SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Next generation sequencing oncology panel, somatic or germline variant detection system
Device Trade Name:	oncoReveal™ CDx
Device Procode:	PQP
Applicant's Name and Address:	Pillar Biosciences, Inc. 9 Strathmore Road Natick, MA 01760
Date(s) of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P200011/S001
Date of FDA Notice of Approval:	April 18, 2024

The original PMA (P200011) for Pillar Biosciences was approved on July 30, 2021, for the detection of single nucleotide variants (SNVs) and deletions in two genes (*EGFR* and *KRAS*) in patients who may benefit from FDA-approved therapies for non-small cell lung cancer (NSCLC), and colorectal cancer (CRC).

The current supplement was submitted to expand the intended use and indication for use of oncoReveal CDx Assay to include tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for cancer patients with solid malignant neoplasms.

II. INTENDED USE/INDICATIONS FOR USE

The oncoReveal™ CDx is a qualitative next generation sequencing based *in vitro* diagnostic test that uses amplicon-based target enrichment technology for detection of single nucleotide variants (SNVs), insertions and deletions in 22 genes using DNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor tissue specimens and using the Illumina MiSeqDx®. The test is intended as a companion diagnostic to identify patients who may benefit from treatment with the targeted therapies listed in Table 1 in accordance with the approved therapeutic product labeling.

Additionally, oncoReveal™ CDx is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for previously diagnosed cancer patients with solid malignant neoplasms. Genomic findings other than those listed in Table 1 are not prescriptive or conclusive for labeled use of any specific therapeutic product.

Table 1. List of Somatic Variants for Therapeutic Use

Indication	Gene	Variant	Targeted therapy
Colorectal Cancer (CRC)	*KRAS*	*KRAS* wild-type (absence of mutations in codons 12 and 13)	ERBITUX® (cetuximab), or VECTIBIX® (panitumumab)
Non-Small Cell Lung Cancer (NSCLC)	*EGFR*	Exon 19 Deletions and Exon 21 L858R Substitution Mutations	EGFR Tyrosine Kinase Inhibitors approved by FDA*

*For the most current information about the therapeutic products in this group, go to: https://www.fda.gov/medicaldevices/productsandmedicalprocedures/invitrodiagnostics/ucm301431.htm

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings/precautions and limitations can be found in the oncoReveal™ CDx assay labeling.

V. DEVICE DESCRIPTION

The oncoReveal CDx is an NGS *in vitro* diagnostic Test that uses amplicon-based target enrichment technology for detection of SNVs, insertions and deletions in 22 genes using DNA isolated from FFPE tumor tissue specimens and using the Illumina MiSeqDx instrument. In addition to the companion diagnostic (CDx) claims noted in Table 1 of the intended use/indications for use, the oncoReveal CDx also reports SNV, insertions and deletions in the 22 genes listed Table 2 to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for previously diagnosed cancer patients with solid malignant neoplasms.

Table 2. Genes Targeted by the oncoReveal™ CDx

AKT1	*CTNNB1*	*ERBB2*	*FGFR1*	*KRAS*	*NOTCH1*	*PTEN*	*TP53*
ALK	*DDR2*	*ERBB4*	*FGFR2*	*MAP2K1*	*NRAS*	*SMAD4*	
BRAF	*EGFR*	*FBXW7*	*FGFR3*	*MET*	*PIK3CA*	*STK11*	

Test Output

The output of the test includes:

Level 1: Companion Diagnostic (CDx) Claims noted in Table 1 of the Intended Use
Level 2: Cancer Mutations with Evidence of Clinical Significance
Level 3: Cancer Mutations with Potential Clinical Significance

Test Kit Contents

The Assay Kit is composed of 7 reagents to allow the processing of 48 reactions (46 patient samples and required controls), refer to Table 3. The reagents are liquids or suspensions, stored in individual vials, and segregated into four labeled sub-containers. The sub-containers allow kit components to be stored at the recommended temperature, which may be room temperature, 4°C, or -20°C, depending on the components. Safety Data Sheets are available from Pillar Biosciences. Outer packaging supports international frozen shipment.

Table 3. Assay Kit Reagents

Kit Box 1: GS-PCR Reagent	**Quantity**	**Storage**
Gene Specific PCR Master Mix	1 tube (red cap)	-25°C to -15°C
LC Oligo Pool	1 tube (yellow cap)	-25°C to -15°C
Positive Control (PosCtrl)	1 tube (clear cap)	-25°C to -15°C
Uracil-DNA glycosylase (UDG)	1 tube (blue cap)	-25°C to -15°C
Kit Box 2: Indexing PCR Reagent	**Quantity**	**Storage**
Indexing PCR Master Mix	1 tube (green cap)	-25°C to -15°C
Forward indexing primers (A501-A508)	8 tubes (white caps)	-25°C to -15°C
Reverse indexing primers (A701-A706)	6 tubes (orange caps)	-25°C to -15°C
Kit Box 3: PCR Product Purification Reagent	**Quantity**	**Storage**
Purification Beads	1 bottle	2°C to 8°C
Kit Box 4: Index Tube Caps	**Quantity**	**Storage**
White caps (for A501-A508 primers)	24 caps	Ambient
Orange caps (for A701-A706 primers)	18 caps	Ambient

Materials and equipment and software that are required for the test but are not provided with the assay kit are listed in Tables 4, 5 and 6.

Table 4. Materials required but not provided in the oncoReveal™ CDx Kit

Material	**Source/Part Number**
Reagent kit for extraction and purification of DNA from formalin-fixed paraffin-embedded (FFPE) tissues used in clinical diagnostic applications.	See DNA EXTRACTION equivalency section 5 below. Column- or bead-based kits for extraction and purification of DNA from FFPE tissues. Proteinase K treatment and final elution volume 25µL are recommended for optimal results with this assay.

Material	Source/Part Number
Reagent kit for quantification of double-stranded DNA (dsDNA) in biological samples used in clinical diagnostic applications.	The assay should: • accurately measure dsDNA for initial sample concentrations from 0.2 ng/µL to 15 ng/µL. • be compatible with a variety of biological samples, including purified genomic DNA from FFPE tissues, and PCR products. • contain a fluorescent dsDNA-binding dye, appropriate buffer, and DNA standards for calibration. • be designed for use with a fluorometer instrument.
Reaction vessels intended for use with a fluorometer instrument for the quantification of dsDNA used in clinical diagnostic applications.	The reaction vessels should be compatible for use with dsDNA quantification assay and fluorometer instrument to provide consistent and accurate fluorescence measurements.
PhiX Library Control intended as a control in nucleic acid sequencing workflows used in clinical diagnostic applications.	Library of bacteriophage PhiX DNA fragment at or above 20 pM. The fragments should have an average size of 500 bp and consist of base composition at ~45% GC and ~55% AT. The control should be used as directed by the
MiSeqDx® Reagent Kit v3 (600 cycles)	Illumina/20037174
Ethanol, 200 proof for molecular biology	General lab supplier
Nuclease-free water	General lab supplier
10 mM Tris-HCl w/ 0.1% Tween-20, pH 8.5	General lab supplier
10 N NaOH or 1 N NaOH	General lab supplier
1.5 mL microcentrifuge tubes	General lab supplier
96-well PCR plates, 0.2 mL	General lab supplier
Microplate sealing film	General lab supplier
Conical tubes, 15 mL	General lab supplier
Conical tubes, 50 mL	General lab supplier
Aerosol filter pipette tips	General lab supplier
Solution basin (trough or reservoir)	General lab supplier

Table 5. Equipment Required But Not Provided

Equipment	Source/Part Number
MiSeqDx® Instrument†	Illumina/DX-410-1001

Fluorometer instrument††	A fluorometer instrument compatible for use with DNA quantification kit.
Vortexer	General lab supplier
Magnetic stand intended for use with PCR product purification workflow.	General lab supplier
Microfuge	General lab supplier
Thermal cycler† with heated lid capability	General lab supplier
Single- and multi-channel pipettes†, 0.5 to 1000 µ1	General lab supplier
Centrifuge adapted for PCR plates	General lab supplier

† Equipment should be maintained and/or calibrated according to the manufacturer's instructions

Table 6. Software Provided During System Set up.

Equipment	Source/Part Number
Pillar LC-HS module v2.1 or higher	Pillar Biosciences/SFW-2008
oncoReveal™ CDx PiVAT® Workstation with software version 2.1 or higher	Pillar Biosciences/SFW-2012
Pillar Sample Sheet Tool version 3.2 or higher	Pillar Biosciences/TL-0059

The PiVAT® software is for use with oncoReveal™ CDx. PiVAT® performs secondary analysis and report generation from sequencing runs that use the oncoReveal CDx.

PiVAT® is installed on a stand-alone computer system configured with an Ubuntu operating system and a Chromium browser. The system is configured with no network connectivity.

Test Process

1. **Specimen Preparation/DNA extraction**
   Specimens must be deparaffinized and digested with protease to liberate the DNA target before purification.
   All tissues must be formalin fixed and embedded in paraffin according to accepted histological methods. FFPE sections with ≥ 30% tumor content by area for processing without macro dissection. For FFPE sections that are less than 30% tumor content by area, tumor content is enriched by macro dissecting multiple sections to obtain ≥ 30% tumor content by area. Column-based DNA extraction kits with Proteinase K treatment with agitation and final elution with 25 µL volume per section are recommended for DNA extractions intended for use with this assay. Extracted DNA giving a dsDNA quantification of > 4.5 ng/µl can be used for the oncoReveal™ CDx assay.

2. **Library Preparation**

   Library preparation is performed using the oncoReveal CDx Kit. Briefly, purified DNA samples are treated with Uracil-DNA Glycosylase to render formalin damaged DNA non-amplifiable. The samples are then amplified using polymerase chain reaction (PCR) and a gene-specific primer pool to enrich the number of assay target

sequences. The amplification products are purified from remaining primers and each sample is "barcoded" in a second PCR reaction using a unique pair of indexing primers to prepare the samples for pooling and multiplexed analysis. After purification of the amplification products from residual indexing primers, the indexed libraries are quantified, normalized, and pooled for sequencing.

3. Sample Sequencing

Sequence information is extracted from the sample library pool using Illumina's MiSeqDx NGS analyzer and corresponding reagents according to the manufacturer's protocols. The user selects the appropriate analysis panel type (oncoReveal™ CDx labeled as LC-HS within Local Run Manager) from the Pillar Module on the MiSeqDx to initiate the sequence analysis utilizing the preset parameters for that panel. The Pillar Module is an interface designed for the MiSeqDx by Illumina to interface with collaborator assays. Its main function is to automatically configure assay-specific sequencing parameters on the instrument, such as read length, when a particular assay is selected from the Module's drop-down menu. As additional assays are developed, they can be added to extend the capabilities of the Module.

The user must also create a Sample Sheet in Illumina's format that holds information about the samples such as name, whether the sample is a control sample (positive, negative, or no template), and what indices were used to tag that sample. Pillar Biosciences provides a tool that facilitates the aggregation of batches of libraries prepared across multiple days onto a single MiSeqDx v3 flow cell, provided that each batch has the required positive and No Template Control (NTC) controls. Up to 48 libraries may be multiplexed onto a single MiSeqDx v3 flow cell. A successful sequencing run will produce sequence data in Illumina's proprietary Binary Base Call format that is converted to a more universal FASTQ sequence format by the Pillar Module.

The oncoReveal CDx requires the user to run positive control (PosCtrl) and NTC for each "Batch" of up to 46 samples (processed on the same plate). Up to 6 batches may be included in a single sequencing run and analyzed through the PiVAT software. PosCtrl is a cell line DNA containing the CDx variants with expected variant allele frequencies as shown in Table 7 below. The PosCtrl must generate expected mutations to be valid. If the PosCtrl is invalid, the PiVAT software will fail the entire batch and no results will be reported for all samples within the batch.

Table 7. Positive Control (PosCtrl)

Gene	Variant	Expected Allelic Frequency, %
EGFR	ΔE746 - A750	2.0%
EGFR	L858R	3.0%
KRAS	G13D	15.0%
KRAS	G12D	6.0%

The non template control (NTC) reaction is setup using DNA diluent or nuclease-free water with no template or DNA input. The NTC should not detect any mutations. If the

NTC is invalid, the PiVAT software will fail the entire batch and no results will be reported for all samples within the batch.

4. Bioinformatic Analysis

The PiVAT IVD bioinformatics pipeline is used to convert the raw FASTQ output from the MiSeqDx into genetic variation observed for each sample. The PiVAT IVD software is provided on a standalone workstation and raw sequence data are transferred from the MiSeqDx to the PiVAT IVD workstation for analysis using a USB drive. Once the required FASTQ files are uploaded to the PiVAT IVD workstation, the user can select sequence data to be analyzed from the browser-based PiVAT interface and begin the analysis workflow. The software will deconvolute the mixed sample sequence data using the unique sample index and consolidate matching forward and reverse sequence reads. Filters are used to minimize the result of random variation introduced during sample amplification steps and sequencing.

The resulting sequences are aligned to the hg19 human genome sequence framework using the BWA-MEM aligner. Local re-alignments are performed to identify longer insertions and deletions (indels). Filters are applied to isolate likely variation from sequencing noise (Table 8). oncoReveal CDx is designed to detect and report somatic variants in three levels:

Level 1: CDx variants listed in Table 1 of Indications for Use
Level 2: Cancer Mutations with Evidence of Clinical Significance
Level 3: Cancer Mutations with Potential Clinical Significance

Non-targeted variants including germline variants are not reported. After this step, the remaining variants are annotated using Human Genome Variation Society (HGVS) standards, and a PDF format summary report is generated in two parts.

- The Run Summary section of the PiVAT Customer PDF Report output file contains various statistics that reflect run quality and an overview of all variation to be reported across all samples analyzed within that run. Samples that fail to meet certain NGS quality criteria are reported as not valid and no genetic variants are reported for these samples.

- The Patient Report section of the PiVAT Patient Report output file aggregates any variation observed at the patient level along with previously established clinical validity. Samples that fail to meet certain NGS quality thresholds are reported as not valid and no genetic variants are reported for these samples. The user can interact with the PiVAT IVD pipeline utilizing a browser-based visual interface. At the end of analysis, all intermediate data files and reports may be downloaded to a USB drive and transferred to another location for permanent storage.

Table 8. NGS-QC in PiVAT®: Run, Sample and Variant Calling Passing Criteria

a. NGS run level quality control		
Category	**QC Metrics**	**Passing Criteria**
Run - Invalid if any QC metric(s)	PosCtrl	Expected mutations are detected
		No unexpected mutation(s) detected

fails	NTC	No mutation detected
		Maximum coverage <50x or <0.5% of median within-run sample coverage

b. NGS sample level quality control

Category	QC Metrics	Passing Criteria
Sample - NOT valid if any QC metric(s) fails	Sequencing base quality	Bases (with Q Score ≥Q30) ≥75%
	Amplification specificity	Effective On-Target-Rate[1]≥70%
	Coverage[2]	Minimum depths of the three amplicons covering CDx mutations ***≥1000x***
CDx mutations thresholds	non-C>T|G>A	1. Variant coverage ≥*10x* AND Total coverage ≥ ***1000x*** 2.Average variant base Q-score ≥ 30 AND VAF ≥ **1%**
	C>T|G>A	1. Variant coverage ≥*10x* AND Total coverage ≥ ***1000x*** 2.Average variant base Q-score ≥ 30 AND VAF ≥ **1.5%**
[a]Group 1 non-CDx mutation thresholds**	non-C>T|G>A	1. Variant coverage ≥*10x* AND Total coverage ≥ ***1000x*** 2.Average variant base Q-score ≥ 30 AND VAF ≥ **1%**
	C>T|G>A	1. Variant coverage ≥*10x* AND Total coverage ≥ ***1000x*** 2.Average variant base Q-score ≥ 30 AND VAF ≥ **1.5%**
	No Call*	Coverage **< 1000x**
[a]Group 2 non-CDx mutation thresholds**	**Variant**	1. Variant coverage ***≥10x*** AND Total coverage ≥ ***500x*** 2.Average variant base Q-score ≥ 30 AND **VAF ≥ 3.2%**
	No Call*	Coverage **< 500 x**

[1] Effective On-Target Rate = Mapping rate * On-target rate

[2] Coverage: the coverage after paired-end assembly by PiVAT®. All markers in the assay are bi-directional sequenced with 2x150bp sequencing protocol due to the short amplicon sizes (144-162bp including primers). 1x coverage = 1x forward + 1x reverse of sequencing reads. Only uniquely mapped reads are analyzed.

a: Group 1 non-CDx mutations includes: EGFR G719X, T790M; KRAS A59X, Q61X, K117N, A146X; and BRAF V600E; all other non-CDx mutations in Group 2.

* ***No calls*** are only applicable to non-CDx variants. Variants on amplicons with coverage below the threshold are at risk of being false-negatives.

** Non-CDx variants may be detected and reported although variant coverage requirement are not met. Positive non-CDx variant calls for variants that did not meet the variant coverage requirement are at risk of being false positive calls.

5. Processes for database and variant annotation

Database: For the oncoReveal CDx distributed kit, the PIVAT software includes database information regarding the variants and their assignment to either Level 1 CDx Mutations', 'Level 2 Mutations = (Variants with Evidence of Clinical Significance)' and 'Level 3 Mutations (Variants with Potential Clinical Significance)'. A description of the assignment and curation process was provided.

Report Generation in oncoReveal CDx: The PiVAT Run Summary Report provides a run summary of the applicable run. The PiVAT software generates reports for each batched run and sample processed. The report includes tumor type, detected variants reported in HGVS format as either Level 1, 2 or 3 mutations (CDRH's approach to tumor profiling), and pertinent no call regions. The PIVAT Sample Test Report is the primary report of identified alterations for a sample. The Test Report divides variants into 3 sections: 'Level 1 CDx Mutations', 'Level 1 Mutations = (Variants with Evidence of Clinical Significance)' and 'Level 3 Mutations (Variants with Potential Clinical Significance)'. The variants listed in the section 'Level 2 Mutations - Variants with Evidence of Clinical Significance' are determined based on the selected tumor type. Only variants clinically associated with the selected tumor type will appear on this Level 2 – Variants with Evidence of Clinical Significance. Any remaining variants, meeting the SOP requirements for Level 3, will appear in the 'Variants with Potential Clinical Significance' section. A qualified healthcare professional selects the appropriate tumor type to ensure the corresponding tumor profiling variants appear in the report.

6. Determination of PiVAT NGS Calling and QC Threshold

a. Requirements on Amplicon and Base Coverage:

Depth of coverage (coverage): defined as the number of aligned reads that contain a given nucleotide position. In PiVAT, the sequencing reads are first aligned to human reference hg19, then go through local re-alignment to remove alignment errors. After local-realignments, the paired-reads (forward and reverse reads) for each pair are assembled into a single read for coverage assessment and variant calling.

Base quality score (Q-score): The quality score of each base within an assembled read is adjusted by the PiVAT software by considering the Q-scores from both sequencing directions. The reassigned base Q-scores are subject to a threshold of 30, corresponding to a 1/1000 chance of error.

b. Statistical Determination of Depth Coverage Requirements

Assuming that alleles reported by reads at a given genomic position follow a Bernoulli random process, with each read representing an independent event, the total number of reads supporting the mutant allele is expected to follow a binomial distribution. The 95% confidence interval (CI) representing the range of observed variant allele frequencies (VAFs) from the true underlying VAFs greater than 2% was computed. Based on the power analysis, the observed VAFs for a true underlying VAF of 2% are estimated to fall between 1.2% to 3.1% with 1000x coverage. When the mutation is

present at 5% with a coverage of 500x, the 95% CI ranges from 3.3% to 7.3%, refer to Table 9.

Based on a requirement of a minimum 10 mutation reads to support a positive call, sequence coverage of ≥ 1000x yields a > 95% probability of detecting a true mutation at 2% VAF (1.2% -3.1% at 95% CI). For a mutation at a 5% VAF, a sequencing coverage of 500x provides close to 100% statistical power for detection (3.3% to 7.3%, 95%CI).

Table 9. Power Analysis Results by Exact Binomial Distribution Model

True_VAF	95% confidence interval for computed observed-VAF, as function of coverage			
	200X	**500x**	800x	**1000x**
1%	(0.1%, 3.6%)	(0.3%, 2.3%)	(0.4%, 2.0%)	(0.5%, 1.8%)
2%	(0.6%, 5.0%)	(1.0%, 3.7%)	(1.2%, 3.2%)	***1.2%, 3.1%)***
3%	(1.1%, 6.4%)	(1.7%, 4.9%)	(1.9%, 4.4%)	(2.0%, 4.3%)
3.7%	(1.4%, 7.1%)	(2.3%, 5.9%)	(2.5%, 5.3%)	**(2.6%, 5.1%)**
4%	(1.7%, 7.7%)	(2.5%, 6.1%)	(2.8%, 5.6%)	(2.9%, 5.4%)
5%	(2.4%, 9.0%)	***3.3%, 7.3%)***	(3.6%, 6.8%)	(3.7%, 6.5%)
6%	(3.1%, 10.3%)	(4.1%, 8.5%)	(4.5%, 7.9%)	(4.6%, 7.7%)
7%	(3.9%, 11.5%)	(4.9%, 9.6%)	(5.3%, 9.0%)	(5.5%, 8.8%)
8%	(4.6%, 12.7%)	(5.8%, 10.7%)	(6.2%, 10.1%)	(6.4%, 9.9%)
9%	(5.4%, 13.9%)	(6.6%, 11.9%)	(7.1%, 11.2%)	(7.3%, 11.0%)
10%	(6.2%, 15.0%)	(7.5%, 13.0%)	(8.0%, 12.3%)	(8.2%, 12.0%)

c. Confirmation of Theoretical VAF Estimates and Coverage Requirements

To evaluate the actual NGS VAFs compared to those estimated from the power analysis, NGS data was obtained from two sequencing runs. These runs yielded a total of 536 VAF measurements across 15 unique hotspot mutations at 10 expected VAF levels, derived from a total of 55 libraries encompassing 8 different DNA or FFPE samples. These samples consisted of mixed cell lines covering multiple endogenous SNPs and deletions, with mutation frequencies confirmed by ddPCR. The coverages for the 15 hotspot mutations typically varied within ranges that greatly exceeded 500x and 1000x in the samples. To evaluate the observed VAF distribution as function of coverage, the data was down-sampled in silico to approximate mean coverages of 500x and 1000x for hotspot mutations with ≥5% VAF and ≥2% VAF, respectively.

i. Direct Comparison of the NGS-Derived VAF with Expected ddPCR VAFs

The detailed variant and sample information, along with the high-level results, are presented in Tables 10a and 10b. The VAFs detected by NGS were highly correlated with and similar to those measured by droplet digital PCR (ddPCR), yielding an R-squared value of 0.99 (see Figures 1 and 2 below).

Table 10a. Sample, Variant, and General Run Information

Sample	Horizon Cat#	Note	Library-prep #	Gene covered	Targeted Somatic mutations per sample	Total VAF measurements	ddPCR VAFs	Mean Base coverage (Range)
1	HD850	FFPE	1	*EGFR*	5	5	1%	Run1: mean = **4302** (3382 – 4992)
2	HD300	FFPE	1	*EGFR*		5	5%	
3	HD301	FFPE	1	*KRAS, NRAS*	6	6		
4	HD701	gDNA	8	*BRAF, EGFR, KRAS, NRAS, PIK3CA*	10	80	1%, 2%, 3%*, 6%, 9%, 10.5%, 12.5%, 15%, 17.5%, 24.5%	
5	HD-C749	Formalin-Compromised DNA I (Mild)	8			80		
6	HD803	Formalin-Compromised DNA I (Severe)	6			60		Run2: mean = **6475** (5523 – 7254)
7	HD799	Formalin-Compromised DNA I (moderate)	24			240		
7	HD701	gDNA	6			60		
Total			55			536		

*Intended to be 3% VAF, but the actual lot is 3.5%

Table 10b. Summary of Results for 15 Unique Mutations Across 536 Observations at Each Tested VAF Level

Expected ddPCR VAF	NGS observations	Mean NGS VAF (%)	%CV of NGS VAFs	Mutations
1%-VAF	57	1.1	31%	*EGFR*: T790M; *EGFR*: G719S, L858R, E746_A750del, T790M, L861Q
2%-VAF	52	2.0	24%	*EGFR*: E746_A750del
3%-VAF	52	3.5	22%	*EGFR*: L858R
5%-VAF	11	5.4	16%	*KRAS*: G13D, G12D, A146T, Q61H |*NRAS*: Q61K, G12V|*EGFR*: G719S, L858R, E746_A750del, T790M, L861Q
6%-VAF	52	6.3	15%	*KRAS*: G12D
9%-VAF	52	8.5	12%	*PIK3CA*: E545K
10.5%-VAF	52	12.6	14%	*BRAF*: V600E
12.5%-VAF	52	12.2	12%	*NRAS*: Q61K

Expected ddPCR VAF	NGS observations	Mean NGS VAF (%)	%CV of NGS VAFs	Mutations
15%-VAF	52	15.4	11%	*KRAS*: G13D
17.5%-VAF	52	18.4	10%	*PIK3CA*: H1047R
24.5%-VAF	52	24.7	6%	*EGFR*: G719S
Total	536			

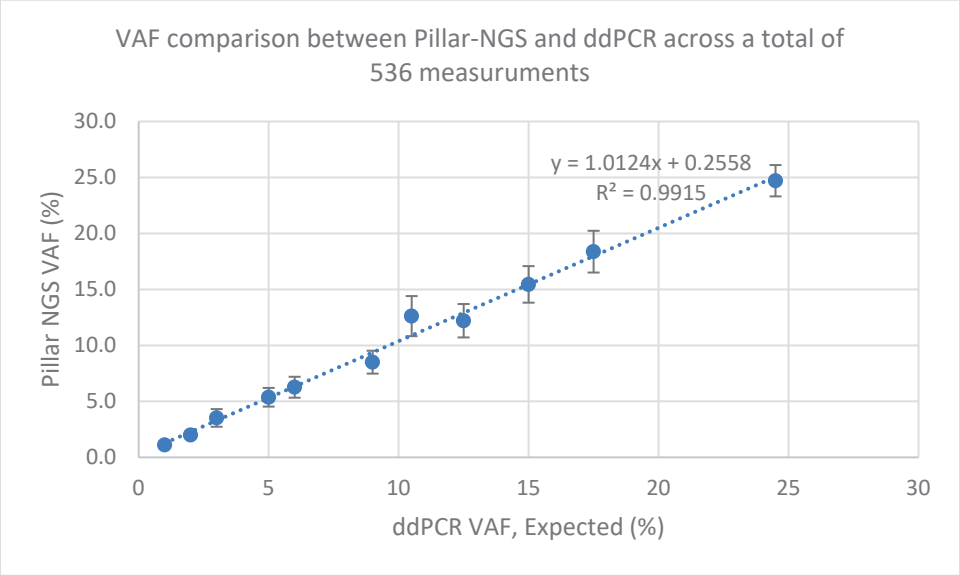


Figure 1: Correlation between NGS and ddPCR VAFs. Comparison between variant allele frequencies (VAF) measured by NGS or ddPCR. Samples used in analysis were eight different FFPE or mixed cell line-derived DNA reference materials. A total of 55 libraries were prepared and sequenced from two independent sequencing runs. 536 observations across 15 unique hotspot mutations estimated to be at 10 different VAF levels were compared. (n=11-57 NGS observations per ddPCR determined VAF level) A. Correlation between VAFs measured by oncoReveal NGS and ddPCR method. Error bars represent SD in NGS VAF measurement.

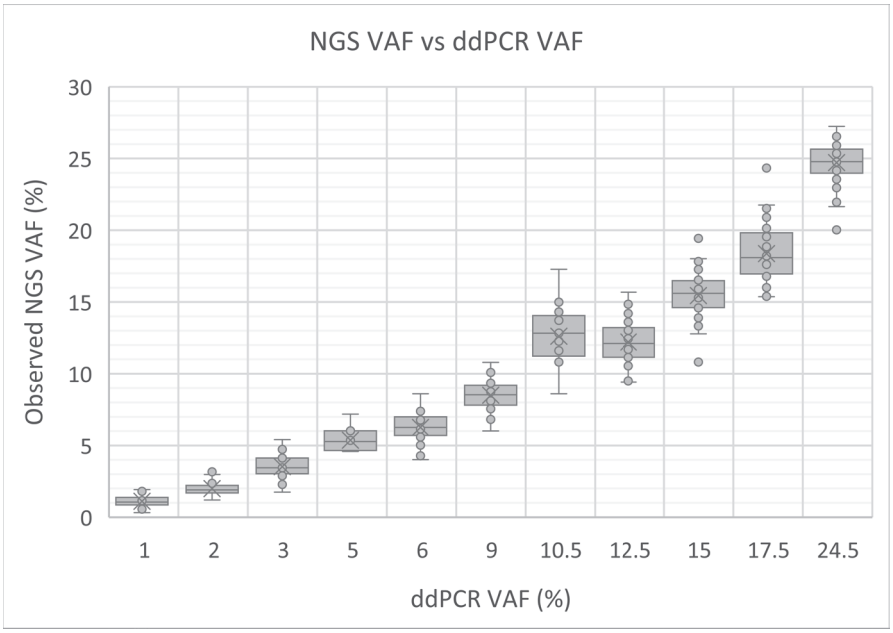


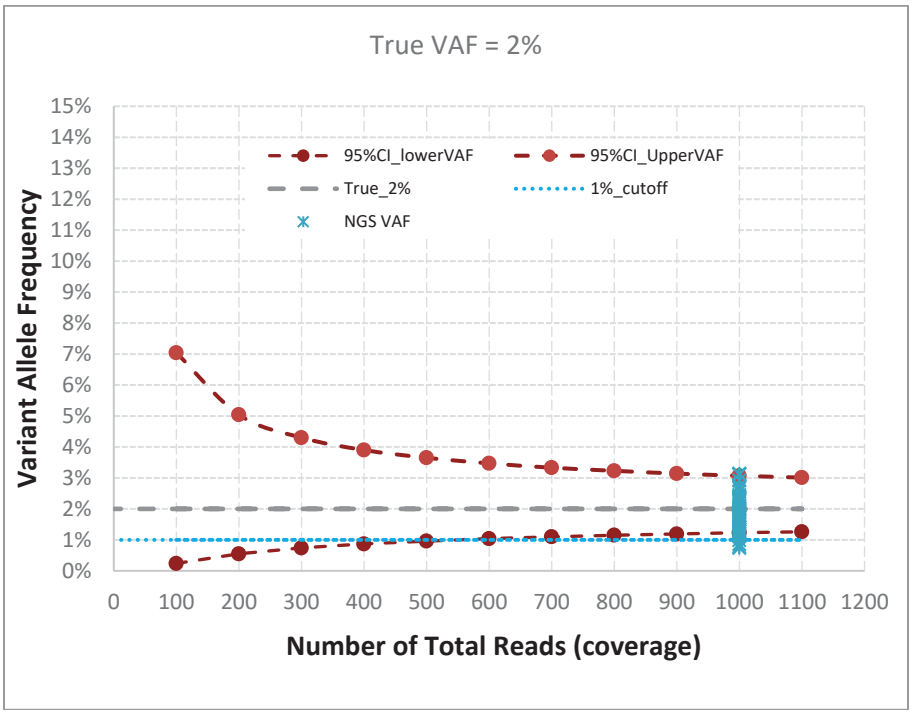
Figure 2: Boxplot of NGS VAF distribution across a total of 536 VAF measurements. Box and whisker plot of NGS determined VAF across all 536 observations (Figure 1), binned by ddPCR VAF. Box and whisker plot with outliers: the boxes represent the interquartile ranges (IQR) from the first quartiles to the third quartiles. The vertical lines go through the boxes at the median with mean value marked. The whiskers extend down or up from each quartile to the minimum data value or maximum data value within 1.5 times the IQR. Values outside of this range are considered to be outliners and represented by a small filled-in circle.

ii. Variant Total Coverage Requirements

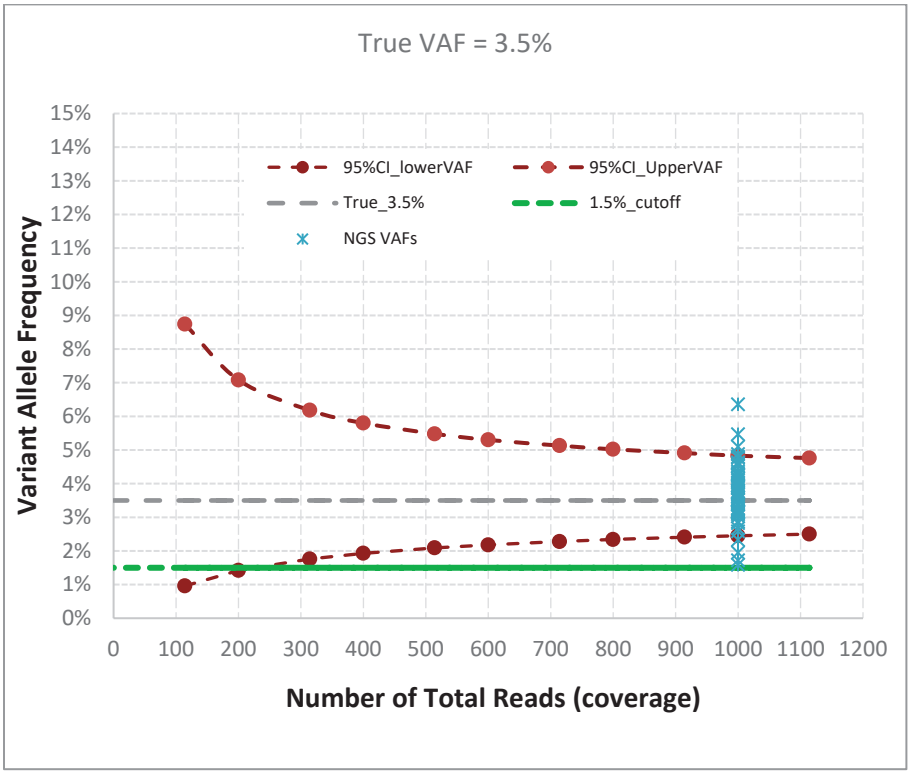
In silico down sampling analysis was conducted on the 55 libraries listed in Table 10a to normalize hotspot coverage to approximately 500x or 1000x. For the expected underlying VAFs of 2% and 3.5%, at a coverage of ~1000x, the observed VAFs were detected at 1.94% (ranging from 0.7% - 3.1%) and 3.68% (ranging from 1.6% to 6.4%) respectively. At a coverage of ~500x, the observed VAFs for an expected 5% VAF ranged from 3.9% to 8.4% with a mean VAF of 5.48%; for an expected 6% VAF, the observed range was 2.7% to 9.1% with a mean VAF at 6.31%. These ranges are roughly in agreement with theoretical statistical estimates (see Figure 3 below). The data support the use of a 1 – 1.5% VAF threshold to detect CDx and Group1 variants with true underlying VAFs of 2-4% at a coverage of ≥ 1000x; a threshold of 3.2% is recommended to report Group 2 variants with true underlying VAFs of 5-6% at a coverage of ≥ 500x.

For Group-2 variants with a VAF cut-off of 3.2%, a "No call" is reported if the position coverage is sequenced at less than 500x and no positive variant is detected at this position. Similarly, for Group-1 variants with a VAF cut-off of 1-1.5%, a "No call" is reported if the position coverage is sequenced at less than 1000x. A "No call" designation indicates that there is a risk of false-negative calls, especially for true variants at VAFs levels around the Limit of detection (LoD).

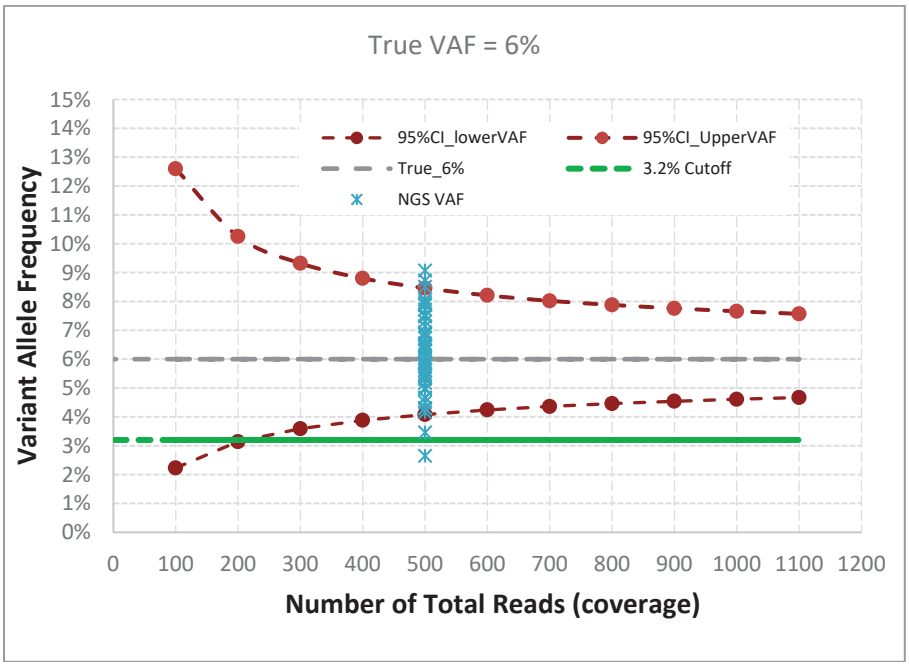
A.


True VAF = 2%
95%CI_lowerVAF
95%CI_UpperVAF
True_2%
1%_cutoff
NGS VAF
Variant Allele Frequency
15%
14%
13%
12%
11%
10%
9%
8%
7%
6%
5%
4%
3%
2%
1%
0%
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
Number of Total Reads (coverage)

B.


True VAF = 3.5%
95%CI_lowerVAF
95%CI_UpperVAF
True_3.5%
1.5%_cutoff
NGS VAFs
Variant Allele Frequency
15%
14%
13%
12%
11%
10%
9%
8%
7%
6%
5%
4%
3%
2%
1%
0%
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
Number of Total Reads (coverage)

C.



D.

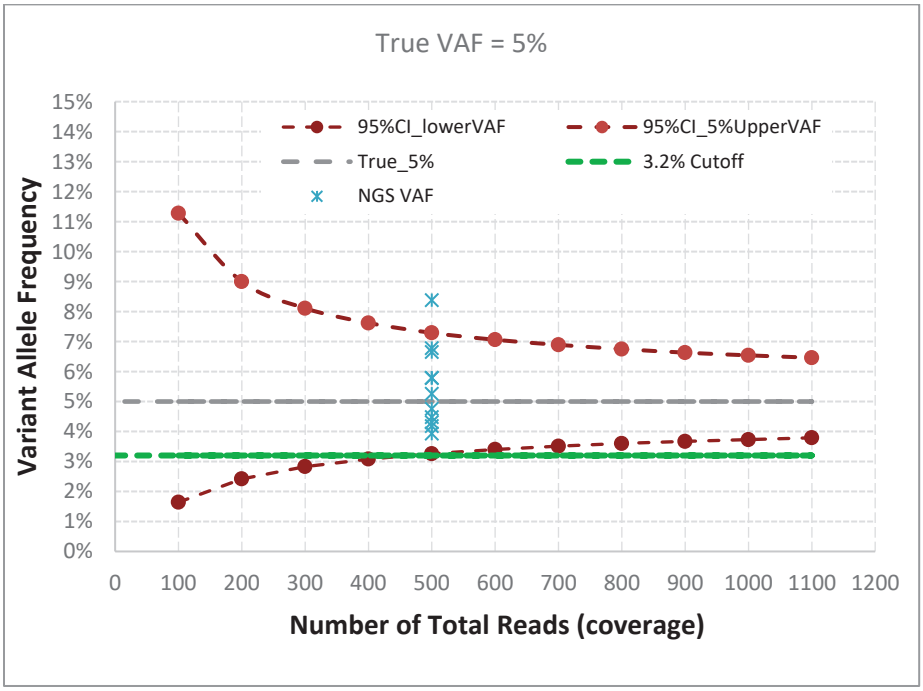


Figure 3. Comparison of observed and theoretical statistically estimated VAFs. *In silico* down sampling analysis of 55 sequenced libraries was used to determine cutoffs for variants with true VAF values of (A) 2%, (B) 3.5%, (C) 6%, and (D) 5%. Observed VAFs after down-sampling libraries to 1000x read coverage with variants at 2% or 3.5% are shown in A and B, respectively. Observed VAFs after down-sampling libraries to 500x read coverage with variants at 6% or 5% are shown in C and D, respectively. The computed lower bound (dark orange) and upper bound (light orange) 95% CI of VAF measurements as a function of coverage as well as VAF cutoffs (green dashed lines) are shown.

iii. Requirements on Sample Quality and Sequencing Metrics for Confirmed Coverage Requirements:

Fifty-four (54) normal (diploid) FFPE samples from 9 different tissue types were tested with normalized 10ng of DNA input to provide the assessment for the pre-NGS library yield, effective on-target rate and amplicon/base coverage (Table 11).

Table 11. Normal FFPE from 9 Tissue Types

FFPE Tissue type	Count
Normal Bladder	5
Normal Breast	8
Normal Cervix	2
Normal Kidney	6
Normal Liver	7
Normal Pancreas	7
Normal Skin	6
Normal Thyroid	9
Normal Uterus	4

The results shown in Figure 4 demonstrate that a lower mean coverage depth and a higher percentage of bases with less than 500x coverage are associated with low library yield and low effective on-target rate (calculated as Mapping rate x on-target rate). The minimal requirement of 3.5nM for library yield and 70% for the effective on-target rate effectively removed low-quality libraries characterized by insufficient base coverage. The 36 normal samples that passed the filter yielded a mean base coverage of 6359x, ranging from 4619x to 10487x, with 100% of bases covered at least 200x. On average, 99.5% of bases achieved coverage exceeding 500x, with a range from 96.9 % to 100%. Statistical analysis indicates that a minimum of 10 mutation reads at a 95% power level, and 200x coverage, can detect a true underlying variant frequency of 8.5% with a 95% CI ranging from 5% to 13.3%.

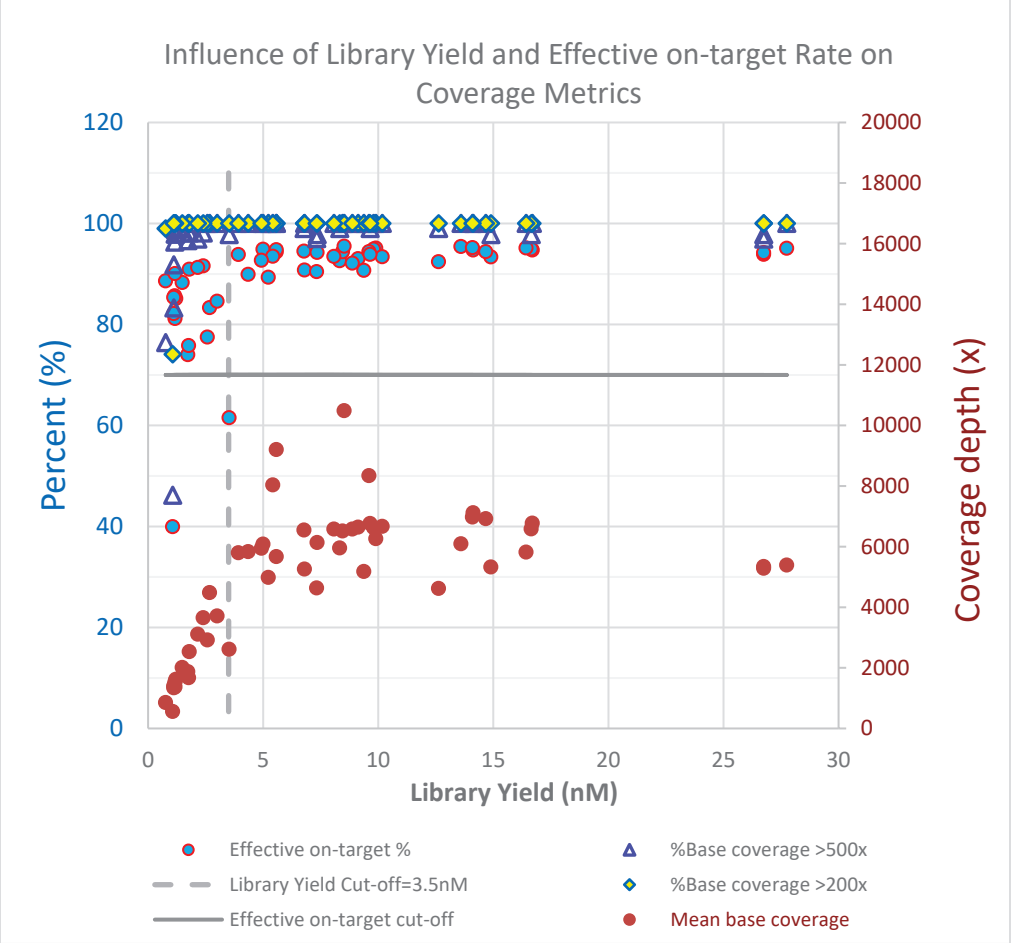


Figure 4. Establishing QC Thresholds Using 54 Normal FFPE Samples
Library yield and effective on-target rate are associated with mean coverage depth and percentage of bases below 500x coverage. Library yield cut off value of 3.5 nM (dash grey line) and effective on-target rate cut off value of 70% (solid grey line) can filter out poor quality libraries.

With the established threshold of 3.5 nM for pre-NGS library yield, sequence coverage was then evaluated across a range of FFPE samples (n= 373), with DNA input varying from 6.5 ng to 513 ng with median input of approximately 30ng. Among these, three samples had effective on-target rates below the 70% threshold and were thus deemed failures. The remaining 370 samples achieved a median average-base-coverage of 5971x with an effective on-target rate of 96.1+-2.59 (mean ± SD). Notably, only two samples did not attain 100% base coverage at a depth of 200x (Table 12).

Table 12. Base Coverage Distribution for 370 Valid FFPE samples

Base coverage stats	Sample	% sample
100% base coverage>200x	368	99.5%
100% base coverage>300x	356	96.2%
100% base coverage>500x	303	81.9%

iv. Requirements on Variant Coverage, Allele Depth and Frequency for Positive Calls:

Variant filtering parameters, as detailed in Table 8, which include coverage, variant allele depth, and variant allele frequency, were established to maximize the probability of true positive calls and minimize false positive calls.

The variant calling thresholds were empirical supported by the 57 libraries, which contained various known level of variants, and the cohort of 36 normal FFPE samples that passed the sample level QC as described in the previous sections.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are several FDA-approved CDx alternatives for the detection of genetic alterations using FFPE tumor specimens, as listed in the oncoReveal™ CDx intended use statement. The approved CDx tests are listed in Table 13 below; for additional details see FDA List of Cleared or Approved CDx Devices at https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools. Each alternative has its own advantages and disadvantages. A patient should fully discuss these alternatives with his/her physician to select the method that best meets expectations and lifestyle.

Table 13. FDA-approved companion diagnostic (CDx) Alternatives to oncoReveal CDx

Indicatio	Gene	Device	Company	Technolog	Therapy
CRC	*KRAS, NRAS*	xT CDx (P210011)	Tempus Labs, Inc.	NGS	ERBITUX (cetuximab) or VECTIBIX (panitumumab).
CRC	*KRAS*	**cobas®** KRAS Mutation Test (P140023)	Roche Molecular Systems, Inc.	PCR	ERBITUX® (cetuximab) VECTIBIX (panitumumab)
CRC	*KRAS*	*Therascreen®* KRAS RGQ PCR Kit (P110030; P110027)	QIAGEN Manchester Ltd.	PCR	ERBITUX® (cetuximab) VECTIBIX (panitumumab)
CRC	*KRAS*	FoundationOne® CDx (P170019)	Foundation Medicine, Inc.	NGS	ERBITUX® (cetuximab) VECTIBIX® (panitumumab)

NSCLC	*EGFR*	*Therascreen*® EGFR RGQ PCR Kit (P120022/S018)	QIAGEN Manchester Ltd.	PCR	IRESSA® (gefitinib) GILOTRIF® (afatinib) VIZIMPRO® (dacomitinib)
NSCLC	*EGFR*	**cobas**® EGFR Mutation Test v2 (P120019/S01	Roche Molecular Systems, Inc.	PCR	EGFR Tyrosine Kinase Inhibitors approved by FDA
NSCLC	*EGFR*	FoundationOne® CDx (P170019)	Foundation Medicine, Inc.	NGS	GILOTRIF® (afatinib) IRESSA® (gefitinib) TARCEVA® (erlotinib) TAGRISSO® (osimertinib)
NSCLC	*EGFR*	Oncomine™ Dx Target Test (P160045;	Life Technologies Corp.	NGS	IRESSA® (gefitinib)

Abbreviations: NSCLC = Non-Small Cell Lung Cancer, CRC=Colorectal Cancer

VII. MARKETING HISTORY

The oncoReveal CDx Premarket Approval (P200011) was originally approved on July 30, 2021, by FDA and has been commercially available in the U.S. since August 21, 2021.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect test results, and subsequently, inappropriate patient management decisions. Patients with false positive results may undergo treatment with one of the therapies listed in the above intended use statement without clinical benefit and may experience adverse reactions associated with the therapy. Patients with false negative results may not be considered for treatment with the indicated therapy. There is also a risk of delayed results, which may lead to delay of treatment with the indicated therapy.

For the specific adverse events related to the approved therapeutics, please see approved drug product labels.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

Performance characteristics of the oncoReveal™ CDx were established using DNA derived from a wide range of FFPE tumor tissue specimens. Studies included reportable CDx variants indicated in Table 1 of the intended use statement and a wide range of representative variant types (SNV, deletion and insertion) across 22 genes.

1. Analytical Accuracy

Analytical accuracy was performed to demonstrate the concordance between the oncoReveal CDx and two externally validated NGS (evNGS) comparator methods (A & B) to support the accuracy of the oncoReveal CDx to detect reportable SNVs, deletions and insertions for tumor profiling in 22 genes. The concordance analysis was done for overall agreement, by variant types, and per gene.

a. Comparator Method A

A total of 271 samples represented by 10 tumor types (colorectal cancer, pancreatic adenocarcinoma, non-small cell lung cancer, breast cancer, melanoma, bladder cancer, uterine corpus endometrial cancer, hepatocellular carcinoma, clear cell renal cell carcinoma, and papillary thyroid cancer) were tested across 15 genes using comparator method A. Of these samples, 257 samples yielded valid results (181 positive and 65 negative) by both assays and were included in the agreement analysis. There was a total of 11 discordant samples including 7 oncoReveal™ CDx positive (+) evNGS A negative (-), and 4 samples positive concordant call in both assays for one mutation but contained an additional variant(s) called by only one assay. In 3 samples an additional low VAF variant (<3%) was reported by oncoReveal™ CDx and not comparator A and in 1 sample, a low VAF variant (<5%) was reported in comparator A but not oncoReveal™ CDx. The three discordant variants with high VAFs (33%- 75%), two 15bp deletions and one 6-bp insertion, were confirmed positive in a third orthogonal method. All other discordant mutations are expected due to the low allelic fractions and the differences in VAF cut-offs applied by oncoReveal CDx and comparator method A. The overall sample level concordance was 95.7% (246/257).

The aggregated results at the variant-type level are shown in Table 14 and gene level is shown in Table 15 below. As the accuracy study samples were enrolled by the oncoReveal™ CDx, the positive predictive value (PPV) and negative predictive value (NPV) were direct calculations; however, the positive percent agreement (PPA) and negative percent agreement (NPA) values were adjusted using the proportion of positive variants detected by oncoReveal CDx assay.

Table 14. Comparator Method A: Overall Variant-level Agreement and Binned by Variant Type

Variant Type	# Samples	# Variants	PPA (n/N) (95% CI)	NPA (n/N) (95% CI)	PPV (n/N) (95% CI)	NPV (n/N) (95% CI)	Adjusted PPA (95% CI)	Adjusted NPA (95% CI)
All	257	243636	99.6% (245/246) (97.7%, 99.9%)	99.9% (243380/243390) (99.9%, 99.9%)	96.1% (245/255) (92.9%, 97.9%)	99.9% (243380/243381) (99.9%, 99.9%)	99.0% (94.4%, 99.8%)	99.9% (99.9%, 99.9%)
SNV	257	141864	99.6% (228/229) (97.6%, 99.9%)	99.9% (141629/141635) (99.9%, 99.9%)	97.4% (228/234) (94.5%, 98.8%)	99.9% (141629/141630) (99.9%, 99.9%)	99.3% (95.9%, 99.9%)	99.9% (99.9%, 99.9%)
MNV	257	31354	100.0% (4/4) (51.0%, 100.0%)	100.0% (31350/31350) (99.9%, 100.0%)	100.0% (4/4) (51.0%, 100.0%)	100.0% (31350/31350) (99.9%, 100.0%)	100.0% (16.5%, 100.0%)	100.0% (99.9%, 100.0%)
Deletion	257	42148	100.0% (11/11) (74.1%, 100.0%)	99.9% (42134/42137) (99.9%, 99.9%)	78.6% (11/14) (52.4%, 92.4%)	100.0% (42134/42134) (99.9%, 100.0%)	100.0% (31.0%, 100.0%)	99.9% (99.9%, 99.9%)
Insertion	257	28270	100.0% (2/2) (34.2%, 100.0%)	99.9% (28267/28268) (99.9%, 99.9%)	66.7% (2/3) (20.8%, 93.9%)	100.0% (28267/28267) (99.9%, 100.0%)	100.0% (6.4%, 100.0%)	99.9% (99.9%, 99.9%)

SNV=single nucleotide variants, MNV=multi-nucleotide variant

Table 15. Comparator Method A: Variant-level Agreement by Gene

Gene	PPA (n/N) (95% CI)	NPA (n/N) (95% CI)	PPV (n/N) (95% CI)	NPV (n/N) (95% CI)
AKT1	100.0% (4/4) (51.0%, 100.0%)	100.0% (767/767) (99.5%, 100.0%)	100.0% (4/4) (51.0%, 100.0%)	100.0% (767/767) (99.5%, 100.0%)
ALK	Not Evaluable	100.0% (8995/8995) (99.9%, 100.0%)	Not Evaluable	100.0% (8995/8995) (99.9%, 100.0%)
BRAF	100.0% (34/34) (89.8%, 100.0%)	99.9% (21296/21297) (99.9%, 99.9%)	97.1% (34/35) (85.5%, 99.5%)	100.0% (21296/21296) (99.9%, 100.0%)
CTNNB1	100.0% (11/11) (74.1%, 100.0%)	100.0% (44964/44964) (99.9%, 100.0%)	100.0% (11/11) (74.1%, 100.0%)	100.0% (44964/44964) (99.9%, 100.0%)
DDR2	Not Evaluable	100.0% (514/514) (99.3%, 100.0%)	Not Evaluable	100.0% (514/514) (99.3%, 100.0%)

Gene	PPA (n/N) (95% CI)	NPA (n/N) (95% CI)	PPV (n/N) (95% CI)	NPV (n/N) (95% CI)
EGFR	100.0% (30/30) (88.6%, 100.0%)	99.9% (68586/68589) (99.9%, 99.9%)	90.9% (30/33) (76.4%, 96.9%)	100.0% (68586/68586) (99.9%, 100.0%)
ERBB2	80.0% (4/5) (37.6%, 96.4%)	99.9% (12330/12331) (99.9%, 99.9%)	80.0% (4/5) (37.6%, 96.4%)	99.9% (12330/12331) (99.9%, 99.9%)
ERBB4	Not Evaluable	Not Evaluable	Not Evaluable	Not Evaluable
FBXW7	Not Evaluable	Not Evaluable	Not Evaluable	Not Evaluable
FGFR1	Not Evaluable	100.0% (771/771) (99.5%, 100.0%)	Not Evaluable	100.0% (771/771) (99.5%, 100.0%)
FGFR2	100.0% (4/4) (51.0%, 100.0%)	100.0% (4365/4365) (99.9%, 100.0%)	100.0% (4/4) (51.0%, 100.0%)	100.0% (4365/4365) (99.9%, 100.0%)
FGFR3	100.0% (5/5) (56.6%, 100.0%)	100.0% (4621/4621) (99.9%, 100.0%)	100.0% (5/5) (56.6%, 100.0%)	100.0% (4621/4621) (99.9%, 100.0%)
KRAS	100.0% (93/93) (96.0%, 100.0%)	99.9% (25603/25607) (99.9%, 99.9%)	95.9% (93/97) (89.9%, 98.4%)	100.0% (25603/25603) (99.9%, 100.0%)
MAP2K1	100.0% (1/1) (20.7%, 100.0%)	99.9% (7194/7195) (99.9%, 99.9%)	50.0% (1/2) (9.5%, 90.5%)	100.0% (7194/7194) (99.9%, 100.0%)
MET	Not Evaluable	100.0% (2827/2827) (99.9%, 100.0%)	Not Evaluable	100.0% (2827/2827) (99.9%, 100.0%)
NOTCH1	Not Evaluable	Not Evaluable	Not Evaluable	Not Evaluable
NRAS	100.0% (10/10) (72.2%, 100.0%)	100.0% (12069/12069) (99.9%, 100.0%)	100.0% (10/10) (72.2%, 100.0%)	100.0% (12069/12069) (99.9%, 100.0%)
PIK3CA	100.0% (49/49) (92.7%, 100.0%)	100.0% (28478/28478) (99.9%, 100.0%)	100.0% (49/49) (92.7%, 100.0%)	100.0% (28478/28478) (99.9%, 100.0%)
PTEN	Not Evaluable	Not Evaluable	Not Evaluable	Not Evaluable
SMAD4	Not Evaluable	Not Evaluable	Not Evaluable	Not Evaluable
STK11	Not Evaluable	Not Evaluable	Not Evaluable	Not Evaluable
TP53	Not Evaluable	Not Evaluable	Not Evaluable	Not Evaluable

b. Comparator Method B

A second validated Comparator Method B (evNGS B) was used to include 6 additional genes not targeted by Method A by testing samples from 10 cancer types (colorectal cancer, pancreatic adenocarcinoma, non-small cell lung cancer, breast cancer, melanoma, bladder cancer, uterine corpus endometrial cancer, hepatocellular carcinoma, clear cell renal cell carcinoma, and papillary thyroid cancer). From the total enrolled 212 samples, 187 samples yielded valid results (158 positive and 10 negative) for both assays and included in the agreement study. There was a total of 19 discordant samples consisting of 2 oncoReveal™

CDx positive (+) evNGS B negative (-) and 17 samples that showed positive concordance in the targeted variant(s) but contained additional positive variant calls in either oncoReveal CDx or comparator B. The overall sample level concordance was 90% (168//187).

The aggregated results at the variant-level is shown in Table 16 and gene level is shown in Table 17 below. As the accuracy study samples were enrolled by the oncoReveal™ CDx, the PPV and NPV were direct calculations; however, the PPA and NPA values were adjusted using the proportion of positive variants detected by oncoReveal CDx assay.

Table 16. Comparator B: Overall Variant-level Agreement and Binned by Variant Type

Variant Type	# Samples	# Variants	PPA (n/N) (95% CI)	NPA (n/N) (95% CI)	PPV (n/N) (95% CI)	NPV (n/N) (95% CI)	Adjusted PPA (95% CI)	Adjusted NPA (95% CI)
All	187	661045	98.6% (345/350) (96.7%, 99.4%)	99.9% (660677/660695) (99.9%, 99.9%)	95.0% (345/363) (92.3%, 96.8%)	99.9% (660677/660682) (99.9%, 99.9%)	94.3% (91.1%, 96.3%)	99.9% (99.9%, 99.9%)
SNV	187	250954	98.7% (308/312) (96.8%, 99.5%)	99.9% (250627/250642) (99.9%, 99.9%)	95.4% (308/323) (92.5%, 97.2%)	99.9% (250627/250631) (99.9%, 99.9%)	94.4% (90.9%, 96.5%)	99.9% (99.9%, 99.9%)
MNV	187	37587	100.0% (6/6) (61.0%, 100.0%)	100.0% (37581/37581) (99.9%, 100.0%)	100.0% (6/6) (61.0%, 100.0%)	100.0% (37581/37581) (99.9%, 100.0%)	100.0% (27.5%, 100.0%)	100.0% (99.9%, 100.0%)
Deletion	187	238051	100.0% (21/21) (84.5%, 100.0%)	99.9% (238028/238030) (99.9%, 99.9%)	91.3% (21/23) (73.2%, 97.6%)	100.0% (238028/238028) (99.9%, 100.0%)	89.3% (66.0%, 96.8%)	100.0% (99.9%, 100.0%)
Insertion	187	134453	90.9% (10/11) (62.3%, 98.4%)	99.9% (134441/134442) (99.9%, 99.9%)	90.9% (10/11) (62.3%, 98.4%)	99.9% (134441/134442) (99.9%, 99.9%)	86.7% (44.1%, 97.6%)	99.9% (99.9%, 99.9%)

SNV=single nucleotide variants, MNV=multi-nucleotide variant

Table 17. Comparator Method B: Variant-level Agreement by Gene

Gene	PPA (n/N) (95% CI)	NPA (n/N) (95% CI)	PPV (n/N) (95% CI)	NPV (n/N) (95% CI)
AKT1	100.0% (4/4) (51.0%, 100.0%)	100.0% (370/370) (99.0%, 100.0%)	100.0% (4/4) (51.0%, 100.0%)	100.0% (370/370) (99.0%, 100.0%)

Gene	PPA (n/N) (95% CI)	NPA (n/N) (95% CI)	PPV (n/N) (95% CI)	NPV (n/N) (95% CI)
ALK	100.0% (1/1) (20.7%, 100.0%)	100.0% (6357/6357) (99.9%, 100.0%)	100.0% (1/1) (20.7%, 100.0%)	100.0% (6357/6357) (99.9%, 100.0%)
BRAF	100.0% (37/37) (90.6%, 100.0%)	99.9% (15296/15297) (99.9%, 99.9%)	97.4% (37/38) (86.5%, 99.5%)	100.0% (15296/15296) (99.9%, 100.0%)
CTNNB1	94.7% (18/19) (75.4%, 99.1%)	100.0% (32706/32706) (99.9%, 100.0%)	100.0% (18/18) (82.4%, 100.0%)	99.9% (32706/32707) (99.9%, 99.9%)
DDR2	Not Evaluable	100.0% (561/561) (99.3%, 100.0%)	Not Evaluable	100.0% (561/561) (99.3%, 100.0%)
EGFR	100.0% (22/22) (85.1%, 100.0%)	100.0% (49533/49533) (99.9%, 100.0%)	100.0% (22/22) (85.1%, 100.0%)	100.0% (49533/49533) (99.9%, 100.0%)
ERBB2	100.0% (7/7) (64.6%, 100.0%)	100.0% (8782/8782) (99.9%, 100.0%)	100.0% (7/7) (64.6%, 100.0%)	100.0% (8782/8782) (99.9%, 100.0%)
ERBB4	100.0% (6/6) (61.0%, 100.0%)	100.0% (3734/3734) (99.9%, 100.0%)	100.0% (6/6) (61.0%, 100.0%)	100.0% (3734/3734) (99.9%, 100.0%)
FBXW7	100.0% (13/13) (77.2%, 100.0%)	100.0% (16443/16443) (99.9%, 100.0%)	100.0% (13/13) (77.2%, 100.0%)	100.0% (16443/16443) (99.9%, 100.0%)
FGFR1	100.0% (1/1) (20.7%, 100.0%)	100.0% (934/934) (99.6%, 100.0%)	100.0% (1/1) (20.7%, 100.0%)	100.0% (934/934) (99.6%, 100.0%)
FGFR2	100.0% (7/7) (64.6%, 100.0%)	100.0% (2985/2985) (99.9%, 100.0%)	100.0% (7/7) (64.6%, 100.0%)	100.0% (2985/2985) (99.9%, 100.0%)
FGFR3	100.0% (3/3) (43.9%, 100.0%)	99.9% (3362/3363) (99.8%, 99.9%)	75.0% (3/4) (30.1%, 95.4%)	100.0% (3362/3362) (99.9%, 100.0%)
KRAS	96.8% (30/31) (83.8%, 99.4%)	99.9% (17919/17921) (99.9%, 99.9%)	93.8% (30/32) (79.9%, 98.3%)	99.9% (17919/17920) (99.9%, 99.9%)
MAP2K1	100.0% (2/2) (34.2%, 100.0%)	100.0% (5608/5608) (99.9%, 100.0%)	100.0% (2/2) (34.2%, 100.0%)	100.0% (5608/5608) (99.9%, 100.0%)
MET	Not Evaluable	100.0% (2431/2431) (99.8%, 100.0%)	Not Evaluable	100.0% (2431/2431) (99.8%, 100.0%)
NOTCH1	Not Evaluable	Not Evaluable	Not Evaluable	Not Evaluable
NRAS	100.0% (21/21) (84.5%, 100.0%)	100.0% (8394/8394) (99.9%, 100.0%)	100.0% (21/21) (84.5%, 100.0%)	100.0% (8394/8394) (99.9%, 100.0%)

Gene	PPA (n/N) (95% CI)	NPA (n/N) (95% CI)	PPV (n/N) (95% CI)	NPV (n/N) (95% CI)
PIK3CA	100.0% (57/57) (93.7%, 100.0%)	99.9% (20699/20700) (99.9%, 99.9%)	98.3% (57/58) (90.9%, 99.7%)	100.0% (20699/20699) (99.9%, 100.0%)
PTEN	100.0% (40/40) (91.2%, 100.0%)	99.9% (131789/131795) (99.9%, 99.9%)	87.0% (40/46) (74.3%, 93.9%)	100.0% (131789/131789) (99.9%, 100.0%)
SMAD4	100.0% (10/10) (72.2%, 100.0%)	99.9% (32713/32715) (99.9%, 99.9%)	83.3% (10/12) (55.2%, 95.3%)	100.0% (32713/32713) (99.9%, 100.0%)
STK11	100.0% (6/6) (61.0%, 100.0%)	100.0% (15702/15702) (99.9%, 100.0%)	100.0% (6/6) (61.0%, 100.0%)	100.0% (15702/15702) (99.9%, 100.0%)
TP53	95.2% (60/63) (86.9%, 98.4%)	99.9% (284359/284364) (99.9%, 99.9%)	92.3% (60/65) (83.2%, 96.7%)	99.9% (284359/284362) (99.9%, 99.9%)

The results of the accuracy study support the accuracy of variant (SNVs, insertions and deletions) calling by the oncoReveal CDx assay.

2. Analytical Sensitivity

a. Limit of Blank (LoB)

An LoB study was conducted by evaluating DNA samples extracted from 16 FFPE specimens from normal tissues for ten cancer types. The following normal tissue were evaluated: lung, colon, bladder, breast, uterus, kidney, liver, pancreas, skin, and thyroid. Each sample was tested with 4 to18 replicates at the maximum specified DNA input for oncoReveal™ CDx which is 80 ng, with two reagent lots, two to three replicates over two to three sequencing runs. All 105 replicate measurements yielded valid results. As shown in Table 18, no false positive calls were observed confirming the false positive rate at 80 ng DNA input as zero.

Table 18. LoB Study Results

Tissue Type	Number of Valid Samples	CDx Positive	Level 2 Positive	Level 3 Positive
Normal Lung	18	0/18	0/18	0/18
Normal Colon	18	0/18	0/18	0/18
Normal Bladder	10	0/10	0/10	0/10
Normal Breast	8	0/8	0/8	0/8
Normal Uterus	10	0/10	0/10	0/10
Normal Kidney	9	0/9	0/9	0/9

Tissue Type	Number of Valid Samples	CDx Positive	Level 2 Positive	Level 3 Positive
Normal Liver	10	0/10	0/10	0/10
Normal Pancreas	9	0/9	0/9	0/9
Normal Skin	4	0/4	0/4	0/4
Normal Thyroid	9	0/9	0/9	0/9
Positive calls/Valid Results	**105**	**0/105**	**0/105**	**0/105**
Percent False Positive Rate		**0%**	**0%**	**0%**

b. Limit of Detection (LoD)

Eleven (11) NSCLC and CRC specimens containing 14 tumor profiling variants (13 SNVs, and 1 insertion) were evaluated in this study. The LoD for tumor profiling variants were estimated using the hit rate approach where LoD is defined as the VAF detected at ≥95% hit rate. A minimum of 5 titration levels were tested with 20 replicates per level with two reagent lots (10 replicates per lot) using the minimum specified DNA input for the oncoReveal CDx, which is 30 ng.

The estimated LoD of each variant is summarized in Table 19 below. LoD of CDx variants were previously reported in P200011, which included a deletion (refer to Section IX.A.2b for the P200011 SSED) for which an LoD of 1.7% VAF was established. Based on the established LoD results presented in Table 20, the LoD ranges from 1.4% to 4.8% VAF.

Table 19. Summary of oncoReveal CDx assay non-CDx Variant Limit of Detection

Gene	Nucleotide Change	Amino Acid Change	Variant Type	LoD (%VAF)
EGFR	c.2155G>T	p.Gly719Cys	SNV	1.6
EGFR	c.2369C>T	p.Thr790Met	SNV	3.0
KRAS	c.182A>T	p.Gln61Leu	SNV	2.2
KRAS	c.436G>A	p.Ala146Thr	SNV	2.8
PIK3CA	c.1624G>A	p.Glu542Lys	SNV	4.4
SMAD4	c.533C>G	p.Ser178Ter	SNV	3.7
TP53	c.880G>T	p.Glu294Ter	SNV	4.5
TP53	c.818G>A	p.Arg273His	SNV	4.7
PIK3CA	c.3140A>G	p.His1047Arg	SNV	4.1
EGFR	c.2303G>T	p.Ser768Ile	SNV	4.8
TP53	c.817C>T	p.Arg273Cys	SNV	4.1
TP53	c.892G>T	p.Glu298Ter	SNV	4.7
BRAF	c.1799T>A	p.Val600Glu	SNV	1.4
EGFR	c.2314_2319dup	p.Pro772_His773dup	Insertion	2.2

LoD confirmation

LoD established using NSCLC and CRC specimens for the 3 variant types above (SNVs, insertion and deletion) were subsequently confirmed using six replicates across 20 samples which included 10 tumor types (specimens from bladder, breast, renal, colon, liver, skin, lung, pancreatic, thyroid and uterine/endometrial cancer). Each specimen had one or more variants between 1x-1.5x LoD and was confirmed with six replicates. Some variants were at 1.5x -2x LoD in the LoD confirmation study. The LoD of 10 SNVs, seven deletions and seven insertions were confirmed as shown in Table 20 below.

Table 20. Observed Hit Rate of LoD Confirmation Stratified by Variant Type.

Variant Type	Positive Detected	Total Positive	Negative Detected	Total Negative	Positive call Rate (n/N) 95% CI	Negative call Rate (n/N) 95% CI
SNV	60	60	8340	8340	100.0% (60/60) (94.0%, 100.0%)	100.0% (8340/8340) (100.0%, 100.0%)
Insertion	41	42[1]	4344	4344	97.6% (41/42) (87.7%, 99.6%)	100.0% (4344/4344) (99.9%, 100.0%)
Deletion	41	42[1]	7830	7830	97.6% (41/42) (87.7%, 99.6%)	100.0% (7830/7830) (100.0%, 100.0%)
ALL	142	144	20514	20514	98.6% (142/144) (95.1%, 99.6%)	100.0% (20514/20514) (100.0%, 100.0%)

[1] One replicate from a sample that contained an insertion, and a deletion was reported as invalid by PiVAT software. When invalid sample s are excluded from the concordance estimation, total insertions and deletions are 41each.

c. DNA Input

The recommended DNA input range of the oncoReveal™ CDx is between 30 ng to 80 ng. A DNA input study testing DNA inputs ranging from 5 ng to 160 ng was previously conducted with CRC and NSCLC tumor samples positive for CDx variants which included SNVs and insertions to assess the robustness of the oncoReveal™ CDx to variations in DNA input, refer to Section IX.A.2.d. of the P200011 SSED.

To assess the robustness of detection insertion variants in the recommend DNA input, five clinical samples representing 3 tumor types, NSCLC, CRC and melanoma were tested at DNA input outside the recommended DNA input range (13 to 318 ng). Concordance at each DNA input level evaluated was determined

against an evNGS comparator. Insertions 1 – 9 bases in length in four genes, *EGFR, ERBB2, TP53* and *PTEN* at 2.5 – 77% VAFs were evaluated. All insertion were detected at the DNA inputs evaluated by both oncoReveal CDx and the evNGS assay, except for a *PTEN* insertion evaluated at 5.1% VAF and 21 ng DNA input level, which was not detected by the evNGS comparator. The discordance may be explained by the variant being below the LoD of the evNGS comparator.

In addition, a supplemental evaluation was performed to confirm performance at 30 ng DNA input extracted from FFPE of eight additional tumor types (other than CRC and NSCLC). The tissues include melanoma, bladder, breast, endometrial, liver, melanoma, pancreatic, cancer, and thyroid cancers. Eighty-four (84) libraries were prepared with 100% valid results that included a total of 106 positive mutation calls confirmed using evNGS comparator methods for evaluation. These results confirm that the assay produced accurate results across eight additional tumor types using minimum DNA input at 30 ng with PPA 100% and NPA of 99.97%.

3. Analytical Specificity

a. Interfering Substances

Potential impact of interfering substances on the performance of the oncoReveal™ CDx were evaluated in three tumor types (from melanoma, breast cancer and thyroid cancer). One hundred eighty-one (181) libraries were analyzed with seven exogenous substances and 142 libraries were analyzed with three endogenous substances (refer to Table 21 for the potential interfering substances evaluated and levels tested).

Table 21. Potential Interfering Substances Tested

Exogenous/ Endogenous	Int Substance	Min/Max	Value
Exogenous	Xylene	Min	0.000002% (v/v)
Exogenous	Xylene	Max	0.000267% (v/v)
Exogenous	Qiagen Proteinase K	Min	0.000004 mg/mL
Exogenous	Qiagen Proteinase K	Max	0.000043 mg/mL
Exogenous	Buffer ATL	Min	0.0002% (v/v)
Exogenous	Buffer ATL	Max	0.0019% (v/v)
Exogenous	Buffer AL	Min	0.0002% (v/v)
Exogenous	Buffer AL	Max	0.0021% (v/v)
Exogenous	Qiagen AW1	Min	0.06% (v/v)
Exogenous	Qiagen AW1	Max	0.33% (v/v)
Exogenous	Qiagen AW2	Min	5.7% (v/v)
Exogenous	Qiagen AW2	Max	16.7% (v/v)
Exogenous	Ethanol	Min	4.0% (v/v)
Exogenous	Ethanol	Max	11.9% (v/v
Exogenous	Control	Control	N/A
Endogenous	Hemoglobin	Min	2% (g/g)

Exogenous/ Endogenous	Int Substance	Min/Max	Value
Endogenous	Hemoglobin	Max	4% (g/g)
Endogenous	Melanin	Min	0.009% (g/g)
Endogenous	Melanin	Max	4% (g/g)
Endogenous	Triglycerides	Min	2% (g/g)
Endogenous	Triglycerides	Max	12% (g/g)
Endogenous	Control	Control	N/A

Ten (10) clinical samples representing 11 variants (4 CDx, 7 tumor profiling variants) were evaluated in the study. Agreement analysis of valid samples against no substance controls showed PPA and NPA at 100% for all substances and levels tested. No impact on the performance of the oncoReveal CDx was observed for each substance and at each level tested.

The impact of necrosis on the performance of oncoReveal CDx was also evaluated by assessing the valid rate of the samples processed in the accuracy study. Of the 312 samples with necrotic tissue content (0 - 60%) available, 284 samples (with 9 insertion variants) passed oncoReveal and comparator QC metrics and were included in the concordance analysis. For samples with 0-10% necrosis, the concordance was 92%, For samples with 10-20% necrosis, the concordance was 83%. For this group, there were 4 discordant. One sample had low VAF levels near LoD and one had low quality by the comparator, not the oncoReveal CDx. The reason for discordance of two out of the 4 samples are likely not due to necrosis but the reason is unknown. For samples with 20-30%, 30-40%, 40-50% and 50-60% necrosis, the concordances were 100%, respectively. Overall concordance was about 93%.

4. Precision and Reproducibility

a. Three (3)-site reproducibility study

A multi-site reproducibility study was performed to support oncoReveal™ CDx performance to detect tumor profiling mutations from different cancer indications. The reproducibility of the oncoReveal™ CDx was evaluated using 10 clinical samples with target tumor profiling variants adjusted to %VAF in the range of 1-1.5x LoD. The sample panel included FFPE tissues from six tumor types, including: bladder, colorectal, melanoma, NSCLC, pancreatic, and uterine/endometrial cancers and is summarized in Table 22. The study was conducted at three sites performing 3 runs on non-consecutive days. One sequencing instrument and one reagent lot were used at each site. Each sample was tested with up to 4 replicates in each run for a total of up to 36 possible results (3 sites by 3 runs by 4 replicates). The study produced a total of 348 test results.

Table 22. Multi-site Reproducibility Study 31 Variants (12 genes) 10 Clinical Samples.

Sample	Gene	Exon	Nucleotide Change	Amino Acid Change	Variant type	Variant level***	Ratio Mean VAF/LoD	Mean VAF	Median	SD	CV %
1	*BRAF*	15	1799T>A	V600E	SNV	T-2	6.76	12.84	12.88	0.53	4.1
1	*FBXW7*	10	1436G>A	R479Q	SNV	T-3	1.97	8.85	8.76	0.67	7.6
1	*PIK3CA*	10	1634A>G	E545G	SNV	T-3	2.82	12.71	12.72	0.49	3.8
1	*PTEN*	1	17_18del	K6RfsTer4	Del	T-3	2.11	9.50	9.44	0.63	6.6
1**	*PTEN*	7	710dup	F238VfsTer5	Ins	T-3	***0.93***	4.18	4.12	0.35	8.4
1	*PTEN*	7	800del	K267RfsTer9	Del	T-3	1.15	5.16	5.11	0.58	11.2
1	*PTEN*	8	968del	N323MfsTer21	Del	T-3	1.54	6.95	6.91	0.38	5.5
1	*TP53*	7	714dup	N239Ter	Ins	T-3	1.39	6.26	6.25	0.54	8.7
2	*FBXW7*	9	1417dup	R473KfsTer4	Ins	T-3	1.52	6.84	6.78	0.68	9.9
2	*NRAS*	3	182A>G	Q61R	SNV	T-3	1.08	4.88	4.83	0.60	12.3
2	*TP53*	5	455del	P152RfsTer18	Del	T-3	2.23	10.02	10.19	1.42	14.1
3	*EGFR*	20	2300_2308dup	A767_V769dup	Ins	T-2	1.57	2.98	3.03	0.69	23.0
4**	*ERBB2*	20	.2321_2326dup	A775_G776insVA	Ins	T-3	***0.94***	4.23	4.24	0.52	12.4
5	*BRAF*	15	1798_1799delinsAG	V600R	MNV	T-2	1.76	7.93	7.93	0.50	6.3
6	*FGFR3*	9	1118A>G	Y373C	SNV	T-2	1.08	4.86	4.87	0.82	16.8
7	*FGFR3*	9	1118A>G	Y373C	SNV	T-2	1.44	6.49	6.50	0.88	13.5
8	*EGFR*	20	2303_2311dup	S768_D770dup	Ins	T-3	2.80	5.32	5.39	0.53	10.
8	*FGFR2*	12	1647T>G	N549K	SNV	T-3	1.17	5.27	5.26	0.58	11.
8	*PIK3CA*	10	1637A>G	N546R	SNV	T-3	1.02	4.60	4.61	0.32	6.9
8	*PTEN*	5	313del	C105VfsTer8	Del	T-3	1.03	4.64	4.58	0.41	8.8
8	*PTEN*	8	968del	N323MfsTer21	Del	T-3	1.08	4.85	4.87	0.33	6.8
9	*EGFR*	20	2303_2311dup	S768_D770dup	Ins	T-3	1.83	3.47	3.58	0.49	14.2
9*	*PIK3CA*	10	1637A>G	Q546R	SNV	T-3	***0.76***	3.19	3.17	0.27	8.0
9*	*PTEN*	5	313del	C105VfsTer8	Del	T-3	***0.72***	3.24	3.18	0.29	9.0
9*	*PTEN*	8	968del	N323MfsTer21	Del	T-3	***0.81***	3.63	3.60	0.36	10.0
9*	*FGFR2*	12	1647T>G	N549Lys	SNV	T-3	***0.82***	3.64	3.60	0.31	8.5
10	*KRAS*	2	34G>T	G12C	SNV	T-1	1.96	3.72	3.68	0.42	11.3
10	*KRAS*	2	35G>A	G12D	SNV	T-1	6.57	17.07	16.91	1.01	5.9
10	*ERBB2*	20	2321_2326dup	A775_G776insVA	Ins	T-3	7.46	33.59	34.48	3.27	9.7
10	*SMAD4*	6	778dup	Y260LfsTer4	Ins	T-3	2.54	11.42	11.38	0.74	6.5
10	*TP53*	5	.378dup	S127LfsTer22	Ins	T-3	1.34	6.03	5.99	0.72	11.9

*Mean observed VAF falls in 0.7 – 0.8x LoD were analyzed with inclusion and exclusion.

** Mean observed VAF is > 0.9x LoD and is included for agreement analysis.
*** Variant Level refer to tumor profiling levels 1 through 3
Ins=Insertion, Del=Deletion
C= colorectal, P= Pancreas, L= non-small cell lung cancer, M= Melanoma, BL= Bladder, U= Uterine/Endometrial

Site to Site Reproducibility:

Site to site reproducibility was assessed via positive and negative call rate for each test site (Table 23). The concordance analysis was performed with and without variants with allele frequencies 0.7-0.9x below the LoD of the device. The overall positive agreement across all sites was 96.0% (1044/1088; 94.6-97.0% CI) when assessed using all 31 variants detected in the sample panel and 100% (944/944; 99.6-100.0% CI) when assessed excluding four variants below the LoD (0.7 – 0.9x LoD) of the device. Negative call rate agreement was 100% when assessed both with (1263916/1263936; 99.998-100.0% CI) and without (1263916/1263936; 99.998-100.0% CI) variants below LoD.

Table 23. Multi-site Agreement by Site.

SITE	# samples	# libraries	# variants	Positive Call Rate (n/N) (2-sided 95% CI)	Negative Call Rate (n/N) (2-sided 95% CI)
ALL	10	348	31	96.0% (1044/1088) (94.6%, 97.0%)	100.0% (1263916/1263936) (99.998%, 100.0%)
Site 1	10	120	31	96.0% (357/372) (93.5%, 97.5%)	100.000% (435840/435840) (99.999%, 100.0%)
Site 2	10	108	31	95.6% (329/344) (92.9%, 97.3%)	99.995% (392236/392256) (99.992%, 100.0%)
Site 3	10	120	31	96.2% (358/372) (93.8%, 97.7%)	100.0% (435840/435840) (100.0%, 100.0%)
Excluded 4 variants with allele frequencies between 0.7-0.9x LoD					
ALL	10	348	27	100.0% (944/944) (99.6%, 100.0%)	100.0% (1263916/1263936) (99.998%, 100.0%)
Site 1	10	120	27	100.0% (324/324) (98.8%, 100.0%)	100.000% (435840/435840) (99.999%, 100.0%)
Site 2	10	108	27	100.0% (296/296) (98.7%, 100.0%)	99.995% (392236/392256) (99.992%, 100.0%)
Site 3	10	120	27	100.0% (324/324) (98.8%, 100.0%)	100.0% (435840/435840) (100.0%, 100.0%)

Agreements Per Variant Type:

The positive call rates for SNV, MNV, insertions, and deletions, stratified by variant allele frequency (VAF) relative to the LoD of the device, are summarized in Table 24. Overall positive call rate for variants above LoD (1x - >5x) was 100% for all variant types, including variants 1x-2x above the LoD (592/592; 99.4-100.0% CI). Variants detected by the oncoReveal CDx below the LoD of the device had an overall positive call rate of 79.2

(168/212; 73.3-84.2% CI). Number of variants and VAF range for each stratum is reported.

Table 24. Multi-site Agreement Analysis by Variant Type.

Mutation type	Mean VAF range	# variants	Positive Call Rate (n/N) (2-sided 95% CI)	Mean VAF range
All	0.7 – 1x LoD	6	79.2% (168/212) (73.3%, 84.2%)	3.2 – 4.2
	1 – 2x LoD	17	100.0% (592/592) (99.4%, 100.0%)	3.0 – 8.9
	2 – 5x LoD	5	100.0% (176/176) (97.9%, 100.0%)	5.3 – 12.7
	>5x LoD	3	100.0% (108/108) (96.6%, 100.0%)	12.8 – 33.6
SNV	0.7 – 1x LoD	2	70.8% (51/72) (59.5%, 80.1%)	3.2 – 3.6
	1 – 2x LoD	7	100.0% (240/240) (98.4%, 100.0%)	3.7 – 8.9
	2 – 5x LoD	1	100.0% (36/36) (90.4%, 100.0%)	12.7 – 12.7
	>5x LoD	2	100.0% (72/72) (94.9%, 100.0%)	12.8 – 17.1
Insertion	~1x LoD	2	100.0% (68/68) (94.7%, 100.0%)	4.2 – 4.2
	1 – 2x LoD	5	100.0% (180/180) (97.9%, 100.0%)	3.0 – 6.8
	2 – 5x LoD	2	100.0% (68/68) (94.7%, 100.0%)	5.3 – 11.4
	>5x LoD	1	100.0% (36/36) (90.4%, 100.0%)	33.6 – 33.6
Deletion	0.7 – 1x LoD	2	68.1% (49/72) (56.6%, 77.7%)	3.2 – 3.6
	1 – 2x LoD	4	100.0% (136/136) (97.3%, 100.0%)	4.6 – 6.9
	2 – 5x LoD	2	100.0% (72/72) (94.9%, 100.0%)	9.5 – 10.0
MNV	1 – 2x LoD	1	100.0% (36/36) (90.4%, 100.0%)	7.9 – 7.9

Analysis of Source of Variance:

Variance due to site, operator, or day of run was assessed using Average Positive Agreement (APA) and Average Negative Agreement (ANA). Results are summarized in Table 25.

Table 25. Inter-site, Inter-operator, and Inter-day/Run Analysis of the Multi-site Reproducibility Study.

	INTER-SITE		INTER-OPERATOR		INTER-DAY/RUN	
Pair Name	**APA (2-sided 95%CI)**	**ANA (2-sided 95%CI)**	**APA (2-sided 95%CI)**	**ANA (2-sided 95%CI)**	**APA (2-sided 95%CI)**	**ANA (2-sided 95%CI)**
ALL	99.0% (98.5%, 99.4%)	100.0% (100.0%, 100.0%)	99.5% (99.1%, 99.7%)	100.0% (100.0%, 100.0%)	98.3% (98.0%, 98.6%)	100.0% (100.0%, 100.0%)
SNV	100.0% (98.5%, 100.0%)	100.0% (100.0%, 100.0%)	99.1% (98.3%, 99.5%)	100.0% (100.0%, 100.0%)	97.8% (97.2%, 98.2%)	100.0% (100.0%, 100.0%)
Insertion	100.0% (98.3%, 100.0%)	100.0% (100.0%, 100.0%)	100.0% (99.6%, 100.0%)	100.0% (100.0%, 100.0%)	100.0% (99.9%, 100.0%)	100.0% (100.0%, 100.0%)
Deletion	100.0% (97.7%, 100.0%)	100.0% (100.0%, 100.0%)	99.3% (98.3%, 99.7%)	100.0% (100.0%, 100.0%)	96.7% (95.8%, 97.4%)	100.0% (100.0%, 100.0%)
MNV	100.0% (86.2%, 100.0%)	100.0% (100.0%, 100.0%)	100.0% (96.2%, 100.0%)	100.0% (100.0%, 100.0%)	100.0% (98.7%, 100.0%)	100.0% (100.0%, 100.0%)

Agreement Per Sample

Positive and negative call rates were assessed for each of the 10 samples within the sample panel used in the multi-site reproducibility study. Samples contained between 1-8 variants per sample. One sample contained five total variants, four of which were below the LoD of the device. Positive call rate and negative call rate were assessed with and without including the four variants below LoD. Results are summarized in Table 26.

Table 26. Positive and Negative Call Rates per Sample used in Multi-Site Reproducibility.

Sample	# libraries	# variants	Positive Call Rate (n/N) (2-sided 95% CI)	Negative Call Rate (n/N) (2-sided 95% CI)
1	36	8	100.0% (288/288) (98.7%, 100.0%)	100.0% (131580/131580) (99.997%, 100.0%)
2	36	3	100.0% (108/108) (96.6%, 100.0%)	99.998% (131758/131760) (99.994%, 100.0%)
3	36	1	100.0% (36/36) (90.4%, 100.0%)	99.999% (131831/131832) (99.996%, 100.0%)
4	32	1	100.0% (32/32) (89.3%, 100.0%)	100.0% (117184/117184) (100.0%, 100.0%)
5	36	1	100.0% (36/36) (90.4%, 100.0%)	99.998% (131830/131832) (99.994%, 100.0%)

Sample	# libraries	# variants	Positive Call Rate (n/N) (2-sided 95% CI)	Negative Call Rate (n/N) (2-sided 95% CI)
6	36	1	100.0% (36/36) (90.4%, 100.0%)	99.998% (131829/131832) (99.993%, 100.0%)
7	32	1	100.0% (32/32) (89.3%, 100.0%)	99.995% (117178/117184) (99.989%, 100.0%)
8	32	5	100.0% (160/160) (97.7%, 100.0%)	99.998% (117054/117056) (99.994%, 100.0%)
9	36	5	75.6% (136/180) (68.8%, 81.3%)	99.998% (131685/131688) (99.993%, 100.0%)
	36	1[1]	100.0% (36/36) (90.4%, 100.0%)	99.998% (131685/131688) (99.993%, 100.0%)
10	36	5	100.0% (180/180) (97.9%, 100.0%)	99.999% (131687/131688) (99.996%, 100.0%)

[1] 4 variants at 0.7 – 0.9x LoD excluded.

A summary of the panel-wide precision results was presented in Table 27 below. The precision analysis was performed for the 31 variants (as listed in Table 22). A total 13 SNVs, 8 deletions and 10 insertions were evaluated. The results showed that all mutations have 100% concordance in all replicates except for 4 mutations. These 4 mutations were believed to be discordant because they have below the LoD (0.7 – 0.9x LoD) of the device.

The coefficient of variation (%CV) for the mutation allele frequency was also calculated for all 36 replicates. 20 out 31 samples had %CV ≤10%, 10/31 had between 10 and 14% and one sample had 23%. All runs passed the quality metrics criteria.

Table 27. Panel-Wide Precision Summary Results for All Replicates Tested by the 3-sites.

Gene Exon	Mutation (cDNA/Protein Changes)	NC* range	VAF range	VAF mean	VAF median	VAF (SD)	VAF (%CV)	Positive /Total Calls	Positive Call Rate (two-sided 95% CI)
ERBB2 exon20	2321_2326dup A775_G776insVA	1.03 - 1.37	24.16 - 36.8	33.59	34.48	3.27	10%	36/36	100.0% (90.4%, 100.0%)
BRAF exon15	1799T>A V600E	1.22 - 1.58	12.01 - 14.41	12.84	12.88	0.53	4%	36/36	100.0% (90.4%, 100.0%)
KRAS exon2	35G>A G12D	1.03 - 1.19	15.2 - 18.97	17.07	16.91	1.01	6%	36/36	100.0% (90.4%, 100.0%)
PIK3CA exon10	1634A>G E545G	1.5 - 1.8	11.85 - 13.82	12.71	12.72	0.49	4%	36/36	100.0% (90.4%, 100.0%)
EGFR exon20	2303_2311dup S768_D770dup	0.34 - 0.46	4.03 - 6.33	5.32	5.39	0.53	10%	32/32	100.0% (89.3%, 100.0%)
SMAD4	778dup	1.06 -	10.01	11.42	11.38	0.74	6%	36/36	100.0%

Gene Exon	Mutation (cDNA/Protein Changes)	NC* range	VAF range	VAF mean	VAF median	VAF (SD)	VAF (%CV)	Positive /Total Calls	Positive Call Rate (two-sided 95% CI)
exon6	Y260Lfs*4	1.34	- 13.11						(90.4%, 100.0%)
TP53 exon5	455del P152Rfs*18	0.4 - 0.61	7.39 - 12.55	10.02	10.19	1.42	14%	36/36	100.0% (90.4%, 100.0%)
PTEN exon1	17_18del K6Rfs*4	0.94 - 1.19	8.18 - 10.86	9.50	9.44	0.63	7%	36/36	100.0% (90.4%, 100.0%)
FBXW7 exon10	1436G>A R479Q	1.19 - 1.59	7.88 - 10.44	8.85	8.76	0.67	8%	36/36	100.0% (90.4%, 100.0%)
KRAS exon2	34G>T G12C	1.03 - 1.19	2.54 - 4.5	3.72	3.68	0.42	11%	36/36	100.0% (90.4%, 100.0%)
EGFR exon20	2303_2311dup S768_D770dup	0.36 - 0.45	2.5 - 4.45	3.47	3.58	0.49	14%	36/36	100.0% (90.4%, 100.0%)
BRAF exon15	1798_1799delinsAG V600R	1.39 - 1.8	6.88 - 8.88	7.93	7.93	0.50	6%	36/36	100.0% (90.4%, 100.0%)
EGFR exon20	2300_2308dup A767_V769dup	0.51 - 0.66	1.72 - 4.45	2.98	3.03	0.69	23%	36/36	100.0% (90.4%, 100.0%)
PTEN exon8	968del N323Mfs*21	0.84 - 1.25	6.37 - 7.73	6.95	6.91	0.38	5%	36/36	100.0% (90.4%, 100.0%)
FBXW7 exon9	1417dup R473Kfs*4	0.89 - 1.02	5.17 - 8.11	6.84	6.78	0.68	10%	36/36	100.0% (90.4%, 100.0%)
FGFR3 exon9	1118A>G Y373C	0.19 - 0.28	5.03 - 8.72	6.49	6.50	0.88	13%	32/32	100.0% (89.3%, 100.0%)
TP53 exon7	714dup N239*	0.67 - 0.83	5.06 - 7.48	6.26	6.25	0.54	9%	36/36	100.0% (90.4%, 100.0%)
TP53 exon5	378dup S127Lfs*22	0.53 - 0.79	4.66 - 7.65	6.03	5.99	0.72	12%	36/36	100.0% (90.4%, 100.0%)
FGFR2 exon12	1647T>G N549K	0.61 - 0.78	3.96 - 6.56	5.27	5.26	0.58	11%	32/32	100.0% (89.3%, 100.0%)
PTEN exon7	800del K267Rfs*9	1.01 - 1.3	4.21 - 6.86	5.16	5.11	0.58	11%	36/36	100.0% (90.4%, 100.0%)
NRAS exon3	182A>G Q61R	1.05 - 1.58	3.6 - 6.18	4.88	4.83	0.60	12%	36/36	100.0% (90.4%, 100.0%)

Gene Exon	Mutation (cDNA/Protein Changes)	NC* range	VAF range	VAF mean	VAF median	VAF (SD)	VAF (%CV)	Positive /Total Calls	Positive Call Rate (two-sided 95% CI)
FGFR3 exon9	1118A>G Y373C	0.13 - 0.19	3.55 - 7.16	4.82	4.82	0.82	17%	36/36	100.0% (90.4%, 100.0%)
PTEN exon8	968del N323Mfs*21	0.83 - 1.25	4.21 - 5.41	4.85	4.87	0.33	7%	32/32	100.0% (89.3%, 100.0%)
PTEN exon5	313del C105Vfs*8	1.21 - 1.45	3.79 - 5.77	4.64	4.58	0.41	9%	32/32	100.0% (89.3%, 100.0%)
PIK3CA exon10	1637A>G Q546R	1.5 - 1.73	4.09 - 5.19	4.60	4.61	0.32	7%	32/32	100.0% (89.3%, 100.0%)
ERBB2 exon20	2321_2326dup A775_G776ins VA	0.55 - 0.72	3.38 - 5.26	4.23	4.24	0.52	12%	32/32	100.0% (89.3%, 100.0%)
PTEN exon7	710dup F238Vfs*5	1.01 - 1.3	3.64 - 5.34	4.18	4.12	0.35	8%	36/36	100.0% (90.4%, 100.0%)
FGFR2 exon12	1647T>G N549K	0.6 - 0.87	3.2 - 4.31	3.70	3.63	0.26	7%	33/36	91.7% (78.2%, 97.1%)
PTEN exon8	968del N323Mfs*21	0.82 - 1.18	3.2 - 4.49	3.70	3.69	0.33	9%	32/36	88.9% (74.7%, 95.6%)
PIK3CA exon10	1637A>G Q546R	1.44 - 1.73	3.21 - 3.87	3.41	3.37	0.17	5%	18/36	50.0% (34.5%, 65.5%)
PTEN exon5	313del C105Vfs*8	1.18 - 1.52	3.22 - 4.02	3.48	3.46	0.21	6%	17/36	47.2% (32.0%, 63.0%)

* NC= Normalized coverage

b. Lot-to-Lot Reproducibility:

Performance of oncoReveal™ CDx was assessed across 3 reagent lots used to test 14 clinical samples at 10 replicates each for a total of 140 libraries. The testing was performed by different operators using different thermocyclers and assayed over five sequencing runs. Lot-to-lot precision as measured by APA across all variants is >98% (Table 29). The samples used to determine lot-to-lot reproducibility are summarized in Table 28 and results from the pairwise APA and ANA analysis between the three lots used in testing are detailed in Table 29.

Table 28. The Cohort of 14 Samples Used in the Lot-to-Lot Reproducibility Study.

Sample	Tumor Type	No. of observed variants		
		SNV	Insertion	Deletion
1	Colorectal cancer	1	1	1
2	Colorectal cancer	1	1	0
3	Colorectal cancer	0	0	1
4	Colorectal cancer	4	0	1
5	Colorectal cancer	0	1	0
6	Non-small cell lung cancer	0	1	0
7	Colorectal cancer	2	0	2
8	Colorectal cancer	1	1	0
9	Bladder cancer	1	0	0
10	Kidney cancer	1	0	1
11	Thyroid cancer	1	0	0
12	Uterine/ovarian cancer	1	1	0
13	Uterine/ovarian cancer	1	0	1
14	Pancreatic cancer	2	1	1
		16	7	8

Table 29. Pairwise APA and ANA Analysis of the Three Lots Tested in Lot-to-Lot Reproducibility.

Variant Type	Analysis	Between Lot A & B	Between Lot A & C	Between Lot B & C
ALL	**APA**	**98.3% (95.1%, 99.4%)**	**98.3% (95.1%, 99.4%)**	**98.9% (95.9%, 99.7%)**
	ANA	**100.0% (99.997%, 100.0%)**	**100.0% (99.997%, 100.0%)**	**100.0% (99.998%, 100.0%)**
SNV	APA	96.6% (90.3%, 98.8%)	96.5% (90.1%, 98.8%)	97.6% (91.7%, 99.3%)
	ANA	100.0% (99.99%, 100.0%)	100.0% (99.99%, 100.0%)	100.0% (99.99%, 100.0%)
Insertion	APA	100.0% (91.6%, 100.0%)	100.0% (91.6%, 100.0%)	100.0% (91.6%, 100.0%)
	ANA	100.0% (99.99%, 100.0%)	100.0% (99.99%, 100.0%)	100.0% (99.99%, 100.0%)
Deletion	APA	100.0% (92.6%, 100.0%)	100.0% (92.6%, 100.0%)	100.0% (92.6%, 100.0%)
	ANA	100.0% (99.99%, 100.0%)	100.0% (99.99%, 100.0%)	100.0% (99.99%, 100.0%)

Positive and negative call rates were calculated for each of the 14 samples used in lot-to-lot reproducibility testing. Results are summarized in Table 30.

Table 30. Per Sample Analysis of lot-to-lot Reproducibility

Sample	# libraries	Total Unique Variants	Positive Call Rate (n/N) (2-sided 95% CI)	Negative Call Rate (n/N) (2-sided 95% CI)
1	10	3	100.0% (30/30) (88.6%, 100.0%)	100.0% (36600/36600) (100.0%, 100.0%)
2	10	2	100.0% (20/20) (83.9%, 100.0%)	100.0% (36610/36610) (100.0%, 100.0%)
3	10	1	100.0% (10/10) (72.2%, 100.0%)	100.0% (36620/36620) (100.0%, 100.0%)
4	10	5	98.0% (49/50) (89.5%, 99.6%)	100.0% (36580/36580) (100.0%, 100.0%)
5	10	1	100.0% (10/10) (72.2%, 100.0%)	100.0% (36620/36620) (100.0%, 100.0%)
6	10	1	100.0% (10/10) (72.2%, 100.0%)	100.0% (36620/36620) (100.0%, 100.0%)
7	10	4	100.0% (40/40) (91.2%, 100.0%)	100.0% (36590/36590) (100.0%, 100.0%)
8	10	2	100.0% (20/20) (83.9%, 100.0%)	100.0% (36610/36610) (100.0%, 100.0%)
9	10	1	90.0% (9/10) (59.6%, 98.2%)	100.0% (36620/36620) (100.0%, 100.0%)
10	10	2	100.0% (20/20) (83.9%, 100.0%)	100.0% (36610/36610) (100.0%, 100.0%)
11	10	1	100.0% (10/10) (72.2%, 100.0%)	100.0% (36620/36620) (100.0%, 100.0%)
12	10	2	55.0% (11/20)* (34.2%, 74.2%)	100.0% (36610/36610) (100.0%, 100.0%)
13	10	2	100.0% (20/20) (83.9%, 100.0%)	100.0% (36610/36610) (100.0%, 100.0%)
14	9	4	80.6% (29/36)* (65.0%, 90.2%)	100.0% (32931/32931) (100.0%, 100.0%)

*The lower precision values can be attributed to the low VAFs that were <1X LoD for some of the variants included for positive call rate calculation.

5. DNA Extraction Method Equivalence

Three commercially available FFPE tissue extraction kits were evaluated. One column-based kit and one bead-based kit were compared to a reference IVD marked column-based kit (Table 31). Kit performance was evaluated based on the overall passing rate (extraction yield, library yield and PiVAT QC) and agreement of variant calls (Table 32).

Sixteen FFPE samples representing eight tumor types were tested. The column-based test extraction kit has an overall passing rate of 100%, PPA 100% and NPA 100%. The bead-based test extraction kit has an overall passing rate of 93.75%, PPA 100% and NPA 100%. These data demonstrate equivalence between all three extraction methods evaluated for use with the assay.

Table 31. Agreement Analysis by Extraction Method

Extraction Kit	Type	# Libraries	Test+ Reference +	Test + Refernce -	Test - Reference +	Test - Reference -	total n	PPA (95%CI)	NPA (95%CI)
Test Kit 1	Column	32	46	0	0	117170	117216	100.0% (92.3%, 100.0%)	100.0% (100.0%, 100.0%)
Test Kit 2	Bead	30	44	0	0	109846	109890	100.0% (92.0%, 100.0%)	100.0% (100.0%, 100.0%)

Table 32. Passing Criteria used to Determine Equivalence Between the Three Extraction Kits Tested

Extraction Kit	Type	Criteria 1: DNA yield >= 4.6 ng	Criteria 2: Library yield >= 3.5 nM	Criteria 3: PiVAT analysis = Sample valid	Overall Passing rate (%)
Reference Kit	Column	93.75% (15/16)	100% (30/30)	100% (30/30)	93.75% (30/32)
Test Kit 1	Column	100% (16/16)	100% (32/32)	100% (32/32)	100% (32/32)
Test Kit 2	Bead	93.75% (15/16)	100% (30/30)	100% (30/30)	93.75% (30/32)

The results demonstrate that the 3 methods yield DNA with comparable quality and quantity to generate reliable results when used with oncoReveal CDx.

6. Tissue comparability

Many factors can influence overall performance of complex molecular tests, including DNAs extracted from FFPE specimens of different tissue types. This study assessed the performance of the assay with samples from 10 tumor types. Table 33 shows invalid rate by tumor type across the workflow. The most common failure mode seen was low library yield, which indicates low amplifiable input DNA, a result of the extensive DNA damage caused by extended formalin-fixation time during the preparation of FFPE specimens. There are no significant differences of the NGS performance of the samples that passed Library Yield QC.

Table 33. Summary of Tissue Type and Assay Performance

		Library Yield QC		NGS QC		Final Total QC			
Tumor	**Total #**	**# Failed**	**% Pass**	**# Failed**	**% Pass**	**Total Pass**	**Total Fail**	**% pass**	**95% CI**
CRC	254	2	99.2	1	99.6	251	3	98.8	96.6 – 99.6
NSCLC	139	13	90.6	1	99.2	125	14	89.9	83.8 – 93.9
HCC	11	0	100	0	100	11	0	100	74.1 – 100
MEL	21	0	100	0	100	21	0	100	84.5 – 100
CCRCC	11	0	100	0	100	11	0	100	74.1 – 100
UEC	24	1	95.8	0	100	23	1	95.8	79.9 – 99.3
THPA	9	0	100	0	100	9	0	100	70.1 – 100
BRCA	7	0	100	0	100	7	0	100	64.6 – 100
PAAD	13	0	100	1	100	12	1	92.3	66.6 – 98.6
BLCA	13	2	84.6	0	100	11	2	84.6	57.8 – 95.7
Total	502	18		3		481	21	95.8	93.7– 97.2

CRC: colorectal, NSCLC: lung, HCC: hepatic, MEL: melanoma, CCRCC: renal, UEC: uterine/endometrial, THPA: thyroid, BRCA: breast, PAAD: pancreas, BLCA: bladder

7. Stability Studies

a. Reagent Kit Shelf-Life Stability

Refer to the Summary of Safety and Effectiveness Data P200011.

The stability of the reagents was further evaluated in an additional study by testing seven insertion variants adjusted to a VAF% in the range of 1-1.5x of the LoD and at low DNA input (30 ng) with three aged lots to supplement the reagent kit shelf-life stability (Table 34). Three reagent kit lots aged 19 (lot A), 12 (lot B) and 6 (Lot C) months were used as representative assay reagent lots to test the samples for a total of 10 replicates per sample.

Table 34. Performance of Each Reagent Kit lot Across Clinical Samples

Gene Exon	Amino acid Change	Mean VAF (%)	Fold LoD*	Total Calls	Lot	Detection rate (%)
PTEN	p.Phe238ValfsTer5	5.95	1.5	4	A	4/4 (100%)
				3	B	3/3 (100%)
				3	C	3/3 (100%)
TP53	p.Asn239Ter	5.27	1.2	4	A	4/4 (100%)
				3	B	3/3 (100%)
				3	C	3/3 (100%)

Gene Exon	Amino acid Change	Mean VAF (%)	Fold LoD*	Total Calls	Lot	Detection rate (%)
ERBB2	p.Ala775_Gly776insValAla	5.46	1.2	4	A	4/4 (100%)
				3	B	3/3 (100%)
				3	C	3/3 (100%)
EGFR	p.Ala767_Val769dup	4.41	2.3	4	A	4/4 (100%)
				3	B	3/3 (100%)
				3	C	3/3 (100%)
SMAD4	p.Tyr260LeufsTer4	5.64	1.3	4	A	4/4 (100%)
				3	B	3/3 (100%)
				3	C	3/3 (100%)
EGFR	p.Ser768_Asp770dup	3.12	1.6	4	A	4/4 (100%)
				3	B	3/3 (100%)
				3	C	3/3 (100%)
FBXW7	p.Arg473LysfsTer4	5.41	1.2	4	A	4/4 (100%)
				3	B	3/3 (100%)
				3	C	3/3 (100%)

The data currently support a shelf life of 13 months for Kit 1 box (GS-PCR reagent), Kit 2 box (indexing PCR reagent), Kit 3 box (PCR product purification reagent) when stored at -25°C to -15°C, -25°C to -15°C and 2°C to 8°C, respectively. For Kit 4 box (index tube caps) when stored at ambient temperature

b. Reagent Interchangeability

The interchangeability of oncoReveal™ CDx kit components was assessed using clinical samples and three independent manufactured lots of reagents. The gene specific PCR and first cleanup steps were performed using reagents from a given reagent kit lot while the subsequent indexing PCR and second cleanup steps were performed using a second reagent kit lot. A total of three unique combinations of GS-PCR + cleanup and Indexing PCR + cleanup using three independent reagent lots were used to demonstrate interchangeability between multiple lots.

The positive and negative call rates were measured by PPA and NPA analysis. The PPA across all three combinations was 98.9% (87/88) with a minimum hit rate of 95.8% (23/24) for a single combination. The NPA was 100.0% (11,000/11,000) across all combinations with a minimum negative hit rate of 100.0% (3,000/3,000) for a single combination. The results demonstrate that components of different lots of oncoReveal CDx can be used interchangeability and does not impact results of the assay.

c. Supplemental FFPE (Section and Block) and DNA Sample Stability

A study was designed to test the stability of FFPE blocks, FFPE curls, and extracted FFPE DNA corresponding to eight additional tumor types, including bladder, breast, endometrial, liver, pancreatic, renal, and thyroid cancers, and melanoma. FFPE blocks and FFPE curls were stored at room temperature while extracted DNA was stored at -20°C for the duration of testing. Additionally, extracted DNA was subject to multiple rounds of freeze/thaw cycles to simulate repeat usage of the sample prior to testing using oncoReveal™ CDx. Baseline measurements and two (2) subsequent time points were assessed for each stability

claim and sample integrity was measured by PPA and NPA analysis by comparing variants called at each time point to baseline variant calls. Freeze/thaw stability was assessed by PPA and NPA analysis comparing variant calls after one (1) round of freeze/thaw to five (5) rounds of freeze/thaw. A total of 16 unique FFPE samples from eight tumor types were used in each stability study and were minimally tested in duplicate.

For DNA stability, the age of samples tested ranged from 16 to 17 weeks at the first time point (T1) and 21 to 38 weeks at the second time point (T2). The resulting PPA and NPA analyses showed 100% agreement at both T1 and T2 with lower bounds of the 95% CI for both were 92.3% and 100%, respectively, across all tissues tested.

PPA and NPA analysis of DNA samples subject to five (5) rounds of freeze/thaw cycles showed 100% agreement and lower bound of the 95% CI to be 92.3% and 100.0% respectively across all tissues tested. Together, this data supports a stability claim for FFPE DNA isolated from bladder, breast, endometrial, liver, pancreatic, renal, and thyroid cancers, and melanoma of 16 weeks and 5 freeze/thaw cycles.

For FFPE block and FFPE curl stability, samples tested ranged from 16 to 49 weeks at the first time point (T1) and 21 to 51 weeks at the second time point (T2) for both stability experiments. The resulting PPA and NPA analyses for block stability showed 100% agreement at both T1 and T2 and lower bounds of the 95% CI for were 92.0% and 100% respectively for T1 and 92.1% and 100% respectively for T2.

The resulting PPA and NPA analyses for curl stability showed 100% agreement at both T1 and T2 and lower bounds of the 95% CI for both were 92.1% and 100% respectively for T1 and 92.0% and 100% respectively for T2. Taken together, these data support a stability claim for FFPE blocks and FFPE curls derived from bladder, breast, endometrial, liver, pancreatic, renal, and thyroid cancers, and melanoma of 12 month.

B. Animal Studies

No animal studies were conducted using the oncoReveal CDx.

C. Additional Studies

The clinical performance of the oncoReveal™ CDx previously concluded in P200011 was analyzed using PiVAT software version 1.0 (CDx variants indicated in Table 1 of the Indications for Use). Clinical validation dataset was analyzed with PiVAT software version 2.0 (CDx and non-CDx tumor profiling variants) to assess equivalence. It was concluded that PiVAT version 2.0 outputs resulted in no change to 2x2x2 concordance matrix, and thus there was no impact to clinical outcome for *EGFR* in NSCLC and KRAS in CRC.

Software verification and validation activities, including unit testing, integration testing, and system testing were performed for the PiVAT® Software.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

No clinical study was conducted in support of the tumor profiling indication. Refer to the Summary of Safety and Effectiveness Data P200011 for a summary of the clinical studies conducted in support of the comparison diagnostic indications in Table 1 of the Intended

Use/Indications for Use.

Pediatric Extrapolation

In this premarket application, the tumor profiling indication is for adult patients 22 years or older. Therefore, pediatric extrapolation was not applicable.

XI. FINANCIAL DISCLOSURE

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The clinical concordance study included 2 investigators of which 1 was full-time or part-time employees of the sponsor and 1 had disclosable financial interests/arrangements as defined in 21 CFR 54.2(a), (b), (c) and (f) and described below:

- Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: 0
- Significant payment of other sorts: 0
- Proprietary interest in the product tested held by the investigator: 1
- Significant equity interest held by investigator in sponsor of covered study: 1

The applicant has adequately disclosed the financial interest/arrangements with clinical investigators. The information provided does not raise any questions about the reliability of the data.

XII. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Not applicable.

XIII. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Molecular and Clinical Genetics Panel of Medical Devices, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XIV. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusion

The analytical performance of oncoReveal™ CDx for the detection of SNVs, insertions and deletions in 22 genes to support a tumor profiling indication across solid tumors was established in the analytical validation studies reported above. Analytical accuracy, sensitivity, specificity, and precision are reported in Section IX establishes the effectiveness of the device for the detection of the variants reported under the tumor profiling Levels 2 and 3 in patients with solid tumors.

B. Safety Conclusions

The risks of the device are based on data collected in the non-clinical laboratory studies conducted to support PMA approval as described above. The oncoReveal CDx is an *in vitro* diagnostic test, which involves testing of DNA extracted from FFPE tumor tissue. The assay can be performed using DNA extracted from an existing (archival) tissue sample routinely collected as part of the diagnosis and patient care.

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect test results, and subsequently, inappropriate patient management decisions in cancer treatment. The main risks of this device for tumor profiling are the risks of false positive and false negative results. However, these risks are sufficiently mitigated by the analytical performance of this device. There is also a risk of delayed results, which may have clinical ramifications. However, for tumor profiling results reported under Levels 2 and 3, this test is not conclusive or prescriptive for the use of any specific therapeutic product and results provided under Levels 2 and 3 of the tumor profiling indication should not be viewed as a formal treatment recommendation. These tumor profiling results provided under Levels 2 and 3 are intended to be used with professional guidelines and are not conclusive or prescriptive for the use of any specific therapeutic product and should not be viewed as a formal treatment recommendation.

C. Benefit-Risk Determination

The probable benefit of the oncoReveal™ CDx, which is a qualitative next generation sequencing based *in vitro* diagnostic test that uses amplicon-based target enrichment technology for detection of single nucleotide variants (SNVs), insertions and deletions in 22 genes using DNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor tissue specimens, for tumor profiling, in patients with solid malignant neoplasms, was demonstrated via a series of analytical validation studies. Previously, this oncoReveal CDx was approved to select patients with EGFR Exon 19del/L858R in NSCLC for treatment with EGFR tyrosine kinase inhibitors approved by the FDA and KRAS wild-type (absence of mutation in codons 12 and 13) patients in CRC for treatment with cetuximab or panitumumab. The oncoReveal™ CDx is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for previously diagnosed cancer patients with solid malignant neoplasms. Genomic findings other than those listed in Table 1, as companion diagnostics, are not prescriptive or conclusive for labeled use of any specific therapeutic product.

Analytical accuracy studies were performed to demonstrate the concordance between the oncoReveal™ CDx and two externally validated comparator methods (externally validated NGS (evNGS), Method A & B) to support the probable benefit of accurately detecting SNVs, deletions and insertions for tumor profiling in 22 genes. The concordance analysis was done for overall agreement, by variant types, per gene and at the sample level. For comparator method A, a total of 271 samples represented by 10 tumor types were tested across 15 genes. Of these samples, 257 samples yielded valid

results (181 positive and 65 negative) by both assays and were included in the agreement analysis. At the variant level, PPA was 99.6% overall (245/246), 99.6% for SNVs (228/229), 100% for MNVs (4/4), 100% (11/11) for deletions and 100.0% (2/2) for insertions. The NPA was 99.9% for all variant categories. For the comparator method B, a second evNGS was used to include 6 additional genes not targeted by Method A. From the total enrolled 212 samples, 187 samples yielded valid results (158 positive and 10 negative) for both assays and were included in the agreement study. At the variant level, PPA was 98.6% overall (345/350), 98.7% for SNVs (308/312), 100% for MNVs (6/6), 100% (21/21) for deletions and 90.9% (10/11) for insertions. The NPA was 99.9% for all variant categories. The sum of these two analytical accuracy studies, indicate a high level of accuracy for the assay; the performance of this device was further supported by key analytical validation studies, like precision, LOD and other supportive studies. All in all, this data indicates probable benefit of this device for tumor profiling.

For the tumor profiling claim, there are risks associated with the use of this device, mainly due to 1) false positive, false negatives, or failure to provide a result, and 2) incorrect interpretation of test results by the user. For tumor profiling results, this test is not conclusive or prescriptive for the use of any specific therapeutic product and should not be viewed as a formal treatment recommendation. These tumor profiling results are intended to be used with professional guidelines and do have risks associated with false negativity and false positivity, as well as a failure to provide results or issues with incorrect interpretation. These risks for the tumor profiling are partly mitigated by the analytical performance of the device. Additional factors considered in determining probable risks and benefits for oncoReveal CDx included the representation of the variants in the analytical studies.

1. Patient Perspective

This submission did not include specific information on patient perspectives for this device.

In conclusion, given the available information above, the data support that for oncoReveal CDx, and the indications noted in the intended use statement, the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indication for use. Data from the analytical validation and clinical concordance studies, described in the Summary of Safety and Effectiveness Data (SSED) for P200011, support the performance of oncoReveal™ CDx as an aid for the identification of NSCLC and CRC patients for whom the therapies listed Table 1 of the Intended Use statement may be indicated. In addition, the validation studies described in the current SSED for P200011/S001 support the performance of oncoReveal CDx includes tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for cancer patients with solid malignant neoplasms.

XV. CDRH DECISION

CDRH issued an approval order on April 18, 2024.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XVI. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.