelic frequency near the limit of detection (LoD).
- The OncoPrint™ Dx Target Test has not been validated for the detection of RET insertions.
- Users are cautioned that DNA variant-positive calls in the RET genomic region have been observed to produce multiple variant calls, even when only one variant is present. These RET variants are all activating and do not change the patient's clinical appropriateness for seliparitinib.
- High variation in fusion reads can be observed with fusion-positive samples. A decrease in fusion reads over time has been observed when testing slides from TC tissue under storage.
- For non-small cell lung cancer (NSCLC), the OncoPrint™ Dx Target Test assay definition file includes prevalent but not all rare or newly identified RET isoforms, ROS1 isoforms, EGFR exon 20 insertions, EGFR exon 19 deletions, and ERBB2/HER2 activating mutations. The OncoPrint™ Dx Target Test may miss rare, complex, or newly identified:
 - RET isoforms carried by a subset of patients who may derive benefit from pralsetinib or seliparitinib
 - ROS1 isoforms carried by a subset of patients who may derive benefit from crizotinib
 - EGFR exon 20 insertions carried by a subset of patients who may derive benefit from amivantamab-vmjw
 - EGFR exon 19 deletions carried by a subset of patients who may derive benefit from gefitinib
 - ERBB2/HER2 activating mutations carried by a subset of patients who may derive benefit from fam-trastuzumab deruxtecan-nxki
- For TC, the OncoPrint™ Dx Target Test assay definition file includes the most prevalent but not all rare or newly identified RET isoforms. The OncoPrint™ Dx Target Test may miss a subset of patients carrying these rare or newly identified RET isoforms who may derive benefit from seliparitinib.
- For medullary thyroid cancer (MTC), the OncoPrint™ Dx Target Test assay definition file includes the most prevalent but not all rare or newly identified RET SNVs, MNVs and deletions. The OncoPrint™ Dx Target Test may miss a subset of patients carrying these rare or newly identified RET SNVs, MNVs and deletions who may derive benefit from seliparitinib.
- For astrocytoma (AC) and oligodendroglioma (OG), the OncoPrint™ Dx Target Test included prevalent but not all rare IDH2 variant clinical specimens in the assay reproducibility study. The OncoPrint™ Dx Target Test may miss rare IDH2 variants carried by patients who may derive benefit from vorasidenib.
- The OncoPrint™ Dx Target Test has only been validated for use with FFPE tumor slide specimens. The validation of the use of derivative core needle biopsy (CNB) samples and stereotactic biopsy (STB) samples for astrocytoma (AC) and oligodendroglioma (OG), with the OncoPrint™ Dx Target Test to support inclusion of these type of samples has not been performed.
- The safe and effective use of the variants reported in Table 2 has not been established for selecting therapy using this device. The variants for KRAS (COSM512/p.Gly12Phe/c.34_35delGGinsTT and COSM516/p.Gly12Cys/c.34G>T), MET (COSM707/p.Thr1010Ile/c.3029C>T) and PIK3CA (COSM754/p.Asn345Lys/c.1035T>A) have been analytically validated. Performance of all other variants identified by the test, other than the clinically validated therapeutic variants and analytically validated variants, has not been directly demonstrated.



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

Contraindications

There are no known contraindications.

Software compatibility and requirements

The procedures in this guide are designed for use with Torrent Suite™ Dx Software version 5.14 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

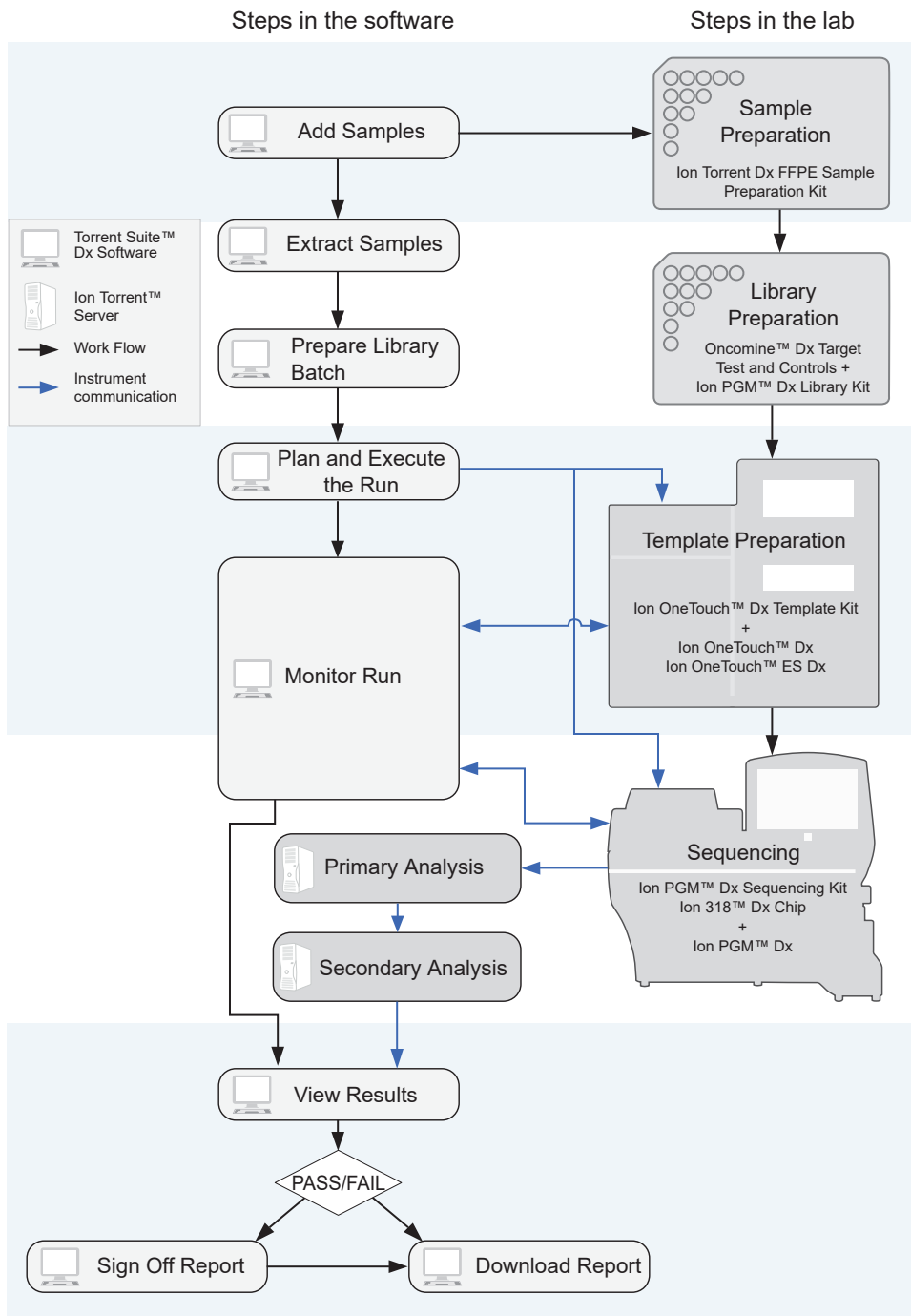
Oncomine™ Dx Target Test Kit

The Oncomine™ Dx Target Test Kit (Cat. No. A55462) 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	A55463
	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	A55464

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	
COSM12558_AF	Variant called and AF ≥0.05
COSM6223_AF	Variant called and AF ≥0.05
COSM6224_AF	Variant called and AF ≥0.05
COSM683_AF	Variant called and AF ≥0.05
COSM48358_AF	Variant called and AF ≥0.05
COSM760_AF	Variant called and AF ≥0.05
COSM476_AF	Variant called and AF ≥0.05
COSM12376_AF	Variant called and AF ≥0.05
COSM516_AF	Variant called and AF ≥0.05
COSM28747_AF	Variant called and AF ≥0.05
Mean AQ20 Read Length (bp)	≥98

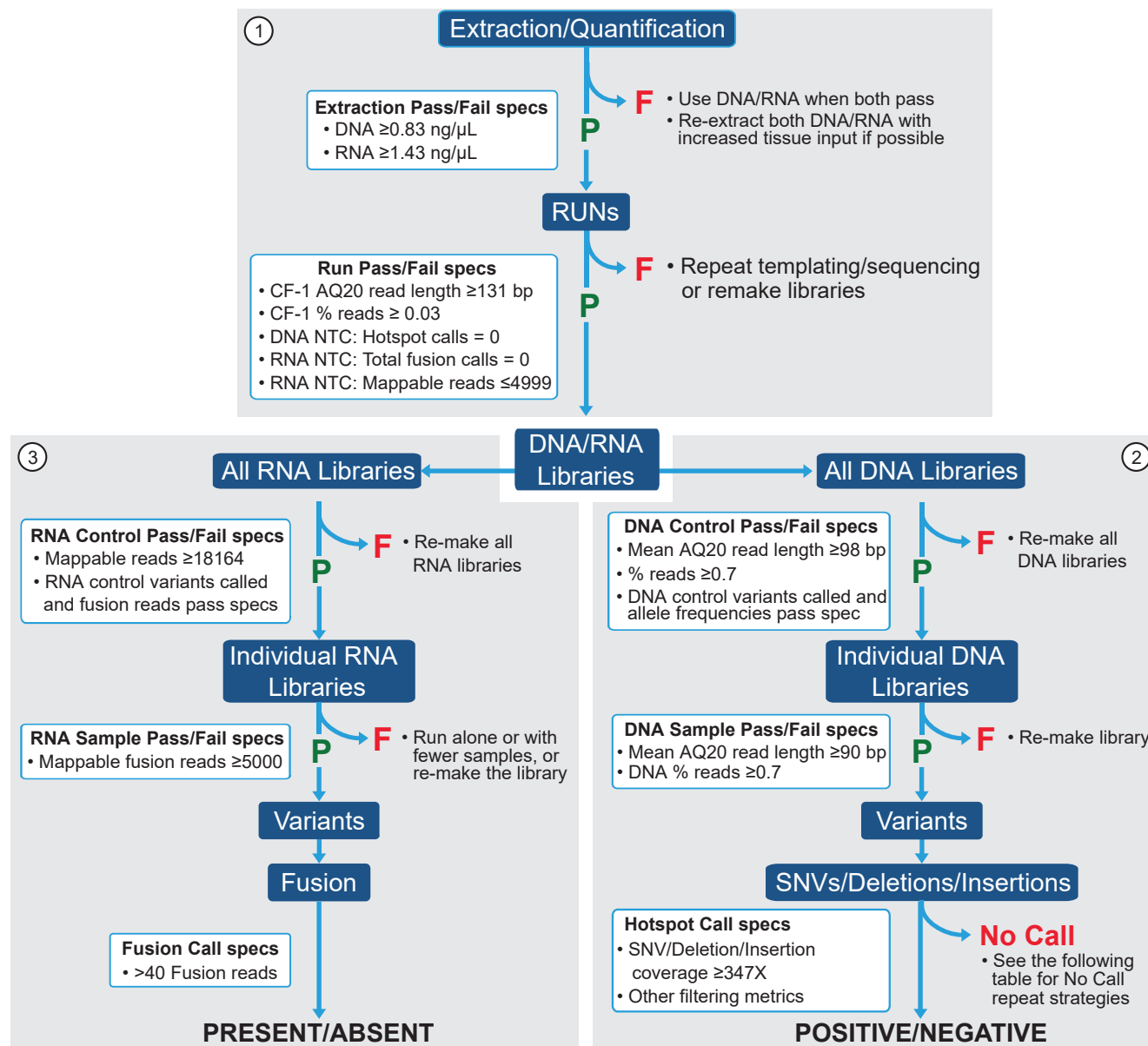
(continued)

Metric	Criteria
Percent Reads (%)	≥ 0.7
RNA Control	
Mappable Reads	≥ 18164
ROS1 Fusion Reads	Variant called and fusion reads ≥ 349
RET Fusion Reads	Variant called and fusion reads ≥ 59

Pass/fail specifications and repeat strategy

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

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



Point of failure		Tissue type	Recommended repeat strategy
1	RUNs	NSCLC, CC, AC, OG, ATC, MTC, and TC	Repeat templating and sequencing or remake the libraries.
2	DNA Library	NSCLC, CC, AC, OG, ATC, MTC, and TC	Remake all DNA libraries.
3	RNA library	NSCLC and TC	Remake all RNA libraries.
		CC, AC, OG, ATC, and MTC	Not applicable 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: <ul style="list-style-type: none"> 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: <ul style="list-style-type: none"> 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: <ul style="list-style-type: none"> 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). 	<p>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.</p> <p>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.</p> <p>If both the DNA NTC and RNA NTC fail, remake all of the DNA and RNA controls and sample libraries.</p>

(continued)

Point of failure	Specification type	Passing criteria	Repeat strategy
2	DNA Control	<p>The DNA control must pass the following specifications in order for any DNA samples within the run to have any reportable results:</p> <ul style="list-style-type: none"> • AQ20 Mean Read Length (bp) must be ≥ 98. • Percent Reads must be $\geq 0.7\%$. • 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. 	<p>If any of these specifications fail, the operator must remake all DNA control and DNA sample libraries.</p>
3	RNA Control	<p>The RNA control must pass the following specifications for any RNA samples within the run to have any reportable results:</p> <ul style="list-style-type: none"> • 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. 	<p>If either of these specifications fails, the operator must remake all the RNA control and RNA sample libraries.</p>
2	Library DNA Sample	<p>Any individual DNA sample library must meet the following specifications to have reportable results for the DNA sample library:</p> <ul style="list-style-type: none"> • Mean AQ20 Read Length (bp) ≥ 90. • Percent Reads ≥ 0.7. 	<p>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.</p> <p>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.</p>

(continued)

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

No Call repeat strategies

Point of failure	Observation (Example FR tag) ^[1]	Reason ^[2]	Repeat strategy
2	<i>MINCOV</i> <347, <i>PosCov</i> <2, <i>NegCov</i> <2*	Coverage	Repeat the run with fewer samples per chip may improve coverage.
2	<i>NODATA</i>	No data	Repeating the run with fewer samples per chip may improve coverage.
2	<i>QualityScore</i> <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	<i>STDBIAS</i> 0.99034>0.96, <i>STDBIASPVAL</i> 0.299<=1	Strand bias	Remake the DNA and/or RNA libraries starting from nucleic acid sample to improve strand coverage.

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

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

Pass/fail specifications and repeat strategy—mixed runs

If you combine different tissue types (for example, NSCLC and CC samples) in a single OncoPrint™ 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 17.

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



Performance characteristics

Cholangiocarcinoma (CC)—Analytical studies

Tissue input study

Fifteen (15) slide-mounted FFPE samples were analyzed to determine if samples extracted using the Ion Torrent Dx Total Nucleic Acid Isolation Kit yield DNA and RNA at the concentrations that are required by the Oncomine™ Dx Target Test when tissue input requirements are met. The test requires DNA at a concentration of ≥ 0.83 ng/ μ L and RNA at a concentration of ≥ 1.43 ng/ μ L.

Five (5) resection samples with $\geq 20\%$ tumor content were prepared without macrodissection, 5 resection samples with $< 20\%$ to $\geq 10\%$ tumor cell content were macrodissected, and 5 samples were collected by core needle biopsy (CNB). For the resection samples with ≥ 100 mm² surface area, 1 x 5 μ m section was used per extraction. For resection samples with < 100 mm², 4 x 5 μ m sections were used per extraction. For CNBs, all of which had a surface area < 30 mm², 9 x 5 μ m 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/ μ L and an RNA concentration of ≥ 1.43 ng/ μ L. One CNB sample failed the minimum DNA and RNA concentration specifications, with values of 0.72 ng/ μ L and 0.81 ng/ μ L respectively. The low concentrations were likely caused by insufficient tissue input as the sample only had a single core biopsy per slide.

Guard band testing study

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

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

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

FFPE block stability study

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

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

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

FFPE slide stability study

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

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

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

Sample processing reproducibility study

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

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

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

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

Table 5 Reproducibility call rates

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

Interfering substances studies

Interfering substances—Study I

Two (2) potentially interfering substances that can be found in cholangiocarcinoma (CC) clinical 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 absence or presence of the excess endogenous substance (Table 6) 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. These data support the claim that hemoglobin and bile acids do not affect Oncomine™ Dx Target Test assay performance at the level tested.

Interfering substances—Study II

A further study was performed to evaluate whether the potential endogenous interfering substances cholic acid, chenodeoxycholic acid, and triglyceride can affect IDH1 variant calling by the Oncomine™ Dx Target Test using the Ion PGM™ Dx System. The substances were added during extraction to cholangiocarcinoma (CC) clinical FFPE samples with IDH1 variants near the Limit of Detection (LoD).

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.

Table 7 Interfering substances and amounts

Potential interfering substance	Step	Amount of substance
Cholic acid	After deparaffinization, cholic acid was added to the Digestion Buffer used to pre-wet the tissue section.	30 nmol/mL
Chenodeoxycholic acid	After deparaffinization, chenodeoxycholic acid was added to the Digestion Buffer used to pre-wet the tissue section.	30 nmol/mL
Triglyceride	After deparaffinization, triglyceride was added to the Digestion Buffer used to pre-wet the tissue section.	37 µmol/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 absence or presence of the endogenous substance at the concentration shown (Table 7). Clinical blends were prepared near LoD (3.7-5.5%) from these samples after extraction 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.

The results of testing with cholic acid, chenodeoxycholic acid, and triglyceride showed 100% concordance with the control condition with both IDH1 R132 variant-positive and WT FFPE CC samples near LoD. However, a no call and negative result were seen when clinical blends were tested below LoD (1.8–2.5%), suggesting there can be interference at 30 nmol/mL chenodeoxycholic acid for samples below the assay cutoff. Interference was not observed at lower chenodeoxycholic acid concentrations.

These data support the claim that cholic acid and triglyceride do not affect the Oncomine™ Dx Target Test assay performance at the level tested. However, interference can be observed at higher concentrations of chenodeoxycholic acid (≥ 30 nmol/mL) in IDH1 variant-positive cholangiocarcinoma samples when AF is near the LoD.

Limit of Detection (LoD) study

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

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

Table 8 LoD of clinical IDH1 variants

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

Assay reproducibility study

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

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

The negative call rate, positive call rate, and within-run repeatability were calculated for each IDH1 R132 variant-positive sample at the expected IDH1 R132 variant location. The results are shown in Table 9.

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 9 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 10.

Table 10 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 10 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 11.

Table 11 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.

Astrocytoma and oligodendroglioma—Analytical studies

Limit of Blank (LoB) study

A study was performed to test the frequency of false positive calls for IDH1 and IDH2 SNVs detected by the OncoPrint™ Dx Target Test in wild-type samples. In this study, operators used DNA extracted from 4 WT FFPE glioma clinical samples and carried it through from library preparation to sequencing. Samples that are WT at IDH1 R132 and IDH2 R172 locations should produce a "variant not detected" call at each location. By definition (EP17-A2), the 95th percentile of test results on blank samples equals zero.

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

The result at each variant location for each sample was tabulated. For all 4 samples, there were no positive calls at either of the IDH1 or IDH2 variant locations analyzed by the test. The false positive rate was therefore zero.

Tissue input study

A study was performed to determine if oligodendroglioma (OG) and astrocytoma (AC) samples extracted using the Ion Torrent Dx Total Nucleic Acid Isolation Kit yield DNA at the concentration that is required by the OncoPrint™ Dx Target Test when tissue input requirements are met. The test requires DNA at a concentration of ≥ 0.83 ng/ μ L. A total of 17 FFPE OG and AC samples were analyzed, including resection, resection with macrodissection, derivative core needle biopsy (CNB), and stereotactic biopsy (STB) samples. Fine needle aspirate (FNA) samples are not in scope for glioma and were not tested. RNA was also out of scope and was not analyzed in the study.

Six resection samples with $\geq 20\%$ tumor content were prepared without macrodissection, and 1 resection sample with $\geq 20\%$ tumor content was prepared with macrodissection. Five resection samples with $< 20\%$ but $\geq 10\%$ tumor cell content were macrodissected, and the 3 CNB and 2 STB samples were prepared without macrodissection. For all resection samples, 2 × 5 μ m slides were used per extraction. For the CNB and STB samples, 9 × 5 μ m slides were used per extraction. DNA concentrations were determined using the Ion Torrent Dx DNA Quantification Kit.

Of the samples tested, 100% (17/17) yielded a DNA concentration of ≥ 0.83 ng/ μ L, meeting the minimum concentration requirements.

Guard band testing study

Guard band testing was performed to define OncoPrint™ Dx Target Test tolerances by testing assay steps at important points of the library preparation workflow. Two clinical sample blends were tested near the limit of detection (1X–1.5X LoD), as determined in the "Limit of Detection (LoD) study". Blend 1 included one IDH1 (R132H / COSM28746) variant-positive and one IDH2 (R172K / COSM33733) variant-positive clinical FFPE oligodendroglioma (OG) sample blended near LoD with a wild-type (WT) clinical FFPE glioma sample. Blend 2 included an IDH1 (R132H / COSM28746) variant-positive derivative glioma sample blended near LoD with a WT clinical FFPE glioblastoma grade 4 sample.

The study tested the conditions listed in Table 12 at 3 levels to test the tolerance to variation in reagent volume, incubation timing, mixing, residual ethanol volume, and thermal cycling temperature during library preparation using the IDH1/IDH2 variant-positive blends.

Table 12 Conditions tested

Study no.	Library preparation step	Condition	SOP (control)	Low and High tested
1	Target amplification—DNA	DNA panel volume	4 µL	2 µL, 6 µL
2	Target amplification—DNA	LIB HiFi Mix volume	4 µL	2 µL, 6 µL
3	Digest DNA	LIB FuPa volume	2 µL	1 µL, 3 µL
4	Ligate barcode adapters—DNA	LIB Switch Soln volume	4 µL	2 µL, 6 µL
5	Ligate barcode adapters—DNA	Barcode adapter volume	2 µL	1 µL, 3 µL
6	Library purification with LIB AMPure™ Reagent—DNA	Incubation time after adding LIB AMPure™ Reagent	5 minutes	4 minutes, 6 minutes
7	Library purification with LIB AMPure™ Reagent—DNA	Bubble formation after adding LIB AMPure™ Reagent	5 times ^[1]	4 times, 6 times ^[1]
8	Library purification with LIB AMPure™ Reagent—DNA	Residual ethanol	0 µL	1.0 µL, 1.75 µL ^[2]
9	All thermal cycling steps—DNA	Thermal cycling temperature offset	±0°C	-1.0°C, +0.5°C

^[1] The number of times each library is pipetted up and down to mix after addition of LIB AMPure™ Reagent.

^[2] Two high conditions were tested: 1 µL ethanol and 1.75 µL ethanol.

For each test condition, 3 levels were tested to determine the tolerance range for each condition: Low, Standard Operating Protocol (SOP), and High. The Low and High volume levels were set at 50% below and 50% above the SOP volume, respectively. Other condition ranges are described in Table 12. For each blend, 18 replicates were tested per condition, generating 162 data points per blend for analysis.

The results of the study indicated that IDH1 and IDH2 variant calls were generated at 100% concordance for all libraries across each sample blend tested for the 9 conditions.

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

Stability of assay intermediates study

The stability of assay intermediates study was performed to test whether hold times in stopping points specified in the Oncomine™ Dx Target Test user guides affect test performance. All samples used in the study were oligodendroglioma samples. A dual variant IDH1/IDH2 SNV-positive DNA blend was prepared at target allele frequency (AF) levels of 1X–1.5X the limit of detection. The blend contained DNA from the most prevalent IDH1 variant (R132H / COSM28746) and IDH2 variant (R172K / COSM33733) along with DNA from one IDH1/IDH2 wild-type clinical sample. The DNA blend was used in each part of the study to test assay performance at baseline, then compare it to performance after the stopping point holds that are specified in the Oncomine™ Dx Target Test user guides are included

in the workflow. The OncoPrint™ Dx Target Test workflow allows a total of 9 stopping points in library preparation, each with a maximum hold time, in addition to stopping points and holds in template preparation and sequencing. The no-hold/hold performance was tested in 3 conditions:

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

Twenty-four replicates per condition were tested using 1 reagent lot, generating a total of 72 data points for the study.

The study demonstrated that the assay intermediates were stable after predefined hold times and OncoPrint™ Dx Target Test performance was not affected by the hold times. Each IDH1 or IDH2 SNV-positive variant was called correctly 100% of the time in the clinical sample blends across both hold conditions and the control condition. In addition, the relative percent change in the mean DNA variant AF at each hold condition from the AF at the nominal condition was less than 20%, meeting study acceptance criteria.

DNA input study

For this study, a dual variant IDH1/IDH2 SNV-positive DNA blend was prepared near the limit of detection from two FFPE clinical variant-positive oligodendroglioma (OG) specimens and one clinical wild-type (WT) OG specimen. The blend contained IDH1 R132H / COSM28746 and IDH2 R172K / COSM33733, each blended to 1X–1.5X variant LoD with DNA from the WT glioma sample. Five DNA input-levels (5 ng, 7.5 ng, 10 ng (SOP), 12.5 ng, and 15 ng) were tested using 6 replicate libraries for each input level, generating a total of 30 data points for each variant in the study.

The results from the study demonstrated a 100% positive variant call rate for the blend for all DNA input level combinations tested within the input range of 5–15 ng of DNA.

FFPE block stability study

Stability of formalin-fixed paraffin-embedded (FFPE) glioma tissue blocks stored at room temperature (15°C to 30°C) was established when tested with the OncoPrint™ Dx Target Test.

Three clinical samples positive for an IDH1 or IDH2 single nucleotide variant (SNV), were tested at baseline (T0), 3 months + 1 week (T1), 6 months + 1 week (T2), 9 months + 1 week (T3) and 12 months + 1 week (T4) time points.

The summary results of the extracted DNA from samples containing an IDH1 or IDH2 SNV are shown in Table 13. The allelic frequency (AF) of IDH1 and IDH2 SNV's present in the DNA samples tested at each time-point (T0–T4) was determined using the OncoPrint™ Dx Target Test. Linear least squares regression analysis was performed on the allele frequency of SNVs for each sample across all time points.

At the 12 months + 1 week (T4) time-point and all previous time points, DNA extracted from FFPE glioma samples harboring an IDH1 or IDH2 SNV met the acceptance criteria for maximum drift from baseline, $\leq 30\%$ for allele frequency of DNA variants. In addition, one derivative stereotactic biopsy AC sample was shown to have at least 6 months of stability.

Table 13 Summary results for baseline to 12 months + 1 week time point for IDH1 and IDH2

Sample	Gene	Variant ID	Average baseline AF	Observed average AF at 12 months	Overall drift from baseline at 12 months	Maximum allowable drift AF range	Pass/Fail
AD4168	IDH1	COSM28746	0.479	0.450	1.06%	30% (range: 0.335–0.623)	Pass
AN2638	IDH2	COSM33733	0.376	0.385	0.97%	30% (range: 0.263–0.488)	Pass

FFPE slide stability study

Stability of paraffin undipped formalin-fixed paraffin-embedded (FFPE) glioma tissue sections mounted on positively-charged slides, and stored at room temperature (15°C to 30°C), was established when tested with the OncoPrint™ Dx Target Test.

Three clinical samples positive for an IDH1 or IDH2 single nucleotide variant (SNV), were tested at baseline (T0), 3 months + 1 week (T1), 6 months + 1 week (T2), 9 months + 1 week (T3) and 12 months + 1 week (T4) time points.

The summary results of the extracted DNA from samples containing an IDH1 or IDH2 SNV are shown in Table 14. The allele frequency (AF) of IDH1 and IDH2 SNV's present in the DNA samples tested at each time-point (T0–T4) was determined using the OncoPrint™ Dx Target Test. Linear least squares regression analysis was performed on the allele frequency of SNVs for each sample across all time points.

At the 12 months + 1 week (T4) time-point and all previous time points, DNA extracted from FFPE glioma samples harboring an IDH1 or IDH2 SNV met the acceptance criteria for maximum drift from baseline, $\leq 30\%$ for allele frequency of DNA variants. In addition, one derivative stereotactic biopsy astrocytoma sample was shown to have at least 3 months of stability.

Table 14 Summary results for baseline to 12 months + 1 week time point for IDH1 and IDH2

Sample	Gene	Variant ID	Average baseline AF	Observed average AF at 12 months	Overall drift from baseline at 12 months	Maximum allowable drift AF range	Pass/Fail
AD4168	IDH1	COSM28746	0.444	0.461	0.96%	30% (range: 0.311–0.577)	Pass
AN2638	IDH2	COSM33733	0.393	0.391	1.01%	30% (range: 0.275–0.511)	Pass

Extracted nucleic acid stability study

Stability of DNA extracted from formalin-fixed paraffin-embedded (FFPE) glioma slide-mounted tissue sections and stored at -30°C to -10°C for up to 12 months was established when tested using the Oncomine™ Dx Target Test.

Three clinical samples positive for an IDH1 or IDH2 single nucleotide variant (SNV), were tested at baseline (T0), 3 months + 1 week (T1), 6 months + 1 week (T2), 9 months + 1 week (T3) and 12 months + 1 week (T4) time points.

The summary results of the extracted DNA from samples containing an IDH1 or IDH2 SNV are shown in Table 15. The allelic frequency (AF) of IDH1 and IDH2 SNV's present in the DNA samples tested at each time-point (T0–T4) was determined using the Oncomine™ Dx Target Test. Linear least squares regression analysis was performed on the allele frequency of SNVs for each sample across all time points.

At the 12 months + 1 week (T4) time-point and all previous time points, DNA extracted from FFPE glioma samples harboring an IDH1 or IDH2 SNV met the acceptance criteria for maximum drift from baseline, $\leq 30\%$ for allele frequency of DNA variants. In addition, one derivative stereotactic biopsy astrocytoma sample was shown to have at least 6 months of stability -30°C to -10°C .

Table 15 Summary results for baseline to 12 months + 1 week time point for IDH1 and IDH2

Sample	Gene	Variant ID	Average baseline AF	Observed average AF at 12 months	Overall drift from baseline at 12 months	Maximum allowable drift AF range	Pass/Fail
AD4168	IDH1	COSM28746	0.470	0.457	1.02%	30% (range: 0.329–0.611)	Pass
AN2638	IDH2	COSM33733	0.347	0.385	0.90%	30% (range: 0.243–0.451)	Pass

Assay reproducibility study

The reproducibility and repeatability of IDH1 and IDH2 variant detection in clinical astrocytoma (AC) and oligodendroglioma (OG) samples were determined with the Oncomine™ Dx Target Test. Seven IDH1 and IDH2 variant-positive and 5 WT FFPE clinical samples were used in the study. For 3 rare IDH2 variants not detected in our screening, 2 adenocarcinoma FFPE clinical samples and 1 cell line FFPE block containing IDH2 variants were used. Extracted DNA from IDH1 and IDH2 variant-positive samples was blended with IDH1/IDH2 wild-type (WT) DNA from 5 samples to prepare 12 sample blends targeting 2 allele frequency (AF) levels per variant or variant pair, 0.9X–1.3X the assay limit of detection (LoD), and 1.8X–2.5X LoD. Thirteen DNA sample blends were prepared, 12 blends positive for either IDH1 or IDH2, or positive for both, and one blend negative for IDH1 and IDH2. Testing was performed at 3 test sites by 2 operators using 2 Ion PGM™ Dx instrument systems per site. Three different reagent lots were used across the 3 test sites, with each site using 2 reagent lots.

Each operator tested 12 replicates per sample blend (6 replicates \times 2 lots) and samples were tested in triplicate on the same chip for obtaining intra-run test points for the same sample. In total, each site performed 52 valid sequencing runs (26 runs per operator \times 2 systems) for a total of 156 valid sequencing runs (72 replicates per sample blend) across the 3 sites.

The correct call rate was calculated for each IDH1 and IDH2 variant-positive sample at the expected variant location. Between-run reproducibility results are shown in Table 16 and Table 17.

The overall positive call rate for IDH1 variants was 100% including or excluding no calls. For IDH2 variants, the overall positive call rate was 97.5% including no calls, and 99.9% excluding no calls. The negative call rate was 100% at all IDH1 and IDH2 variant locations (Table 18).

Table 16 Reproducibility results for correct IDH1 call rate

IDH1 variant	Variant ID	Number of valid results	Number of positive calls	Number of negative calls	Number of no calls	Correct call rate		Observed AF (X LoD)
						Excluding no calls	Including no calls	
R132H	COSM28746	72	72	0	0	100%	100%	1.1X
R132H	COSM28746	72	72	0	0	100%	100%	1.9X
R132C	COSM28747	72	72	0	0	100%	100%	1.2X
R132C	COSM28747	72	72	0	0	100%	100%	2.1X
R132S	COSM28748	72	72	0	0	100%	100%	1.1X
R132S	COSM28748	72	72	0	0	100%	100%	2.1X
R132G	COSM28749	72	72	0	0	100%	100%	1.1X
R132G	COSM28749	72	72	0	0	100%	100%	2.2X
R132L	COSM28750	72	72	0	0	100%	100%	1.1X
R132L	COSM28750	72	72	0	0	100%	100%	2.0X

Table 17 Reproducibility results for correct IDH2 call rate

IDH2 variant	Variant ID	Number of valid results	Number of positive calls	Number of negative calls	N of no calls	Correct call rate		Observed AF (X LoD)
						Excluding no calls	Including no calls	
R172G	COSM33731	72	72	0	1	100%	98.6%	1.1X
R172G	COSM33731	72	71	0	0	100%	100%	1.9X
R172M	COSM33732	72	70	0	2	100%	97.2%	1.1X
R172M	COSM33732	72	72	0	0	100%	100%	2.2X
R172K	COSM33733	72	72	0	0	100%	100%	1.1X
R172K	COSM33733	72	72	0	0	100%	100%	2.1X
R172S	COSM34090	72	69	0	3	100%	95.8%	1.1X
R172S	COSM34090	72	72	0	0	100%	100%	2.0X

Table 17 Reproducibility results for correct IDH2 call rate (continued)

IDH2 variant	Variant ID	Number of valid results	Number of positive calls	Number of negative calls	N of no calls	Correct call rate		Observed AF (X LoD)
						Excluding no calls	Including no calls	
R172W	COSM34039	72	60	1	11	98.4%	83.3% ^[1]	0.9X
R172W	COSM34039	72	72	0	0	100%	100%	2.2X

^[1] Many no calls were seen because the observed AF was below the LoD for this variant.

Table 18 Reproducibility results for negative call rates

IDH Variant	Variant ID	Number of valid results	Number of positive calls	Number of negative calls	Number of no calls	Correct call rate		Observed AF (X LoD)
						Excluding no calls	Including no calls	
R132H	COSM28746	72	0	72	0	100%	100%	0.000
R132C	COSM28747	72	0	72	0	100%	100%	0.000
R132S	COSM28748	72	0	72	0	100%	100%	0.000
R132G	COSM28749	72	0	72	0	100%	100%	0.000
R132L	COSM28750	72	0	72	0	100%	100%	0.000
R172G	COSM33731	72	0	72	0	100%	100%	0.000
R172M	COSM33732	72	0	72	0	100%	100%	0.000
R172K	COSM33733	72	0	72	0	100%	100%	0.000
R172W	COSM34039	72	0	72	0	100%	100%	0.000
R172S	COSM34090	72	0	72	0	100%	100%	0.000

Estimates of within-run repeatability for each sample (10 variant-positive samples and 3 WT samples for each gene), calculated as the percentage of runs with concordant replicates, was 100% across all IDH1 variants, including or excluding no calls (Table 19). For IDH2 variants, the repeatability estimates ranged from 95.8% to 100% with no calls excluded. Including no calls, repeatability estimates for most samples ranged from 87.5% to 100% except for S11, which was lower due to no calls resulting from an AF that was below LoD, as noted in Table 17.

Table 19 Repeatability estimates for IDH1 and IDH2 variant calling by sample

Gene	Number of runs per sample ^[1]	% Concordant runs excluding no calls		% Concordant runs including no calls	
		Mean	Median	Mean	Median
IDH1	24	100%	100%	100%	100%
IDH2	24	99.9%	100%	94.9%	100%

^[1] Ten variant-positive samples and 3 WT samples were tested for each gene.

Sample processing reproducibility study

The sample processing reproducibility and repeatability of variant detection using the OncoPrint™ Dx Target Test workflow were evaluated with clinical FFPE glioma and contrived FFPE hybrid cell line samples. Five samples were tested, including 2 unique IDH1 variant-positive cell line samples (R132H/C), 2 unique IDH2 variant-positive cell line samples (R172W/K), each harboring a single nucleotide variant (SNV), and one wild-type (WT) sample (Table 20).

Table 20 Sample used in the study

Sample ID	Sample type	Gene	Variant ID	Amino acid change	Allelic frequency
S1	Hybrid cell line block	IDH1	COSM28746	c.395G>A	5.5%
S2	Hybrid cell line block	IDH2	COSM34039	c.514A>T	4.5%
S4	Hybrid cell line block	IDH1	COSM28747	c.394C>T	6.5%
S5	Hybrid cell line block	IDH2	COSM33733	c.515G>A	5.6%
S6	Clinical	WT	—	—	—

The samples were extracted using 3 lots of the Ion Torrent Dx FFPE Sample Preparation Kit and processed through the workflow by 2 operators at each of 3 test sites (2 operators and 2 Ion PGM™ Dx instrument systems at each site). Each operator performed the steps from deparaffinization and nucleic acid extraction through sequencing. Operators extracted each sample twice per run using the same sample preparation kit lot, then prepared libraries and templates, and sequenced them together in a single system run. Each operator completed 8 runs using 2 lots of reagents.

Between-run correct call rate reproducibility was computed for each gene and sample. The results at the positive variant locations tested are shown in Table 21. Including no calls, correct call rate ranged from 93.8% to 100%. Excluding no calls, correct call rate was 100% for all samples.

Within-run correct call rate repeatability was computed as the percentage of runs with concordant replicates (24 runs per sample). Including no calls, repeatability of correct call rate estimates ranged from 87.5% to 100%. Excluding no calls, repeatability estimates were 100% for all samples.

Table 21 Reproducibility and repeatability summary for correct call rate by sample

Sample	Gene	Number of replicates	Number of positive calls	Number of negative calls	Number of no calls	Reproducibility Correct call rate (95% CI)		Repeatability Concordant run rate (95% CI)	
						Including no calls	Excluding no calls	Including no calls	Excluding no calls
S1	IDH1	48	47	0	1	97.9% (88.9%, 99.9%)	100% (92.5%, 100.0%)	95.8% (78.9%, 99.9%)	100% (85.8%, 100.0%)
S1	IDH2	48	0	47	1	97.9% (88.9%, 99.9%)	100% (92.5%, 100.0%)	95.8% (78.9%, 99.9%)	100% (85.8%, 100.0%)
S2	IDH1	48	0	47	1	97.9% (88.9%, 99.9%)	100% (92.5%, 100.0%)	95.8% (78.9%, 99.9%)	100% (85.8%, 100.0%)
S2	IDH2	48	45	0	3	93.8% (82.8%, 98.7%)	100% (92.1%, 100.0%)	87.5% (67.6%, 97.3%)	100% (85.8%, 100.0%)
S4	IDH1	48	48	0	0	100% (92.6%, 100.0%)	100% (92.6%, 100.0%)	100% (85.8%, 100.0%)	100% (85.8%, 100.0%)
S4	IDH2	48	0	47	1	97.9% (88.9%, 99.9%)	100% (92.5%, 100.0%)	95.8% (78.9%, 99.9%)	100% (85.8%, 100.0%)
S5	IDH1	48	0	48	0	100% (92.6%, 100.0%)	100% (92.6%, 100.0%)	100% (85.8%, 100.0%)	100% (85.8%, 100.0%)
S5	IDH2	48	47	0	1	97.9% (88.9%, 99.9%)	100% (92.5%, 100.0%)	95.8% (78.9%, 99.9%)	100% (85.8%, 100.0%)
S6	IDH1	48	0	48	0	100% (92.6%, 100.0%)	100% (92.6%, 100.0%)	100% (85.8%, 100.0%)	100% (85.8%, 100.0%)
S6	IDH2	48	0	47	1	97.9% (88.9%, 99.9%)	100% (92.5%, 100.0%)	95.8% (78.9%, 99.9%)	100% (85.8%, 100.0%)

Interfering substances study

Six potentially interfering substances or conditions that can be found in clinical FFPE astrocytoma (AC) and oligodendroglioma (OG) 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.

In this study, two precharacterized clinical FFPE AC and OG samples containing the most prevalent IDH1 (R132H) and IDH2 (R172K) variants were blended with an IDH1/IDH2 wild-type (WT) clinical OG sample to dilute the variant allele frequency (AF) to a level near the limit of detection (1X–1.5X range LoD) as determined in the “Limit of Detection (LoD) study”. A second WT sample with 70% necrotic tissue content was blended with normal tissue for the necrotic tissue content condition.

The samples were spiked with additional concentrations or amounts of the interferent substances at the relevant processing step, as listed in Table 22. For the necrotic tissue content condition, each variant sample and the normal WT sample was blended with a necrotic WT sample to give a necrotic tissue content >30%. For this study, only the DNA workflow was tested. Premade RNA libraries were used as a placeholder for all the runs performed during the study.

Table 22 Interfering substances and amounts

Potential interfering substance	Step	Amount of substance
Hemoglobin	After deparaffinization, hemoglobin was added to the 1X Digestion Buffer used to prewet the tissue sections.	4 mg/mL
Paraffin	Paraffin was dissolved into the xylene bath during the deparaffination step	0.72 g
Ethanol	To simulate ethanol carryover, ethanol was added into the Protease digestion step before digestion.	16 µL
Protease	Extra Protease was added into the reaction after the digestion step and before column purification.	2.5 µL
Wash 2 Buffer	Wash 2 Buffer used to isolate DNA and RNA from deparaffinized and digested samples was added to an aliquot of Dilution Solution, which was later used to dilute the RNA and DNA to the appropriate concentration before library preparation.	1% Wash 2 Buffer (equivalent to ~10% wash buffer carried over into eluate)
Necrotic tissue content	DNA extracted from highly necrotic clinical wild-type tissue was blended with variant positive DNA	>30% necrotic tissue
Control SOP	Tissue sections were processed using the standard operating protocol (SOP), without the addition of any potentially interfering substances.	N/A

Six replicates per condition per sample (IDH1 variant-positive, IDH2 variant-positive, WT) generated a total of 126 data points (6 replicates × 7 conditions (including SOP) × 3 samples) in the study. The concordance between variant calls in samples with and without interfering substances was computed for each substance or condition studied.

Including or excluding no calls, 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%.

Limit of Detection (LoD) study

The LoD of the OncoPrint™ Dx Target Test was calculated for 5 IDH1 R132 and 5 IDH2 R172 SNVs in SNV-positive oligodendroglioma and astrocytoma FFPE clinical samples using the OncoPrint™ Dx Target Test workflow. Seven IDH1 and IDH2 variant-positive and 5 WT FFPE clinical samples were used in the study. For 3 rare IDH2 variants not detected in our screening, 2 adenocarcinoma FFPE clinical samples and 1 cell line FFPE block containing IDH2 variants were used.

To test the 10 IDH1 and IDH2 variants, 6 DNA sample blends, including 3 blends that contained one IDH1 and one IDH2 variant, were created by blending IDH1 and/or IDH2 variant-positive DNA with DNA from a WT sample. Six dilution levels were then made from each sample blend. Ten replicates per dilution level were tested with 2 reagent lots to obtain a total of 20 replicates per dilution level, giving a total of 10 variants × 6 dilution levels × 20 replicates = 1,200 total data points.

The LoD was determined as the lowest mean allelic frequency (AF) among the 6 dilution levels at which at least 95% of the replicates yield a positive result. The LoD estimates of the assay for the 5 IDH1 SNVs ranged from 4.6% to 7.0% AF. The LoD estimates for the 5 IDH2 SNVs ranged from 4.1% to 5.8% AF.

Contrived sample functional equivalency study

A study was performed to show that the performance of the OncoPrint™ Dx Target Test in the detection of representative IDH1 and IDH2 single nucleotide variants (SNVs) from contrived FFPE cell line samples is comparable to the detection of these SNVs in clinical glioma FFPE samples. A contrived cell line sample was used in the glioma Limit of Detection (LoD) study due to the rarity of clinical IDH2 variant-positive glioma samples. Contrived sample functional equivalency was determined by comparing OncoPrint™ Dx Target Test limit of detection (LoD) sequencing results from contrived cell line FFPE blocks with results using clinical FFPE samples.

To establish equivalency, the study used nucleic acid extracted from two IDH1 (R132C and R132H) variant-positive contrived FFPE cell lines, one IDH2 (R172K) variant-positive contrived FFPE cell line, and two WT glioma clinical samples. The DNA extracted from the contrived cell line FFPE samples was blended with DNA from WT samples at 6 dilution levels of varying allele frequencies with 20 replicates/level (10 replicates/reagent lot). This resulted in 120 replicates/sample and 120 data points/IDH variant, which created sufficient data points to estimate the LoD (with 95% confidence interval) of the targeted variants.

In two variants (COSM28746 and COSM33733), the LoD was so close to the variant calling threshold that the Probit model failed to adequately describe the data as there were less than 3 dilution levels with hit rates between 10% and 90% for the IDH variants. In these cases where the Probit model did not adequately describe the data, estimates were made using the empirical result of hit rate at each sample dilution level, where the LoD was determined as the lowest mean value among the 6 dilution levels at which at least 95% of the replicates yield a positive result. The final LoD for each IDH1 or IDH2 variant, as estimated by Probit analysis or empirical results, was then based on the results from both reagent lots combined. For each variant, the average allelic frequency (AF) associated with each

test blend was computed, and the corresponding proportion of correct calls was determined. Results comparing contrived and clinical samples are summarized in Table 23.

Table 23 Comparison of contrived and clinical LoD estimates for each variant

Gene	COSMIC ID	Contrived sample		Clinical sample	
		Empirical LoD	Probit LoD	Empirical LoD	Probit LoD
IDH1	COSM28746	5.44%	4.67%	7.0%	3.92%
	COSM28747	5.42%	4.67%	5.5%	3.71%
IDH2	COSM33733	5.07%	4.56%	5.8%	5.45%

All differences in C25, C50, C75 and C95 estimates between the two sample types fell between 1.04% and 0.51% for each variant tested. The comparable C25, C50, C75 and C95 estimates between sample types support the functional equivalency of contrived cell line samples and clinical samples for IDH1 and IDH2 variants. The study results support the functional equivalency of contrived cell line samples and clinical samples for IDH1 and IDH2 variants.

Tumor content study

The tumor cell content in FFPE glioma samples used as input material was calculated for 437 FFPE clinical samples. The tumor cell content of each specimen and region of interest was estimated before the study by an external pathology laboratory.

A total of 327 IDH1/IDH2 variant-positive and 103 IDH1/IDH2 variant-negative samples, along with 7 unknowns, were included in the study analysis. All samples gave valid results for both the Oncomine™ Dx Target Test (Passing Run, DNA Control, and DNA Sample QC criteria) and the reference method test. These valid samples were used for the tumor content study analysis. The observed tumor content for the IDH1/IDH2 variant-positive and variant-negative samples, including unknowns, had the following distribution:

- 6 samples with tumor content <30%
- 11 samples with tumor content 30–39%
- 40 samples with tumor content 40–59%
- 124 samples with tumor content 60–79%
- 256 samples with tumor content 80–100%

Tumor content range for the IDH1/IDH2 variant-positive samples is shown in Table 24.

Table 24 Tumor content range in IDH1/IDH2 variant-positive samples used in clinical studies

IDH1/IDH2 variant-positive samples	Tumor-content range
4	<30%
6	30–39%
26	40–59%
94	60–79%
197	80–100%

The corresponding 95% Clopper Pearson Exact CIs of the PPA, NPA, and OPA overlapped between tumor content levels. This shows that the IDH1/IDH2 variant detection performance of the OncoPrint™ Dx Target Test was equivalent at all ranges of tumor content level. The tumor content level of the clinical samples had no effect on the performance of the OncoPrint™ Dx Target Test.

Analytical accuracy study

An analytical accuracy study was performed with the OncoPrint™ Dx Target Test and an orthogonal next generation sequencing test method representative of local laboratory tests (LLT) as a comparator (referred to henceforth as NGS representative assay) using 437 FFPE astrocytoma (AC) and oligodendroglioma (OG) clinical tumor samples. The AC and OG IDH-positive clinical samples were obtained from the Servier Pharmaceuticals clinical trial (AG881-C-004) and IDH1 and IDH2-negative clinical samples were sourced from commercial vendors.

The 327 IDH1 and IDH2 mutation-positive samples and 2 IDH1 and IDH2 negative samples were derived from the clinical study (cohort 1). The remaining 108 negative samples were commercially-procured AC and OG – staged matched IDH1 and IDH2 mutation negatives screened by the clinical trial assay (CTA). The concordance between the results from the OncoPrint™ Dx Target Test and the NGS representative assay evaluated for positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) is shown in Table 25, Table 26, and Table 27.

Table 25 IDH1/IDH2 SNV—concordance between the OncoPrint™ Dx Target Test and the NGS representative assay, unadjusted

Agreement measure	Excluding unknowns ^[1]		Including unknowns ^[1]	
	Percent agreement	95% CI	Percent agreement	95% CI
PPA	100% (317/317)	(98.8%, 100%)	100% (317/317)	(98.8%, 100%)
NPA	96.2% (100/104)	(90.5%, 98.5%)	90.1% (100/111)	(83.1%, 94.4%)
OPA	99.0% (417/421)	(97.6%, 99.6%)	97.4% (417/428)	(95.5%, 98.6%)

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

Table 26 IDH1 SNV—concordance between the Oncomine™ Dx Target Test and the NGS representative assay, unadjusted

Agreement measure	Excluding unknowns ^[1]		Including unknowns ^[1]	
	Percent agreement	95% CI	Percent agreement	95% CI
PPA	100% (301/301)	(98.7%, 100%)	100% (301/301)	(98.7%, 100%)
NPA	96.7% (118/122)	(91.9%, 98.7%)	92.9% (118/127)	(87.1%, 96.2%)
OPA	99.1% (419/423)	(97.6%, 99.6%)	99.1% (419/428)	(96.1%, 98.9%)

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

Table 27 IDH2 SNV—concordance between the Oncomine™ Dx Target Test and the NGS representative assay, unadjusted

Agreement measure	Excluding unknowns ^[1]		Including unknowns ^[1]	
	Percent agreement	95% CI	Percent agreement	95% CI
PPA	100% (16/16)	(80.6%, 100%)	100% (16/16)	(80.6%, 100%)
NPA	100% (398/398)	(99.0%, 100%)	96.6% (398/412)	(94.4%, 98.0%)
OPA	100% (414/414)	(99.1%, 100%)	96.7% (414/428)	(94.6%, 98.0%)

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

The analytical concordance between Oncomine™ Dx Target Test and NGS representative assay for IDH1/IDH2 mutation was adjusted to account for patient selection in which 327 Oncomine™ Dx Target Test+ samples, 69 Oncomine™ Dx Target Test– samples, 4 Oncomine™ Dx Target Test IDH unknown samples, and 37 reference NGS representative assay samples were evaluated in the accuracy study. The updated concordance is shown in Table 28.

Table 28 Adjusted agreements between Oncomine™ Dx Target Test and the NGS representative assay for IDH1 and IDH2

Agreement measure	Percent agreement	
	Excluding unknowns ^[1]	Including unknowns ^[1]
PPA ^[2]	100%	100%
NPA ^[2]	93.2%	89.7%

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

^[2] Adjusted for Oncomine™ Dx Target Test and NGS representative assay enrollment in this study.

Astrocytoma and oligodendroglioma—Clinical studies

IDH1/IDH2 clinical concordance and sensitivity analysis

The clinical validation of the Oncomine™ Dx Target Test was performed by using FFPE glioma specimens (AC and OG) with an IDH1 or IDH2 mutation obtained from the Servier pivotal clinical trial (AG881-C-004). Prescreened positive subjects tested with local laboratory tests (LLT) were retested and confirmed for enrollment by the Oncomine™ Dx Target Test. Therefore, the clinical trial enrolled Oncomine™ Dx Target Test and LLT double positive patients. A total of 444 samples were evaluated for this study. Out of these 444 samples, 410 were identified as IDH variant-positive, 3 were identified as IDH variant-negative, and 31 were identified as unknown by the enrolling CTA (Oncomine™ Dx Target Test). Out of the 410 IDH variant-positive subjects, 79 were not enrolled in the AG881-C-004 clinical trial, leaving a total of 331 CTA IDH-variant positive subjects were enrolled in the AG881-C-004 clinical trial. Out of these 331, samples from 4 subjects were not included (randomized) for this study due to insufficient material.

To supplement the IDH variant-negative population, 108 commercially-procured stage-matched variant-negative samples were identified using two different NGS-based screening assays representative of local prescreening tests. A total of 71 of the commercially-procured samples included in the clinical concordance were excluded due to prescreening bias, leaving 39 evaluable samples in the negative cohort.

To evaluate the impact of prescreening due to the usage of LLT during the patient enrollment, the concordance between the Oncomine™ Dx Target Test and the NGS LLT assay for IDH1/IDH2 mutations was determined. The concordance results and agreement estimates are shown in Table 29 and Table 30, respectively.

Table 29 Concordance between Oncomine™ Dx Target Test and NGS LLT assay for IDH1/IDH2 mutation

Oncomine™ Dx Target Test (IDH1/IDH2)	Concordance of Oncomine™ Dx Target Test by NGS LLT assay			
	NGS LLT assay (IDH1/IDH2)			
Frequency	Positive	Negative	Unknown	Total
Positive	306	0	21	327
Negative	1	34	1	36
Unknown	0	3	0	3
Total	307	37	22	366

Table 30 Clinical concordance of the Oncomine™ Dx Target Test in reference to NGS LLT assay for IDH1/IDH2 mutation

Agreement measure	Estimate	95% CI
PPA	99.7%	(98.2%, 99.9%)
NPA	100%	(89.8%, 100.0%)
Adjusted PPV	100%	(98.8%, 100.0%)
Adjusted NPV	100%	(89.8%, 100.0%)

IDH1/IDH2 study—clinical effectiveness

The clinical effectiveness of vorasidenib (VORANIGO®) for treating IDH1/IDH2 variant-positive astrocytoma (AC) or oligodendroglioma (OG) patients was evaluated in the Servier AG881-C-004 clinical trial. A total of 331 subjects out of 410 IDH1/2 variant-positive patients were enrolled in the AG881-C-004 trial based on the central confirmation of mutation status by the clinical trial assay (CTA, Oncomine™ Dx Target Test) and randomized to either placebo (163 subjects) or vorasidenib (168 subjects). Out of the 410 IDH variant-positive patients, 79 were not enrolled in the AG881-C-004 clinical trial.

Progression-free survival (PFS), defined as the time from date of randomization to date of death or documented radiographic progressive disease (PD), was used as the primary endpoint. Hazard ratio (HR) was determined from the Cox regression model stratified by the randomization strata with placebo as the denominator. The Kaplan-Meier survival rate (%) was calculated based on survival distribution function estimates from the product-limit method. Overall, 75/163 patients in the placebo group and 121/168 patients in the vorasidenib treatment group were censored, the majority of which were due to ongoing without an event with placebo (70/75) or vorasidenib (115/121).

Key efficacy results from the clinical study are the following:

- The median progression-free survival time was 27.7 months (95% CI: 17.0 months, NE) for patients treated with vorasidenib, versus 11.1 months (95% CI: 11.0 months, 13.7 months) for the placebo group. (NE: not estimable)
- The hazard ratio for patients treated with vorasidenib was 0.39 (95% CI: 0.27, 0.56). This indicates a 61% decrease in risk of progression or death compared with placebo.
- The Kaplan-Meier survival rate at cutoff readout (24 months) was 50.7% (95% CI: 36.2%, 63.5%) for patients treated with vorasidenib, versus 17.6% (95% CI: 7.1%, 31.9%) for the placebo group.

The results support use of the Oncomine™ Dx Target Test for the identification of IDH1/IDH2 variant-positive AC and OG patients for treatment with vorasidenib.

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) studies

Limit of Blank (LoB)—Study I

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

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

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

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

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

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

Limit of Blank (LoB)—Study II

A second study was performed to test the frequency of false positive calls for EGFR exon 20 insertion variants detected by the Oncomine™ Dx Target Test in wild-type samples. In this study, nucleic acid was extracted from 4 wild-type (WT) FFPE NSCLC clinical samples and carried through from library preparation to sequencing. Samples that are WT at all EGFR locations should produce a "variant not detected" call at each location. By definition (EP17-A2), the 95th percentile of test results on blank samples equals zero.

Operators extracted and sequenced nucleic acid from the 4 WT samples prepared as FFPE sections on slides. The study was conducted using two different lots of the Oncomine™ Dx Target Test Kit. Operators made 18 library replicates for each sample and kit lot, giving a total of 4 samples × 18 replicates × 2 lots = 144 libraries sequenced.

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

Limit of Blank (LoB)—Study III

A further study was performed to test the frequency of false positive calls for EGFR exon 20 insertions detected by the OncoPrint™ Dx Target Test in wild-type samples. In this study, operators used pre extracted DNA from 4 WT FFPE NSCLC clinical samples and carried it through from library preparation to sequencing. Samples that are WT at all EGFR locations should produce a "variant not detected" call at each location. By definition (EP17-A2), the 95th percentile of test results on blank samples equals zero.

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

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

Limit of Blank (LoB)—Study IV

A study was performed to test the frequency of false positive calls for ERBB2/HER2 exon 20 insertions detected by the OncoPrint™ Dx Target Test in wild-type samples. In this study, operators used pre extracted DNA from 4 WT FFPE NSCLC clinical samples and carried it through from library preparation to sequencing. Samples that are WT at all ERBB2/HER2 locations should produce a "variant not detected" call at each location. By definition (EP17-A2), the 95th percentile of test results on blank samples equals zero.

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

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

Limit of Blank (LoB)—Study V

A study was performed to test the frequency of false positive calls for ERBB2/HER2 SNVs detected by the OncoPrint™ Dx Target Test in wild-type samples. In this study, operators used DNA extracted from 4 WT FFPE NSCLC clinical samples and carried it through from library preparation to sequencing. Samples that are WT at all ERBB2/HER2 locations should produce a "variant not detected" call at each location. By definition (EP17-A2), the 95th percentile of test results on blank samples equals zero.

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

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

Tissue input study

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

Thirty resection samples with $\geq 20\%$ tumor content were prepared without macrodissection, 15 resection samples with $< 20\%$ to $\geq 10\%$ tumor cell content were macrodissected, and 15 samples were collected by core needle biopsy (CNB). For the resection samples, 2×5 μ m sections were used per extraction. For CNBs, 9×5 μ m 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/ μ L and an RNA concentration of ≥ 1.43 ng/ μ L. One CNB sample failed the minimum DNA and RNA concentration specifications, with values of 0.52 ng/ μ L and 1.23 ng/ μ L 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 OncoPrint™ Dx Target Test when tissue input requirements are met.

For FNAs, 7×5 μ m 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/ μ L and RNA at a concentration of ≥ 1.43 ng/ μ L.

Guard band testing studies

Guard band testing—initial studies

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

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

Guard band testing—Study II

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

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

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

Guard band testing—Study III

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

Tolerances were tested across 11 test conditions across 3 test levels: Low, Standard Operating Protocol (SOP)/Nominal, and High. A DNA blend of nucleic acids isolated from clinical FFPE samples with the ERBB2/HER2 exon 20 insertion was used in 10 test conditions related to volumes, temperature, and time, while a DNA control was used in a test condition related to DNA control volume. For a test condition to be considered acceptable, 6 library replicates must pass sample validity metrics (Sample AQ20 mean read length and % reads), and the ERBB2/HER2 exon 20 insertion variant within the clinical blended sample must be called present using the OncoPrint™ Dx Target Test. Following initial testing, a narrower acceptable range was established for EtOH (1.0–1.75 µL) and thermal cycling temperature offset (–1.0°C to +0.5°C).

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

Guard band testing—Study IV

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

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

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

FFPE block stability studies

FFPE block stability—Study I

Stability of non-small cell lung cancer (NSCLC) formalin-fixed paraffin-embedded (FFPE) tissue blocks stored at room temperature (15°C to 30°C) was evaluated when tested with the OncoPrint™ Dx Target Test.

One RET fusion isoform (KIF5B-RET) clinical sample block was tested with 6 replicates per time point at baseline, 6 months + 2 weeks, and 12 months + 2 weeks time points.

Linear regression analyses and stability estimates of log-fusion reads across the time points showed that NSCLC FFPE RET fusion-positive tissue blocks are stable for up to 12 months when stored at 15°C to 30°C (Table 31). The overall drift of mean log-fusion reads from baseline of 34% met the study acceptance criterion of drift from baseline of ≤40%.

Table 31 Summary of analysis of fusion reads

AD1460	KIF5B-RET	Baseline (T0)	615.3	2.784
		6 months + 2 weeks	3435	3.365
		12 months + 2 weeks	5417.6	3.723

FFPE block stability—Study II

In a further study, the stability of NSCLC FFPE ROS1 fusion-positive tissue stored at room temperature (15°C to 30°C) was evaluated when tested with the OncoPrint™ Dx Target Test.

One ROS1 fusion isoform (SLC34A2-ROS1.S13R32.COSF1259) clinical sample block was tested with 6 replicates per time point at baseline, 6 months + 2 weeks, and 12 months + 2 weeks time points.

Linear regression analyses and stability estimates of log-fusion reads across the time points showed that NSCLC FFPE ROS1 fusion-positive tissue blocks are stable for up to 12 months when stored at 15°C to 30°C (Table 32). The overall drift of mean log-fusion reads from baseline of –3.1% met the study acceptance criterion of drift from baseline of ≤40%.

Table 32 Summary of analysis of fusion reads

Sample ID	Fusion	Time point	Mean fusion reads (n=6)	Mean log-fusion reads (n=6)
BN1654	SLC34A2- ROS1.S13R32.COSF1259	Baseline (T0)	19,577.3	4.2825
		6 months + 2 weeks	12,726.7	4.0930
		12 months + 2 weeks	14,439.2	4.1512

FFPE slide stability studies

FFPE slide stability—Study I

Stability of FFPE tissue sections mounted on slides (undipped in paraffin) stored at room temperature (15°C to 30°C) was evaluated when tested with the Oncomine™ Dx Target Test.

RNA extracted from tissue sections of a non-small cell lung cancer (NSCLC) clinical sample harboring a prevalent RET fusion isoform (CCDC6-RET) was 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 of log-fusion reads from the sample across the time points showed that FFPE tissue sections mounted on slides are stable for up to 12 months when stored at 15°C to 30°C (Table 33). The overall drift of mean log-fusion reads from baseline of 12% met the study acceptance criterion of drift from baseline $\leq 40\%$.

Table 33 Summary of analysis of fusion reads

Sample ID	Fusion	Time point	Mean fusion reads (n=6)	Mean log-fusion reads (n=6)
AD3217	CCDC6-RET	Baseline (T0)	651.5	2.7806
		3 months + 1 week	1900	3.2575
		6 months + 1 week	1903	3.2408
		9 months + 1 week	1457.5	3.1531
		12 months + 1 week	1446.5	3.1107

FFPE slide stability—Study II

In a further study, the stability of FFPE ROS1 fusion-positive tissue sections mounted on slides (undipped in paraffin) stored at room temperature (15°C to 30°C) was evaluated when tested with the Oncomine™ Dx Target Test.

RNA extracted from tissue sections of a non-small cell lung cancer (NSCLC) clinical sample harboring a prevalent ROS1 fusion isoform (SLC34A2-ROS1.S13R32.COSF1259) was 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 of log-fusion reads from the sample across the time points showed that FFPE tissue sections mounted on slides are stable for up to 12 months when stored at 15°C to 30°C (Table 34). The overall drift of mean log-fusion reads from baseline of -10.2% met the study acceptance criterion of drift from baseline $\leq 40\%$.

Table 34 Summary of analysis of fusion reads

Sample ID	Fusion	Time point	Mean fusion reads (n=6)	Mean log-fusion reads (n=6)
BN1654	SLC34A2-ROS1.S13R32.COSF1259	Baseline (T0)	23,271.7	4.3452
		3 months + 1 week	9,516.2	3.9658
		6 months + 1 week	17,983.8	4.2448
		9 months + 1 week	15,217.7	4.1735
		12 months + 1 week	8,213.3	3.9016

Extracted RNA stability studies

Extracted RNA stability—Study I

A study was performed to test the storage stability of RNA extracted from a RET fusion-positive formalin-fixed, paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) sample. Testing was performed to establish stability when extracted RNA is prepared using the OncoPrint™ Dx Target Test protocol and stored at -90°C to -60°C.

One clinical sample harboring a KIF5B-RET fusion isoform was tested with the OncoPrint™ Dx Target Test. RNA samples were prepared to obtain a ~2.5X limit of detection (LoD) level. Each RNA sample was taken through the OncoPrint™ Dx Target Test workflow at baseline (T0) within a week of extraction, and then at 6 months + 1 week and 12 months + 1 week to show the stability of extracted RNA when stored at -90°C to -60°C. Testing 1 week beyond the required stability time point was allowed to provide sufficient time to take the RNA sample through the entire workflow.

Linear regression analyses and stability estimates of log-fusion reads across the time points showed that RNA extracted from an NSCLC FFPE RET fusion-positive clinical sample is stable for up to 12 months when stored at -90°C to -60°C (Table 35). The overall drift of mean log-fusion reads from baseline of 30% met the study acceptance criterion of drift from baseline of $\leq 40\%$.

Table 35 Summary of analysis of fusion reads

Sample ID	Fusion	Time point	Mean fusion reads (n=6)	Mean log-fusion reads (n=6)
RL1036	KIF5B-RET	Baseline (T0)	675.1	2.8127
		6 months + 2 weeks	733.1	2.8505
		12 months + 2 weeks	1456.3	3.1085

Extracted RNA stability—Study II

A further study was performed to test the storage stability of RNA extracted from a ROS1 fusion-positive formalin-fixed, paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) sample. Testing was performed to establish stability when extracted RNA is prepared using the Oncomine™ Dx Target Test protocol and stored at -90°C to -60°C .

One clinical sample harboring a SLC34A2-ROS1.S13R32.COSF1259 fusion was tested with the Oncomine™ Dx Target Test. RNA samples were prepared to obtain a $\sim 2.5\text{X}$ limit of detection (LoD) level. Each RNA sample was taken through the Oncomine™ Dx Target Test workflow at baseline (T0) within a week of extraction, and then at 6 months + 1 week and 12 months + 1 week to show the stability of extracted RNA when stored at -90°C to -60°C . Testing 1 week beyond the required stability time point was allowed to provide sufficient time to take the RNA sample through the entire workflow.

Linear regression analyses and stability estimates of log-fusion reads across the time points showed that RNA extracted from an NSCLC FFPE ROS1 fusion-positive clinical sample is stable for up to 12 months when stored at -90°C to -60°C (Table 36). The overall drift of mean log-fusion reads from baseline of -15.7% met the study acceptance criterion of drift from baseline of $\leq 40\%$.

Table 36 Summary of analysis of fusion reads

Sample ID	Fusion	Time point	Mean fusion reads (n=6)	Mean log-fusion reads (n=6)
BN1654	SLC34A2-ROS1.S13R32.COSF1259)	Baseline (T0)	23,437.2	4.3486
		6 months + 2 weeks	4,549.7	3.4762
		12 months + 2 weeks	5528.8	3.6675

Stability of assay intermediates studies

Stability of assay intermediates—Study I

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

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

For DNA, allelic frequency and the log-transformed median absolute pairwise difference [$\log(\text{MAPD})$] were used as metrics to evaluate stability. For RNA, the log-transformed fusion reads and the log-transformed normalized read ratio [for example, $\log(\text{fusion reads}/\text{total mapped reads})$] were used as metrics to evaluate stability. In all of the evaluations, the results of the test conditions with the incorporated hold times were compared to the samples tested without the hold times. The study results

support the conclusion that the 30-day library hold and combo hold conditions did not result in a decrease in OncoPrint™ Dx Target Test performance relative to the nominal test condition.

Stability of assay intermediates—Study II

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

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

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

Stability of assay intermediates—Study III

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

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

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

Stability of assay intermediates—Study IV

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

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

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

DNA and RNA input studies

DNA and RNA input—Study I

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

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

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

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

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

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

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

DNA and RNA input—Study II

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

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

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

DNA input—Study III

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

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

DNA input—Study IV

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

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

In silico specificity study

An *in silico* cross-reactivity analysis was performed that evaluated the 832 primers in the Oncomine™ Dx Target Test Kit DNA and RNA panels to determine the specificity of the primers to their targeted sequences. The primers were checked for specificity to the human genome, the human transcriptome, and genomes from representative bacteria, fungi, and viruses frequently found in human tissue and lung specimens.

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

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

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

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

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

Cross-contamination study

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

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

Tissue fixation study

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

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

Sample processing reproducibility studies

Sample processing reproducibility—Study I

The reproducibility and repeatability of variant detection using the OncoPrint™ 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 37. 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 37 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 37 Call rates at positive variant locations (continued)

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

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

Sample processing reproducibility—Study II

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

The call rate, no call rate, positive call rate, negative call rate, and within-run repeatability were computed for each EGFR exon 20 insertion variant. Including no calls and excluding known positive variant locations, the negative call rate at each clinical variant location for all samples was 100%.

The results at positive variant locations are shown in Table 38. Including no calls, all positive call rates from positive variant locations were 100%.

Including or excluding no calls, and combining data across all study samples, the estimate of repeatability was 100% for both EGFR exon 20 insertion variants. The lower limit of the 95% two-sided confidence interval (CI) for repeatability exceeded 66.4% at all variant locations.

Table 38 Call rates at positive variant locations

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

Sample processing reproducibility—Study III

In a further study, the sample processing reproducibility and repeatability of variant detection using the OncoPrint™ Dx Target Test were evaluated with a 12 bp EGFR exon 20 insertion variant-positive NSCLC sample. The sample was extracted in quadruplicate at 3 sites and tested using 3 lots of reagents by 2 operators at each site, for a total of $4 \times 3 \times 3 \times 2 = 72$ replicates for the sample.

A hybrid cell line sample was created for the study by blending wild-type (WT) and 12 bp EGFR exon 20 insertion variant-positive NSCLC-derived cell lines (Table 39) to generate a contrived FFPE sample with an allele frequency of 1–1.5X LoD (7.4%).

Table 39 Cell lines used in the study

GM24385	WT	N/A	N/A	N/A	NSCLC
BID-007	INS	COSM26720	p.A763_Y764insFQEA	64% ^[1]	NSCLC

^[1] Cell line used to generate a hybrid cell line with allele frequency of 7.4%.

The mean and median call rates, and between-run reproducibility were computed. The results at the positive variant location tested are shown in Table 40. Including or excluding no calls, the positive call rate was 100%.

Table 40 Summary of reproducibility results—12 bp EGFR exon 20 insertion

12 bp EGFR exon 20 insertion (positive calls)	1	100%	100%	100%	100%
12 bp EGFR exon 20 insertion (negative calls)	1	0%	0%	0%	0%

The estimate of reproducibility was 100% at the tested EGFR exon 20 insertion variant location. The lower limit of the 95% two-sided confidence interval for repeatability was 95.0%.

The call rate, no call rate, positive call rate, negative call rate, and within-run repeatability were computed. The results at the positive variant location tested are shown in Table 41. Including or excluding no calls, the positive call rate was 100%.

Table 41 Within-run repeatability estimate—12 bp EGFR exon 20 insertion

Sample ID	Variant ID	Total calls	Positive calls	Negative calls	No calls	Within-run repeatability(95% CI)	
						No calls included	No calls excluded
S1	COSM26720	72	72	0	0	100.0% (95.0%–100.0%)	100.0% (95.0%–100.0%)

The estimate of repeatability was 100% at the tested EGFR exon 20 insertion variant location. The lower limit of the 95% two-sided confidence interval for repeatability was 95.0%.

Sample processing reproducibility—Study IV

In a further study, the sample processing reproducibility and repeatability of variant detection using the OncoPrint™ Dx Target Test were evaluated with a 12 bp ERBB2 exon 20 insertion variant-positive NSCLC sample. A sample FFPE block was extracted 12 times per site at 3 sites by 1 operator per site (2 sequencing runs/site), giving a total of 36 data points for the sample. The sample contained a 12 bp ERBB2 exon 20 insertion present at 1.5X–2X the limit of detection (LoD) for the variant (Table 42).

Table 42 Sample used in the study

Nucleic acid type	Variant type	ERBB2 variant ID	Variant amino acid change	LoD	Tissue type
DNA	INS	COSM20959	p.Y772_A775YVMA	1.5X–2X	NSCLC

The mean and median call rates, and between-run reproducibility were computed. The results at the positive variant location tested are shown in Table 43. Including or excluding no calls, the positive call rate was 100%.

Table 43 Summary of reproducibility results—12 bp ERBB2 exon 20 insertion

12 bp ERBB2 exon 20 insertion (positive calls)	1	100%	100%
12 bp ERBB2 exon 20 insertion (negative calls)	1	0%	0%

The estimate of reproducibility was 100% at the tested ERBB2 exon 20 insertion variant location. The lower limit of the 95% two-sided confidence interval for repeatability was 90.0%.

The call rate, no call rate, positive call rate, negative call rate, and within-run repeatability were computed. The results at the positive variant location tested are shown in Table 44. Including or excluding no calls, the positive call rate was 100%.

Table 44 Within-run repeatability estimate—12 bp ERBB2 exon 20 insertion

Sample ID	Variant ID	Total calls ^[1]	Positive calls	Negative calls	No calls	Within-run repeatability (95% CI)	
						No calls included	No calls excluded
S1	COSM20959	35	35	0	0	100.0% (80.5%–100.0%)	100.0% (80.5%–100.0%)

^[1] One sample replicate did not generate a valid result.

The estimate of repeatability was 100% at the tested ERBB2 exon 20 insertion variant location. The lower limit of the 95% two-sided confidence interval for repeatability was 80.5%.

Interfering substances studies

Interfering substances—Study I

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

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

Table 45 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 45) 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 45) on assay performance, and the results were compared to the control (no interferents) condition. For the 6 interferents tested, both the positive concordance and the overall concordance for all samples was 100%. These data support the claim that paraffin, xylene, ethanol, hemoglobin, protease, or wash buffer do not affect assay performance at the level tested in detection of the RET fusions.

Interfering substances—Study IV

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

A total of 3 FFPE samples (2 EGFR exon 20 insertion-positive, and 1 WT) with 2 replicates each were used to evaluate the impact of the listed interferents (Table 45) on assay performance, and the results were compared to the control (no interferents) condition. For the 6 interferents tested, both the positive concordance and the overall concordance for all samples was 100%. These data support the claim that paraffin, xylene, ethanol, hemoglobin, protease, or wash buffer do not affect assay performance at the level tested in detection of EGFR exon 20 insertion-positive variants.

Interfering substances—Study V

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

One ERBB2/HER2 exon 20 insertion-positive and one variant negative (WT) FFPE samples with 2 replicates each were used to evaluate the impact of the listed interferents (Table 45) on assay performance, and the results were compared to the control (no interferents) condition. For the interferents tested, the positive, negative, and the overall concordance for all samples was 100%. These data support the claim that paraffin, xylene, ethanol, protease, or wash buffer do not affect assay performance at the level tested in detection of ERBB2/HER2 exon 20 insertion-positive variants.

Interfering substances—Study VI

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

Three ERBB2/HER2 SNV-positive and one variant negative (WT) FFPE samples with 2 replicates each were used to evaluate the impact of interferents (Table 45) on assay performance, and the results were compared to the control (no interferents) condition. For the interferents tested, the positive, negative, and the overall concordance for all samples was 100%. These data support the claim that paraffin, xylene, ethanol, hemoglobin, protease, or wash buffer do not affect assay performance at the level tested in detection of ERBB2/HER2 SNVs.

Interfering substances—Study VII

An analysis was performed to show that the performance of the Oncomine™ Dx Target Test in detecting EGFR exon 20 insertion variants in FFPE non-small cell lung cancer (NSCLC) clinical samples is not affected by the presence of necrotic tissue.

Two different EGFR exon 20 insertion variant-positive samples, each with a 9 bp insertion, with necrosis percentage between 0–10% were used. DNA was extracted from each sample and blended with DNA extracted from a necrotic wild-type (WT) NSCLC sample to obtain approximately 28% and 43% necrotic tissue content, with a target allelic frequency (AF) of $\leq 9.6\%$. This resulted in a total of 4 samples: 2 unblended samples and 2 blended samples. Each sample and sample blend were tested in triplicate giving a total of 12 data points per variant.

Analysis showed a 100% positive percent agreement (PPA) and negative percent agreement (NPA) in EGFR exon 20 insertion variant calling between necrotic samples (28% and 43% necrotic tissue) and control samples (0–10% necrotic tissue) in the detection of EGFR exon 20 insertion variants using the Oncomine™ Dx Target Test.

These results show that the Oncomine™ Dx Target Test can generate correct EGFR exon 20 insertion variant calling results in the presence of necrotic tissue, at levels up to 43%.

Interfering substances—Study VIII

A study was performed to show that the performance of the Oncomine™ Dx Target Test in detecting EGFR exon 20 insertion variants (3 bp and 12 bp) is not affected by the presence of the potentially interfering substance hemoglobin in clinical FFPE non-small cell lung cancer (NSCLC) samples.

Two NSCLC clinical samples containing EGFR exon 20 insertion variants (3 bp and 12 bp) and 1 wild-type (WT) sample were used in the study. For each sample, 2 test conditions (1 interferent condition and 1 control non-interferent condition) were tested at 3 replicates per condition. For the interferent tested (4 mg/ml hemoglobin), both the positive concordance and the overall concordance for all samples was 100%. These data support the claim that hemoglobin does not affect assay performance at the level tested in detection of EGFR exon 20 insertion-positive variants.

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 allelic frequency (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 AFs of 6–8%.

Limit of Detection (LoD)—Study II

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

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

Limit of Detection (LoD)—Study III

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

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

Limit of Detection (LoD)—Study IV

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

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

Limit of Detection (LoD)—Study V

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

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

Limit of Detection (LoD)—Study VI

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

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

Limit of Detection (LoD)—Study VII

In a further study, the LoD of the OncoPrint™ Dx Target Test was calculated by testing 2 EGFR exon 20 insertion variants (3 bp and 12 bp) in 2 clinical non-small cell lung cancer (NSCLC) FFPE samples using the OncoPrint™ Dx Target Test DNA workflow.

DNA extracted from EGFR exon 20 insertion-positive clinical FFPE specimens was blended with DNA extracted from WT clinical FFPE specimens to obtain 6 dilution levels. For each EGFR exon 20 insertion variant, 10 replicates per dilution level were tested with 2 reagent lots, giving a total of $6 \times 10 \times 2 = 120$ data points per variant.

The LoD of the assay for the 3 bp EGFR exon 20 insertion, determined via hit rate, was 6.48% AF. For the 12 bp EGFR exon 20 insertion, the LoD of the assay was determined to be 5.54% AF.

Limit of Detection (LoD) confirmation study—Study VIII

In a study to confirm limit of detection (LoD) results for 3 EGFR exon 20 insertion variants (3 bp, 9 bp, and 12 bp), 3 clinical non-small cell lung cancer (NSCLC) FFPE samples positive for these variants were tested using the OncoPrint™ Dx Target Test DNA workflow.

DNA extracted from EGFR exon 20 insertion-positive clinical FFPE specimens was blended with DNA extracted from WT clinical FFPE specimens to target 1X–1.5X LoD using the LoD established in earlier studies. For each EGFR exon 20 insertion variant, 4 replicates were tested with 3 reagent lots at 3 testing sites by 2 operators per site, giving a total of $4 \times 3 \times 3 \times 2 = 72$ data points per variant.

Allelic frequency for the 3 insertion variants compiled from run results confirmed the established LoD estimates (Table 46).

Table 46 LoD results summary

Variant ID	Variant information	Established LoD (AF)	Study result (AF)
COSM1238030	EGFR exon 20, 3 bp Insertion (p.D770_N771insY)	6.5%	6.61%
COSM12376	EGFR exon 20, 9 bp Insertion (p.M766_A767insASV)	5.4%	6.82%
COSM26720	EGFR exon 20, 12 bp Insertion (p.A763_Y764insFQEA)	5.54%	9.2%

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 OncoPrint™ 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 OncoPrint™ 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 47 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 OncoPrint™ 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 OncoPrint™ 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 OncoPrint™ Dx Target Test (passing Run, RNA Control, and RNA Sample QC criteria) and the reference method test. These valid samples were used for the tumor content study analysis. The observed tumor content had the following distribution:

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

Table 48 Tumor content range in ROS1-positive samples used in clinical studies

ROS1-positive samples	Tumor-content range
0	<20%
1	20–29%
1	30–39%
0	40–49%
4	50–69%
3	70–90%

The corresponding 95% Clopper Pearson Exact CIs of the PPA, NPA, and OPA overlapped between tumor content levels. This shows that the ROS1 fusion detection performance of the OncoPrint™ 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 OncoPrint™ Dx Target Test.

Tumor content—Study III

In a further study, the tumor cell content in FFPE samples used as input material was calculated for 149 FFPE clinical samples. The tumor cell content of each specimen and region of interest was estimated before the study by an external pathology laboratory. The samples were analyzed with and without macrodissection.

A total of 54 EGFR exon 20 insertion-positive and 95 EGFR exon 20 insertion-negative samples were included in the study analysis. All samples gave valid results for both the OncoPrint™ Dx Target Test (passing Run, DNA Control, and DNA Sample QC criteria) and the reference method test. These valid

samples were used for the tumor content study analysis. The observed tumor content had the following distribution:

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

Table 49 Tumor content range in EGFR exon 20 insertion-positive samples used in clinical studies

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

The corresponding 95% Clopper Pearson Exact CIs of the PPA, NPA, and OPA overlapped between tumor content levels. This shows that the EGFR exon 20 insertion detection performance of the Oncomine™ Dx Target Test was equivalent at all ranges of tumor content level. The tumor content level of the clinical samples had no impact on the performance of the Oncomine™ Dx Target Test.

Tumor content—Study IV

To determine the minimum tumor cell content required in FFPE samples used as input material, 110 pre-characterized clinical samples were analyzed using the Oncomine™ Dx Target Test. The tumor cell content of each specimen and region of interest was estimated before the study by an external pathology lab.

A total of 47 EGFR exon 20 insertion-positive and 63 EGFR exon 20 insertion-negative samples were included in the study analysis. All samples gave valid results for both the Oncomine™ Dx Target Test and the reference method test. These valid samples were used for the tumor content study analysis. The observed tumor content had the following distribution:

- 21 samples with tumor content >0–30%
- 8 samples with tumor content ≥30–40%
- 29 samples with tumor content ≥40–60%
- 52 samples with tumor content ≥60–100%

Table 50 Tumor content range in EGFR exon 20 insertion-positive samples used in clinical studies

14	>0–30%
3	≥30–40%
7	≥40–60%
23	≥60–100%

The corresponding two-sided 95% CIs of the PPA, NPA, and OPA overlapped between tumor content levels. This shows that the EGFR exon 20 insertion detection performance of the Oncomine™ Dx Target Test was similar at all ranges of tumor content level. The tumor content level of the clinical samples had no impact on the performance of the Oncomine™ Dx Target Test, all samples were accurately called 100% of the time.

Tumor content—Study V

In a further study, the tumor cell content in FFPE samples used as input material was calculated for 147 FFPE clinical samples. The tumor cell content of each specimen and region of interest was estimated before the study by an external pathology laboratory. The samples were analyzed with and without macrodissection.

A total of 38 ERBB2/HER2 exon 20 insertion-positive or ERBB2/HER2 SNV-positive samples and 109 ERBB2/HER2 variant-negative samples were included in the study analysis. All samples gave valid results for both the Oncomine™ Dx Target Test (passing Run, DNA Control, and DNA Sample QC criteria) and the reference method test. These valid samples were used for the tumor content study analysis. The observed tumor content had the following distribution:

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

Table 51 Tumor content range in ERBB2/HER2 exon 20 insertion-positive and SNV-positive samples used in clinical studies

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

The corresponding 95% Clopper Pearson Exact CIs of the PPA, NPA, and OPA overlapped between tumor content levels. This shows that the ERBB2/HER2 SNV and exon 20 insertion detection performance of the Oncomine™ Dx Target Test was equivalent at all ranges of tumor content level. The tumor content level of the clinical samples had no impact on the performance of the Oncomine™ Dx Target Test.

Tumor content—Study VI

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

A total of 91 RET fusion-positive and 125 RET fusion-negative samples were included in the study analysis. All samples gave valid results for both the Oncomine™ Dx Target Test (Passing Run, RNA Control, and RNA Sample QC criteria) and the reference method test. These valid samples were used for the tumor content study analysis. The observed tumor content had the following distribution:

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

Table 52 Tumor content range in RET fusion-positive samples used in clinical studies

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

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

Assay reproducibility studies

Assay reproducibility—Study I

The reproducibility and repeatability of the OncoPrint™ 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 53 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 54 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 $\geq 98.8\%$ (95% CI lower limit of $\geq 97.5\%$). 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 OncoPrint™ 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 OncoPrint™ 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 55 Representative variant approach—Study II

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

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

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

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

^[4] Each WT RNA sample was tested twice.

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

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

The reproducibility results are summarized in the following table.

Table 56 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 $\geq 94.4\%$ (95% CI lower limit of $\geq 72.7\%$). 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 57 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 OncoPrint™ 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 OncoPrint™ 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 OncoPrint™ 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 58 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 59 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 OncoPrint™ Dx Target Test.

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

Assay reproducibility—Study IV

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

Table 60 Sample description—Study IV

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

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

Six pre-extracted DNA sample blends (4 insertion positive blends and 2 WT specimens) and placeholder RNA libraries were sequenced at 3 sites by 6 operators. At each site, 2 operators were assigned to 2 instrument systems and were responsible for testing the 6 DNA sample blends. Samples were run in duplicate using 2 different reagent lots at all study sites. The study design resulted in a total of 24 test determinations per sample blend tested.

The reproducibility results are summarized in the following table.

Table 61 Reproducibility results (EGFR exon 20 insertions)—Study IV

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

^[1] Analysis includes invalid results.

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

Assay reproducibility—Study V

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

Table 62 Sample description—Study V

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

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

Six pre-extracted DNA samples (4 insertion positive blends and 2 WT specimens) and placeholder RNA libraries were sequenced at 3 sites by 6 operators. At each site, 2 operators were assigned to 2 instrument systems and were responsible for testing the 6 DNA sample blends. Samples were run in duplicate using 2 different reagent lots at all study sites. The study design resulted in a total of 72 test determinations per sample blend tested.

The reproducibility results are summarized in the following table.

Table 63 Reproducibility results (ERBB2/HER2 exon 20 insertions)—Study V

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

^[1] Analysis includes invalid results.

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

Assay reproducibility—Study VI

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

Table 64 Sample description—Study VI

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

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

Seven pre-extracted DNA sample blends (3 SNV positive specimens and 4 WT specimens) and placeholder RNA libraries were sequenced at 3 sites by 6 operators. At each site, 2 operators were assigned to 2 instrument systems and were responsible for testing the 6 DNA sample blends. Samples were run in duplicate using 2 different reagent lots at all study sites. The study design resulted in a total of 72 test determinations per sample blend tested.

The reproducibility results are summarized in the following table.

Table 65 Reproducibility results (ERBB2/HER2 SNVs)—Study VI

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

^[1] Analysis includes invalid results.

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

Assay reproducibility—Study VII

A supplemental study was performed to evaluate the reproducibility and repeatability of the OncoPrint™ Dx Target Test for detecting RET fusions near the limit of detection (1X LoD) in FFPE RNA extracted from RET fusion-positive non-small cell lung cancer (NSCLC) samples. Reproducibility for 2X LoD and wild-type (WT) samples was established in previous studies. The study was designed to evaluate within-run precision performance (repeatability) and between-run variability across operators, reagent kit lots, and instrument platforms (reproducibility).

Table 66 Sample description—Study VII

Sample ID	RET fusion	Number of clinical specimens used	Number of levels tested	1X and 1.1X LoD targeted (reads)
RL1036	KIF5B-RET.K15R12.COSF1232	1	2	321, 353
BT1001	CCDC6-RET.C1R12.COSF1271	1	2	405, 445
AD1483 (WT for blending)	NA	1	NA	NA

Four RET fusion-positive sample blends (R1-R4) were prepared by blending 2 RET fusion positive specimens with FFPE WT / fusion-negative RNA to obtain levels ~1.1X and ~1X LoD (estimated as mean fusion reads).

The 4 fusion-positive sample blends and placeholder DNA libraries were sequenced at 1 site by 2 operators using 2 instrument systems and 3 reagent kit lots. Each operator performed full runs starting from library preparation through sequencing such that library preparation for the same sample and sequencing run for each lot of reagent was performed on non-consecutive days. The study design resulted in a total of 36 test determinations per sample blend (2 operators × 6 replicates × 3 reagent kit lots).

The reproducibility results are summarized in Table 67.

Table 67 Reproducibility results (RET fusions)—Study VII

Sample	Sample variant ID	Mean fusion reads	Number of valid sample results	Number of positive calls	Number of negative calls	Positive call rate (95% CI)	Negative call rate (95% CI)
R1	KIF5B-RET.K15R12.COSF1232	243.6	36	28	8	77.8% (60.8%, 89.9%)	22.2% (10.1%,39.2%)
R2	KIF5B-RET.K15R12.COSF1232	157.6	36	24	12	66.7% (49.0%, 81.4%)	33.3% (18.6%,51.0%)
R3	CCDC6-RET.C1R12.COSF1271	456.9	36	36	0	100.0% (90.3%,100.0%)	0.0% (0.0%,9.7%)
R4	CCDC6-RET.C1R12.COSF1271	373.1	36	34	2	94.4% (81.3%, 99.3%)	5.6% (0.7%,18.7%)

Estimates of repeatability were 55.6% (95% CI: 30.8%, 78.5%) and 33.3% (95% CI: (13.3%, 59.0%)) for the R1 and R2 KIF5B-RET.K15R12.COSF1232 isoform blends, respectively, and 100% (95% CI: 81.5%, 100.0%) and 88.9% (95% CI: 65.3%, 98.6%) for the R3 and R4 CCDC6-RET.C1R12.COSF1271 isoform blends, respectively.

The study met the acceptance criteria listed in the study protocol for the RET fusion isoform CCDC6-RET.C1R12.COSF1271, but not for KIF5B-RET.K15R12.COSF1232. The study was therefore repeated for this sample (see “Assay reproducibility—Study VIII”).

- The reproducibility of the Oncomine™ Dx Target Test showed 100% positive call rate for RET fusion isoform CCDC6-RET.C1R12.COSF1271 and met the acceptance criteria of ≥93% positive call rate.
- The reproducibility of the Oncomine™ Dx Target Test showed 77.8% positive call rate for RET fusion isoform KIF5B-RET.K15R12.COSF1232. The sample blends prepared were below the 1X LoD level, which resulted in a positive call rate below 93%. The samples yielded the expected results at the observed LoD (0.79X for R1 and 0.60X for R2 samples) based on the LoD probit curve analysis, which indicates that the study showed reproducibility for the KIF5B-RET fusion isoform at the levels tested (below 1X LoD). The study was repeated (“Assay reproducibility—Study VIII”) to show the reproducibility and repeatability of the KIF5B-RET fusion isoform when tested at 1X LoD.

Assay reproducibility—Study VIII

Assay reproducibility—Study VII was repeated to determine the reproducibility and repeatability of the Oncomine™ Dx Target Test for detecting the KIF5B-RET fusion at the limit of detection (1X LoD). The study had used sample blends for the KIF5B-RET fusion at a level below 1X LoD, resulting in a positive call rate that failed the acceptance criterion cutoff of ≥93%. The study was repeated to evaluate within-run precision performance (repeatability) and between-run variability across operators, reagent kit lots, and instrument platforms (reproducibility) using 2 KIF5B-RET fusion blends that targeted a slightly higher fusion read level at 1X to 1.5X LoD.

Two RET fusion positive sample blends (R1 and R2) were created by blending FFPE KIF5B-RET (AV-04-056) fusion-positive specimen RNA with FFPE wild type (WT) fusion-negative RNA (BON1093) to obtain levels ~1.0X to ~1.5X LoD, estimated as the mean fusion reads (Table 68). The KIF5B-RET fusion (KIF5B-RET.K15R12.COSF1232) LoD for ODxTT was previously established as 321 fusion reads. The

R1 blend was titrated to the lower half of the targeted range of ~1.0x to~1.5x LoD, whereas R2 blend was titrated to upper half of the targeted ~1.0x to~1.5x LoD range.

Table 68 Sample blend description—Study VIII

AV-04-056 and BON1093	R1	KIF5B-RET.K15R12.COSF1232	1.0X to 1.5X LoD	353
	R2	KIF5B-RET.K15R12.COSF1232	1.0X to 1.5X LoD	321
BON1093	R3	WT	NA	NA

Testing was performed at 1 site by 2 operators using 2 instrument systems and 3 reagent kit lots. Each operator performed full runs starting from library preparation through sequencing such that library preparation for the same sample and sequencing run for each lot of reagent was performed on non-consecutive days. The study design resulted in a total of 36 test determinations per sample blend (2 operators × 6 replicates × 3 reagent kit lots). The study acceptance criteria were met with the completion of testing of sample blend R2. Therefore, sample blend R1 was not tested.

The reproducibility results are summarized in Table 69.

Table 69 Reproducibility results (RET fusions)—Study VIII

Sample	Sample variant ID	Mean fusion reads	Number of valid sample results	Number of positive calls	Number of negative calls	Positive call rate	Negative call rate
R2	KIF5B-RET.C1R12.COSF1232	235	36	34	2	94.4%	5.6%

The estimate of repeatability was 91.67% for the R2 KIF5B-RET.K15R12.COSF1232 blend.

The average positive percent agreement (PPA) was determined for the RET fusion-positive R2 sample, and the negative percent agreement (NPA) of the WT R3 sample are listed in Table 70.

Table 70 Average percent agreement—Study VIII

Sample	RET variant ID	Variant type	Percent agreement	95% CI
R2	KIF5B-RET.C1R12.COSF1232	Fusion	PPA = 94.1%	80.32%–99.28%
R3	WT	WT	NPA = 100%	90.26%–100.0%

The study met the acceptance criteria listed in the study protocol for the RET fusion isoform KIF5B-RET.K15R12.COSF1232.

Assay reproducibility—Study IX

In a study to confirm and extend previous assay reproducibility and repeatability results for 3 EGFR exon 20 insertion variants (3 bp, 9 bp, and 12 bp), 3 clinical non-small cell lung cancer (NSCLC) FFPE samples positive for these variants were tested using the OncoPrint™ Dx Target Test DNA workflow.

DNA extracted from EGFR exon 20 insertion-positive clinical FFPE specimens was blended with DNA extracted from WT clinical FFPE specimens to target 1X–1.5X LoD using the LoD established in earlier studies. For each EGFR exon 20 insertion variant, 4 replicates were tested with 3 reagent lots at 3 testing sites by 2 operators per site, giving a total of $4 \times 3 \times 3 \times 2 = 72$ data points per variant.

Reproducibility results (between-run variability) are summarized in Table 71.

Table 71 Reproducibility results

Description	Variants evaluated across the samples	Call rate including no calls		Call rate excluding no calls	
		Mean	Median	Mean	Median
EGFR exon 20 insertion (positive calls)	3	99.5%	100%	100%	100%

Repeatability results (within-run variability) are summarized in Table 72. For the 3 bp and 9 bp variants, there were 72 positive calls out of 72 total calls per variant. For the 12 bp variant, there were 71 positive calls out of 72 total calls and 1 no call.

Table 72 Repeatability results

Blend ID	Variant ID	Amino acid change (Insertion size)	Total calls	Positive calls	Negative calls	No calls	Within-run repeatability (95% CI)	
							Including no calls	Excluding no calls
D1	COSM1238030	p.Asp770_Asn771 insTyr (3 bp)	72	72	0	0	100% (95.0%–100.0%)	100% (95.0%–100.0%)
D2	COSM12376	p.Met766_Ala767 insAlaSerVal (9 bp)	72	72	0	0	100% (95.0%–100.0%)	100% (95.0%–100.0%)
D3	COSM26720	p.Ala763_Tyr764 insPheGlnGluAla (12 bp)	72	71	0	1	98.6% (92.5%–100.0%)	100% (94.9%–100.0%)

The study met the acceptance criteria set by the study protocol demonstrating $\geq 95\%$ positive call rate for the 3 EGFR exon 20 insertion variants tested using 3 lots of reagents.

Panel accuracy study

To evaluate the ability of the OncoPrint™ Dx Target Test DNA and RNA panels to identify somatic variants in human specimens, 290 FFPE tumor samples were analyzed using the OncoPrint™ 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
- A RET FISH reference test, to detect RET fusions

Variants detected by the OncoPrint™ Dx Target Test that were not covered by the reference methods were not included in the PPA/NPA concordance calculation. Variants detected by the OncoPrint™ 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 73 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 74 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 75 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 76 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 OncoPrint™ test. The concordances by variant and overall concordance are shown in the following tables:

Table 77 Exon 19 deletion—Concordance

Agreement measure	Excluding no calls		Including no calls	
	Percent agreement	95% CI	Percent agreement	95% CI
PPA	97.6% (41/42)	(87.43%, 99.94%)	74.5% (41/55)	(61.00%, 85.33%)
NPA	99.3% (147/148)	(96.29%, 99.98%)	94.2% (147/156)	(89.33%, 97.33%)
OPA	99.0% (188/190) ^[1]	(96.25%, 99.87%)	89.1% (188/211)	(84.09%, 92.96%)

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

Table 78 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 79 Overall concordance

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

EGFR exon 20 insertions clinical studies

EGFR exon 20 insertions concordance evaluation—Study I

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

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

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

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

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

Table 80 EGFR exon 20 insertions—Concordance

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

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

EGFR exon 20 insertions concordance evaluation—Study II

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

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

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

In all, 116 samples were used to evaluate concordance between the Oncomine™ Dx Target Test as an investigational method and the reference NGS assay. A total of 83 samples were excluded due to insufficient material, invalid results, or no calls by both assays.

The PPA was defined as the proportion of EGFR exon 20 insertion-positive specimens as called by the reference NGS assay that were also EGFR exon 20 insertion-positive as called by the Oncomine™ Dx Target Test, and the NPA was defined as the proportion of EGFR exon 20 insertion-negative specimens as called by the reference NGS assay that were also EGFR exon 20 insertion-negative as called by the Oncomine™ Dx Target Test. The concordances by variant and overall concordance are shown in the following table:

Table 81 EGFR exon 20 insertions—Concordance

Agreement measure	Excluding unknowns ^[1]		Including unknowns ^[1]	
	Percent agreement	95% CI	Percent agreement	95% CI
PPA	100% (46/46)	(92.3%, 100%)	97.9% (46/47)	(88.7%, 99.9%)
NPA	100% (63/63)	(94.3%, 100%)	91.3% (63/69)	(82.0%, 96.7%)
OPA	100% (109/109)	(97.6%, 100%)	94.0% (109/116)	(88.0%, 97.5%)

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

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

In the efficacy population, the ORR for the NSCLC patients with prior platinum treatment was 47.4% (95% CI: 31.0, 64.2).

The Oncomine™ Dx Target Test clinical bridging study included 46 (57%) of the 81 subjects from primary efficacy population. Because 43% of the efficacy population could not be evaluated in the clinical bridging study, additional clinical data were provided from the CHRYSALIS clinical study (non-efficacy population) to mitigate the absence, and to demonstrate the clinical effectiveness of the Oncomine™ Dx Target Test. The study included an additional cohort of 23 samples that tested positive for EGFR exon 20 insertions, from which 5 samples were excluded because of insufficient material or invalid results. Of the 18 patients, 15 samples were positive and 3 samples were EGFR exon 20 insertion-negative. The observed ORR for the additional patients supported the efficacy conclusions from the amivantamab primary efficacy population.

Refer to the [Drugs@FDA database](#) for the most recent therapeutic product labeling.

ERBB2/HER2 clinical studies

ERBB2/HER2 concordance evaluation—Study I

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

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

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

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

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

Table 82 ERBB2/HER2 SNV and exon 20 insertions—concordance

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

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

ERBB2/HER2 clinical effectiveness—Study I

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

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

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

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

Refer to the [Drugs@FDA database](#) for the most recent therapeutic product labeling.

RET clinical studies

RET concordance evaluation—Study I

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

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

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

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

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

The PPA was defined as the proportion of RET fusion-positive specimens as called by the Archer assay that were also RET fusion-positive as called by the Oncomine™ Dx Target Test, and the NPA was defined as the proportion of RET fusion-negative specimens as called by the Archer assay that were also RET fusion-negative as called by the Oncomine™ Dx Target Test. Concordance between the Oncomine™ Dx Target Test and the Archer assay is shown in Table 83.

Table 83 Concordance between the Archer assay and the Oncomine™ Dx Target Test

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

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

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

RET clinical effectiveness—Study I

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

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).

Refer to the [Drugs@FDA database](#) for the most recent therapeutic product labeling.

RET concordance evaluation—Study II

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

Of the 203 RET fusion-positive samples, 161 were positive by the Oncomine™ Dx Target Test, 30 samples were negative, and 12 samples yielded an invalid result. For the reference assay, 92 samples were positive, 12 samples were negative, and 19 samples yielded an invalid result. Eighty samples were not part of the analytical accuracy sample set and/or were not tested by the reference method.

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

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

The PPA was defined as the proportion of RET fusion-positive specimens as called by the reference assay that were also RET-positive as called by the Oncomine™ Dx Target Test, and the NPA was defined as the proportion of RET fusion-negative specimens as called by the reference assay that were also RET fusion-negative as called by the Oncomine™ Dx Target Test. Concordance between the Oncomine™ Dx Target Test and the reference assay is shown in Table 84.

Table 84 Concordance between the Oncomine™ Dx Target Test and the reference assay

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

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

RET clinical effectiveness—Study II

The clinical effectiveness of the Oncomine™ Dx Target Test was evaluated by measuring the overall response rate (ORR) for patients with NSCLC that tested positive for a RET fusion by both LLTs and the Oncomine™ Dx Target Test. The ORR was calculated for patients selected for treatment with the RET inhibitor selpercatinib, with prior platinum treatment, and with no prior systemic treatment. In total, 77 Oncomine™ Dx Target Test RET fusion-positive patients from the LIBRETTO-001 clinical study (58 from the prior platinum treatment cohort, 19 from a treatment-naive cohort) were included in the evaluation. The ORR for patients testing positive for RET fusions with the Oncomine™ Dx Target Test was compared with the ORR for patients in the full drug efficacy population (LLT-positive; n = 144).

- ORR for the Oncomine™ Dx Target Test RET fusion-positive NSCLC patients with prior platinum treatment (n = 58) was 67.2% (95% CI: 53.7%, 79.0%) with 39 patients having achieved best confirmed response of complete response (CR) or partial response (PR).
- ORR for the Oncomine™ Dx Target Test RET fusion-positive NSCLC patients in the treatment-naive cohort (n = 19) was 78.9% (95% CI: 54.4%, 93.95%) with 15 patients having achieved best confirmed response of CR or PR.

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

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 85 Concordance between the ROS1 FISH assay and the OncoPrint™ 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 OncoPrint™ 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 OncoPrint™ 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 OncoPrint™ 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 86 Overall concordance between the ROS1 FISH assay replicates and the updated OncoPrint™ 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 OncoPrint™ 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 87 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.

Anaplastic thyroid cancer (ATC)—Analytical studies

Guard band testing

Guard band testing was performed to define Oncomine™ Dx Target Test tolerances for 3 critical conditions of the proteinase K digestion step of the workflow, using three BRAF V600E positive ATC samples and two BRAF V600E variant-negative samples.

Tolerances were tested across 3 test conditions across 3 test levels: Low, Standard Operating Protocol (SOP)/Control, and High. One BRAF V600E variant-positive and one variant-negative FFPE ATC sample were used for each test condition: proteinase K enzyme volume, incubation temperature, and incubation time. For a test condition to be considered acceptable, the average DNA and RNA concentrations observed at high and low levels were (\pm) 50% from the DNA and RNA concentrations observed for the control (SOP) level. All DNA sample library replicates passed QC and met the study acceptance criteria for BRAF V600E variant calling (100% overall call rate). In addition, all DNA sample library replicates for each ATC BRAF V600E positive sample produced similar mean AF values across all condition levels tested using the Oncomine™ Dx Target Test.

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

Interfering substances study

A retrospective analysis was performed to demonstrate that the performance of the Oncomine™ Dx Target Test in detecting BRAF V600E mutations is not affected by the presence of highly necrotic tissue (defined as having \geq 10% necrotic tissue present).

A statistical analysis showed that there was 100% agreement for positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) between the Oncomine™ Dx Target Test and the clinical trial PCR assay for the detection of BRAF V600E mutations in highly necrotic FFPE thyroid cancer tissue samples.

These results demonstrate that the Oncomine™ Dx Target Test is capable of generating the correct results in the presence of highly necrotic tissue, at levels up to 60%.

Limit of Detection (LoD) confirmation study

The LoD was evaluated for the BRAF V600E variant that is detected by the Oncomine™ Dx Target Test in clinical samples. DNA from a BRAF V600E variant-positive thyroid cancer sample was blended with DNA from a BRAF V600E variant-negative sample and used as input DNA for the test. Twenty-four (24) data points were generated by testing 2 reagent lots and 12 replicates (two operators performed 6 runs each). The results confirmed that the LoD of the Oncomine™ Dx Target Test for BRAF V600E mutation in thyroid cancer FFPE tissue was at the LoD of 6.4% allele frequency established for detection of BRAF V600E mutation in NSCLC tissues.

Table 88 LoD confirmation study results

Operator	Lot # Tested	# of Positive/ # of Tested	Point Estimates %	Upper Bound %
1	1	6/6	100%	100%
	2	6/6	100%	100%
2	1	6/6	100%	100%
	2	6/6	100%	100%
Total		24/24	100%	100%

Limit of Detection (LoD) confirmation study supplement

The LoD was evaluated for the BRAF V600E variant that is detected by the OncoPrint™ Dx Target Test in ATC clinical samples. Three DNA sample blends (S1, S2, S3) near the previously established LoD of 6.4% allelic frequency (AF) were prepared from DNA from a BRAF V600E variant-positive ATC sample and a BRAF V600E variant-negative ATC sample, and used as input DNA for the test. A total of 60 replicates, 20 replicates of each DNA sample blend were tested by 2 operators across 2 instrument systems and 2 reagent lots (10 replicates per blend for each lot) to confirm the LoD. LoD was determined by the lowest mean AF among the 3 DNA sample blends that produced a BRAF V600E mutation detection rate of at least 95% across both reagent lots combined. The results confirmed that the LoD of the OncoPrint™ Dx Target Test for BRAF V600E mutation in ATC FFPE tissue was 6.4% as previously established for detection of BRAF V600E mutation in NSCLC tissues.

Table 89 Anaplastic thyroid cancer LoD confirmation study results

Lot # Tested	DNA Blend	# of Positive/ # of Tested	Positive Hit Rate	Mean AF
L1	S1	10/10	100%	6.27%
	S2	10/10	100%	6.93%
	S3	8/10	80%	5.54%
L2	S1	10/10	100%	6.48%
	S2	10/10	100%	6.27%
	S3	10/10	100%	5.51%
L1 + L2 Combined	S1	20/20	100%	6.38%
	S2	20/20	100%	6.60%
	S3	18/20	90%	5.52%

Assay precision study

The reproducibility and repeatability of the OncoPrint™ Dx Target Test was evaluated for the BRAF V600E variant in two variant-positive and one variant-negative (wild-type) thyroid cancer DNA samples.

The study was designed to evaluate the point estimate of average positive agreement (APA) and the point estimate of average negative agreement (ANA) for within-run precision performance (repeatability) and variability across operators, and instrument platforms (reproducibility).

Three thyroid cancer FFPE DNA samples, consisting of two BRAF V600E variant-positive DNA blends with high and low allele frequency and one BRAF V600E variant-negative (wild-type) thyroid cancer FFPE sample were used for the study. Each sample was sequenced in duplicate by 2 operators on 2 Ion PGM™ Dx Instrument Systems with 2 lots of reagents over a test period of more than 20 operational days, generating a total of 72 data points.

The assay precision was successfully assessed with 72 valid results from 72 replicates tested. For assay repeatability, the point estimate of the average positive agreement (APA) for each positive sample and the average negative agreement (ANA) for each negative sample, assessed for the BRAF V600E mutation status between replicates within a run were 100%. For total precision (within-laboratory precision), the point estimate of the APA for each positive sample and the ANA for each negative sample assessed for the BRAF V600E mutation status between replicates within laboratory was 100%. For operator-to-operator, instrument-to-instrument, and reagent lot-to-lot precision, the point estimate of the APA for each positive sample and the ANA for each negative sample assessed for the BRAF V600E mutation status were all 100%. The point estimate of the APA between two instruments for each positive sample and the ANA for each negative sample on BRAF V600E mutation status were both 100% and the OPA was 100% for all samples. The two-sided bootstrap confidence intervals for APA, ANA, and OPA were not calculated and reported due to the 100% agreement between replicates within each sample. Results showing precision/reproducibility by operator, instrument, and reagent lot are summarized in Table 90.

Table 90 Results for the assay precision study (operator-to-operator, instrument-to-instrument, and lot-to-lot)

BRAF V600E mutation status	Sample	Operator, instrument, or lot	# of positives/total (call rate)	# of negatives/total (call rate)
BRAF V600E-positive	S2	1	12/12 (100%)	0/12 (0%)
		2	12/12(100%)	0/12 (0%)
		Subtotal	24/24 (100%)	0/24 (0%)
BRAF V600E-positive	S3	1	12/12 (100%)	0/12 (0%)
		2	12/12 (100%)	0/12 (0%)
		Subtotal	24/24 (100%)	0/24 (0%)
Wild-type	S1	1	0/12 (0%)	12/12 (100%)
		2	0/12 (0%)	12/12 (100%)
		Subtotal	0/24 (0%)	24/24 (100%)

Anaplastic thyroid cancer (ATC)—Clinical studies

BRAF clinical study

BRAF study—concordance evaluation

Concordance of the Oncomine™ Dx Target Test with a clinical trial PCR assay in the detection of BRAF V600E variants in ATC samples was evaluated with patient samples from the ATC clinical trial (BRF117019/DRB436X2201) and commercially sourced thyroid cancer tissue samples. The commercially sourced samples included several thyroid cancer histologies other than ATC, such as follicular thyroid cancer (FTC), papillary thyroid cancer (PTC), and medullary thyroid cancer (MTC). A total of 199 samples (32 ATC trial samples and 167 commercial samples) were tested in the evaluation.

Of the 206 patients enrolled in the ATC clinical trial, 36 patients with BRAF V600E-positive status were enrolled in the ATC cohort either by a local test result (N=30) or by the clinical trial PCR assay (N=6). All 30 patient samples enrolled by a local test result were retrospectively tested with the PCR assay, however 1 sample had insufficient material for further PCR assay testing. Of the resulting 35 ATC samples, 3 samples had insufficient material for testing with the Oncomine™ Dx Target Test. Thirty-two samples with sufficient material were tested by the Oncomine™ Dx Target Test, and yielded 29 BRAF V600E-positive results, 1 BRAF V600E-negative result, and 2 invalid results. With the PCR assay, the 35 ATC samples yielded 33 BRAF V600E-positive results and 2 BRAF V600E-negative results.

Of the 211 commercially sourced samples, 167 samples had valid PCR assay results (94 BRAF V600E-positive results and 73 BRAF V600E-negative results) and were included in Oncomine™ Dx Target Test testing. With the Oncomine™ Dx Target Test, these samples yielded 68 BRAF V600E-positive results, 57 BRAF V600E-negative results, and 42 invalid results.

The positive percent agreement (PPA) was defined as the proportion of BRAF-positive samples called by the clinical trial 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 PPA, NPA, and overall percent agreement (OPA) results are shown in Table 91.

Table 91 Concordance between the clinical trial PCR assay and the Oncomine™ Dx Target Test

Agreement measure	Excluding invalid results and No Calls		Including invalid results and No Calls	
	Percent agreement	95% CI ^[1]	Percent agreement	95% CI ^[1]
PPA	99.0% (97/98)	(94.4%, 99.8%)	77.6% (97/125)	(69.5%, 84.0%)
NPA	100% (57/57)	(93.7%, 100.0%)	77.0% (57/74)	(66.3%, 85.1%)
OPA	99.4% (154/155)	(96.4%, 99.9%)	77.4% (154/199)	(71.1%, 82.6%)

^[1] The 95% CI was calculated using the Wilson Score method.

BRAF study—clinical effectiveness

The drug effectiveness in the patient population testing positive for BRAF V600E variants with the Oncomine™ Dx Target Test was estimated using samples from ATC patients enrolled in the ROAR study (BRF11079/DRB436X220). The endpoints for the efficacy analysis are the ORR of TAFINLAR® (dabrafenib) and MEKINIST® (trametinib) anti-cancer combination therapy by investigator assessment, and independent radiology review, where ORR is defined as the percentage of subjects with the best overall response (BOR) of confirmed complete response (CR) or partial response (PR) based on Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1.

The primary analysis set included patients in the full analysis set with valid (positive or negative) clinical trial assay (CTA) test results (N = 35 from ROAR, and N = 167 from commercial samples).

Based on investigator assessment, the ORR was 65.5% (95% CI: 45.7%, 82.1%) in CTA+, ODxT Test+ patients (19/29) and 60.6% (95% CI: 42.1%, 77.7%) in CTA+, ODxT Test– patients (20/33).

Based on independent radiology review, the ORR was 58.6% (95% CI: 38.9%, 76.5%) in CTA+, ODxT Test+ patients (17/29) and 57.6% (95% CI: 39.2%, 74.5%) in CTA+, ODxT Test– patients (19/33).

To address the potential bias of bridging the CTA and the Oncomine™ Dx Target Test due to prescreening patient samples with a laboratory developed test (LDT), an additional analysis was performed to evaluate the concordance between the LDT and CTA using the samples from locally enrolled ATC patients (N = 30) and a set of commercially sourced LDT-negative samples (N = 58). The high concordance between LDT+ and CTA+ patients in this analysis showed the impact of prescreening with the LDT was negligible.

Complete Case Analysis: The ORR in the ODxT Test+ population was then estimated as the ORR in CTA+, ODxT Test+ patients, which was 65.5% (95% CI: 45.7%, 82.1%) based on investigator assessment, and 58.6% (95% CI: 38.9%, 76.5%) based on independent radiology review.

Sensitivity Analysis: To evaluate the robustness of the clinical efficacy, estimate against the missing ODxT Test results including 27 CTA-positives tested by ODxT Test with invalid results and 17 CTA-negatives tested by ODxT Test with invalid results, the sensitivity analysis employed the multiple imputation method using fully conditional specification method to impute the missing ODxT Test results. In the sensitivity analysis for the efficacy in the ODxT Test-positive population based on investigator assessment and independent radiology review, the ORR estimates ranged from 58.6% to 61.9% for investigator assessment, and 54.8% to 57.9% for independent radiology review (data not shown). For comparison, the ORR in the CTA+ patients enrolled in ATC cohort of the ROAR trial was 60.6% (95% CI: 42.1%, 77.7%) based on investigator assessment, and 57.6% (95% CI: 39.2%, 74.5%) based on independent radiology review.

Refer to the [Drugs@FDA database](#) for the most recent therapeutic product labeling.

Medullary thyroid cancer and thyroid cancer—Analytical studies

Limit of Blank (LoB) study

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

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

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

Tissue input study

A study was performed to determine if thyroid cancer samples extracted using the Ion Torrent Dx Total Nucleic Acid Isolation Kit yield DNA and RNA at the concentrations that are required by the Oncomine™ Dx Target Test when tissue input requirements are met. The test requires DNA at a concentration of ≥ 0.83 ng/ μ L and RNA at a concentration of ≥ 1.43 ng/ μ L. A total of 25 FFPE thyroid samples were analyzed, including 15 resection, 5 core needle biopsy (CNB), and 5 fine needle aspirate (FNA) samples.

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

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

Guard band testing study

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

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

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

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

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

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

RNA input study

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

Sample RNA and DNA libraries were prepared with input ratios corresponding to the range of levels shown in Table 92. Six replicates of each input ratio were run, and the RET fusion reads were tabulated. The results showed a 100% call rate for RET fusions across the RNA:DNA input ratios tested, and further showed that mapped reads and log of fusion reads were not impacted by varying the input ratio from the standard RNA:DNA ratio of 10 ng:10 ng.

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

Table 92 RNA:DNA input ratio

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

FFPE tissue slide stability study

A study was performed to evaluate the stability of FFPE slide sections as a tissue source for the detection of RET variants in medullary thyroid cancer (MTC) and thyroid cancer (TC) with the OncoPrint™ Dx Target Test. FFPE sections from 4 clinical samples, each containing a unique RET DNA variant or RNA fusion, were tested with the OncoPrint™ Dx Target Test workflow at baseline (T0) and 4 time points after slide preparation: 3 months, 6 months, 9 months, and 12 months. The RET variant samples tested in the study, which represent prevalent DNA variants and RNA fusions found in MTC and TC, are listed in Table 93. Slide-mounted 5-µM tissue sections (non-paraffin dipped) from each sample were prepared from FFPE tissue blocks at the start of the study and stored at room temperature (15°C to 30°C) during the study.

Table 93 Samples tested

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

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

The mean allelic frequency (AF) of the SNV and deletion in the DNA samples, and the mean log₁₀-fusion reads in the RNA samples were determined by results analysis with Torrent Suite™ Dx Software. Study results are shown in Table 94 and Table 95.

Table 94 DNA variants

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

Table 95 RNA fusions

RET variant ID	Mean log ₁₀ fusion reads					
	T0 baseline	3 mo.	6 mo.	9 mo.	12 mo.	Lower threshold (0.6 × T0 baseline)
CCDC6-RET.C1R12.COSF1271	3.298	2.903	2.488	2.873	2.865	1.979
NCOA4-RET.N7R12	3.664	3.348	3.149	3.258	3.309	2.198

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

Extracted RNA stability study

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

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

Two aliquots of each RNA sample blend were tested at each timepoint to evaluate the effect of freeze-thaw cycles on the ability to obtain valid sequencing results using the Oncomine™ Dx Target Test. One aliquot (Aliquot R1) was tested after going through a single freeze-thaw cycle where the frozen sample was allowed to thaw at room temperature until no ice crystals were present, then kept on ice until use. A second aliquot (Aliquot R2) was tested after going through 3 freeze-thaw cycles. In this case, a freeze-thaw cycle was defined as the frozen sample being thawed at room temperature until no ice crystals were present, kept on ice for one hour, then returned to the freezer for a minimum of 24 hours before beginning another freeze-thaw cycle. At each time point, 3 replicates of Aliquot R1 and 3 replicates of Aliquot R2 were tested in a single run for each RNA sample blend (2 total runs per time point). Sequencing results from each timepoint were compared to baseline results for each RNA sample blend.

As shown in Table 96, the percent positive calls are 100% across all timepoints for both RNA sample blends, and no statistically significant difference in percent fusion reads was observed in any resulting RET RNA fusion data.

Table 96 Extracted RNA stability

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

Sample processing reproducibility

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

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

The results at positive variant locations are shown in Table 97. Including no calls, all positive call rates from positive variant locations were 100%.

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

Table 97 Call rates at positive variant locations

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

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

Interfering substances study

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

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

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

Limit of Detection (LoD) study

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

Table 98 LoD study variants

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

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

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

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

Tumor content study

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

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

All samples gave valid results for both the Oncomine™ Dx Target Test (Passing Run, Control, and Sample QC criteria) and the reference method test. These valid samples were used for the tumor content study analysis. The observed tumor content had the following distribution:

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

Table 99 Tumor content range in RET mutation-positive samples used in clinical studies

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

Table 100 Tumor content range in RET fusion-positive samples used in clinical studies

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

The PPA, NPA, and OPA agreement between the Oncomine™ Dx Target Test and the reference method test was 100% across all tumor content ranges. The corresponding 95% Clopper Pearson Exact CIs of the PPA, NPA, and OPA overlapped between tumor content levels. This result shows that the RET mutation and RET fusion detection performance of the Oncomine™ Dx Target Test was equivalent at all ranges of tumor content level. The tumor content level of the clinical samples had no impact on the performance of the Oncomine™ Dx Target Test.

Assay reproducibility study

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

Table 101 Sample description

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

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

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

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

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

The reproducibility results are summarized in Table 102 and Table 103.

Table 102 Reproducibility results (RET DNA variants)

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

Table 103 Reproducibility results (RET RNA fusions)

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

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

Medullary thyroid cancer and thyroid cancer—Clinical studies

RET clinical studies

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

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

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

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

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

The PPA was defined as the proportion of RET DNA variant-positive specimens as called by the reference assay that were also RET DNA variant-positive as called by the Oncomine™ Dx Target Test, and the NPA was defined as the proportion of RET DNA variant-negative specimens as called by the reference assay that were also RET DNA variant-negative as called by the Oncomine™ Dx Target Test. Concordance between the Oncomine™ Dx Target Test and the reference assay is shown in Table 104.

Table 104 Concordance between Oncomine™ Dx Target Test and the reference assay—RET DNA variants (MTC)

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

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

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

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

- ORR for the Oncomine™ Dx Target Test RET DNA variant-positive MTC patients with prior cabozantinib and/or vandetanib treatment (n = 38) was 68.4% (95% CI: 51.4%, 82.5%) with 26 patients having achieved best confirmed response of CR or PR.
- ORR for the Oncomine™ Dx Target Test RET DNA variant-positive MTC patients in the treatment-naive cohort (n = 59) was 78.0% (95% CI: 65.3%, 87.7%) with 46 patients having achieved best confirmed response of CR or PR.

For comparison, the results for the primary efficacy endpoint of ORR observed in the corresponding drug efficacy set (LLT RET DNA variant-positive) of the prior treatment cohort (N= 55) was 69.1% (95% CI: 55.2%, 80.9%), with 38 patients having achieved best confirmed response of CR or PR. The ORR observed in the corresponding drug efficacy set (LLT RET DNA variant-positive) for the treatment-naive cohort (n = 87) was 73.6% (95% CI: 63.0%, 82.45%), with 64 patients having achieved best confirmed response of CR or PR.

Refer to the [Drugs@FDA database](#) for the most recent therapeutic product labeling.

RET fusion concordance evaluation—Study I

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

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

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

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

The PPA was defined as the proportion of RET fusion-positive specimens as called by the reference assay that were also RET fusion-positive as called by the Oncomine™ Dx Target Test, and the NPA was defined as the proportion of RET fusion-negative specimens as called by the reference assay that

were also RET fusion-negative as called by the Oncomine™ Dx Target Test. Concordance between the Oncomine™ Dx Target Test and the reference assay is shown in Table 105.

Table 105 Concordance between Oncomine™ Dx Target Test and the reference assay—RET fusions (TC)

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

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

RET fusion clinical effectiveness—Study I

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

- ORR for the Oncomine™ Dx Target Test RET fusion-positive TC patients with prior treatment (n = 13) was 69.2% (95% CI: 38.6%, 90.9%) with 9 patients having achieved best confirmed response of CR or PR.
- ORR for the Oncomine™ Dx Target Test RET fusion-positive TC patients in the treatment-naive cohort (n = 10) was 100.0% (95% CI: 69.15%, 100.0%) with all 10 patients having achieved best confirmed response of CR or PR.

For comparison, the ORR for RET fusion-positive TC patients observed in the corresponding drug efficacy set (LLT RET fusion-positive), in the prior treatment cohort (n = 24), was 75.0% (95% CI: 53.3%, 90.2%) with 18 patients having achieved best confirmed response of CR or PR. The ORR for RET fusion-positive TC patients observed in the corresponding drug efficacy population (LLT RET fusion-positive) for the treatment-naive cohort (n = 12), was 100.0% (95% CI: 73.5%, 100.0%) with 12 patients having achieved best confirmed response of CR or PR.

Refer to the [Drugs@FDA database](#) for the most recent therapeutic product labeling.

RET fusion concordance evaluation—Study II

In a supplemental study, the ability of the OncoPrint™ Dx Target Test to identify RET fusions in FFPE TC specimens was evaluated with additional clinical samples. For this study, a clinical validation of the OncoPrint™ Dx Target Test was performed by retrospectively testing RET fusion-positive TC samples collected from the additional patients enrolled into the LIBRETTO-001 clinical trial along with additional procured RET fusion-negative samples. Test samples from the combined data sets included de-identified samples categorized as RET fusion-positive by local laboratory tests (LLTs) derived from patients enrolled in the LIBRETTO-001 clinical trial, and TC samples obtained from commercial vendors that were selected as RET fusion-negative using a representative next-generation sequencing LLT. The LLTs served as the reference test in the concordance analysis.

In the supplemental study sample set of 65 RET fusion-positive samples, 42 were positive by the OncoPrint™ Dx Target Test, 3 samples were negative, and 20 samples yielded an invalid result, or were not tested due to failed RNA extraction, or failed pathology review.

Of the 152 LLT-negative samples, 133 were negative with the OncoPrint™ Dx Target Test, 17 samples yielded an invalid result, and 2 samples were not tested due to insufficient RNA, or were otherwise excluded.

In all, 217 samples were used to evaluate concordance between the OncoPrint™ Dx Target Test as an investigational method and the reference test (LLT).

The PPA was defined as the proportion of RET fusion-positive specimens as called by the LLT that were also RET fusion-positive as called by the OncoPrint™ Dx Target Test, and the NPA was defined as the proportion of RET fusion-negative specimens as called by the LLT that were also RET fusion-negative as called by the OncoPrint™ Dx Target Test. Concordance between the OncoPrint™ Dx Target Test and the reference assay is shown in Table 106.

Table 106 Concordance between OncoPrint™ Dx Target Test and LLT—RET fusions (TC)

Agreement measure	Excluding unknowns ^[1]		Including unknowns ^[1]	
	Percent agreement	95% CI	Percent agreement	95% CI
PPA	93.33% (42/45)	(81.73%, 98.60%)	64.62% (42/65)	(51.77%, 76.08%)
NPA	100.0% (133/133)	(97.26%, 100.00%)	87.5% (133/152)	(81.17%, 92.30%)
OPA	98.31% (175/178)	(95.15%, 99.65%)	80.65% (175/217)	(74.75%, 85.68%)

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

RET fusion clinical effectiveness—Study II

For the supplemental analysis incorporating the additional patient samples, RET fusion-positive and -negative samples were tested by the Oncomine™ Dx Target Test to evaluate the clinical efficacy of selpercatinib based on Oncomine™ Dx Target Test results. The clinical effectiveness of the Oncomine™ Dx Target Test was evaluated by measuring the overall response rate (ORR), the percentage of patients with either a complete response (CR) or partial response (PR), for patients with thyroid cancer (TC) that tested positive for a RET fusion by both local laboratory tests (LLTs) and the Oncomine™ Dx Target Test. The ORR was calculated for patients selected for treatment with the RET inhibitor selpercatinib, with prior systemic treatment, and with no prior systemic treatment. In total, 42 Oncomine™ Dx Target Test RET fusion-positive patients from the LIBRETTO-001 clinical study (24 from the prior treatment cohort, 18 from the treatment-naive cohort) were included in the evaluation. The ORR for patients testing positive for RET fusions with the Oncomine™ Dx Target Test was compared with the ORR for patients in the full drug efficacy population (LLT-positive; n = 65).

- ORR for the Oncomine™ Dx Target Test RET fusion-positive TC patients with prior treatment (n = 24) was 83.3% (95% CI: 62.6%, 95.3%) with 20 patients having achieved best confirmed response of CR or PR.
- ORR for the Oncomine™ Dx Target Test RET fusion-positive TC patients in the treatment-naive cohort (n = 18) was 94.4% (95% CI: 72.7%, 100.0%) with 17 patients having achieved best confirmed response of CR or PR.

For comparison, the ORR for RET fusion-positive TC patients observed in the corresponding drug efficacy population (LLT RET fusion-positive) for the prior treatment cohort (n = 41), was 85.4% (95% CI: 70.8%, 94.4%) with 35 patients having achieved best confirmed response of CR or PR. The ORR for RET fusion-positive TC patients observed in the corresponding drug efficacy population (LLT RET fusion-positive) for the treatment-naive cohort (n = 24), was 95.8% (95% CI: 78.9%, 99.9%) with 23 patients having achieved best confirmed response of CR or PR.

Refer to the [Drugs@FDA database](#) for the most recent therapeutic product labeling.



Variants detected by the Oncomine™ Dx Target Test

DNA variants detected in non-small cell lung cancer (NSCLC)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
AKT1	3	p.Glu17Lys	c.49G>A	COSM33765
ALK	21	p.Gly1128Ala	c.3383G>C	COSM98475
ALK	22	p.Leu1152Pro	c.3455T>C	COSM1407659
ALK	22	p.Leu1152Arg	c.3455T>G	COSM97185
ALK	22	p.Cys1156Tyr	c.3467G>A	COSM99136 Note: Some "no calls" were observed for this analytical variant due to strand bias with plasmid targets. This does not impact clinical test results.
ALK	22	p.Ile1171Asn	c.3512T>A	COSM28498
ALK	22	p.Ile1171Thr	c.3512T>C	COSM4381100 Note: Some "no calls" were observed for this analytical variant due to strand bias with plasmid targets. This does not impact clinical test results.
ALK	23	p.Phe1174Ile	c.3520T>A	COSM28491
ALK	23	p.Phe1174Leu	c.3520T>C	COSM28057
ALK	23	p.Phe1174Val	c.3520T>G	COSM28054
ALK	23	p.Phe1174Ser	c.3521T>C	COSM53063
ALK	23	p.Phe1174Cys	c.3521T>G	COSM28059
ALK	23	p.Phe1174Leu	c.3522C>A	COSM28055

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
ALK	23	p.Phe1174Leu	c.3522C>G	COSM28061
ALK	23	p.Val1180Leu	c.3538G>C	COSM4381101
ALK	23	p.Leu1196Met	c.3586C>A	COSM99137
ALK	23	p.Leu1196Gln	c.3587T>A	COSM1169447
ALK	23	p.Gly1202Arg	c.3604G>A	COSM144250
ALK	23	p.Ser1206Tyr	c.3617C>A	COSM144251
ALK	24	p.Phe1245Ile	c.3733T>A	COSM28492
ALK	24	p.Phe1245Val	c.3733T>G	COSM28499
ALK	24	p.Phe1245Cys	c.3734T>G	COSM28500
ALK	24	p.Phe1245Leu	c.3735C>A	COSM28493
ALK	24	p.Phe1245Leu	c.3735C>G	COSM28062
ALK	25	p.Arg1275Gln	c.3824G>A	COSM28056
ALK	25	p.Arg1275Leu	c.3824G>T	COSM28060
BRAF	11	p.Gly466Glu	c.1397G>A	COSM453
BRAF	11	p.Gly466Val	c.1397G>T	COSM451
BRAF	11	p.Gly469Arg	c.1405G>A	COSM457
BRAF	11	p.Gly469Ala	c.1406G>C	COSM460
BRAF	11	p.Gly469Val	c.1406G>T	COSM459
BRAF	15	p.Asp594Asn	c.1780G>A	COSM27639
BRAF	15	p.Asp594Gly	c.1781A>G	COSM467
BRAF	15	p.Val600Lys	c.1798_1799delGTinsAA	COSM473
BRAF	15	p.Val600Arg	c.1798_1799delGTinsAG	COSM474
BRAF	15	p.Val600Glu	c.1799_1800delTGinsAA	COSM475
BRAF	15	p.Val600_Lys601delinsGlu	c.1799_1801delTGA	COSM1133
BRAF	15	p.Val600Glu	c.1799T>A	COSM476
BRAF	15	p.Lys601Glu	c.1801A>G	COSM478
CDK4	2	p.Lys22Gln	c.64A>C	OM3153
CDK4	2	p.Lys22Arg	c.65A>G	COSM232013

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
CDK4	2	p.Lys22Met	c.65A>T	COSM3463915
CDK4	2	p.Arg24Ser	c.70C>A	COSM3463914
CDK4	2	p.Arg24Cys	c.70C>T	COSM1677139
CDK4	2	p.Arg24His	c.71G>A	COSM1989836
CDK4	2	p.Arg24Leu	c.71G>T	COSM363684
DDR2	5	p.Arg124Trp	c.370C>T	COSM4024594
DDR2	5	p.Arg124Leu	c.371G>T	COSM400880
EGFR	3	p.Arg108Gly	c.322A>G	COSM1451536
EGFR	3	p.Arg108Lys	c.323G>A	COSM21683
EGFR	7	p.Ala289Thr	c.865G>A	COSM21686
EGFR	7	p.Ala289Asp	c.866C>A	COSM21685
EGFR	7	p.Ala289Val	c.866C>T	COSM21687
EGFR	12	p.Ser492Arg	c.1474A>C	COSM236671
EGFR	12	p.Ser492Arg	c.1476C>A	COSM236670
EGFR	15	p.Gly598Ala	c.1793G>C	COSM3412196
EGFR	15	p.Gly598Val	c.1793G>T	COSM21690
EGFR	18	p.Glu709Lys	c.2125G>A	COSM12988
EGFR	18	p.Glu709Ala	c.2126A>C	COSM13427
EGFR	18	p.Glu709Gly	c.2126A>G	COSM13009
EGFR	18	p.Glu709Val	c.2126A>T	COSM12371
EGFR	18	p.Gly719Ser	c.2155G>A	COSM6252
EGFR	18	p.Gly719Cys	c.2155G>T	COSM6253
EGFR	18	p.Gly719Asp	c.2156G>A	COSM18425
EGFR	18	p.Gly719Ala	c.2156G>C	COSM6239
EGFR	19	p.Lys745_Glu749del	c.2233_2247delAAGGAATTAA GAGAA	COSM26038
EGFR	19	p.Lys745_Ala750delinsThr	c.2234_2248delAGGAATTAAG AGAAG	COSM1190791

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Gene	Exon	Amino acid change	Nucleotide change	Variant ID
EGFR	19	p.Glu746_Glu749del	c.2235_2246delGGAATTAAGA GA	COSM28517
EGFR	19	p.Glu746_Ala750del	c.2235_2249delGGAATTAAGA GAAGC	COSM6223
EGFR	19	p.Glu746_Thr751delinsIle	c.2235_2252delGGAATTAAGA GAAGCAACinsAAT	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_2253delGAATTAAGAG AAGCAACA	COSM12728
EGFR	19	p.Glu746_Ala750del	c.2236_2250delGAATTAAGAG AAGCA	COSM6225
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.Glu746_Ser752delinsVal	c.2237_2255delAATTAAGAGAA GCAACATCinsT	COSM12384
EGFR	19	p.Glu746_Thr751delinsValAla	c.2237_2253delAATTAAGAGAA GCAACAinsTTGCT	COSM12416
EGFR	19	p.Glu746_Ser752delinsAsp	c.2238_2255delATTAAGAGAA GCAACATC	COSM6220
EGFR	19	p.Leu747_Thr751delinsGln	c.2238_2252delATTAAGAGAA GCAACinsGCA	COSM12419
EGFR	19	p.Leu747_Ala750delinsPro	c.2238_2248delATTAAGAGAA GinsGC	COSM12422
EGFR	19	p.Glu746_Arg748del	c.2239_2247delTTAAGAGAA	COSM6218
EGFR	19	p.Leu747_Thr751delinsPro	c.2239_2251delTTAAGAGAAG CAAinsC	COSM12383
EGFR	19	p.Leu747_Ala750delinsPro	c.2239_2248delTTAAGAGAAG insC	COSM12382

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Gene	Exon	Amino acid change	Nucleotide change	Variant ID
EGFR	19	p.Leu747_Ser752del	c.2239_2256delTTAAGAGAAG CAACATCT	COSM6255
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_Thr751del	c.2240_2254delTAAGAGAAGC AACAT	COSM12369
EGFR	19	p.Leu747_Pro753delinsSer	c.2240_2257delTAAGAGAAGC AACATCTC	COSM12370
EGFR	19	p.Leu747_Thr751delinsSer	c.2240_2251delTAAGAGAAGC AA	COSM6210
EGFR	—	NA	NA	COSM26720 ^[1]
EGFR	20	p.Met766_Ala767insAlaSerVal	c.2296_2297insTGGCCAGTG	COSM20884 Note: A false negative call was observed for this variant when tested with plasmid targets for 1 out of 6 of the replicates tested.
EGFR	20	p.Ala767_Ser768insTyrValMet	c.2301_2302insTACGTGATG	COSM1651740
EGFR	20	p.Met766_Ala767insAlaThrLeu	c.2302_2303insCGCTGGCCA	COSM12425
EGFR	20	p.Met766_Ala767insAlaIle	c.2302_2303insTAGCCA	COSM13559
EGFR	20	p.Ser768Ile	c.2303G>T	COSM6241
EGFR	20	p.Ser768_Val769insValAlaAsn	c.2303_2304insTGTGGCCAA	COSM1651741
EGFR	20	p.Ser768_Val769delinsIleLeu	c.2303_2305delGCGinsTCC	COSM6984779
EGFR	20	p.Ser768_Val769delinsIleLeu	c.2303_2305delGCGinsTCT	COSM85750
EGFR	20	p.V769_D770insGG	c.2306_2307insGGGGGG	MATNV07
EGFR	20	p.Ser768_Val769insValCys	c.2307_2308insTGCGTG	COSM12379
EGFR	20	p.Met766_Ala767insAlaSerVal	c.2308_2309insCCAGCGTGG	COSM12376
EGFR	20	p.Ala767_Ser768insSerValGly	c.2308_2309insGCAGCGTGG	COSM18429

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
EGFR	20	p.Ala767_Ser768insSerValGly	c.2308_2309insGGAGCGTGG	COSM1235344
EGFR	20	p.Ser768_Val769insValGlyVal	c.2308_2309insGGGTCGTGG	COSM18430
EGFR	20	p.Val769dup	c.2308_2309insTGG	COSM6506514
EGFR	20	p.Asp770delinsGlyTyr	c.2308_2309insGTT	COSM12427
EGFR	20	p.D770_N771insH	c.2308_2309insACC	OMINDEL1081
EGFR	20	p.D770delinsNNPH	c.2308_2308delGinsAACAAACC CCC	OMINDEL1078
EGFR	20	p.D770_N771insNH	c.2308_2309insACAACC	OMINDEL1084
EGFR	20	p.Asp770delinsGlyThrHis	c.2308_2309insGCACAC	COSM6983510
EGFR	20	p.Val769_Asp770insGluArgGly	c.2309_2310insGCGTGGAGA	COSM1651742
EGFR	20	p.Ala767_Val769dup	c.2309_2310delACinsCCAGCG TGGAT	COSM13558
EGFR	20	p.Asp770_Asn771delinsAlaGlyGly	c.2309_2312delACAAinsCTGG TGG	COSM12737
EGFR	20	p.D770_N771delInsAGH	c.2309_2311delACAinsCTGGC C	MATNV09
EGFR	20	p.Val769_Asp770insAspGly	c.2310_2311insGGGGAC	COSM85795
EGFR	20	p.Val769_Asp770insAspGly	c.2310_2311insGGCGAC	COSM22955
EGFR	20	p.Asp770_Asn771insTyr	c.2310_2311insTAC	COSM1238030
EGFR	20	p.Asp770_Asn771insGlnArgGly	c.2310_2311insCAGCGTGGC	COSM4970107
EGFR	20	p.Asp770_Asn771insGly	c.2310_2311insGGC	COSM13004
EGFR	20	p.Asp770_Asn771insAlaProTrp	c.2310_2311insGCACCGTGG	COSM20886
EGFR	20	p.Asp770_Asn771insGlyThr	c.2310_2311insGGCACA	COSM1238029
EGFR	20	p.Asp770_Asn771insGlyLeu	c.2310_2311insGGGTTA	COSM48921
EGFR	20	p.Asp770_Asn771insGlyPhe	c.2310_2311insGGGTTT	COSM655155
EGFR	20	p.Asp770_Asn771insGly	c.2310_2311insGGT	COSM12378
EGFR	20	p.D770_N771insG	c.2310_2311insGGG	MATNV05
EGFR	20	p.Ala767_Ser768insSerValAsp	c.2311_2312insGCGTGGACA	COSM13428
EGFR	20	p.Asp770_Asn771insThr	c.2311_2312insCCA	COSM5023008

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Gene	Exon	Amino acid change	Nucleotide change	Variant ID
EGFR	20	p.Asp770_Asn771insMetAlaThr Pro	c.2311_2312insTGGCCACCCC CA	COSM26719
EGFR	20	p.Asp770_Asn771insSerValGlu	c.2311_2312insGCGTCGAAA	COSM1651743
EGFR	20	p.Asn771delinsThrHis	c.2311_2312insCAC	COSM22946
EGFR	20	p.Asn771delinsSerGlyHis	c.2311_2312insGTGGCC	COSM1651744
EGFR	20	p.Asn771delinsValHis	c.2311_2311delAinsGTCC	COSM5023007
EGFR	20	p.N771delinsKH	c.2311_2312insAAC	OMINDEL1123
EGFR	20	p.N771delinsPH	c.2311_2311delAinsCCCC	MATNV04
EGFR	20	p.Asn771delinsSerHis	c.2311_2312insGTC	COSM24434
EGFR	20	p.Asn771delinsSerThrHis	c.2311_2312insGCACCC	COSM6920147
EGFR	20	p.Asn771delinsGlyPhe	c.2311_2312delAAinsGGGTT	COSM18431
EGFR	20	p.Asn771delinsGlyTyr	c.2311_2311delAinsGGTT	COSM53189
EGFR	20	p.Asn771delinsLysLeu	c.2312_2313insACT	COSM6438147
EGFR	20	p.Val769_Asp770insAspLys	c.2312_2313insGGACAA	D770_N771insKD
EGFR	20	p.Ser768_Val769insValAspAsn	c.2313_2314insGTGGACAAC	COSM20885
EGFR	20	p.Asn771dup	c.2313_2314insAAC	COSM13003
EGFR	20	p.Asn771_Pro772insThr	c.2313_2314insACA	c.2313_2314insACA
EGFR	20	p.Asn771_Pro772insLeu	c.2313_2314insTTG	c.2313_2314insTTG
EGFR	20	p.Asn771_Pro772insVal	c.2313_2314insGTC	COSM6922328
EGFR	20	p.Asn771delinsLysGly	c.2313_2313delCinsGGGG	N771delinsKG
EGFR	20	p.Asn771_Pro772insHis	c.2314_2315insACC	COSM1238031
EGFR	20	p.Asn771_Pro772insArgHis	c.2314_2315insGGCACC	COSM166390
EGFR	20	p.P772_H773insGT	c.2314_2315insCCGGCA	MATNV02
EGFR	20	p.Asn771_Pro772insHisHis	c.2314_2315insACCACC	COSM6931207
EGFR	20	p.Asn771_Pro772insLeu	c.2314_2315insTCC	N771_P772insL
EGFR	20	p.Asn771_Pro772insProThrHis	c.2315_2316insGACACACCC	COSM48923
EGFR	20	p.Val769_Asp770insAspAsnPro	c.2315_2316insGGACAACCC	COSM6845099
EGFR	20	p.Val769_Asp770insAspAsnPro	c.2316_2317insGACAACCCC	COSM1651745
EGFR	20	p.Asp770_Asn771insAsnPro	c.2316_2317insAACCCC	MAN123

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Gene	Exon	Amino acid change	Nucleotide change	Variant ID
EGFR	20	p.Pro772_His773insGlyAsnPro	c.2316_2317insGGCAACCCC	c.2316_2317insGGCAACCC CC
EGFR	20	p.Pro772_His773insThrProAsnPro	c.2316_2317insACACCCAACC CC	COSM6977296
EGFR	20	p.Asp770_Asn771insAsnProGly	c.2316_2317insGGAAACCCC	P772_H773insGNP
EGFR	20	p.Ser768_Val769insValAspAsnPro	c.2316_2317insGTGGACAACC CC	V769_P772dup
EGFR	20	p.His773delinsProAsnProTyr	c.2317_2318insCTAACCCCT	COSM1735761
EGFR	20	p.H773delinsRY	c.2317_2318insGTT	MATNV08
EGFR	20	p.His773delinsAsnProTyr	c.2317_2317delCinsAACCCCT	H773delinsNPY
EGFR	20	p.Asp770_Asn771insAsnProPro	c.2317_2318insCCAACCCCC	P772_H773insPNP
EGFR	20	p.Asp770_Asn771insAsnProHis	c.2319_2320insAACCCCCAC	COSM12381
EGFR	20	p.Asn771_Pro772insProHis	c.2319_2320insCCCCAC	COSM12380
EGFR	20	p.His773dup	c.2319_2320insCAC	COSM12377
EGFR	20	p.Pro772_His773insHisAsn	c.2319_2320insAACCCAC	COSM5023006
EGFR	20	p.His773_Val774insThrGlnProPro	c.2319_2320insACACAACCCC CC	COSM3727813
EGFR	20	p.His773_Val774insGln	c.2319_2320insCAG	COSM131552
EGFR	20	p.Asp770_Asn771insAsnProHis Gly	c.2320_2321insGCAACCCCCA CG	COSM51544
EGFR	20	p.Pro772_His773insHisAla	c.2320_2321insCCCACG	COSM1238028
EGFR	20	p.Pro772_His773insHisVal	c.2321_2322insCCACGT	COSM18432
EGFR	20	NA	NA	COSM12388 ^[1]
EGFR	20	NA	NA	COSM22948 ^[1]
EGFR	20	NA	NA	COSM255205 ^[1]
EGFR	20	NA	NA	COSM4170223 ^[1]
EGFR	20	p.Asn771_Pro772insProHisVal	c.2322_2323insCCCCACGTG	COSM6845098
EGFR	20	p.Cys797Ser	c.2389T>A	COSM6493937
EGFR	20	p.Cys797Ser	c.2390G>C	COSM5945664
EGFR	21	p.Leu858Met	c.2572C>A	COSM12366



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Gene	Exon	Amino acid change	Nucleotide change	Variant ID
EGFR	21	p.Leu858Arg	c.2573T>G	COSM6224
EGFR	21	p.Leu861Gln	c.2582T>A	COSM6213
EGFR	21	p.Leu861Arg	c.2582T>G	COSM12374
ERBB2	8	p.Ser310Tyr	c.929C>A	COSM94225
ERBB2	8	p.Ser310Phe	c.929C>T	COSM48358
ERBB2	17	p.Arg678Gln	c.2033G>A	COSM436498
ERBB2	18	p.Thr733Ile	c.2198C>T	COSM14059
ERBB2	19	p.Leu755Pro	c.2263_2264delTTinsCC	COSM683
ERBB2	19	p.Leu755Ala	c.2263_2264delTTinsGC	COSM6906940
ERBB2	19	p.Leu755Met	c.2263T>A	COSM1205571
ERBB2	19	p.Leu755Ser	c.2264T>C	COSM14060
ERBB2	19	p.Leu755Trp	c.2264T>G	COSM436499
ERBB2	19	p.Ile767Met	c.2301C>G	COSM51317
ERBB2	19	p.Asp769Asn	c.2305G>A	COSM1302747
ERBB2	19	p.Asp769His	c.2305G>C	COSM13170
ERBB2	19	p.Asp769Tyr	c.2305G>T	COSM1251412
ERBB2	20	p.Glu770_Ala771insAlaTyrValMet	c.2324_2325insATACGTGATG GC	COSM20959
ERBB2	20	p.Tyr772_Val773insValMetAlaThr	c.2325_2326insACCGTGATGG CT	MAN302
ERBB2	20	p.Ala771_Tyr772insTyrValMetAla	c.2325_2326insTACGTGATGG CT	COSM12558
ERBB2	20	p.Gly776delinsLeuCys	c.2326_2326delGinsCTTT	COSM12554
ERBB2	20	p.Gly776delinsLeuCys	c.2326_2326delGinsTTGT	COSM19875
ERBB2	20	p.Ala775_Gly776insVal	c.2326_2327insTAG	MAN309
ERBB2	20	p.Gly776delinsValCys	c.2326_2327insTAT	OMINDEL612
ERBB2	20	p.Ala775_Gly776insVal	c.2326_2327insTCG	MAN308
ERBB2	20	p.Gly776delinsValCys	c.2326_2327insTCT	COSM85995
ERBB2	20	p.Ala775_Gly776insVal	c.2326_2327insTGG	MAN300

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
ERBB2	20	p.Gly776delinsValCys	c.2326_2327insTGT	COSM12553
ERBB2	20	p.Ala775_Gly776insVal	c.2326_2327insTTG	MAN307
ERBB2	20	p.Gly776delinsValCys	c.2326_2327insTTT	COSM12552
ERBB2	20	p.Gly776Ser	c.2326G>A	COSM685
ERBB2	20	p.Gly776Cys	c.2326G>T	COSM303938
ERBB2	20	p.Gly776Val	c.2327G>T	COSM18609
ERBB2	20	p.Gly776_Val777insLeu	c.2328_2329insCTT	COSM6438151
ERBB2	20	p.Val777Met	c.2329G>A	COSM14064
ERBB2	20	p.Val777Leu	c.2329G>C	COSM436500
ERBB2	20	p.Val777Leu	c.2329G>T	COSM14062
ERBB2	20	p.Gly776_Val777insValGlyCys	c.2330_2331insAGGTTGTGT	MAN311
ERBB2	20	p.Val777_Gly778insCysGly	c.2331_2332insTGTGGG	COSM303939
ERBB2	20	p.Gly778_Ser779insLeuProSer	c.2333_2334insGCTCCCCAG	COSM5802314
ERBB2	20	p.Val777_Gly778insGly	c.2333_2334insGGG	COSM26681
ERBB2	20	p.Val777_Gly778insGlyCysPro	c.2335_2336insGCCCAGGCT	MAN310
ERBB2	20	p.Gly776_Val777insValGlySer	c.2336_2337insTGTGGGCTC	COSM681
ERBB2	20	p.Val777_Gly778insGlySerPro	c.2339_2340insCGGCTCCCC	COSM6865893
ERBB2	20	p.Val777_Gly778insGlySerPro	c.2339_2340insGGGCTCCCC	COSM12555
ERBB2	20	p.Val777_Gly778insGlySerPro	c.2339_2340insTGGCTCCCC	COSM303948
ERBB2	20	p.Val777_Gly778insGlySerPro	c.2340_2341insGGCTCCCCA	COSM12556
ERBB2	21	p.Val842Ile	c.2524G>A	COSM14065
ERBB2	21	p.Thr862Ile	c.2585C>T	MAN306
ERBB2	21	p.Leu869Arg	c.2606T>G	COSM249793
ERBB2	22	p.Arg896Cys	c.2686C>T	COSM14066
ERBB2	22	p.Arg896His	c.2687G>A	COSM119971
ERBB3	2	p.Met60Leu	c.178A>T	COSM1606366
ERBB3	2	p.Met60Lys	c.179T>A	COSM254678
ERBB3	2	p.Met60Arg	c.179T>G	COSM941484

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Gene	Exon	Amino acid change	Nucleotide change	Variant ID
ERBB3	3	p.Met91Ile	c.273G>A	COSM122890
ERBB3	3	p.Met91Ile	c.273G>C	COSM1299636
ERBB3	3	p.Val104Met	c.310G>A	COSM172423
ERBB3	3	p.Val104Leu	c.310G>C	COSM160824
ERBB3	3	p.Val104Leu	c.310G>T	COSM191840
ERBB3	6	p.Ala232Thr	c.694G>A	COSM4043440
ERBB3	6	p.Ala232Val	c.695C>T	COSM1242239
ERBB3	8	p.Asp297Tyr	c.889G>T	COSM160822
ERBB3	8	p.Asp297Val	c.890A>T	COSM941490
ERBB3	9	p.Glu332Lys	c.994G>A	COSM254677
FGFR2	7	p.Ser252Trp	c.755C>G	COSM36903
FGFR2	7	p.Pro253Arg	c.758C>G	COSM49170
FGFR2	7	p.Pro253Leu	c.758C>T	COSM537801
FGFR2	8	p.Ala314Asp	c.941C>A	COSM49171
FGFR2	9	p.Tyr375His	c.1123T>C	COSM1560916
FGFR2	9	p.Tyr375Cys	c.1124A>G	COSM36904
FGFR2	9	p.Cys382Arg	c.1144T>C	COSM36906
FGFR2	9	p.Cys382Tyr	c.1145G>A	COSM915493
FGFR2	12	p.Asn549His	c.1645A>C	COSM250083
FGFR2	12	p.Asn549Ser	c.1646A>G	COSM3665553
FGFR2	12	p.Asn549Lys	c.1647T>A	COSM36912
FGFR2	12	p.Asn549Lys	c.1647T>G	COSM36902
FGFR2	14	p.Lys659Glu	c.1975A>G	COSM36909
FGFR2	14	p.Lys659Met	c.1976A>T	COSM49175
FGFR2	14	p.Lys659Asn	c.1977G>C	COSM683054
FGFR2	14	p.Lys659Asn	c.1977G>T	COSM49173
FGFR3	7	p.Arg248Cys	c.742C>T	COSM714
FGFR3	7	p.Ser249Cys	c.746C>G	COSM715

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
FGFR3	14	p.Lys650Gln	c.1948A>C	COSM726
FGFR3	14	p.Lys650Glu	c.1948A>G	COSM719
FGFR3	14	p.Lys650Asn	c.1950G>T	COSM1428730
FGFR3	16	p.Gly697Cys	c.2089G>T	COSM24802
HRAS	2	p.Gly12Ser	c.34G>A	COSM480
HRAS	2	p.Gly12Arg	c.34G>C	COSM482
HRAS	2	p.Gly12Cys	c.34G>T	COSM481
HRAS	2	p.Gly12Asp	c.35G>A	COSM484
HRAS	2	p.Gly12Ala	c.35G>C	COSM485
HRAS	2	p.Gly12Val	c.35G>T	COSM483
HRAS	2	p.Gly13Ser	c.37G>A	COSM487
HRAS	2	p.Gly13Arg	c.37G>C	COSM486
HRAS	2	p.Gly13Cys	c.37G>T	COSM488
HRAS	2	p.Gly13Asp	c.38G>A	COSM490
HRAS	2	p.Gly13Val	c.38G>T	COSM489
HRAS	3	p.Gln61Lys	c.181C>A	COSM496
HRAS	3	p.Gln61Pro	c.182A>C	COSM500
HRAS	3	p.Gln61Arg	c.182A>G	COSM499
HRAS	3	p.Gln61Leu	c.182A>T	COSM498
HRAS	3	p.Gln61His	c.183G>C	COSM503
HRAS	3	p.Gln61His	c.183G>T	COSM502
KIT	8	p.Asp419del	c.1255_1257delGAC	COSM29014
KIT	8	p.Asp419_Arg420del	c.1255_1260delGACAGG	COSM1578132
KIT	11	p.Trp557_Lys558del	c.1669_1674delTGGAAG	COSM1217
KIT	11	p.Trp557Arg	c.1669T>A	COSM1216
KIT	11	p.Trp557Arg	c.1669T>C	COSM1219
KIT	11	p.Trp557Gly	c.1669T>G	COSM1221
KIT	11	p.Trp557_Val559delinsPhe	c.1670_1675delGGAAGG	COSM1226

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
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.Val559del	c.1679_1681delTTG	COSM1247
KIT	11	p.Val560Asp	c.1679T>A	COSM1257
KIT	11	p.Leu576Pro	c.1727T>C	COSM1290
KIT	11	p.Asp579del	c.1735_1737delGAT	COSM1294
KIT	13	p.Lys642Glu	c.1924A>G	COSM1304
KIT	13	p.Val654Ala	c.1961T>C	COSM12706
KIT	17	p.Arg796Lys	c.2387G>A	COSM1600411
KIT	17	p.Asp816His	c.2446G>C	COSM1311
KIT	17	p.Asp816Tyr	c.2446G>T	COSM1310
KIT	17	p.Asp816Val	c.2447A>T	COSM1314
KIT	17	p.Asn822Lys	c.2466T>A	COSM1321
KIT	17	p.Asn822Lys	c.2466T>G	COSM1322
KIT	17	p.Val825Ala	c.2474T>C	COSM1323
KRAS	2	p.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.Gly12Ser	c.34G>A	COSM517
KRAS	2	p.Gly12Arg	c.34G>C	COSM518
KRAS	2	p.Gly12Cys	c.34G>T	COSM516
KRAS	2	p.Gly12Asp	c.35G>A	COSM521
KRAS	2	p.Gly12Ala	c.35G>C	COSM522
KRAS	2	p.Gly12Val	c.35G>T	COSM520
KRAS	2	p.Gly13Ser	c.37G>A	COSM528

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
KRAS	2	p.Gly13Arg	c.37G>C	COSM529
KRAS	2	p.Gly13Cys	c.37G>T	COSM527
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.Gly13Asp	c.38G>A	COSM532
KRAS	2	p.Gly13Ala	c.38G>C	COSM533
KRAS	2	p.Gly13Val	c.38G>T	COSM534
KRAS	3	p.Ala59Thr	c.175G>A	COSM546
KRAS	3	p.Ala59Glu	c.176C>A	COSM547
KRAS	3	p.Ala59Gly	c.176C>G	COSM28518
KRAS	3	p.Gln61Lys	c.180_181delTCinsAA	COSM87298
KRAS	3	p.Gln61Lys	c.181C>A	COSM549
KRAS	3	p.Gln61Glu	c.181C>G	COSM550
KRAS	3	p.Gln61Pro	c.182A>C	COSM551
KRAS	3	p.Gln61Arg	c.182A>G	COSM552
KRAS	3	p.Gln61Leu	c.182A>T	COSM553
KRAS	3	p.Gln61His	c.183A>C	COSM554
KRAS	3	p.Gln61His	c.183A>T	COSM555
KRAS	4	p.Lys117Asn	c.351A>C	COSM19940
KRAS	4	p.Lys117Asn	c.351A>T	COSM28519
KRAS	4	p.Ala146Thr	c.436G>A	COSM19404
KRAS	4	p.Ala146Pro	c.436G>C	COSM19905
KRAS	4	p.Ala146Val	c.437C>T	COSM19900
MAP2K1	2	p.Phe53Ile	c.157T>A	COSM3503329
MAP2K1	2	p.Phe53Leu	c.157T>C	COSM555604

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
MAP2K1	2	p.Phe53Val	c.157T>G	COSM1562837 Note: The base change c.157T>G in MAP2K1 is associated with Mutation ID COSM5077832 in the COSMIC v.76 database, even though it has been given the Variant HotSpot ID COSM1562837 in the software. This does not impact the test results.
MAP2K1	2	p.Phe53Leu	c.159T>A	COSM1725008
MAP2K1	2	p.Phe53Leu	c.159T>G	COSM2257208
MAP2K1	2	p.Lys57Thr	c.170A>C	COSM4756761
MAP2K1	2	p.Lys57Met	c.170A>T	MAN124
MAP2K1	2	p.Lys57Asn	c.171G>C	COSM5520914
MAP2K1	2	p.Lys57Asn	c.171G>T	COSM1235478
MAP2K1	3	p.Pro124Ser	c.370C>T	COSM235614
MAP2K1	3	p.Pro124Gln	c.371C>A	COSM1167912
MAP2K1	3	p.Pro124Leu	c.371C>T	COSM1315861
MAP2K1	6	p.Glu203Lys	c.607G>A	COSM232755
MAP2K1	6	p.Glu203Val	c.608A>T	COSM3386991
MAP2K2	2	p.Phe57Leu	c.169T>C	COSM1235618
MAP2K2	2	p.Phe57Val	c.169T>G	COSM3534171
MAP2K2	2	p.Phe57Leu	c.171T>A	COSM3389034
MAP2K2	2	p.Phe57Leu	c.171T>G	OM3158
MAP2K2	2	p.Gln60Pro	c.179A>C	COSM145610
MET	14	p.Thr1010Ile	c.3029C>T	COSM707
MET	14	p.Tyr1021Asn	c.3061T>A	COSM48564
MET	14	p.Tyr1021Phe	c.3062A>T	COSM339515
MET	14	NA	c.3082+1G>T	COSM24687 ^[1]
MET	14	NA	c.3082+1G>A	COSM29633 ^[1]

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
MET	14	NA	c.3082+2T>C	COSM35468 ^[1]
MET	16	p.His1112Tyr	c.3334C>T	COSM696
MET	16	p.His1112Arg	c.3335A>G	COSM703
MET	16	p.His1112Leu	c.3335A>T	COSM698
MET	19	p.Tyr1248His	c.3742T>C	COSM690
MET	19	p.Tyr1248Cys	c.3743A>G	COSM699
MET	19	p.Tyr1253Asp	c.3757T>G	COSM700
MET	19	p.Met1268Thr	c.3803T>C	COSM691
MET	19	p.Met1268Ile	c.3804G>A	COSM694
MTOR	30	p.Cys1483Arg	c.4447T>C	COSM3747775
MTOR	30	p.Cys1483Tyr	c.4448G>A	COSM462615
MTOR	30	p.Cys1483Phe	c.4448G>T	COSM462616
MTOR	30	p.Cys1483Trp	c.4449C>G	OM3149
MTOR	39	p.Glu1799Lys	c.5395G>A	COSM180789
MTOR	40	p.Phe1888Ile	c.5662T>A	COSM3358968
MTOR	40	p.Phe1888Leu	c.5662T>C	COSM3358967
MTOR	40	p.Phe1888Val	c.5662T>G	COSM893814
MTOR	40	p.Phe1888Leu	c.5664C>A	COSM893813
MTOR	40	p.Phe1888Leu	c.5664C>G	COSM462604
MTOR	43	p.Thr1977Ser	c.5929A>T	COSM1289945 Note: Some "no calls" were observed for this analytical variant due to strand bias with plasmid targets. This does not impact clinical test results.
MTOR	43	p.Thr1977Lys	c.5930C>A	COSM462601

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
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.Val2006Ile	c.6016G>A	COSM893804
MTOR	43	p.Val2006Leu	c.6016G>C	COSM1134662
MTOR	43	p.Val2006Phe	c.6016G>T	COSM249481
MTOR	47	p.Ser2215Pro	c.6643T>C	COSM1560108
MTOR	47	p.Ser2215Tyr	c.6644C>A	COSM20417
MTOR	47	p.Ser2215Phe	c.6644C>T	COSM1686998
MTOR	53	p.Leu2427Gln	c.7280T>A	COSM1185313
MTOR	53	p.Leu2427Arg	c.7280T>G	COSM2119114
NRAS	2	p.Gly12Ser	c.34G>A	COSM563
NRAS	2	p.Gly12Arg	c.34G>C	COSM561
NRAS	2	p.Gly12Cys	c.34G>T	COSM562
NRAS	2	p.Gly12Asp	c.35G>A	COSM564
NRAS	2	p.Gly12Ala	c.35G>C	COSM565
NRAS	2	p.Gly12Val	c.35G>T	COSM566
NRAS	2	p.Gly13Ser	c.37G>A	COSM571
NRAS	2	p.Gly13Arg	c.37G>C	COSM569
NRAS	2	p.Gly13Cys	c.37G>T	COSM570
NRAS	2	p.Gly13Asp	c.38G>A	COSM573
NRAS	2	p.Gly13Ala	c.38G>C	COSM575
NRAS	2	p.Gly13Val	c.38G>T	COSM574
NRAS	3	p.Ala59Thr	c.175G>A	COSM578
NRAS	3	p.Gln61Lys	c.181C>A	COSM580
NRAS	3	p.Gln61Glu	c.181C>G	COSM581

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
NRAS	3	p.Gln61Pro	c.182A>C	COSM582
NRAS	3	p.Gln61Arg	c.182A>G	COSM584
NRAS	3	p.Gln61Leu	c.182A>T	COSM583
NRAS	3	p.Gln61His	c.183A>C	COSM586
NRAS	3	p.Gln61His	c.183A>T	COSM585
NRAS	4	p.Lys117Asn	c.351G>T	MAN13
NRAS	4	p.Ala146Thr	c.436G>A	COSM27174
NRAS	4	p.Ala146Val	c.437C>T	COSM4170228
PDGFRA	12	p.Val561Asp	c.1682T>A	COSM739
PDGFRA	14	p.Asn659Tyr	c.1975A>T	COSM22416
PDGFRA	14	p.Asn659Lys	c.1977C>A	COSM22415
PDGFRA	14	p.Asn659Lys	c.1977C>G	COSM22414
PDGFRA	18	p.Asp842_Met844del	c.2524_2532delGACATCATG	COSM12401
PDGFRA	18	p.Asp842Tyr	c.2524G>T	COSM12396
PDGFRA	18	p.Asp842Val	c.2525A>T	COSM736
PDGFRA	18	p.Asp842_His845del	c.2526_2537delCATCATGCATGA	COSM737
PDGFRA	18	p.Ile843_Asp846del	c.2527_2538delATCATGCATGAT	COSM12400
PDGFRA	18	p.Ile843_Ser847delinsThr	c.2528_2539delTCATGCATGAT	COSM12407
PIK3CA	2	p.Arg38Ser	c.112C>A	COSM87310
PIK3CA	2	p.Arg38Gly	c.112C>G	COSM40945
PIK3CA	2	p.Arg38Cys	c.112C>T	COSM744
PIK3CA	2	p.Arg38His	c.113G>A	COSM745
PIK3CA	2	p.Glu39Lys	c.115G>A	COSM30625
PIK3CA	2	p.Glu81Lys	c.241G>A	COSM27502
PIK3CA	2	p.Arg88Gln	c.263G>A	COSM746
PIK3CA	2	p.Arg93Trp	c.277C>T	COSM27493



(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
PIK3CA	2	p.Arg93Gln	c.278G>A	COSM86041
PIK3CA	2	p.Gly106Val	c.317G>T	COSM748
PIK3CA	2	p.Arg108His	c.323G>A	COSM27497
PIK3CA	2	p.Lys111Glu	c.331A>G	COSM13570
PIK3CA	5	p.Val344Ala	c.1031T>C	COSM86951
PIK3CA	5	p.Val344Gly	c.1031T>G	COSM22540
PIK3CA	5	p.Asn345Ile	c.1034A>T	COSM94978
PIK3CA	5	p.Asn345Lys	c.1035T>A	COSM754
PIK3CA	6	p.Glu365Lys	c.1093G>A	COSM86044
PIK3CA	6	p.Glu365Gly	c.1094A>G	COSM1420797
PIK3CA	6	p.Glu365Val	c.1094A>T	COSM1484860
PIK3CA	6	p.Cys378Arg	c.1132T>C	COSM756
PIK3CA	6	p.Cys378Tyr	c.1133G>A	COSM1041478
PIK3CA	6	p.Cys378Phe	c.1133G>T	COSM21450
PIK3CA	8	p.Cys420Arg	c.1258T>C	COSM757
PIK3CA	10	p.Pro539Arg	c.1616C>G	COSM759
PIK3CA	10	p.Glu542Lys	c.1624G>A	COSM760
PIK3CA	10	p.Glu542Val	c.1625A>T	COSM762
PIK3CA	10	p.Glu545Lys	c.1633G>A	COSM763
PIK3CA	10	p.Glu545Gln	c.1633G>C	COSM27133
PIK3CA	10	p.Glu545Ala	c.1634A>C	COSM12458
PIK3CA	10	p.Glu545Gly	c.1634A>G	COSM764
PIK3CA	10	p.Glu545Asp	c.1635G>C	COSM27374
PIK3CA	10	p.Glu545Asp	c.1635G>T	COSM765
PIK3CA	10	p.Gln546Lys	c.1636C>A	COSM766
PIK3CA	10	p.Gln546Glu	c.1636C>G	COSM6147
PIK3CA	10	p.Gln546Pro	c.1637A>C	COSM767
PIK3CA	10	p.Gln546Arg	c.1637A>G	COSM12459

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
PIK3CA	10	p.Glu547Lys	c.1639G>A	COSM29315
PIK3CA	14	p.His701Pro	c.2102A>C	COSM778
PIK3CA	14	p.His701Arg	c.2102A>G	COSM1420881
PIK3CA	14	p.Glu726Lys	c.2176G>A	COSM87306
PIK3CA	14	p.Glu726Gly	c.2177A>G	COSM1420887
PIK3CA	19	p.Cys901Arg	c.2701T>C	COSM1420899
PIK3CA	19	p.Cys901Tyr	c.2702G>A	COSM1420901
PIK3CA	19	p.Cys901Phe	c.2702G>T	COSM769
PIK3CA	21	p.Tyr1021Cys	c.3062A>G	COSM12461
PIK3CA	21	p.Thr1025Ala	c.3073A>G	COSM771
PIK3CA	21	p.Met1043Val	c.3127A>G	COSM12591
PIK3CA	21	p.Met1043Ile	c.3129G>A	COSM29313
PIK3CA	21	p.Met1043Ile	c.3129G>T	COSM773
PIK3CA	21	p.Asn1044Lys	c.3132T>A	COSM12592
PIK3CA	21	p.His1047Tyr	c.3139C>T	COSM774
PIK3CA	21	p.His1047Arg	c.3140A>G	COSM775
PIK3CA	21	p.His1047Leu	c.3140A>T	COSM776
PIK3CA	21	p.Gly1049Ser	c.3145G>A	COSM777
PIK3CA	21	p.Gly1049Arg	c.3145G>C	COSM12597
RAF1	7	p.Ser257Trp	c.770C>G	COSM581519
RAF1	7	p.Ser257Leu	c.770C>T	COSM181063
RAF1	12	p.Thr421Met	c.1262_1263delCCinsTG	MAN9
RET	10	p.Cys618Arg	c.1852T>C	COSM29803
RET	10	p.Cys618Tyr	c.1853G>A	COSM980
RET	10	p.Cys620Arg	c.1858T>C	COSM29804
RET	11	p.Cys634Arg	c.1900T>C	COSM966
RET	13	p.Glu768Gly	c.2303A>G	COSM1347811
RET	13	p.Glu768Asp	c.2304G>C	COSM21338

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
RET	15	p.Ala883Phe	c.2646_2648delAGCinsTTT	COSM981
RET	15	p.Ala883Ser	c.2647G>T	COSM133167
RET	15	p.Asp898_Glu901del	c.2694_2705delTGTTTATGAAG A	COSM962
RET	16	p.Met918Thr	c.2753T>C	COSM965
ROS1	36	p.Leu1951Met	c.5851C>A	COSM1072521
ROS1	38	p.Gly2032Arg	c.6094G>A	MAN10
ROS1	38	p.Gly2032Arg	c.6094G>C	MAN11

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

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

RET fusion isoforms (N=44)	
ACBD5-RET.A11R12	KIF5B-RET.K16R12.COSF1230
AFAP1-RET.A3R12	KIF5B-RET.K18R12
AKAP13-RET.A35R12	KIF5B-RET.K22R12.COSF1253
AKAP13-RET.A36R12	KIF5B-RET.K23R11
CCDC6-RET.C1R11	KIF5B-RET.K23R11mid
CCDC6-RET.C1R11.1	KIF5B-RET.K23R12.COSF1234
CCDC6-RET.C1R12	KIF5B-RET.K24R8.COSF1236
CCDC6-RET.C1R12.COSF1271	KIF5B-RET.K24R10
CCDC6-RET.C2R12	KIF5B-RET.K24R11.COSF1262
CCDC6-RET.C8R11	KTN1-RET.K29R12.COSF1513
CCDC6-RET.C8R12full	NCOA4-RET.N7R12.COSF1491
CUX1-RET.C10R12	NCOA4-RET.E6R12.COSF1340
ERC1-RET.E12R12	PCM1-RET.P29R12.COSF1481
ERC1-RET.E17R12	PRKAR1A-RET.P7R12.COSF1511
ERC1-RET.E7R12	RUFY2-RET.R9R12
ERC1_ELKS-RET.E11R12.COSF1507	SPECC1L-RET.S10R11.NGS.1
FKBP15-RET.F25R12	SPECC1L-RET.S10R12
GOLGA5-RET.G7R12.COSF1503	TBL1XR1-RET.T9R11.NGS.1
HOKK3-RET.H11R12.COSF1509	TBL1XR1-RET.T9R12
KIAA1468-RET.K10R12	TRIM24-RET.T9R12.COSF1521
KIF5B-RET.K15R11.COSF1255.1	TRIM27-RET.T3R12.COSF1519
KIF5B-RET.K15R12.COSF1232	TRIM33-RET.T16R12.COSF1525

ROS1 fusion isoforms (N=34)	
CCDC6-ROS1.C5R35	MSN-ROS1.M9R34
CD74-ROS1.C4R33.NGS	MYO5A-ROS1.M23R35
CD74-ROS1.C6R32.COSF1202	PPFIBP1-ROS1.P9R35
CD74-ROS1.C6R34.COSF1200	PWWP2A-ROS1.P1R36
CD74-ROS1.C6R35.COSF1478	SDC4-ROS1.S2R32.COSF1265
CD74-ROS1.C7R34	SDC4-ROS1.S2R34
CEP85L-ROS1.C8R36	SDC4-ROS1.S4R32.COSF1278
CLIP1-ROS1.C19R36	SDC4-ROS1.S4R34.COSF1280
CLTC-ROS1.C31R35	SHTN1-ROS1.S11R36
ERC1-ROS1.E11R36	SLC34A2-ROS1.S13R32.COSF1259
EZR-ROS1.E10R34.COSF1267	SLC34A2-ROS1.S13R34.COSF1261
EZR-ROS1.E10R35	SLC34A2-ROS1.S4R32.COSF1196
GOPC-ROS1.G4R36.COSF1188	SLC34A2-ROS1.S4R34.COSF1198
GOPC-ROS1.G8R35.COSF1139	TFG-ROS1.T4R35
HLA_A-ROS1.H7R34	TPM3-ROS1.T3R36
KDEL2-ROS1.K5R35	TPM3-ROS1.T7R35.COSF1273
LRIG3-ROS1.L16R35.COSF1269	ZCCHC8-ROS1.Z2R36

IDH1 DNA variants detected in cholangiocarcinoma (CC)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
IDH1	4	p.Arg132Cys	c.394C>T	COSM28747
IDH1	4	p.Arg132Ser	c.394C>A	COSM28748
IDH1	4	p.Arg132Gly	c.394C>G	COSM28749
IDH1	4	p.Arg132Leu	c.395G>T	COSM28750
IDH1	4	p.Arg132His	c.395G>A	COSM28746

IDH1 and IDH2 DNA variants detected in astrocytoma (AC) and oligodendroglioma (OG)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
IDH1	4	p.Arg132Cys	c.394C>T	COSM28747
IDH1	4	p.Arg132Ser	c.394C>A	COSM28748
IDH1	4	p.Arg132Gly	c.394C>G	COSM28749
IDH1	4	p.Arg132Leu	c.395G>T	COSM28750
IDH1	4	p.Arg132His	c.395G>A	COSM28746
IDH2	4	p.Arg172Trp	c.514A>T	COSM34039
IDH2	4	p.Arg172Gly	c.514A>G	COSM33731
IDH2	4	p.Arg172Met	c.515G>T	COSM33732
IDH2	4	p.Arg172Lys	c.515G>A	COSM33733
IDH2	4	p.Arg172Ser	c.516G>T	COSM34090
IDH2	4	p.Arg172Ser	c.516G>C	COSM133672

BRAF DNA variants detected in anaplastic thyroid cancer (ATC)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
BRAF	15	p.Val600Glu	c.1799T>A	COSM476
BRAF	15	p.Val600Glu	c.1799_1800delTGinsAA	COSM475

RET DNA variants detected in medullary thyroid cancer (MTC)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
RET	10	p.Cys609Ser	c.1825T>A	RETC609S2
RET	10	p.Cys609Arg	c.1825T>C	RETC609R
RET	10	p.Cys609Gly	c.1825T>G	RETC609G
RET	10	p.Cys609Tyr	c.1826G>A	COSM967

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
RET	10	p.Cys609Ser	c.1826G>C	RETC609S
RET	10	p.Cys609Phe	c.1826G>T	RETC609F
RET	10	p.Cys609Trp	c.1827C>G	RETC609W
RET	10	p.Cys611Ser	c.1831T>A	COSM6984745
RET	10	p.Cys611Arg	c.1831T>C	COSM87269
RET	10	p.Cys611Gly	c.1831T>G	RETC611G
RET	10	p.Cys611Tyr	c.1832G>A	COSM4440700
RET	10	p.Cys611Tyr	c.1832_1833delGCinsAT	RETC611Y
RET	10	p.Cys611Ser	c.1832G>C	RETC611S
RET	10	p.Cys611Phe	c.1832G>T	RETC611F
RET	10	p.Cys611Phe	c.1832_1833delGCinsTT	RETC611F2
RET	10	p.Cys618Thr	c.1852_1853delTGinsAC	RETC618T Note: The nucleotide change of RETC618T overlaps that of RETC618S, so a positive RETC618T sample will also result in a positive call for RETC618S.
RET	10	p.Cys618Ser	c.1852T>A	COSM87267
RET	10	p.Cys618Gly	c.1852T>G	RETC618G
RET	10	p.Cys618Arg	c.1852T>C	COSM29803
RET	10	p.Cys618Tyr	c.1853G>A	COSM980
RET	10	p.Cys618Ser	c.1853G>C	RETC618S
RET	10	p.Cys618Phe	c.1853G>T	RETC618F
RET	10	p.Cys618Trp	c.1854C>G	RETC618W
RET	10	p.Cys620Arg	c.1858T>C	COSM29804
RET	10	p.Cys620Ser	c.1858T>A	COSM5946160
RET	10	p.Cys620Gly	c.1858T>G	RETC620G
RET	10	p.Cys620Tyr	c.1859G>A	COSM7403807
RET	10	p.Cys620Ser	c.1859G>C	COSM29805

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
RET	10	p.Cys620Phe	c.1859G>T	RETC620F
RET	10	p.Cys620Trp	c.1860C>G	COSM249815
RET	—	NA	NA	RETD627_L633delinsAH ^[1]
RET	11	p.Pro628_Leu633del	c.1882_1899delICCACTGTGCG ACGAGCTG	RETP628_L633del
RET	11	p.Leu629_Glu632del	c.1891_1902delGACGAGCTGT GC	RETD631_C634del
RET	11	p.Leu629_Ile638delinsCysAsp	c.1884_1913delIACTGTGCGAC GAGCTGTGCCGCACGGTGATi nsGTGCGA	RETL629_I638delinsCD
RET	11	p.Cys630Ser	c.1888T>A	RETC630S
RET	11	p.Cys630Arg	c.1888T>C	COSM964
RET	11	p.Cys630Gly	c.1888T>G	COSM29806
RET	11	p.Cys630Tyr	c.1889G>A	RETC630Y
RET	11	p.Cys630Phe	c.1889G>T	RETC630F
RET	11	p.Asp631del	c.1893_1895delCGA	RETD631del
RET	11	p.Asp631_Leu633delinsVal	c.1892_1897delACGAGC	RETD631_L633delinsV
RET	11	p.Asp631Tyr	c.1891G>T	RETD631Y
RET	11	p.Asp631_Leu633delinsGlu	c.1893_1898delICGAGCT	COSM983
RET	11	p.Glu632_Ala639delinsHisArg	c.1894_1917delGAGCTGTGCC GCACGGTGATCGCAinsCACC GT	COSM5945861
RET	11	p.Glu632_Leu633del	c.1894_1899delGAGCTG	COSM968
RET	11	p.Glu632_Thr636delinsSerSer	c.1894_1906delGAGCTGTGCC GCAinsAGCT	COSM1048
RET	11	p.Glu632_Cys634delinsGly	c.1895_1900delAGCTGT	COSM973
RET	11	p.Glu632_Leu633delinsVal	c.1895_1897delAGC	COSM982
RET	11	p.Glu632_Ala640delinsValArgPro	c.1895_1918delAGCTGTGCCG CACGGTGATCGCAGinsTGCG GC	COSM1049

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
RET	11	p.Glu632_Cys634delinsAspValArg	c.1896_1900delGCTGTinsCGTGC	RETE632_C634insDVR Note: The nucleotide change of RETE632_C634insDVR overlaps that of COSM966, so a positive RETE632_C634insDVR sample will also result in a positive call for COSM966.
RET	11	p.Cys634Ser	c.1900T>A	COSM1237918
RET	11	p.Cys634Gly	c.1900T>G	COSM1738369
RET	11	p.Cys634Arg	c.1900T>C	COSM966
RET	11	p.Cys634Tyr	c.1901G>A	COSM974
RET	11	p.Cys634Ser	c.1901G>C	COSM1666664
RET	11	p.Cys634Phe	c.1901G>T	COSM1237919
RET	11	p.Cys634Leu	c.1901_1902delGCinsTG	RETC634L Note: The nucleotide change of RETC634L overlaps that of COSM975, so a positive RETC634L sample will also result in a positive call for COSM975.
RET	11	p.Cys634Trp	c.1902C>G	COSM975
RET	11	p.Ala640Gly	c.1919C>G	RETA640G
RET	11	p.Val642Ile	c.1924G>A	COSM6005497
RET	11	p.Ser649Leu	c.1946C>T	COSM4170226
RET	13	p.Glu768Gln	c.2302G>C	COSM1716312
RET	13	p.Glu768Gly	c.2303A>G	COSM1347811
RET	13	p.Glu768Asp	c.2304G>T	RETE768D
RET	13	p.Glu768Asp	c.2304G>C	COSM21338
RET	13	p.Arg770Gln	c.2309G>A	RETR770Q
RET	13	p.Asn777Ser	c.2330A>G	RETN777S
RET	13	p.Val778Ile	c.2332G>A	COSM3807173

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
RET	13	p.Gln781Arg	c.2342A>G	COSM87265
RET	15	p.Leu881Val	c.2641C>G	RETL881V
RET	15	p.Ala883Phe	c.2646_2648delAGCinsTTT	COSM981 Note: The nucleotide change of COSM981 overlaps that of COSM4594154, so a positive COSM981 sample will also result in a positive call for COSM4594154.
RET	15	p.Ala883Ser	c.2647G>T	COSM133167
RET	15	p.Ala883Thr	c.2647G>A	COSM100081
RET	15	p.Ala883Phe	c.2647_2648delGCinsTT	COSM977 Note: The nucleotide change of COSM977 overlaps that of COSM4594154, so a positive COSM977 sample will also result in a positive call for COSM4594154.
RET	15	p.Ala883Tyr	c.2647_2648delGCinsTA	RETA883Y
RET	15	p.Ala883Val	c.2648C>T	COSM4594154
RET	15	p.Glu884Val	c.2651A>T	COSM1570338
RET	15	p.Arg886Trp	c.2656C>T	COSM6942691
RET	15	p.Ser891Ala	c.2671T>G	COSM5945860
RET	15	p.Asp898_Glu901del	c.2694_2705delTGTTTATGAAGA	COSM962
RET	15	p.Asp898_Glu902del	c.2695_2709delGTTTATGAAGAGGAT	COSM5991595
RET	15	p.Asp903_Ser904delinsGluPro	c.2709_2710delTTinsAC	RETD903_S904EP1
RET	15	p.Asp903_Ser904delinsGluPro	c.2709_2710delTTinsGC	RETD903_S904EP2
RET	15	p.Ser904Cys	c.2711C>G	RETS904C
RET	15	p.Ser904Phe	c.2711C>T	COSM6438204
RET	16	p.Gly911Asp	c.2732G>A	COSM20888

(continued)

Gene	Exon	Amino acid change	Nucleotide change	Variant ID
RET	16	p.Arg912Trp	c.2734C>T	COSM3415038
RET	16	p.Arg912Pro	c.2735G>C	RETR912P
RET	16	p.Arg912Leu	c.2735G>T	COSM188545
RET	16	p.Met918Val	c.2752A>G	RETM918V
RET	16	p.Met918Thr	c.2753T>C	COSM965
RET	16	p.Ser922Pro	c.2764T>C	COSM26636

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

RET fusion isoforms detected in thyroid cancer (TC)

RET fusion isoforms (N=44)	
ACBD5-RET.A11R12	KIF5B-RET.K16R12.COSF1230
AFAP1-RET.A3R12	KIF5B-RET.K18R12
AKAP13-RET.A35R12	KIF5B-RET.K22R12.COSF1253
AKAP13-RET.A36R12	KIF5B-RET.K23R11
CCDC6-RET.C1R11	KIF5B-RET.K23R11mid
CCDC6-RET.C1R11.1	KIF5B-RET.K23R12.COSF1234
CCDC6-RET.C1R12	KIF5B-RET.K24R8.COSF1236
CCDC6-RET.C1R12.COSF1271	KIF5B-RET.K24R10
CCDC6-RET.C2R12	KIF5B-RET.K24R11.COSF1262
CCDC6-RET.C8R11	KTN1-RET.K29R12.COSF1513
CCDC6-RET.C8R12full	NCOA4-RET.N7R12.COSF1491
CUX1-RET.C10R12	NCOA4-RET.E6R12.COSF1340
ERC1-RET.E12R12	PCM1-RET.P29R12.COSF1481
ERC1-RET.E17R12	PRKAR1A-RET.P7R12.COSF1511
ERC1-RET.E7R12	RUFY2-RET.R9R12
ERC1_ELKS-RET.E11R12.COSF1507	SPECC1L-RET.S10R11.NGS.1
FKBP15-RET.F25R12	SPECC1L-RET.S10R12
GOLGA5-RET.G7R12.COSF1503	TBL1XR1-RET.T9R11.NGS.1
HOOK3-RET.H11R12.COSF1509	TBL1XR1-RET.T9R12
KIAA1468-RET.K10R12	TRIM24-RET.T9R12.COSF1521
KIF5B-RET.K15R11.COSF1255.1	TRIM27-RET.T3R12.COSF1519
KIF5B-RET.K15R12.COSF1232	TRIM33-RET.T16R12.COSF1525



Customer and technical support

Visit [thermofisher.com/support](https://www.thermofisher.com/support) for the latest in services and support, including:

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

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

Obtaining Certificates of Analysis

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

Obtaining Certificates of Conformance

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

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

for use with Torrent Suite™ Dx Software 5.14

Publication Number MAN0018949

Revision C.02



For In Vitro Diagnostic Use. | Rx Only

ThermoFisher
S C I E N T I F I C



Life Technologies Holdings Pte Ltd |
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Marsiling Industrial Estate Road 3 |
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Products manufactured at this site:

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



Life Technologies Corporation |
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Frederick, Maryland 21704 USA

Products manufactured at this site:

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

For descriptions of symbols on product labels or product documents, go to [thermofisher.com/symbols-definition](https://www.thermofisher.com/symbols-definition).

Revision history: **MAN0018949 C.02 (English)**

Revision	Date	Description
C.02	27 September 2024	Updated with tissue input requirements for astrocytoma and oligodendroglioma samples on page 15.
C.01	8 December 2023	Draft for FDA review. <ul style="list-style-type: none"> • Updated with tissue input requirements for anaplastic thyroid cancer samples on page 15. • Updated for Oncomine™ Dx Target DNA Control v3 and Oncomine™ Dx Target RNA Control v2. • Updated guidance for storage of extracted RNA at -90°C to -60°C from up to 5 months to up to 12 months.
C.0	18 April 2023	<ul style="list-style-type: none"> • Updated tissue input requirements for thyroid cancer and medullary thyroid cancer samples on page 15. • Updated for Torrent Suite™ Dx Software 5.14.
B.0	28 November 2022	<ul style="list-style-type: none"> • Updated sample storage and stability guidelines. • Clarified guidance for control tube reuse. • Updated labeling and workflow formatting.
A.0	2 December 2021	New Oncomine™ Dx Target Test user guide for commercial release.

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

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

Oncomine™ Dx Target Test Kit user guides

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

- *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide*
- *Oncomine™ Dx Target Test Part II: Sample and Library Preparation User Guide*
- *Oncomine™ Dx Target Test Part III: Template Preparation User Guide*
- *Oncomine™ Dx Target Test Part IV: Sequencing and Results Reports User Guide*
- *Oncomine™ Dx Target Test Part V: Torrent Suite™ Dx Software 5.14 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

Product description

Oncomine™ Dx Target Test

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

For a complete product description of the Oncomine™ Dx Target Test, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide*.

Sample preparation components

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

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

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

Library preparation components

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

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

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

Intended use

For the intended use statement for the Oncomine™ Dx Target Test, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide*.

Theory of operation

For a complete description of the theory of operation of the system, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide*.

Software compatibility and requirements

The procedures in this guide are designed for use with Torrent Suite™ Dx Software version 5.14 or later. For a complete description of software compatibility and requirements, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide*.

Materials provided

Oncomine™ Dx Target Test Kit

The Oncomine™ Dx Target Test Kit (Cat. No. A55462) 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	A55463
	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	A55464

Subkits used in this guide

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

Ion Torrent Dx FFPE Sample Preparation Kit

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

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

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

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

Oncomine™ Dx Target Test, Controls, and Diluent Kit

The Oncomine™ Dx Target Test, Controls, and Diluent Kit (Part No. A55463) provides the following panels and controls.

✓	Component	Amount	Storage
Oncomine™ Dx Target Test DNA and RNA Panel (Part No. A32441)			
	Oncomine™ Dx Target Test—DNA panel (blue cap)	6 × 32 µL	-30°C to -10°C
	Oncomine™ Dx Target Test—RNA panel (yellow cap)	6 × 32 µL	
Oncomine™ Dx Target DNA Control v3 (Part No. A53248)			
	Oncomine™ Dx Target DNA Control v3 (brown cap)	8 × 7 µL (single-use tubes)	-30°C to -10°C
Oncomine™ Dx Target RNA Control v2 (Part No. A53247)			
	Oncomine™ Dx Target RNA Control v2 (white cap)	8 × 7 µL (single-use tubes)	-90°C to -60°C
Oncomine™ Dx Target RNA Control Diluent (Part No. A38872)			
	Oncomine™ Dx Target RNA Control Diluent (blue cap)	8 × 88 µL (single-use tubes)	-90°C to -60°C
Ion Torrent Dx No Template Control Kit (Part No. A32444)			
	No Template Control (purple cap)	8 × 30 µL	15°C to 30°C

Ion PGM™ Dx Library Kit

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

IMPORTANT! Do not mix components from other library kits.

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

Materials and equipment required but not provided

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

Description	Source
Veriti™ Dx 96-well Thermal Cycler, 0.2 mL	4452300
Laminar flow hood	MLS
Dry-bath heaters and aluminum heat blocks (quantity = 3), for use with 1.5-mL tubes	MLS
1.5-mL snap-cap low-retention polypropylene microcentrifuge tubes	MLS
1.5-mL tube rack	MLS

(continued)

Description	Source
Aluminum cold blocks for use with 96-well plates	MLS
Benchtop cold box for use with 1.5-mL tubes	MLS
Microcentrifuge (must accommodate standard 1.5-mL and 0.2-mL microcentrifuge tubes, and generate 20,000 rcf)	MLS
0.2-mL tube adapters	MLS
Mini centrifuge	MLS
96-well plate centrifuge	MLS
Vortex mixer with a rubber platform	MLS
Fluorometer/fluorescence reader (see additional specifications following)	MLS
Tubes or plates for the fluorometer/fluorescence reader	MLS
DynaMag™ Dx 96-Well Plate Magnet magnet	A31347
DynaMag™ Dx 16 2-mL Magnet	A31346
Slide rack, able to hold standard 3" × 1" (75 × 25 mm) slides	MLS
Staining dish or jar, able to hold sufficient liquid to fully submerge the slide rack	MLS
Disposable scalpel with a sterile #10 blade	MLS
RNase decontamination solution	MLS
Absolute ethanol (ACS grade)	MLS
Xylene (ACS grade, ≥98.5%)	MLS
Nuclease-free water	MLS
Single- and multi-channel pipettes (2-, 20-, 200-, 1000-μL)	MLS
Aerosol-barrier pipette tips (2-, 10-, 20-, 200-, 1000-μL)	MLS
Troughs for multi-channel pipettors	MLS
MicroAmp™ Optical 96-well Reaction Plates	4481191 4481192 (with barcode)
Adhesive PCR Plate Seals	AB0558
15-mL and 50-mL conical tubes and tube holders (for preparing bulk solutions)	MLS
5-mL and 25-mL serological pipettes, and pipette controller (for preparing bulk solutions)	MLS

Fluorometer/ fluorescence reader specifications

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

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

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

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

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

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

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

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



Before you begin

Tissue input requirements for FFPE sample extraction

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

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

Sample collection method	Recommended number of sections
Non-small cell lung cancer (NSCLC) and anaplastic thyroid cancer (ATC)	
Resection or surgical biopsies	2 × 5-micron sections
Core needle biopsies	9 × 5-micron sections
Fine needle aspirates	7 × 5-micron sections
Cholangiocarcinoma (CC)	
Resection or surgical biopsies	4 × 5-micron sections
Core needle biopsies	9 × 5-micron sections
Thyroid cancer (TC) and medullary thyroid cancer (MTC)	
Resection or surgical biopsies	2 × 5-micron sections
Core needle biopsies	9 × 5-micron sections
Astrocytoma (AC) and oligodendroglioma (OG)	
Resection or surgical biopsies	2 × 5-micron sections
Core needle biopsies	9 × 5-micron sections

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

Sample storage and stability

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

Blocks and slides (paraffin-dipped or undipped) are stable for up to 12 months at 15–30°C. Stability studies for blocks and slides with DNA insertions are ongoing.

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

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

Procedural guidelines

Definitions

Throughout this guide:

- Room temperature is defined as the temperature range 15–30°C (59–86°F).
- A pulse centrifugation consists of a 3–5 second centrifugation at maximum speed in a mini centrifuge.

Guidelines to prevent cross-contamination



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

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

Reagent contamination

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

Guidelines for FFPE samples

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

Guidelines for RNA

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

Guidelines for mixing reagents

Immediately before each use:

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

Guidelines for pipetting

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

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

Guidelines for freezing and thawing samples

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

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

Guidelines for library preparation

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

Equilibrate materials

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

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

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

Reagent management

Follow the guidelines below for proper reagent storage and use.

Storage

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

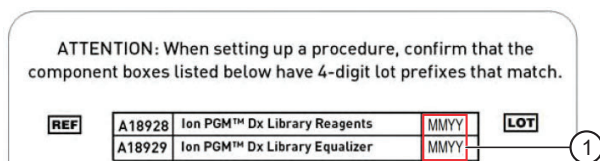
Kit interchangeability and component box lot matching

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

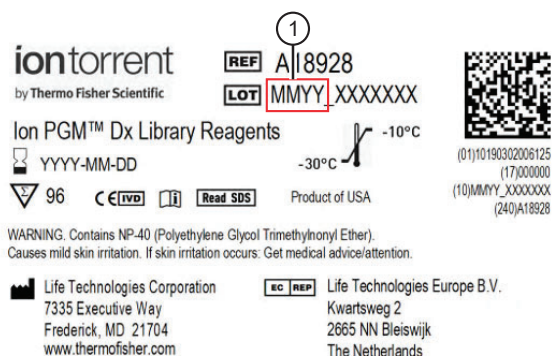
Note: The Oncomine™ Dx Target RNA Control and Oncomine™ Dx Target RNA Control Diluent in the Oncomine™ Dx Target Test, Controls, and Diluent Kit must be lot-matched. However, the controls do not require lot-matching with the Oncomine™ Dx Target Test DNA and RNA Panel.

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

An example inside box lid label is shown below:

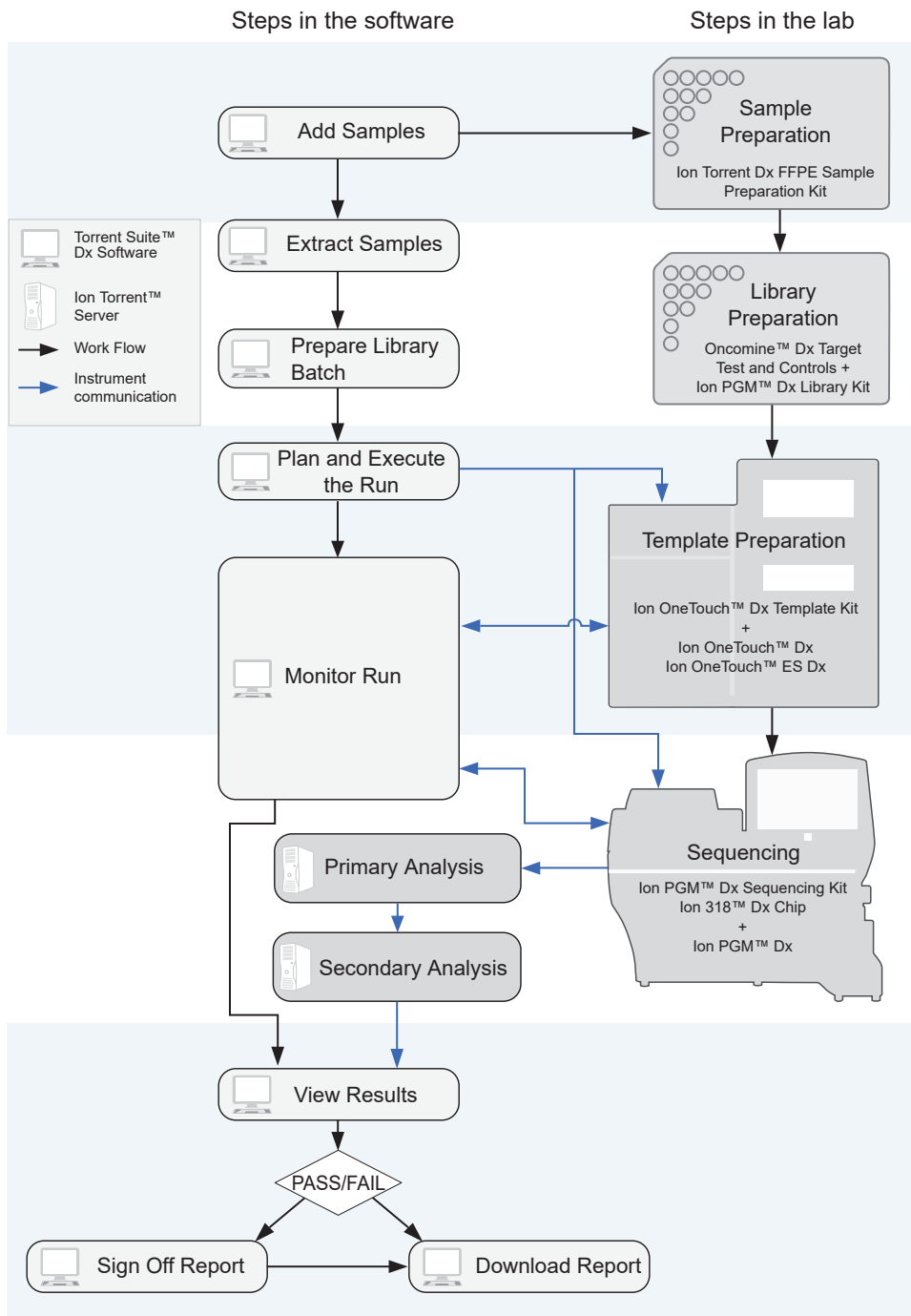


An example box label with lot information is shown below:



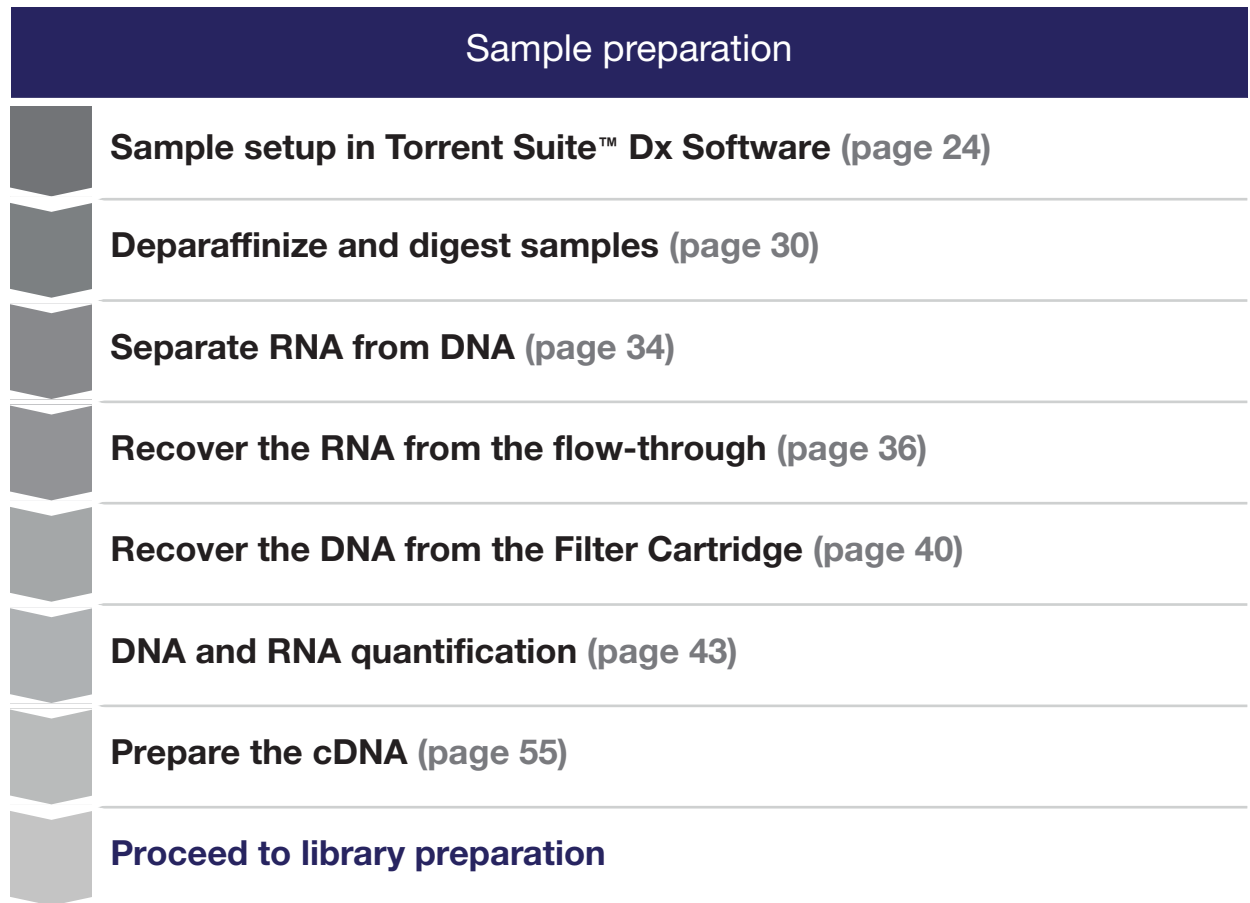
① Lot prefix

Oncomine™ Dx Target Test system diagram

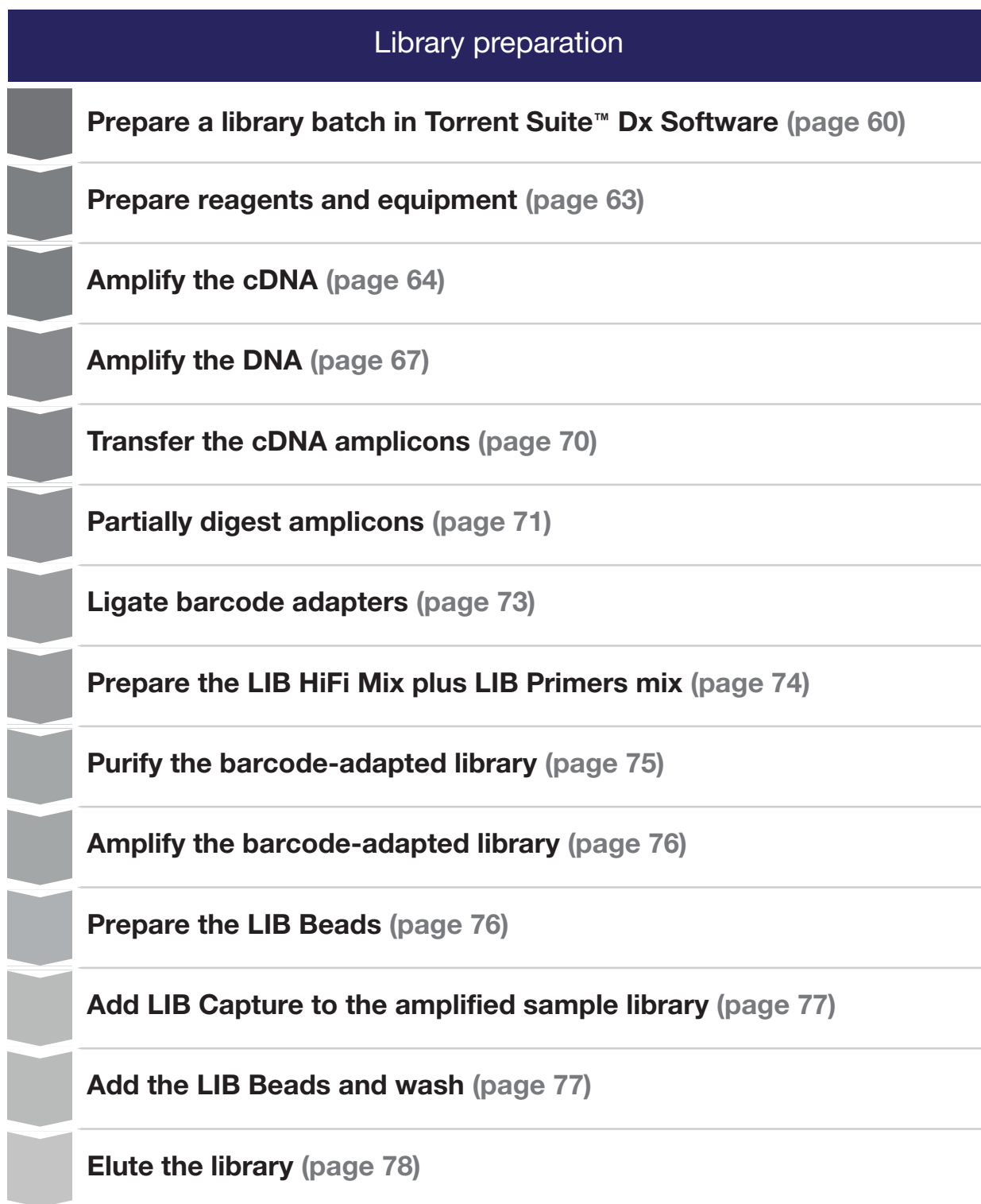


Sample preparation workflow

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



Library preparation workflow



Library preparation

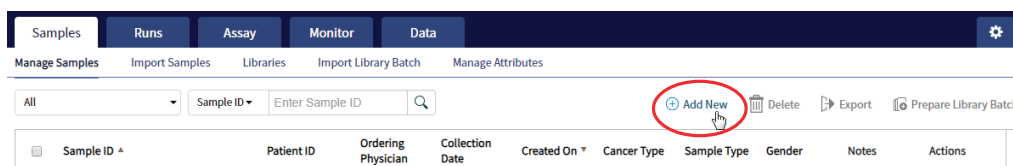
Proceed to *Oncomine™ Dx Target Test Part III: Template Preparation User Guide*

3



Sample setup in Torrent Suite™ Dx Software

Add a new sample

- Under the **Samples** tab, in the **Manage Samples** screen, click **+ Add New**.



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

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

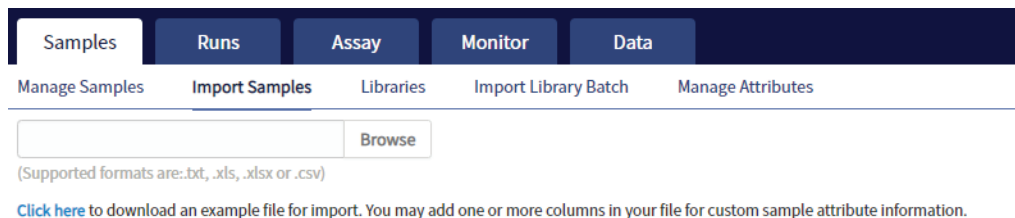
(continued)

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

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

Import samples

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



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

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

2. In the template file, fill in the information for each sample, one sample per row. See “Predefined sample attributes” on page 26 for more information.
3. Save the file.
4. Click **Browse**, navigate to the saved file, then select it.
5. Click **Import**.
A progress bar followed by an import report displays. If the import process fails, an error message indicates the reason for failure (for example, an invalid character was used). For additional troubleshooting, see “Batch sample import fails” on page 81.
6. Click **Manage Samples** to return to the sample list. Successfully imported samples are listed.

Predefined sample attributes

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

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

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

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

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

Enter the Ion Torrent Dx Total Nucleic Acid Isolation Kit barcode


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

1. Above the samples list, select **To Be Extracted** from the **Filter Samples by...** dropdown list to display only those samples that do not have a kit barcode that is associated with them.
2. Select the checkbox of the sample to be extracted. Select multiple samples if you are using the same kit to process them.

The screenshot shows the 'Manage Samples' interface. At the top, there are tabs for 'Samples', 'Runs', 'Assay', 'Monitor', and 'Data'. Below the tabs, there are navigation options: 'Manage Samples', 'Import Samples', 'Libraries', 'Import Library Batch', and 'Manage Attributes'. A dropdown menu is set to 'To Be Extracted'. To the right, there are buttons for 'Add New', 'Delete', 'Export', 'Extract' (circled in red), and 'Prepare Library Batch'. Below this, a table displays sample information:

Sample ID	Patient ID	Ordering Physician	Collection Date	Created On	Cancer Type	Sample Type	Gender	Notes	Actions
<input checked="" type="checkbox"/> BC1	BC1	Smith	2018-09-05	2018-10-06 02:17	Non-small Cell Lung Cancer	DNA	Male		Edit Audit

At the bottom of the table, there is a pagination control showing '1' of 1 items and a '20 items per page' dropdown.

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

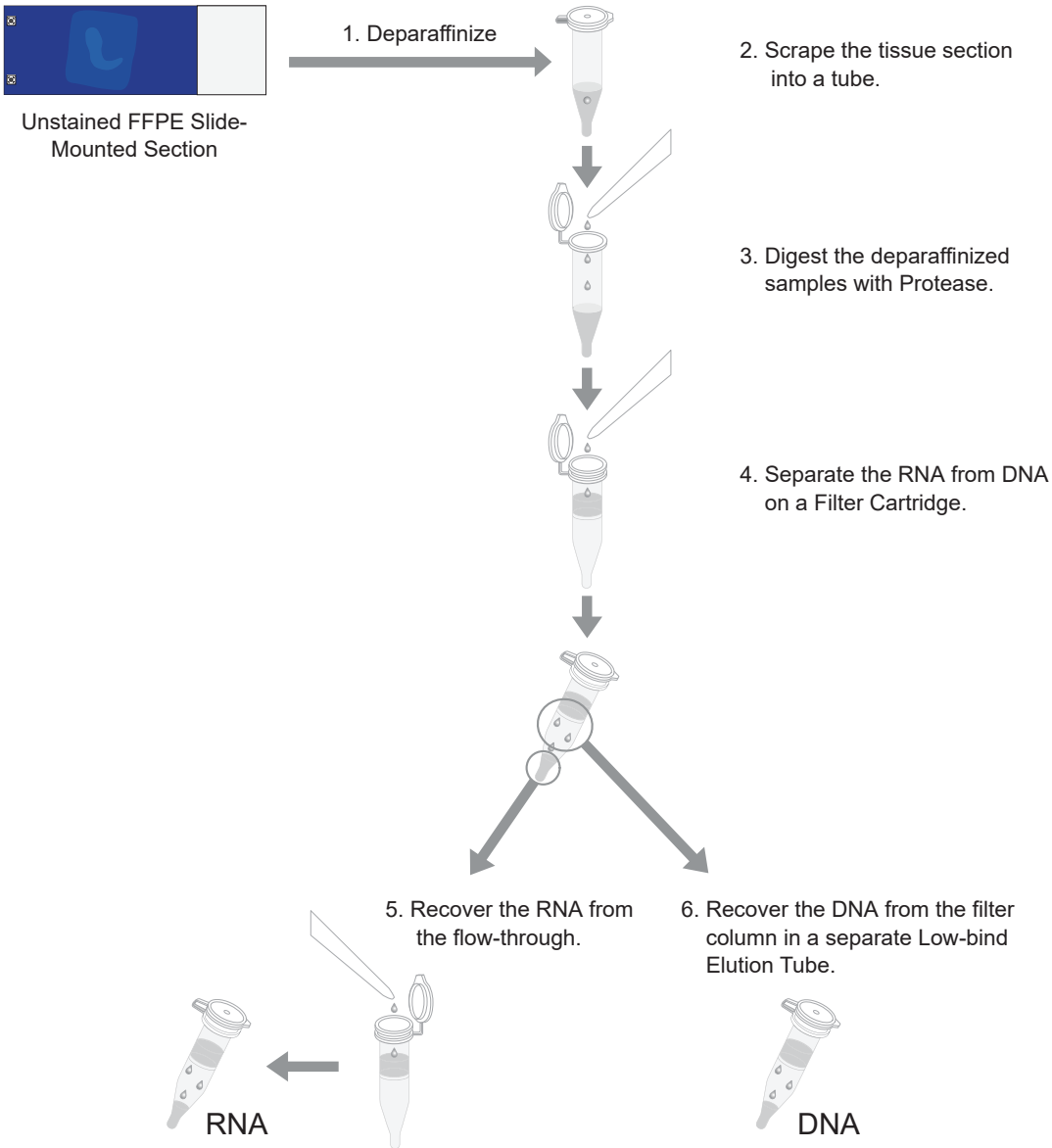
IMPORTANT! Check the expiration date on the box. If the kit is expired, select another kit.

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

4

Isolate RNA and DNA from FFPE samples

Sample extraction workflow



Review the procedural guidelines

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

Ion Torrent Dx FFPE Sample Preparation Kit component lot matching

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

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

Equilibrate the equipment and reagents

Before starting the following procedure:

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

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

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



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

Note: Ensure that the heaters are calibrated.

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

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

Prepare wash buffers

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

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

Deparaffinize and digest samples

Kit components used in this procedure

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

Prepare 1X Digestion Buffer

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

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

	Component	Volume per reaction	
		For ≤6 samples	For ≥7 samples
<input type="checkbox"/>	Digestion Buffer (green cap)	$(n+1) \times 25 \mu\text{L}$	$(n+2) \times 25 \mu\text{L}$
<input type="checkbox"/>	Dilution Solution (black cap)	$(n+1) \times 75 \mu\text{L}$	$(n+2) \times 75 \mu\text{L}$
<input type="checkbox"/>	Total 1X Digestion Buffer	$(n+1) \times 100 \mu\text{L}$	$(n+2) \times 100 \mu\text{L}$

4. Vortex the 1X Digestion Buffer for ~5 seconds to mix, then pulse centrifuge to collect.
5. Add 100 μL of 1X Digestion Buffer to each labeled tube from step 1.

Deparaffinize dipped FFPE slides



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

IMPORTANT! These instructions are only for paraffin-dipped FFPE slides. For slides that have not been dipped in paraffin, see “Deparaffinize undipped FFPE slides” on page 32.

Note:

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

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

IMPORTANT! Use light pressure to prevent cracking the slide.

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

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

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

Note: Properly discard used scalpels.

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

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

15. Proceed to “Collect the tissue” on page 33.

Deparaffinize undipped FFPE slides



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

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

Note:

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

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

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

10. Proceed to “Collect the tissue”.

Collect the tissue

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

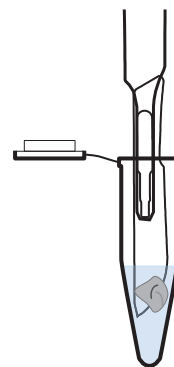
In this procedure, scrape each tissue section into the appropriate labeled 1.5-mL low-retention microcentrifuge tube containing 1X Digestion Buffer (prepared in “Prepare 1X Digestion Buffer” on page 30).

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

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

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

- Carefully insert the scalpel blade with the tissue mass into the 1X Digestion Buffer in the 1.5-mL low-retention microcentrifuge tube. Rinse the tissue from the blade into the buffer, then ensure that the entire mass is in solution.
- Remove and inspect the blade to ensure that no tissue remains on it.
- Inspect the slide to ensure that all the tissue has been removed (the slide should be translucent). Discard the scalpel in a waste container for sharp objects.



Digest the deparaffinized samples

- Flick-mix the Protease (blue cap) 4 times with your finger, then pulse centrifuge to collect the contents.
- Add 4 μ L of Protease to each tissue sample tube.
- Flick-mix each sample tube 4 times, then pulse centrifuge.
- Incubate the samples at 55°C in a calibrated heat block for 1 hour.

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

- Pulse centrifuge to collect any condensation droplets.
- Incubate the samples at 90°C in a calibrated heat block for 1 hour.
- Pulse centrifuge to collect any condensation droplets, then proceed immediately to “Separate RNA from DNA on a Filter Cartridge” on page 36.

Separate RNA from DNA

Kit components used in this procedure

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

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

Label the Filter Cartridges and Collection Tubes

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

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

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

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

Preheat the Elution Solution

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



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

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

Separate RNA from DNA on a Filter Cartridge

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

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

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

IMPORTANT! Do not discard the flow-through in the Collection Tube (labeled with Sample ID and "RNA"). The flow-through contains the RNA.

5. Place the filter column with the bound DNA in a new Collection Tube (B), then store it at 2–8°C for later DNA purification in “Recover the DNA from the Filter Cartridge” on page 40.
6. Proceed to “Recover the RNA from the flow-through” on page 36.

Recover the RNA from the flow-through

Kit components used in this procedure

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

Bind the RNA to the Filter Cartridge

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

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

1. Place the new Filter Cartridge (C) in a tube rack.
2. Add 275 μL of ACS-grade 100% ethanol to the flow-through containing RNA (the tube labeled with the Sample ID and "RNA") from "Separate RNA from DNA on a Filter Cartridge" on page 36.
3. Mix well by pipetting up and down 5 times, then transfer the sample (~450 μL) to the new Filter Cartridge (C).
4. Centrifuge the Filter Cartridge at 10,000 rcf for 30 seconds in a microcentrifuge.
5. Discard the flow-through in the Collection Tube, then reinsert the filter column into the same Collection Tube.
6. Add 600 μL of Wash 1 Buffer (prepared in "Prepare wash buffers" on page 30) to the Filter Cartridge.
7. Centrifuge the Filter Cartridge at 10,000 rcf for 30 seconds.
8. Discard the flow-through, then reinsert the filter column in the same Collection Tube.
9. Centrifuge the Filter Cartridge at 10,000 rcf for 30 seconds to remove any remaining fluid.

Treat the RNA bound to the Filter Cartridge with DNase

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

	Component	Volume per reaction	
		For ≤ 6 samples	For ≥ 7 samples
<input type="checkbox"/>	Dilution Solution (black cap)	$(n+1) \times 50 \mu\text{L}$	$(n+2) \times 50 \mu\text{L}$
<input type="checkbox"/>	10X DNase Buffer (white cap)	$(n+1) \times 6 \mu\text{L}$	$(n+2) \times 6 \mu\text{L}$
<input type="checkbox"/>	DNase (purple cap)	$(n+1) \times 4 \mu\text{L}$	$(n+2) \times 4 \mu\text{L}$
<input type="checkbox"/>	Total Volume	$(n+1) \times 60 \mu\text{L}$	$(n+2) \times 60 \mu\text{L}$

2. Flick the 1X DNase Solution tube 4 times to mix, then pulse centrifuge to collect.
3. Pipet 60 μL of the 1X DNase Solution into the center of each filter column (previously labeled with Sample ID and "RNA").

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

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

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

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

Wash the RNA bound to the Filter Cartridge

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

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

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

Elute the RNA

Use the following components for this procedure.

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

IMPORTANT!

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

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



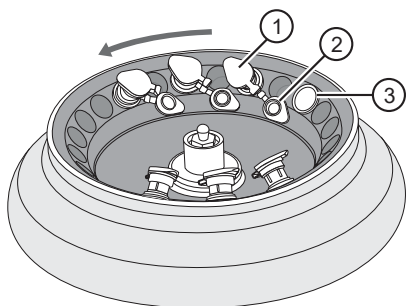
CAUTION! The heat block and Elution Solution are hot. Use care when handling tubes and tube contents to avoid being burned.

2. Wet the pipette tip by slowly pipetting up and down 3 times in the pre-heated Elution Solution.
3. Slowly pipet up 30 µL of Elution Solution, then confirm that there are no large air gaps in the tip (a small air gap at the bottom of the tip is acceptable). Pipet the solution into the center of the filter column in the Low-bind Elution Tube (D) (from step 9 in “Wash the RNA bound to the Filter Cartridge”).

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

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

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



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

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

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

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

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

Recover the DNA from the Filter Cartridge

Wash the DNA bound to the Filter Cartridge

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

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

1. Retrieve the Filter Cartridge (A) with bound DNA and Collection Tube (B) from 2–8°C storage (previously stored in “Separate RNA from DNA on a Filter Cartridge” on page 36).
2. Add 600 µL of Wash 1 Buffer to the filter column.
3. Hold the Filter Cartridge for 30 seconds at room temperature, then centrifuge at 10,000 rcf for 30 seconds.
4. Discard the flow-through, then reinsert the filter column into the same Collection Tube (B).
5. Add 500 µL of Wash 2 Buffer to the filter column, then centrifuge at 10,000 rcf for 30 seconds.
6. Discard the flow-through, then reinsert the filter column into the same Collection Tube (B).
7. Repeat steps 5 and 6 for a second wash.
8. Centrifuge the Filter Cartridge at 20,000–21,000 rcf for 2 minutes to remove any remaining fluid.
9. Remove the filter column from the tube, then touch the bottom of the column with a clean laboratory wipe to wick off any remaining wash buffer.
10. Transfer the filter column to the pre-labeled Low-bind Elution Tube (E).

Elute the DNA

Use the following components for this procedure.

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

IMPORTANT!

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

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



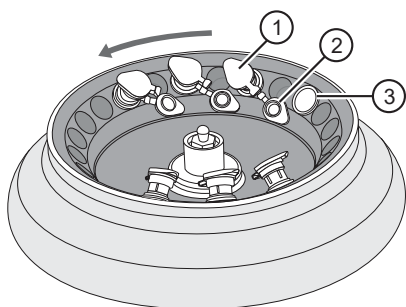
CAUTION! The heat block and Elution Solution are hot. Use care when handling tubes and tube contents to avoid being burned.

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

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

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

4. Close the cap on the filter column, then hold the filter column/Low-bind Elution Tube assembly at room temperature for 1 minute. Close the cap on the Elution Solution tube in the heat block.
5. Insert the filter column/Low-bind Elution Tube assembly in the microcentrifuge in the orientation shown below. To prevent the Low-bind Elution Tube caps from breaking, place a 0.2-mL tube adapter in the position shown.



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

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

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

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

STOPPING POINT If you are not quantifying on the same day, store the recovered DNA aliquots at –30°C to –10°C for up to 12 months. Stability studies for extracted DNA (insertions) are ongoing, but a minimum stability of 5 months, including one freeze-thaw cycle, has been established.



DNA and RNA quantification

Review the procedural guidelines

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

Prepare the reagents and equipment

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

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

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

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

Set up the DNA quantification assay

Kit components used in this procedure

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

Prepare the DNA Working Solution

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

IMPORTANT! We recommend using 4 standards. If your quantification system does not allow the use of 4 standards, use the maximum allowed by the system. At a minimum, you must use the 0 ng/μL and 10 ng/μL DNA standards. Note that R² values should only be evaluated when 3 or more standards are used.

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

$$\mathbf{S \text{ (\# of standards)} + N \text{ (\# of samples)} + 1 = \# \text{ of reactions}}$$

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

$$\mathbf{\# \text{ reactions} \times 1 \text{ } \mu\text{L (DNA Dye Reagent)} = \text{total volume of DNA Dye Reagent}}$$

$$\mathbf{\# \text{ reactions} \times 199 \text{ } \mu\text{L (DNA Buffer)} = \text{total volume of DNA Buffer}}$$

4. Mix the DNA Buffer and DNA Dye Reagent bottles by inverting 5 times.
5. Prepare the DNA Working Solution: Pipet the calculated volume of DNA Buffer into a pre-labeled tube, then add the calculated volume of DNA Dye Reagent into the same tube.
6. Vortex the tube for ~5 seconds, then proceed to the next steps.

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

Prepare the DNA standards

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

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

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

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

Prepare the DNA samples

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

Set up the RNA quantification assay

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

Kit components used in this procedure

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

Prepare the RNA working solution

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

IMPORTANT! We recommend using 4 standards. If your quantification system does not allow the use of 4 standards, use the maximum allowed by the system. At a minimum, you must use the 0 ng/μL and 10 ng/μL RNA standards. Note that R² values should only be evaluated when 3 or more standards are used.

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

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

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

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

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

4. Mix the RNA Buffer and RNA Dye Reagent bottles by inverting 5 times.
5. Prepare the RNA Working Solution: Pipet the total volume of RNA Buffer into a pre-labeled tube, then add the total volume of RNA Dye Reagent into the same tube.
6. Vortex the tube for ~5 seconds, then proceed to the next steps.

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

Prepare the RNA standards

1. Add 190 μL of RNA Working Solution to each well or tube that will contain an RNA standard.
2. Flick mix each RNA Standard 4 times, then pulse centrifuge. Refer to the following table of RNA Standards and concentrations.

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

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

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

Prepare the RNA samples

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

Run the quantification assays

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

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

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

Sample type	Required concentration	Required R^2 value ^[1]
DNA	$\geq 0.83 \text{ ng}/\mu\text{L}$	≥ 0.99
RNA	$\geq 1.43 \text{ ng}/\mu\text{L}$	≥ 0.98

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

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

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

STOPPING POINT If you do not dilute the sample on the same day, store the quantified DNA sample at -30°C to -10°C for up to 12 months (SNVs and deletions) and the quantified RNA sample at -90°C to -60°C for up to 5 months. Stability studies for extracted DNA with insertions are ongoing, but a minimum stability of 5 months has been established.

Dilute the samples

Kit components used in this procedure

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

Sample Dilution Calculator

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

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

IMPORTANT!

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

Dilution volume guidelines

- Unless the test or study protocol dictates that multiple libraries must be made from a single sample (for example, replicate libraries), dilute only sufficient sample to prepare a single library.
- The Sample Dilution Calculator includes a 10% overage in its calculations. For manually calculated dilutions, include a 10% overage, except for low-concentration DNA (0.83–0.97 ng/μL) and RNA (1.43–1.93 ng/μL) samples where there is insufficient sample to include an overage.
- The default **Sample Volume (X1)** in the Sample Dilution Calculator is 2 μL. If needed, increase the **Sample Volume (X1)** until the **Volume of Dilution Solution (Y1)** is ≥2 μL. When all volumes are ≥2 μL, red highlighted table cells turn white, indicating sufficient volumes for accurate pipetting.
- In the following procedure, we recommend that you do not pipet volumes <2.0 μL. For samples that are ≥6.01 ng/μL, perform a normalization dilution to an intermediate concentration of 5 ng/μL, then further dilute the sample to a final concentration of 0.83 ng/μL for DNA and 1.43 ng/μL for RNA.
- Low-concentration DNA (0.83–0.97 ng/μL) and RNA (1.43–1.93 ng/μL) samples require pipetting volumes <2 μL to achieve the correct concentration. When necessary, only pipet volumes <2 μL with a pipette designed and calibrated to dispense volumes <2 μL.
- Do not use the Sample Dilution Calculator for low-concentration DNA (0.83–0.97 ng/μL) and RNA (1.43–1.93 ng/μL) samples. Instead, follow the detailed procedures that are provided in the second row of the tables on page 49 and page 51.
- Samples that do not meet the minimum concentration specification (DNA samples <0.83 ng/μL and RNA samples <1.43 ng/μL) must not be used for library preparation.

Thaw frozen samples

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

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

Dilute DNA samples

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

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

DNA concentration	Dilution procedure
<0.83 ng/ μL	Samples do not meet the minimum concentration specification and must not be used for library preparation.
0.83–0.97 ng/ μL	<p>Dilute the DNA sample to 0.83 ng/μL in a total volume of 12 μL.</p> <ol style="list-style-type: none"> Determine the volume of DNA sample required ($10 \text{ ng} \div \text{Sample concentration ng}/\mu\text{L} = N \mu\text{L}$). Add Dilution Solution if required: <ul style="list-style-type: none"> If N is <12 μL, pipet $N \mu\text{L}$ of DNA sample into a new labeled 1.5-mL low-retention microcentrifuge tube, then add $(12 - N \mu\text{L})$ of Dilution Solution, for a total volume of 12 μL. If $N = 12 \mu\text{L}$, skip to step 4. Pipet up and down 5 times to mix, then pulse centrifuge. Proceed directly to “Dilute RNA samples” on page 51. Do not store the diluted DNA samples for longer than necessary. <p>Note: Store the remaining undiluted DNA sample at -30°C to -10°C for up to 12 months. Stability studies for extracted DNA (insertions) are ongoing, but a minimum stability of 5 months, including one freeze-thaw cycle, has been established.</p>
0.98–6.00 ng/ μL	<p>Perform the Direct Dilution to 0.83 ng/μL.</p> <ol style="list-style-type: none"> Open <code>SFW0000786_ODxTT_SampleDilution_Calculator_3.02.xlsm</code> in Microsoft™ Excel™, select the Calculator worksheet tab, then enter the Operator and Date Completed information. In column B, enter the Sample ID. <p>Note: Only alphanumeric characters (0–9 and A to Z), full stops/periods (.), underscores (_), or hyphens (-) are allowed.</p> In column C, enter the DNA sample concentration (C1) in ng/μL determined in the quantification assay. Pipet the indicated volume (X3) of undiluted DNA sample into a new labeled 1.5-mL low-retention microcentrifuge tube, then add the indicated volume of Dilution Solution (Y3). Pipet up and down 5 times to mix, then pulse centrifuge. Proceed directly to “Dilute RNA samples” on page 51. Do not store the diluted DNA samples for longer than necessary. <p>Note: Store the remaining undiluted DNA sample at -30°C to -10°C for up to 12 months. Stability studies for extracted DNA (insertions) are ongoing, but a minimum stability of 5 months, including one freeze-thaw cycle, has been established.</p>

(continued)

DNA concentration	Dilution procedure
6.01–9.99 ng/μL	<p>Perform Normalization Dilution #1 and Normalization Dilution #2 to a final concentration of 0.83 ng/μL.</p> <p>Note: The default Sample Volume (X1) is 2 μL. When all volumes are ≥2 μL, red highlighted table cells turn white, indicating sufficient volumes for accurate pipetting have been met.</p> <ol style="list-style-type: none"> 1. Open SFW0000786_ODxTT_SampleDilution_Calculator_3.02.xlsm in Microsoft™ Excel™, select the Calculator worksheet tab, then enter the Operator and Date Completed information. 2. In column B, enter the Sample ID. <p>Note: Only alphanumeric characters (0–9 and A to Z), full stops/periods (.), underscores (_), or hyphens (-) are allowed.</p> 3. In column C, enter the DNA sample concentration (C1) in ng/μL determined in the quantification assay. 4. Increase the value for Sample Volume (X1) until the Volume of Dilution Solution (Y1) is ≥2 μL. 5. Pipet the indicated volume (X1) of undiluted DNA sample into a new labeled 1.5-mL low-retention microcentrifuge tube, then add the indicated volume of Dilution Solution (Y1). 6. Pipet up and down 5 times to mix, then pulse centrifuge. 7. Pipet the indicated volume (X2) of diluted DNA sample (5 ng/μL) into a new labeled 1.5-mL low-retention microcentrifuge tube, then add the indicated volume of Dilution Solution (Y2). 8. Pipet up and down 5 times to mix, then pulse centrifuge. 9. Proceed directly to “Dilute RNA samples” on page 51. Do not store the diluted DNA samples for longer than necessary. <p>Note: Store the remaining Normalization Dilution #1 (5 ng/μL) and undiluted DNA sample at –30°C to –10°C for up to 12 months. Stability studies for extracted DNA (insertions) are ongoing, but a minimum stability of 5 months, including one freeze-thaw cycle, has been established.</p>

(continued)

DNA concentration	Dilution procedure
≥10 ng/μL	<p>Perform Normalization Dilution #1 and Normalization Dilution #2 to a final concentration of 0.83 ng/μL.</p> <p>Note: The default Sample Volume (X1) is 2 μL. When all volumes are ≥2 μL, red highlighted table cells turn white, indicating sufficient volumes for accurate pipetting have been met.</p> <ol style="list-style-type: none"> 1. Open SFW0000786_ODxTT_SampleDilution_Calculator_3.02.xlsm in Microsoft™ Excel™, select the Calculator worksheet tab, then enter the Operator and Date Completed information. 2. In column B, enter the Sample ID. <p>Note: Only alphanumeric characters (0–9 and A to Z), full stops/periods (.), underscores (_), or hyphens (-) are allowed.</p> 3. In column C, enter the DNA sample concentration (C1) in ng/μL determined in the quantification assay. 4. Pipet the indicated volume (X1) of undiluted DNA sample into a new labeled 1.5-mL low-retention microcentrifuge tube, then add the indicated volume of Dilution Solution (Y1). 5. Pipet up and down 5 times to mix, then pulse centrifuge. 6. Pipet the indicated volume (X2) of diluted DNA sample (5 ng/μL) into a new labeled 1.5-mL low-retention microcentrifuge tube, then add the indicated volume of Dilution Solution (Y2). 7. Pipet up and down 5 times to mix, then pulse centrifuge. 8. Proceed directly to “Dilute RNA samples” on page 51. Do not store the diluted DNA samples for longer than necessary. <p>Note: Store the remaining Normalization Dilution #1 (5 ng/μL) and undiluted DNA sample at –30°C to –10°C for up to 12 months. Stability studies for extracted DNA (insertions) are ongoing, but a minimum stability of 5 months, including one freeze-thaw cycle, has been established.</p>

Dilute RNA samples

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

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

RNA concentration	Dilution procedure
<1.43 ng/μL	Samples do not meet the minimum concentration specification and must not be used for library preparation.
1.43–1.93 ng/μL	<p>Dilute the RNA sample to 1.43 ng/μL in a total volume of 7 μL.</p> <ol style="list-style-type: none"> Determine the volume of RNA sample required ($10 \text{ ng} \div \text{Sample concentration ng/}\mu\text{L} = \text{N } \mu\text{L}$). Add Dilution Solution if required: <ul style="list-style-type: none"> If N is <7 μL, pipet N μL of RNA sample into a new labeled 1.5-mL low-retention microcentrifuge tube, then add (7 – N μL) of Dilution Solution, for a total volume of 7 μL. If N = 7 μL, skip to step 4. Pipet up and down 5 times to mix, then pulse centrifuge. Proceed directly to “Reverse transcribe the RNA” on page 56. Do not store the diluted RNA samples for longer than necessary. <p>Note: Store the remaining undiluted RNA sample at –90°C to –60°C for up to 12 months.</p>
1.94–6.00 ng/μL	<p>Perform the Direct Dilution to 1.43 ng/μL.</p> <ol style="list-style-type: none"> Open SFW0000786_ODxTT_SampleDilution_Calculator_3.02.xlsm in Microsoft™ Excel™, select the Calculator worksheet tab, then enter the Operator and Date Completed information. In column B, enter the Sample ID. <p>Note: Only alphanumeric characters (0–9 and A to Z), full stops/periods (.), underscores (_), or hyphens (-) are allowed.</p> In column C, enter the RNA sample concentration (C1) in ng/μL determined in the quantification assay. Pipet the indicated volume (X3) of undiluted RNA sample into a new labeled 1.5-mL low-retention microcentrifuge tube, then add the indicated volume of Dilution Solution (Y3). Pipet up and down 5 times to mix, then pulse centrifuge. Proceed directly to “Reverse transcribe the RNA” on page 56. Do not store the diluted RNA samples for longer than necessary. <p>Note: Store the remaining undiluted RNA sample at –90°C to –60°C for up to 12 months.</p>

(continued)

RNA concentration	Dilution procedure
6.01–9.99 ng/μL	<p>Perform Normalization Dilution #1 and Normalization Dilution #2 to a final concentration of 1.43 ng/μL.</p> <p>Note: The default Sample Volume (X1) is 2 μL. When all volumes are ≥2 μL, red highlighted table cells turn white, indicating sufficient volumes for accurate pipetting have been met.</p> <ol style="list-style-type: none"> 1. Open SFW0000786_ODxTT_SampleDilution_Calculator_3.02.xlsm in Microsoft™ Excel™, select the Calculator worksheet tab, then enter the Operator and Date Completed information. 2. In column B, enter the Sample ID. <p>Note: Only alphanumeric characters (0–9 and A to Z), full stops/periods (.), underscores (_), or hyphens (-) are allowed.</p> 3. In column C, enter the RNA sample concentration (C1) in ng/μL determined in the quantification assay. 4. Increase the value for Sample Volume (X1) until the Volume of Dilution Solution (Y1) is ≥2 μL. 5. Pipet the indicated volume (X1) of undiluted RNA sample into a new labeled 1.5-mL low-retention microcentrifuge tube, then add the indicated volume of Dilution Solution (Y1). 6. Pipet up and down 5 times to mix, then pulse centrifuge. 7. Pipet the indicated volume (X2) of diluted RNA sample (5 ng/μL) into a new labeled 1.5-mL low-retention microcentrifuge tube, then add the indicated volume of Dilution Solution (Y2). 8. Pipet up and down 5 times to mix, then pulse centrifuge. 9. Proceed directly to “Reverse transcribe the RNA” on page 56. Do not store the diluted RNA samples for longer than necessary. <p>Note: Store the remaining Normalization Dilution #1 (5 ng/μL) and undiluted RNA sample at –90°C to –60°C for up to 12 months.</p>

(continued)

RNA concentration	Dilution procedure
≥10 ng/μL	<p>Perform Normalization Dilution #1 and Normalization Dilution #2 to a final concentration of 1.43 ng/μL.</p> <p>Note: The default Sample Volume (X1) is 2 μL. When all volumes are ≥2 μL, red highlighted table cells turn white, indicating sufficient volumes for accurate pipetting have been met.</p> <ol style="list-style-type: none"> 1. Open SFW0000786_ODxTT_SampleDilution_Calculator_3.02.xlsm in Microsoft™ Excel™, select the Calculator worksheet tab, then enter the Operator and Date Completed information. 2. In column B, enter the Sample ID. <p>Note: Only alphanumeric characters (0–9 and A to Z), full stops/periods (.), underscores (_), or hyphens (-) are allowed.</p> 3. In column C, enter the RNA sample concentration (C1) in ng/μL determined in the quantification assay. 4. Pipet the indicated volume (X1) of undiluted RNA sample into a new labeled 1.5-mL low-retention microcentrifuge tube, then add the indicated volume of Dilution Solution (Y1). 5. Pipet up and down 5 times to mix, then pulse centrifuge. 6. Pipet the indicated volume (X2) of diluted RNA sample (5 ng/μL) into a new labeled 1.5-mL low-retention microcentrifuge tube, then add the indicated volume of Dilution Solution (Y2). 7. Pipet up and down 5 times to mix, then pulse centrifuge. 8. Proceed directly to “Reverse transcribe the RNA” on page 56. Do not store the diluted RNA samples for longer than necessary. <p>Note: Store the remaining Normalization Dilution #1 (5 ng/μL) and undiluted RNA sample at –90°C to –60°C for up to 12 months.</p>

6

Prepare the cDNA

Review the procedural guidelines

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

Kit components used in this procedure

Kit component	Box
5X Reaction Mix (red cap) 10X Enzyme Mix (green cap)	Ion Torrent Dx cDNA Synthesis Kit (Part No. A32436, stored at -30°C to -10°C)
Oncomine™ Dx Target RNA Control v2 (white cap; single-use tubes)	Oncomine™ Dx Target RNA Control v2 (Part No. A53247, stored at -90°C to -60°C)
Oncomine™ Dx Target RNA Control Diluent (blue cap; single-use tubes)	Oncomine™ Dx Target RNA Control Diluent (Part No. A38872, stored at -90°C to -60°C)
No Template Control (purple cap)	Ion Torrent Dx No Template Control Kit (Part No. A32444, stored at 15°C to 30°C)

IMPORTANT! Control tubes have significant volume overage to compensate for evaporation or absorption by the tube. Tubes containing control reagents are single-use. Controls should be thawed once and used immediately after thawing—do not re-freeze unused volume. Multiple freeze-thaws of unused volume can reduce the performance of the controls.

Oncomine™ Dx Target Test Controls lot matching

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

Component	Part No.	Storage
Oncomine™ Dx Target RNA Control v2	A53247	-90°C to -60°C
Oncomine™ Dx Target RNA Control Diluent	A38872	-90°C to -60°C

Thaw then dilute frozen samples

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

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

Reverse transcribe the RNA

Perform the following steps in a laminar flow hood.

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

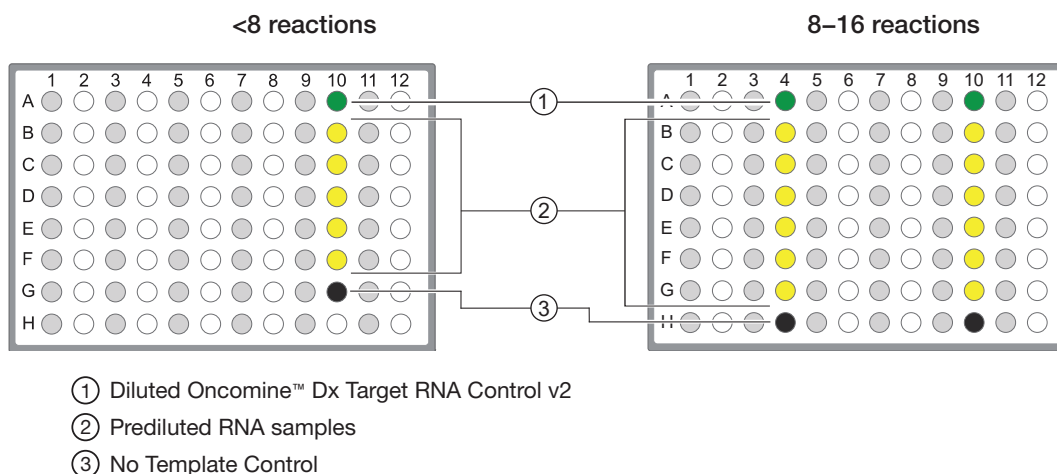
IMPORTANT! For every 6 samples, there must be one No Template Control (NTC) and one Oncomine™ Dx Target RNA Control v2.

1. Vortex the No Template Control (purple cap) and Dilution Solution (black cap) for ~5 seconds each, then pulse centrifuge.
2. Flick the prediluted RNA sample (1.43 ng/μL), the single-use Oncomine™ Dx Target RNA Control v2 tube (white cap), and the 10X Enzyme Mix (green cap) 4 times each to mix, then pulse centrifuge.

IMPORTANT! Do not vortex the prediluted RNA sample.

3. Label a MicroAmp™ Optical 96-well Reaction Plate with "RNA/cDNA".
4. Place the labeled 96-well plate on a 2–8°C aluminum cold block, then set up the reactions in the designated wells of the plate. Configure the plate for <8 reactions or 8–16 reactions as shown in the figure.

IMPORTANT! Include one No Template Control and one Oncomine™ Dx Target RNA Control v2 well for each column, as illustrated.



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

5. To each No Template Control well, add 7 μ L of No Template Control.
6. Flick the Oncomine™ Dx Target RNA Control v2 and Oncomine™ Dx Target RNA Control Diluent tubes 4 times to mix, then pulse centrifuge to collect.
7. Dilute the Oncomine™ Dx Target RNA Control v2. Add the following components to a 1.5-mL low-retention microcentrifuge tube in the order indicated

Note:

- When preparing multiple replicates of the control, create a separate dilution for each replicate.
- The Oncomine™ Dx Target RNA Control v2 and Oncomine™ Dx Target RNA Control Diluent tubes are single-use only. Discard unused volume.

	Order	Component	Volume per reaction
<input type="checkbox"/>	1	Oncomine™ Dx Target RNA Control v2 (white cap)	3 μ L
<input type="checkbox"/>	2	Oncomine™ Dx Target RNA Control Diluent (blue cap)	72 μ L

8. Flick the diluted Oncomine™ Dx Target RNA Control v2 tube 4 times to mix, then pulse centrifuge to collect.
9. To each Oncomine™ Dx Target RNA Control v2 well, add the following components in the order indicated:

	Order	Component	Volume per reaction
<input type="checkbox"/>	1	Diluted Oncomine™ Dx Target RNA Control v2	3 μ L
<input type="checkbox"/>	2	Dilution Solution (black cap)	4 μ L

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

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

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

	Component	Volume per reaction
<input type="checkbox"/>	5X Reaction Mix (red cap)	(n+1) \times 2 μ L
<input type="checkbox"/>	10X Enzyme Mix (green cap)	(n+1) \times 1 μ L
<input type="checkbox"/>	Total	(n+1) \times 3 μL

12. Flick the master mix tube 4 times to mix, then pulse centrifuge to collect.
13. Pipet 3 μ L of the master mix into each RNA sample, No Template Control, and Oncomine™ Dx Target RNA Control v2 well in the 96-well plate.

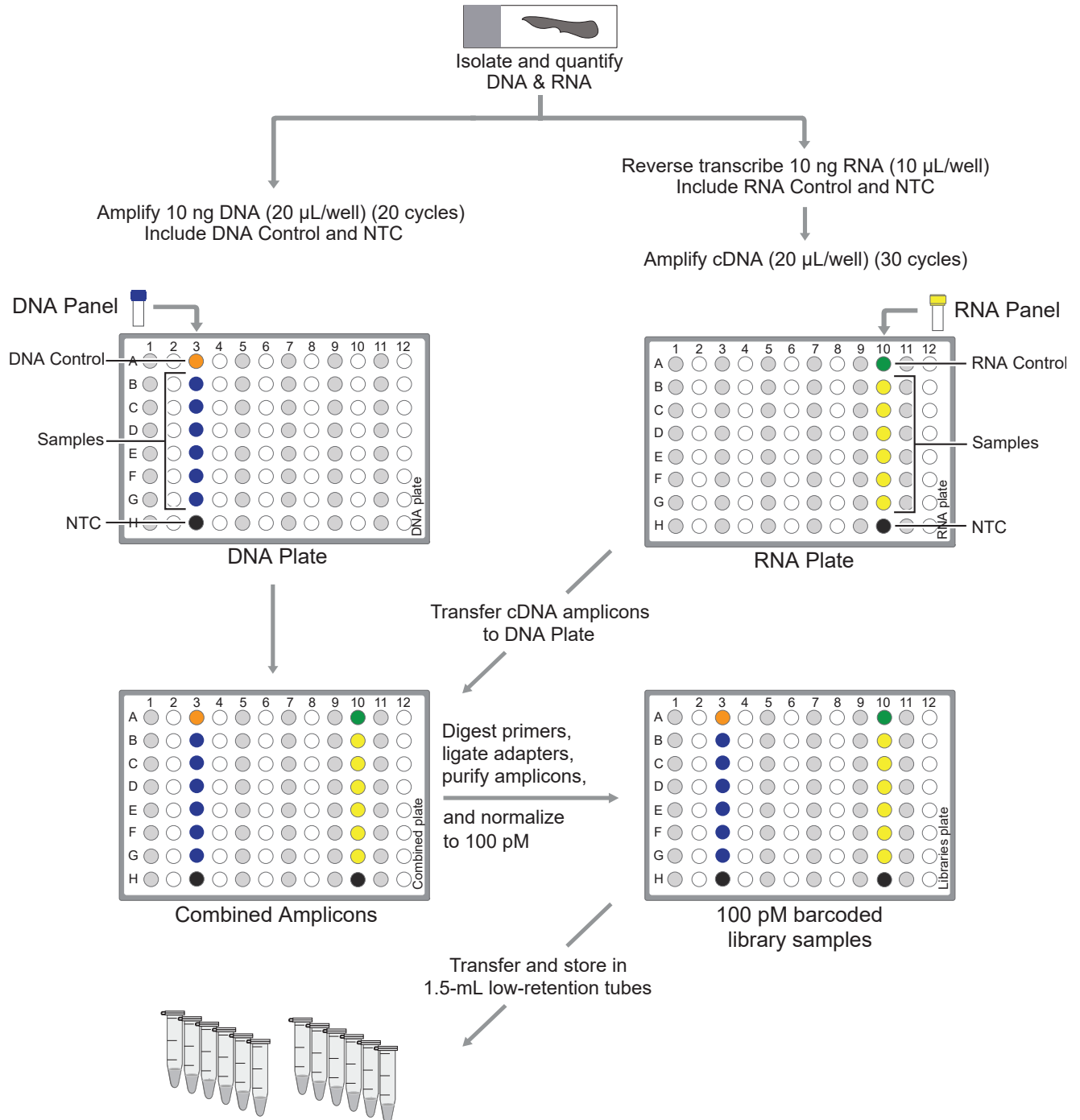
14. Set a pipette to 8 μ L, then pipet the contents of each reaction well up and down 5 times to mix.
15. Seal the plate with an Adhesive PCR Plate Seal, then centrifuge the plate at 100 rcf for 30 seconds.
16. Load the plate in the Veriti™ Dx 96-well Thermal Cycler, then select the **1 ODxTT cDNA Synthesis** program. Select **View**, then confirm that the steps in the program match those in the following table.

	Temperature	Time
<input type="checkbox"/>	42°C	30 minutes
<input type="checkbox"/>	85°C	5 minutes
<input type="checkbox"/>	10°C	Hold (up to 1 hour)

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

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

Library preparation workflow diagram



Prepare a library batch in Torrent Suite™ Dx Software

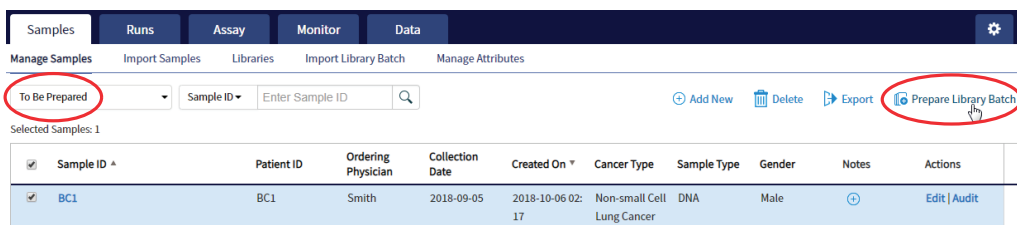
In Torrent Suite™ Dx Software, samples that are entered into the software are placed in library batches for processing and tracking. A library batch consists of a group of libraries that are prepared at the same time.

Note:

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

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

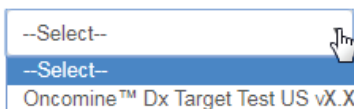
IMPORTANT! Samples that have not been queued for extraction in the software also appear on this tab. Ensure that the samples have been queued for extraction before queuing them for library batch preparation.



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

Prepare Library Batch




Select Assay:



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

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

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

Barcode field	Kit	Kit box	Storage	Label scanned
Library Kit Barcode	Ion PGM™ Dx Library Kit	Ion PGM™ Dx Library Reagents	-30°C to -10°C	
Panel Kit Barcode	Oncomine™ Dx Target Test Panel	Oncomine™ Dx Target Test DNA and RNA Panel (box 1 of 3)	-30°C to -10°C	
Control Kit Barcode	Oncomine™ Dx Target Test, Controls, and Diluent Kit	Oncomine™ Dx Target DNA Control v3 (box 2 of 3)	-30°C to -10°C	

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

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

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

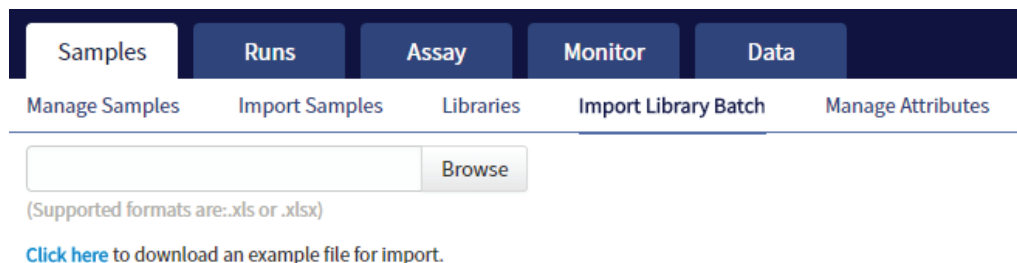
Note: Each library in a library batch must have a different Barcode ID. When preparing the physical libraries, we recommend swapping barcodes between DNA and RNA libraries in consecutive sequencing runs to prevent carryover contamination. See “Alternating barcodes” on page 72.

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

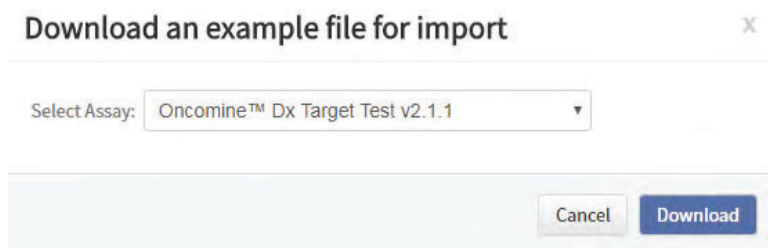
- In the **Input Quantity** field, enter 10 ng for each library.
- Click **Save** to save your selections and close the dialog. The **Libraries** screen opens, listing the libraries that you created. Libraries that are prepared in the same batch have the same **Library Batch ID**.

Import a library batch

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



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



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

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

6. Click **Import.**

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

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

Prepare reagents and equipment

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

Ion PGM™ Dx Library Kit component lot matching

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

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

Amplify the cDNA

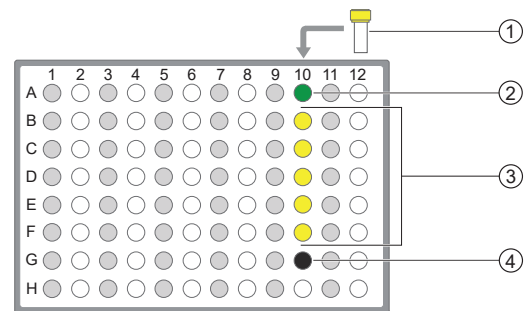
Kit components used in this procedure

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

Set up the cDNA amplification reaction (<8 reactions)

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

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



- ① Oncomine™ Dx Target Test—RNA panel
- ② Oncomine™ Dx Target RNA Control v2
- ③ cDNA samples
- ④ No Template Control

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

	Component	Volume
<input type="checkbox"/>	Nuclease-free Water	4 µL
<input type="checkbox"/>	Oncomine™ Dx Target Test—RNA panel (yellow cap)	2 µL
<input type="checkbox"/>	LIB HiFi Mix (red cap)	4 µL
<input type="checkbox"/>	Total volume per well (includes 10 µL from cDNA synthesis)	20 µL

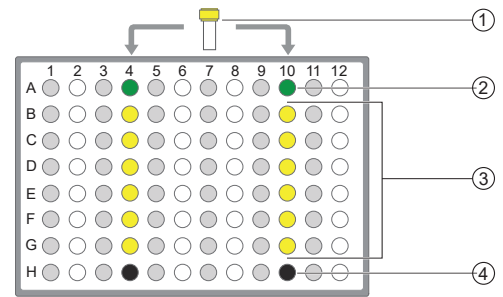
5. With the pipettor set to 15 µL, pipet up and down 5 times to mix the contents of each reaction well.
6. Proceed to “Amplify the cDNA targets” on page 66.

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

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

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

1. Remove the “RNA/cDNA” plate from the thermal cycler, then centrifuge the plate at 100 rcf for 30 seconds.
2. Transfer the plate to a chilled (2–8°C) 96-well aluminum block.
3. Vortex the Oncomine™ Dx Target Test—RNA panel for ~5 seconds, then pulse centrifuge. Flick the tube of LIB HiFi Mix 4 times to mix, then pulse centrifuge.
4. Calculate the amounts of the following components needed for n+1 reactions, then add the components to a single 1.5-mL low-retention microcentrifuge tube.



- ① Oncomine™ Dx Target Test—RNA panel
- ② Oncomine™ Dx Target RNA Control v2
- ③ cDNA samples
- ④ No Template Control

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

	Component	Volume per reaction
<input type="checkbox"/>	Nuclease-Free Water	(n+1) × 4 μL
<input type="checkbox"/>	Oncomine™ Dx Target Test—RNA panel (yellow cap)	(n+1) × 2 μL
<input type="checkbox"/>	LIB HiFi Mix (red cap)	(n+1) × 4 μL
<input type="checkbox"/>	Total	(n+1) × 10 μL

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

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

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

Amplify the cDNA targets

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

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

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

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

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

Amplify the DNA

Kit components used in this procedure

Kit component	Box
No Template Control (purple cap)	Ion Torrent Dx No Template Control Kit (Part No. A32444, stored at 15°C to 30°C)
Oncomine™ Dx Target Test—DNA panel (blue cap)	Oncomine™ Dx Target Test DNA and RNA Panel (Part No. A32441, stored at –30°C to –10°C)
LIB HiFi Mix (red cap)	Ion PGM™ Dx Library Reagents (Part No. A18928, stored at –30°C to –10°C)
Dilution Solution (black cap)	Ion Torrent Dx Sample Dilution Kit (Part No. A32439, stored at 15°C to 30°C)
Oncomine™ Dx Target DNA Control v3 (brown cap; single-use tubes)	Oncomine™ Dx Target DNA Control v3 (Part No. A53248, stored at –30°C to –10°C)

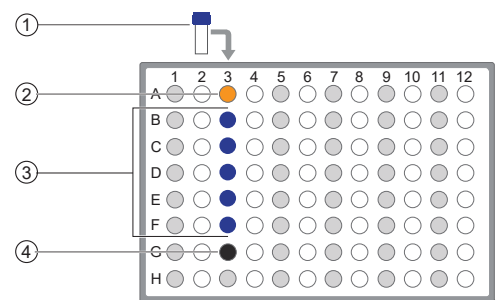
IMPORTANT! Control tubes have significant volume overage to compensate for evaporation or absorption by the tube. Tubes containing control reagents are single-use. Controls should be thawed once and used immediately after thawing—do not re-freeze unused volume. Multiple freeze-thaws of unused volume can reduce the performance of the controls.

Set up the DNA amplification reaction (<8 reactions)

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

For <8 reactions, set up individual reactions, including a No Template Control (purple cap), an Oncomine™ Dx Target DNA Control v3 (brown cap), and up to 5 clinical samples.

1. Label a 96-well plate "DNA".
2. Place the labeled 96-well plate on a 2–8°C chilled 96-well aluminum block, then set up individual reactions in an odd-numbered column. For every run, include the No Template Control and the Oncomine™ Dx Target DNA Control v3.
3. Vortex the Oncomine™ Dx Target Test—DNA panel for ~5 seconds, then pulse centrifuge. Flick the tube of LIB HiFi Mix 4 times to mix, then pulse centrifuge.



- ① Oncomine™ Dx Target Test—DNA panel
- ② Oncomine™ Dx Target DNA Control v3
- ③ Prediluted FFPE DNA sample
- ④ No Template Control

4. To the No Template Control well, add the following components in the order indicated:

	Order	Component	Volume
<input type="checkbox"/>	1	No Template Control (purple cap)	12 μ L
<input type="checkbox"/>	2	Oncomine™ Dx Target Test—DNA panel (blue cap)	4 μ L
<input type="checkbox"/>	3	LIB HiFi Mix (red cap)	4 μ L
<input type="checkbox"/>	—	Total	20 μL

5. To the Oncomine™ Dx Target DNA Control v3 well, add the following components in the order indicated:

	Order	Component	Volume
<input type="checkbox"/>	1	Dilution Solution (black cap)	9 μ L
<input type="checkbox"/>	2	Oncomine™ Dx Target DNA Control v3 (brown cap)	3 μ L
<input type="checkbox"/>	3	Oncomine™ Dx Target Test—DNA panel (blue cap)	4 μ L
<input type="checkbox"/>	4	LIB HiFi Mix (red cap)	4 μ L
<input type="checkbox"/>	—	Total	20 μL

Note: The Oncomine™ Dx Target DNA Control v3 tube is single-use only. Discard unused volume.

6. Vortex the prediluted FFPE DNA sample (0.83 ng/ μ L) for ~5 seconds, then pulse centrifuge to collect.
7. To each sample well, add the following components in the order indicated.

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

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

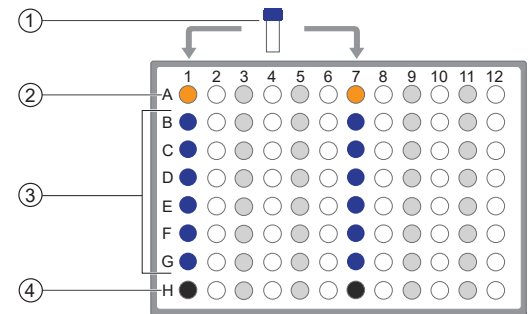
	Order	Component	Volume
<input type="checkbox"/>	1	Prediluted sample FFPE DNA (0.83 ng/ μ L)	12 μ L
<input type="checkbox"/>	2	Oncomine™ Dx Target Test—DNA panel (blue cap)	4 μ L
<input type="checkbox"/>	3	LIB HiFi Mix (red cap)	4 μ L
<input type="checkbox"/>	—	Total	20 μL

8. Set a 20- μ L pipettor to 15 μ L, and pipet the contents of each well up and down 5 times to mix.
9. Proceed to “Amplify the DNA targets” on page 70.

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

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

For 8–16 reactions (for example, 12 clinical samples plus 4 controls), make a master mix containing every component except prediluted FFPE DNA as follows, where "n" is the number of reactions you are preparing. Include one No Template Control (purple cap) and one Oncomine™ Dx Target DNA Control v3 (brown cap) for each column of reactions as illustrated.



1. Label a 96-well plate "DNA".
2. Place the labeled 96-well plate on a 2–8°C chilled aluminum block, then set up reactions in individual wells in odd-numbered columns of the plate. Skip columns to prevent cross-contamination.
3. To each No Template Control well, add 12 µL of No Template Control (purple cap).
4. To each Oncomine™ Dx Target DNA Control v3 well, add the following components in the order indicated:

- ① Oncomine™ Dx Target Test—DNA panel
- ② Oncomine™ Dx Target DNA Control v3
- ③ Prediluted FFPE DNA samples
- ④ No Template Control

	Order	Component	Volume per reaction
<input type="checkbox"/>	1	Dilution Solution (black cap)	9 µL
<input type="checkbox"/>	2	Oncomine™ Dx Target DNA Control v3 (brown cap)	3 µL

Note: The Oncomine™ Dx Target DNA Control v3 tube is single-use only. Discard unused volume.

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

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

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

	Order	Component	Volume
<input type="checkbox"/>	1	Oncomine™ Dx Target Test—DNA panel (blue cap)	(n+1) × 4 µL
<input type="checkbox"/>	2	LIB HiFi Mix (red cap)	(n+1) × 4 µL
<input type="checkbox"/>	—	Total	(n+1) × 8 µL

- Vortex for ~5 seconds, then pulse centrifuge.

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

- Pipet 8 µL of master mix into each DNA sample, No Template Control, and OncoPrint™ Dx Target DNA Control v3 well in the labeled 96-well plate.
- Set a pipettor to 15 µL, then pipet the contents of each well up and down 5 times to mix.
- Proceed to “Amplify the DNA targets”.

Amplify the DNA targets

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

- Seal the 96-well plate with a new adhesive film, then centrifuge the plate at 100 rcf for 30 seconds.
- Load the 96-well plate in the thermal cycler, then select the **3 ODxTT DNA Target Amp** program. Select **View**, and confirm that the program steps match those in the following table:

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

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

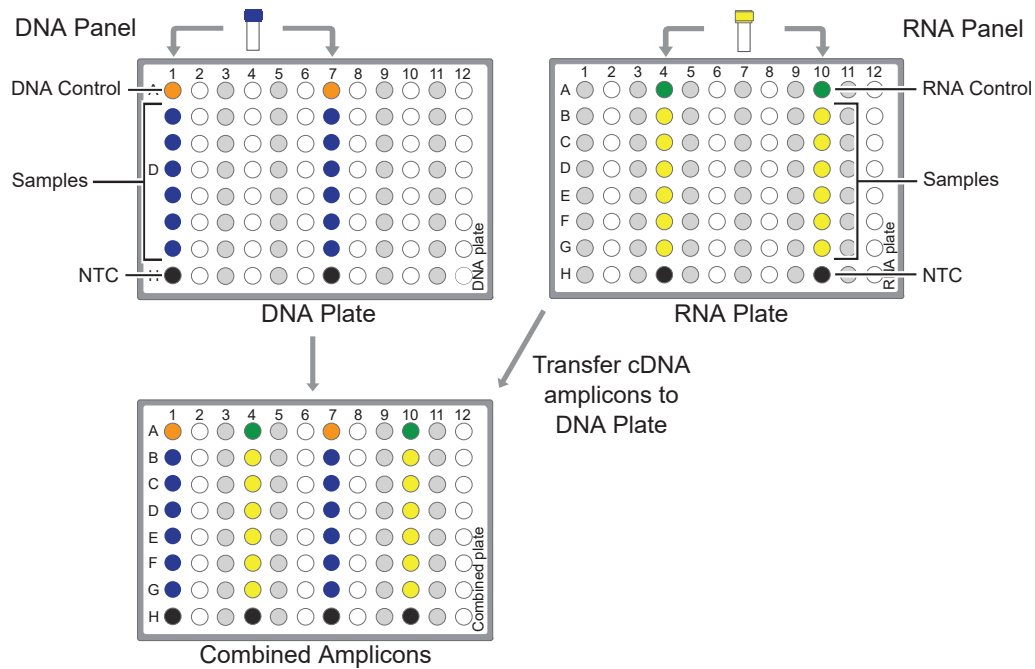
STOPPING POINT Amplicons can be held in the thermal cycler for up to 24 hours or stored at 2–8°C for up to 1 week. If stored longer than 1 week, prepare new amplicons.

Transfer the cDNA amplicons

- After thermal cycling, transfer the 96-well plate to a designated post-PCR preparation area.
- Centrifuge the plates containing amplified cDNA and DNA at 100 rcf for 30 seconds.
- Carefully remove the adhesive film from the plates.

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

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



Proceed to “Partially digest amplicons” on page 71.

Partially digest amplicons

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

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

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

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

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

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

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

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

Alternating barcodes

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

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

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

Ligate barcode adapters

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

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

IMPORTANT! LIB Switch Soln is highly viscous and must be thoroughly mixed before use. There should be no visible precipitate after vortexing. Inspect the tube and cap carefully for precipitate. If precipitate is visible, secure the cap, invert the tube, then vortex upside down for ~5 seconds or until no visible precipitate is present. Use caution to ensure that the correct volume is delivered while pipetting.

3. Flick the tube of LIB DNA Ligase (clear cap) 4 times to mix, then pulse centrifuge to collect.
4. Ensure that the barcode adapters (BC 1–16) are thawed such that no visible ice is present. Vortex for ~5 seconds, then pulse centrifuge to collect.
5. Carefully remove the adhesive film from the plate, then add the following components to each well containing digested sample in the order shown:

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

	Order	Component	Volume
<input type="checkbox"/>	1	LIB Switch Soln (orange cap)	4 µL
<input type="checkbox"/>	2	Barcode adapter (white cap) ^[1]	2 µL
<input type="checkbox"/>	3	LIB DNA Ligase (clear cap)	2 µL
<input type="checkbox"/>	—	Total volume per well (includes 22 µL of sample)	30 µL

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

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

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

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

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

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

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

Prepare the LIB HiFi Mix plus LIB Primers mix

- Flick the LIB HiFi Mix (red cap) 4 times to mix, then pulse centrifuge. Keep the LIB HiFi Mix in a -30°C to -10°C chilled benchtop cold box.
- Vortex the LIB Primers (blue cap) for ~5 seconds, then pulse centrifuge.
- Prepare the LIB HiFi Mix plus LIB Primers master mix:
 - <8 libraries—For each library, add components to individual 1.5-mL low-retention microcentrifuge tubes on ice or in a 2–8°C chilled benchtop cold box in the following order:

	Order	Component	Volume
<input type="checkbox"/>	1	Nuclease-free Water	40 µL
<input type="checkbox"/>	2	LIB HiFi Mix (red cap)	10 µL
<input type="checkbox"/>	3	LIB Primers (blue cap)	2 µL
<input type="checkbox"/>	—	Total	52 µL

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

	Order	Component	Volume
<input type="checkbox"/>	1	Nuclease-free Water	(n+1) × 40 µL
<input type="checkbox"/>	2	LIB HiFi Mix (red cap)	(n+1) × 10 µL
<input type="checkbox"/>	3	LIB Primers (blue cap)	(n+1) × 2 µL
<input type="checkbox"/>	—	Total	(n+1) × 52 µL

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

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

Purify the barcode-adapted library

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

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

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

15. Repeat steps 10–14 one more time.
16. Ensure that all the ethanol droplets are removed from the wells. Keeping the plate in the magnet, air-dry the beads at room temperature for 5 minutes.

Amplify the barcode-adapted library

1. Flick the LIB HiFi Mix/LIB Primers master mix (prepared in “Prepare the LIB HiFi Mix plus LIB Primers mix” on page 74) 4 times to mix, then pulse centrifuge.
2. Remove the 96-well plate from the DynaMag™ Dx 96-Well Plate Magnet, then add 52 µL of LIB HiFi Mix/LIB Primers master mix to each well.
3. Set the pipettor to 40 µL, then pipet up and down 10 times to mix until the beads are resuspended.

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

4. Seal the 96-well plate with a new adhesive film. Centrifuge the plate at 100 rcf for 30 seconds.
5. Load the plate in the Veriti™ Dx 96-well Thermal Cycler, then select the **6 ODxTT Library Amplification** program. Select **View**, and confirm that the program steps match those in the table below.

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

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

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

Prepare the LIB Beads

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

1. Equilibrate the LIB Beads (yellow cap) to room temperature, vortex for 10 seconds or until resuspended, then pulse centrifuge to collect.
2. For each library, combine 3 µL of LIB Beads and 6 µL of LIB Wash Soln (clear cap) in a 1.5-mL low-retention microcentrifuge tube, as follows:
 - For 1–3 libraries, prepare a separate tube of beads and wash solution per library.

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

Number of libraries	Number of reactions to prepare in master mix	Volume of LIB Beads to add	Volume of LIB Wash Soln to add
4–7	$n + 0.5$	$(n + 0.5) \times 3 \mu\text{L}$	$(n + 0.5) \times 6 \mu\text{L}$
8	9	27 μL	54 μL
9–16	$n + 2$	$(n + 2) \times 3 \mu\text{L}$	$(n + 2) \times 6 \mu\text{L}$

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

Add LIB Capture to the amplified sample library

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

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

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

Add the LIB Beads and wash

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

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

Elute the library

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

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

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

Temperature	Time
35°C	5 minutes

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

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

6. During cycling, label a 1.5-mL low-retention microcentrifuge tube for each library. Alternatively, if you are proceeding directly to pooling libraries, label a tube for each library pool.
7. Remove the plate from the thermal cycler, then centrifuge the plate at 100 rcf for 30 seconds.



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

8. Place the plate in the DynaMag™ Dx 96-Well Plate Magnet, then hold at room temperature for 3 minutes. Confirm that the solution is clear.
9. Carefully remove the adhesive film, then transfer the supernatant containing the equalized library (~100- μ L total volume) to a labeled 1.5-mL low-retention microcentrifuge tube. The final concentration of each library is ~100 pM.

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



Troubleshooting

Troubleshooting—Sample preparation

Observation	Possible cause	Recommended action
Slide cracked during scraping	Too much pressure was used during scraping.	Repeat the extraction using a fresh slide if possible. Processing a cracked or broken slide can pose a safety hazard to the operator.
Low-bind Elution tube cap breaks off	Low-bind Elution tubes were not properly aligned in the microcentrifuge prior to centrifugation.	Transfer eluted samples to new, prelabeled Low-bind Elution tubes. Extra Low-bind Elution tubes are provided, however 1.5-mL snap-cap low-retention microcentrifuge tubes may also be used.
DNA/RNA quantification values are not returned	The signal for the sample was oversaturated.	Dilute the DNA and RNA samples with Dilution Solution. Prepare new standards and repeat the quantification assay.
	The signal from the sample was too low.	Prepare new standards and repeat the quantification assay. If the low signal persists, repeat the extraction with increased tissue input (for example, more sections) if available.
DNA/RNA samples do not meet the minimum concentration requirement	Quantification assays performed incorrectly.	Prepare new standards and repeat the quantification assay.
	Insufficient tissue was used in the extraction.	Repeat the extraction with increased tissue input (i.e., more sections) if possible. If the tissue was collected via macrodissection of a resection/surgical biopsy sample, repeat the macrodissection and DNA/RNA extraction with more than two 5-micron sections if available. If only two 5-micron sections remain from the sample, repeat the DNA/RNA extraction with the remaining sections without macrodissection. Note: To proceed with library preparation, both the DNA and RNA from a single sample extraction must meet the minimum concentration requirement. Do not use DNA from one extraction with RNA from a different extraction.
	Elution Solution cooled below 95°C.	Keep the Elution Solution in a 95°C heat block throughout the procedure, including when pipetting.
R ² values do not meet minimum requirement	Standards were not prepared correctly.	Prepare new standards and repeat the quantification assay.

Observation	Possible cause	Recommended action
Batch sample import fails	One or more entries in the sample-import spreadsheet contains special characters, lines breaks, unexpected spaces, incorrect entry length, incorrect date formatting, or other formatting errors.	Check each entry for correct formatting, correct any errors, and repeat the import.
	Blank rows were copied into the sample-import template file from a different source.	Rows that appear empty may contain hidden formatting that conflicts with the import function. Start with a clean sample-import template file, and be careful to copy only those rows that contain actual data.
	The sample import spreadsheet contains a nonunique Sample ID.	Every Sample ID in the software must be unique. Make sure the spreadsheet does not contain any duplicate IDs, and repeat the import. Note that the system check is not case-sensitive, so a Sample ID of ABC1 conflicts with abc1.
	The headings in the sample import spreadsheet do not match the sample attributes in the software.	The headings must match the sample attributes in the software exactly. Check the headings for spelling or other errors.
Library batch import fails	One or more entries in the library batch import spreadsheet contains special characters, lines breaks, unexpected spaces, incorrect entry length, incorrect date formatting, or other formatting errors.	Check each entry for correct formatting, correct any errors, and repeat the import.
	Blank rows were copied into the library batch import template file from a different source.	Rows that appear empty can contain hidden formatting that conflicts with the import function. Start with a clean library batch import template file, and be careful to copy only those rows that contain actual data.
	The library batch import spreadsheet contains a nonunique Library Batch ID.	Every Library Batch ID in the software must be unique. Ensure that the spreadsheet does not contain any duplicate IDs, and repeat the import. Note that the system check is not case-sensitive, so a Library Batch ID of ABC1 conflicts with abc1.
	A Sample ID entered in the library batch import spreadsheet does not match a Sample ID listed in the Manage Samples screen.	Ensure that the Sample IDs entered into the spreadsheet are correct and match an existing sample ID added to the software.
	The Barcode ID name format does not exactly match the format that is used in the Prepare Library Batch dialog box.	Use the following Barcode ID name format: IonDx-1 through IonDx-16.



Observation	Possible cause	Recommended action
Library batch import fails <i>(continued)</i>	An invalid library, control, or panel kit barcode has been entered in the spreadsheet.	Ensure that you have correctly entered a valid kit barcode in the appropriate cell of the spreadsheet.

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

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



Performance characteristics

For performance characteristics of the Oncomine™ Dx Target Test Kit, see the *Oncomine™ Dx Target Test Part I: Test Description and Performance Characteristics User Guide*.

For performance characteristics of the Ion PGM™ Dx System, see the *Ion PGM™ Dx System Performance Characteristics User Guide* (Pub. No. MAN0024930).



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

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit [thermofisher.com/support](https://www.thermofisher.com/support).

Biological hazard safety



WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.



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

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
www.who.int/publications/i/item/9789240011311



Chemical safety



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

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



WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.



WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Precaution—strong magnet

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









The DynaMag™ Dx 96-Well Plate Magnet and DynaMag™ Dx 16 2-mL Magnet contain very strong permanent magnets. People wearing a pacemaker or any other medical magnetized implant should not use this product unless advised by a health professional; the implant could be affected or damaged by exposure to a strong magnetic field. Keep tools and objects that could be damaged by the magnetic



field out of the working area. This includes, but is not restricted to, credit cards and other products containing magnetic recording devices. Keep away from delicate instruments, watches, electronic equipment, displays and monitors. The magnet may attract steel or other magnetic material with high mechanical forces. Take care during handling. Avoid contact between two magnets. Do not pull the magnets apart if contact has been made; twist off to prevent damage to the unit or fingers. The Health and Safety Officer should take all necessary steps and full responsibility to ensure that the precautions and statements are followed and adhered to.

Definition of symbols on product labels or product documents

The following table describes symbols that are present on product labels or product documents. Symbols conform to applicable international regulatory standards.

Symbol	Description
	MANUFACTURER
	DATE OF MANUFACTURE (YYYY-MM-DD)
	CONSULT ELECTRONIC INSTRUCTIONS FOR USE
	CATALOGUE NUMBER
	UNIQUE DEVICE IDENTIFIER
	BATCH CODE
	SERIAL NUMBER
	CONTAINS SUFFICIENT FOR <n> TESTS






(continued)

Symbol	Description
	DO NOT REUSE
	USE-BY DATE
	TEMPERATURE LIMIT
	UPPER LIMIT OF TEMPERATURE
	LOWER LIMIT OF TEMPERATURE
	UPPER AND LOWER LIMITS OF HUMIDITY
	KEEP AWAY FROM SUNLIGHT, KEEP AWAY FROM HEAT
	IN VITRO DIAGNOSTIC MEDICAL DEVICE
	PRESCRIPTION USE ONLY
	READ SAFETY DATA SHEET (SDS)
	BIOLOGICAL RISKS



(continued)

Symbol	Description
	OBSERVE PRECAUTIONS FOR HANDLING ELECTROSTATIC SENSITIVE DEVICES
	CAUTION, CONSULT ACCOMPANYING DOCUMENTS
	FRAGILE, HANDLE WITH CARE



Supplemental sample dilution information

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

Dilute the samples (manual calculation)

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

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

1. Label two new 1.5-mL low-retention microcentrifuge tubes, one for the DNA sample and the other for the RNA sample. Place the tubes in a pre-chilled benchtop cold box until needed.
2. Use the DNA and RNA sample concentrations (ng/μL) determined in the quantification assays to calculate the volume (X) of each RNA and DNA sample required for 10 ng of sample plus 10% overage. Use the following formula:
$$1.1 \times (10 \text{ ng/DNA or RNA sample concentration in ng/}\mu\text{L}) = X \text{ }\mu\text{L of DNA or RNA}$$

Note: See “Example dilution calculations” on page 90.

3. Calculate the volume (Y) of Dilution Solution required to yield a correctly diluted sample using the following formulas:
DNA samples: $(11 \text{ ng}/0.83 \text{ ng/}\mu\text{L}) - X \text{ }\mu\text{L of DNA} = Y \text{ }\mu\text{L of Dilution Solution}$
RNA samples: $(11 \text{ ng}/1.43 \text{ ng/}\mu\text{L}) - X \text{ }\mu\text{L of RNA} = Y \text{ }\mu\text{L of Dilution Solution}$

IMPORTANT!

- If the sample volume (X) from step 2 is <2.0 μL, use 2.0 μL of the sample and adjust the volume of Dilution Solution accordingly.
 - If volume of Dilution Solution (Y) from step 3 is <2.0 μL, increase the amount of the DNA and/or RNA sample volume until the required volume of Dilution Solution is ≥2 μL. See “Example dilution calculations” on page 90.
-
4. For each DNA or RNA sample, pipet the calculated Y μL of Dilution Solution into the appropriate labeled 1.5-mL low-retention microcentrifuge tube from step 1.
 5. Add the calculated X μL of DNA or RNA sample into the appropriate labeled tube.

6. Pipet up and down 5 times to mix, then pulse centrifuge.
7. Place the diluted DNA and RNA samples back in the chilled benchtop cold box or in a 2–8°C refrigerator, then proceed immediately to “Reverse transcribe the RNA” on page 56.

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

STOPPING POINT Store the remaining undiluted DNA sample at –30°C to –10°C for up to 12 months, and the remaining undiluted RNA sample at –90°C to –60°C for up to 12 months. Stability studies for extracted DNA with insertions are ongoing, but a minimum stability of 5 months has been established.

Example dilution calculations

Table 2 Example calculation if the sample volume is $\geq 2 \mu\text{L}$

	Calculation	DNA concentration = 3 ng/ μL	RNA concentration = 4 ng/ μL
1	Sample volume calculation	$1.1 \times [10 \text{ ng}/(3 \text{ ng}/\mu\text{L})] = 3.67 \mu\text{L DNA sample volume}$	$1.1 \times [10 \text{ ng}/(4 \text{ ng}/\mu\text{L})] = 2.75 \mu\text{L RNA sample volume}$
2	Dilution Solution calculation	$(11 \text{ ng}/0.83 \text{ ng}/\mu\text{L}) - 3.67 \mu\text{L DNA sample} = 9.58 \mu\text{L of Dilution Solution}$	$(11 \text{ ng}/1.43 \text{ ng}/\mu\text{L}) - 2.75 \mu\text{L RNA sample} = 4.90 \mu\text{L of Dilution Solution}$
3	Final concentration check	$(3.67 \mu\text{L} \times 3 \text{ ng}/\mu\text{L}) / (3.67 \mu\text{L} + 9.58 \mu\text{L}) = 0.83 \text{ ng}/\mu\text{L}$	$(2.75 \mu\text{L} \times 4 \text{ ng}/\mu\text{L}) / (2.75 \mu\text{L} + 4.94 \mu\text{L}) = 1.43 \text{ ng}/\mu\text{L}$

Table 3 Example calculation if the sample volume is $< 2 \mu\text{L}$

	Calculation	DNA concentration = 15 ng/ μL	RNA concentration = 14 ng/ μL
1	Sample volume calculation	$1.1 \times [10 \text{ ng}/(15 \text{ ng}/\mu\text{L})] = 0.73 \mu\text{L DNA sample volume}$	$1.1 \times [10 \text{ ng}/(14 \text{ ng}/\mu\text{L})] = 0.79 \mu\text{L RNA sample volume}$
2	Sample volume adjustment ($\times 3$)	$0.73 \mu\text{L of sample} \times 3 = 2.19 \mu\text{L DNA sample volume}$	$0.79 \mu\text{L of sample} \times 3 = 2.37 \mu\text{L RNA sample volume}$
3	Dilution Solution calculation with adjustment	$[(11 \text{ ng}/0.83 \text{ ng}/\mu\text{L}) \times 3] - 2.19 \mu\text{L DNA sample} = 37.6 \mu\text{L of Dilution Solution}$	$[(11 \text{ ng}/1.43 \text{ ng}/\mu\text{L}) \times 3] - 2.37 \mu\text{L RNA sample} = 20.7 \mu\text{L of Dilution Solution}$
4	Final concentration check	$(2.19 \mu\text{L} \times 15 \text{ ng}/\mu\text{L}) / (2.19 \mu\text{L} + 37.6 \mu\text{L}) = 0.83 \text{ ng}/\mu\text{L}$	$(2.37 \mu\text{L} \times 14 \text{ ng}/\mu\text{L}) / (2.37 \mu\text{L} + 20.7 \mu\text{L}) = 1.43 \text{ ng}/\mu\text{L}$



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

	Calculation	DNA concentration = 0.9 ng/µL	RNA concentration = 1.8 ng/µL
1	Sample volume calculation	$1.1 \times [10 \text{ ng}/(0.9 \text{ ng}/\mu\text{L})] = 12.22 \text{ }\mu\text{L DNA sample volume}$	$1.1 \times [10 \text{ ng}/(1.8 \text{ ng}/\mu\text{L})] = 6.11 \text{ }\mu\text{L RNA sample volume}$
2	Dilution Solution calculation	$(11 \text{ ng}/0.83 \text{ ng}/\mu\text{L}) - 12.22 \text{ }\mu\text{L DNA sample} = 1.03 \text{ }\mu\text{L of Dilution Solution}$	$(11 \text{ ng}/1.43 \text{ ng}/\mu\text{L}) - 6.11 \text{ }\mu\text{L RNA sample} = 1.58 \text{ }\mu\text{L of Dilution Solution}$
3	Dilution Solution adjustment (× 2)	$1.03 \text{ }\mu\text{L of Dilution Solution} \times 2 = 2.06 \text{ }\mu\text{L of Dilution Solution}$	$1.58 \text{ }\mu\text{L of Dilution Solution} \times 2 = 3.16 \text{ }\mu\text{L of Dilution Solution}$
4	Sample volume adjustment (× 2)	$12.22 \text{ }\mu\text{L of sample} \times 2 = 24.44 \text{ }\mu\text{L DNA sample volume}$	$6.11 \text{ }\mu\text{L of sample} \times 2 = 12.22 \text{ }\mu\text{L RNA sample volume}$
5	Dilution Solution calculation with adjustment	$[(11 \text{ ng}/0.83 \text{ ng}/\mu\text{L}) \times 2] - 24.44 \text{ }\mu\text{L DNA sample} = 2.06 \text{ }\mu\text{L of Dilution Solution}$	$[(11 \text{ ng}/1.43 \text{ ng}/\mu\text{L}) \times 2] - 12.22 \text{ }\mu\text{L RNA sample} = 3.16 \text{ }\mu\text{L of Dilution Solution}$
6	Final concentration check	$(24.44 \text{ }\mu\text{L} \times 0.9 \text{ ng}/\mu\text{L}) / (24.44 \text{ }\mu\text{L} + 2.06 \text{ }\mu\text{L}) = 0.83 \text{ ng}/\mu\text{L}$	$(12.22 \text{ }\mu\text{L} \times 1.8 \text{ ng}/\mu\text{L}) / (12.22 \text{ }\mu\text{L} + 3.16 \text{ }\mu\text{L}) = 1.43 \text{ ng}/\mu\text{L}$



Customer and technical support

Visit [thermofisher.com/support](https://www.thermofisher.com/support) for the latest in services and support, including:

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

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

Obtaining Certificates of Analysis

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

Obtaining Certificates of Conformance

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

Oncomine Dx Target Test

Epidemiology of lung cancer

Lung cancer is the leading cause of cancer deaths in the United States [1]. In 2022, an estimated 236,740 new cases (117,910 in men and 118,830 in women) of lung and bronchial cancer will be diagnosed, and 130,180 deaths (68,820 in men and 61,360 in women) are estimated to occur because of the disease [2]. Only 22.9% of all patients with lung cancer live 5 years or more after diagnosis [3].

Genetic companion diagnostic testing for targeted therapy selection for NSCLC

Lung cancer comprises two main histologic subtypes: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Over the past decade, several biomarkers associated with therapeutic benefit have emerged for NSCLC. To obtain comprehensive molecular biomarker profiling, given the limited tissue, multiplexing technology such as next-generation sequencing (NGS) is recommended by the International Association for the Study of Lung Cancer (IASLC) and Association for Molecular Pathology (AMP) NSCLC testing guidelines.

For the most current information concerning the essential biomarkers for lung cancer and their association with therapeutic outcomes, refer to the therapeutic labels available at [Drugs@FDA](https://www.fda.gov/drugs) on the FDA website.

EGFR: *EGFR* exon 19 deletions and the L858R mutation are found in approximately 12.8% of European NSCLC patients and up to 49.1% of Asian NSCLC patients [4]. These mutations result in activation of the tyrosine kinase domain, and are associated with sensitivity to small-molecule tyrosine kinase inhibitors (TKIs), such as erlotinib, gefitinib, and afatinib [5]. Data show that erlotinib, gefitinib, or afatinib (instead of standard first-line chemotherapy) should be used in patients with *EGFR* exon 19 deletions and the L858R mutation [6–11]. *EGFR* companion diagnostic tests have been approved by the FDA for specific drug indications, including the *therascreen*[™] *EGFR* RGQ PCR Kit by Qiagen for gefitinib and afatinib, the *cobas*[™] *EGFR* Mutation Test v2 by Roche for erlotinib, and the Ion Torrent[™] Oncomine[™] Dx Target Test by Thermo Fisher Scientific for gefitinib.

EGFR: *EGFR* exon 20 insertions are much less common and seen in approximately 2% of Caucasian patients with non-squamous NSCLC, or 12% of all *EGFR* mutations [12]. *EGFR* exon 20 insertions are typically represented by in-frame insertion of 3 to 21 base pairs, or 1 to 7 amino acids, involving

codons 761 to 775 [13]. NSCLC with *EGFR* exon 20 insertions, with the exception of the A763_Y764insFQEA variant, do not typically respond to first- and second-generation TKIs or anti-PD-L1 treatments [12]. Over 60 unique variants of *EGFR* exon 20 insertions have been identified through comprehensive genomic profiling, the majority of which are rare variants [12]. amivantamab-vmjw is approved by the FDA to treat metastatic NSCLC patients with *EGFR* exon 20 insertions who have received prior platinum-based chemotherapy. The Oncomine Dx Target Test is approved by the FDA as a companion diagnostic test for detection of *EGFR* exon 20 insertions.

ALK: It is estimated that 2–7% of patients with NSCLC have an *ALK* gene rearrangement [14]. Crizotinib is approved by the FDA to treat people with NSCLC that has spread to other parts of the body and is caused by either an *ALK* fusion or a *ROS1* fusion. Molecular diagnostic testing using fluorescence *in situ* hybridization (FISH) and immunohistochemistry (IHC) has been approved by the FDA for detecting *ALK* fusions and *ALK* expression, respectively [15,16]. While NGS can also be used to assess the presence of an *ALK* fusion, the Oncomine Dx Target Test does not detect *ALK* fusions. Two *ALK* companion diagnostic tests have been approved by the FDA for use with crizotinib: the Vysis[™] *ALK* Break Apart FISH Probe Kit by Abbott Molecular and the VENTANA[™] *ALK* (D5F3) CDx Assay by Roche.

ROS1: It is estimated that *ROS1* fusions occur in about 1–2% of patients with NSCLC [17]. *ROS1* is very similar to *ALK* (77% amino acid sequence homology in the ATP binding sites of the tyrosine kinase domain), and both are members of the insulin receptor family. Crizotinib is very effective for NSCLC patients with *ROS1* rearrangements [18]. The Oncomine Dx Target Test is approved by the FDA as a companion diagnostic test for detection of *ROS1* fusions.

BRAF: It is estimated that *BRAF* mutations occur in about 3–5% of patients with NSCLC [19]. Dabrafenib in combination with trametinib is approved by the FDA to treat NSCLC patients with a *BRAF* V600E mutation. The Oncomine Dx Target Test is approved by the FDA as a companion diagnostic test for detection of the *BRAF* V600E mutation.

RET: *RET* fusions occur in ~1–2% of lung carcinomas [20]. Pralsetinib is approved by the FDA to treat NSCLC patients with *RET* fusions. The Oncomine Dx Target Test is approved by the FDA as a companion diagnostic test for detection of *RET* fusions.

ERBB2/HER2: ERBB2/HER2 mutations, largely exon 20 in-frame insertions, have been described as an oncogenic driver alterations in 2–4% of NSCLC [21–23], associated with the initiation and progression of adenocarcinoma [23]. Fam-trastuzumab deruxtecan-nxki has been approved by the FDA to treat NSCLC patients with ERBB2/HER2 activating mutations. The Oncomine Dx Target Test is approved by the FDA as a companion diagnostic test for detection of ERBB2/HER2 activating mutations.

Epidemiology of cholangiocarcinoma

Cholangiocarcinoma (CC), i.e., bile duct cancer, is classified according to its anatomical location relative to the liver, either intrahepatic or extrahepatic. Extrahepatic CC, also known as perihilar (or referred to as a Klatskin tumor) or distal bile duct cancers, are more common than intrahepatic CC [24]. Each year, about 8,000 people in the United States are diagnosed with CC [25]. The average 5-year survival rate is estimated at about 9% for intrahepatic bile duct cancers, with 25% for localized and as low as 2% for metastatic; and 10% for extrahepatic bile duct cancers, with 15% for localized and only 2% for metastatic tumors [26].

IDH1/2: *IDH1/2* mutations are the most commonly observed alteration found in intrahepatic cholangiocarcinomas (10–23% of cases) [27–33]. Mutations in *IDH1* have been found in approximately 70% of grade 2 to 3 gliomas [34], 50% of chondrosarcomas [35], and up to 20% of cholangiocarcinomas [36]. Ivosidenib has been approved by the FDA for the treatment of adult patients who have been previously treated with gemcitabine- or fluorouracil (5-FU)-based regimens with an *IDH1* mutation as detected by an FDA-approved test. For more information on the biomarker and therapeutic outcomes, refer to the therapeutic labels available at [Drugs@FDA](#) on the FDA website.

Epidemiology of Astrocytomas and Oligodendrogliomas Low Grade Gliomas arise from glial cells of central nervous system [49]. They include Astrocytomas (AC) and Oligodendrogliomas (OG) [49]. 70% of gliomas are astrocytic tumors and about 9% are oligodendroglioma [50]. In US, about 15,000 new Astrocytomas are diagnosed every year [52] and about 1200 new Oligodendroglioma [53].

IDH1 and *IDH2*: Isocitrate Dehydrogenase (*IDH*) enzymes participate in essential metabolic processes [51]. Mutation in *IDH1* and *IDH2* are elevated in gliomas. 80% of WHO grade II and III have *IDH* mutations [51]. VORANIGO® (vorasidenib) has been approved by FDA for treatment of advanced grade 2 glioma with *IDH1* or *IDH2* mutations. For more information of the biomarker and therapeutic outcomes, refer to the therapeutic labels available at [Drugs@FDA](#) on the FDA website

Epidemiology of Anaplastic Thyroid Cancer

Thyroid cancer is a malignant tumor of the thyroid gland, with an estimate of 64,000 new patients a year in the United States [45]. Anaplastic thyroid cancer (ATC) is the most advanced and aggressive thyroid cancer, found in 2% of patients with thyroid cancer, with an average survival rate of 6 months [45].

Genomic alternations for targeted therapies in ATC

Comprehensive genomic profiling has identified a number of genomic alterations in ATC, including *TP53*, *BRAF*, *TERT*, *CDKN2A*, and *NRAS* mutations and *BRAF*, *RET*, *ALK*, and *NTRK* fusions [46].

BRAF: The activating *BRAF* V600E mutation occurs in 20-50% of patients with ATC [47]. Dabrafenib in combination with Trametinib has been approved by FDA for the treatment of patients with locally advanced or metastatic ATC with *BRAF* V600E mutation. For more information of the biomarker and therapeutic outcomes, refer to the therapeutic labels available at [Drugs@FDA](#) on the FDA website.

Epidemiology of thyroid cancer

Thyroid cancer (TC) is a cancer of the thyroid gland and is classified based on the types of cells found in the tumor as papillary (PTC), follicular (FTC), medullary (MTC), anaplastic (ATC), and other rare types of thyroid cancer [37–39]. In 2022, it is estimated that 43,800 adults will be diagnosed with thyroid cancer in the United States [40]. The most common form of thyroid cancer is the papillary variety, accounting for ~80% of all thyroid cancer cases [41]. The estimated 5-year survival rate for metastatic papillary thyroid cancer is approximately 75%, with the survival rate for metastatic follicular cancer being close to 63%. The 5-year survival rate for advanced metastatic medullary thyroid cancer is approximately 40%, with the estimated survival rate for anaplastic thyroid cancer being only 4% [40].

RET fusions are found in 10–20% of papillary thyroid cancer patients, while *RET* mutations are found in ~60% of sporadic MTC patients and more than 90% of patients with familial MTC [42–44]. Selpercatinib has been approved for use in adult and pediatric patients ≥12 years of age with advanced or metastatic *RET* mutant MTC that requires systemic therapy, or with advanced or metastatic *RET* fusion–positive thyroid cancer who require systemic therapy and who are radioactive iodine-refractory. The Oncomine Dx Target Test is approved by the FDA as a companion diagnostic test for detection of *RET* mutations in MTC and *RET* fusions in TC.

Test intended use and indications for use

The Oncomine™ Dx Target Test is a qualitative in vitro diagnostic test that uses targeted high-throughput, parallel-sequencing technology to detect single nucleotide variants (SNVs), deletions, and insertions in 23 genes from DNA and fusions in *ROS1* and *RET* from RNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor tissue samples from patients with non-small cell lung cancer (NSCLC), *IDH1* SNVs from FFPE tumor tissue samples from patients with cholangiocarcinoma (CC), *BRAF* V600E mutations from FFPE tumor tissue samples from patients with anaplastic thyroid cancer (ATC), *IDH1* and *IDH2* SNVs from FFPE tumor tissue samples from patients with astrocytoma (AC) or oligodendroglioma (OG), *RET* SNVs, multi-nucleotide variants (MNVs), and deletions from DNA isolated from FFPE tumor tissue samples from patients with medullary thyroid cancer (MTC), and *RET* fusions from RNA isolated from FFPE tumor tissue samples from patients with thyroid cancer (TC) using the Ion PGM™ Dx System.

The test is indicated to aid as companion diagnostics in selecting NSCLC, CC, ATC, AC, OG and TC patients for treatment with the targeted therapies listed in Tables 1–6 in accordance with the approved therapeutic product labeling.

Safe and effective use has not been established for selecting therapies using this test for the variants other than those listed in Tables 1–6. Results other than those listed in Tables 1–6 are indicated for use only in patients who have already been considered for all appropriate therapies (including those listed in Tables 1–6).

Analytical performance using NSCLC specimens has been established for the variants listed in Table 7.

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

Table 1. Variants for therapeutic use for NSCLC.

Gene	Variant status	Targeted therapy
	<i>BRAF</i> V600E	TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib)
	<i>EGFR</i> L858R and exon 19 deletions	IRESSA® (gefitinib)
	<i>EGFR</i> exon 20 insertions	RYBREVANT™ (amivantamab-vmjw)
	<i>ERBB2/HER2</i> activating mutations (SNVs and exon 20 insertions)	ENHERTU® (fam-trastuzumab deruxtecan-nxki)
	<i>RET</i> fusions	GAVRETO™ (pralsetinib) RETEVMO® (selpercatinib)
	<i>ROS1</i> fusion	XALKORI® (crizotinib)

Table 2. Variants for therapeutic use for CC.

Gene	Variant status	Targeted therapy
	<i>IDH1</i> R132C <i>IDH1</i> R132G <i>IDH1</i> R132H <i>IDH1</i> R132L <i>IDH1</i> R132S	TIBSOVO® (ivosidenib)

Test performance and characteristics—NSCLC

The Oncomine Dx Target Test detects more than 300 variants in 23 genes, all of which have an active clinical trial and/or have demonstrated association with NSCLC in the literature. A summary of reported variants in patients with NSCLC:

DNA: *AKT1*, *ALK*, *BRAF*, *CDK4*, *DDR2*, *EGFR*, *ERBB2*, *ERBB3*, *FGFR2*, *FGFR3*, *HRAS*, *KIT*, *KRAS*, *MAP2K1*, *MAP2K2*, *MET*, *MTOR*, *NRAS*, *PDGFRA*, *PIK3CA*, *RAF1*, *RET*, and *ROS1*

RNA: *RET* and *ROS1*

Analytical validation of the Oncomine Dx Target Test was established by a series of studies to assess the accuracy, sensitivity, specificity, and reproducibility of the assay for the detection of SNVs, deletions, insertions, and fusions [48].

Based on the data observed, the test demonstrated a limit of detection (LoD) of between 6–8% allele frequencies for DNA variants, 4.8–5.2% allele frequencies for *EGFR* insertions, 6.5% allele frequencies for *EGFR* exon 20 three base pair insertion variation, 5.5% allele frequencies for *EGFR* exon 20 twelve base pair insertion variation, 4.8–5.0% allele frequencies for *ERBB2/HER2* exon 20 insertions, 4.5–5.8% allele frequencies for *ERBB2/HER2* SNVs, 516 fusion reads for *ROS1* fusions, and 405 fusion reads for *RET* fusions, with 95% confidence.

Table 3. Variants for therapeutic use for ATC.

Gene	Variant status	Targeted therapy
<i>BRAF</i>	<i>BRAF</i> V600E	TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib)

Table 4. Variants for therapeutic use for TC.

Gene	Variant status	Targeted therapy
<i>RET</i>	<i>RET</i> fusions	RETEVMO® (selpercatinib)

Table 5. Variants for therapeutic use for MTC.

Gene	Variant status	Targeted therapy
<i>RET</i>	<i>RET</i> mutations (SNVs, MNVs, and deletions)	RETEVMO® (selpercatinib)

Table 6. Variants for therapeutic use for ATC. Variants for therapeutic use for AC and OG.

Gene	Variant ID	Targeted therapy
<i>IDH1</i>	<i>IDH1</i> R132C <i>IDH1</i> R132G <i>IDH1</i> R132H	VORANIGO® (vorasidenib)
<i>IDH2</i>	<i>IDH2</i> R172M <i>IDH2</i> R172K <i>IDH2</i> R172W	
	<i>IDH1</i> R132L <i>IDH1</i> R132S	
	<i>IDH2</i> R172S <i>IDH2</i> R172F	

Table 7. Variants with established analytical performance only.

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

Additionally, based on the representative variants that were tested in the accuracy study, the test detected variants with 98.5% positive percent agreement (PPA) and 100% negative percent agreement (NPA) against validated reference methods (excluding no-calls).

Nine studies were conducted to evaluate the repeatability and reproducibility of the test for DNA variants, EGFR exon 20 insertions, *ERBB2/HER2* SNVs and exon 20 insertions, *ROS1* fusions, and *RET* fusions. Repeatability was >94% for DNA variants, 100% for *EGFR* exon 20 insertions, 100% for *ERBB2/HER2* SNVs and exon 20 insertions, 100% for *ROS1* fusions, and >98% for *RET* fusions. Reproducibility was >99% for DNA variants, *EGFR* exon 20 insertions, *ERBB2/HER2* SNVs and exon 20 insertions, *ROS1* fusions, and *RET* fusions (excluding no-calls and unknowns). Supplementary study for repeatability and reproducibility near LOD for *RET* fusions was performed and see details in User Guide. Additional study for *EGFR* exon 20 insertions demonstrated repeatability and reproducibility of 100% excluding no calls.

Method comparison studies evaluated the concordance of the Oncomine Dx Target Test for the detection of *BRAF* V600E; *EGFR* exon 19 deletions, L858R, and exon 20 insertions; *ROS1* fusions; and *RET* fusions, using a *BRAF* V600E PCR assay, the *therascreen™ EGFR* PCR kit, a *ROS1* FISH assay, and validated NGS assays, respectively. The following agreements between the Oncomine Dx Target Test and reference methods were observed, excluding invalids and no-calls:

- 100% overall percent agreement (OPA), PPA, and NPA for *BRAF*, *EGFR* exon 20 insertions, and *ROS1* fusions
- 99% OPA, PPA, and NPA for *EGFR* exon 19 deletions and L858R
- 92% OPA, 91% PPA, and 92% NPA for *RET* fusions
- 99% OPA, 100% PPA, and 99% NPA for *ERBB2/HER2* activating mutations (SNVs and exon 20 insertions)

Test performance and characteristics—CC

The Oncomine Dx Target Test only reports *IDH1* R132 variants in patients with CC. Analytical validation of the Oncomine Dx Target Test for CC was established by LoD and precision studies [34]. Based on the data observed, the test confirmed a LoD of 4.5–5.7% allele frequencies for five *IDH1* R132 mutations, including 4.5% for R132C, 5.7% for R132G, 4.9% for R132H, 5.1% for R132L, and 5.3% for R132S. Repeatability and reproducibility studies demonstrated an overall positive call rate of 92.6% for the *IDH1* R132 variants when including no-calls and 97.1% when excluding no-calls. The negative call rate for the *IDH1* wild-type sample was 100%.

A clinical concordance study was conducted to evaluate the ability of the Oncomine Dx Target Test to identify five *IDH1* biomarkers in FFPE CC tumor specimens compared to a validated Sanger assay. The study shows 99.4% PPA, 96.5% NPA, and 97.9% OPA excluding invalid results and no-calls.

Test performance and characteristics—AC and OG

The Oncomine Dx Target Test reports *IDH1* R132 SNVs and *IDH2* R172 SNVs in AC and OG. Analytical validation of the Oncomine Dx Target Test in AC and OG was established through LoD and reproducibility studies [48]. Based on data observed, LoD of the 5 *IDH1* R132 variants ranged from 4.6%–7.0% allele frequencies and of 5 *IDH2* variants from 4.1% to 5.8% allele frequencies. The reproducibility of *IDH1* and *IDH2* variant detection using the Oncomine Dx Target Test was assessed with clinical AC and OG samples at 2 allelic frequency levels. The overall positive call rate for *IDH1* and *IDH2* variants was 100% and 97.5% respectively including no calls. The negative call rate was 100% at all *IDH1* and *IDH2* variant locations. A method comparison study evaluated the concordance of the test for the detection of *IDH1* R132 SNVs and *IDH2* R172 SNVs compared to validated NGS assay. The study demonstrated PPA of 100%, NPA of 96.2%, and OPA of 99% excluding unknowns. See User guide for details.

Test performance and characteristics - ATC

Oncomine Dx Target Test only reports *BRAF* V600E in ATC. Analytical validation of the Oncomine Dx Target Test in ATC was established through LoD and reproducibility studies [48]. Based on the data observed, the test confirmed a limit of detection (LoD) of 6.4% allele frequencies for *BRAF* V600E mutation, same as previously established in NSCLC tissues. Repeatability and reproducibility were demonstrated at 100%. A method comparison study evaluated the concordance of the test for the detection of *BRAF* V600E, using a *BRAF* V600E PCR assay. The study shows overall percent agreement (OPA) of 99% excluding invalids and no-calls.

Test performance and characteristics—TC

The Oncomine Dx Target Test reports *RET* DNA mutations (SNVs, MNVs, and deletions) in patients with medullary thyroid cancer (MTC) and *RET* fusions in patients with thyroid cancer (TC).

Analytical validation of the Oncomine Dx Target Test was established through a series of studies to assess the LoD and reproducibility of the assay for the detection of *RET* mutations and fusions [45].

Based on the data observed, the test confirmed a limit of detection (LoD) of 4.9–5.5% allele frequencies for *RET* DNA variants and 236 fusion reads for *RET* fusions. Repeatability and reproducibility studies demonstrated within-run repeatability of 100% for the *RET* DNA variants tested, with one wild-type blend showing a 97.9% repeatability with no-calls included. Repeatability estimates for the *RET* RNA fusion blends tested ranged from 88.9% to 100%.

A method comparison study was conducted to evaluate the accuracy of the test for the detection of *RET* DNA variants in MTC using a validated NGS assay. The study showed an OPA of 99% between the Oncomine Dx Target Test and the validated NGS assay.

A method comparison study was conducted to evaluate the accuracy of the test for the detection of *RET* fusions in TC using a validated NGS assay. The study showed an OPA of 100% between the Oncomine Dx Target Test and the validated NGS assay. Supplementary study to identify *RET* fusions in TC was performed using 217 samples to evaluate for concordance between the Oncomine Dx Target Test and a validated NGS assay. The study demonstrated a variant level PPA of 93.3%, NPA of 100.0%, and OPA of 98.3%, excluding unknowns.

Guide to interpreting results

Test results should be interpreted in the context of pathological evaluation of tumors, treatment history, clinical findings, and other laboratory data.

All clinical interpretations of the variants detected should be made by a board-certified pathologist or equivalent. It is recommended that the physician ordering the test consult with a board-certified pathologist. Patients are advised to seek information from their oncologist or certified health care provider.

Additional information may be obtained from NCCN Guidelines™ and IASLC/AMP NSCLC testing guidelines.

The molecular profile of a tumor can vary between primary and metastatic sites, as well as change over time in response to treatment, leading to the development of mutations that could confer resistance to therapeutic agents.

Test limitations

The test is designed to interrogate over 300 variants in 23 genes associated with NSCLC and one gene in CC. However, when certain quality metrics and controls established for the specimen testing are not met, accuracy of the test cannot be assured, and therefore mutation status in the exons is not reported. Variants detected by the panel that are not clinically or analytically validated should not be used for selecting treatment for NSCLC.

- This test does not detect genomic copy number variants.
- This test does not detect ALK fusions.
- This test does not detect structural variants in genes other than ROS1 and RET.
- Rare polymorphisms exist that could lead to false-negative or false-positive results.
- A negative (wild-type) result does not rule out the presence of a mutation that is below the limits of detection of this assay (6–8% allele frequencies).
- This test is designed to detect a targeted set of known variants in the genes. New variants that are not included in the test may be discovered in the future.

- The OncoPrint Dx Target Test has not been validated for the detection of *RET* insertions.
- Users are cautioned that DNA variant–positive calls in the *RET* genomic region have been observed to produce multiple variant calls, even when only one variant is present. These *RET* variants are all activating and do not change the patient’s clinical appropriateness for seliperatinib.
- High variation in fusion reads can be observed in fusion-positive samples. A decrease in fusion read over time has been observed when testing slides from TC tissue under storage.
- For NSCLC, the OncoPrint Dx Target Test assay definition file includes prevalent but not all rare or newly identified *RET* isoforms, *ROS1* isoforms, and *EGFR* exon 20 insertions, *EGFR* exon 19 deletions and *ERBB2/HER2* activating mutations (SNVs and exon 20 insertions). The OncoPrint Dx Target Test may miss rare, complex or newly identified:
 - *RET* isoforms carried by a subset of patients who may derive benefit from GAVRETO™ (pralsetinib) or RETEVMO® (seliperatinib)
 - *ROS1* isoforms carried by a subset of patients who may derive benefit from XALKORI® (crizotinib)
 - *EGFR* exon 20 insertions carried by a subset of patients who may derive benefits from RYBREVANT™ (amivantamab-vmjw)
 - *EGFR* exon 19 deletions carried by a subset of patients who may derive benefit from IRESSA® (gefitinib)
 - *ERBB2/HER2* activating mutations (SNVs and exon 20 insertions) carried by a subset of patients who may derive benefit from ENHERTU® (fam-trastuzumab deruxtecan-nxki)
- For TC, the OncoPrint Dx Target Test assay definition file includes the most prevalent but not all rare or newly identified *RET* isoforms. The OncoPrint Dx Target Test may miss a subset of patients carrying these rare or newly identified *RET* isoforms who may derive benefit from RETEVMO® (seliperatinib).
- For MTC, the OncoPrint Dx Target Test assay definition file includes the most prevalent but not all rare or newly identified *RET* SNVs, MNVs and deletions. The OncoPrint Dx Target Test may miss a subset of patients carrying these rare or newly identified *RET* SNVs, MNVs and deletions who may derive benefit from RETEVMO® (seliperatinib).
- For astrocytoma (AC) and oligodendroglioma (OG), the OncoPrint™ Dx Target Test included prevalent but not all rare *IDH2* variant clinical specimens in the assay reproducibility study. The OncoPrint™ Dx Target Test may miss rare *IDH2* variants carried by patients who may derive benefit from vorasidenib.
- The OncoPrint™ Dx Target Test has only been validated for use with FFPE tumor slide specimens. The validation of the use of derivative core needle biopsy (CNB) samples and stereotactic biopsy (STB) samples for astrocytoma (AC) and oligodendroglioma (OG), with the OncoPrint™ Dx Target Test to support inclusion of these type of samples has not been performed.

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