

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY**

**I. Background Information**

**A. 510(k) Number**

K250003

**B. Applicant**

Geneseeq Technology Inc.

**C. Proprietary and Established Names**

GENESEEQPRIME NGS Tumor Profiling Assay (FFPE)

**D. Regulatory Information**

Product Code:	PZM
Device Class:	Class II
Classification Regulation:	21 CFR 866.6080 – Next Generation Sequencing Based Tumor Profiling Assay
Classification Panel:	Pathology

**II. Submission/Device Overview**

**A. Purpose for submission**

New device

**B. Measurand**

Somatic single nucleotide variants (SNVs), insertions and deletions (Indels), select amplifications and translocations, microsatellite instability (MSI), and tumor mutation burden (TMB) in human genomic DNA obtained from formalin-fixed paraffin embedded tumor tissue. A complete list of genes and their corresponding regions of interest covered by the assay can be found in Appendix A.

**C. Type of Test:**

Next-Generation Sequencing Tumor Profiling Test

### III. Intended Use/Indications for Use

#### A. Intended Use(s)

The GENESEEQPRIME NGS Tumor Profiling Assay (FFPE) is a qualitative in vitro diagnostic test kit that uses next generation sequencing of DNA isolated from formalin-fixed paraffin-embedded tumor tissue from previously diagnosed patients with solid malignant neoplasms to detect tumor gene alterations in a broad multi gene panel. This test is intended to provide tumor mutation profiling information on somatic variants, including single nucleotide variants (SNVs), insertions and deletions (indels), one amplification, four translocations, microsatellite instability (MSI), and tumor mutation burden (TMB).

Information provided by GENESEEQPRIME NGS Tumor Profiling Assay (FFPE) is intended to be used by qualified health care professionals in accordance with professional guidelines in oncology. Results from GENESEEQPRIME NGS Tumor Profiling Assay (FFPE) are not intended to be prescriptive or conclusive for labeled use of any specific therapeutic product.

#### B. Indication(s) for Use:

Same as above

#### C. Special Conditions for Use Statement(s)

Rx - For prescription use  
For *in vitro* diagnostic use

#### D. Special Instrument Requirements

Illumina NextSeq® 550Dx Sequencer

### IV. Device/System Characteristics

#### A. Device Description

##### 1. Reagents

The GENESEEQPRIME NGS Tumor Profiling Assay (FFPE) (hereafter referred to as “GENESEEQPRIME”) is for use as part of a test system with the Illumina NextSeq 550Dx Sequencer and reagents. The included components of the GENESEEQPRIME assay are listed in Table 1. Geneseeq provided components include reagent kits and software for data analysis. The assay contains reagents for two full sequencing runs (i.e. 28 samples plus two external controls). Materials required but not provide are described below. A detailed list of required instruments, software reagents, consumables, and storage conditions are described in the product labeling (GENESEEQPRIME NGS Tumor Profiling Assay User Manual).

**Table 1. GENESEEQPRIME Kit: Reagents and Storage Condition**

<i>Library Preparation and Target Enrichment Kit, Box 1 of 3 (Store at -25°C to -15°C)</i>			
<b>Cap Label</b>	<b>Component Name</b>	<b>Cap Color</b>	<b>Volume (µL)</b>
LB1	ER/AT Buffer	Yellow	130
LE1	ER/AT Enzyme Mix	Yellow	55
LE2	DNA Ligase	Green	180
LB2	Ligation Buffer	Green	540
PM	PCR Master Mix	Pink	1200
PR	PCR Primers	Pink	240
HP	Hybridization Probes	Red	15
BL1	DNA Blockers	Red	120
BL2	Adaptor Blocker	Red	12
HB1	Hybridization Buffer 1	Red	45
HB2	Hybridization Buffer 2	Red	18
WB1	Wash Buffer 1	White	180
WB2	Wash Buffer 2	White	120
WB3	Wash Buffer 3	White	120
WB4	Wash Buffer 4	White	240
BW	Beads Wash Buffer	White	1500
NC	Negative Control	Blue	55
PC	Positive Control	Blue	55
<i>UDI Adaptor for Illumina, Box 2 of 3 (Store at -25°C to -15°C)</i>			
<b>Cap Label</b>	<b>Component Name</b>	<b>Volume</b>	<b>Concentration</b>
DA01-DA30	UDI Adaptor 1-30	6.5 µL/tube	15 µM
<i>Purification and Capture Beads, Box 3 of 3 (Store at 2 to 8°C)</i>			
<b>Cap Label</b>	<b>Component Name</b>	<b>Volume</b>	
CB	Capture Beads	300 µL	
PB	Purification Beads	9.0 mL	

## 2. **Material Required but Not Provided**

A list of materials required for upstream preparation of samples for sequencing, but not provided, is included as part of the GENESEEQPRIME assay is shown in Table 2. For a detailed list of reagents and consumables refer to the product labeling (GENESEEQPRIME NGS Tumor Profiling Assay User Manual).

**Table 2. Materials Required but Not Provided**

Name	Recommendations
FFPE DNA extraction kit	User's choice (column-based or beads-based)
dsDNA quantification kit	User's choice (Fluorometric method)
Library quantification kit	User's choice
Molecular biology grade nuclease-free water	User's choice
TE Buffer (10 mM Tris, 1 mM EDTA, pH 8.0)	User's choice
Low EDTA TE Buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0)	User's choice
1N NaOH	User's choice
100% Ethanol, molecular biology grade	User's choice
200 mM Tris-HCl pH 8.0	User's choice
NextSeq 550Dx High Output Reagent Kits v2.5 (300 Cycles)	Illumina, 20024908/20024905 or 20028871
Disposable Pipet Basin	User's choice
Aerosol Barrier, Nuclease-free, Low Retention Sterile Pipette Tips (1000ul, 200ul, 20ul, 10ul)	User's choice
Nuclease-free, microcentrifuge tubes for preparing master mixes	User's choice
dsDNA quantification assay tubes	User's choice
Appropriate PPE	User's choice

### 3. **GENESIS Software**

The GENESIS by GENESEQ software necessary for the GENESEQPRIME assay (software version is displayed on the user interface and on reports) is provided by Geneseeq Technology Inc. (Geneseeq) to perform sample information management, sequencing data analysis and test report generation. The software is only compatible with Illumina's NextSeq550 Dx sequencers. The raw data is maintained on the system during data analysis and report generation using redundant disk storage, and the system does not automatically delete or modify the raw data in any way. However, the minimal-requirement server necessary to run the software stores only sample information and reports; it does not provide long-term storage or backup of raw

sequencing data. The software saves sample information and reports only and does not provide backup of raw sequencing data.

GENESIS Software is designed to prevent the use of unqualified instruments and reagent kits. Improper use will result in no report being generated.

#### 4. **Instrument**

The GENESEEQPRIME is validated for use on the Illumina NextSeq 550Dx sequencing platform.

Other required equipment and specifications for the specific equipment not included in the GENESEEQPRIME assay are described in Table 3.

**Table 3. Other Required Equipment, Not Provided**

<b>Equipment</b>	<b>Notes</b>
Centrifugal Vacuum Concentrator	Uses vacuum centrifugal force to evaporate liquid and concentrate DNA.
Sonicator	Mechanically shears DNA to the appropriate size.
Fluorometer	Uses detection of target-specific fluorescence to provide quantification of samples prior to library preparation and sequencing. Separate fluorometers are required in pre- PCR and post-PCR areas.
DNA fragment analyzer	Automated sample processing determines size, quantity, and purity for quick library QC.
Magnetic stand	Designed for paramagnetic bead precipitation from standard and deep 96-well microplates. Separate magnetic stands are required in pre-PCR and post-PCR areas.
qPCR machine	For library quantification.
Thermal cyclers	One 96-well dual-block thermal cycler (or two 96-well single block thermal cyclers) is required in the post-PCR areas.
Vortex mixer	Separate vortex mixers are required in pre-PCR and post-PCR areas.
Thermomixer	Thermomixer capable of temperatures ranging from 20 °C to 70 °C and shaking at 1700 rpm. Two thermomixers or two thermal cyclers (or one thermal cycler with multiple thermal blocks) are required in the pre-PCR area and one thermomixer is required in the post-PCR area.

Equipment	Notes
Microcentrifuge	Tabletop micro-centrifuge or mini-centrifuge capable of holding 0.5 mL to 2.0 mL tubes. Separate micro- or mini-centrifuges are required in pre-PCR and post-PCR areas.
Single-channel pipettors (P-2, P-10, P-20, P-200, P-1000)	Separate sets of pipettors are required in pre-PCR and post-PCR areas. Pipettors should be calibrated regularly and verified accurate within 5% of stated volume.
Multi-channel pipettor (P-20, P-200)	Separate sets of pipettors are required in pre-PCR and post-PCR areas. Pipettors should be calibrated regularly and verified accurate within 5% of stated volume.

## 5. Sample Preparation

The GENESEEQPRIME assay requires genomic DNA isolated from formalin- fixed paraffin-embedded (FFPE) tissue specimens using a validated commercially available DNA extraction method (column-based or beads-based). The GENESEEQPRIME assay has been validated with FFPE sample stored at room temperature (15 – 25°C) for up to 5 years. The recommended number of FFPE sections of each sample for DNA extraction are shown in Table 4.

**Table 4. Tissue Sample Size and FFPE Section Specifications for GENESEEQPRIME Assay**

Surface Area of Tissue Sample (A)	Unstained FFPE Slides or Curls
$A \geq 1.0 \text{ cm} * 1.0 \text{ cm}$	2-5 slides/curls at 5-10 $\mu\text{m}$
$0.5 \text{ cm} * 0.5 \text{ cm} \leq A < 1.0 \text{ cm} * 1.0 \text{ cm}$	5-10 slides/curls at 5-10 $\mu\text{m}$
$A < 0.5 \text{ cm} * 0.5 \text{ cm}$	10-15 slides/curls at 5-10 $\mu\text{m}$

The tumor volume and minimum tumor content needed to obtain sufficient DNA for testing to achieve stated performance is at least 20% (Table 5). If the specimen contains less than 20% tumor content, the tissue should be macro-dissected to select as much viable tumor as possible to minimize the amount of adjacent non-tumor tissue.

**Table 5. Specimen Handling and Processing for Validated Specimen Types**

<b>Tissue Type</b>	<b>Volume</b>	<b>Minimum Tumor Proportion</b>	<b>Macrodissection Requirements (Based on tumor proportion)</b>	<b>Limitations</b>	<b>Storage</b>
FFPE Sections	2-15 unstained sections, 10 microns thick	≥ 20% tumor proportion based on proportion of tumor nuclei in total viable nuclei in the selected tumor area	Any sample containing less than 20% tumor content can be macro-dissected before use.	Archival FFPE material >5 years post-resection is not suitable for analysis	Room Temperature

## 6. DNA Extraction

The GENESEEQPRIME assay requires genomic DNA isolated from FFPE tissue using an appropriate commercially available DNA extraction method (magnetic bead-based and spin column-based). DNA extraction kits should be able to yield 50 ng of DNA with a minimum concentration of 1 ng/μL. The concentration of the extracted genomic DNA can be measured by a fluorescence quantification method. The recommended DNA input for GENESEEQPRIME is 100 ng of total DNA recovered from tissue with a minimum 20% viable tumor nuclei. While recommended DNA input for the assay is 100 ng, results can be obtained with DNA inputs down to 50 ng. The assay has been validated with extracted DNA stored at -20°C – 15°C for up to 12 months.

## 7. Library Preparation

Illumina compatible DNA adaptors need to be added onto the end of fragmented DNA. Each adaptor contains unique index composed of eight nucleotides to distinguish one sample from another, which enables multiplexing and sequencing multiple libraries in one sequencing run.

The GENESEEQPRIME assay workflow begins with genomic DNA. Genomic DNA is quantified using a fluorometer. DNA molecules are mechanically sheared to a target size of ~300 bp and subjected to a magnetic bead purification step to remove smaller fragments and perform an exchange of buffer. Fragmented DNA is end-repaired, phosphorylated, and adenylated. Index adaptors are then ligated to the A-tailed DNA molecules. Unincorporated adaptors and reagents are removed by magnetic bead purification. Adaptor-ligated DNA is enriched by PCR amplification. Primer dimers and residual reagents are removed by magnetic bead purification. Sample libraries must be ≥ 10 ng/μL (≥ 200 ng total amount) prior to proceeding to hybridization / target enrichment.

## 8. **Hybrid Capture NGS**

The adapter-ligated libraries are pooled together, denatured by heating, and subjected to hybridization with biotinylated probes (single-stranded DNA oligonucleotides) in a length of ~120 bp. Targeted regions are captured using magnetic streptavidin coated beads. Captured libraries are enriched by PCR amplification. Primer dimers and residual reagents are removed by magnetic bead purification. Final library quality is assessed using a library quantification kit the prior to sequencing. A total output volume of > 9 ng at a concentration of  $\geq 0.5$  ng/ $\mu$ L indicates a successful targeted enrichment.

## 9. **Sequencing**

Sequencing libraries prepared from this kit are sequenced on Illumina NextSeq 550Dx sequencing platforms. Sample libraries are quantified and normalized into a sequencing pool of up to eight samples and the controls. A maximum of 28 samples with one positive control and one negative control can be run in one sequencing batch. Pooled sample libraries are quantified using a qPCR method, loaded on a sequencing flow cell, and sequenced.

## 10. **Data Analysis**

- a) **Data Management System (DMS):** Sequencing data is automatically using the GENESIS software that tracks sample names, sample metadata and processing status from sequencing through to analysis and reporting. Reports of identified alterations are available in a web-based user interface for download. Sequencing and sample metrics, including sample and sequencing quality, are available in the final report output.
- b) **Demultiplexing and FASTQ Generation:** Demultiplexing software generates FASTQ files containing sequence reads and base quality score information. The FASTQ formatted files are used for subsequent processing of samples.
- c) **Run QC Check:** Cluster density and the Q30 read proportion are used to determine the quality control for each sequencing run. Cluster density measures the number of clusters on a flow cell, with a sequencer cluster density threshold of  $\geq 135$ . The proportion of total reads with a Q score of at least 30 (Q30) must be  $\geq 80\%$  for each run.
- d) **Read Alignment and BAM Generation:** Genomic alignment is performed to map sequence reads for each sample to the human reference genome (hg19/GRCh37). Alignments are saved as Binary Alignment Map (BAM) formatted files, which contain read placement information relative to the reference genome with quality scores. Aligned BAM files are further processed in a pipeline to identify genomic alterations.
- e) **Sample QC Checks:** Samples are checked for potential contamination through a bioinformatic analysis of genome haplotypes, based on analysis of pre-defined SNP sites that are representative of populations and individuals. Samples containing multiple haplotypes are considered potentially contaminated. Samples with  $\geq 4\%$  contamination are flagged as failed. Sequence coverage is evaluated across the panel requiring  $\geq 90\%$  of targeted regions have a minimum coverage > 100x.

- f) **Mutation Calling:** A fully automated pipeline for bioinformatics analysis is used to identify genomic alterations, including SNVs, indels, select amplification and translocations, MSI, and TMB.
- i) **SNVs and Indels:** Identification of variants, insertions and deletions are filtered according to variant allele frequency, allele depth, and variant coverage. For non-hotspot SNV and indel, at least 2% allele frequency and five mutant reads is required. For a hotspot variant, at least a 1% variant allele frequency (VAF) and four supporting mutant reads is required.
  - ii) **Amplifications:** The assay is validated to detect only *ERBB2* amplifications. *ERBB2* amplifications are reported when a > 1.8 change is observed.
  - iii) **Translocations:** The assay is validated to report four translocations only, *ALK*, *RET*, *ROS1*, and *NTRK1*. At least six fusion supporting reads are required for translocation with a hotspot gene and its canonical partner; and 12 fusion supporting reads are required for translocations with a hotspot gene and its non-canonical partner.
  - iv) **Microsatellite Status:** Microsatellite instability is assessed from the mutation status of 61 microsatellite sites from within the region of interest. A sample is deemed to have microsatellite instability (MSI-H) when at least 16% of the detected sites are unstable or contain specific mutations signatures. A sample is deemed to be microsatellite stable (MSS) when < 16% of the detected sites are unstable or contain specific mutations signatures.
  - v) **Tumor Mutation Burden (TMB):** TMB is calculated based on detected sequence mutations and indels. Filtering a sequence mutation is performed to exclude low mutant allele fraction mutations (< 2% VAF, non-hotspot variants, < 1% VAF, hotspot variants, < 0.4% VAF clinically significant variants), common somatic driver mutations, and common germline mutations. Both synonymous and non-synonymous alterations are considered for the mutation load. TMB is reported as the number of mutations per megabase (Muts/Mb).

## 11. **Controls**

- i) **Negative Control:** An external control that is provided in the GENESEQPRIME assay reagent kit consists of non-cancerous cell line (NA18535) derived DNA with no variants of interest. The external control is processed from library preparation through sequencing to serve as an end-to-end control to validate the quality of the sequencing run. Failure of either external control to meet the quality control thresholds will result in all test samples on the run being reported as invalid.
- ii) **Positive Control:** An external control that is provided in the GENESEQPRIME assay reagent kit consists of cell line derived-DNA with multiple verified sequence mutations (*BRAF* V600E, *EGFR* L858R, *EGFR* Exon 19 Deletion, *KRAS* G13D, *TPM3~NTRK1* translocation, *CD74~ROS1* translocation, and *ERBB2* amplification). The external control is processed from library preparation through sequencing to serve as an end-to-end control to demonstrate assay performance. All seven alterations must be detected for a sequencing run to pass quality control. Failure of either external control to meet the quality control thresholds will result in all test samples on the run being reported as invalid.

## 12. Result Reporting

The GENESEQPRIME assay reports SNV and indels in protein coding regions across all genes in the panel. In addition, amplifications are reported for *ERBB2* as well as translocations for *ALK*, *RET*, *ROSI*, and *NTRK1*. Germline mutations, including common polymorphisms, in the population present in 1000g (version 201508), ExAC (version 0.3nontcga), and gnomAD (version r2.0.1), are filtered and excluded from the final report. The assay also reports on two genomic signatures, MSI and TMB.

Variants are reported in one of two levels of evidence<sup>1</sup>: Variants with Evidence of Clinical Significance and Variants with Potential Clinical Significance. Variants reporting as having evidence of clinical significance are defined by AMP/ASCO/CAP guidelines (Li et al., 2017)<sup>2</sup>. The variants listed in the section Variants with Evidence of Clinical Significance are determined based on the selected tumor type. Only variants clinically associated with the tested tumor type will appear in the Variants with Evidence of Clinical Significance section. Any remaining detected variants will appear as the Variants with Potential Clinical Significance. A list of all 425 genes is provided in Appendix A; and a list of excluded regions in the genes or genes with variants that are excluded due to challenging regions (e.g., low complexity/repeats) is provided in Appendix B and Appendix C, respectively.

## 13. Quality Metrics

Reporting of variants considers the quality metrics outlined in Table 6. Quality metrics are assessed across the following categories.

- Batch-Level: Quality metrics that are quantified per sequencing run; failing batch-level metrics will prevent all reports for samples in the run from generating. If the positive or negative control fails these criteria, all samples in the sequencing run will not generate IVD reports.
- Sample-Level: Metrics that are quantified per sample; generates no IVD report for any sample failing these QC metrics.
- Analyte-Level: Metrics that are quantified for individual alteration types and loci. Only variants that pass analyte-level QC are reported.

**Table 6. Summary of GENESEQPRIME Post-Sequencing Quality Control Metrics**

Quality Metric	Level of Qualification	Passing Criteria
Cluster Density	Batch-Level	Sequencer Cluster Density $\geq$ 130

<sup>1</sup> Refer to <https://www.fda.gov/media/109050/download>

<sup>2</sup> Li MM et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn. 2017 Jan;19(1):4-23.

Quality Metric	Level of Qualification	Passing Criteria
Q30 Reads	Batch-Level	%Q30 (Total) $\geq$ 80%
External Control	Batch-Level	Seven known mutations in positive control are detected; No level 2 or hotspot mutations detected in negative control
Percent Regions Covered	Sample-Level	$\geq$ 90% exon region with $>$ 100X Dedup Depth
Contamination QC	Sample-Level	Estimated contamination levels $<$ 4%
Select SNVs and Indels with Evidence of Clinical Significance	Analyte-Level	Mutant reads $\geq$ 4 VAF $\geq$ 0.4% $\geq$ 50x Dedup Depth
Hotspot SNVs and Indels	Analyte-Level	Mutant reads $\geq$ 4 VAF $\geq$ 1% $\geq$ 50x Dedup Depth
Non-hotspot SNVs and Indels	Analyte-Level	Mutant reads $\geq$ 5 VAF $\geq$ 2% $\geq$ 100x Dedup Depth
MSI Detection	Analyte-Level	MSI-H: MSI Score $\geq$ 16 MSS: MSI Score $<$ 16
<i>ERBB2</i> Amplification	Analyte-Level	Fold change $\geq$ 1.8
Translocations ( <i>ALK</i> , <i>NTRK1</i> , <i>RET</i> , and <i>ROS1</i> )	Analyte-Level	Fusion reads $\geq$ 6 for canonical partner genes; Fusion reads $\geq$ 12 for non-canonical partner genes

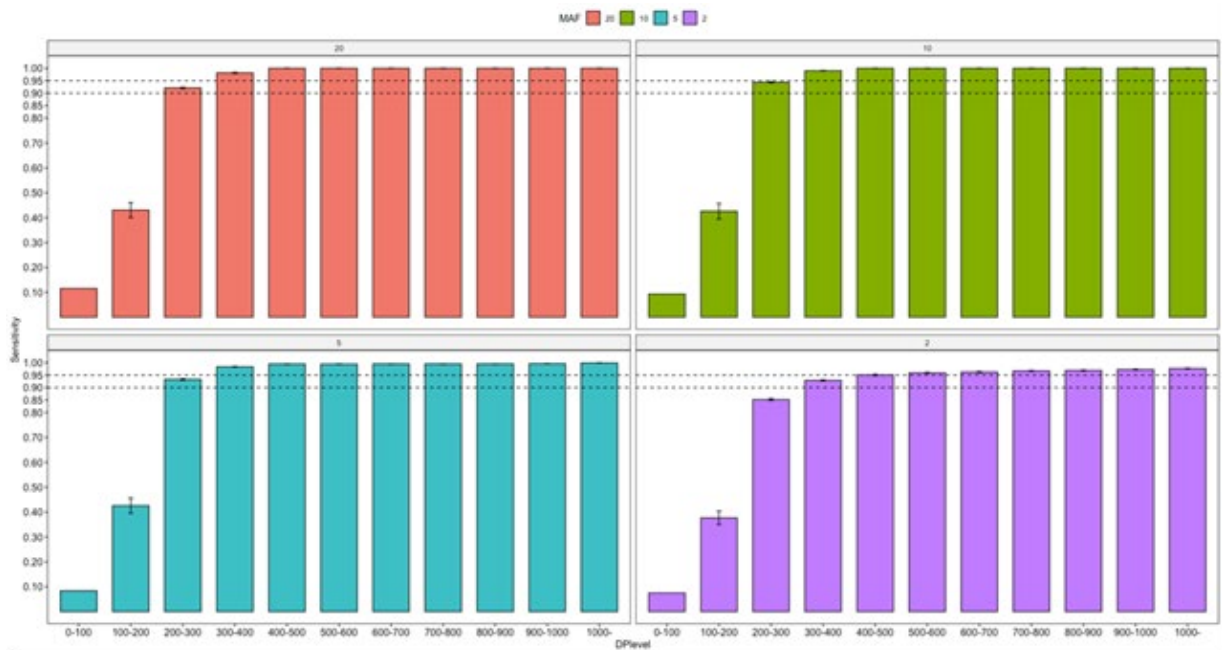
## B. Principle of Operation

The GENESEQPRIME assay kit is an in vitro diagnostic assay that uses targeted next generation sequencing to detect tumor gene alterations in genomic DNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor tissue in a 425 gene panel. GENESEQPRIME targets cancer-associated genes that are enriched from genomic libraries using a hybrid capture-based chemistry. Genomic libraries are prepared and captured. Samples are pooled for sequencing. After sequencing, automated software executes a bioinformatics analytics pipeline to identify genomic alterations in sequence data. The GENESEQPRIME assay workflow does not use a patient-matched normal sample but filters polymorphisms using databases. A summary of the alterations found, including a PDF report, are reported in output files and provided in a user interface as part of the GENESIS software.

## C. Determination of Assay Thresholds

### 1. Requirements on Exon Coverage

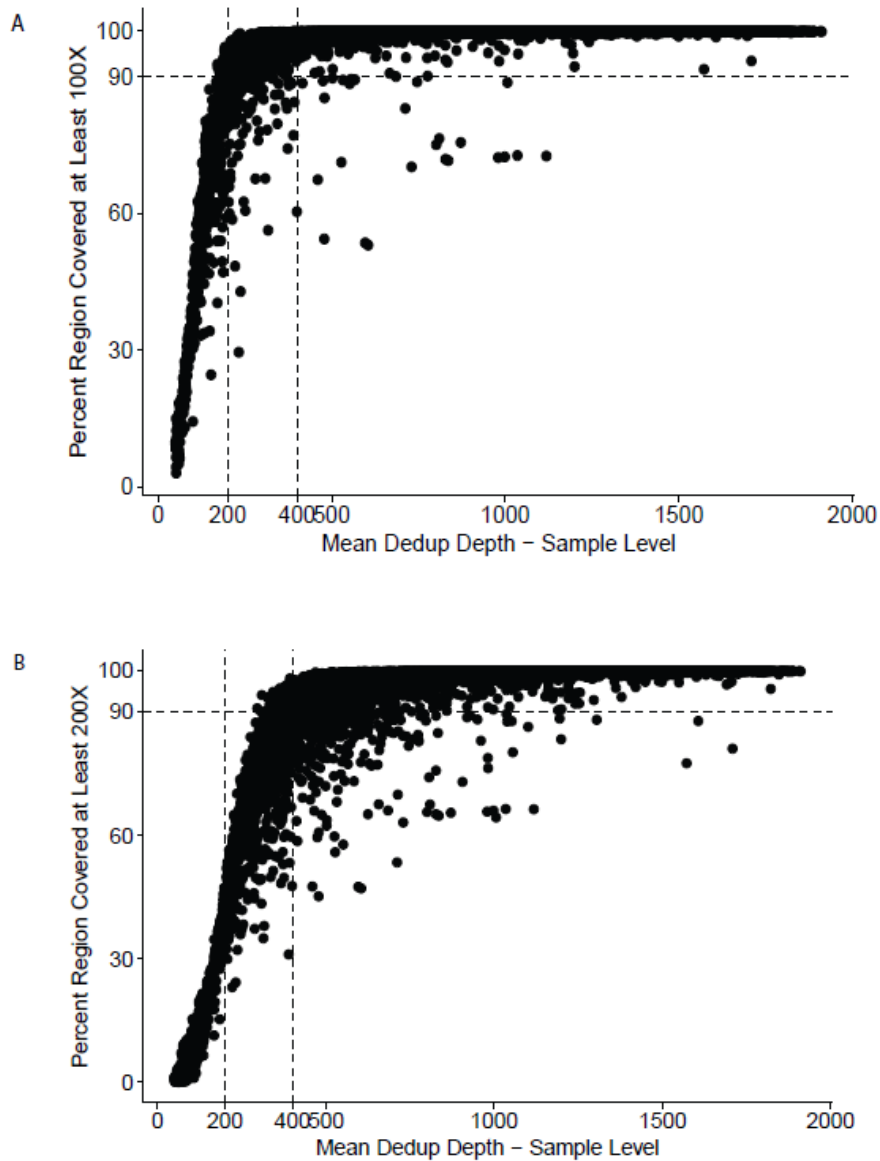
A power analysis was conducted to determine the minimum sequence coverage necessary to detect mutations with true underlying VAFs as low as 2% (Figure 1). Statistical power was estimated based on a requirement of four mutant observations to make a positive call. Sequence coverage of > 400x coverage provided 95% statistical power for detection of true mutations at 2% VAF (95% CI: 1.02%-3.90%). For mutations with > 5% underlying VAF, sequence coverage of > 300x provides 95% statistical power for detection (mean VAF = 17.0%, 95% CI: 3.05%-8.10%).



**Figure 1. Power Analysis for Mutations Detected based on Exon Coverage.** Mutations were separated into four types based on variant allele frequency (True VAF = 20%, 10%, 5%, and 2%). Lower dotted line indicates a 90% sensitivity in detecting variants and the upper dotted line indicates the 95% sensitivity threshold.

Summary statistics were calculated for individual exons across a cohort of samples to identify exons with consistent below-target coverage. These specific regions were removed from the GENESEQPRIME assay and are not included in variant analysis or reporting. The excluded regions are listed in Appendix B and Appendix C. No Variants with Evidence of Clinical Significance or somatic hotspot mutations are masked from the report.

Sequence coverage was evaluated in the remaining regions across a cohort of 200 FFPE samples, and 90% of targeted regions (4,156 of 5,286 regions) were sequenced to a depth of 100x or greater with 90% of regions of interest achieved a sequencing depth of 200x or greater (Figure 2).



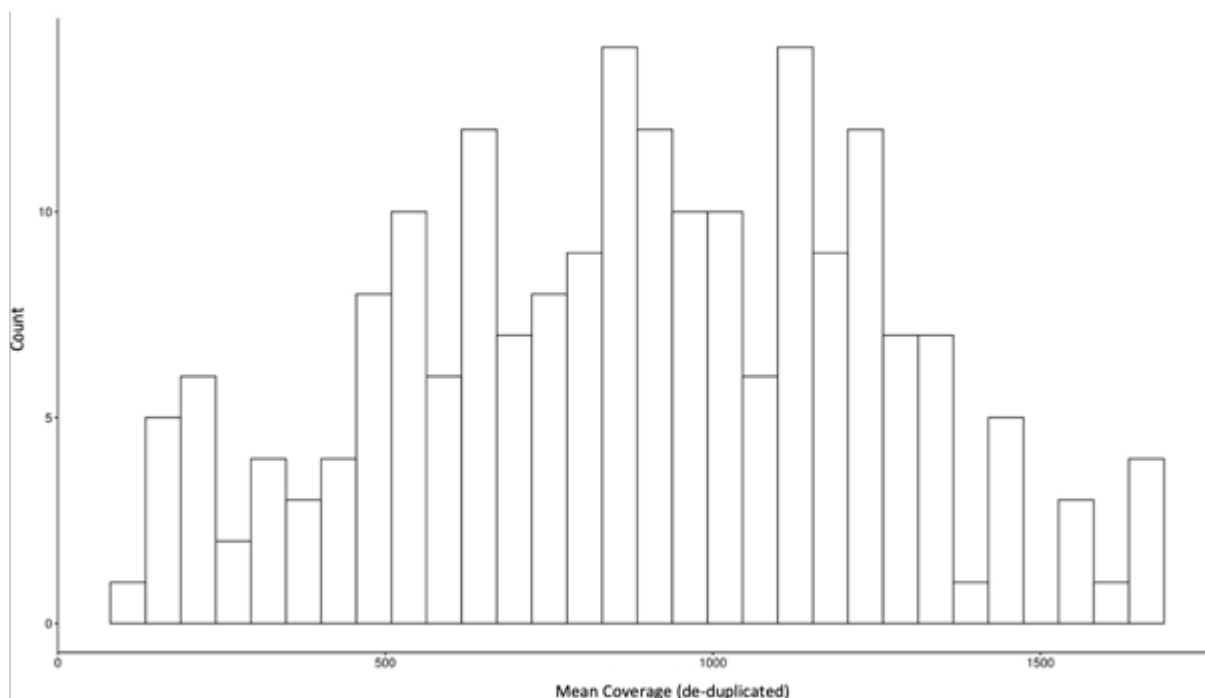
**Figure 2. Percent of Regions of Interest.** Achieving A) 100x or B) 200x coverage in correlation to sample level depth. Horizontal dotted line indicates 90% of regions. The leftmost

vertical dotted line indicates the sample level average deduplicated depth of 200x. The rightmost vertical dotted line indicates the sample level average deduplicated depth of 400x.

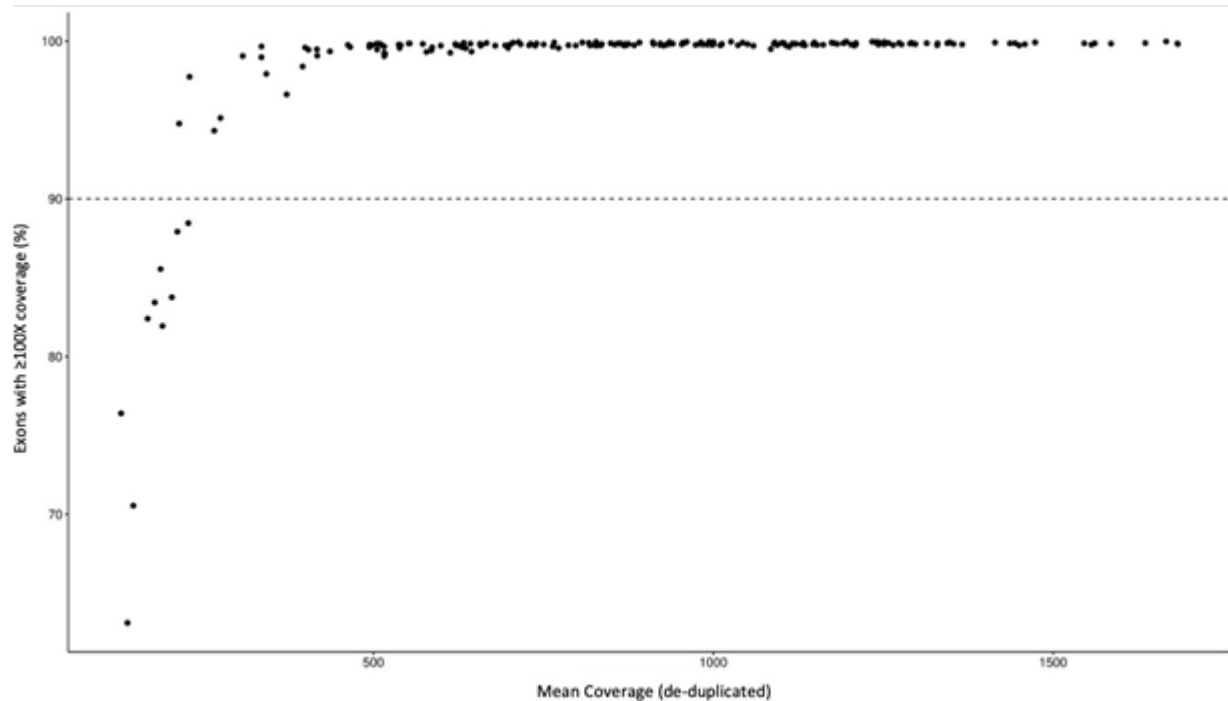
## 2. Requirements on Sample Coverage

Sample coverage was evaluated across a range of FFPE samples (n=200 across 10 different tumor types) to obtain sample summary statistics. Overall sample coverage was high in targeted regions of interest. The mean coverage across all targeted regions for the FFPE samples was 978x (SD = ± 245x).

Sequence coverage was further evaluated to establish the minimum requirements and reporting of variants. Based on a power analysis, a minimum sequence coverage of 100x is essential to call mutations with true underlying mutation frequency of 2% or higher. The number of exons for all individual samples meeting this coverage threshold was evaluated to establish a per sample threshold. The samples evaluated included a range of DNA quality estimates. Of the 200 samples evaluated, >96.5% of samples (193 of 200 samples) demonstrated ≥ 100x coverage across at least 90% of targeted regions of interest (Figure 3 and Figure 4). The consistently high coverage supports tolerance of occasional low coverage regions that may be seen with varying sample quality. A threshold of 90% of evaluated regions with at least 100x coverage was selected and is used to determine if a sample sequence to sufficient depth for analysis and reporting.



**Figure 3. Distribution of mean coverage per sample in GENESEQPRIME across 200 FFPE Samples.**



**Figure 4. Distribution of mean coverage values per sample (x-axis) and percent of regions with more than 100x coverage (y-axis).** Dotted line indicates the cut off of 90% regions with more than 100x coverage.

### 3. Requirements on Mutation Coverage, Allele Depth, and Frequency

Variant calling parameters were established at multiple cut offs for each variant type using 30 non-cancerous FFPE samples. An average of 8 mutations were detected per sample and 273 (209 non-hotspot, 58 hotspot, and 6 clinically significant variants) false positive variants were identified using a less stringent cut off. After inclusion of the filter criteria the rejection rate of false positive variants was 100% (Table 7).

**Table 7. Sample Error Correction by Allele Depth and Allele Frequency**

<b>Variant Category:</b>	<b>Non-hotspot Variants</b>	<b>Hotspot Variants</b>	<b>Clinically Significant Variants</b>
<b>Filter Criteria:</b>	<b>AD ≥ 5, AF ≥ 2%</b>	<b>AD ≥ 4, AF ≥ 1%</b>	<b>AD ≥ 4, AF ≥ 0.4%</b>
Pre-Filter Variant Count	209	58	6
Post-Filter Variant Count	0	0	0
Rejection Rate (%)	100	100	100

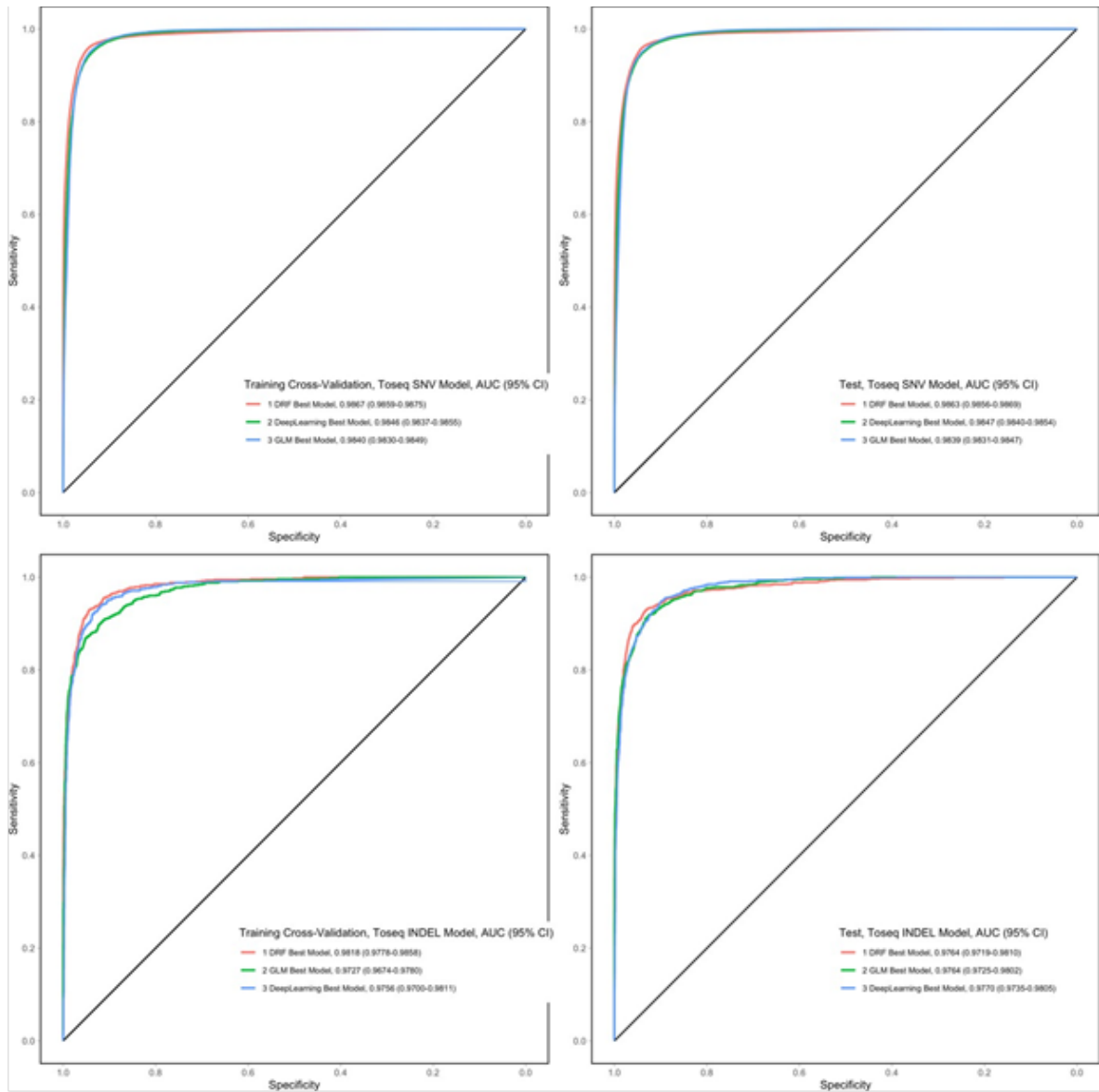
**a) Variants with Evidence of Clinical Significance**

The GENESEQPRIME assay reports variants with Evidence of Clinical Significance and Variants with Potential Clinical Significance. A database was utilized for clinical evidence curation according to a three-tiered approach for reporting biomarkers in tumor profiling NGS tests. Variants classified as Level 2 were grouped as Variants with Evidence of Clinical Significance, and variants classified as Level 3 were grouped as Variants with Potential Evidence of Clinical Significance. Variants belonging to the Level 2 category are detected by the GENESEQPRIME assay if the variant allele frequency is  $\geq 0.4\%$  and has at least four supporting reads.

**b) Somatic Mutation Detection by Tumor Only Sequencing (ToSeq) Module**

GENESIS software includes a machine learning module developed by Geneseeq called “ToSeq” to distinguish germline SNVs and indels from somatic SNV and indels without the need for a matched normal sample. A set of 848 fixed features are used to evaluate variants. Features fall into one of the following categories: 1) location of variant, 2) variant allele frequency, 3) pathogenicity based on the InvtterVar classification, 4) identity of the annotated reference and alternate alleles, 5) variant function classification, 6) sample copy number status, 7) frequency of occurrence within publicly available germline variant databases, and 8) internally established mutational hotspot database. The culmination of distinguishing features will correspond to a binary output status for each variant as either “Somatic” or “Germline”.

The performance of the somatic mutation calling was evaluated using tumor-normal sample pairs ( $n = 7,970$ ). Matched pairs were analyzed using Geneseeq’s variant calling algorithm targeting regions covered by the GENESEQPRIME assay. The machine learning model was trained using a set of 3,189 matched pairs and contained 53,664 SNVs and 4,338 indels; and independently tested using 4,781 matched pairs containing 79,070 SNVs and 6,748 indels. The reference list of germline and somatic mutations was identified by comparing the results from each matched pair. Out of the three training models used, the Distributed Random Forest model produced the best performance for ToSeq SNV and INDEL models (Figure 5) with a sensitivity of 95.59% (41,027/42,918, 95% CI: 95.40% - 95.78%) and a PPV of 95.63% (41,027/42,902, 95% CI: 95.43% - 95.82%).



**Figure 5. ROC Curve Analysis of ToSeq Performance.** The curve is graphed using the best performance model of one algorithm on training and test datasets. The AUC and associated 95% CI is provided in the bottom right corner of each graph. Red line – Distributed forest (DRF) model. Green line – Generalized Linear Model. Blue line – Deep Learning Model

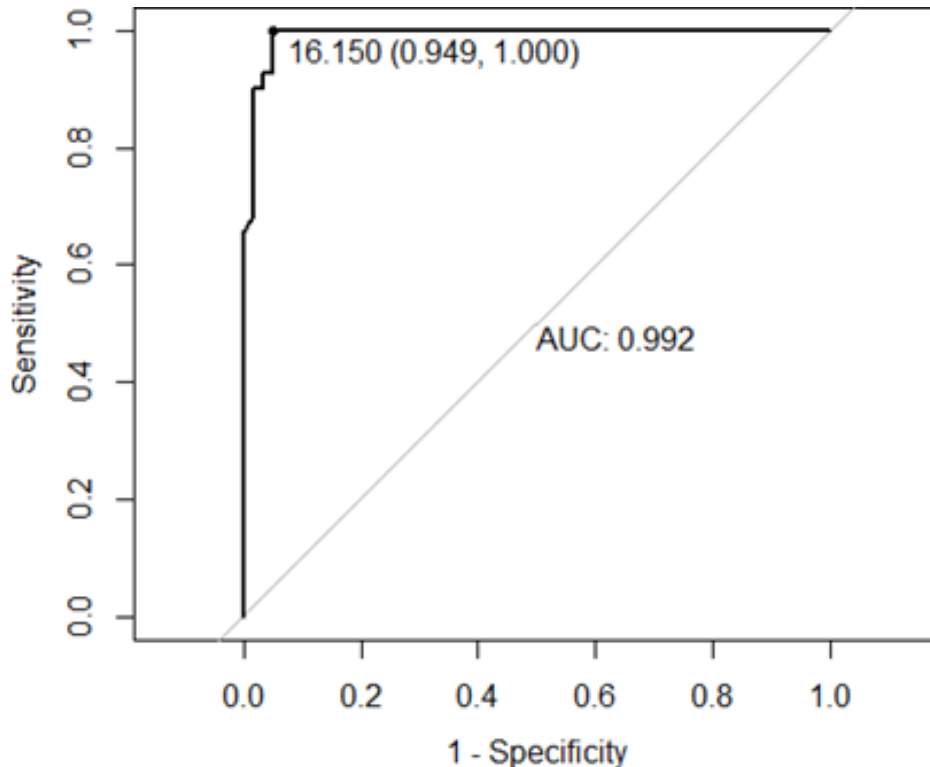
**c) *ERBB2* Copy Number Variation Detection Cut Off**

The GENESEEQPRIME assay cut off value for reporting *ERBB2* copy number variation was established using 46 FFPE *ERBB2*-negative samples. Amplification status for was determined by comparing the normalized coverage of the region of interest against a panel of normal controls. A threshold at four times the standard deviation from the mean of the copy number was chosen for the assay. Amplification of the *ERBB2*

gene is reported when a fold change (in relation to the diploid state) of over 1.8x is detected.

**d) MSI-H Cut Off Determination**

Microsatellite instability is evaluated in terms of frequency of somatic sites out of the total number of tracked sites. The threshold for separating MSS from MSI-H samples was determined using 81 FFPE and 19 standard samples (obtained from the National Institutes for Food and Drug Control (NIFDC) in China). A reference MSI status was provided by the supplier for each sample and served as the true MSI status. Variant calls from GENESEEQPRIME assay were compared to the true MSI status (Figure 6). The greatest AUROC was observed when somatic sites occupied between 15% to 17% of the total sites. The MSI score cut off for the GENESEEQPRIME assay is set to 16%.



**Figure 6. Receiver Operator Characteristic Curve for Microsatellite Score Determined by GENESEEQPRIME.**

e) **Structural Variant Cut Off Determination**

The baseline fusion read count of gene translocations was established using a total of 39 FFPE samples without the targeted translocations (*ALK*, n = 10; *RET*, n = 8; *ROSI*, n = 10; and *NTRK1*, n = 11). Structural variants with canonical and non-canonical gene partners were used in the analysis. Gene translocation cut off values with a canonical and non-canonical gene partner was determined to be 6 and 12, respectively and are shown in Table 8.

**Table 8. Detection Limits for Gene Translocation Negative Samples**

<b>Major Gene</b>	<b>Partner Gene</b>	<b>Range of Supporting Reads for Negative Samples</b>
<i>ALK</i> <sup>1</sup>	Canonical (n = 4)	2 – 5
	Non-Canonical (n = 6)	6 – 11
<i>RET</i>	Canonical (n = 3)	2 – 4
	Non-Canonical (n = 5)	6 – 11
<i>ROSI</i>	Canonical (n = 5)	2 – 5
	Non-Canonical (n = 5)	3 – 11
<i>NTRK1</i>	Canonical (n = 5)	4 – 5
	Non-Canonical (n = 6)	2– 11

**D. Substantial Equivalence Information:**

1. **Predicate Device Name(s)**  
PGDx elio Tissue Complete
2. **Predicate 510(k) Number(s)**  
K192063

3. Comparison with Predicate(s)

Characteristics	Predicate device: <b>PGDx elio tissue complete</b>	<b>Subject Device:</b> <b>GENESEQPRIME NGS Tumor Profiling Assay (FFPE)</b>
<b>Similarities</b>		
<b>Indications for Use</b>	<p>The PGDx elio™ tissue complete assay is a qualitative in vitro diagnostic device that uses targeted next generation sequencing of DNA isolated from formalin-fixed, paraffin-embedded tumor tissue from patients with solid malignant neoplasms to detect tumor gene alterations in a broad multi-gene panel.</p> <p>PGDx elio tissue complete is intended to provide tumor mutation profiling information on somatic alterations (SNVs, small insertions and deletions, one amplification and four translocations), microsatellite instability (MSI) and tumor mutation burden (TMB) for use by qualified healthcare professionals in accordance with professional guidelines in oncology for previously diagnosed cancer patients and is not conclusive or prescriptive for labeled use of any specific therapeutic product.</p>	<p>The GENESEQPRIME NGS Tumor Profiling Assay (FFPE) is a qualitative in vitro diagnostic test kit that uses next generation sequencing of DNA isolated from formalin-fixed paraffin-embedded tumor tissue from previously diagnosed patients with solid malignant neoplasms to detect tumor gene alterations in a broad multi gene panel. This test is intended to provide tumor mutation profiling information on somatic variants, including single nucleotide variants (SNVs), insertions and deletions (indels), one amplification, four translocations, microsatellite instability (MSI), and tumor mutation burden (TMB).</p> <p>Information provided by GENESEQPRIME NGS Tumor Profiling Assay (FFPE) is intended to be used by qualified health care professionals in accordance with professional guidelines in oncology. Results from GENESEQPRIME NGS Tumor Profiling Assay (FFPE) are not intended to be prescriptive or conclusive for labeled use of any specific therapeutic product.</p>
<b>Technology</b>	Hybrid Capture	Same
<b>Specimen Type</b>	Formalin-fixed, paraffin-embedded (FFPE) tumor tissue from patients with solid malignant neoplasms	Same

<b>Characteristics</b>	<b>Predicate device: PGDx elio tissue complete</b>	<b>Subject Device: GENESEEQPRIME FFPE tissue tumor profiling assay</b>
<b>Similarities</b>		
<b>Target Population</b>	Patients with malignant solid neoplasms	Same
<b>Instrument</b>	Illumina NextSeq 550Dx (qualified by PGDx)	Illumina NextSeq 550Dx
<b>Test Environment</b>	Kit	Same
<b>Differences</b>		
<b>Genes on Panel</b>	505	425
<b>Variant Types</b>	Somatic Variants including point mutations, small insertions, and small deletions, <i>ERBB2</i> amplification, 4 gene translocations ( <i>RET</i> , <i>NTRK2</i> , <i>ALK</i> , and <i>NTRK3</i> ), MSI and TMB information.	Somatic Variants including point mutations, small insertions, and small deletions, <i>ERBB2</i> amplification, 4 gene translocations ( <i>ROS1</i> , <i>ALK</i> , <i>RET</i> , and <i>NTRK1</i> ), MSI and TMB information.
<b>Black List</b>	58 genes/exons excluded from reporting due to consistently low coverage and low complexity, and repeat genomic regions in 254 genes	7 regions from 5 genes and 268 variants from 135 genes are excluded from reporting as recurrent artifacts based on next generation sequencing results of normal samples (blood or FFPE)
<b>Determination of Pipeline Threshold</b>	Sequence coverage of >400x provides 95% statistical power for detection of true mutations at 2% MAF (95% CI, 0.8% - 3.5% MAF).  For mutations with 5% underlying MAF, sequence coverage of >150x provides 95% statistical power for detection (95% CI, 2.0% - 8.6% MAF).	Sequencing coverage of >400x provides 95% statistical power for detection of true mutations at 2% VAF (95% CI, 1.9% - 2.5% VAF)  For mutations with 5% underlying VAF, sequencing coverage of >300x provides 95% statistical power for detection (95% CI, 12.0%, 22.0% VAF, Mean VAF = 17.0%).

<b>Characteristics</b>	<b>Predicate device: PGDx elio tissue complete</b>	<b>Subject Device: GENESEEQPRIME FFPE tissue tumor profiling assay</b>
<b>Differences</b>		
<b>Assay cut-off</b>	<p>A minimum of 4 or 6 mutant observations and 0.4%, 2%, or 5% mutant allele fraction (MAF) are required depending on sequence coverage and status of the variant as a Variant with Evidence of Clinical Significance, somatic hotspot, or a Variant with Potential Clinical Significance.</p> <p>SNVs with lower bound 95% Confidence Interval &lt;5% MAF based on sequence coverage are excluded from reporting.</p> <p>Common germline mutations present in dbSNP, ExAC, and gnomAD are identified and excluded from reporting.</p> <p>Additional germline mutations with <math>\geq 3</math> matches in ExAC and <math>MAF \geq 20\%</math> are also excluded from reporting.</p>	<p>A minimum of 4 or 5 mutant observations and 0.4%, 1%, or 2% variant allele fraction (VAF) are required depending on sequence coverage and status of the variant as a Variant with Evidence of Clinical Significance, somatic hotspot, or a Variant with Potential Clinical Significance.</p> <p>SNVs and Indels &lt;2% VAF based on sequence coverage are excluded from reporting.</p> <p>Common germline mutations present in 1000g, ExAC, and gnomAD are identified and excluded from reporting.</p> <p>GENESIS software includes a sponsor-developed machine learning model used to distinguish germline SNVs and Indels from somatic SNVs and Indels without the need for a matched normal sample</p>
<b>Controls</b>	<ul style="list-style-type: none"> <li>• Positive Control</li> <li>• No template control (NTC)</li> <li>• Normalized to database of common germline SNPs</li> </ul>	<ul style="list-style-type: none"> <li>• Positive Control</li> <li>• Negative Control</li> <li>• Normalized to database of common germline SNPs</li> </ul>
<b>Samples per Run (controls excluded)</b>	15	28

<b>Characteristics</b>	<b>Predicate device: PGDx elio tissue complete</b>	<b>Subject Device: GENESEEQPRIME FFPE tissue tumor profiling assay</b>
<b>Differences</b>		
<b>Clinical Evidence Curation</b>	<p>Variant calls are organized into Variants with Evidence of Clinical Significance or Variants with Potential Clinical Significance; with Variants with Evidence of Clinical Significance aligning with Tier 1A of the AMP/ASCO/CAP guidelines, based on the selected tumor type for use in tumor profiling.</p> <p>Tumor type selection should align with the clinical diagnosis and all available information. In the case of metastasis of unknown origin, unknown primary site, or uncertainty of the tumor type, 'Other' should be selected.</p>	<p>Variant calls are organized into Variant with Evidence of Clinical Significance or Variant with Potential Clinical Significance depending on the designated cancer type.</p> <p>Classification of variants adhere to the three-tiered approach for reporting biomarkers as outlined by the CDRH. Variants with Evidence of Clinical Significance fall within Tier 1A of the AMP/ASCO/CAP guidelines.</p>

**E. Standards/Guidance Documents Referenced**

The following FDA guidance documents were consulted:

1. FDA Fact Sheet - CDRH’s Approach to Tumor Profiling Next Generation Sequencing Tests (2017),
2. General Principles of Software Validation; Final Guidance for Industry and FDA Staff (January 11, 2002),
3. Guidance for Industry Cybersecurity for Networked Medical Devices Containing Off-the-Shelf (OTS) Software (January 14, 2005),
4. Guidance for Industry and FDA Staff Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests (March 13, 2007),
5. Content of Premarket Submissions for Management of Cybersecurity in Medical Devices Guidance for Industry and Food and Drug Administration Staff (October 2, 2014),
6. Postmarket Management of Cybersecurity in Medical Devices Guidance for Industry and Food and Drug Administration Staff (December 28, 2016),
7. Content of Premarket Submissions for Device Software Functions Guidance for Industry and Food and Drug Administration Staff (June 14, 2023), and

8. Off-The-Shelf Software Use in Medical Devices Guidance for Industry and Food and Drug Administration Staff (August 11, 2023).

**F. Performance Characteristics:**

**1. Analytical Performance - General**

The GENESEEQPRIME kit is a targeted NGS panel with 425 genes. The targeted regions of interest in GENESEEQPRIME are designed to detect SNVs, small indels < 30 bp in length in the coding exons of the targeted genes, as well as *ERBB2* amplifications, *ALK*, *RET*, *ROS*, and *NTRK1* translocations, MSI, and TMB. For SNVs and indels, a representative approach to validation of the targeted genes in the panel was submitted with data representing variant types for SNVs and indels, and at the gene levels for amplifications and translocations indicated with this assay. In addition, the assay was evaluated for performance regarding the panel-wide quality metrics.

*a) Invalid Rates*

Performance throughout verification and validation of the device was tracked and a summary of the rates for first pass (no repeat) is presented below. The data shown represents the first pass rate of each sample. Repeat testing was not conducted for this analysis. Data were aggregated for clinical cases from > 40 tumor types. Resulting pass rates by tumor type across the workflow are shown in Table 9.

**Table 9. Comparability of Tumor Pass Rates for the GENESEEQPRIME Assay**

<b>Tumor Type</b>	<b>Total Samples</b>	<b>Total Failures</b>	<b>Total Passes</b>	<b>Failed Pre-Sequencing QC</b>	<b>Failed Post-Sequencing QC</b>	<b>Pass Rate (%)</b>
Bladder cancer	55	6	49	4	2	89.09
Breast cancer	564	52	512	28	24	90.78
Triple negative breast cancer	96	8	88	4	4	91.67
Adenocarcinoma	23	2	21	1	1	91.30
Cervical cancer	119	9	110	0	9	92.44
Squamous	20	1	19	0	1	95.00
Colon cancer	1389	36	1353	19	17	97.41
Rectum cancer	1061	27	1034	15	12	97.46
Colorectal cancer, NOS*	76	2	74	1	1	97.37
Endometrial cancer	133	9	124	0	9	93.23

<b>Tumor Type</b>	<b>Total Samples</b>	<b>Total Failures</b>	<b>Total Passes</b>	<b>Failed Pre-Sequencing QC</b>	<b>Failed Post-Sequencing QC</b>	<b>Pass Rate (%)</b>
Adenocarcinoma	21	4	17	0	4	80.95
Squamous	118	15	103	0	15	87.29
Esophageal cancer, NOS*	10	1	9	0	1	90.00
Adenocarcinoma	303	27	276	10	17	91.09
Gastric cancer	402	30	372	14	16	92.54
Gastrointestinal stromal tumor (GIST)	94	10	84	7	3	89.36
Brain glioma	67	8	59	5	3	88.06
Nasopharyngeal carcinoma	27	3	24	2	1	88.89
Head and neck cancer, Other	121	14	107	8	6	88.43
Cholangiocarcinoma	119	4	115	4	0	96.64
Liver cancer	429	13	416	7	6	96.97
Lung adenocarcinoma	3964	317	3647	178	139	92.00
Lung squamous cell carcinoma	427	25	402	10	15	94.15
Non-small cell lung cancer	197	15	182	10	5	92.39
Small cell lung cancer	80	4	76	0	4	95.00
Mediastinal	10	0	10	0	0	100.00
Melanoma	167	19	148	7	12	88.62
Neuroendocrine	49	2	47	0	2	95.92
High Grade Serous	378	20	358	4	16	94.71
Ovarian cancer	138	8	130	2	6	94.20
Pancreatic cancer	311	13	298	8	5	95.82
Prostate cancer	116	9	107	9	0	92.24
Renal cancer	294	13	281	6	7	95.58
Soft tissue sarcoma	140	13	127	2	11	90.71

<b>Tumor Type</b>	<b>Total Samples</b>	<b>Total Failures</b>	<b>Total Passes</b>	<b>Failed Pre-Sequencing QC</b>	<b>Failed Post-Sequencing QC</b>	<b>Pass Rate (%)</b>
Thyroid cancer	46	3	43	1	2	93.48
Bone cancer	17	1	16	0	1	94.12
Parathyroid	9	1	8	0	1	88.89
Skin cancer	17	3	14	0	3	82.35
Urinary tract	6	0	6	0	0	100.00
Uterine	29	2	27	0	2	93.10
<b>Sum</b>	<b>11642</b>	<b>749</b>	<b>10893</b>	<b>366</b>	<b>383</b>	<b>93.57</b>

\*NOS – Not otherwise specified; samples that was not categorized into any of the other subtypes.

## 2. Precision/Reproducibility

### a) *Interlaboratory Reproducibly*

Interlaboratory reproducibility of the GENESEEQPRIME assay was assessed across three different sites, using DNA extracted from 28 FFPE tissue specimens. The samples represented a range of SNVs, indels, *ERBB2* amplifications, *ALK*, *ROS1*, *RET*, and *NTRK1* translocations, MSI, and TMB. Each of the 28 samples were tested in duplicate by two different operators on 12 sequencing runs across three non-consecutive days at each of the three independent laboratory sites using a single kit lot (36 total sequencing runs and 1,008 total replicates). Allele frequencies for the variants in the specimens spanned all ranges. Each replicate began with the workflow post-DNA extraction.

Reproducibility was assessed by: 1) the average positive and negative agreement between all possible replicates within each test condition listed above were used to analyze sources of variance; 2) coefficient of variance of TMB score between all replicates within each test condition listed above were used to analyze sources of variance; 3) concordance of MSI status calls between all replicates of each samples was used; 4) positive call rate for variants that were detected in over 50% of replicates for each sample (Modal PCR) were used to determine per specimen reproducibility; 5) modal positive call rate (PCR) and negative call rate (NCR) of variants stratified by allele frequency and variant type (SNV, INS, DEL) were determined to show the reproducibility of GENESEEQPRIME assay performance across all levels and types of variants.

The sample used in the multi-site reproducibility study, along with their expected variants, are presented in Table 10 below. In terms of invalid rate, the first pass rate was 94.35% (951/1008).

**Table 10. Samples used in the Multi-Site Reproducibility Study**

Tissue Type	Expected SNVs with Evidence of Clinical Significance	Number of Variants with Protanal Clinical Significance	Translocation (Trans) or Amplification (Amp)	Mean TMB Score (Muts/Mb)	MSI-H Status
Colorectal Cancer	0	39	<i>NTRK1</i> (Trans)	46.0	Yes
Lung Adenocarcinoma	0	8	<i>ALK</i> (Trans)	1.4	
Thyroid Cancer	<i>BRAF</i> V600E	7		0.3	
Melanoma	<i>BRAF</i> V600E	31		16.6	
Endometrial Cancer	0	50		44.5	Yes
Breast Cancer	<i>PIK3CA</i> H1047R & <i>PIK3CA</i> E545K	7	<i>ERBB2</i> (Amp)	2.3	
Lung Adenocarcinoma	0	16		13.7	
Colorectal Cancer	0	43	<i>ERBB2</i> (Amp)	10.6	
Lung Adenocarcinoma	0	35		29.5	
Head and Neck Cancer	0	17		3.5	
Esophageal Cancer	0	13	<i>ERBB2</i> (Amp)	9.8	
Lung Adenocarcinoma	0	16	<i>ROS1</i> (Trans)	3.5	
Colorectal Cancer	<i>BRAF</i> V600E	71		75.3	Yes

Tissue Type	Expected SNVs with Evidence of Clinical Significance	Number of Variants with Protanal Clinical Significance	Translocation (Trans) or Amplification (Amp)	Mean TMB Score (Muts/Mb)	MSI-H Status
Lung Adenocarcinoma	<i>EGFR</i> L858R	9		7.2	
Lung Adenocarcinoma	0	34		5.9	
Prostate Cancer	0	8		1.0	
Prostate Cancer	0	4		2.3	
Breast Cancer	<i>PIK3CA</i> H1047R	17	<i>ERBB2</i> (Amp)	7.7	
Lung Adenocarcinoma	<i>EGFR</i> e19del	9		7.1	
Endometrial Cancer	0	14		11.7	
Ovarian Cancer	0	13		9.6	
Gastric Cancer	0	98		109.5	Yes
Endometrial Cancer	0	49		54.1	Yes
Bladder Cancer	0	37		18.8	
Melanoma	<i>BRAF</i> V600K	43		56.8	
Colorectal Cancer	0	19	<i>ERBB2</i> (Amp)	10.7	
Lung Adenocarcinoma	<i>EGFR</i> e20dup	30		11.0	

Tissue Type	Expected SNVs with Evidence of Clinical Significance	Number of Variants with Protanal Clinical Significance	Translocation (Trans) or Amplification (Amp)	Mean TMB Score (Muts/Mb)	MSI-H Status
Endometrial Cancer	0	68		74.4	Yes

b) **Panel-wide Reproducibility**

Reproducibility was assessed for each variant across all 36 replicates. The modal positive and negative call rates were calculated along with the two-sided 95% confidence interval.

Table 11 summarizes the PCR and NCR stratified by mutation type (SNV, insertions, and deletions) and variant allele frequency (VAF). An overall modal PCR of 97.46% across all samples and replicates (16137/16558, 95% CI: 97.21%, 97.69%, average VAF range: 0.4% - 77.06%), with an increase in PCR at higher VAFs observed, and an overall NCR of 94.27% (10506/11144, 95% CI: 93.83%,94.69%). Repeat testing for the failed samples was not performed.

The positive call rates for individual sequence mutations assessed in the Interlaboratory Reproducibility study, along with the VAF range, mean, SD, and CV per variant per specimen tested are presented in **Appendix D**. A total of 671 SNVs and 144 indels (23 insertions, 121 deletions) are provided. Variants are listed by specimen with each specimen separated by a gray line. Discordant cases are denoted in light grey.

**Table 11. Interlaboratory Reproducibility Positive (PCR) and Negative (NCR) Call Rates**

Variant Type	VAF Level (%)	Unique Mutations	PCR (%) (n/N)	NCR (%) (n/N)	Mean Allele Frequency Range (%)	Mean Allele Depth Range	Mean Loci Depth Range
All	AF $\geq$ 0	815	97.46 (16137/16558)	94.27 (10506/11144)	0.4- 77.06	4-1527	113- 13236
	AF $\geq$ 2.0	590	97.51 (16080/16490)	90.36 (3224/3568)	2- 77.06	5-1527	113- 13236
	AF $\geq$ 5.0	457	98.9 (14425/14586)	81.58 (775/950)	5.16- 77.06	14-1527	113- 11822
	AF $\geq$ 10.0	389	99.24 (12349/12444)	81.15 (633/780)	10.01- 77.06	24-1527	113- 2710
	AF $\geq$ 15.0	288	99.13 (8999/9078)	83.43 (594/712)	15.06- 77.06	24-1527	113- 2710

Variant Type	VAF Level (%)	Unique Mutations	PCR (%) (n/N)	NCR (%) (n/N)	Mean Allele Frequency Range (%)	Mean Allele Depth Range	Mean Loci Depth Range
Variants with Evidence of Clinical Significance	AF $\geq$ 0	26	100 (306/306)	95.14 (548/576)	0.4-52.65	4-1425	250-2710
	AF $\geq$ 2.0	9	100 (306/306)	-	10.19-52.65	76-1425	453-2710
	AF $\geq$ 5.0	9	100 (306/306)	-	10.19-52.65	76-1425	453-2710
	AF $\geq$ 10.0	9	100 (306/306)	-	10.19-52.65	76-1425	453-2710
	AF $\geq$ 15.0	8	100 (272/272)	-	16.03-52.65	81-1425	453-2710
Hotspot Variants	AF $\geq$ 0	266	99.23 (1417/1428)	95.99 (7305/7610)	0.4-75.84	4-1425	206-2710
	AF $\geq$ 2.0	41	100 (1360/1360)	67.65 (23/34)	3.23-75.84	17-1425	267-2710
	AF $\geq$ 5.0	39	100 (1292/1292)	67.65 (23/34)	6.1-75.84	52-1425	267-2710
	AF $\geq$ 10.0	37	100 (1224/1224)	67.65 (23/34)	10.19-75.84	52-1425	267-2710
	AF $\geq$ 15.0	32	100 (1054/1054)	67.65 <sup>1</sup> (23/34)	15.07-75.84	52-1425	267-2710
Non-Hotspot Variants	AF $\geq$ 0	549	97.29 (14720/15130)	90.58 (3201/3534)	2-77.06	5-1527	113-13236
	AF $\geq$ 2.0	549	97.29 (14720/15130)	90.58 (3201/3534)	2-77.06	5-1527	113-13236
	AF $\geq$ 5.0	418	98.79 (13133/13294)	82.1 (752/916)	5.16-77.06	14-1527	113-11822
	AF $\geq$ 10.0	352	99.15 (11125/11220)	81.77 (610/746)	10.01-77.06	24-1527	113-2437
	AF $\geq$ 15.0	256	99.02 (7945/8024)	84.22 (571/678)	15.06-77.06	24-1527	113-2345
Single Nucleotide Variations	AF $\geq$ 0	671	97.52 (11936/12240)	94.65 (10001/10566)	0.4-75.84	4-978	113-13236
	AF $\geq$ 2.0	454	97.59 (11879/12172)	91.45 (2983/3262)	2-75.84	5-978	113-13236
	AF $\geq$ 5.0	326	99.28 (10329/10404)	82.89 (562/678)	5.16-75.84	18-978	113-11822
	AF $\geq$ 10.0	287	99.29 (9014/9078)	82.89 (562/678)	10.19-75.84	24-978	113-2498
	AF $\geq$ 15.0	218	99.2 (6678/6732)	82.89 (562/678)	15.06-75.84	24-978	113-2498

Variant Type	VAF Level (%)	Unique Mutations	PCR (%) (n/N)	NCR (%) (n/N)	Mean Allele Frequency Range (%)	Mean Allele Depth Range	Mean Loci Depth Range
Insertions	AF $\geq$ 0	23	97.19 (760/782)	-	3.6-77.06	20-1527	399-2026
	AF $\geq$ 2.0	23	97.19 (760/782)	-	3.6-77.06	20-1527	399-2026
	AF $\geq$ 5.0	21	96.92 (692/714)	-	5.24-77.06	27-1527	399-2026
	AF $\geq$ 10.0	16	96.88 (527/544)	-	11.77-77.06	69-1527	399-2026
	AF $\geq$ 15.0	12	96.81 (395/408)	-	15.99-77.06	69-1527	399-2026
Deletions	AF $\geq$ 0	121	97.31 (3441/3536)	87.37 (505/578)	1.11-52.65	4-1425	267-2710
	AF $\geq$ 2.0	113	97.31 (3441/3536)	78.76 (241/306)	2.11-52.65	12-1425	267-2710
	AF $\geq$ 5.0	110	98.15 (3404/3468)	78.31 (213/272)	5.18-52.65	14-1425	267-2710
	AF $\geq$ 10.0	86	99.5 (2808/2822)	69.61 (71/102)	10.01-52.65	37-1425	285-2710
	AF $\geq$ 15.0	58	99.38 (1926/1938)	94.12 (32/34)	15.13-52.65	48-1425	299-2710
<i>ERBB2</i> Amplification		5	100 (170/170)	-	N/A	N/A	N/A
<i>ALK</i> Translocation		1	100 (33/33)	-	N/A	67-269	403-1114
<i>RET</i> Translocation		1	100 (34/34)	-	N/A	14-285	896-1942
<i>ROSI</i> Translocation		1	100 (34/34)	-	N/A	422-1458	75-212
<i>NTRK1</i> Translocation		1	100 (34/34)	-	N/A	28-129	533-1270

<sup>1</sup>For hotspot variants above 2% VAF, only one mutation in one specimen was found in fewer than 50% of its replicates. This variant is expected to be an outlier due to being deemed a germline mutation and is not shown in the final report due to lack of clinical evidence supporting targeted drug use. It is expected with a larger sample size and more mutations, the modal NCR for hotspot variants will approach that of the non-hotspot variants.

c) **Per Specimen**

The modal positive and negative call rates for sequence mutations (SNVs and indels) in each specimen are summarized in Table 12. A total of 651 unique SNV, 23 unique insertions, 121 unique deletions, and 144 indels were identified. A modal analysis yielded a 97.46% (16137/16558, 95% CI: 92.94% - 100%) positive call rate among all positives and an NCR of 94.27% (10506/11144, 95% CI: 83.82% - 96.64%).

One replicate for Sample 2 did not pass the QC cut-off and was removed from analysis. R02 and R16 were found to have no modal PCR due to all detected variants in the samples having allele frequencies below the limit of detection of the GENESEEQPRIME assay, thus resulting in inconsistent variant calls.

**Table 12. Interlaboratory Reproducibility Modal Call Rates per Specimen**

<b>Specimen</b>	<b>Total Unique Mutations* Detected Across All Replicates</b>	<b>Modal Positive Call Rate (n/N) (95% CI)</b>	<b>Modal Negative Call Rate (n/N) (95% CI)</b>
1	39	95.35% (1005/1054) (93.91%,96.47%)	91.54% (249/272) (87.63%,94.30%)
2	8	-	94.32% (249/264) (90.84%,96.53%)
3	8	100% (34/34) (89.85%,100%)	96.22% (229/238) (92.97%,98.00%)
4	32	100% (374/374) (98.98%,100%)	96.64% (690/714) (95.05%,97.73%)
5	50	99.59% (1219/1224) (99.05%,99.83%)	91.81% (437/476) (89.00%,93.95%)
6	9	100% (68/68) (94.65%,100%)	92.44% (220/238) (88.36%,95.16%)
7	16	96.73% (296/306) (94.09%,98.22%)	92.02% (219/238) (87.87%,94.83%)
8	43	94.41% (321/340) (91.44%,96.39%)	95.10% (1067/1122) (93.67%,96.21%)
9	35	98.47% (837/850) (97.4%,99.1%)	96.18% (327/340) (93.57%,97.75%)
10	17	100% (68/68) (94.65%,100%)	96.08% (490/510) (94.02%,97.45%)
11	13	95.8% (228/238) (92.44%,97.7%)	90.20% (184/204) (85.34%,93.56%)
12	16	100% (102/102) (96.37%,100%)	96.61% (427/442) (94.48%,97.93%)
13	72	95.86% (1923/2006) (94.9%,96.65%)	96.38% (426/442) (94.20%,97.76%)
14	10	94.12% (160/170) (89.51%,96.77%)	94.12% (160/170) (89.51%,96.77%)
15	34	83.82% (57/68) (73.31%,90.72%)	96.60% (1051/1088) (95.35%,97.52%)
16	8	-	87.50% (238/272) (83.04%,90.92%)
17	4	100% (68/68) (94.65%,100%)	83.82% (57/68) (73.31%,90.72%)

Specimen	Total Unique Mutations* Detected Across All Replicates	Modal Positive Call Rate (n/N) (95% CI)	Modal Negative Call Rate (n/N) (95% CI)
18	18	99.51% (203/204) (97.28%,99.97%)	96.57% (394/408) (94.32%,97.95%)
19	10	95.59% (195/204) (91.83%,97.66%)	95.59% (130/136) (90.71%,97.96%)
20	14	100% (306/306) (98.76%,100%)	95.29% (162/170) (90.99%,97.60%)
21	13	92.94% (158/170) (88.07%,95.92%)	95.96% (261/272) (92.90%,97.73%)
22	98	97.63% (2888/2958) (97.02%,98.12%)	89.57% (335/374) (86.06%,92.28%)
23	49	98.67% (1409/1428) (97.93%,99.15%)	94.12% (224/238) (90.37%,96.46%)
24	37	99.26% (540/544) (98.12%,99.71%)	95.94% (685/714) (94.23%,97.16%)
25	44	99.92% (1223/1224) (99.54%,100%)	89.71% (244/272) (85.52%,92.78%)
26	19	100% (340/340) (98.88%,100%)	95.75% (293/306) (92.87%,97.50%)
27	31	98.04% (200/204) (95.07%,99.23%)	93.88% (798/850) (92.07%,95.30%)
28	68	95.46% (1915/2006) (94.46%,96.29%)	84.97% (260/306) (80.53%,88.54%)

\*"Unique mut" indicates the union of all unique mutations from all repeats

d) *Analysis of Source of Variance*

The Average Positive Agreement (APA) and Average Negative Agreement (ANA) was assessed to analyze the imprecision caused by different sources of variance across all 3 sites. Data analysis is presented stratified by variant type and presented for 1) overall, 2) site to site, 3) operator to operator, 4) day to day), and 5) within run concordance.

TMB was assessed using %CV of the TMB score across test sample replicates for samples. The results are shown in Table 13.

**Table 13. Interlaboratory Reproducibility of GENESEEQPRIME Assay**

<b>Alteration Type</b>	<b>Metric</b>	<b>Overall (95% CI)</b>	<b>Inter-Site (95% CI)</b>	<b>Inter-Operator (95% CI)</b>	<b>Inter-Day (95% CI)</b>	<b>Repeatability (Within-Run) (95% CI)</b>
Variants with Evidence of Clinical Significance	APA	92.49% (91.98%, 92.96%)	92.43% (91.81%, 93.01%)	92.28% (91.00%, 93.40%)	93.00% (91.46%, 94.29%)	92.99% (89.62%, 95.33%)
	ANA	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)
Hotspot Variants	APA	82.90% (82.59%, 83.21%)	82.86% (82.48%, 83.23%)	83.00% (82.25%, 83.73%)	82.98% (82.05%, 83.88%)	83.14% (81.24%, 84.88%)
	ANA	99.98% (99.98%, 99.98%)	99.98% (99.98%, 99.98%)	99.98% (99.98%, 99.98%)	99.98% (99.98%, 99.98%)	99.98% (99.98%, 99.99%)
Non-Hotspot Variants	APA	96.25% (96.20%, 96.31%)	96.23% (96.17%, 96.29%)	96.30% (96.17%, 96.42%)	96.32% (96.17%, 96.48%)	96.26% (95.93%, 96.56%)
	ANA	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)
SNVs	APA	94.22% (94.15%, 94.29%)	94.19% (94.1%, 94.28%)	94.25% (94.08%, 94.42%)	94.33% (94.12%, 94.54%)	94.28% (93.84%, 94.68%)
	ANA	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)
Insertions (All)	APA	97.38% (97.17%, 97.57%)	97.40% (97.15%, 97.63%)	97.42% (96.9%, 97.85%)	97.17% (96.49%, 97.71%)	97.34% (95.89%, 98.29%)
	ANA	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)
Insertions 1 – 5 bp	APA	97.12% (96.89%, 97.33%)	97.15% (96.87%, 97.4%)	97.16% (96.6%, 97.64%)	96.89% (96.15%, 97.49%)	97.08% (95.49%, 98.12%)
	ANA	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)
Insertions 5 – 10 bp	APA	100% (99.66%, 100%)	100% (99.5%, 100%)	100% (98.06%, 100%)	100% (97.09%, 100%)	100% (89.28%, 100%)
	ANA	100% (100%, 100%)	100% (100%, 100%)	100% (100%, 100%)	100% (100%, 100%)	100% (100%, 100%)

Alteration Type	Metric	Overall (95% CI)	Inter-Site (95% CI)	Inter-Operator (95% CI)	Inter-Day (95% CI)	Repeatability (Within-Run) (95% CI)
Insertions 21 – 30 bp	APA	100% (99.66%, 100%)	100% (99.5%, 100%)	100% (98.06%, 100%)	100% (97.09%, 100%)	100% (89.28%, 100%)
	ANA	100% (100%,100 %)	100% (100%,100 %)	100% (100%,100 %)	100% (100%,100 %)	100% (100%,100%)
Deletions (All)	APA	96.70% (96.60%, 96.81%)	96.68% (96.55%, 96.80%)	96.83% (96.57%, 97.06%)	96.68% (96.36%, 96.97%)	96.64% (95.97%, 97.2%)
	ANA	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)
Deletions 1 – 5 bp	APA	96.61% (96.5%, 96.71%)	96.58% (96.45%, 96.71%)	96.73% (96.47%, 96.97%)	96.58% (96.25%, 96.88%)	96.54% (95.85%, 97.12%)
	ANA	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)
Deletions 5 – 10 bp	APA	100% (99.66%, 100%)	100% (99.5%, 100%)	100% (98.06%, 100%)	100% (97.09%, 100%)	100% (89.28%, 100%)
	ANA	100% (100%, 100%)	100% (100%, 100%)	100% (100%, 100%)	100% (100%, 100%)	100% (100%, 100%)
Deletions 11 – 20 bp	APA	100% (99.83%, 100%)	100% (99.75%, 100%)	100% (99.02%, 100%)	100% (98.52%, 100%)	100% (94.34%, 100%)
	ANA	100% (100%, 100%)	100% (100%, 100%)	100% (100%, 100%)	100% (100%, 100%)	100% (100%, 100%)
MSI	APA	100% (99.94%, 100%)	100% (99.92%, 100%)	100% (99.67%, 100%)	100% (99.75%, 100%)	100% (98.04%, 100%)
	ANA	100% (99.99%, 100%)	100% (99.98%, 100%)	100% (99.91%, 100%)	100% (99.93%, 100%)	100% (99.46%, 100%)
<i>ERBB2</i> Amplification	APA	100% (99.93%, 100%)	100% (99.90%, 100%)	100% (99.61%, 100%)	100% (99.70%, 100%)	100% (97.66%, 100%)
	ANA	100% (99.99%, 100%)	100% (99.98%, 100%)	100% (99.91%, 100%)	100% (99.94%, 100%)	100% (99.48%, 100%)

Alteration Type	Metric	Overall (95% CI)	Inter-Site (95% CI)	Inter-Operator (95% CI)	Inter-Day (95% CI)	Repeatability (Within-Run) (95% CI)
<i>ALK</i> Translocation	APA	100% (99.64%, 100%)	100% (99.47%, 100%)	100% (97.93%, 100%)	100% (98.44%, 100%)	100% (88.65%, 100%)
	ANA	100% (99.99%, 100%)	100% (99.98%, 100%)	100% (99.93%, 100%)	100% (99.94%, 100%)	100% (99.56%, 100%)
<i>RET</i> Translocation	APA	100% (99.66%, 100%)	100% (99.50%, 100%)	100% (98.06%, 100%)	100% (98.53%, 100%)	100% (89.28%, 100%)
	ANA	100% (99.99%, 100%)	100% (99.98%, 100%)	100% (99.93%, 100%)	100% (99.94%, 100%)	100% (99.56%, 100%)
<i>ROSI</i> Translocation	APA	100% (99.66%, 100%)	100% (99.50%, 100%)	100% (98.06%, 100%)	100% (98.53%, 100%)	100% (89.28%, 100%)
	ANA	100% (99.99%, 100%)	100% (99.98%, 100%)	100% (99.93%, 100%)	100% (99.94%, 100%)	100% (99.56%, 100%)
<i>NTRK1</i> Translocation	ANA	100% (99.66%, 100%)	100% (99.50%, 100%)	100% (98.06%, 100%)	100% (98.53%, 100%)	100% (89.28%, 100%)
	APA	100% (99.99%, 100%)	100% (99.98%, 100%)	100% (99.93%, 100%)	100% (99.94%, 100%)	100% (99.56%, 100%)
TMB	CV	8.18%	1.16%	0.54%	0.17%	7.17%

e) **Precision for MSI**

Precision for MSI was evaluated across 22 microsatellite stable (MSS) and six microsatellite instability-high (MSI-H) samples with a range of MSI scores. The mean MSI score, MSI range, SD, and %CV for the score along with positive call rates are provided in Table 14.

**Table 14. MSI Performance in the Interlaboratory Reproducibility Study**

Case No.	Modal Status	Total Replicates	Mean MSI Score (%)	MSI Score Range (%)	SD	%CV	Positive Call Rate (%) (95% CI)
1	MSI-H	34	25.58	(19.64, 29.51)	2.60	10.17	100 (89.85, 100)

Case No.	Modal Status	Total Replicates	Mean MSI Score (%)	MSI Score Range (%)	SD	%CV	Positive Call Rate (%) (95% CI)
2	MSS	33	6.05	(1.69, 10)	1.91	31.62	100 (89.57, 100)
3	MSS	34	5.35	(1.64, 11.48)	2.18	40.75	100 (89.85, 100)
4	MSS	34	5.84	(1.75, 10.00)	2.17	37.15	100 (89.85, 100)
5	MSI-H	34	56.96	(51.67, 63.33)	2.68	4.70	100 (89.85, 100)
6	MSS	34	6.05	(1.69, 11.67)	2.53	41.85	100 (89.85, 100)
7	MSS	34	7.03	(3.28, 10.00)	1.71	24.38	100 (89.85, 100)
8	MSS	34	3.55	(0, 8.62)	2.02	56.84	100 (89.85, 100)
9	MSS	34	4.04	(0, 9.84)	2.41	59.60	100 (89.85, 100)
10	MSS	34	5.21	(0, 8.77)	2.06	39.47	100 (89.85, 100)
11	MSS	34	4.56	(1.67, 8.33)	1.91	41.92	100 (89.85, 100)
12	MSS	34	5.18	(1.75, 8.93)	1.94	37.37	100 (89.85, 100)
13	MSI-H	34	77.10	(66.67, 81.97)	3.40	4.41	100 (89.85, 100)
14	MSS	34	5.13	(1.72, 10.17)	2.00	38.91	100 (89.85, 100)
15	MSS	34	6.80	(3.51, 14.81)	2.74	40.27	100 (89.85, 100)
16	MSS	34	3.11	(0, 6.90)	1.85	59.47	100 (89.85, 100)
17	MSS	34	4.50	(1.67, 8.33)	2.05	45.64	100 (89.85, 100)
18	MSS	34	7.09	(3.33, 13.79)	2.59	36.55	100 (89.85, 100)
19	MSS	34	4.56	(0, 8.33)	1.71	37.48	100 (89.85, 100)
20	MSS	34	5.71	(1.82, 10.00)	1.85	32.38	100 (89.85, 100)
21	MSS	34	9.11	(5.17, 15.25)	2.35	25.83	100 (89.85, 100)

Case No.	Modal Status	Total Replicates	Mean MSI Score (%)	MSI Score Range (%)	SD	%CV	Positive Call Rate (%) (95% CI)
22	MSI-H	34	76.40	(70.49, 81.97)	2.94	3.85	100 (89.85, 100)
23	MSI-H	34	55.81	(48.21, 62.30)	3.15	5.64	100 (89.85, 100)
24	MSS	34	6.35	(1.79, 10.53)	2.46	38.74	100 (89.85, 100)
25	MSS	34	5.20	(1.69, 9.84)	2.16	41.64	100 (89.85, 100)
26	MSS	34	5.22	(1.72, 10.17)	2.25	43.17	100 (89.85, 100)
27	MSS	34	3.76	(0, 8.77)	2.00	53.31	100 (89.85, 100)
28	MSI-H	34	75.09	(68.85, 80.33)	2.70	3.59	100 (89.85, 100)

*f) Precision for Tumor Mutational Burden (TMB)*

Precision of TMB was evaluated across 28 FFPE samples (with samples near the analytical borderline value of 1.1 Muts/Mb) using the coefficient of variance (%CV) to measure the degree of variation. The overall variance between the samples was 8.18% (Table 15). The data demonstrates the precision of TMB scores.

**Table 15. TMB Performance across all Repeats**

Sample	Replicates	Range	Mean	SD	%CV
1	34	43.4-48.6	45.97	1.24	2.70
2	33	0-3.2	1.44	0.72	50.10
3*	34	0 – 2.1	031	0.67	212.10
4	34	15.9-19	16.62	1.02	6.16
5	34	42.3-47.6	44.52	1.43	3.22
6	34	1.1-4.2	2.29	0.68	29.56
7	34	11.6-15.9	13.72	1.15	8.35
8	34	8.5-13.7	10.63	1.28	12.01
9	34	28.6-31.7	29.47	0.92	3.14
10	34	3.2-5.3	3.50	0.63	18.07
11	34	8.5-10.6	9.77	0.78	8.00
12	34	3.2-5.3	3.51	0.75	21.29
13	34	70.9-78.3	75.32	1.68	2.24
14	34	4.2-9.5	7.24	1.33	18.42
15	34	4.2-9.5	5.93	1.35	22.73

Sample	Repeats	Range	Mean	SD	%CV
16*	34	0 - 3.2	0.95	0.82	85.65
17	34	2.1-3.2	2.26	0.48	21.31
18	34	7.4-8.5	7.69	0.56	7.32
19	34	5.3-8.5	7.15	0.70	9.77
20	34	10.6-13.7	11.73	0.59	5.03
21	34	7.4-11.6	9.59	1.12	11.70
22	34	106.8-113.1	109.54	1.93	1.76
23	34	48.6-57.1	54.08	1.95	3.61
24	34	16.9-22.2	18.78	1.29	6.85
25	34	55-60.3	56.78	1.33	2.35
26	34	10.6-11.6	10.72	0.35	3.30
27	34	8.5-23.3	10.99	2.70	24.62
28	34	70.9-79.3	74.38	2.04	2.74
<b>TMB Median %CV (All Samples)</b>					<b>8.18</b>
<b>TMB Mean CV% (All Samples)</b>					<b>21.58</b>
<b>TMB Median %CV (All Samples with the Mean TMB greater than LoB)</b>					<b>7.66</b>
<b>TMB Mean %CV (All Samples with the Mean TMB greater than LoB)</b>					<b>11.78</b>

\*Sample 3 and Sample 16 have a mean TMB below the LoB (1.1Muts/Mb) of the GENESEEQPRIME Assay.

g) *Lot-to-Lot Precision*

Performance of the GENESEEQPRIME assay was assessed across three unique kit lots by determining the concordance of variant calls in five FFPE tissue samples. The three unique kit lots were used to process five test cases in triplicate for a total of 45 observations. All batches were sequenced on the same instrument. Table 16. lists the APA and ANA used to assess lot to lot performance. The overall all panel-wide variants (SNVs, Insertions, and Deletions) APA is  $\geq 98.11\%$  and the NPA did not fall below 99.99%. *ERBB2* amplification, *RET* translocation, and MSI status had a concordance of 100% across all comparisons. The %CV for TMB analyses is  $< 2.46\%$ .

**Table 16. Lot-to-Lot Precision of GENESEEQPRIME**

Variant Type	Performance	Between Lot 1 & 2	Between Lot 2 & 3	Between Lot 1 & 3
Variants with Evidence of Clinical Significance	APA	100% (93.36%, 100%)	100% (93.36%, 100%)	100% (93.36%, 100%)
	ANA	100% (99.99%, 100%)	100% (99.99%, 100%)	100% (99.99%, 100%)

<b>Variant Type</b>	<b>Performance</b>	<b>Between Lot 1 &amp; 2</b>	<b>Between Lot 2 &amp; 3</b>	<b>Between Lot 1 &amp; 3</b>
Panel-Wide Variant (SNVs + Insertions+ Deletions)	APA	98.48% (98.15%, 98.75%)	98.77% (98.47%, 99.01%)	98.11% (97.75%, 98.41%)
	ANA	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)
Hotspot SNV (including Clinically Significant Variants)	APA	99.03% (97.18%, 99.67%)	100% (98.76%, 100%)	99.10% (97.18%, 99.67%)
	ANA	99.99% (99.99%, 99.99%)	100% (99.99%, 100%)	99.99% (99.99%, 99.99%)
Non-Hotspot SNVs (including Clinically Significant Variants)	APA	98.35% (97.99%, 98.65%)	98.63% (98.3%, 98.90%)	97.94% (97.54%, 98.27%)
	ANA	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)
MSI	APA	100% (82.41%, 100%)	100% (82.41%, 100%)	100% (82.41%, 100%)
	ANA	100% (94.93%, 100%)	100% (94.93%, 100%)	100% (94.93%, 100%)
SNVs (Hotspot + Non-Hotspot)	APA	98.43% (98.09%, 98.72%)	98.70% (98.38%, 98.95%)	98.04% (97.66%, 98.36%)
	ANA	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)	99.99% (99.99%, 99.99%)
Insertions	APA	100% (94.93%, 100%)	100% (94.93%, 100%)	100% (94.93%, 100%)
	ANA	100% (99.99%, 100%)	100% (99.99%, 100%)	100% (99.99%, 100%)
Deletions	APA	99.03% (97.18%, 99.67%)	100% (98.76%, 100%)	99.03% (97.18%, 99.67%)
	ANA	99.99% (99.99%, 99.99%)	100% (99.99%, 100%)	99.99% (99.99%, 99.99%)
<i>ERBB2</i> Amplification	APA	100% (82.41%, 100%)	100% (82.41%, 100%)	100% (82.41%, 100%)
	ANA	100% (94.93%, 100%)	100% (94.93%, 100%)	100% (94.93%, 100%)
<i>RET</i> Translocation	APA	100% (82.41%, 100%)	100% (82.41%, 100%)	100% (82.41%, 100%)
	ANA	100% (94.93%, 100%)	100% (94.93%, 100%)	100% (94.93%, 100%)
TMB	%CV	2.46%	1.55%	2.46%

3. **Analytical Sensitivity – Limit of Detection (LoD)**

The recommended DNA input for GENESEEQPRIME assay is 100 ng of total DNA recovered from tissue with a minimum 20% viable tumor nuclei. The LoD study was comprised of two steps: LoD establishment using cell lines and LoD confirmation with FFPE clinical tumor samples from clinical cases across a diverse set of cancers. Select specimens were used to evaluate specific mutations with evidence of clinical significance. Specimens were selected for allele frequencies near the claimed cut offs. Details of the data are discussed and shown below.

a) ***LoD – SNVs, Insertions, and Deletions***

The LoD of the GENESEEQPRIME assay is defined as the lowest measured mean allele frequency at which 95% of replicates for a variant type are reliably detected. Target levels for detection were first established in a dilution series from cell lines with up to five targeted VAF levels. The analytical sensitivity and LoD was then confirmed in seven clinical FFPE specimens (six samples for SNVs, four for insertions, and four for deletions). The data was aggregated across two reagent kit lots. Cell lines were used to establish the LoD VAF range for 461 unique targeted and non-targeted variants (Hotspot SNVs, Non-Hotspot SNVs, SNVs, Insertions, and Deletions) across the panel (Table 17). A total of 300 observations were generated (three samples with 20 replicates at five dilution levels). Positive call status and VAF was evaluated from select variants identified in seven FFPE clinical specimens diluted with normal DNA derived from FFPE tissue. Each specimen was processed with two kit lot of GENESEEQPRIME across 20 replicates for a for a total of 140 observations.

The established analytical sensitivity ranges were confirmed at  $\geq 95\%$  call rate with FFPE clinical cases on a per variant level (Table 18) and using all somatic variants identified in FFPE clinical cases representing a range of VAFs for hotspot and non-hotspot positions (Table 18). The observed sequencing depth (DP), allele depth (AD), variant allele frequency (VAF), and average VAF are included. A summary of the established analytical sensitivity for the represented SNVs and Indels in FFPE tumor tissue is provided in Table 19.

**Table 17. Established LoD VAF of Representative SNVs, Insertions, and Deletions**

<b>Variant Type</b>	<b>Mean AF Range in Cell Lines (%)</b>	<b>Mean LoD in Cell Lines (%)</b>	<b>Unique Variants in Cell Lines</b>	<b>Mean LoD in FFPE Samples (%)</b>	<b>Unique Variants in FFPE in the Established Range</b>
HS SNV	1.15 - 6.73	2.33	26	2.14	22
NHS SNV	2.23 - 10.13	3.60	384	2.95	265
INS	0.93 - 6.37	2.82	9	2.93	4
DEL	0.89 - 15.18	5.32	42	5.44	9

Variant Type	Mean AF Range in Cell Lines (%)	Mean LoD in Cell Lines (%)	Unique Variants in Cell Lines	Mean LoD in FFPE Samples (%)	Unique Variants in FFPE in the Established Range
Indels at Homopolymer Context*	2.70 - 15.18	6.98	25	7.94	5
Indels at Non-Homopolymer Context	0.89 - 6.36	3.15	26	2.62	8

\*Homopolymer context - a region containing  $\geq 5$  consecutive identical nucleotides.

**Table 18. Analytical Sensitivity of Representative SNVs and Indels in FFPE Tumor Tissue**

Variant Type	Gene	AA Chane	DP Range	AD Range	AF Range (%)	Mean AF (%)	Call Rate (%)
INS	<i>ERBB2</i>	Y772_A775dup	1515 - 1592	22 - 23	1.4 - 1.46	1.43	100 (20/20)
INS	<i>KMT2B</i>	P2259Sfs*44	530 - 552	29 - 29	5.25 - 5.47	5.33	100 (20/20)
INS	<i>RAD51</i>	G45Wfs*20	688 - 738	17 - 20	2.34 - 2.71	2.45	100 (20/20)
INS	<i>GATA</i>	H333dup	584-613	15 - 15	2.45-2.57	2.51	100 (20/20)
DEL	<i>EGFR</i>	E746_A750del	858 - 935	7 - 7	0.75 - 0.82	0.78	100 (20/20)
DEL	<i>CDH1</i>	T467Hfs*15	1188 - 1248	28 - 30	2.32 - 2.4	2.35	100 (20/20)
DEL	<i>B2M</i>	V69Wfs*34	975 - 1044	76 - 82	7.79 - 8.02	7.90	100 (20/20)
DEL	<i>TP53</i>	R209Kfs*6	1362 - 1438	28 - 30	2 - 2.14	2.08	100 (20/20)
SNV	<i>APC</i>	R1450*	672-721	16 - 17	2.32-2.45	2.38	100 (20/20)
SNV	<i>EGFR</i>	L858R	973 - 1076	15 - 15	1.39 - 1.54	1.47	100 (20/20)
SNV	<i>TP53</i>	Q104*	971-1040	14 - 17	1.38-1.65	1.46	100 (20/20)
SNV	<i>PTEN</i>	Q245*	852 - 901	13 - 16	1.49 - 1.8	1.63	100 (20/20)
SNV	<i>NRAS</i>	G12D	771 - 823	18 - 19	2.21 - 2.33	2.29	100 (20/20)
SNV	<i>KRAS</i>	G12C	1101 - 1177	18 - 19	1.55 - 1.63	1.60	100 (20/20)

**Table 19. Analytical Sensitivity (LoD VAF) for SNVs and Indels in FFPE Tumor Tissue**

Variant	Established VAF Range (%)	Number of Variants in Clinical Cases in the Established Range
Hotspot SNVs	0.90-6.47	22
Non-Hotspot SNVs	2.03-10.07	265
Insertions	1.43-5.33	4
Deletions	0.78 - 14.17	9

**b) LoD – MSI, Translocations, and Amplifications (Tumor Purity)**

The analytical sensitivity of *ALK*, *RET*, *ROSI*, *NTRK1* was confirmed by testing four clinical FFPE specimens diluted with normal FFPE DNA to achieve the targeted detection levels. One clinical FFPE specimen was diluted with normal FFPE DNA to achieve the targeted detection levels. Each unique case was confirmed at  $\geq 95\%$  call rate with 10 replicates per kit lot, across two unique lots for amplification and translocations. For MSI, three clinical FFPE specimens (colorectal cancer, endometrial cancer, and gastric cancer) were confirmed at five tumor purity levels with 20 replicates each. The established analytical sensitivity for specific translocations, amplifications, and MSI are summarized in Table 20. Analytical sensitivity results of each translocation and amplification are summarized in Table 21 – Table 26.

**Table 20. Analytical Sensitivity (LoD Tumor Purity) of GENESEEQPRIME – Translocations, Amplifications, and MSI**

Variant	Confirmed LoD Tumor Purity (%)	Positive Call Rate (%)	Mean Coverage Range
Microsatellite Status	20.00	19/20 (95)	558 - 1791
<i>ERBB2</i> Amplification	5.00	20/20 (100)	739 - 990
<i>ALK</i> Translocation	7.50	20/20 (100)	723 - 990
<i>RET</i> Translocation	10.00	20/20 (100)	679 - 1519
<i>ROSI</i> Translocation	7.50	20/20 (100)	721 - 2044
<i>NTRK1</i> Translocation	15.00	20/20 (100)	594 - 1957

**Table 21. Limit of Detection for *ALK* Translocation with Clinical FFPE Specimens**

Gene	Tumor Purity (%)	Detected Mean Variant Reads	Call Rate (%)
<i>ALK</i>	50.00	196.5	20/20 (100)
<i>ALK</i>	20.00	55.5	20/20 (100)
<i>ALK</i>	10.00	17.3	20/20 (100)
<i>ALK</i>	7.50	18.9	20/20 (100)
<i>ALK</i>	5.00	11.8	15/20 (75)

**Table 22. Limit of Detection for *RET* Translocation with Clinical FFPE Specimens**

Gene	Tumor Purity (%)	Detected Mean Variant Reads	Call Rate (%)
<i>RET</i>	50.00	132.7	20/20 (100)
<i>RET</i>	20.00	27	20/20 (100)
<i>RET</i>	15.00	23.5	20/20 (100)
<i>RET</i>	10.00	13.7	20/20 (100)
<i>RET</i>	7.50	12.5	15/20 (75)
<i>RET</i>	5.00	n.d.	0/20 (0)

n.d. – Not Detected

**Table 23. Limit of Detection for *ROS1* Translocation with Clinical FFPE Specimens**

Gene	Tumor Purity (%)	Detected Mean Variant Reads	Call Rate (%)
<i>ROS1</i>	50.00	178.7	20/20 (100)
<i>ROS1</i>	20.00	41.9	20/20 (100)
<i>ROS1</i>	10.00	20	20/20 (100)
<i>ROS1</i>	7.50	16.9	20/20 (100)
<i>ROS1</i>	5.00	10.3	6/20 (30)

**Table 24. Limit of Detection for *NTRK1* Translocation with Clinical FFPE Specimens**

Gene	Tumor Purity (%)	Detected Mean Variant Reads	Call Rate (%)
<i>NTRK1</i>	50.00	71.1	20/20 (100)
<i>NTRK1</i>	30.00	50.5	20/20 (100)
<i>NTRK1</i>	20.00	32.6	20/20 (100)
<i>NTRK1</i>	15.00	26.3	20/20 (100)
<i>NTRK1</i>	12.50	10.1	16/20 (80)
<i>NTRK1</i>	10.00	n.d.	0/20 (0)

n.d. – Not Detected

**Table 25. Limit of Detection for *ERBB2* Amplification with Clinical FFPE Specimens**

Gene	Tumor Purity (%)	Detected Mean Variant Reads	Call Rate (%)
<i>ERBB2</i>	50.00	11.97	20/20 (100)
<i>ERBB2</i>	20.00	5.54	20/20 (100)
<i>ERBB2</i>	10.00	3.01	20/20 (100)
<i>ERBB2</i>	5.00	1.89	20/20 (100)
<i>ERBB2</i>	4.00	1.83	18/20 (90)
<i>ERBB2</i>	2.50	n.d.	0/20 (0)

n.d. – Not Detected

**Table 26. Limit of Detection for MSI with Clinical FFPE Specimens**

MSI Sample	Tumor Purity (%)	Detected Mean Variant Reads (%)	Call Rate (%)
1	50.00	70.57	20/20 (100)
	40.00	57.95	20/20 (100)
	30.00	41.88	20/20 (100)
	20.00	17.48	19/20 (95)
	10.00	8.86	0/20 (0)

MSI Sample	Tumor Purity (%)	Detected Mean Variant Reads (%)	Call Rate (%)
2	40.00	35.49	20/20 (100)
	35.00	31.97	20/20 (100)
	30.00	28.94	20/20 (100)
	25.00	24.59	20/20 (100)
	10.00	10.90	0/20 (0)
3	50.00	44.26	20/20 (100)
	40.00	35.66	20/20 (100)
	30.00	26.48	20/20 (100)
	20.00	21.39	19/20 (95)
	10.00	6.97	0/20 (0)

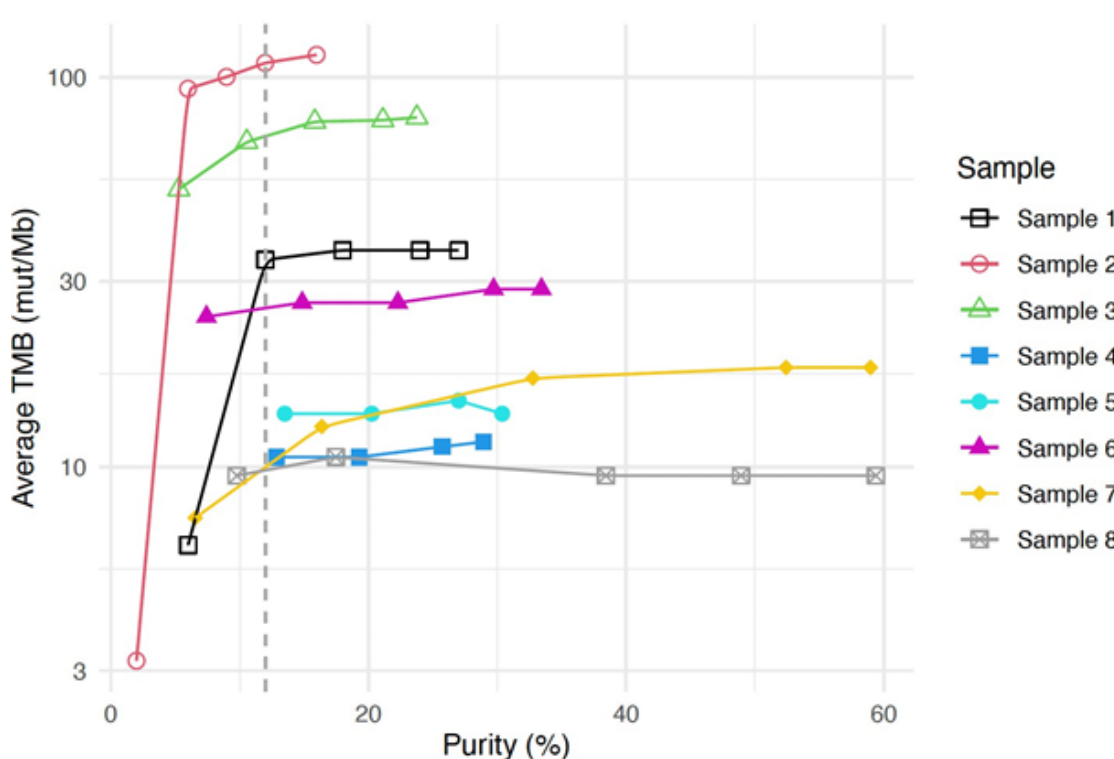
**c) LoD – Tumor Mutation Burden (TMB) and DNA Input**

The minimum tumor purity requirement for input into the GENESEEQPRIME assay is 20%. The minimum tumor purity required for robust reporting of TMB scores by GENESEEQPRIME was confirmed using eight FFPE specimens. A total of 162 replicates were used for analysis. Samples 1 – 3 and 8 were serially diluted across five levels with five replicates per level (n = 25). Sample 4 was serially diluted across four levels with three replicates per level (n = 12). Sample 5 was serially diluted across four levels with 5 replicates per level (n = 15). Samples 6 and 7 were serially diluted across five levels with three replicates per level (n = 15). The total number of replicates per sample and the %CV of all replicates with  $\geq 12\%$  tumor purity are shown in Table 27. Together these data show the GENESEEQPRIME assay has consistent TMB performance across tumor purities at or above 12% (Figure 7), which falls within the minimum sample tumor purity of 20% input for the GENESEEQPRIME assay.

**Table 27. TMB Calls for FFPE Samples  $\geq 12\%$  Tumor Purity**

Sample Number	Undiluted TMB Score	%CV of Replicates $\geq 12\%$ Tumor Purity (%)	Number of Replicates $\geq 12\%$ Tumor Purity
1	35.0	2.56	20
2	130.8	<0.01	5
3	74.1	1.24	15
4	10.3	4.67	12

Sample Number	Undiluted TMB Score	%CV of Replicates $\geq$ 12% Tumor Purity (%)	Number of Replicates $\geq$ 12% Tumor Purity
5	13.4	3.50	20
6	26.8	6.22	15
7	22.7	13.90	12
8	11.3	5.00	20



**Figure 7. Fluctuation of TMB Score with Tumor Purity in GENESEQPRIME Assay.** The x-axis shows the percent of tumor purity for each replicate. The y-axis shows the average TMB score for the replicates, measured in mutations per megabase (Muts/Mb). The dotted line denotes the lowest recommended tumor purity of 12% for consistent TMB scores.

#### 4. Linearity/Assay Reportable Range

Not applicable

5. **Traceability, Stability, Expected Values (Controls, Calibrators, or Methods)**

a) ***Traceability***

The GENESEEQPRIME assay is not traceable to any known standard. Controls and quality metrics are described in the device description section.

b) ***Stability/Shelf-Life***

Product expiration dating is based on testing at multiple time points with specimens representative of variant types with three lots of the GENESEEQPRIME assay reagent kit. The current shelf life for the GENESEEQPRIME assay reagent kit is established as 10 months when stored according to the temperatures indicated on the label.

The GENESEEQPRIME can be used commercially for up to four freeze/thaw cycles.

c) ***Transport Conditions***

The GENESEEQPRIME assay kits are shipped using an insulated container with dry ice (all -25°C to -15°C reagents) and a controlled room temperature container (with predetermined configuration of gel (cold and/or frozen) packs, meant for 2°C to 8°C reagents) to maintain the product for up to three days when stored at ambient temperature.

d) ***Expected Values (Controls, Calibrators, or Methods)***

The external controls (Negative and Positive) are provided in the GENESEEQPRIME assay reagent kit. A negative external control contains a non-cancerous cell line derived-DNA with no variants of interest; and the positive external control contains cell line derived-DNA with multiple verified sequence mutations. Both external controls are processed from library preparation through sequencing to serve as an end-to-end control to demonstrate assay performance. Failure of either external control to meet the quality control thresholds will result in all test samples on the run being reported as invalid. In addition, several quality metrics are established as threshold for reporting results to provide for high confidence data.

6. **Analytical Specificity**

a) ***Cut-Off/ False Positive Range (Limit of Blank in DNA NGS)***

Non-cancerous FFPE tissues and a reference material were evaluated for analytical specificity to assess the risk of false positives in normal tissue when detecting SNVs, indels, amplifications, translocations, MSI, and TMB using the GENESEEQPRIME assay.

- i) *Reference Standard*: One reference standard from the National Institute of Standards and Technology (NIST), NA18535, was evaluated by GENESEEQPRIME assay for variants reported. Specificity was observed at 100% with no unverified mutations reported across the 15 replicates. For MSI, all replicates were classified as microsatellite stable (MSS). For TMB, values ranged from 0 to 1.1 Muts/Mb with a mean TMB score of 0.073 Muts/Mb.

ii) *FFPE Clinical Specimens – 500 ng Input*: Analytical specificity of SNVs, indels, *ALK*, *RET*, *ROSI*, and *NTRK1* translocations, *ERBB2* amplifications, MSI, and TMB was further assessed with non-cancerous FFPE tissues. Unique test cases from 42 normal FFPE samples were processed with the maximum DNA input (500 ng) across two different lots of the GENESEEQPRIME assay. A total of 14 variants were detected across the 84 FFPE samples (14/84, 16.7%). The detected variants were all classified as non-hotspot variants. Hot-Spot variants and variants with evidence of clinical significance the false positive rate was determined to be < 0.01% (0/84). The false positive rate was determined to be < 0.01% (0/84) for insertions and deletions. For MSI, all samples were correctly classified as microsatellite stable (MSS). For TMB, values above 1.1 Muts/Mb were observed in two out of the 84 replicates (2/84 = 2.38%) setting the LoB for TMB at 1.1 Muts/Mb.

**b) *Index Cross-Contamination***

In NGS assays, technical artifacts, such as index hopping, where sequencing reads are incorrectly assigned to the wrong sample due to index misassignment can lead to cross-contamination. To evaluate the extent of index hopping in the GENESEEQPRIME assay, five sequencing runs were performed. In each sequencing run, one synthetic DNA library was spiked into a pool containing 27 clinical samples, a positive control, and a negative control. A synthetic index-pair was randomly selected and excluded from assignment to any clinical samples. Detection of synthetic reads in non-synthetic libraries was considered evidence of index hopping; and contamination levels were quantified as the number of synthetic reads detected per 1,000 sequencing reads.

Synthetic reads were predominantly restricted to the intended synthetic DNA samples across all five sequencing runs demonstrating correct index assignment. Trace levels (0.453 – 4.708 reads per million sequence reads, < 0.001% of total reads) of low-level synthetic reads were observed in a few cases, which is consistent with rare index hopping events. The data indicated cross-contamination from index hopping was rare in the GENESEEQPRIME assay and occurred at levels below the threshold.

**c) *Necrotic Tissue***

The impact of necrosis on the performance of the GENESEEQPRIME assay was evaluated by assessing the quality control (QC) pass rate and the performance of the GENESEEQPRIME assay in detecting variants of samples processed in the clinical accuracy study (see Method Comparison (Accuracy) section below). All 503 samples utilized in the accuracy study were evaluated for necrosis over a range of 0% – 53% and pass rates were examined. The assay performance was assessed and compared to the predicate device. The QC pass rates ranged from 92.16% to 100% (Table 28). The PPA for overall variant (SNV, insertions, and deletions) detection ranged from 82.14% to 98.62%, while an NPA of 99.99% was observed (Table 29). The data indicated there is no correlation between necrosis and the pass/fail rate from necrotic tissue content up to ~50%.

**Table 28. Necrotic Tissue QC Pass Rate for the GENESEEQPRIME Assay**

<b>Cancer Types</b>	<b>Sample Count</b>	<b>Necrotic Tissue Content (%)</b>	<b>QC Pass Rate (%) (n/N)</b>
33	370	0 to 5	92.16 (341/370)
21	115	5 to 20	94.78 (109/115)
4	14	21 to 40	100 (14/14)
3	4	41 to 53	100 (4/4)

**Table 29. Concordance of Overall Variant (SNV + Insertions + Deletions) Detection**

<b>Necrotic Tissue Content (%)</b>	<b>Sample Count</b>	<b>PPA (%) (95% CI) (n/N)</b>	<b>NPA (%) (95% CI) (n/N)</b>
0 to 5	370	92.54 (91.31, 93.61) (1862/2012)	99.99 (99.99, 99.99) (273312652/273313741)
5 to 20	115	90.74 (88.62, 92.50) (784/864)	99.99 (99.99, 99.99) (88450335/88450836)
21 to 40	14	98.62 (95.11, 99.62) (143/145)	99.99 (99.99, 99.99) (10613919/10614059)
41 to 53	4	82.14 (64.41, 92.12) (23/28)	99.99 (99.99, 99.99) (1768996/1769006)
<b>Overall</b>	<b>423</b>	<b>92.23</b> <b>(91.22, 93.13)</b> <b>(2812/3049)</b>	<b>99.99</b> <b>(99.99, 99.99)</b> <b>(374145902/374147642)</b>

**d) Interfering Substances**

The impact of interfering substances on the performance of the GENESEEQPRIME assay was assessed by processing DNA from FFPE samples tested in the presence of each interfering substance at varying amounts (Table 30). The samples were evaluated for concordance of variant call when compared to samples processed without the interfering substances. Replicates for four test cases were analyzed for eight experimental and one baseline condition. Performance was evaluated across four samples x nine conditions x 12 replicates. Samples were selected to be near the LoD.

Analysis of all variant types tested (SNVs, indels, amplifications, translocations, and MSI, showed no effect of exogenous interferent for all conditions (PPA  $\geq$  95.14% and NPA  $\geq$  99.99%, Table 31). The TMB mean absolute percent error (MAPE) ranged from 0% to 9.7% across conditions (Table 32). The results show minimal risk to assay performance from interfering substances.

**Table 30. Exogenous Interfering Substances Tested**

Substance	Amount in Excess of Standard Conditions
Proteinase K	2X and 3X
Ethanol	2.5% and 5%
Index Adapter	15% and 30%
Melanin	0.2 $\mu\text{g/mL}$ and 1.6 $\mu\text{g/mL}$

**Table 31. Exogenous Interfering Substances Concordance by Test Condition**

Test Condition	PPA % (n/N) (2-sided 95% CI)	NPA % (n/N) (2-sided 95% CI)
Proteinase K 2X	96.08% (1029/1071) (94.74%, 97.09%)	99.99% (52609387/52609473) (99.99%, 99.99%)
Proteinase K 3X	96.08% (1029/1071) (94.74%, 97.09%)	99.99% (52609389/52609473) (99.99%, 99.99%)
Ethanol 2.5%	95.99% (1028/1071) (94.64%, 97.01%)	99.99% (52609372/52609473) (99.99%, 99.99%)
Ethanol 5%	95.14% (1019/1071) (93.69%, 96.28%)	99.99% (52609389/52609473) (99.99%, 99.99%)
Index Adapter 15%	96.55% (1034/1071) (95.27%, 97.48%)	99.99% (52609391/52609473) (99.99%, 99.99%)
Index Adapter 30%	95.70% (1025/1071) (94.32%, 96.76%)	99.99% (52609395/52609473) (99.99%, 99.99%)
Melanin 0.2 $\mu\text{g/mL}$	95.89% (1027/1071) (94.53%, 96.93%)	99.99% (52609384/52609473) (99.99%, 99.99%)
Melanin 1.6 $\mu\text{g/mL}$	95.24% (1020/1071) (93.79%, 96.36%)	99.99% (52609385/52609473) (99.99%, 99.99%)

**Table 32. Exogenous Interfering Substances Concordance of TMB Mean Absolute Percent Error Reported**

FFPE Sample No.	Condition	Observed TMB Score	Absolute Percent Error (%)	Mean Absolute Percent Error (%)
1	Proteinase K 2X	23.3	4.1	4.1
		23.3	4.1	
		23.3	4.1	
2		77.2	4.0	4.4
		77.2	4.0	
		76.1	5.3	
3	Proteinase K 2X	358.5	3.9	4.8
		363.8	5.4	
		362.7	5.1	
4		1.1	0.0	0.0
		1.1	0.0	
		1.1	0.0	
1	Proteinase K 3X	21.1	13.2	7.1
		23.3	4.1	
		23.3	4.1	
2		77.2	4.0	4.9
		76.1	5.3	
		76.1	5.3	
3		358.5	3.9	4.6
		361.7	4.8	
		362.7	5.1	
4		1.1	0.0	0.0
		1.1	0.0	
		1.1	0.0	
1	Ethanol 2.5%	23.3	4.1	4.1
		23.3	4.1	
		23.3	4.1	
2		76.1	5.3	5.3
		76.1	5.3	
		76.1	5.3	
3		365.9	6.0	5.4
		361.7	4.8	
		363.8	5.4	
4		1.1	0.0	0.0
		1.1	0.0	
		1.1	0.0	

FFPE Sample No.	Condition	Observed TMB Score	Absolute Percent Error (%)	Mean Absolute Percent Error (%)	
1	Ethanol 5%	24.3	0.0	2.7	
		23.3	4.1		
		23.3	4.1		
2		74	8.0	9.7	
		73	9.2		
		70.9	11.8		
3		361.7	4.8	4.8	
		361.7	4.8		
		361.7	4.8		
4		Ethanol 5%	1.1	0.0	0.0
			1.1	0.0	
			1.1	0.0	
1	Adapter 15%	23.3	4.1	4.1	
		23.3	4.1		
		23.3	4.1		
2		77.2	4.0	4.4	
		77.2	4.0		
		76.1	5.3		
3		361.7	4.8	5.0	
		361.7	4.8		
		363.8	5.4		
4		1.1	0.0	0.0	
		1.1	0.0		
		1.1	0.0		
1	Adaptor 30%	22.2	8.6	8.6	
		22.2	8.6		
		22.2	8.6		
2		76.1	5.3	5.3	
		76.1	5.3		
		76.1	5.3		
3		355.3	3.0	3.5	
		355.3	3.0		
		360.6	4.5		
4		1.1	0.0	0.0	
		1.1	0.0		
		1.1	0.0		

FFPE Sample No.	Condition	Observed TMB Score	Absolute Percent Error (%)	Mean Absolute Percent Error (%)			
1	Melanin 0.2 µg/mL	21.1	13.2	7.1			
		23.3	4.1				
		23.3	4.1				
2		Melanin 0.2 µg/mL	76.1	5.3	5.3		
			76.1	5.3			
			76.1	5.3			
3			Melanin 0.2 µg/mL	359.5	4.2	4.6	
				360.6	4.5		
				362.7	5.1		
4				Melanin 0.2 µg/mL	1.1	0.0	0.0
					1.1	0.0	
					1.1	0.0	
1	Melanin 1.6 µg/mL				21.1	13.2	7.1
					23.3	4.1	
					23.3	4.1	
2		Melanin 1.6 µg/mL			70.9	11.8	7.9
					75.1	6.6	
					76.1	5.3	
3			Melanin 1.6 µg/mL		362.7	5.1	4.3
					359.5	4.2	
					357.4	3.6	
4				Melanin 1.6 µg/mL	1.1	0.0	0.0
					1.1	0.0	
					1.1	0.0	

*e) Sample Carryover and Cross-Contamination*

Cross-contamination (contamination from one sample to another within the same batch) and sample carryover (contamination from a previous sequencing run when using the same instrument) were assessed by evaluating the false positive and false negative variant calls in 46 FFPE samples. Twenty-seven (27) of the 46 cases had known positive variants, the remaining samples were known negative samples. All FFPE samples were assessed across two batches to test for contamination within and between runs. In batch 1, a checkerboard pattern within a 96-well plate was created by altering the samples with representative positive variants and known negative samples. Batch 2 contained 24 known negative samples and three positive variants and was sequenced directly after completion of batch 1, following standard instrument cleaning procedures. No positive variant results were observed in known negative samples tested.

## 7. Robustness Studies

### a) *Sample Stability (FFPE Block)*

The stability of FFPE blocks was established using 21 FFPE blocks stored at under appropriate conditions (15 – 20°C, dry conditions, relative humidity (below 60%), protected from light) for 1 – 7 years by comparing the detection of the intended variants within these samples to the baseline timepoint (T0). FFPE characteristics are summarized in Table 33. Two curls from each block were immediately removed after block preparation for DNA extraction and subsequent analysis. At each timepoint, three FFPE blocks were assessed as biological replicates. One FFPE sample passed all quality metrics at T0, but failed library preparation QC after being stored for 7 years. FFPE blocks stored up to five years showed concordant variant calls (PPA of 98.49% and NPA  $\geq$  99.99%). Results are summarized in Table 34.

**Table 33. FFPE Characteristics for T0**

Sample ID	Lot Used	Variant Count	HS SNV Count	<i>ERBB2</i> Amplification	SV	TMB	MSI Status
1	1	2	2	n.d.	n.d.	1.1	MSS
2		2	1	n.d.	n.d.	1.1	MSS
3		5	2	n.d.	n.d.	3.2	MSS
4		5	2	n.d.	n.d.	3.2	MSS
5		4	3	n.d.	n.d.	2.1	MSS
6		12	1	n.d.	n.d.	13.7	MSS
7		2	2	n.d.	n.d.	0	MSS
8		2	2	n.d.	n.d.	0	MSS
9		3	1	n.d.	<i>ALK</i>	4.2	MSS
10		4	1	n.d.	<i>ALK</i>	4.2	MSS
11		5	1	n.d.	<i>ALK</i>	7.4	MSS
12		6	1	n.d.	<i>ALK</i>	7.4	MSS
13	2	2	0	Yes	n.d.	1.1	MSS
14		1	0	Yes	n.d.	0	MSS
15		3	1	Yes	n.d.	3.2	MSS
16		4	2	Yes	n.d.	4.2	MSS
17		53	5	n.d.	n.d.	70.9	MSI-H
18		57	4	n.d.	n.d.	71.9	MSI-H
19		53	3	n.d.	n.d.	69.8	MSI-H
20*		40	0	n.d.	n.d.	32.1	MSI-H
21		35	2	n.d.	n.d.	70.9	MSI-H

\*This sample did not pass the QC metrics after seven years of storage.  
n.d. – Not Detected

**Table 34. Panel-Wide (SNV + INS + DEL) Concordance**

<b>Timepoint (Years)</b>	<b>PPA % (n/N) (95% CI)</b>	<b>NPA % (n/N) (95% CI)</b>
1	98.44 (63/64) (91.67, 99.92)	99.99 (21928583/21928584) (99.99, 99.99)
3	98.73 (78/79) (93.17, 99.94)	99.99 (21928570/21928573) (99.99, 99.99)
5	98.48 (65/66) (91.90, 99.92)	100 (21928593/21928593) (99.99, 100)
7	94.44 (51/54) (84.89, 98.09)	99.99 (21928594/21928595) (99.99, 99.99)

**b) Sample Stability (Extracted DNA)**

DNA stability was assessed for extracted DNA stored  $\leq -20^{\circ}\text{C}$  prior to processing through the GENESEEQPRIME assay. The duration of DNA storage at the time of evaluation in this study ranged from six to 14 months. DNA was extracted from 10 FFPE clinical specimens and sequenced at the time of extraction to determine the baseline (T0) status of the variants. GENESEEQPRIME demonstrated a robust analytical performance and the concordant results for all variants assessed (*ERBB2* amplification, *ALK* translocation, sequence mutations, MSI, and TMB) using DNA specimens stored for various times. Performance was maintained across 6 months and 12 months post extraction with a PPA of 100% (95% CI: 95.07% - 100%) and an NPA of 100% (95% CI: 99.99% - 100%) and 99.99% (95% CI: 99.99% - 99.99%) for six months and 12 months, respectively. A concordance of 100% was observed for MSI status across all timepoints tested, and TMB scores for each sample showed a coefficient of variance of  $\leq 0.79\%$ . Based on these results, DNA stored for up to 12 months at  $\leq -20^{\circ}\text{C}$  does not affect the performance of the GENESEEQPRIME assay.

**c) DNA Extraction**

Four DNA extraction methods were evaluated. The four commonly used, commercially available DNA extraction kits include a column-based extraction method and a magnetic bead-based extraction method. DNA extraction method concordance was evaluated using eight FFPE clinical specimens selected to contain all variant types assessed by GENESEEQPRIME, including borderline variants near the LoD. Each of the samples were extracted in duplicate by two operators for each of the four DNA extraction kits. Method 2 (magnetic-bead based), Method 3 (column-based), and Method 4 (column-based) were compared to the reference, Method 1 (column-based). A total of 168 DNA samples [(five samples x two extraction kit lots x two operators x three extraction methods x two assay replicates) + (three samples x two extraction kit lots x two operators x two extraction methods x two assay replicates)] were processed with the GENESEEQPRIME assay. The GENESEEQPRIME assay yielded concordant analytical performance for variant calls across the DNA extraction method with a PPA  $\geq$

99.02% and an NPA of 99.99%. TMB coefficient of variance for mutational burden is  $\leq$  6.13%. Results are summarized in Table 36 – Table 40.

**Table 36. Overall Variant (SNV + IND +DEL) Concordance**

<b>Extraction Method</b>	<b>PPA % (n/N) (95% CI)</b>	<b>NPA % (n/N) (95% CI)</b>
Method 1 vs Method 2	99.16 (1420/1432) (98.54, 99.52)	99.99 (175367008/175367048) (99.99, 99.99)
Method 1 vs Method 3	99.02 (1418/1432) (98.37, 99.42)	99.99 (175367009/175367048) (99.99, 99.99)
Method 1 vs Method 4	99.78 (926/928) (99.22, 99.94)	99.99 (105220096/105220160) (99.99, 99.99)

**Table 37. ERB2 Amplification Concordance**

<b>Extraction Method</b>	<b>PPA % (n/N) (95% CI)</b>	<b>NPA % (n/N) (95% CI)</b>
Method 1 vs Method 2	100 (8/8) (67.56, 100)	100 (24/24) (86.20, 100)
Method 1 vs Method 3	100 (8/8) (67.56, 100)	100 (24/24) (86.20, 100)
Method 1 vs Method 4	100 (8/8) (67.56, 100)	100 (16/16) (80.64, 100)

**Table 38. Translocation Concordance**

<b>Extraction Method</b>	<b>PPA % (n/N) (95% CI)</b>	<b>NPA % (n/N) (95% CI)</b>
Method 1 vs Method 2	100 (8/8) (67.56, 100)	100 (24/24) (86.20, 100)
Method 1 vs Method 3	100 (8/8) (67.56, 100)	100 (24/24) (86.20, 100)

<b>Extraction Method</b>	<b>PPA % (n/N) (95% CI)</b>	<b>NPA % (n/N) (95% CI)</b>
Method 1 vs Method 4	100 (8/8) (67.56, 100)	100 (16/16) (80.64, 100)

**Table 39. MSI Concordance**

<b>Extraction Method</b>	<b>PPA % (n/N) (95% CI)</b>	<b>NPA % (n/N) (95% CI)</b>
Method 1 vs Method 2	100 (8/8) (67.56, 100)	100 (24/24) (86.20, 100)
Method 1 vs Method 3	100 (8/8) (67.56, 100)	100 (24/24) (86.20, 100)
Method 1 vs Method 4	100 (8/8) (67.56, 100)	100 (16/16) (80.64, 100)

**Table 40. TMB Coefficient of Variation**

<b>Comparison</b>	<b>Sample</b>	<b>Mean TMB</b>	<b>TMB Standard Deviation</b>	<b>CV (%)</b>
Method 1 vs Method 2 vs Method 3	1	7.5	0.311	4.15
	2	76.7	2.036	2.66
	3	93.9	1.532	1.72
	4	56.1	0.879	1.57
	5	3.2	0	0
Method 1 vs Method 4	6	53.7	0.447	0.83
	7	87.0	1.194	1.37
	8	16.5	1.011	6.13

d) **DNA Input**

The optimal and recommended amount of input DNA for the assay is 100 ng. Minimum (50 ng) and recommended (100 ng) DNA requirements were established by measuring assay performance with different inputs from FFPE tumor tissues (25 – 1000 ng). To evaluate assay performance across a range of DNA inputs, seven FFPE samples with known variants were prepared in triplicate at 25, 50, 100, 250, and 1000 ng DNA input levels. The seven FFPE cases assessed contained representative SNVs, indels, amplifications, translocations, MSI, and TMB.

The variant calls for these samples were compared to the respective reference DNA input of 100 ng for each case to assess concordance. Table 41 - Table 47 lists the PPA and NPA for each input level where aggregated variants were analyzed, including SNVs, indels, amplifications, translocations, and MSI. For TMB, the mean absolute percent error rate at each DNA input levels was compared to the reference level of 100 ng (Table 48). These data indicate the GENESEQPRIME assay is robust around the recommended 100 ng DNA input.

**Table 41. Panel-wide Variant (SNVs + INS + DEL) Concordance**

<b>DNA Input Level (ng)</b>	<b>PPA % (n/N) (95% CI)</b>	<b>NPA % (n/N) (95% CI)</b>
25	95.00 (228/240) (91.47, 97.12)	99.99 (92068184/92068212) (99.99, 99.99)
50	96.25 (231/240) (93.03, 98.01)	99.99 (92068180/92068212) (99.99, 99.99)
250	97.50 (234/240) (94.65, 98.85)	99.99 (92068188/92068212) (99.99, 99.99)
500	97.92 (235/240) (95.22, 99.11)	99.99 (92068193/92068212) (99.99, 99.99)
1000	98.33 (236/240) (95.79, 99.35)	99.99 (92068186/92068212) (99.99, 99.99)

**Table 42. ERBB2 Amplification Concordance**

<b>DNA Input Level (ng)</b>	<b>PPA % (n/N) (95% CI)</b>	<b>NPA % (n/N) (95% CI)</b>
25	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)
50	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)
250	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)
500	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)

DNA Input Level (ng)	PPA % (n/N) (95% CI)	NPA % (n/N) (95% CI)
1000	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)

**Table 43. *ALK* Translocation Concordance**

DNA Input Level (ng)	PPA % (n/N) (95% CI)	NPA % (n/N) (95% CI)
25	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)
50	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)
250	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)
500	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)
1000	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)

**Table 44. *RET* Translocation Concordance**

DNA Input Level (ng)	PPA % (n/N) (95% CI)	NPA % (n/N) (95% CI)
25	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)
50	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)
250	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)
500	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)
1000	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)

**Table 45. *NTRK1* Translocation Concordance**

DNA Input Level (ng)	PPA % (n/N) (95% CI)	NPA % (n/N) (95% CI)
25	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)
50	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)

DNA Input Level (ng)	PPA % (n/N) (95% CI)	NPA % (n/N) (95% CI)
250	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)
500	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)
1000	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)

**Table 46. *ROS1* Translocation Concordance**

DNA Input Level (ng)	PPA % (n/N) (95% CI)	NPA % (n/N) (95% CI)
25	100% (3/3) (43.85%, 100%)	100% (6/6) (60.97%, 100%)
50	100% (3/3) (43.85%, 100%)	100% (6/6) (60.97%, 100%)
250	100% (3/3) (43.85%, 100%)	100% (6/6) (60.97%, 100%)
500	100% (3/3) (43.85%, 100%)	100% (6/6) (60.97%, 100%)
1000	100% (3/3) (43.85%, 100%)	100% (6/6) (60.97%, 100%)

**Table 47. MSI Concordance**

DNA Input Level (ng)	PPA % (n/N) (95% CI)	NPA % (n/N) (95% CI)
25	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)
50	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)
250	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)
500	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)
1000	100 (3/3) (43.85, 100)	100 (6/6) (60.97, 100)

**Table 48. TMB Mean Absolute Percent Error**

Case No.	Mean Expected TMB Score	DNA Input Level (ng)	Observed TMB Score	Absolute Percent Error (%)	Mean Absolute Percent Error (%)
1	2.1	25	2.1	0.00	0.00
			2.1	0.00	
			2.1	0.00	
		50	2.1	0.00	0.00
			2.1	0.00	
			2.1	0.00	
		250	2.1	0.00	0.00
			2.1	0.00	
			2.1	0.00	
		500	2.1	0.00	0.00
			2.1	0.00	
			2.1	0.00	
1000	2.1	0.00	0.00		
	2.1	0.00			
	2.1	0.00			
2	11.6	25	10.6	8.62	8.91
			11.6	0.00	
			9.5	18.10	
		50	11.6	0.00	0.00
			11.6	0.00	
			11.6	0.00	
		250	11.6	0.00	0.00
			11.6	0.00	
			11.6	0.00	
		500	11.6	0.00	0.00
			11.6	0.00	
			11.6	0.00	
1000	11.6	0.00	0.00		
	11.6	0.00			
	11.6	0.00			
3	99.1	25	99.4	0.34	2.07
			97.3	1.82	
			95.2	4.06	
		50	98.3	0.78	0.98
			99.4	0.34	
			97.3	1.82	

Case No.	Mean Expected TMB Score	DNA Input Level (ng)	Observed TMB Score	Absolute Percent Error (%)	Mean Absolute Percent Error (%)
3	99.1	250	97.3	1.82	1.19
			99.4	0.34	
			100.5	1.43	
		500	97.3	1.82	2.20
			97.3	1.82	
			96.2	2.98	
		1000	97.3	1.82	2.20
			97.3	1.82	
			96.2	2.98	

## 8. Comparison Studies

### a) *Method Comparison (Accuracy)*

The analytical accuracy of the GENESEEQPRIME assay as a tumor profiling device was evaluated using 503 clinical FFPE samples, obtained from patients with a variety of tumor types (n = 40). The clinical FFPE samples were primarily selected based on the ethnicity and distribution of new cancer cases in the United States in 2023 (Table 49). For rare variants not commonly represented in consecutive sampling, samples were identified based on the variant information provided by the Biobank’s pre-existing test results. No samples were pre-selected using the GENESEEQPRIME assay.

**Table 49. Distribution of Ethnic Background of Clinical Samples**

Ethnicity	Accuracy	Proportion (%)
White or Caucasian	345	68.59
Asian	108	21.47
Black or African American	29	5.77
Hispanic or Latino	10	1.99
Native American or Alaskan Native	1	0.20
Other	8	1.59
Unknown	2	0.40
<b>Sum</b>	<b>503</b>	<b>100.00</b>

Data was aggregated at the variant level for SNVs and indels, gene level for amplification and translocations, and case level for MSI and TMB. The accuracy is summarized for the entire cohort of 503 samples for each of the assessed types (SNVs, indels, structural variants, MSI, and TMB). Orthogonal methods used consisted of validated Next Generation Sequencing (NGS), Fluorescence In Situ Hybridization (FISH), immunohistochemistry (IHC), and whole exome sequencing (WES). For all analyses, the PPA and NPA were calculated by comparing the concordance between the GENESEEQPRIME Assay to the appropriate comparator to evaluate the degree of concordance between the assays (Table 50).

**Table 50. Accuracy of GENESEEQPRIME**

<b>Variant Category</b>	<b>Orthogonal Method</b>	<b>Analysis Category</b>	<b>PPA (95% CI)</b>	<b>NPA (95% CI)</b>
Overall (SNVs and indels)	Predicate Device (NGS)	All	92.44% (91.45%, 93.33%)	99.99% (99.99%, 99.99%)
		SNV	91.53% (90.39%, 92.55%)	99.99% (99.99%, 99.99%)
		Insertions	96.64% (91.68%, 98.69%)	99.99% (99.99%, 99.99%)
		Deletions	97.31% (95.12%, 98.53%)	99.99% (99.99%, 99.99%)
Variants with Evidence of Clinical Significance	Predicate Device (NGS)	All	96.53% (92.64%, 98.4%)	99.98% (99.97%, 99.98%)
		SNV	96.15% (91.86%, 98.23%)	99.98% (99.97%, 99.98%)
		Insertions	100% (51.01%, 100%)	99.99% (99.96%, 99.99%)
		Deletions	100% (77.19%, 100%)	99.97% (99.96%, 99.98%)
Variants with Potential of Clinical Significance	Predicate Device (NGS)	All	92.19% (91.15%, 93.12%)	99.99% (99.99%, 99.99%)
		SNV	91.23% (90.03%, 92.30%)	99.99% (99.99%, 99.99%)
		Insertions	96.52% (111/115) (91.40%, 98.64%)	99.99% (99.99%, 99.99%)
		Deletions	97.21% (349/359) (94.95%, 98.48%)	99.99% (99.99%, 99.99%)
Hotspot Variants	Predicate Device (NGS)	All	98.08% (512/522) (96.51%, 98.96%)	99.98% (99.97%, 99.98%)
		SNV	97.89% (96.16%, 98.85%)	99.98% (99.97%, 99.98%)

Variant Category	Orthogonal Method	Analysis Category	PPA (95% CI)	NPA (95% CI)
Hotspot Variants	Predicate Device (NGS)	Insertions	100% (79.61%, 100%)	99.99% (99.96%, 99.99%)
		Deletions	100% (89.57%, 100%)	99.97% (99.96%, 99.98%)
Non-Hotspot Variants	Predicate Device (NGS)	All	91.27% (90.10%, 92.31%)	99.99% (99.99%, 99.99%)
		SNV	90.08% (88.72%, 91.29%)	99.99% (99.99%, 99.99%)
		Insertions	96.15% (90.53%, 98.49%)	99.99% (99.99%, 99.99%)
		Deletions	97.05% (94.65%, 98.39%)	99.99% (99.99%, 99.99%)
<i>ERBB2</i>	FISH	Amplification	93.75% (83.16%, 97.85%)	100% (88.30%, 100%)
<i>ALK</i>	FISH	Translocation	88.89% (56.50%, 99.43%)	90.91% (62.26%, 99.53%)
<i>RET</i>	FISH	Translocation	100% (56.55%, 100%)	66.67% (41.71%, 84.82%)
<i>ROS1</i>	FISH	Translocation	100% (67.56%, 100%)	100% (72.24%, 100%)
<i>NTRK1</i>	NGS Comparator Assay	Translocation	100% (43.85%, 100%)	99.76% (98.66%, 99.99%)
MSI	IHC/dMMR	All Tumor Types	97.50% (87.12%, 99.87%)	90.38% (79.39%, 95.82%)
MSI	IHC/dMMR	CRC or Endometrial	100% (89.85%, 100%)	96.88% (84.26%, 99.84%)

i) ***Germline Mutation Filtering Pipeline***

Eleven FFPE tumor samples with matching normal samples were used to evaluate the performance of the GENESEEQPRIME assay at filtering out non-pathogenic germline mutations in comparison to only using databases. The matched pairs originated from six different tumor types, including colorectal cancer, gastric cancer, head and neck cancer, non-small cell lung cancer, breast cancer, and endometrial cancer. The mutation results of each paired (tumor-normal) sample were compared to identify the reference list of germline and somatic mutations. A total of 292 somatic variants were reported with 8 variants being germline variants producing a false positive rate of 2.74% compared to the orthogonal device false positive rate of 13.77%.

ii) *Accuracy – SNVs and Indels*

The GENESEQPRIME accuracy study included a total of 4789 variants including 4015 SNVs, 196 insertions, and 570 deletions. Out of 503 FFPE tumor specimens, 423 had both predicate and GENESEQPRIME results. Performance was stratified by mutation type and gene for positive percent agreement (PPA) and negative percent agreement (NPA) with two-sided 95% confidence interval (CI). Overall, the GENESEQPRIME assay yielded concordance analytical performance for variant calls across the SNVs and Indels with a PPA  $\geq$  91.53% and an NPA  $\geq$  99.00%. The concordance between detected mutations for each category of variants (Variants with Evidence of Clinical Significance Variants with Potential Clinical Significance, Hotspot, and Non-Hotspot) are shown in Table 51. The complete listing of data can be found in Appendix E (1-3).

**Table 51. SNV and Indel Concordance between GENESEQPRIME and Predicate Orthogonal Device**

Variant Category	Analysis Category	PPA (n/N) (95% CI)	NPA (n/N) (95% CI)
Overall	All	92.44% (2812/3042) (91.45%, 93.33%)	99.99% (1120688450/1120690189) (99.99%, 99.99%)
	SNV	91.53% (2335/2551) (90.39%, 92.55%)	99.99% (372648833/372650297) (99.99%, 99.99%)
	Insertions	96.64% (115/119) (91.68%, 98.69%)	99.99% (374079851/374079928) (99.99%, 99.99%)
	Deletions	97.31% (362/372) (95.12%, 98.53%)	99.99% (373959766/373959964) (99.99%, 99.99%)
Variants with Evidence of Clinical Significance	All	96.53% (167/173) (92.64%, 98.4%)	99.94% (21811/21823) (99.90%, 99.97%)
	SNV	96.15% (150/156) (91.86%, 98.23%)	99.95% (17601/17610) (99.90%, 99.97%)
	Insertions	100% (4/4) (51.01%, 100%)	99.94% (1687/1688) (99.67%, 99.99%)
	Deletions	100% (13/13) (77.19%, 100%)	99.92% (2523/2525) (99.71%, 99.98%)

<b>Variant Category</b>	<b>Analysis Category</b>	<b>PPA (n/N) (95% CI)</b>	<b>NPA (n/N) (95% CI)</b>
Variants with Potential Clinical Significance	All	92.19% (2645/2869) (91.15%, 93.12%)	99.99% (1122425051/1122426777) (99.99%, 99.99%)
	SNV	91.23% (2185/2395) (90.03%, 92.30%)	99.99% (374129075/374130530) (99.99%, 99.99%)
	Insertions	96.52% (111/115) (91.40%, 98.64%)	99.99% (374148805/374148881) (99.99%, 99.99%)
	Deletions	97.21% (349/359) (94.95%, 98.48%)	99.99% (374147171/374147368) (99.99%, 99.99%)
Hotspot Variants	All	98.08% (512/522) (96.51%, 98.96%)	99.98% (780573/780758) (99.97%, 99.98%)
	SNV	97.89% (464/474) (96.16%, 98.85%)	99.98% (706191/706359) (99.97%, 99.98%)
	Insertions	100% (15/15) (79.61%, 100%)	99.99% (20286/20289) (99.96%, 99.99%)
	Deletions	100% (33/33) (89.57%, 100%)	99.97% (54096/54110) (99.96%, 99.98%)
Non-Hotspot Variants	All	91.27% (2300/2520) (90.10%, 92.31%)	99.99% (1121666711/1121668264) (99.99%, 99.99%)
	SNV	90.08% (1871/2077) (88.72%, 91.29%)	99.99% (373440485/373441781) (99.99%, 99.99%)
	Insertions	96.15% (100/104) (90.53%, 98.49%)	99.99% (374130206/374130280) (99.99%, 99.99%)
	Deletions	97.05% (329/339) (94.65%, 98.39%)	99.99% (374096020/374096203) (99.99%, 99.99%)

Variant Category	Analysis Category	PPA (n/N) (95% CI)	NPA (n/N) (95% CI)
Insertions	1 – 5 bp	96.43% (108/112) (91.18%,98.60%)	99.99% (374079866/374079935) (99.99%, 99.99%)
	6 -10 bp	100% (5/5) (56.55%,100%)	99.99% (374080038/374080042) (99.99%, 99.99%)
	11 – 20 bp	100% (2/2) (34.24%,100%)	99.99% (374080044/374080045) (99.99%, 99.99%)
Deletions	1 – 5 bp	97.60% (325/333) (95.33%,98.78%)	99.98% (373959827/373960003) (99.97%, 99.98%)
	6 -10 bp	90.91% (10/11) (62.26%,99.53%)	99.99% (373960319/373960325) (99.99%, 99.99%)
	11 – 20 bp	95.83% (23/24) (79.76%,99.79%)	99.99% (373960301/373960312) (99.99%, 99.99%)
	21 – 30 bp	100% (4/4) (51.01%,100%)	99.99% (373960327/373960332) (99.99%, 99.99%)

iii) **Accuracy - Amplification and Translocations**

*ERBB2* amplifications and *ALK*, *RET*, and *ROSI* translocations results of GENESEEQPRIME assay were compared to the corresponding FISH analysis, and additionally to the predicate device or validated orthogonal NGS assay results, if available. The microsatellite status obtained by the GENESEEQPRIME assay was compared to predicate device results and IHC testing for the deficient mismatch repair (dMMR) repair pathway.

a. ***ERBB2* Amplification Concordance**

In total, 79 different FFPE samples representing eight different tumor types, including breast cancer, colorectal cancer, endometrial cancer, stomach cancer, ovarian cancer, non-small cell lung cancer, esophagus cancer, and biliary tract cancer, were analyzed for concordance between FISH status and GENESEEQPRIME *ERBB2* status. Two samples did not pass the FISH quality controls due to high background noise and were removed from the analysis. Three samples had a positive *ERBB2* FISH results but were negative for *ERBB2* in the

GENESEEQPRIME assay. These samples were also found to be negative for *ERBB2* when tested with an orthogonal NGS method. The PPA and NPA values for *ERBB2* amplification reflect the totals across borderline and non-borderline samples (Table 52). In non-borderline cases (excluding all cases of a FISH ratio 1.5 – 2.5), a PPA of 95.56% (95% CI: 85.14%, 98.77%) and an NPA, PPV, and NPV at or above 91.67% (95%CI: 74.15%, 97.68%) (Table 53) was observed.

**Table 52. *ERBB2* Amplification Concordance between GENESEEQPRIME and FISH**

GENESEEQPRIME	FISH		
	<i>ERBB2</i> (+)	<i>ERBB2</i> (-)	Total
<i>ERBB2</i> (+)	45	0	45
<i>ERBB2</i> (-)	3	29	32
<b>Total</b>	48	29	77
PPA (2-sided 95% CI)	93.75% (83.16%, 97.85%)		
NPA (2-sided 95% CI)	100% (88.30%, 100%)		

Cancer types include breast cancer (n=48), colorectal cancer (n=12), endometrial cancer (n=4), stomach cancer (n=4), ovary cancer (n=3), NSCLC (n=3), esophagus cancer (n=2), and biliary tract cancer (n=1).

**Table 53. *ERBB2* Amplification Concordance between GENESEEQPRIME and FISH Borderline Samples**

Category	Total Cases	TP	FP	FN	TN	PPA (2-sided 95% CI)	NPA (2-sided 95% CI)
All	77	45	0	3	29	93.75% (83.16%, 97.85%)	100% (88.30%, 100%)
Excluding FISH 1.5 - 2.5	67	53	00	2	22	95.56% (85.17%, 98.77%)	100% (85.13%, 100%)
Excluding FISH 1.8 – 2.2	75	45	0	3	27	93.75% (83.16%, 97.85%)	100% (87.54%, 100%)
Only FISH 1.5 - 2.5	10	2	0	1	7	66.66% (20.77%, 98.29%)	100% (64.57%, 100%)
Only FISH 1.8 – 2.2	2	0	0	0	2	NA (NA)	100% (32.24%, 100%)

TP – True Positive, FP – False Positive, FN – False Negative, TN – True Negative

In addition to comparing the analytical performance of the GENESEEQPRIME assay to results of the medically established method, the results were also compared to the predicate device results. A total of 34 *ERBB2* positive samples representing seven different tumor types including, breast cancer, colorectal cancer, endometrial cancer, ovarian cancer, esophagus cancer, non-small cell lung cancer, and gallbladder cancer, were used in this concordance study to detect *ERBB2* amplifications. *ERBB2* amplifications were detected in 32 out of 34 samples in both the GENESEEQPRIME assay and the orthogonal device. Two samples with *ERBB2* amplifications were detected but were not reported by the orthogonal device due to a difference in reporting thresholds between the two devices. Out of a total of 423 samples, 421 samples (99.53%) showed an agreement between the GENESEEQPRIME assay and the orthogonal device. The concordance between GENESEEQPRIME assay and comparator NGS-based assay in detected *ERBB2* amplifications is shown in Table 54.

**Table 54. *ERBB2* Amplification Concordance between GENESEEQPRIME and Predicate Device**

GENESEEQPRIME	Predicate Device		
	<i>ERBB2</i> (+)	<i>ERBB2</i> (-)	Total
<i>ERBB2</i> (+)	32	2	34
<i>ERBB2</i> (-)	0	389	389
<b>Total</b>	32	391	423
PPA (2-sided 95% CI)	100% (89.28%, 100%)		
NPA (2-sided 95% CI)	99.49% (98.15%, 99.86%)		

**b. *ALK* Translocation Concordance**

A total of 20 FFPE samples from four tumor types, including non-small cell lung cancer, renal carcinoma, colorectal cancer, and lung adenocarcinoma, were included in the analysis. Out of nine *ALK* positive samples, eight were detected for *ALK* translocations using both FISH and the GENESEEQPRIME assay. One *ALK* positive sample by FISH was not reported as positive by the GENESEEQPRIME assay due to a low number of supporting reads. The concordance between GENESEEQPRIME assay and FISH is shown in Table 55.

**Table 55. *ALK* Translocation Concordance between GENESEQPRIME and FISH**

GENESEQPRIME	FISH		
	<i>ALK</i> (+)	<i>ALK</i> (-)	Total
<i>ALK</i> (+)	8	1	9
<i>ALK</i> (-)	1	10	11
<b>Total</b>	9	11	20
PPA (2-sided 95% CI)	88.89% (56.50%, 99.43%)		
NPA (2-sided 95% CI)	90.91% (62.26%, 99.53%)		

Cancer types include NSCLC (n=15), renal carcinoma (n=3), colorectal cancer (n=1), and lung adenocarcinoma (n=1)

In addition, a total of 10 *ALK* positive samples representing three different tumor types including, non-small cell lung cancer (8 samples), renal cancer (1), and colorectal cancer (1), were used to assess the concordance of the GENESEQPRIME assay to predicate device to detect *ALK* translocations. *ALK* translocations were detected in nine out of 10 samples in both the GENESEQPRIME and the orthogonal comparator (predicate device). One *ALK* translocation was unreported by the GENESEQPRIME assay due to the assay cut off, which was below the six reads required for reporting, as further investigation into the GENESEQPRIME analysis pipeline indicated this translocation event was at five reads. The concordance between the detected *ALK* translocations is shown in Table 56.

**Table 56. *ALK* Translocation Concordance between GENESEQPRIME and Predicate Device**

GENESEQPRIME	Predicate Device		
	<i>ALK</i> (+)	<i>ALK</i> (-)	Total
<i>ALK</i> (+)	9	0	9
<i>ALK</i> (-)	1	413	414
<b>Total</b>	10	413	423
PPA (2-sided 95% CI)	90.00% (59.59%, 99.49%)		
NPA (2-sided 95% CI)	100% (99.08%, 100%)		

**c. *RET* Translocation Concordance**

Twenty (20) FFPE specimens from four tumor types, including non-small cell lung cancer, thyroid cancer, and colorectal cancer, were included in the analysis. The concordance between the detected *RET* translocations is shown in Table 57.

**Table 57. *RET* Translocation Concordance between GENESEEQPRIME and FISH**

GENESEEQPRIME	FISH		
	<i>RET</i> (+)	<i>RET</i> (-)	Total
<i>RET</i> (+)	5	5	10
<i>RET</i> (-)	0	10	10
<b>Total</b>	5	15	20
PPA (2-sided 95% CI)	100% (56.55%, 100%)		
NPA (2-sided 95% CI)	66.67% (41.71%, 84.82%)		

Cancer types include NSCLC (n=11), thyroid cancer (n=6), colorectal cancer (n=2), and sarcoma (n=1).

Five out of 10 samples were reported with *RET* translocation by the GENESEEQPRIME assay and were reported negative by FISH. Three of the five discordant results were confirmed as positive for *RET* translocation by three different NGS methods (Table 58). One discordant sample was detected by the GENESEEQPRIME assay and was labeled as positive when obtained from the biorepository, but failed QC for the orthogonal method. Another discordant sample was detected as *RET* positive by both the orthogonal assay and GENESEEQPRIME but was not originally labeled as positive when obtained from the biorepository. The false positive rate for *RET* translocation might be due to the differences in sensitivity between the GENESEEQPRIME assay and FISH. Based on the NGS *RET* detection results, the five samples that were discordant with FISH were positive for *RET* translocation using validated NGS comparators.

**Table 58. *RET* Translocation Status in Discordant FFPE Samples from Orthogonal Methods**

Sample ID	Cancer Type	<i>RET</i> FISH Test Result	GENESEEQPRIME SV Call	Orthogonal NGS Method SV Call	Original Biorepository SV Call
1	Lung-NSCLC	Negative	Detected <i>RET</i> : exon6~ <i>RET</i> : exon12 (23 supporting reads)	Detected <i>RET-RET</i> (21 supporting reads)	Not detected
2	Lung-NSCLC	Negative	Detected <i>CCDC6</i> : exon1~ <i>RET</i> : exon12 (84 supporting reads)	Failed QC	Detected <i>CCDC6</i> :exon1 ~ <i>RET</i> :exon12

Sample ID	Cancer Type	<i>RET</i> FISH Test Result	GENESEEQPRIME SV Call	Orthogonal NGS Method SV Call	Original Biorepository SV Call
3	Thyroid	Negative	Detected NCOA4: exon7~ <i>RET</i> : exon12 (239 supporting reads)	Detected NCOA4- <i>RET</i> (186 supporting reads)	Detected NCOA4:exon7 ~ <i>RET</i> :exon12
4	Colorectal	Negative	Detected NCOA4: exon7~ <i>RET</i> : exon12 (549 supporting reads)	Detected NCOA4- <i>RET</i> (60 supporting reads)	Detected NCOA4:exon9 ~ <i>RET</i> :exon12
5	Colorectal	Negative	Detected NCOA4: exon7~ <i>RET</i> : exon12 (172 supporting reads)	Detected NCOA4- <i>RET</i> (21 supporting reads)	Detected NCOA4:exon9 ~ <i>RET</i> :exon12

In addition, a total of 10 *RET* positive samples representing five different tumor types including, non-small cell lung cancer (4 samples), thyroid cancer (2), colorectal cancer (2), and neuroendocrine tumor (1), were used to compare the results of the GENESEEQPRIME assay to the results of the validated orthogonal NGS assay (predicate device) to detect *RET* translocations. The GENESEEQPRIME detected *RET* translocations in seven out of seven samples that were positive by the orthogonal NGS comparator. Three samples were reported with *RET* translocation by the GENESEEQPRIME assay and were reported negative by the orthogonal assay. The concordance between the detected *RET* translocations is shown in Table 59.

**Table 59. *RET* Translocation Concordance between GENESEEQPRIME and Predicate Device**

GENESEEQPRIME	Predicate Device		
	<i>RET</i> (+)	<i>RET</i> (-)	Total
<i>RET</i> (+)	7	3	10
<i>RET</i> (-)	0	413	413
<b>Total</b>	7	416	423
PPA (2-sided 95% CI)	100% (64.57%, 100%)		
NPA (2-sided 95% CI)	99.28% (97.90%, 99.75%)		

**d. *ROS1* Translocation Concordance**

Eighteen (18) FFPE specimens from one tumor type, non-small cell lung cancer (NSCLC), were included in the analysis. Eight out of eight *ROS1* positive samples and 10 out of 10 negative *RET* samples were concordant between the GENESEQPRIME and FISH assay. The concordance between the detected *RET* translocations is shown in Table 60.

**Table 60. *ROS1* Translocation Concordance between GENESEQPRIME and FISH**

GENESEQPRIME	FISH		
	<i>ROS1</i> (+)	<i>ROS1</i> (-)	Total
<i>ROS1</i> (+)	8	0	8
<i>ROS1</i> (-)	0	10	10
<b>Total</b>	8	10	18
PPA (2-sided 95% CI)	100% (67.56%, 100%)		
NPA (2-sided 95% CI)	100% (72.24%, 100%)		
OPA (2-sided 95% CI)	100% (82.41%, 100%)		

In addition, *ROS1* positive samples representing two different tumor types including, non-small lung cancer (8) and breast cancer (1), were used to evaluate concordance between the GENESEQPRIME assay and the orthogonal validated NGS comparator assay to detect *ROS1* translocations. *ROS1* translocations were detected by the GENESEQPRIME in all six samples that were positive by the orthogonal assay. Three additional samples were reported with *ROS1* translocation by the GENESEQPRIME assay and were reported negative by the orthogonal comparator. Positive FISH results were obtained for two out of the three discordant results that were negative by orthogonal NGS but positive by the GENESEQPRIME assay. The concordance between the detected *ROS1* translocations is shown in Table 61.

**Table 61. *ROS1* Translocation Concordance between GENESEQPRIME and Orthogonal NGS Comparator**

GENESEQPRIME	Orthogonal NGS Comparator		
	<i>ROS1</i> (+)	<i>ROS1</i> (-)	Total
<i>ROS1</i> (+)	6	3	9
<i>ROS1</i> (-)	0	414	414
<b>Total</b>	6	417	423
PPA (2-sided 95% CI)	100% (60.97%, 100%)		
NPA (2-sided 95% CI)	99.28% (97.91%, 99.76%)		

**e. *NTRK1* Translocation Concordance**

A total of four *NTRK1* positive samples representing two different tumor types including, colorectal cancer, were used to assess the analytical accuracy of the GENESEEQPRIME assay to detect *NTRK1* translocations. *NTRK1* translocations were detected by the GENESEEQPRIME assay and the orthogonal NGS comparator in three samples. One sample was reported with *NTRK1* translocation by the GENESEEQPRIME assay and was reported negative by the orthogonal comparator. The concordance between the detected *NTRK1* translocations is shown in Table 62.

**Table 62. *NTRK1* Translocation Concordance between GENESEEQPRIME and Orthogonal NGS Comparator**

GENESEEQPRIME	NGS Comparator		
	<i>NTRK1</i> (+)	<i>NTRK1</i> (-)	Total
<i>NTRK1</i> (+)	3	1	4
<i>NTRK1</i> (-)	0	419	419
<b>Total</b>	3	420	423
PPA (2-sided 95% CI)	100% (43.85%, 100%)		
NPA (2-sided 95% CI)	99.76% (98.66%, 99.99%)		

**iv) Accuracy – MSI Concordance**

The accuracy of the GENESEEQPRIME assay calling of MSI status in tumor tissue was evaluated in a method comparison study against a validated IHC test (Table 63). A total of 92 cases of which 44 were colorectal, 22 of endometrial, six of stomach cancer, two of cervix, melanoma, skin, bladder, and thyroid, one of prostate, kidney, pancreas, NSCLC, ovary, breast, hand and neck, and small intestine cancer.

As colorectal cancer (CRC) and endometrial cancer tend to be overrepresented in the MSI high (MSI-H) population, samples were categorized on whether or not the tumor types were CRC or endometrial. The concordance specifically for colorectal and endometrial cancer samples was evaluated. One false positive MSI-H was detected in the colorectal and endometrial cancer samples. The false positive sample had a score of 19.64%, which is close to the assay cutoff of 16%. The MSI status concordance between the two assays is shown in Table 63.

**Table 63. MSI Status\* Concordance between GENESEEQPRIME and IHC Assay**

GENESEEQPRIME	IHC		
	dMMR	Not Detected	Total
<b>MSI-H</b>	39	5	44
<b>MSS</b>	1	47	48
<b>Total</b>	40	52	92
PPA (2-sided 95% CI)	97.50% (87.12%,99.87%)		
NPA (2-sided 95% CI)	90.38% (79.39%, 95.82%)		
PPA (CRC + Endometrial) (2-sided 95% CI)	100% (89.85%, 100%)		
NPA (CRC + Endometrial) (2-sided 95% CI)	96.88% (84.26%, 99.84%)		

\*Cancer types include colorectal (n=44), endometrial (n=22), stomach cancer (n=6), sarcoma cancer (n=2), cervix cancer (n=2), melanoma cancer (n=2), skin cancer (n=2), bladder cancer (n=2), thyroid cancer (n=2), prostate cancer (n=1), kidney cancer (n=1), pancreas cancer (n=1), NSCLC (n=1), ovary cancer (n=1), breast cancer (n=1), hand and neck cancer (n=1), and small intestine cancer (n=1).

In addition, 423 samples representing 10 different tumor types including, colorectal cancer, endometrial cancer, cervical cancer, gastric cancer, skin cancer, thyroid cancer, bladder cancer, melanoma, and brain-glioma, were part of the study samples used to assess the concordance of the GENESEEQPRIME assay to the predicate device to detect MSI status. Samples with an “Indeterminate” result for MSI status were removed and only those samples with reliable MSI calls were included in the analysis. Additionally, samples were categorized on whether or not the tumor types were CRC or endometrial, and the concordance specifically for colorectal and endometrial cancer samples was evaluated. Out of a total of 5 discordant samples, 4 samples (3 MSI-H and one MSS) were found to have IHC results matching the GENESEEQPRIME MSI status call. The remaining discordant sample that was called MSI-H by GENESEEQPRIME but was MSS by orthogonal comparator method produced a GENESEEQPRIME MSI score of 19.64%, which is near the threshold of 16%. The concordance in MSI status is shown in Table 63 to Table 65.

**Table 63. MSI Status Concordance between GENESEEQPRIME and Predicate Device**

GENESEEQPRIME	Predicate Device			
	MSI-H	MSS	Indeterminate	Total
<b>MSI-H</b>	30	9	1	40
<b>MSS</b>	1	377	1	379
<b>Indeterminate</b>	0	4	0	4
<b>Total</b>	31	390	2	423
PPA (2-sided 95% CI) (Indeterminate Samples Excluded)	96.77% (83.81%, 99.83%)			
NPA (2-sided 95% CI) (Indeterminate Samples Excluded)	97.67% (95.63%, 98.77%)			
PPA (2-sided 95% CI) (Indeterminate Samples Included)	96.77% (83.81%, 99.83%)			
NPA (2-sided 95% CI) (Indeterminate Samples Included )	96.67% (94.38%, 98.04%)			

\*Cancer type for samples determined to be MSI-H by GENESEEQPRIME include colorectal (n=18), endometrial (n=12), cervix (n=2), gastric (n=2), sarcoma (n=1), skin (n=1), thyroid (n=1), bladder (n=1), melanoma (n=1), brain-glioma (n=1)

**Table 64. MSI Status\* Concordance for CRC or Endometrial Cancer Samples between GENESEEQPRIME and Predicate Device**

GENESEEQPRIME	Predicate Device			
	MSI-H	MSS	Indeterminate	Total
<b>MSI-H</b>	25	4	1	30
<b>MSS</b>	1	54	1	56
<b>Indeterminate</b>	0	0	0	0
<b>Total</b>	26	58	2	86
PPA (2-sided 95% CI) (Indeterminate Samples Excluded)	96.15% (81.11%, 99.8%)			
NPA (2-sided 95% CI) (Indeterminate Samples Excluded)	93.10% (83.57%, 97.29%)			
PPA (2-sided 95% CI) (Indeterminate Samples Included)	92.59% (76.63%, 97.94%)			
NPA (2-sided 95% CI) (Indeterminate Samples Included)	91.53% (81.65%, 96.33%)			

\* Cancer type for samples determined to be MSI-H by orthogonal device include colorectal (n=17) and endometrial (n=9). Cancer type for samples determined to be MSI-H by GENESEEQPRIME include colorectal (n=18) and endometrial (n=12)

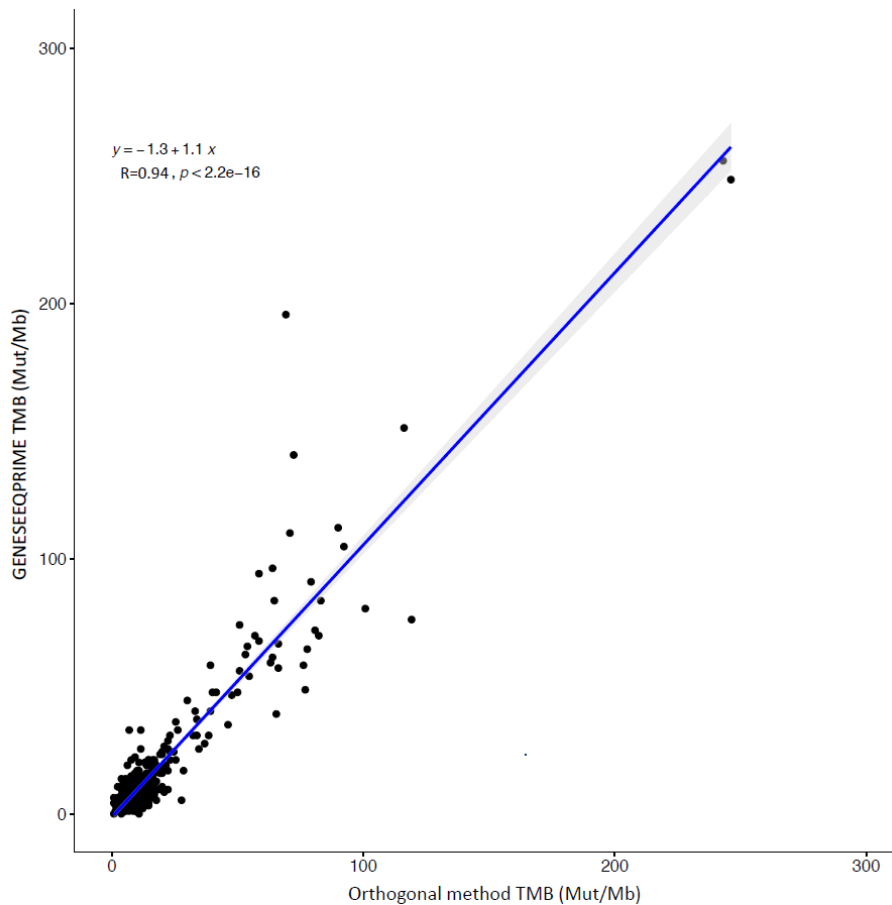
**Table 65. MSI Status\* Concordance for Non-CRC or Non-Endometrial Cancer Samples between GENESEEQPRIME and Predicate Device**

GENESEEQPRIME	Predicate Device			
	MSI-H	MSS	Indeterminate	Total
<b>MSI-H</b>	5	5	0	10
<b>MSS</b>	0	323	0	323
<b>Indeterminate</b>	0	4	0	4
<b>Total</b>	5	332	0	337
PPA (2-sided 95% CI) (Indeterminate Samples Excluded)	100% (56.55%, 100%)			
NPA (2-sided 95% CI) (Indeterminate Samples Excluded)	98.48% (96.48%, 99.35%)			
PPA (2-sided 95% CI) (Indeterminate Samples Included)	100% (56.55%, 100%)			
NPA (2-sided 95% CI) (Indeterminate Samples Included)	97.29% (94.93%, 98.57%)			

\*Cancer type for samples determined to be MSI-H by GENESEEQPRIME include cervix (n=2), gastric (n=2), sarcoma (n=1), skin (n=1), thyroid (n=1), bladder (n=1), melanoma (n=1), brain-glioma (n=1)

v) **TMB Accuracy**

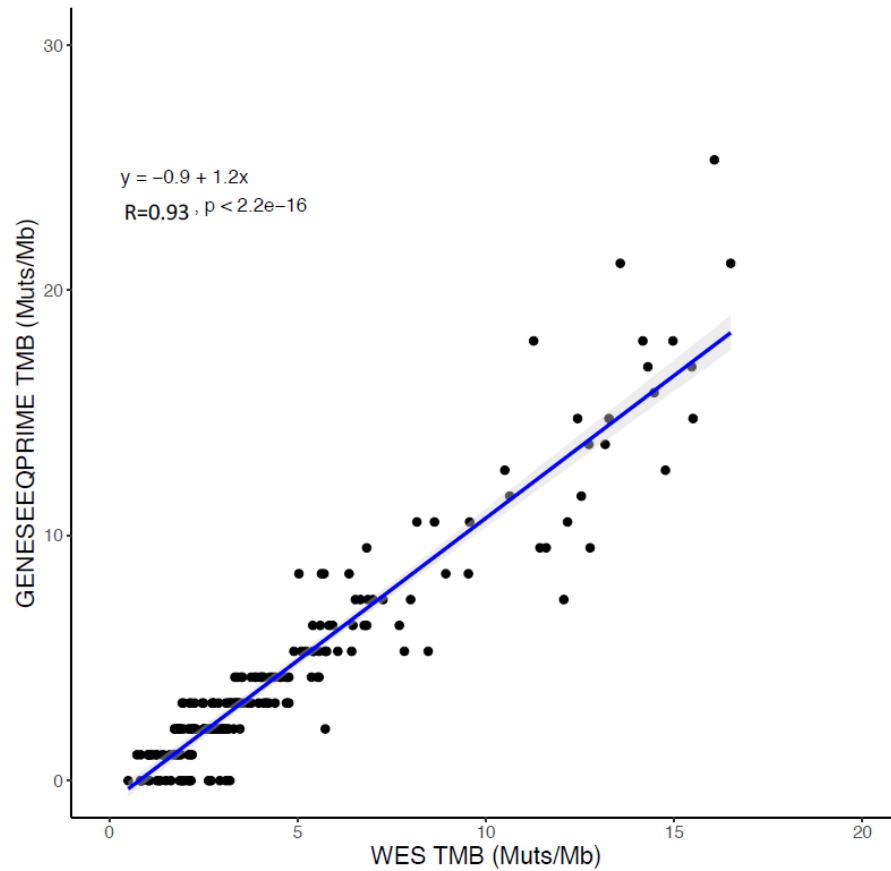
The GENESEEQPRIME assay reports a TMB score calculated based on the detected sequence mutations and indels across the entire coding region of interest per sample. A total of 423 samples were used to assess the analytical accuracy of the GENESEEQPRIME assay to determine TMB scores. GENESEEQPRIME assay calculates the TMB score by removing variants classified as germline variants and well-known drivers of mutations from the total detected variant count for each sample. Only variants with an allele frequency > 2% are included in the count. The TMB score is reported as the normalized variant count per megabase of coding region (Muts/Mb). TMB scores were determined for the 423 samples by the GENESEEQPRIME assay and compared to the results by the predicate device. The concordance of the scores was measured using the Pearson correlation between the two assays, and a linear regression analysis. Assessment of all 423 cases resulted in a Pearson correlation coefficient of 0.9408 and a linear slope of 1.1 (Figure 8).



**Figure 8 TMB Concordance between GENESEQPRIME and Orthogonal Device**

vi) ***TMB Method Comparison – Whole Exon Sequencing (WES)***

The GENESEQPRIME assay reports a TMB score calculated as the number of non-drive variants (both synonymous and non-synonymous) measured as a proportion of the entire coding regions of the sequencing coverage length. A total of 208 matched FFPE specimens from 3 tumor types, including lung adenocarcinoma (n = 117), lung squamous cell carcinoma (n = 12), and colorectal cancer (n = 79), were included in the analysis. TMB scores were detected for the 208 samples by the GENESEQPRIME assay and WES. The Pearson correlation coefficient and a linear regression analysis was used to determine the relationship between the two assays. Assessment of all 208 cases resulted in a Pearson correlation coefficient of 0.9298. The GENESEQPRIME TMB scores displayed a linear correlation to WES with a slope of 1.2. The results in Figure 9 show concordance between the GENESEQPRIME assay TMB scores and tumor-normal WES.



**Figure 9. TMB Concordance between GENESSEQPRIME and WES with Matched Normal Samples**

vi. *Method Comparison – Wild-Type Calls*

A study was conducted to assess accuracy for 52 clinically significant loci within 15 genes. A total of 167 specimens were tested, the accuracy of the GENESSEQPRIME assay results were compared to an orthogonal method (predicate device). Of the 167 specimens, variants were concordantly detected between the two assays, resulting in a PPA of 96.53% (95% CI: 92.64% - 98.40%). Within the 167 specimens, there were 8,499 wild-type calls. The GENESSEQPRIME assay identified all 8,499 wild type variants corresponding to an NPA of 99.93% (95% CI: 99.85% - 99.97%).

**V. Instrument Name:**

Illumina NextSeq 550Dx

**VI. Systems Descriptions:**

1. Modes of Operation:

Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

Yes \_\_\_✓\_\_\_ or No \_\_\_\_\_

Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

Yes \_\_\_\_\_ or No \_\_\_✓\_\_\_

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes \_\_\_\_\_ or No \_\_\_✓\_\_\_

3. Level of Concern:

Moderate

4. Specimen Handling:

Refer to Device Description section above.

5. Calibration and Quality Controls:

Refer to Device Description section above.

**VII. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:**

Not applicable

**VIII. Proposed Labeling:**

The labeling is sufficient, and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable.

**IX. Other Supportive Instrument Performance Characteristics Data:**

**Database:** The bioinformatics software includes database information regarding the assignment of variants to either Variants with Evidence of Clinical Significance or Variants with Potential of Clinical Significance.

**Report Generation:** Reports for each sequencing run sample processed are generated by the GENESIS software. The report includes tumor type, allele frequency, and functional information; and is broken down into the IVD Report, IVD Record, and Batch Summary.

**Expired Sequencing Reagents:** The GENESIS software checks the expiration date of the sequencing reagents based on the information extracted from the sequencing output files. The software will produce a warning if expired reagents were used and prohibit report generation from the sequencing data.

**X. Conclusion:**

The submitted information in this premarket notification is complete and supports a substantial equivalence conclusion.

## XI. Appendix

### Appendix A. GENESEEQPRIME Targeted Genes of Interest

Gene ID (Alias)*	Transcript ID	Chromosome	CDS Region Covered
<i>ABCB1</i>	NM_000927.4	7	All CDS Region Covered
<i>ABCC2</i>	NM_000392.5	10	ex10
<i>ADH1B</i>	NM_000668.6	4	ex3
<i>AIP</i>	NM_001302959.1	11	All CDS Region Covered
<i>AKT1</i>	NM_005163.2	14	All CDS Region Covered
<i>AKT2</i>	NM_001626.6	19	All CDS Region Covered
<i>AKT3</i>	NM_181690.2	1	All CDS Region Covered
<i>ALDH2</i>	NM_000690.4	12	ex12
<i>ALK</i>	NM_004304.5	2	All CDS Region Covered
<i>AMER1</i>	NM_152424.4	X	All CDS Region Covered
<i>APC</i>	NM_000038.6	5	All CDS Region Covered
<i>AR</i>	NM_000044.6	X	All CDS Region Covered
<i>ARAF</i>	NM_001256196.1	X	All CDS Region Covered
<i>ARID1A</i>	NM_139135.4	1	All CDS Region Covered
<i>ARID1B</i>	NM_017519.2	6	All CDS Region Covered
<i>ARID2</i>	NM_152641.4	12	All CDS Region Covered
<i>ASCL4</i>	NM_203436.2	12	All CDS Region Covered
<i>ASXL1</i>	NM_015338.6	20	All CDS Region Covered
<i>ATF1</i>	NM_005171.5	12	ex7
<i>ATIC</i>	NM_004044.7	2	ex12
<i>ATM</i>	NM_000051.3	11	All CDS Region Covered
<i>ATR</i>	NM_001184.4	3	All CDS Region Covered
<i>ATRX</i>	NM_000489.5	X	All CDS Region Covered
<i>AURKA</i>	NM_003600.4	20	All CDS Region Covered
<i>AURKB</i>	NM_004217.4	17	All CDS Region Covered
<i>AXIN2</i>	NM_004655.4	17	All CDS Region Covered
<i>AXL</i>	NM_001278599.1	19	All CDS Region Covered
<i>B2M</i>	NM_004048.3	15	All CDS Region Covered
<i>BAD</i>	NM_032989.3	11	All CDS Region Covered
<i>BAI3</i> ( <i>ADGRB3</i> )	NM_001704.3	6	ex3,ex26,ex29
<i>BAK1</i>	NM_001188.4	6	All CDS Region Covered
<i>BAP1</i>	NM_004656.4	3	All CDS Region Covered
<i>BARD1</i>	NM_000465.4	2	All CDS Region Covered
<i>BAX</i>	NM_138763.4	19	All CDS Region Covered

<b>Gene ID (Alias)*</b>	<b>Transcript ID</b>	<b>Chromosome</b>	<b>CDS Region Covered</b>
<i>BCL2</i>	NM_000633.2	18	All CDS Region Covered
<i>BCR</i>	NM_004327.4	22	ex13,ex14,ex15,ex19,ex20
<i>BLM</i>	NM_000057.4	15	All CDS Region Covered
<i>BMPRIA</i>	NM_004329.2	10	All CDS Region Covered
<i>BRAF</i>	NM_004333.6	7	All CDS Region Covered
<i>BRCA1</i>	NM_007294.4	17	All CDS Region Covered
<i>BRCA2</i>	NM_000059.3	13	All CDS Region Covered
<i>BRD4</i>	NM_058243.2	19	All CDS Region Covered
<i>BRIP1</i>	NM_032043.3	17	All CDS Region Covered
<i>BTG2</i>	NM_006763.3	1	All CDS Region Covered
<i>BTK</i>	NM_000061.2	X	All CDS Region Covered
<i>BUB1B</i>	NM_001211.5	15	All CDS Region Covered
<i>CASP8</i>	NM_001228.4	2	ex3,ex8,ex9
<i>CBL</i>	NM_005188.4	11	All CDS Region Covered
<i>CBLB</i>	NM_170662.5	3	All CDS Region Covered
<i>CCND1</i>	NM_053056.3	11	All CDS Region Covered
<i>CCNE1</i>	NM_001238.4	19	All CDS Region Covered
<i>CD274</i>	NM_014143.4	9	All CDS Region Covered
<i>CD74</i>	NM_004355.3	5	ex6,ex7,ex8
<i>CDA</i>	NM_001785.3	1	All CDS Region Covered
<i>CDC73</i>	NM_024529.4	1	All CDS Region Covered
<i>CDH1</i>	NM_004360.5	16	All CDS Region Covered
<i>CDK12</i>	NM_016507.4	17	All CDS Region Covered
<i>CDK4</i>	NM_000075.4	12	All CDS Region Covered
<i>CDK6</i>	NM_001145306.1	7	All CDS Region Covered
<i>CDK8</i>	NM_001260.3	13	All CDS Region Covered
<i>CDKN1A</i>	NM_078467.2	6	All CDS Region Covered
<i>CDKN1B</i>	NM_004064.4	12	All CDS Region Covered
<i>CDKN1C</i>	NM_000076.2	11	All CDS Region Covered
<i>CDKN2A</i>	NM_000077.4	9	All CDS Region Covered
<i>CDKN2B</i>	NM_004936.4	9	All CDS Region Covered
<i>CDKN2C</i>	NM_001262.2	1	All CDS Region Covered
<i>CEBPA</i>	NM_004364.4	19	All CDS Region Covered
<i>CEP57</i>	NM_014679.5	11	All CDS Region Covered
<i>CHD4</i>	NM_001273.5	12	All CDS Region Covered
<i>CHD8</i>	NM_020920.4	14	All CDS Region Covered
<i>CHEK1</i>	NM_001274.5	11	All CDS Region Covered
<i>CHEK2</i>	NM_001005735.2	22	All CDS Region Covered

<b>Gene ID (Alias)*</b>	<b>Transcript ID</b>	<b>Chromosome</b>	<b>CDS Region Covered</b>
<i>CREBBP</i>	NM_004380.3	16	All CDS Region Covered
<i>CRKL</i>	NM_005207.4	22	All CDS Region Covered
<i>CSF1R</i>	NM_005211.3	5	All CDS Region Covered
<i>CTCF</i>	NM_006565.4	16	All CDS Region Covered
<i>CTLA4</i>	NM_005214.5	2	All CDS Region Covered
<i>CTNNB1</i>	NM_001098210.2	3	All CDS Region Covered
<i>CUL3</i>	NM_001257197.1	2	All CDS Region Covered
<i>CXCR4</i>	NM_001008540.2	2	All CDS Region Covered
<i>CYLD</i>	NM_001042355.2	16	All CDS Region Covered
<i>CYP2A13</i>	NM_000766.5	19	ex1, ex2, ex4, ex7, ex8, ex9
<i>CYP2A6</i>	NM_000762.6	19	All CDS Region Covered
<i>CYP2A7</i>	NM_030589.2	19	ex3, ex4, ex5, ex6, ex8
<i>CYP2B6</i>	NM_000767.5	19	ex4,ex5
<i>CYP2C19</i>	NM_000769.4	10	ex5
<i>CYP2C9</i>	NM_000771.4	10	ex7
<i>CYP2D6</i>	NM_000106.6	22	All CDS Region Covered
<i>CYP3A4</i>	NM_001202855.3	7	ex5
<i>CYP3A5</i>	NM_000777.5	7	ex4
<i>CYSLTR2</i>	NM_001308465.3	13	All CDS Region Covered
<i>DAXX</i>	NM_001141969.2	6	All CDS Region Covered
<i>DDR2</i>	NM_006182.4	1	All CDS Region Covered
<i>DENND1A</i>	NM_024820.3	9	All CDS Region Covered
<i>DICER1</i>	NM_030621.4	14	All CDS Region Covered
<i>DLL3</i>	NM_016941.4	19	All CDS Region Covered
<i>DNMT3A</i>	NM_022552.4	2	All CDS Region Covered
<i>DOT1L</i>	NM_032482.3	19	All CDS Region Covered
<i>DPYD</i>	NM_000110.4	1	All CDS Region Covered
<i>DTL</i>	NM_016448.4	1	All CDS Region Covered
<i>DUSP2</i>	NM_004418.4	2	All CDS Region Covered
<i>EGFR</i>	NM_005228.5	7	All CDS Region Covered
<i>EIF1AX</i>	NM_001412.4	X	All CDS Region Covered
<i>EMSY</i> ( <i>C11orf30</i> )	NM_020193.4	11	All CDS Region Covered
<i>EP300</i>	NM_001429.4	22	All CDS Region Covered
<i>EPAS1</i>	NM_001430.5	2	All CDS Region Covered
<i>EPCAM</i>	NM_002354.3	2	All CDS Region Covered
<i>EPHA2</i>	NM_004431.5	1	All CDS Region Covered
<i>EPHA3</i>	NM_005233.6	3	All CDS Region Covered

<b>Gene ID (Alias)*</b>	<b>Transcript ID</b>	<b>Chromosome</b>	<b>CDS Region Covered</b>
<i>EPHA5</i>	NM_004439.8	4	ex3,ex4,ex5,ex8,ex15
<i>ERBB2</i>	NM_004448.3	17	All CDS Region Covered
<i>ERBB2IP</i> ( <i>ERBIN</i> )	NM_018695.4	5	ex3,ex18,ex21,ex22
<i>ERBB3</i>	NM_001982.3	12	All CDS Region Covered
<i>ERBB4</i>	NM_001042599.1	2	All CDS Region Covered
<i>ERCC1</i>	NM_001983.4	19	All CDS Region Covered
<i>ERCC2</i>	NM_000400.3	19	All CDS Region Covered
<i>ERCC3</i>	NM_000122.2	2	All CDS Region Covered
<i>ERCC4</i>	NM_005236.3	16	All CDS Region Covered
<i>ERCC5</i>	NM_000123.3	13	All CDS Region Covered
<i>ESR1</i>	NM_000125.3	6	All CDS Region Covered
<i>ETV5</i>	NM_004454.3	3	ex6,ex7
<i>ETV6</i>	NM_001987.5	12	ex4,ex5,ex6,ex7
<i>EWSR1</i>	NM_005243.4	22	ex7,ex8,ex9,ex10,ex11,ex12,ex13,ex14
<i>EXT1</i>	NM_000127.2	8	All CDS Region Covered
<i>EXT2</i>	NM_000401.3	11	All CDS Region Covered
<i>EZH2</i>	NM_004456.5	7	All CDS Region Covered
<i>EZR</i>	NM_003379.5	6	ex8,ex9,ex10,ex11
<i>FANCA</i>	NM_000135.4	16	All CDS Region Covered
<i>FANCC</i>	NM_000136.3	9	All CDS Region Covered
<i>FANCD2</i>	NM_001018115.2	3	All CDS Region Covered
<i>FANCE</i>	NM_021922.3	6	All CDS Region Covered
<i>FANCF</i>	NM_022725.4	11	All CDS Region Covered
<i>FANCG</i>	NM_004629.1	9	All CDS Region Covered
<i>FANCI</i>	NM_018193.3	15	All CDS Region Covered
<i>FANCL</i>	NM_018062.3	2	All CDS Region Covered
<i>FANCM</i>	NM_020937.4	14	ex1,ex14,ex20,ex21,ex22
<i>FAT1</i>	NM_005245.4	4	All CDS Region Covered
<i>FBXW7</i>	NM_001013415.2	4	All CDS Region Covered
<i>FGF19</i>	NM_005117.3	11	All CDS Region Covered
<i>FGFR1</i>	NM_023110.3	8	All CDS Region Covered
<i>FGFR2</i>	NM_000141.4	10	All CDS Region Covered
<i>FGFR3</i>	NM_000142.4	4	All CDS Region Covered
<i>FGFR4</i>	NM_001291980.2	5	All CDS Region Covered
<i>FH</i>	NM_000143.3	1	All CDS Region Covered
<i>FLCN</i>	NM_144997.7	17	All CDS Region Covered
<i>FLT1</i>	NM_002019.4	13	All CDS Region Covered

<b>Gene ID (Alias)*</b>	<b>Transcript ID</b>	<b>Chromosome</b>	<b>CDS Region Covered</b>
<i>FLT3</i>	NM_004119.3	13	All CDS Region Covered
<i>FLT4</i>	NM_001354989.2	5	All CDS Region Covered
<i>FOXA1</i>	NM_004496.5	14	All CDS Region Covered
<i>FOXL2</i>	NM_023067.4	3	All CDS Region Covered
<i>FOXP1</i>	NM_032682.5	3	ex15,ex18
<i>FRG1</i>	NM_004477.3	4	ex6,ex8
<i>GATA1</i>	NM_002049.4	X	All CDS Region Covered
<i>GATA2</i>	NM_032638.5	3	All CDS Region Covered
<i>GATA3</i>	NM_001002295.2	10	All CDS Region Covered
<i>GATA4</i>	NM_002052.5	8	All CDS Region Covered
<i>GATA6</i>	NM_005257.5	18	All CDS Region Covered
<i>GNAI1</i>	NM_002067.5	19	All CDS Region Covered
<i>GNAQ</i>	NM_002072.5	9	All CDS Region Covered
<i>GNAS</i>	NM_000516.6	20	All CDS Region Covered
<i>GRIN2A</i>	NM_000833.5	16	All CDS Region Covered
<i>GRM3</i>	NM_000840.2	7	All CDS Region Covered
<i>GRM8</i>	NM_000845.3	7	ex2,ex4,ex5,ex6,ex9
<i>GSTM1</i>	NM_000561.4	1	All CDS Region Covered
<i>GSTM4</i>	NM_000850.5	1	ex4, ex8
<i>GSTP1</i>	NM_000852.4	11	ex5,ex6,ex7
<i>GSTT1</i>	NM_000853.3	22	All CDS Region Covered
<i>HDAC2</i>	NM_001527.4	6	All CDS Region Covered
<i>HDAC9</i>	NM_001204144.3	7	ex5,ex12,ex13
<i>HGF</i>	NM_000601.6	7	All CDS Region Covered
<i>HLA-A</i>	NM_002116.8	6	All CDS Region Covered
<i>HNF1A</i>	NM_000545.6	12	All CDS Region Covered
<i>HRAS</i>	NM_176795.4	11	All CDS Region Covered
<i>IDH1</i>	NM_005896.3	2	All CDS Region Covered
<i>IDH2</i>	NM_002168.3	15	All CDS Region Covered
<i>IFNA6</i>	NM_021002.2	9	All CDS Region Covered
<i>IFNB1</i>	NM_002176.4	9	All CDS Region Covered
<i>IFNE</i>	NM_176891.4	9	All CDS Region Covered
<i>IFNG</i>	NM_000619.3	12	All CDS Region Covered
<i>IFNGR1</i>	NM_000416.2	6	All CDS Region Covered
<i>IFNGR2</i>	NM_001329128.1	21	All CDS Region Covered
<i>IGF1R</i>	NM_000875.5	15	All CDS Region Covered
<i>IGF2</i>	NM_000612.6	11	All CDS Region Covered
<i>IKBKE</i>	NM_001193321.2	1	All CDS Region Covered

<b>Gene ID (Alias)*</b>	<b>Transcript ID</b>	<b>Chromosome</b>	<b>CDS Region Covered</b>
<i>IL7R</i>	NM_002185.5	5	All CDS Region Covered
<i>INPP4B</i>	NM_003866.3	4	All CDS Region Covered
<i>IRF2</i>	NM_002199.4	4	All CDS Region Covered
<i>JAK1</i>	NM_002227.4	1	All CDS Region Covered
<i>JAK2</i>	NM_001322194.1	9	All CDS Region Covered
<i>JAK3</i>	NM_000215.3	19	All CDS Region Covered
<i>JARID2</i>	NM_004973.4	6	ex7,ex8
<i>JUN</i>	NM_002228.4	1	All CDS Region Covered
<i>KDM5A</i>	NM_001042603.3	12	All CDS Region Covered
<i>KDR</i>	NM_002253.3	4	All CDS Region Covered
<i>KEAP1</i>	NM_012289.4	19	All CDS Region Covered
<i>KIF1B</i>	NM_001365951.2	1	ex4,ex21
<i>KIT</i>	NM_000222.2	4	All CDS Region Covered
<i>KITLG</i>	NM_003994.5	12	All CDS Region Covered
<i>KLLN</i>	NM_001126049.1	10	All CDS Region Covered
<i>KMT2A</i>	NM_005933.4	11	All CDS Region Covered
<i>KMT2B</i>	NM_014727.2	19	All CDS Region Covered
<i>KMT2C</i>	NM_170606.3	7	ex7,ex8,ex16
<i>KMT2D</i>	NM_003482.3	12	ex6,ex14
<i>KRAS</i>	NM_033360.4	12	All CDS Region Covered
<i>LHCGR</i>	NM_000233.4	2	All CDS Region Covered
<i>LMO1</i>	NM_002315.3	11	All CDS Region Covered
<i>LRP1B</i>	NM_018557.3	2	All CDS Region Covered
<i>LYN</i>	NM_002350.4	8	All CDS Region Covered
<i>LZTR1</i>	NM_006767.4	22	All CDS Region Covered
<i>MAP2K1</i>	NM_002755.3	15	All CDS Region Covered
<i>MAP2K2</i>	NM_030662.3	19	All CDS Region Covered
<i>MAP2K4</i>	NM_001281435.2	17	All CDS Region Covered
<i>MAP3K1</i>	NM_005921.2	5	All CDS Region Covered
<i>MAP3K4</i>	NM_005922.3	6	ex2, ex3
<i>MAX</i>	NM_197957.3	14	All CDS Region Covered
<i>MCL1</i>	NM_021960.5	1	All CDS Region Covered
<i>MDM2</i>	NM_002392.5	12	All CDS Region Covered
<i>MDM4</i>	NM_002393.5	1	All CDS Region Covered
<i>MECOM</i>	NM_004991.4	3	ex8
<i>MED12</i>	NM_005120.3	X	All CDS Region Covered
<i>MEF2B</i>	NM_001145785.2	19	ex7
<i>MEN1</i>	NM_000244.3	11	All CDS Region Covered

<b>Gene ID (Alias)*</b>	<b>Transcript ID</b>	<b>Chromosome</b>	<b>CDS Region Covered</b>
<i>MET</i>	NM_000245.4	7	All CDS Region Covered
<i>MGMT</i>	NM_002412.5	10	All CDS Region Covered
<i>MITF</i>	NM_198159.3	3	All CDS Region Covered
<i>MLH1</i>	NM_000249.3	3	All CDS Region Covered
<i>MLH3</i>	NM_014381.3	14	All CDS Region Covered
<i>MLLT1</i>	NM_005934.4	19	ex4
<i>MLLT3</i>	NM_004529.4	9	ex5
<i>MLLT4</i> ( <i>AFDN</i> )	NM_001207008.1	6	ex2,ex14
<i>MPL</i>	NM_005373.3	1	All CDS Region Covered
<i>MRE11A</i> ( <i>MRE11</i> )	NM_005591.3	11	All CDS Region Covered
<i>MSH2</i>	NM_000251.3	2	All CDS Region Covered
<i>MSH6</i>	NM_000179.2	2	All CDS Region Covered
<i>MTHFR</i>	NM_005957.5	1	ex5
<i>MTOR</i>	NM_004958.4	1	All CDS Region Covered
<i>MUTYH</i>	NM_012222.2	1	All CDS Region Covered
<i>MYC</i>	NM_002467.6	8	All CDS Region Covered
<i>MYCL</i>	NM_001033082.3	1	All CDS Region Covered
<i>MYCN</i>	NM_005378.6	2	All CDS Region Covered
<i>MYD88</i>	NM_002468.5	3	All CDS Region Covered
<i>MYH9</i>	NM_002473.5	22	ex31,ex41
<i>NAT1</i>	NM_000662.8	8	All CDS Region Covered
<i>NBN</i>	NM_001024688.2	8	All CDS Region Covered
<i>NCOR1</i>	NM_006311.4	17	ex5,ex19,ex22
<i>NF1</i>	NM_000267.3	17	All CDS Region Covered
<i>NF2</i>	NM_000268.3	22	All CDS Region Covered
<i>NFE2L2</i>	NM_006164.5	2	All CDS Region Covered
<i>NFKBIA</i>	NM_020529.2	14	All CDS Region Covered
<i>NKX2-1</i>	NM_003317.4	14	All CDS Region Covered
<i>NOTCH1</i>	NM_017617.5	9	All CDS Region Covered
<i>NOTCH2</i>	NM_024408.4	1	All CDS Region Covered
<i>NOTCH3</i>	NM_000435.3	19	ex27
<i>NPM1</i>	NM_002520.6	5	All CDS Region Covered
<i>NQO1</i>	NM_000903.3	16	ex4,ex6
<i>NRAS</i>	NM_002524.5	1	All CDS Region Covered
<i>NRG1</i>	NM_013956.5	8	All CDS Region Covered
<i>NSD1</i>	NM_172349.2	5	All CDS Region Covered

Gene ID (Alias)*	Transcript ID	Chromosome	CDS Region Covered
<i>NTRK1</i>	NM_002529.3	1	All CDS Region Covered
<i>NTRK2</i>	NM_001007097.3	9	All CDS Region Covered
<i>NTRK3</i>	NM_001012338.2	15	All CDS Region Covered
<i>PAK3</i>	NM_002578.5	X	All CDS Region Covered
<i>PALB2</i>	NM_024675.4	16	All CDS Region Covered
<i>PALLD</i>	NM_016081.4	4	All CDS Region Covered
<i>PARK2</i> ( <i>PRKN</i> )	NM_004562.3	6	All CDS Region Covered
<i>PARP1</i>	NM_001618.4	1	All CDS Region Covered
<i>PARP2</i>	NM_001042618.1	14	All CDS Region Covered
<i>PAX5</i>	NM_001280547.2	9	All CDS Region Covered
<i>PBRM1</i>	NM_018313.5	3	All CDS Region Covered
<i>PDCD1</i>	NM_005018.3	2	All CDS Region Covered
<i>PDCDILG2</i>	NM_025239.4	9	All CDS Region Covered
<i>PDE11A</i>	NM_001077196.2	2	All CDS Region Covered
<i>PDGFRA</i>	NM_006206.6	4	All CDS Region Covered
<i>PDGFRB</i>	NM_002609.4	5	All CDS Region Covered
<i>PDK1</i>	NM_002610.5	2	All CDS Region Covered
<i>PGR</i>	NM_000926.4	11	All CDS Region Covered
<i>PHOX2B</i>	NM_003924.4	4	All CDS Region Covered
<i>PIK3C3</i>	NM_002647.4	18	All CDS Region Covered
<i>PIK3CA</i>	NM_006218.4	3	All CDS Region Covered
<i>PIK3CD</i>	NM_005026.5	1	ex7
<i>PIK3R1</i>	NM_181504.4	5	All CDS Region Covered
<i>PIK3R2</i>	NM_005027.4	19	All CDS Region Covered
<i>PKHD1</i>	NM_138694.4	6	All CDS Region Covered
<i>PLAG1</i>	NM_002655.3	8	All CDS Region Covered
<i>PLCB4</i>	NM_000933.3	20	All CDS Region Covered
<i>PLK1</i>	NM_005030.6	16	All CDS Region Covered
<i>PMS1</i>	NM_000534.4	2	All CDS Region Covered
<i>PMS2</i>	NM_000535.7	7	All CDS Region Covered
<i>POLD1</i>	NM_001256849.1	19	All CDS Region Covered
<i>POLE</i>	NM_006231.4	12	All CDS Region Covered
<i>POLH</i>	NM_006502.3	6	All CDS Region Covered
<i>POT1</i>	NM_001042594.1	7	All CDS Region Covered
<i>PPARD</i>	NM_006238.5	6	All CDS Region Covered
<i>PPP2R1A</i>	NM_014225.6	19	All CDS Region Covered
<i>PRDMI</i>	NM_001198.4	6	All CDS Region Covered

<b>Gene ID (Alias)*</b>	<b>Transcript ID</b>	<b>Chromosome</b>	<b>CDS Region Covered</b>
<i>PREX2</i>	NM_025170.6	8	All CDS Region Covered
<i>PRF1</i>	NM_005041.5	10	All CDS Region Covered
<i>PRKACA</i>	NM_002730.4	19	All CDS Region Covered
<i>PRKARIA</i>	NM_001276289.1	17	All CDS Region Covered
<i>PRKCI</i>	NM_002740.6	3	All CDS Region Covered
<i>PRKDC</i>	NM_001081640.2	8	ex29,ex54,ex55,ex70,ex75
<i>PRSSI</i>	NM_002769.5	7	All CDS Region Covered
<i>PTCH1</i>	NM_000264.5	9	All CDS Region Covered
<i>PTEN</i>	NM_000314.8	10	All CDS Region Covered
<i>PTK2</i>	NM_005607.5	8	All CDS Region Covered
<i>PTPN11</i>	NM_001330437.1	12	All CDS Region Covered
<i>PTPN13</i>	NM_006264.3	4	All CDS Region Covered
<i>QKI</i>	NM_006775.3	6	All CDS Region Covered
<i>RAC1</i>	NM_018890.4	7	All CDS Region Covered
<i>RAC3</i>	NM_001316307.2	17	All CDS Region Covered
<i>RAD50</i>	NM_005732.4	5	All CDS Region Covered
<i>RAD51</i>	NM_002875.5	15	All CDS Region Covered
<i>RAD51B</i>	NM_001321809.1	14	All CDS Region Covered
<i>RAD51C</i>	NM_058216.3	17	All CDS Region Covered
<i>RAD51D</i>	NM_002878.3	17	All CDS Region Covered
<i>RAD54L</i>	NM_003579.4	1	All CDS Region Covered
<i>RAF1</i>	NM_002880.3	3	All CDS Region Covered
<i>RARA</i>	NM_000964.4	17	All CDS Region Covered
<i>RASGEF1A</i>	NM_145313.4	10	ex12
<i>RBI</i>	NM_000321.2	13	All CDS Region Covered
<i>RECQL4</i>	NM_004260.3	8	All CDS Region Covered
<i>RELN</i>	NM_005045.4	7	ex33,ex35
<i>RET</i>	NM_020975.6	10	All CDS Region Covered
<i>RHOA</i>	NM_001664.4	3	All CDS Region Covered
<i>RICTOR</i>	NM_152756.5	5	All CDS Region Covered
<i>RNF43</i>	NM_017763.5	17	All CDS Region Covered
<i>ROSI</i>	NM_002944.2	6	All CDS Region Covered
<i>RPTOR</i>	NM_020761.3	17	All CDS Region Covered
<i>RRM1</i>	NM_001033.5	11	All CDS Region Covered
<i>RUNX1</i>	NM_001001890.3	21	All CDS Region Covered
<i>RUNX1T1</i>	NM_004349.4	8	All CDS Region Covered
<i>SBDS</i>	NM_016038.4	7	All CDS Region Covered
<i>SDC4</i>	NM_002999.4	20	ex2,ex3

<b>Gene ID (Alias)*</b>	<b>Transcript ID</b>	<b>Chromosome</b>	<b>CDS Region Covered</b>
<i>SDHA</i>	NM_004168.4	5	All CDS Region Covered
<i>SDHB</i>	NM_003000.3	1	All CDS Region Covered
<i>SDHC</i>	NM_003001.3	1	All CDS Region Covered
<i>SDHD</i>	NM_003002.4	11	All CDS Region Covered
<i>SEPT9</i> ( <i>SEPTIN9</i> )	NM_006640.4	17	ex2
<i>SETBP1</i>	NM_001130110.2	18	All CDS Region Covered
<i>SETD2</i>	NM_014159.6	3	All CDS Region Covered
<i>SF3B1</i>	NM_012433.3	2	All CDS Region Covered
<i>SGK1</i>	NM_001143676.1	6	All CDS Region Covered
<i>SKP2</i>	NM_005983.4	5	All CDS Region Covered
<i>SMAD2</i>	NM_005901.6	18	All CDS Region Covered
<i>SMAD3</i>	NM_005902.4	15	All CDS Region Covered
<i>SMAD4</i>	NM_005359.6	18	All CDS Region Covered
<i>SMARCA4</i>	NM_003072.4	19	All CDS Region Covered
<i>SMARCB1</i>	NM_003073.5	22	All CDS Region Covered
<i>SMO</i>	NM_005631.5	7	All CDS Region Covered
<i>SOCS1</i>	NM_003745.1	16	All CDS Region Covered
<i>SOS1</i>	NM_005633.3	2	ex10
<i>SOX2</i>	NM_003106.4	3	All CDS Region Covered
<i>SPOP</i>	NM_003563.3	17	All CDS Region Covered
<i>SPRED1</i>	NM_152594.3	15	All CDS Region Covered
<i>SPRY4</i>	NM_001127496.2	5	All CDS Region Covered
<i>SRC</i>	NM_005417.4	20	All CDS Region Covered
<i>SRSF2</i>	NM_003016.4	17	All CDS Region Covered
<i>SRY</i>	NM_003140.3	Y	All CDS Region Covered
<i>STAG2</i>	NM_006603.5	X	All CDS Region Covered
<i>STAT3</i>	NM_139276.2	17	All CDS Region Covered
<i>STK11</i>	NM_000455.5	19	All CDS Region Covered
<i>STMN1</i>	NM_005563.4	1	All CDS Region Covered
<i>SUFU</i>	NM_016169.3	10	All CDS Region Covered
<i>TACC3</i>	NM_006342.3	4	ex5,ex6,ex7,ex8,ex9,ex10,ex11,ex12,ex13
<i>TAP1</i>	NM_000593.5	6	All CDS Region Covered
<i>TAP2</i>	NM_018833.2	6	All CDS Region Covered
<i>TEK</i>	NM_000459.4	9	All CDS Region Covered
<i>TEKT4</i>	NM_144705.4	2	ex2
<i>TERC</i>	NR_001566.1	3	All CDS Region Covered

<b>Gene ID (Alias)*</b>	<b>Transcript ID</b>	<b>Chromosome</b>	<b>CDS Region Covered</b>
<i>TERT</i>	NM_001193376.2	5	All CDS Region Covered
<i>TET2</i>	NM_017628.4	4	All CDS Region Covered
<i>TGFBR2</i>	NM_001024847.2	3	All CDS Region Covered
<i>THADA</i>	NM_022065.4	2	All CDS Region Covered
<i>TMEM127</i>	NM_017849.3	2	All CDS Region Covered
<i>TMPRSS2</i>	NM_001135099.1	21	ex1,ex2,ex3,ex4
<i>TNFAIP3</i>	NM_001270507.2	6	All CDS Region Covered
<i>TNFRSF11A</i>	NM_001270949.1	18	All CDS Region Covered
<i>TNFRSF14</i>	NM_001297605.1	1	All CDS Region Covered
<i>TNFSF11</i>	NM_003701.4	13	All CDS Region Covered
<i>TOP1</i>	NM_003286.4	20	All CDS Region Covered
<i>TOP2A</i>	NM_001067.4	17	All CDS Region Covered
<i>TP53</i>	NM_000546.5	17	All CDS Region Covered
<i>TP63</i>	NM_003722.5	3	ex4,ex8
<i>TPMT</i>	NM_000367.4	6	All CDS Region Covered
<i>TSC1</i>	NM_000368.5	9	All CDS Region Covered
<i>TSC2</i>	NM_000548.5	16	All CDS Region Covered
<i>TSHR</i>	NM_000369.3	14	All CDS Region Covered
<i>TTF1</i>	NM_007344.4	9	All CDS Region Covered
<i>TUBB3</i>	NM_006086.4	16	All CDS Region Covered
<i>U2AF1</i>	NM_006758.2	21	ex2
<i>UGT1A1</i>	NM_000463.3	2	All CDS Region Covered
<i>VAMP2</i>	NM_001330125.1	17	ex4
<i>VEGFA</i>	NM_001025366.3	6	All CDS Region Covered
<i>VHL</i>	NM_000551.3	3	All CDS Region Covered
<i>WAS</i>	NM_000377.3	X	All CDS Region Covered
<i>WISP3 (CCN6)</i>	NM_003880.3	6	All CDS Region Covered
<i>WRN</i>	NM_000553.6	8	All CDS Region Covered
<i>WT1</i>	NM_000378.6	11	All CDS Region Covered
<i>XPA</i>	NM_000380.3	9	All CDS Region Covered
<i>XPC</i>	NM_004628.4	3	All CDS Region Covered
<i>XRCC1</i>	NM_006297.2	19	ex6,ex9,ex10
<i>XRCC2</i>	NM_005431.2	7	All CDS Region Covered

Gene ID (Alias)*	Transcript ID	Chromosome	CDS Region Covered
<i>YAP1</i>	NM_006106.5	11	All CDS Region Covered
<i>ZNF217</i>	NM_006526.2	20	All CDS Region Covered
<i>ZNF703</i>	NM_025069.3	8	All CDS Region Covered

\*Targeted regions on *ARID5B*, *BCL2L11*, *BIRC3*, *CDK10*, *CUX1*, *CYP19A1*, *DHFR*, *ETV1*, *ETV4*, *HNFB1B*, *IKZF1*, *NUTM1*, *POLD3*, *PRSS3*, *RARG*, *SLC34A2*, *SLC3A2*, *SMAD7*, *TNFRSF19*, and *TYMS* were designed to capture translocation partners, some microsatellite markers, and SNP loci.

**Appendix B. List of Regions Excluded from Reporting in GENESEQPRIME due to Consistently Low Coverage**

<b>Gene</b>	<b>Transcript ID</b>	<b>Exon</b>	<b>Black Region</b>
<i>AR</i>	NM_000044.6	1	X:66765147-66765264, X:66766338-66766416
<i>ARID1B</i>	NM_017519.2	1	6:157099164-157099461, 6:157099969-157100434
<i>CDKN1C</i>	NM_000076.2	1	11:2906071-2906257
<i>MLLT3</i>	NM_004529.4	5	9:20414259-20414399
<i>PHOX2B</i>	NM_003924.4	3	4:41747985-41748050

**Appendix C. List of Genes with Variants Excluded from Reporting in GENESEQPRIME due to Consistently Low Coverage**

<b>Gene</b>	<b>Transcript ID</b>	<b>Number of Excluded Variants</b>
<i>ANKRD26</i>	NM_001256053.1	1
<i>APC</i>	NM_000038.6	1
<i>AR</i>	NM_000044.6	15
<i>ARID1A</i>	NM_139135.4	1
<i>ARID1B</i>	NM_017519.2	3
<i>ARID2</i>	NM_152641.4	2
<i>ASXL1</i>	NM_015338.6	1
<i>ASXL2</i>	NM_018263.6	3
<i>ATF1</i>	NM_005171.5	1
<i>ATR</i>	NM_001184.4	1
<i>ATRX</i>	NM_000489.5	1
<i>BAD</i>	NM_032989.3	2
<i>BARD1</i>	NM_000465.4	1
<i>BCL10</i>	NM_003921.5	1
<i>BCL11B</i>	NM_138576.4	4
<i>BCR</i>	NM_004327.4	3
<i>BIVM-ERCC5</i>	NM_001204425.1	1
<i>BLM</i>	NM_000057.4	1
<i>BRD4</i>	NM_058243.2	5
<i>CBL</i>	NM_005188.4	2
<i>CDH1</i>	NM_004360.5	1
<i>CDK12</i>	NM_016507.4	2

<b>Gene</b>	<b>Transcript ID</b>	<b>Number of Excluded Variants</b>
<i>CDK8</i>	NM_001260.3	1
<i>CEBPA</i>	NM_004364.4	2
<i>CEP57</i>	NM_014679.5	1
<i>CHD4</i>	NM_001273.5	1
<i>CHD8</i>	NM_020920.4	4
<i>CHEK1</i>	NM_001274.5	1
<i>CHEK2</i>	NM_001005735.2	3
<i>CREBBP</i>	NM_004380.3	1
<i>CUL3</i>	NM_001257197.1	1
<i>CYP2A6</i>	NM_000762.6	7
<i>CYP2A7</i>	NM_030589.2	1
<i>CYP2D6</i>	NM_000106.6	11
<i>DAXX</i>	NM_001141969.2	1
<i>EGR1</i>	NM_001964.3	2
<i>ERCC4</i>	NM_005236.3	1
<i>ERCC5</i>	NM_000123.3	1
<i>ETV4</i>	NM_001986.4	1
<i>FANCD2</i>	NM_001018115.2	2
<i>FAT1</i>	NM_005245.4	1
<i>FGFR1</i>	NM_023110.3	1
<i>FLCN</i>	NM_144997.7	1
<i>FOXO1</i>	NM_002015.4	1
<i>FOXO3</i>	NM_001455.4	2
<i>GATA1</i>	NM_002049.4	2
<i>GATA2</i>	NM_032638.5	1
<i>GATA3</i>	NM_001002295.2	1
<i>GATA6</i>	NM_005257.5	1
<i>GNAQ</i>	NM_002072.5	3
<i>GNAS</i>	NM_000516.6	1
<i>GRIN2A</i>	NM_000833.5	1
<i>HDAC2</i>	NM_001527.4	2
<i>HNFI1A</i>	NM_000545.6	1
<i>IFNGR2</i>	NM_001329128.1	1

<b>Gene</b>	<b>Transcript ID</b>	<b>Number of Excluded Variants</b>
<i>ITPKB</i>	NM_002221.3	1
<i>JAK1</i>	NM_002227.4	1
<i>JAK3</i>	NM_000215.3	1
<i>JARID2</i>	NM_004973.4	1
<i>JUN</i>	NM_002228.4	1
<i>KDM2B</i>	NM_032590.5	1
<i>KDM5A</i>	NM_001042603.3	2
<i>KDM5C</i>	NM_004187.4	1
<i>KLRC1</i>	NM_002259.5	1
<i>KMT2A</i>	NM_005933.4	2
<i>KMT2B</i>	NM_014727.2	3
<i>KMT2C</i>	NM_170606.3	18
<i>KMT2D</i>	NM_003482.3	7
<i>LEF1</i>	NM_001130713.2	1
<i>LHCGR</i>	NM_000233.4	1
<i>LZTR1</i>	NM_006767.4	1
<i>MAP3K1</i>	NM_005921.2	1
<i>MCL1</i>	NM_021960.5	1
<i>MED12</i>	NM_005120.3	12
<i>MFHAS1</i>	NM_004225.3	3
<i>MGA</i>	NM_001164273.1	2
<i>MLH3</i>	NM_014381.3	1
<i>MLLT3</i>	NM_004529.4	5
<i>MSH3</i>	NM_002439.5	1
<i>MTOR</i>	NM_004958.4	1
<i>MUTYH</i>	NM_012222.2	4
<i>MYBL1</i>	NM_001080416.4	1
<i>MYCL</i>	NM_001033082.3	1
<i>MYCN</i>	NM_005378.6	1
<i>MYH11</i>	NM_001040113.2	1
<i>NBN</i>	NM_001024688.2	1
<i>NCOR2</i>	NM_006312.6	3
<i>NFI</i>	NM_000267.3	1

<b>Gene</b>	<b>Transcript ID</b>	<b>Number of Excluded Variants</b>
<i>NFKB2</i>	NM_001077494.3	1
<i>NOS2</i>	NM_000625.4	1
<i>NOTCH2</i>	NM_024408.4	1
<i>NSD1</i>	NM_172349.2	1
<i>NT5C2</i>	NM_012229.4	1
<i>PAK3</i>	NM_002578.5	1
<i>PALB2</i>	NM_024675.4	1
<i>PALLD</i>	NM_016081.4	1
<i>PDE11A</i>	NM_001077196.2	2
<i>PHOX2B</i>	NM_003924.4	2
<i>PIK3R2</i>	NM_005027.4	1
<i>PLCB4</i>	NM_000933.3	2
<i>POLE</i>	NM_006231.4	1
<i>POT1</i>	NM_001042594.1	1
<i>PRDM6</i>	NM_001136239.4	1
<i>PREX2</i>	NM_025170.6	2
<i>PRSS3</i>	NM_002771.3	1
<i>RAD50</i>	NM_005732.4	1
<i>RARA</i>	NM_000964.4	1
<i>RBI</i>	NM_000321.2	1
<i>RECQL4</i>	NM_004260.3	4
<i>RICTOR</i>	NM_152756.5	1
<i>RRM1</i>	NM_001033.5	1
<i>RUNX1</i>	NM_001001890.3	1
<i>RUNX3</i>	NM_004350.3	1
<i>SDHA</i>	NM_004168.4	4
<i>SDHB</i>	NM_003000.3	1
<i>SETBP1</i>	NM_001130110.2	1
<i>SMARCA4</i>	NM_003072.4	1
<i>SMO</i>	NM_005631.5	3
<i>SPEN</i>	NM_015001.3	1
<i>STAT3</i>	NM_139276.2	1
<i>STAT5B</i>	NM_012448.4	1

<b>Gene</b>	<b>Transcript ID</b>	<b>Number of Excluded Variants</b>
<i>SUFU</i>	NM_016169.3	2
<i>SUZ12</i>	NM_015355.4	1
<i>TAP2</i>	NM_018833.2	1
<i>TERT</i>	NM_001193376.2	1
<i>TGFB1</i>	NM_000660.7	1
<i>TGFBR2</i>	NM_001024847.2	1
<i>TP53</i>	NM_000546.5	1
<i>TSC2</i>	NM_000548.5	1
<i>TTF1</i>	NM_007344.4	7
<i>UGT1A1</i>	NM_000463.3	1
<i>WAS</i>	NM_000377.3	3
<i>XPC</i>	NM_004628.4	1
<i>ZNF703</i>	NM_025069.3	3
<i>ZRSR2</i>	NM_005089.3	1

**Appendix D. Interlaboratory Reproducibility Summary of GENESEEQPRIME per Variant, per Specimen Tested**

<b>Gene</b>	<b>Amino Acid Change</b>	<b>Mutation Type</b>	<b>Mean MAF (%)</b>	<b>MAF Range (%)</b>	<b>SD</b>	<b>%CV</b>	<b>Positive Call Rate % (n/N)</b>
<i>APC</i>	c.6589dup(p.S2197Kfs*10)	INS<15bp	5.49	4.26-6.66	0.62	11.25%	100% (34/34)
<i>ASXL1</i>	c.1231C>T(p.R411C)	SNV	7.02	5.58-8.65	0.81	11.53%	100% (34/34)
<i>B2M</i>	c.35T>C(p.L12P)	SNV	6.75	5.44-8.43	0.73	10.76%	100% (34/34)
<i>B2M</i>	c.238T>C(p.W80R)	SNV	5.28	3.23-7.09	0.96	18.28%	100% (34/34)
<i>B2M</i>	c.204del(p.V69Wfs*34)	DEL<15bp	5.51	5.03-6.53	0.48	8.71%	52.94% (18/34)
<i>CDK12</i>	c.633_634del(p.K211Nfs*18)	DEL<15bp	6.10	4.67-7.22	0.74	12.12%	100% (34/34)
<i>CHEK1</i>	c.131G>A(p.R44H)	SNV	5.60	3.73-9.14	1.13	20.27%	100% (34/34)
<i>CREBBP</i>	c.2849C>T(p.T950M)	SNV	7.56	5.86-9.12	0.79	10.40%	100% (34/34)
<i>DICER1</i>	c.4115C>A(p.S1372*)	SNV	5.68	3.04-7.79	1.13	19.87%	100% (34/34)
<i>DNMT3A</i>	c.2059G>A(p.V687I)	SNV	7.28	5.12-9.15	0.89	12.16%	100% (34/34)
<i>ERBB3</i>	c.1981G>A(p.G661S)	SNV	6.93	4.68-8.83	0.97	13.95%	100% (34/34)
<i>ERBB4</i>	c.2493G>T(p.M831I)	SNV	5.90	2.56-10.23	1.5	25.46%	94.12% (32/34)
<i>ERCC3</i>	c.2183T>A(p.V728E)	SNV	7.91	6.42-9.12	0.7	8.81%	100% (34/34)
<i>FANCC</i>	c.265del(p.I89Ffs*2)	DEL<15bp	6.19	5-8.24	0.85	13.80%	61.76% (21/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>FANCM</i>	c.2589del(p.D864Ifs*12)	DEL<15bp	5.98	5.04-8.21	0.75	12.47%	94.12% (32/34)
<i>FGFR1</i>	c.1981C>T(p.R661*)	SNV	6.84	4.48-8.29	0.98	14.33%	100% (34/34)
<i>SMARCA4</i>	c.4436G>A(p.R1479H)	SNV	4.43	2.92-5.86	0.8	17.97%	100% (34/34)
<i>FLT4</i>	c.1267del(p.Q423Rfs*70)	DEL<15bp	10.54	10.04-11.36	0.39	3.72%	47.06% (16/34)
<i>IGF1R</i>	c.722C>T(p.A241V)	SNV	6.99	5-8.61	0.73	10.38%	100% (34/34)
<i>IGF2</i>	c.298G>A(p.V100M)	SNV	7.68	5.8-9.35	0.85	11.05%	100% (34/34)
<i>KMT2A</i>	c.416del(p.G139Efs*11)	DEL<15bp	6.56	5.1-7.67	0.65	9.97%	100% (34/34)
<i>KRAS</i>	c.216G>A(p.M72I)	SNV	5.84	4-7.72	0.81	13.80%	100% (34/34)
<i>MET</i>	c.1003G>A(p.A335T)	SNV	6.04	3.45-8.91	1.01	16.67%	100% (34/34)
<i>NOTCH2</i>	c.4064A>G(p.E1355G)	SNV	7.10	5.02-8.33	0.7	9.86%	100% (34/34)
<i>RECQL4</i>	c.462del(p.V155Sfs*25)	DEL<15bp	6.86	5.32-9.06	0.86	12.50%	100% (34/34)
<i>RNF43</i>	c.1976del(p.G659Vfs*41)	DEL<15bp	15.30	13.29-16.86	0.9	5.86%	100% (34/34)
<i>SDHA</i>	c.1724C>T(p.A575V)	SNV	7.73	6.26-9.79	0.85	11%	100% (34/34)
<i>SGK1</i>	c.1270G>T(p.E424*)	SNV	6.82	4.13-8.45	0.84	12.31%	100% (34/34)
<i>SMAD4</i>	c.634G>A(p.A212T)	SNV	6.30	4.95-8.54	0.93	14.78%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>SUFU</i>	c.1022C>T(p.P341L)	SNV	7.77	6.37-10	0.91	11.75%	100% (34/34)
<i>TERT</i>	c.581G>A(p.R194Q)	SNV	7.88	5.84-9.88	0.92	11.71%	100% (34/34)
<i>MYD88</i>	c.473C>T(p.A158V)	SNV	36.53	34.73-37.11	0.77	2.10%	24.24% (8/33)
<i>PLCB4</i>	c.607G>A(p.A203T)	SNV	29.27	29.27-29.27	0	0%	3.03% (1/33)
<i>BRAF</i>	c.1799T>A(p.V600E)	SNV	23.78	20.54-26.28	1.52	6.41%	100% (34/34)
<i>RAD51C</i>	c.859A>G(p.T287A)	SNV	28.12	21.93-32.48	5.51	19.59%	8.82% (3/34)
<i>ARID1A</i>	c.1780C>T(p.Q594*)	SNV	34.43	30.85-38.12	1.88	5.45%	100% (34/34)
<i>BRAF</i>	c.1799T>A(p.V600E)	SNV	17.99	13.54-24.91	2.02	11.24%	100% (34/34)
<i>CBLB</i>	c.1642C>T(p.P548S)	SNV	16.25	12.94-18.69	1.4	8.60%	100% (34/34)
<i>CDK12</i>	c.3806C>T(p.P1269L)	SNV	18.30	15.26-20.94	1.39	7.57%	100% (34/34)
<i>ETV6</i>	c.395C>T(p.P132L)	SNV	27.58	23.51-31.14	2.14	7.76%	100% (34/34)
<i>JAK3</i>	c.1258C>T(p.P420S)	SNV	30.30	26.26-32.91	1.88	6.21%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>JAK3</i>	c.377G>A(p.S126N)	SNV	26.01	22.73-29.39	1.86	7.16%	100% (34/34)
<i>LHCGR</i>	c.1837C>T(p.P613S)	SNV	28.94	24.49-33.47	2.35	8.12%	100% (34/34)
<i>MAP2K1</i>	c.371C>A(p.P124Q)	SNV	30.03	24.95-33.6	1.71	5.71%	100% (34/34)
<i>RET</i>	c.2114C>T(p.S705F)	SNV	45.02	41.87-48.41	1.92	4.26%	100% (34/34)
<i>RPTOR</i>	c.3077C>T(p.P1026L)	SNV	27.08	20.6-31.54	2.47	9.11%	100% (34/34)
<i>AR</i>	c.94G>A(p.E32K)	SNV	29.16	25.95-33.37	1.53	5.26%	100% (34/34)
<i>AR</i>	c.853G>A(p.A285T)	SNV	28.70	26.13-31.79	1.26	4.39%	100% (34/34)
<i>ASCL4</i>	c.109del(p.L37Sfs*53)	DEL<15bp	28.27	25.75-30.47	1.38	4.89%	100% (34/34)
<i>ATM</i>	c.8159A>G(p.D2720G)	SNV	28.86	23.62-32.71	2.19	7.58%	100% (34/34)
<i>BRCA1</i>	c.4814T>C(p.L1605S)	SNV	31.32	28.46-36.51	1.85	5.92%	100% (34/34)
<i>CBL</i>	c.1484del(p.P495Rfs*120)	DEL<15bp	27.14	24.6-29.87	1.33	4.92%	100% (34/34)
<i>CCND1</i>	c.718G>A(p.D240N)	SNV	31.34	28.66-34.34	1.67	5.33%	100% (34/34)
<i>CTCF</i>	c.950_951del(p.T317Rfs*91)	DEL<15bp	26.42	20.86-29.56	1.84	6.96%	100% (34/34)
<i>CYP2B6</i>	c.639_641delinsTGA(p.G214D)	SNV	22.49	14.29-26.88	2.75	12.25%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>DICER1</i>	c.3907_3908del(p.L1303Vfs*4)	DEL<15bp	27.06	23.52-29.28	1.37	5.05%	100% (34/34)
<i>DOTIL</i>	c.3195C>G(p.S1065R)	SNV	29.11	25.58-32.66	1.75	6%	100% (34/34)
<i>EP300</i>	c.407T>C(p.M136T)	SNV	31.13	28.61-33.51	1.23	3.95%	100% (34/34)
<i>ERCC5</i>	c.341del(p.L114*)	DEL<15bp	28.28	25-33.33	1.89	6.68%	100% (34/34)
<i>FANCI</i>	c.2326A>G(p.M776V)	SNV	28.93	28.93-28.93	0	0%	2.94% (1/34)
<i>FAT1</i>	c.3784C>T(p.R1262*)	SNV	29.23	26.48-33.44	1.68	5.75%	100% (34/34)
<i>FGFR1</i>	c.1681G>A(p.V561M)	SNV	29.56	23.93-37.67	2.63	8.90%	100% (34/34)
<i>JAK1</i>	c.2580del(p.K860Nfs*16)	DEL<15bp	30.02	26.74-33.78	2.08	6.92%	100% (34/34)
<i>KMT2B</i>	c.521dup(p.T176Dfs*8)	INS<15bp	21.95	17.52-26.2	2.14	9.77%	97.06% (33/34)
<i>MAP3K1</i>	c.1594C>T(p.R532*)	SNV	30.10	25.71-34.06	1.78	5.92%	100% (34/34)
<i>NF1</i>	c.5839C>T(p.R1947*)	SNV	29.92	26.19-35	2.66	8.88%	100% (34/34)
<i>NF1</i>	c.3988G>T(p.E1330*)	SNV	28.44	21.41-34.26	3.05	10.71%	100% (34/34)
<i>NF2</i>	c.1271G>A(p.R424H)	SNV	29.35	26.5-31.89	1.48	5.05%	100% (34/34)
<i>NOTCH1</i>	c.1280G>T(p.G427V)	SNV	21.57	18.48-23.57	1.26	5.83%	100% (34/34)
<i>NSD1</i>	c.3784del(p.M1262Cfs*43)	DEL<15bp	28.26	25.13-32.64	1.64	5.80%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>PIK3CA</i>	c.3140A>G(p.H1047R)	SNV	30.33	27.56-33.58	1.18	3.89%	100% (34/34)
<i>PKHD1</i>	c.104del(p.G35Efs*29)	DEL<15bp	28.13	23.85-32.03	1.91	6.79%	100% (34/34)
<i>PLCB4</i>	c.127G>A(p.E43K)	SNV	27.88	22.03-32.36	2.21	7.92%	100% (34/34)
<i>PREX2</i>	c.1904del(p.K635Rfs*9)	DEL<15bp	22.09	13.47-29.44	3.92	17.73%	97.06% (33/34)
<i>PTCH1</i>	c.3921del(p.R1308Efs*64)	DEL<15bp	32.67	29.41-35.76	1.45	4.43%	100% (34/34)
<i>PTEN</i>	c.688G>T(p.G230*)	SNV	30.01	26.09-33.49	2.11	7.02%	100% (34/34)
<i>PTEN</i>	c.139A>G(p.R47G)	SNV	25.72	6.16-31.83	4.85	18.87%	100% (34/34)
<i>PTEN</i>	c.800del(p.K267Rfs*9)	DEL<15bp	28.15	23.53-31.82	2.12	7.53%	100% (34/34)
<i>TAP2</i>	c.223del(p.L75*)	DEL<15bp	27.35	25.42-30.14	1.14	4.19%	100% (34/34)
<i>TP63</i>	c.1013G>A(p.R338H)	SNV	27.64	23.79-29.68	1.46	5.28%	100% (34/34)
<i>PIK3CA</i>	c.3140A>G(p.H1047R)	SNV	10.19	8.17-12.3	1.03	10.14%	100% (34/34)
<i>TP53</i>	c.772G>T(p.E258*)	SNV	19.93	16.76-23.65	1.43	7.18%	100% (34/34)
<i>CTNNB1</i>	c.134C>T(p.S45F)	SNV	23.39	20.2-27.15	1.73	7.40%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>ERBB3</i>	c.3793C>T(p.R1265W)	SNV	37.90	36.25-39.14	0.88	2.33%	76.47% (26/34)
<i>FANCM</i>	c.5692G>A(p.V1898M)	SNV	33.48	28.77-37.61	2.34	6.98%	94.12% (32/34)
<i>KEAP1</i>	c.1808G>A(p.G603E)	SNV	56.94	52.89-60.49	1.78	3.13%	100% (34/34)
<i>KRAS</i>	c.35G>C(p.G12A)	SNV	42.41	38.24-46.23	2.11	4.98%	100% (34/34)
<i>STK11</i>	c.152_153insA(p.M51Ifs*112)	INS<15bp	48.29	43.98-51.21	1.71	3.55%	100% (34/34)
<i>UGT1A1</i>	c.1364T>C(p.L455P)	SNV	37.90	33.98-39.99	1.33	3.52%	100% (34/34)
<i>AKT3</i>	c.659G>A(p.R220H)	SNV	14.29	5.24-20.67	3.43	23.98%	100% (34/34)
<i>APC</i>	c.3845C>A(p.S1282*)	SNV	30.56	24.09-37.89	3.29	10.75%	100% (34/34)
<i>APC</i>	c.4216C>T(p.Q1406*)	SNV	15.07	10.76-18.89	2.05	13.59%	100% (34/34)
<i>ATM</i>	c.8612G>A(p.R2871K)	SNV	11.65	6.43-17.31	2.55	21.91%	91.18% (31/34)
<i>BRIP1</i>	c.2032G>A(p.A678T)	SNV	19.08	15.05-24.68	2.34	12.27%	100% (34/34)
<i>CYP2A6</i>	c.1366G>A(p.V456I)	SNV	15.86	15.07-18.06	1.02	6.42%	29.41% (10/34)
<i>FOXL2</i>	c.672_674del(p.A234del)	DEL<15bp	12.79	8.75-19.59	3.7	28.97%	38.24% (13/34)
<i>PBRM1</i>	c.232C>T(p.R78*)	SNV	8.27	4.23-12.03	1.86	22.49%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>PREX2</i>	c.1694C>T(p.S565L)	SNV	16.01	9.73-23.49	3.27	20.43%	100% (34/34)
<i>ABCB1</i>	c.1997G>T(p.R666I)	SNV	23.29	18.74-27.42	1.65	7.07%	100% (34/34)
<i>ARID1B</i>	c.4387C>T(p.Q1463*)	SNV	14.25	12.97-16.48	0.86	6.04%	100% (34/34)
<i>ARID1B</i>	c.5759C>G(p.T1920S)	SNV	14.34	12.76-15.55	0.73	5.09%	100% (34/34)
<i>BRAF</i>	c.1391G>T(p.G464V)	SNV	26.30	22.09-31.94	1.91	7.26%	100% (34/34)
<i>BTK</i>	c.769A>T(p.K257*)	SNV	17.03	13.98-19.14	1.23	7.21%	100% (34/34)
<i>CBLB</i>	c.2295G>T(p.K765N)	SNV	22.55	18.27-27.92	2.34	10.38%	100% (34/34)
<i>ERCC5</i>	c.3296G>C(p.G1099A)	SNV	28.28	25.8-31.24	1.54	5.43%	100% (34/34)
<i>ETV5</i>	c.425C>T(p.S142L)	SNV	12.28	9.83-13.92	1.19	9.72%	100% (34/34)
<i>FLT4</i>	c.3682G>C(p.A1228P)	SNV	21.96	19.83-23.75	1.11	5.04%	100% (34/34)
<i>GATA3</i>	c.1280C>T(p.S427F)	SNV	21	19.01-23.21	0.85	4.03%	100% (34/34)
<i>KEAP1</i>	c.1285G>T(p.G429C)	SNV	27.63	23.99-30.62	1.45	5.26%	100% (34/34)
<i>LRP1B</i>	c.1885C>A(p.L629M)	SNV	18.48	15.38-22.66	1.76	9.52%	100% (34/34)
<i>LRP1B</i>	c.10733G>T(p.C3578F)	SNV	14.49	11.05-18.16	1.76	12.15%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>LRP1B</i>	c.3137-1G>C	SNV	13	8.84-17.53	1.74	13.36%	100% (34/34)
<i>MTOR</i>	c.1226-2A>T	SNV	15.06	12.63-17.79	1.2	7.95%	100% (34/34)
<i>PBRM1</i>	c.277G>T(p.D93Y)	SNV	27.84	22.39-33.21	2.61	9.38%	100% (34/34)
<i>PDK1</i>	c.916A>T(p.T306S)	SNV	13.30	10.11-18.23	1.68	12.63%	100% (34/34)
<i>PREX2</i>	c.2077G>A(p.G693R)	SNV	16.54	13.46-18.22	1.03	6.23%	100% (34/34)
<i>RARA</i>	c.830A>T(p.Y277F)	SNV	23.30	18.92-26.21	1.35	5.80%	100% (34/34)
<i>SOX2</i>	c.92G>T(p.G31V)	SNV	11.47	9.68-13.17	0.87	7.55%	100% (34/34)
<i>TAP1</i>	c.1687G>A(p.V563I)	SNV	13.27	10.27-15.95	1.3	9.82%	100% (34/34)
<i>TOP2A</i>	c.482G>T(p.G161V)	SNV	21.13	17.74-25.43	1.75	8.26%	100% (34/34)
<i>TP53</i>	c.775G>T(p.D259Y)	SNV	40.71	37.64-43.07	1.17	2.88%	100% (34/34)
<i>DNMT3A</i>	c.2351_2352delinsTG(p.E784V)	SNV	3.64	2.35-5.06	0.75	20.72%	100% (34/34)
<i>NSD1</i>	c.3715dup(p.A1239Gfs*9)	INS<15bp	31.41	28.47-34.39	1.68	5.34%	100% (34/34)
<i>TP53</i>	c.594_611del(p.G199_E204del)	DEL≥15bp	34.20	28.26-39.52	2.81	8.23%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>GATA4</i>	c.118C>T(p.P40S)	SNV	18.33	15.16-21.03	1.55	8.48%	100% (34/34)
<i>NFE2L2</i>	c.86A>G(p.D29G)	SNV	29.78	21.45-34.25%	2.7	9.05%	100% (34/34)
<i>NFE2L2</i>	c.85G>C(p.D29H)	SNV	3.23%	1.99%-5.45%	0.77	23.74%	100% (34/34)
<i>RAD50</i>	c.511G>T(p.A171S)	SNV	17.83%	14.19%-19.94%	1.59	8.90%	73.53% (25/34)
<i>ROS1</i>	c.4190G>A(p.W1397*)	SNV	36.45%	31.99%-42.44	2.87	7.88%	100% (34/34)
<i>TP53</i>	c.363_375+4delinsG	SNV	54.34	46.53-63.16	3.87	7.12%	97.06% (33/34)
<i>XPC</i>	c.718C>T(p.R240C)	SNV	13.81	12.35-15.55	0.94	6.79%	100% (34/34)
<i>BRD4</i>	c.2957C>A(p.P986H)	SNV	4	4-4	0	0%	2.94% (1/34)
<i>MUTYH</i>	c.713G>A(p.R238Q)	SNV	52.07	49.24-53.91	1.18	2.27%	100% (34/34)
<i>SMAD2</i>	c.779G>A(p.S260N)	SNV	20.95	9.9-34.45	12.46	59.47%	8.82% (3/34)
<i>TP53</i>	c.817C>T(p.R273C)	SNV	12.61	10.46-15.66	1.05	8.34%	100% (34/34)
<i>ABCBI</i>	c.1763G>A(p.R588H)	SNV	15.27	10.03-18.82	1.67	10.92%	100% (34/34)
<i>AKT2</i>	c.1188del(p.S398Afs*177)	DEL<15bp	5.24	5.24-5.24	0	0%	2.94% (1/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>AXIN2</i>	c.1994del(p.G665Afs*24)	DEL<15bp	17.33	14.01-20.02	1.27	7.32%	100% (34/34)
<i>AXIN2</i>	c.2023del(p.R675Vfs*14)	DEL<15bp	17	13.26-23.2	1.58	9.30%	100% (34/34)
<i>BLM</i>	c.1544del(p.N515Mfs*16)	DEL<15bp	14.61	10.88-16.71	1.29	8.84%	100% (34/34)
<i>BRAF</i>	c.1799T>A(p.V600E)	SNV	16.03	11.62-20.53	1.91	11.89%	100% (34/34)
<i>CDKN2A</i>	c.106del(p.A36Rfs*17)	DEL<15bp	19.64	16.94-21.99	1.26	6.42%	100% (34/34)
<i>CHD8</i>	c.2282del(p.N761Tfs*12)	DEL<15bp	7.44	5.56-12.08	1.25	16.82%	100% (34/34)
<i>CYP2A6</i>	c.1047G>A(p.M349I)	SNV	14.53	11.89-17.06	1.33	9.14%	100% (34/34)
<i>DAXX</i>	c.809G>A(p.R270H)	SNV	17.87	15.24-20.03	1.3	7.25%	100% (34/34)
<i>EPHA3</i>	c.1095del(p.K365Nfs*6)	DEL<15bp	14.66	11.11-17.32	1.6	10.93%	100% (34/34)
<i>EPHA5</i>	c.896del(p.N299Mfs*123)	DEL<15bp	7.59	5.45-10.33	1.13	14.90%	100% (34/34)
<i>ERBB3</i>	c.608A>G(p.Q203R)	SNV	10.33	6.47-12.77	1.35	13.11%	100% (34/34)
<i>ERBB4</i>	c.2402A>G(p.H801R)	SNV	16.74	12.5-20.3	1.68	10.01%	100% (34/34)
<i>EWSR1</i>	c.1375C>T(p.P459S)	SNV	15.34	12.21-17.8	1.21	7.92%	100% (34/34)
<i>EXT1</i>	c.747del(p.L251*)	DEL<15bp	13.43	11.14-15.15	1	7.43%	100% (34/34)
<i>EXT2</i>	c.1067G>T(p.G356V)	SNV	14.72	11.52-17.86	1.6	10.84%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>FANCI</i>	c.3016G>A(p.V1006M)	SNV	15.51	12.63-18.25	1.54	9.93%	100% (34/34)
<i>FLT3</i>	c.203G>T(p.R68I)	SNV	12.49	9.84-14.47	1.21	9.67%	100% (34/34)
<i>FLT4</i>	c.3178C>T(p.R1060W)	SNV	18.41	15.74-21.63	1.68	9.14%	100% (34/34)
<i>FLT4</i>	c.3511dup(p.D1171Gfs*43)	INS<15bp	17.24	11.25-20.77	2.19	12.69%	100% (34/34)
<i>GATA4</i>	c.616G>A(p.D206N)	SNV	16.19	14.26-19.08	1.35	8.33%	100% (34/34)
<i>GATA4</i>	c.793C>T(p.R265C)	SNV	15.50	13.32-17.68	1.22	7.88%	100% (34/34)
<i>HNF1A</i>	c.864del(p.P291Qfs*51)	DEL<15bp	17.72	15.1-19.72	1.23	6.95%	100% (34/34)
<i>IDH2</i>	c.435del(p.T146Lfs*15)	DEL<15bp	16.21	13.71-19.44	1.47	9.09%	100% (34/34)
<i>INPP4B</i>	c.442G>A(p.A148T)	SNV	15.81	12.39-20.59	1.85	11.71%	100% (34/34)
<i>JAK1</i>	c.1289del(p.P430Rfs*2)	DEL<15bp	22.99	20.92-26.11	1.12	4.86%	100% (34/34)
<i>KDM5A</i>	c.844C>T(p.R282W)	SNV	14.55	10.26-19.33	1.67	11.46%	100% (34/34)
<i>KDM5A</i>	c.23del(p.G8Afs*58)	DEL<15bp	5.18	5.05-5.47	0.2	3.80%	11.76% (4/34)
<i>KMT2B</i>	c.3799G>T(p.E1267*)	SNV	17.46	14.74-20.27	1.45	8.31%	100% (34/34)
<i>MYCN</i>	c.1061C>T(p.A354V)	SNV	3.90	2.31-5.32	0.8	20.42%	97.06% (33/34)
<i>GRM8</i>	c.499A>G(p.R167G)	SNV	3.79	2.59-5.72	0.72	19%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>MRE11</i>	c.1532del(p.N511Ifs*13)	DEL<15bp	15.18	9.73-19.95	2.11	13.90%	94.12% (32/34)
<i>MYC</i>	c.307G>A(p.G103S)	SNV	15.44	13.48-18.74	1.2	7.74%	100% (34/34)
<i>MYD88</i>	c.109G>A(p.A37T)	SNV	18.16	16.07-20.77	1.25	6.86%	100% (34/34)
<i>NOTCH2</i>	c.1496A>G(p.Q499R)	SNV	14.41	11.57-18.38	1.79	12.46%	100% (34/34)
<i>PDCD1</i>	c.399G>T(p.Q133H)	SNV	17.56	13.74-21.39	1.44	8.18%	100% (34/34)
<i>PDGFRA</i>	c.3244G>T(p.D1082Y)	SNV	14.38	12.19-18.43	1.45	10.10%	100% (34/34)
<i>PRF1</i>	c.1135C>T(p.R379W)	SNV	17.50	14.24-20.62	1.59	9.07%	100% (34/34)
<i>PRKACA</i>	c.322G>A(p.E108K)	SNV	15.74	12.79-20.04	1.68	10.68%	100% (34/34)
<i>PTEN</i>	c.800del(p.K267Rfs*9)	DEL<15bp	14.87	9.8-18.31	1.65	11.10%	100% (34/34)
<i>PTEN</i>	c.867del(p.V290*)	DEL<15bp	15.13	11.96-17.87	1.59	10.48%	97.06% (33/34)
<i>RNF43</i>	c.1976del(p.G659Vfs*41)	DEL<15bp	34.71	32.21-38.8	1.45	4.18%	100% (34/34)
<i>ROS1</i>	c.5230G>T(p.E1744*)	SNV	16.67%	13.4-19.89	1.62	9.69%	94.12% (32/34)
<i>SOX2</i>	c.133A>G(p.M45V)	SNV	19.60	16.75-23.1	1.46	7.47%	100% (34/34)
<i>TAP2</i>	c.646T>C(p.Y216H)	SNV	33.27	30.6-36.35	1.55	4.66%	100% (34/34)
<i>TGFBR2</i>	c.457_458del(p.K153Afs*3)	DEL<15bp	26.38	22.87-29.63	1.61	6.09%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>TP53</i>	c.430C>T(p.Q144*)	SNV	17.75	13.77-21.43	1.68	9.48%	100% (34/34)
<i>TUBB3</i>	c.1070C>T(p.P357L)	SNV	17.84	15.02-21.67	1.43	8.03%	100% (34/34)
<i>ZNF217</i>	c.1939G>A(p.V647I)	SNV	16.16	12.91-19.47	1.66	10.26%	100% (34/34)
<i>EGFR</i>	c.2573T>G(p.L858R)	SNV	39.19	37.95-41.67	0.83	2.11%	100% (34/34)
<i>SF3B1</i>	c.2098A>G(p.K700E)	SNV	4.78	3.61-6.11	0.78	16.25%	100% (34/34)
<i>TP53</i>	c.404G>T(p.C135F)	SNV	25.39	23.21-28.59	1.38	5.44%	100% (34/34)
<i>BAP1</i>	c.375+2_375+4del	DEL<15bp	30.84	29.51-32.18	1.89	6.12%	5.88% (2/34)
<i>PBRM1</i>	c.3478C>T(p.R1160*)	SNV	1.94	1.94-1.94	0	0%	2.94% (1/34)
<i>CTNNB1</i>	c.761del(p.Y254Lfs*22)	DEL<15bp	11.06	7.89-14.78	1.87	16.90%	100% (34/34)
<i>DICER1</i>	c.1258G>A(p.E420K)	SNV	35.61	31.4-40.49	2.29	6.42%	67.65% (23/34)
<i>AXL</i>	c.526C>T(p.P176S)	SNV	17.01	13.73-19.94	1.52	8.92%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>TAP1</i>	c.508C>G(p.L170V)	SNV	12.34	11.07-14.43	0.79	6.44%	100% (34/34)
<i>GATA3</i>	c.198C>G(p.N66K)	SNV	16.20	13.43-19.29	1.25	7.72%	100% (34/34)
<i>KMT2A</i>	c.200_202dup(p.A67dup)	INS<15bp	12.97	10.62-15.52	1.31	10.11%	100% (34/34)
<i>PIK3CA</i>	c.3140A>G(p.H1047R)	SNV	36.13	32.81-40.24	1.75	4.84%	100% (34/34)
<i>PTCH1</i>	c.1230T>A(p.S410R)	SNV	33.38	30.13-36.52	1.62	4.84%	100% (34/34)
<i>TP53</i>	c.652_654del(p.V218del)	DEL<15bp	39.99	35.71-45.73	2.28	5.71%	100% (34/34)
<i>ABCB1</i>	c.229G>C(p.D77H)	SNV	12.72	9.97-18.91	1.86	14.62%	100% (34/34)
<i>EGFR</i>	c.2235_2249del(p.E746_A750del)	DEL≥15bp	52.65	49.97-55.63	1.39	2.63%	100% (34/34)
<i>LRP1B</i>	c.2398G>A(p.V800I)	SNV	22	17.39-26.35	2.25	10.21%	100% (34/34)
<i>LRP1B</i>	c.5228T>A(p.V1743E)	SNV	20.69	16.06-26.19	2.45	11.86%	91.18% (31/34)
<i>PBRM1</i>	c.4250dup(p.P1418Afs*91)	INS<15bp	77.06	75.92-79.33	0.81	1.05%	82.35% (28/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>XPC</i>	c.718C>T(p.R240C)	SNV	13.08	11.01-14.91	0.96	7.34%	100% (34/34)
<i>CTCF</i>	c.1701+1dup	INS<15bp	30.27	28.29-32.95	1.2	3.98%	100% (34/34)
<i>FBXW7</i>	c.1159C>G(p.R387G)	SNV	9.21	8.22-10.7	0.63	6.82%	100% (34/34)
<i>FGFR2</i>	c.1922A>G(p.K641R)	SNV	36.78	31.83-39.69	1.68	4.57%	100% (34/34)
<i>KMT2B</i>	c.4987C>T(p.H1663Y)	SNV	34.41	31.59-38.03	1.49	4.33%	100% (34/34)
<i>KRAS</i>	c.35G>A(p.G12D)	SNV	14.86	11.15-18.28	1.62	10.88%	100% (34/34)
<i>PIK3CA</i>	c.113G>A(p.R38H)	SNV	38.80	32.81-44.11	3.02	7.78%	100% (34/34)
<i>PIK3CA</i>	c.1616C>G(p.P539R)	SNV	38.07	27.78-45.54	3.79	9.95%	100% (34/34)
<i>PKHD1</i>	c.11338C>T(p.P3780S)	SNV	31.55	29.22-33.88	3.3	10.44%	5.88% (2/34)
<i>PTEN</i>	c.406T>C(p.C136R)	SNV	70.31	66-73.18	1.96	2.78%	100% (34/34)
<i>SMAD2</i>	c.1388G>A(p.C463Y)	SNV	33.05	28.97-38.17	2.26	6.83%	100% (34/34)
<i>FLT1</i>	c.779C>T(p.T260M)	SNV	6.48	4.04-11.57	1.55	23.86%	100% (34/34)
<i>MAP3K1</i>	c.233_234delinsCT(p.L78P)	SNV	9.69	6.81-14.29	1.61	16.57%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>RECQL4</i>	c.3314G>A(p.G1105D)	SNV	23.59	21.74-24.8	0.85	3.62%	64.71% (22/34)
<i>TP53</i>	c.949C>T(p.Q317*)	SNV	75.84	70.51-79.55	2.32	3.05%	100% (34/34)
<i>MTOR</i>	c.5485_5505dup(p.T1829_A1835dup)	INS≥15bp	3.60	2.4-4.34	0.52	14.45%	100% (34/34)
<i>AKT2</i>	c.153dup(p.N53Kfs*77)	INS<15bp	13.96	11.98-16.46	1.09	7.80%	100% (34/34)
<i>ARAF</i>	c.628del(p.L210Yfs*85)	DEL<15bp	14.99	13.31-16.92	1	6.65%	100% (34/34)
<i>ARID1A</i>	c.4897del(p.D1633Tfs*33)	DEL<15bp	15.47	13.81-16.8	0.89	5.78%	100% (34/34)
<i>ARID1A</i>	c.5769del(p.F1924Sfs*59)	DEL<15bp	14.76	12.53-16.52	0.82	5.57%	100% (34/34)
<i>ARID1A</i>	c.3220C>T(p.R1074W)	SNV	14.91	11.93-17.41	1.44	9.69%	100% (34/34)
<i>ATR</i>	c.2319_2320del(p.K773Nfs*3)	DEL<15bp	6.02	5.02-8.18	0.78	12.94%	94.12% (32/34)
<i>AXIN2</i>	c.2225T>C(p.L742P)	SNV	16.84	14.09-20.05	1.41	8.39%	100% (34/34)
<i>BAX</i>	c.337C>A(p.L113I)	SNV	15.21	11.51-18.4	1.49	9.81%	100% (34/34)
<i>BLM</i>	c.1544del(p.N515Mfs*16)	DEL<15bp	14.36	10.29-17.4	1.77	12.34%	100% (34/34)
<i>BRCA2</i>	c.5073del(p.K1691Nfs*15)	DEL<15bp	14.04	10.9-16.49	1.32	9.37%	100% (34/34)
<i>BRCA2</i>	c.68-4dup	INS<15bp	5.24	3.4-9.14	1.12	21.45%	97.06% (33/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>CHEK2</i>	c.1738G>A(p.A580T)	SNV	15.68	12.73-18.93	1.15	7.33%	100% (34/34)
<i>CREBBP</i>	c.5100G>T(p.Q1700H)	SNV	16.41	14.61-18.62	1.06	6.43%	100% (34/34)
<i>CREBBP</i>	c.6584G>A(p.R2195K)	SNV	15.83	14.04-17.5	0.96	6.08%	100% (34/34)
<i>CREBBP</i>	c.1280G>T(p.C427F)	SNV	14.39	10.74-17.3	1.47	10.23%	100% (34/34)
<i>CREBBP</i>	c.3250del(p.I1084Sfs*15)	DEL<15bp	5.61	5.06-6.82	0.51	9.07%	32.35% (11/34)
<i>CREBBP</i>	c.4230del(p.F1410Lfs*49)	DEL<15bp	5.69	5.11-6.53	0.44	7.77%	58.82% (20/34)
<i>DAXX</i>	c.1507T>C(p.S503P)	SNV	15.13	12.25-19	1.5	9.91%	100% (34/34)
<i>DLL3</i>	c.493G>A(p.A165T)	SNV	8.28	7.19-9.91	0.72	8.65%	100% (34/34)
<i>EPHA3</i>	c.1067A>G(p.D356G)	SNV	14.69	11.77-17.59	1.38	9.39%	100% (34/34)
<i>ERBB3</i>	c.2353C>T(p.L785F)	SNV	15.48	12.55-17.64	1.25	8.07%	100% (34/34)
<i>ERBB4</i>	c.1del(p.M1?)	DEL<15bp	14.50	11.62-19.27	1.69	11.63%	100% (34/34)
<i>ERCC1</i>	c.321+2T>C	SNV	17.21	14.54-20.8	1.71	9.95%	100% (34/34)
<i>EXT1</i>	c.1670T>C(p.I557T)	SNV	38.40	38.4-38.4	0	0%	2.94% (1/34)
<i>FANCA</i>	c.2915del(p.G972Afs*17)	DEL<15bp	15.28	12.92-17.24	1.35	8.82%	100% (34/34)
<i>FANCF</i>	c.729dup(p.N244Efs*22)	INS<15bp	15.99	13.19-18.41	1.08	6.76%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>FANCI</i>	c.1333C>T(p.L445F)	SNV	33.92	21.17-40.82	7.09	20.91%	17.65% (6/34)
<i>FAT1</i>	c.6301C>T(p.R2101C)	SNV	16.76	15.07-18.67	1.03	6.14%	100% (34/34)
<i>FLT4</i>	c.1924del(p.R642Afs*43)	DEL<15bp	14.36	12.23-16	1.01	7.06%	100% (34/34)
<i>FLT4</i>	c.89dup(p.T31Dfs*15)	INS<15bp	11.77	9.09-14.71	1.35	11.51%	100% (34/34)
<i>FOXL2</i>	c.463C>T(p.P155S)	SNV	15.51	14.36-18.21	0.93	6.02%	100% (34/34)
<i>GATA1</i>	c.1073del(p.P358Qfs*74)	DEL<15bp	14.96	12.79-16.62	0.96	6.41%	100% (34/34)
<i>GRIN2A</i>	c.883G>A(p.G295S)	SNV	14.30	12.46-16.12	0.81	5.64%	100% (34/34)
<i>GRM8</i>	c.1513G>A(p.A505T)	SNV	13.76	11.67-16.71	1.18	8.56%	100% (34/34)
<i>HDAC9</i>	c.1539G>A(p.M513I)	SNV	14.19	13.13-16.36	0.79	5.60%	100% (34/34)
<i>HNF1A</i>	c.864del(p.P291Qfs*51)	DEL<15bp	14.96	13.28-17.15	0.96	6.39%	100% (34/34)
<i>IRF2</i>	c.612G>T(p.E204D)	SNV	17.24	16.21-20.01	0.98	5.71%	100% (34/34)
<i>JAK1</i>	c.2580del(p.K860Nfs*16)	DEL<15bp	7.12	5.75-8.93	0.86	12.10%	100% (34/34)
<i>KDM5A</i>	c.1282C>T(p.R428W)	SNV	13.89	10.56-18.72	2.07	14.91%	100% (34/34)
<i>KDM5A</i>	c.2227del(p.V743Sfs*18)	DEL<15bp	5.72	3.12-8.73	1.13	19.77%	100% (34/34)
<i>KDR</i>	c.1055C>T(p.A352V)	SNV	15.79	12.02-20.05	1.76	11.15%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>KEAP1</i>	c.886C>A(p.R296S)	SNV	17.16	13.98-20.43	1.36	7.90%	100% (34/34)
<i>KMT2A</i>	c.10992A>C(p.K3664N)	SNV	15.39	12.62-18.83	1.55	10.06%	100% (34/34)
<i>KMT2A</i>	c.11443C>T(p.P3815S)	SNV	13.39	10.22-16.22	1.7	12.66%	100% (34/34)
<i>KMT2A</i>	c.60_65del(p.G22_G23del)	DEL<15bp	14.92	11.82-19.1	1.63	10.91%	100% (34/34)
<i>KMT2B</i>	c.4235del(p.G1412Afs*10)	DEL<15bp	14.93	12.64-18.17	1.17	7.84%	100% (34/34)
<i>KMT2B</i>	c.521dup(p.T176Dfs*8)	INS<15bp	11.98	10.58-14.39	0.97	8.09%	88.24% (30/34)
<i>LZTR1</i>	c.800G>A(p.R267H)	SNV	15.16	13.18-17.86	1.09	7.16%	100% (34/34)
<i>MED12</i>	c.5423G>A(p.R1808Q)	SNV	13.37	11.46-15.6	1.03	7.71%	100% (34/34)
<i>MITF</i>	c.299T>C(p.V100A)	SNV	15.67	13.33-17.5	1	6.37%	100% (34/34)
<i>MITF</i>	c.650G>A(p.R217Q)	SNV	14.95	11.9-18.12	1.63	10.90%	100% (34/34)
<i>MSH6</i>	c.3261del(p.F1088Sfs*2)	DEL<15bp	16.72	14.58-19.72	1.39	8.29%	100% (34/34)
<i>MYCL</i>	c.14C>T(p.A5V)	SNV	6.23	4.73-8.51	0.89	14.33%	91.18% (31/34)
<i>NBN</i>	c.1676A>T(p.D559V)	SNV	11.56	7.64-14.96	1.64	14.14%	97.06% (33/34)
<i>NKX2-1</i>	c.97G>T(p.G33C)	SNV	16.99	14.24-19.38	1.2	7.08%	100% (34/34)
<i>NOTCH1</i>	c.146G>A(p.G49D)	SNV	17.03	13.98-19.1	1.14	6.68%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>NRG1</i>	c.1474C>T(p.R492W)	SNV	12.51	11.11-14.07	0.7	5.61%	100% (34/34)
<i>NSD1</i>	c.2666del(p.K889Sfs*6)	DEL<15bp	5.26	5.07-5.56	0.16	3.13%	32.35% (11/34)
<i>PIK3CA</i>	c.1625A>T(p.E542V)	SNV	13.94	9.5-17.88	1.95	14%	100% (34/34)
<i>PKHD1</i>	c.4357C>T(p.P1453S)	SNV	16.01	13.59-17.79	0.95	5.95%	100% (34/34)
<i>PLCB4</i>	c.1883G>A(p.R628Q)	SNV	14.68	11.51-17.99	1.59	10.80%	100% (34/34)
<i>EMSY</i>	c.1849dup(p.T617Nfs*34)	INS<15bp	3.62	2.01-5.22	0.78	21.53%	100% (34/34)
<i>PMS2</i>	c.1239del(p.D414Tfs*34)	DEL<15bp	14.35	12.06-17.36	1.33	9.28%	100% (34/34)
<i>PPP2R1A</i>	c.1540C>T(p.Q514*)	SNV	14.79	12.89-16.97	1.11	7.52%	100% (34/34)
<i>PRF1</i>	c.421A>C(p.S141R)	SNV	15.21	13.49-17.45	0.82	5.41%	100% (34/34)
<i>PTEN</i>	c.97_99del(p.I33del)	DEL<15bp	11.88	2.36-15.61	2.62	22.10%	97.06% (33/34)
<i>PTPN13</i>	c.2548A>G(p.N850D)	SNV	14.56	11.64-18.05	1.85	12.74%	100% (34/34)
<i>PTPN13</i>	c.799G>A(p.D267N)	SNV	13.37	9.51-16.83	1.73	12.92%	100% (34/34)
<i>QKI</i>	c.401del(p.K134Rfs*14)	DEL<15bp	13.26	10.06-16.38	1.64	12.36%	100% (34/34)
<i>RET</i>	c.1763del(p.G588Afs*50)	DEL<15bp	14.45	12.77-15.7	0.76	5.25%	100% (34/34)
<i>RNF43</i>	c.1976del(p.G659Vfs*41)	DEL<15bp	30.48	27.48-35.31	1.44	4.73%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>SETBP1</i>	c.146C>T(p.P49L)	SNV	14.17	12.52-16.05	0.95	6.69%	100% (34/34)
<i>SETD2</i>	c.4219del(p.R1407Gfs*5)	DEL<15bp	13.75	11.98-16.16	1.17	8.49%	97.06% (33/34)
<i>SLC3A2</i>	c.899del(p.K300Rfs*31)	DEL<15bp	15	13-17.41	1.2	7.99%	100% (34/34)
<i>SMARCA 4</i>	c.3727C>T(p.R1243W)	SNV	15.39	13.42-18.07	1.12	7.25%	100% (34/34)
<i>SMARCA 4</i>	c.2899C>T(p.R967C)	SNV	15.45	12.94-19.24	1.45	9.36%	100% (34/34)
<i>SOX2</i>	c.574G>T(p.A192S)	SNV	16.07	12.47-18.43	1.3	8.12%	100% (34/34)
<i>SOX2</i>	c.610G>A(p.A204T)	SNV	15.28	12.53-18.12	1.19	7.81%	100% (34/34)
<i>TEK</i>	c.1766A>G(p.Q589R)	SNV	14.59	12.21-17.91	1.39	9.56%	100% (34/34)
<i>TET2</i>	c.685del(p.T229Hfs*21)	DEL<15bp	5.47	5.47-5.47	0	0%	2.94% (1/34)
<i>TGFBR2</i>	c.457_458delinsG(p.K153Gfs*35)	SNV	10.91	7.82-13.52	1.58	14.52%	100% (34/34)
<i>TNFAIP3</i>	c.1773del(p.S592Lfs*105)	DEL<15bp	13.39	11.86-15.83	0.93	6.95%	100% (34/34)
<i>TOP1</i>	c.265C>T(p.R89*)	SNV	13.91	10.8-18.98	1.58	11.38%	100% (34/34)
<i>TSHR</i>	c.991G>A(p.G331S)	SNV	17.44	14.25-21.34	1.87	10.71%	100% (34/34)
<i>TUBB3</i>	c.1076G>A(p.R359H)	SNV	15.64	14.29-17.3	0.66	4.23%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>ZNF703</i>	c.1238del(p.G413Afs*130)	DEL<15bp	14.60	12.48-17.19	0.97	6.63%	100% (34/34)
<i>AKT3</i>	c.679T>C(p.Y227H)	SNV	13.39	5.77-18.71	3.1	23.18%	88.24% (30/34)
<i>ALK</i>	c.3989A>T(p.Y1330F)	SNV	14.99	12.6-17.78	1.08	7.17%	100% (34/34)
<i>ARID1A</i>	c.3524del(p.P1175Hfs*5)	DEL<15bp	22.01	19.57-24.35	1.21	5.49%	100% (34/34)
<i>CCN6</i>	c.491A>G(p.H164R)	SNV	24.75	22.21-27.04	1.13	4.55%	100% (34/34)
<i>CHEK1</i>	c.676del(p.T226Hfs*14)	DEL<15bp	21.86	16.78-27.6	2.15	9.84%	100% (34/34)
<i>CYP2D6</i>	c.638T>C(p.L213P)	SNV	16.40	15.9-17.14	0.53	3.24%	17.65% (6/34)
<i>DOT1L</i>	c.2438C>A(p.P813H)	SNV	24.57	21.08-28.14	1.5	6.09%	100% (34/34)
<i>DPYD</i>	c.232A>T(p.R78*)	SNV	47.65	41.07-55.17	3.06	6.42%	100% (34/34)
<i>ERBB2</i>	c.1076C>T(p.A359V)	SNV	24.11	21.28-27.22	1.54	6.38%	100% (34/34)
<i>ERBB3</i>	c.1300A>G(p.K434E)	SNV	22.99	20.26-26.67	1.53	6.64%	100% (34/34)
<i>ERBB4</i>	c.2050A>G(p.R684G)	SNV	24.50	19.1-30.28	2.86	11.65%	100% (34/34)
<i>ERCC4</i>	c.897_898del(p.Q300Vfs*2)	DEL<15bp	21.29	13.53-26.05	2.39	11.25%	100% (34/34)
<i>FAT1</i>	c.3757A>G(p.R1253G)	SNV	29.30	26.85-31.88	1.19	4.08%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>FGFR1</i>	c.1636A>G(p.N546D)	SNV	16.05	14.09-17.68	0.94	5.85%	100% (34/34)
<i>HNF1A</i>	c.864del(p.P291Qfs*51)	DEL<15bp	21.60	18.99-24.08	1.07	4.97%	100% (34/34)
<i>JAK1</i>	c.2580del(p.K860Nfs*16)	DEL<15bp	35.66	33.08-38.38	1.17	3.27%	100% (34/34)
<i>JAK2</i>	c.3391G>A(p.A1131T)	SNV	20.52	13.73-27.52	3.17	15.42%	97.06% (33/34)
<i>JARID2</i>	c.2263G>A(p.E755K)	SNV	25.26	22.74-27.97	1.21	4.79%	100% (34/34)
<i>KDM5A</i>	c.3650G>A(p.R1217Q)	SNV	23.45	21.66-27.67	1.17	5%	100% (34/34)
<i>KMT2B</i>	c.7997T>G(p.V2666G)	SNV	25.17	23.33-27.86	1.17	4.65%	100% (34/34)
<i>LRP1B</i>	c.11605T>C(p.C3869R)	SNV	5.16	3.25-9.09	1.51	29.26%	82.35% (28/34)
<i>NF1</i>	c.801G>A(p.W267*)	SNV	21.49	16.38-26.89	2.42	11.27%	100% (34/34)
<i>NF1</i>	c.1882del(p.Y628Tfs*3)	DEL<15bp	15.91	11.28-23.02	2.76	17.34%	97.06% (33/34)
<i>NKX2-1</i>	c.611A>G(p.Q204R)	SNV	24.96	22.84-27.35	1.14	4.55%	100% (34/34)
<i>NOTCH1</i>	c.4343C>A(p.A1448E)	SNV	20.63	18.28-22.77	1.06	5.12%	100% (34/34)
<i>PIK3CA</i>	c.353G>A(p.G118D)	SNV	22.50	17.97-28	2.25	10.02%	100% (34/34)
<i>PIK3CA</i>	c.263G>A(p.R88Q)	SNV	21.37	12.5-26.87	3.25	15.21%	100% (34/34)
<i>PIK3R1</i>	c.206C>G(p.S69W)	SNV	22.17	14.63-28.17	2.92	13.17%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>PIK3R2</i>	c.1322C>T(p.A441V)	SNV	23.91	22.13-25.19	0.77	3.23%	100% (34/34)
<i>PLK1</i>	c.781C>T(p.L261F)	SNV	24.90	24.9-24.9	0	0%	2.94% (1/34)
<i>SDHA</i>	c.1118C>A(p.P373Q)	SNV	4.25	3.47-5.14	0.44	10.46%	100% (34/34)
<i>NFI</i>	c.3721C>T(p.R1241*)	SNV	4.50	2.58-6.68	1.01	22.49%	100% (34/34)
<i>PMS1</i>	c.1001C>T(p.T334M)	SNV	32.16	32.16-32.16	0	0%	2.94% (1/34)
<i>POLD1</i>	c.1360C>A(p.R454S)	SNV	24.17	22.15-26.3	1.12	4.64%	100% (34/34)
<i>PTEN</i>	c.955_958del(p.T319*)	DEL<15bp	39.52	30.97-44.61	2.8	7.07%	85.29% (29/34)
<i>RICTOR</i>	c.3647G>A(p.R1216H)	SNV	21.63	18.95-25.68	1.66	7.66%	100% (34/34)
<i>RUNX1T1</i>	c.1292G>A(p.R431H)	SNV	22.52	20.43-25.46	1.2	5.35%	100% (34/34)
<i>SDHA</i>	c.332T>G(p.L111R)	SNV	23.52	20.01-27.9	1.6	6.81%	100% (34/34)
<i>SLC3A2</i>	c.899del(p.K300Rfs*31)	DEL<15bp	22.60	19.96-25.91	1.48	6.57%	100% (34/34)
<i>TGFBR2</i>	c.1363C>A(p.L455I)	SNV	17.21	15.18-20	1.17	6.81%	100% (34/34)
<i>TOP2A</i>	c.3613del(p.T1205Hfs*19)	DEL<15bp	13.26	10.88-15.92	1.17	8.80%	100% (34/34)
<i>TTF1</i>	c.88C>G(p.Q30E)	SNV	25.64	22.18-29.42	1.75	6.84%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>VHL</i>	c.196G>A(p.V66M)	SNV	16.44	14.23-18.71	1.05	6.36%	100% (34/34)
<i>ARID1A</i>	c.6060_6061del(p.L2021Afs*39)	DEL<15bp	16.26	14.53-17.86	0.92	5.63%	100% (34/34)
<i>ATM</i>	c.4980C>A(p.N1660K)	SNV	33.17	27.78-36.82	3.31	9.97%	26.47% (9/34)
<i>CDKN2A</i>	c.238del(p.R80Dfs*66)	DEL<15bp	14.29	12.67-16.39	0.86	6.05%	100% (34/34)
<i>CDKN2A</i>	c.365del(p.G122Afs*24)	DEL<15bp	18.17	16.85-20.28	0.83	4.55%	100% (34/34)
<i>CREBBP</i>	c.4769A>G(p.N1590S)	SNV	36.09	33.38-39.15	1.19	3.30%	100% (34/34)
<i>CREBBP</i>	c.4628A>T(p.D1543V)	SNV	34.47	29.76-37.97	1.82	5.29%	100% (34/34)
<i>CTNNB1</i>	c.1922C>T(p.T641I)	SNV	8.80	6.02-11.56	1.44	16.35%	100% (34/34)
<i>FANCF</i>	c.635C>A(p.P212Q)	SNV	15.95	13.75-17.38	0.78	4.90%	100% (34/34)
<i>FAT1</i>	c.12932dup(p.P4312Tfs*8)	INS<15bp	8.04	6.94-9.3	0.58	7.16%	88.24% (30/34)
<i>FAT1</i>	c.8766del(p.Y2923Ifs*5)	DEL<15bp	16.08	13.14-21.86	1.88	11.68%	100% (34/34)
<i>FGFR3</i>	c.1138G>A(p.G380R)	SNV	20.52	17.7-22.53	1.18	5.77%	100% (34/34)
<i>FGFR3</i>	c.2005C>G(p.R669G)	SNV	20.09	17.37-22.63	1.15	5.72%	100% (34/34)
<i>GATA3</i>	c.1274del(p.P425Rfs*51)	DEL<15bp	7.54	6.3-8.6	0.56	7.37%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>KEAP1</i>	c.790G>A(p.V264I)	SNV	18.08	15.92-19.48	0.84	4.63%	100% (34/34)
<i>PKHD1</i>	c.12065C>T(p.P4022L)	SNV	15.58	13.77-17.26	0.94	6.02%	100% (34/34)
<i>TERT</i>	c.-146C>T	SNV	18.98	15.83-21.69	1.69	8.89%	100% (34/34)
<i>VHL</i>	c.61G>A(p.E21K)	SNV	9.70	7.85-11.96	1.05	10.83%	100% (34/34)
<i>APC</i>	c.7432C>T(p.Q2478*)	SNV	15.70	13.07-17.55	1.17	7.43%	100% (34/34)
<i>ARID2</i>	c.513T>G(p.N171K)	SNV	14.86	12.59-18.68	1.39	9.38%	100% (34/34)
<i>BRAF</i>	c.1088C>T(p.S363F)	SNV	41.42	39.01-44.46	1.62	3.92%	100% (34/34)
<i>BRAF</i>	c.1798_1799delinsAA(p.V600K)	SNV	40.23	36.82-43.62	1.47	3.65%	100% (34/34)
<i>BRCA2</i>	c.9976A>T(p.K3326*)	SNV	57.94	57.29-58.93	0.65	1.11%	32.35% (11/34)
<i>CDH1</i>	c.1607A>T(p.N536I)	SNV	19.55	16.25-21.66	1.39	7.11%	100% (34/34)
<i>CREBBP</i>	c.6763C>T(p.P2255S)	SNV	28.20	25.35-32.73	1.57	5.56%	100% (34/34)
<i>CUL3</i>	c.61T>A(p.F21I)	SNV	13.40	10.63-16.52	1.54	11.52%	100% (34/34)
<i>DPYD</i>	c.979C>T(p.P327S)	SNV	14.06	11.88-16.72	1.25	8.92%	100% (34/34)
<i>DPYD</i>	c.2228G>A(p.G743E)	SNV	14.02	11.07-17.86	1.7	12.15%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>ERBB4</i>	c.3509C>T(p.P1170L)	SNV	12.70	11.32-15	0.82	6.44%	100% (34/34)
<i>FAT1</i>	c.3929C>T(p.S1310L)	SNV	15.19	13.43-17.37	0.9	5.92%	100% (34/34)
<i>FGFR1</i>	c.995C>T(p.S332F)	SNV	25.30	22.17-30.02	1.73	6.82%	100% (34/34)
<i>FGFR2</i>	c.170C>T(p.S57L)	SNV	14.32	12.23-17.11	1.26	8.79%	100% (34/34)
<i>FGFR3</i>	c.1816G>A(p.E606K)	SNV	38.36	33.94-42.7	1.99	5.19%	100% (34/34)
<i>FGFR4</i>	c.2123C>T(p.S708F)	SNV	15.76	11.75-20.24	2	12.69%	100% (34/34)
<i>FLT4</i>	c.1538G>A(p.G513E)	SNV	29.72	25.94-33.64	1.65	5.57%	100% (34/34)
<i>FLT4</i>	c.758C>T(p.T253I)	SNV	14.54	11.08-18.01	1.89	13.02%	100% (34/34)
<i>GRIN2A</i>	c.1864_1865delinsTT(p.P622F)	SNV	21.84	18.86-25.42	1.75	8.01%	100% (34/34)
<i>GRIN2A</i>	c.2890C>A(p.Q964K)	SNV	12.55	9.58-14.9	1.25	9.94%	100% (34/34)
<i>GRM3</i>	c.1696C>T(p.P566S)	SNV	25.18	22.97-27.46	1.02	4.05%	100% (34/34)
<i>GRM8</i>	c.2125G>A(p.G709R)	SNV	11.84	10.14-13.05	0.74	6.23%	100% (34/34)
<i>KDR</i>	c.3824G>A(p.R1275K)	SNV	13.92	10.69-17.57	1.81	13.03%	100% (34/34)
<i>LRP1B</i>	c.1651C>T(p.P551S)	SNV	25.46	22.15-28.08	1.37	5.39%	100% (34/34)
<i>LRP1B</i>	c.8872C>T(p.Q2958*)	SNV	25.46	21.64-28.74	1.46	5.75%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>LRP1B</i>	c.9556C>T(p.R3186C)	SNV	24.99	21.06-27.64	1.67	6.69%	100% (34/34)
<i>NF1</i>	c.7003C>T(p.P2335S)	SNV	14.12	11.19-18.01	1.57	11.15%	100% (34/34)
<i>NOTCH1</i>	c.2080G>A(p.E694K)	SNV	39.20	38.26-39.58	0.49	1.24%	17.65% (6/34)
<i>PIK3C3</i>	c.1403G>A(p.G468D)	SNV	13.61	10.21-18.01	1.79	13.13%	100% (34/34)
<i>PLCB4</i>	c.2405G>A(p.R802Q)	SNV	23.51	19.86-28.35	1.75	7.45%	100% (34/34)
<i>PRF1</i>	c.805C>T(p.H269Y)	SNV	13.79	11.97-15.6	0.95	6.88%	100% (34/34)
<i>RET</i>	c.392C>T(p.S131F)	SNV	14.49	12.63-18.7	1.23	8.47%	100% (34/34)
<i>ROS1</i>	c.1696G>A(p.V566M)	SNV	13.36	11.68-15.37	0.94	7.02%	100% (34/34)
<i>SPOP</i>	c.718C>T(p.R240*)	SNV	50.26	45.53-53.75	2	3.97%	100% (34/34)
<i>TEK</i>	c.1717G>A(p.D573N)	SNV	26.39	24.38-29.16	1.21	4.57%	100% (34/34)
<i>TERT</i>	c.-124C>T	SNV	32.39	26.04-39.66	2.78	8.58%	97.06% (33/34)
<i>TP53</i>	c.856G>T(p.E286*)	SNV	23.66	18.85-28.79	2.15	9.10%	100% (34/34)
<i>APC</i>	c.1490del(p.L497Qfs*9)	DEL<15bp	25.66	19.51-33.66	3.15	12.29%	100% (34/34)
<i>CDK12</i>	c.700_703delinsCATA (p.D234_D235delins HN)	SNV	5.90	5.35-6.58	0.3	5.11%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>FANCI</i>	c.2044G>A(p.V682I)	SNV	19.24	11.49-24.38	3.09	16.06%	100% (34/34)
<i>SETBP1</i>	c.199C>T(p.R67W)	SNV	6.11	4.78-8.78	0.87	14.29%	100% (34/34)
<i>TOP2A</i>	c.1773C>A(p.S591R)	SNV	11.52	7.43-14.29	1.71	14.85%	100% (34/34)
<i>TP53</i>	c.673-1G>T	SNV	31.12	26.03-36.15	2.18	7%	100% (34/34)
<i>CDK12</i>	c.278G>A(p.R93Q)	SNV	4.51	4.11-4.91	0.21	4.69%	100% (34/34)
<i>SMAD4</i>	c.394C>T(p.H132Y)	SNV	4.29	2.04-6.74	1.08	25.26%	100% (34/34)
<i>CDK12</i>	c.56G>C(p.G19A)	SNV	4.13	3.78-4.51	0.23	5.54%	100% (34/34)
<i>ATM</i>	c.1744T>C(p.F582L)	SNV	18.80	15.17-21.53	2.24	11.93%	41.18% (14/34)
<i>ATR</i>	c.1678G>C(p.E560Q)	SNV	13.73	10-19.48	2.45	17.82%	94.12% (32/34)
<i>CDH1</i>	c.796G>T(p.V266F)	SNV	34.94	29.23-38.97	2.36	6.77%	97.06% (33/34)
<i>DDR2</i>	c.371G>A(p.R124Q)	SNV	16.53	14.04-18.93	1.24	7.47%	100% (34/34)
<i>EGFR</i>	c.2303_2311dup(p.S768_D770dup)	INS<15bp	46.70	43.73-49.52	1.53	3.27%	100% (34/34)
<i>IRF2</i>	c.919C>T(p.P307S)	SNV	37.25	35.58-39.26	0.98	2.64%	38.24% (13/34)
<i>ROSI</i>	c.1885A>G(p.I629V)	SNV	17.94	11.82-22.27	2.3	12.85%	97.06% (33/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>KDR</i>	c.3095G>A(p.R1032Q)	SNV	1.88	1.88-1.88	0	0%	2.94% (1/34)
<i>SMAD4</i>	c.353C>T(p.A118V)	SNV	1.90	1.9-1.9	0	0%	2.94% (1/34)
<i>SBDS</i>	c.287C>A(p.S96*)	SNV	3.81	3.81-3.81	0	0%	2.94% (1/34)
<i>TP53</i>	c.578A>G(p.H193R)	SNV	54.97	49.83-58.47	1.95	3.55%	100% (34/34)
<i>ADGRB3</i>	c.4068G>T(p.M1356I)	SNV	6.60	4.44-8.83	1.03	15.65%	100% (34/34)
<i>APC</i>	c.2544del(p.D849Ifs*12)	DEL<15bp	9.48	7.19-11.24	0.97	10.18%	100% (34/34)
<i>APC</i>	c.4666dup(p.T1556Nfs*3)	INS<15bp	9.06	7.18-10.93	0.88	9.68%	100% (34/34)
<i>ARAF</i>	c.23del(p.P8Lfs*26)	DEL<15bp	25.24	22.62-27.45	1.25	4.96%	100% (34/34)
<i>ARID1A</i>	c.3999_4001dup(p.Q1334dup)	INS<15bp	18.71	16.78-19.93	0.88	4.69%	88.24% (30/34)
<i>ARID1A</i>	c.5769del(p.F1924Sfs*59)	DEL<15bp	24.84	22.87-28.14	1.22	4.89%	100% (34/34)
<i>ARID1B</i>	c.5432del(p.G1811Vfs*27)	DEL<15bp	25.13	23.6-27.45	0.86	3.41%	100% (34/34)
<i>BRCA1</i>	c.2077_2082delinsAACAGT (p.D693N)	SNV	42.20	39.97-43.97	1.36	3.22%	29.41% (10/34)
<i>BRCA2</i>	c.1114A>T(p.N372Y)	SNV	11.11	8.35-13.29	1.26	11.34%	100% (34/34)
<i>CASP8</i>	c.185T>G(p.L62R)	SNV	26.48	24.41-28.82	1.07	4.04%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>CASP8</i>	c.1345C>T(p.R449*)	SNV	9.34	6.68-10.66	0.9	9.69%	100% (34/34)
<i>CBL</i>	c.279G>C(p.Q93H)	SNV	26.51	23.07-29.37	1.59	5.99%	100% (34/34)
<i>CHD8</i>	c.5304del(p.S1769Lfs*28)	DEL<15bp	6.13	5.04-7.31	0.48	7.78%	100% (34/34)
<i>CREBBP</i>	c.5875G>A(p.A1959T)	SNV	5.79	3.92-7.45	0.85	14.71%	100% (34/34)
<i>CTCF</i>	c.610dup(p.T204Nfs*26)	INS<15bp	23.87	21.51-27.16	1.43	5.97%	100% (34/34)
<i>EPHA3</i>	c.1938del(p.E647Rfs*9)	DEL<15bp	6.48	5-9.85	0.94	14.50%	100% (34/34)
<i>FAT1</i>	c.12157G>A(p.V4053I)	SNV	7.90	6.41-10.03	0.65	8.16%	100% (34/34)
<i>FLCN</i>	c.1285dup(p.H429Pfs*27)	INS<15bp	17.77	15.31-20.44	1.12	6.30%	100% (34/34)
<i>JAK1</i>	c.1289del(p.P430Rfs*2)	DEL<15bp	22.36	20.05-24.72	1.24	5.56%	100% (34/34)
<i>JAK1</i>	c.2580del(p.K860Nfs*16)	DEL<15bp	21.75	19.66-24.88	1.12	5.14%	100% (34/34)
<i>KMT2B</i>	c.521dup(p.T176Dfs*8)	INS<15bp	19.19	16.49-21.94	1.29	6.70%	94.12% (32/34)
<i>LHCGR</i>	c.47T>A(p.L16Q)	SNV	15.76	15.1-17.32	0.66	4.20%	26.47% (9/34)
<i>LRP1B</i>	c.8446G>T(p.V2816L)	SNV	26.58	22.67-30	1.83	6.87%	100% (34/34)
<i>LRP1B</i>	c.11447C>T(p.A3816V)	SNV	37.06	37.06-37.06	0	0%	2.94% (1/34)
<i>MAP3K1</i>	c.862del(p.R288Efs*24)	DEL<15bp	6.27	4.39-7.76	0.67	10.71%	100% (34/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>MECOM</i>	c.2013del(p.K671Nfs*45)	DEL<15bp	24.79	21.76-26.86	1.08	4.34%	100% (34/34)
<i>MED12</i>	c.4702G>A(p.G1568S)	SNV	10.53	8.47-12.76	1	9.50%	100% (34/34)
<i>HNF1A</i>	c.1849G>A(p.V617I)	SNV	3.89	2.29-5.1	0.65	16.60%	97.06% (33/34)
<i>MED12</i>	c.5324C>A(p.A1775D)	SNV	10.22	8.65-11.39	0.8	7.84%	100% (34/34)
<i>ATM</i>	c.6581C>T(p.T2194I)	SNV	3.68	2.16-5.26	0.86	23.22%	88.24% (30/34)
<i>NFKB1A</i>	c.91_93del(p.D31del)	DEL<15bp	22.93	18.81-26.03	1.47	6.41%	100% (34/34)
<i>NOTCH2</i>	c.6140G>T(p.R2047L)	SNV	10.54	8.97-11.75	0.64	6.09%	100% (34/34)
<i>NSD1</i>	c.3953G>A(p.R1318H)	SNV	5.98	3.61-8.62	1.11	18.60%	100% (34/34)
<i>NSD1</i>	c.3784del(p.M1262Cfs*43)	DEL<15bp	25.13	22.25-28.07	1.25	4.95%	100% (34/34)
<i>NSD1</i>	c.4590dup(p.G1531Wfs*3)	INS<15bp	9.01	6.88-11.02	0.89	9.91%	100% (34/34)
<i>PDCD1</i>	c.105del(p.T36Pfs*9)	DEL<15bp	10.01	8.01-12.9	1.14	11.37%	100% (34/34)
<i>PIK3CA</i>	c.1636C>A(p.Q546K)	SNV	70.63	48.11-77.18	5.71	8.08%	100% (34/34)
<i>PTEN</i>	c.138C>G(p.Y46*)	SNV	20.34	2.89-27.43	4.45	21.89%	97.06% (33/34)
<i>PTEN</i>	c.377C>T(p.A126V)	SNV	9.64	6.38-13.13	1.41	14.61%	100% (34/34)
<i>PTEN</i>	c.188del(p.N63Tfs*36)	DEL<15bp	9.13	5.87-13.16	1.42	15.53%	91.18% (31/34)

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range (%)	SD	%CV	Positive Call Rate % (n/N)
<i>PTEN</i>	c.800del(p.K267Rfs*9)	DEL<15bp	25.20	21.86-27.88	1.71	6.77%	100% (34/34)
<i>RNF43</i>	c.673del(p.R225Afs*194)	DEL<15bp	25.90	22.83-28.72	1.34	5.18%	100% (34/34)
<i>SLC3A2</i>	c.899del(p.K300Rfs*31)	DEL<15bp	25.40	21.65-28.88	1.65	6.51%	100% (34/34)
<i>SMARCA 4</i>	c.2318T>C(p.L773P)	SNV	24.44	22.41-26.63	1.09	4.45%	100% (34/34)
<i>SPRY4</i>	c.520del(p.R174Gfs*85)	DEL<15bp	6.19	5.09-7.69	0.64	10.34%	100% (34/34)
<i>STMN1</i>	c.46T>G(p.S16A)	SNV	6.80	4.88-8.89	1.07	15.75%	100% (34/34)
<i>TOP1</i>	c.1378C>T(p.Q460*)	SNV	10.75	8.75-12.4	0.89	8.32%	100% (34/34)
<i>TOP2A</i>	c.4303del(p.R1435Gfs*13)	DEL<15bp	23.09	21.24-25.56	1.22	5.28%	100% (34/34)
<i>TP53</i>	c.214_215delinsTG(p.P72C)	SNV	26.20	23.43-28.71	1.22	4.66%	100% (34/34)

## Appendix E. Accuracy

### Appendix E.1. Concordance for SNV by Gene

Gene	Number of Exons	Number of Unique Mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>AKT1</i>	3	5	5	100% (51.01% ,100%)	99.93% (99.61% ,100%)
<i>AKT2</i>	5	7	8	100% (60.97% ,100%)	99.93% (99.61% ,100%)
<i>AKT3</i>	4	5	4	100% (34.24% ,100%)	99.79% (99.37% ,99.93%)
<i>ALK</i>	15	31	30	85% (63.96% ,94.76%)	99.77% (99.59% ,99.87%)
<i>AMER1</i>	2	26	28	94.44% (74.24% ,99.72%)	99.76% (99.53% ,99.88%)
<i>APC</i>	10	76	69	94.83% (85.86% ,98.23%)	99.79% (99.66% ,99.87%)
<i>AR</i>	5	13	14	88.89% (56.5% ,99.43%)	99.85% (99.63% ,99.94%)
<i>ARAF</i>	6	6	9	100% (56.55% ,100%)	99.95% (99.69% ,100%)
<i>ARID1A</i>	14	53	57	100% (90.82% ,100%)	99.76% (99.6% ,99.85%)
<i>ARID1B</i>	10	32	28	100% (79.61% ,100%)	99.75% (99.59% ,99.84%)
<i>ARID2</i>	14	31	24	83.33% (60.78% ,94.16%)	99.76% (99.6% ,99.86%)
<i>ASXL1</i>	6	15	18	88.89% (56.5% ,99.43%)	99.87% (99.72% ,99.94%)
<i>ATM</i>	34	57	55	79.31% (61.61% ,90.15%)	99.69% (99.56% ,99.79%)
<i>ATR</i>	14	22	23	76.92% (49.74% ,91.82%)	99.89% (99.78% ,99.94%)
<i>ATRX</i>	11	23	20	78.57% (52.41% ,92.43%)	99.88% (99.77% ,99.94%)
<i>AURKA</i>	4	3	4	100% (5.13% ,100%)	99.83% (99.4% ,99.95%)
<i>AURKB</i>	2	1	2	100% (5.13% ,100%)	100% (99.63% ,100%)
<i>AXIN2</i>	8	11	14	88.89% (56.5% ,99.43%)	99.92% (99.71% ,99.98%)
<i>AXL</i>	8	8	11	100% (56.55% ,100%)	99.84% (99.53% ,99.95%)

Gene	Number of Exons	Number of Unique Mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>B2M</i>	2	6	5	100% (51.01% ,100%)	99.44% (97.98% ,99.85%)
<i>BAP1</i>	8	9	12	100% (60.97% ,100%)	99.86% (99.6% ,99.95%)
<i>BARD1</i>	4	6	7	100% (51.01% ,100%)	99.91% (99.69% ,99.98%)
<i>BCL2</i>	1	1	1	100% (5.13% ,100%)	100% (99.47% ,100%)
<i>BCR</i>	4	8	7	50% (2.56% ,97.44%)	99.84% (99.66% ,99.93%)
<i>BLM</i>	10	14	20	80% (49.02% ,94.33%)	99.91% (99.76% ,99.96%)
<i>BMPRIA</i>	3	5	4	100% (56.55% ,100%)	100% (99.76% ,100%)
<i>BRAF</i>	8	44	43	97.37% (86.51% ,99.87%)	99.73% (99.42% ,99.88%)
<i>KRAS</i>	4	66	64	98.36% (91.28% ,99.92%)	99.02% (97.72% ,99.58%)
<i>BRCA1</i>	9	36	41	80% (49.02% ,94.33%)	99.53% (99.32% ,99.68%)
<i>BRCA2</i>	10	27	29	92.31% (66.69% ,99.61%)	99.86% (99.77% ,99.92%)
<i>FGFR3</i>	10	33	33	100% (77.19% ,100%)	99.17% (98.72% ,99.46%)
<i>BRD4</i>	9	12	14	100% (64.57% ,100%)	99.88% (99.71% ,99.95%)
<i>BRIP1</i>	10	13	13	71.43% (35.89% ,91.78%)	99.84% (99.65% ,99.93%)
<i>BTG2</i>	2	1	2	100% (5.13% ,100%)	100% (99.2% ,100%)
<i>BTK</i>	5	5	5	66.67% (20.77% ,98.29%)	99.9% (99.63% ,99.97%)
<i>BUB1B</i>	6	7	10	80% (37.55% ,98.97%)	99.94% (99.77% ,99.98%)
<i>CASP8</i>	3	10	9	100% (67.56% ,100%)	99.87% (99.51% ,99.96%)
<i>CBL</i>	6	11	10	100% (60.97% ,100%)	99.82% (99.57% ,99.92%)
<i>CCND1</i>	5	5	6	100% (56.55% ,100%)	100% (99.57% ,100%)
<i>CCNE1</i>	5	5	6	100% (34.24% ,100%)	99.76% (99.29% ,99.92%)

Gene	Number of Exons	Number of Unique Mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>CD274</i>	1	1	1	100% (5.13% ,100%)	100% (99.56% ,100%)
<i>CDC73</i>	5	8	7	100% (60.97% ,100%)	99.87% (99.54% ,99.97%)
<i>CDH1</i>	13	22	25	92.31% (66.69% ,99.61%)	99.66% (99.35% ,99.82%)
<i>CDK12</i>	8	21	19	72.73% (43.44% ,90.25%)	99.78% (99.59% ,99.88%)
<i>CDK4</i>	1	1	1	100% (5.13% ,100%)	100% (99.58% ,100%)
<i>CDK6</i>	4	4	4	100% (43.85% ,100%)	99.9% (99.42% ,99.99%)
<i>CDK8</i>	4	5	4	100% (34.24% ,100%)	99.78% (99.37% ,99.93%)
<i>CDKN1A</i>	3	4	4	100% (51.01% ,100%)	100% (99.22% ,100%)
<i>CDKN1B</i>	2	2	2	0% (0% ,94.87%)	99.83% (99.06% ,99.99%)
<i>CDKN1C</i>	1	2	2	100% (34.24% ,100%)	100% (99.6% ,100%)
<i>CDKN2A</i>	4	13	15	100% (70.09% ,100%)	99.13% (97.8% ,99.66%)
<i>CDKN2B</i>	2	3	4	50% (2.56% ,97.44%)	99.76% (98.65% ,99.99%)
<i>CDKN2C</i>	2	2	2	100% (5.13% ,100%)	99.8% (98.89% ,99.99%)
<i>CEBPA</i>	2	5	5	NaN% (NaN% ,NaN%)	99.54% (98.92% ,99.8%)
<i>CHD4</i>	18	25	25	100% (80.64% ,100%)	99.84% (99.7% ,99.92%)
<i>CHEK1</i>	4	4	4	50% (2.56% ,97.44%)	99.86% (99.49% ,99.96%)
<i>CHEK2</i>	5	6	8	100% (51.01% ,100%)	99.89% (99.59% ,99.97%)
<i>CREBBP</i>	19	45	42	95.45% (78.2% ,99.77%)	99.69% (99.53% ,99.79%)
<i>CRKL</i>	3	3	4	100% (34.24% ,100%)	99.89% (99.38% ,99.99%)
<i>CSF1R</i>	6	8	11	100% (51.01% ,100%)	99.86% (99.65% ,99.95%)
<i>CTCF</i>	8	13	15	100% (70.09% ,100%)	99.82% (99.53% ,99.93%)

Gene	Number of Exons	Number of Unique Mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>CTLA4</i>	1	2	2	100% (34.24% ,100%)	100% (99.43% ,100%)
<i>CTNNB1</i>	8	28	27	95.83% (79.76% ,99.79%)	99.83% (99.56% ,99.93%)
<i>CUL3</i>	7	7	9	100% (56.55% ,100%)	99.9% (99.65% ,99.97%)
<i>CXCR4</i>	2	4	5	100% (43.85% ,100%)	99.91% (99.47% ,100%)
<i>CYLD</i>	2	2	3	0% (0% ,94.87%)	99.97% (99.8% ,100%)
<i>DAXX</i>	5	8	9	83.33% (43.65% ,99.15%)	99.91% (99.67% ,99.98%)
<i>DDR2</i>	7	12	13	100% (70.09% ,100%)	99.88% (99.66% ,99.96%)
<i>DICER1</i>	15	31	27	90.48% (71.09% ,97.35%)	99.83% (99.68% ,99.91%)
<i>DNMT3A</i>	15	29	25	100% (82.41% ,100%)	99.6% (99.28% ,99.77%)
<i>DOTIL</i>	15	28	34	94.44% (74.24% ,99.72%)	99.78% (99.6% ,99.88%)
<i>EGFR</i>	14	36	37	82.61% (62.86% ,93.02%)	99.64% (99.38% ,99.79%)
<i>NTRK1</i>	10	13	15	85.71% (48.69% ,99.27%)	99.75% (99.45% ,99.88%)
<i>PIK3CA</i>	15	109	94	92.31% (84.96% ,96.22%)	99.42% (99.09% ,99.63%)
<i>EP300</i>	19	30	38	89.47% (68.61% ,97.06%)	99.85% (99.73% ,99.91%)
<i>EPAS1</i>	11	14	16	100% (74.12% ,100%)	99.88% (99.66% ,99.96%)
<i>EPCAM</i>	3	2	4	100% (34.24% ,100%)	100% (99.59% ,100%)
<i>EPHA2</i>	8	16	21	72.73% (43.44% ,90.25%)	99.83% (99.6% ,99.93%)
<i>EPHA3</i>	10	17	19	100% (77.19% ,100%)	99.86% (99.65% ,99.95%)
<i>EPHA5</i>	4	6	7	80% (37.55% ,98.97%)	99.97% (99.82% ,100%)
<i>ERBB2</i>	13	23	23	91.67% (64.61% ,99.57%)	99.71% (99.48% ,99.84%)
<i>ERBB3</i>	13	19	20	85.71% (60.06% ,95.99%)	99.88% (99.71% ,99.95%)

Gene	Number of Exons	Number of Unique Mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>ERBB4</i>	16	25	25	83.33% (60.78% ,94.16%)	99.82% (99.63% ,99.91%)
<i>ERCC1</i>	3	4	4	100% (34.24% ,100%)	99.78% (99.19% ,99.94%)
<i>ERCC2</i>	10	12	13	100% (64.57% ,100%)	99.78% (99.49% ,99.91%)
<i>ERCC3</i>	9	10	12	75% (40.93% ,92.85%)	99.91% (99.69% ,99.98%)
<i>ERCC4</i>	5	11	13	100% (60.97% ,100%)	99.82% (99.57% ,99.92%)
<i>ERCC5</i>	11	18	21	100% (72.25% ,100%)	99.77% (99.56% ,99.89%)
<i>ESR1</i>	6	13	15	87.5% (52.91% ,99.36%)	99.72% (99.34% ,99.88%)
<i>ETV1</i>	2	2	3	100% (34.24% ,100%)	100% (99.73% ,100%)
<i>ETV4</i>	3	2	3	NaN% (NaN% ,NaN%)	99.86% (99.5% ,99.96%)
<i>ETV5</i>	1	3	3	100% (34.24% ,100%)	99.93% (99.63% ,100%)
<i>ETV6</i>	3	4	5	100% (43.85% ,100%)	99.93% (99.58% ,100%)
<i>EWSR1</i>	2	1	2	100% (5.13% ,100%)	100% (99.81% ,100%)
<i>EXT1</i>	5	9	9	100% (64.57% ,100%)	99.91% (99.67% ,99.98%)
<i>EXT2</i>	5	6	9	100% (43.85% ,100%)	99.87% (99.61% ,99.95%)
<i>EZH2</i>	8	8	7	80% (37.55% ,98.97%)	99.87% (99.61% ,99.95%)
<i>FANCA</i>	19	23	27	100% (67.56% ,100%)	99.66% (99.43% ,99.79%)
<i>FANCC</i>	6	6	8	100% (5.13% ,100%)	99.7% (99.3% ,99.87%)
<i>FANCD2</i>	8	10	16	60% (23.07% ,88.24%)	99.89% (99.73% ,99.95%)
<i>FANCE</i>	3	3	5	100% (5.13% ,100%)	99.88% (99.55% ,99.97%)
<i>FANCF</i>	2	3	5	100% (34.24% ,100%)	99.91% (99.5% ,100%)
<i>FANCG</i>	9	10	10	100% (60.97% ,100%)	99.79% (99.45% ,99.92%)

Gene	Number of Exons	Number of Unique Mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>FANCI</i>	10	11	16	75% (30.06% ,98.72%)	99.82% (99.62% ,99.91%)
<i>FANCL</i>	4	4	5	100% (43.85% ,100%)	99.91% (99.5% ,100%)
<i>FANCM</i>	4	6	11	66.67% (20.77% ,98.29%)	99.95% (99.86% ,99.98%)
<i>FAT1</i>	16	59	65	96% (80.46% ,99.79%)	99.75% (99.65% ,99.82%)
<i>FBXW7</i>	9	35	30	88.46% (71.02% ,96%)	99.48% (99.02% ,99.73%)
<i>FGF19</i>	2	3	3	100% (34.24% ,100%)	99.85% (99.13% ,99.99%)
<i>FGFR1</i>	8	7	8	100% (60.97% ,100%)	99.96% (99.77% ,100%)
<i>FGFR2</i>	10	17	13	100% (75.75% ,100%)	99.8% (99.52% ,99.91%)
<i>FGFR4</i>	11	17	18	77.78% (45.26% ,93.68%)	99.64% (99.28% ,99.82%)
<i>FH</i>	5	9	7	100% (43.85% ,100%)	99.61% (99.15% ,99.82%)
<i>FLCN</i>	9	10	11	88.89% (56.5% ,99.43%)	99.94% (99.67% ,100%)
<i>FLT1</i>	16	21	17	100% (78.47% ,100%)	99.83% (99.64% ,99.92%)
<i>FLT3</i>	13	21	19	73.33% (48.05% ,89.1%)	99.8% (99.56% ,99.91%)
<i>FLT4</i>	13	19	20	92.86% (68.53% ,99.63%)	99.87% (99.7% ,99.95%)
<i>FOXA1</i>	2	10	12	100% (51.01% ,100%)	99.58% (99.08% ,99.81%)
<i>FOXL2</i>	1	7	7	100% (51.01% ,100%)	99.73% (99.22% ,99.91%)
<i>FOXP1</i>	1	1	1	NaN% (NaN% ,NaN%)	99.95% (99.72% ,100%)
<i>GATA1</i>	3	5	5	100% (34.24% ,100%)	99.76% (99.29% ,99.92%)
<i>GATA2</i>	3	3	3	100% (43.85% ,100%)	100% (99.73% ,100%)
<i>GATA3</i>	5	14	14	100% (74.12% ,100%)	99.77% (99.34% ,99.92%)
<i>GATA4</i>	6	14	14	100% (67.56% ,100%)	99.55% (99.01% ,99.79%)

Gene	Number of Exons	Number of Unique Mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>GATA6</i>	2	3	9	NaN% (NaN% ,NaN%)	99.83% (99.51% ,99.94%)
<i>GNAI1</i>	6	6	7	100% (43.85% ,100%)	99.72% (99.18% ,99.91%)
<i>GNAQ</i>	2	3	3	100% (5.13% ,100%)	99.81% (99.33% ,99.95%)
<i>GNAS</i>	5	9	8	100% (43.85% ,100%)	99.49% (98.9% ,99.77%)
<i>GRIN2A</i>	14	35	29	92.59% (76.63% ,97.94%)	99.82% (99.64% ,99.91%)
<i>GRM3</i>	5	24	24	100% (82.41% ,100%)	99.77% (99.5% ,99.9%)
<i>HDAC2</i>	5	7	7	100% (34.24% ,100%)	99.66% (99.2% ,99.85%)
<i>HGF</i>	5	6	7	60% (23.07% ,88.24%)	99.95% (99.74% ,100%)
<i>HNF1A</i>	6	11	13	83.33% (43.65% ,99.15%)	99.74% (99.38% ,99.89%)
<i>HRAS</i>	3	3	4	100% (34.24% ,100%)	99.8% (98.9% ,99.99%)
<i>IDH1</i>	6	11	10	85.71% (48.69% ,99.27%)	99.68% (99.17% ,99.87%)
<i>IDH2</i>	6	7	8	100% (34.24% ,100%)	99.63% (99.14% ,99.84%)
<i>IFNGR1</i>	2	1	2	100% (5.13% ,100%)	100% (99.74% ,100%)
<i>IGF1R</i>	9	11	12	100% (72.25% ,100%)	99.98% (99.86% ,100%)
<i>IGF2</i>	2	1	3	100% (5.13% ,100%)	100% (99.3% ,100%)
<i>IKBKE</i>	10	9	12	100% (60.97% ,100%)	99.84% (99.53% ,99.95%)
<i>IL7R</i>	8	12	15	90% (59.58% ,99.49%)	99.85% (99.47% ,99.96%)
<i>INPP4B</i>	13	14	13	100% (60.97% ,100%)	99.71% (99.43% ,99.85%)
<i>IRF2</i>	5	4	5	100% (51.01% ,100%)	100% (99.63% ,100%)
<i>JAK1</i>	10	12	15	100% (67.56% ,100%)	99.88% (99.7% ,99.95%)
<i>JAK2</i>	9	11	13	85.71% (48.69% ,99.27%)	99.88% (99.7% ,99.95%)

Gene	Number of Exons	Number of Unique Mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>JAK3</i>	15	20	23	100% (75.75% ,100%)	99.76% (99.53% ,99.88%)
<i>JUN</i>	1	1	1	NaN% (NaN% ,NaN%)	99.9% (99.43% ,99.99%)
<i>KDM5A</i>	14	19	22	100% (67.56% ,100%)	99.78% (99.61% ,99.88%)
<i>KDR</i>	20	29	24	90.48% (71.09% ,97.35%)	99.8% (99.61% ,99.9%)
<i>KEAP1</i>	6	16	18	100% (78.47% ,100%)	99.89% (99.61% ,99.97%)
<i>KIT</i>	11	13	13	100% (67.56% ,100%)	99.83% (99.6% ,99.93%)
<i>KMT2A</i>	13	30	32	76.47% (52.74% ,90.44%)	99.89% (99.81% ,99.94%)
<i>KMT2C</i>	3	8	8	100% (5.13% ,100%)	99.95% (99.9% ,99.98%)
<i>LMO1</i>	3	2	3	100% (5.13% ,100%)	99.79% (98.8% ,99.99%)
<i>LRP1B</i>	63	119	79	84.52% (75.3% ,90.73%)	99.74% (99.65% ,99.82%)
<i>LYN</i>	5	6	7	100% (56.55% ,100%)	99.93% (99.63% ,100%)
<i>LZTR1</i>	10	11	13	75% (40.93% ,92.85%)	99.88% (99.65% ,99.96%)
<i>MAP2K1</i>	3	4	4	100% (43.85% ,100%)	99.92% (99.52% ,100%)
<i>MAP2K2</i>	4	3	4	100% (5.13% ,100%)	99.83% (99.4% ,99.95%)
<i>MAP2K4</i>	6	10	9	100% (67.56% ,100%)	99.84% (99.41% ,99.96%)
<i>MAP3K1</i>	8	24	27	90.91% (62.26% ,99.53%)	99.71% (99.51% ,99.83%)
<i>MAX</i>	2	1	3	100% (5.13% ,100%)	100% (98.49% ,100%)
<i>MCL1</i>	2	4	8	100% (43.85% ,100%)	99.9% (99.46% ,100%)
<i>MDM2</i>	3	3	3	100% (34.24% ,100%)	99.93% (99.62% ,100%)
<i>MDM4</i>	4	5	5	100% (43.85% ,100%)	99.86% (99.51% ,99.96%)
<i>MED12</i>	16	34	33	89.47% (68.61% ,97.06%)	99.77% (99.62% ,99.86%)

Gene	Number of Exons	Number of Unique Mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>MEN1</i>	4	5	6	100% (51.01% ,100%)	99.95% (99.69% ,100%)
<i>MET</i>	9	25	32	100% (75.75% ,100%)	99.69% (99.47% ,99.82%)
<i>MITF</i>	5	6	10	75% (30.06% ,98.72%)	99.87% (99.53% ,99.96%)
<i>MLH1</i>	4	5	8	75% (30.06% ,98.72%)	99.96% (99.75% ,100%)
<i>MLH3</i>	4	8	12	85.71% (48.69% ,99.27%)	99.98% (99.87% ,100%)
<i>MPL</i>	5	5	9	100% (56.55% ,100%)	100% (99.8% ,100%)
<i>MRE11A</i>	1	3	11	0% (0% ,56.15%)	100% (99.82% ,100%)
<i>MSH2</i>	7	13	17	83.33% (43.65% ,99.15%)	99.75% (99.48% ,99.88%)
<i>MSH6</i>	6	8	15	100% (56.55% ,100%)	99.93% (99.78% ,99.97%)
<i>MTOR</i>	23	32	29	82.35% (58.97% ,93.81%)	99.8% (99.68% ,99.88%)
<i>MUTYH</i>	5	8	12	100% (51.01% ,100%)	99.76% (99.37% ,99.9%)
<i>MYC</i>	3	3	7	100% (34.24% ,100%)	99.93% (99.59% ,100%)
<i>MYCL</i>	3	5	9	100% (34.24% ,100%)	99.75% (99.26% ,99.91%)
<i>MYCN</i>	3	7	26	100% (43.85% ,100%)	99.71% (99.26% ,99.89%)
<i>MYD88</i>	3	4	4	100% (34.24% ,100%)	99.78% (99.18% ,99.94%)
<i>NBN</i>	9	11	12	100% (56.55% ,100%)	99.7% (99.35% ,99.86%)
<i>NCOR1</i>	3	4	4	50% (2.56% ,97.44%)	99.97% (99.9% ,99.99%)
<i>NF1</i>	26	37	38	100% (85.13% ,100%)	99.82% (99.71% ,99.89%)
<i>NF2</i>	8	10	12	100% (60.97% ,100%)	99.78% (99.42% ,99.91%)
<i>NFE2L2</i>	3	9	9	71.43% (35.89% ,91.78%)	99.89% (99.6% ,99.97%)
<i>NFKBIA</i>	3	6	6	100% (51.01% ,100%)	99.79% (99.24% ,99.94%)

Gene	Number of Exons	Number of Unique Mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>NKX2-1</i>	3	8	9	100% (56.55% ,100%)	99.73% (99.21% ,99.91%)
<i>NOTCH1</i>	20	37	35	85.71% (65.36% ,95.02%)	99.79% (99.66% ,99.87%)
<i>NOTCH2</i>	14	23	24	100% (79.61% ,100%)	99.89% (99.79% ,99.95%)
<i>NOTCH3</i>	1	1	1	NaN% (NaN% ,NaN%)	99.99% (99.92% ,100%)
<i>NPM1</i>	1	1	1	NaN% (NaN% ,NaN%)	99.89% (99.36% ,99.99%)
<i>NRAS</i>	3	16	17	91.67% (64.61% ,99.57%)	99.28% (98.17% ,99.72%)
<i>NSD1</i>	11	22	24	100% (74.12% ,100%)	99.85% (99.73% ,99.92%)
<i>NTRK2</i>	4	5	8	75% (30.06% ,98.72%)	99.93% (99.6% ,100%)
<i>NTRK3</i>	11	15	13	100% (70.09% ,100%)	99.76% (99.48% ,99.89%)
<i>PAK3</i>	3	3	3	100% (5.13% ,100%)	99.88% (99.55% ,99.97%)
<i>PALB2</i>	7	12	16	66.67% (30% ,90.32%)	99.83% (99.63% ,99.92%)
<i>PARP1</i>	7	8	10	100% (60.97% ,100%)	99.93% (99.76% ,99.98%)
<i>PARP2</i>	3	3	6	66.67% (20.77% ,98.29%)	100% (99.78% ,100%)
<i>PAX5</i>	5	4	5	100% (51.01% ,100%)	100% (99.64% ,100%)
<i>PBRM1</i>	9	19	24	91.67% (64.61% ,99.57%)	99.85% (99.7% ,99.93%)
<i>PDCD1</i>	4	6	7	100% (43.85% ,100%)	99.65% (98.98% ,99.88%)
<i>PDCDILG2</i>	2	2	3	NaN% (NaN% ,NaN%)	99.76% (99.12% ,99.93%)
<i>PDGFRA</i>	11	14	13	90.91% (62.26% ,99.53%)	99.91% (99.73% ,99.97%)
<i>PDGFRB</i>	11	13	16	100% (60.97% ,100%)	99.79% (99.56% ,99.9%)
<i>PDK1</i>	6	6	7	100% (56.55% ,100%)	99.92% (99.57% ,100%)
<i>PHOX2B</i>	3	7	7	100% (43.85% ,100%)	99.58% (98.91% ,99.83%)

Gene	Number of Exons	Number of Unique Mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>PIK3C3</i>	4	5	5	75% (30.06% ,98.72%)	99.96% (99.79% ,100%)
<i>PIK3R1</i>	7	13	14	100% (64.57% ,100%)	99.56% (99.04% ,99.8%)
<i>PIK3R2</i>	7	8	10	100% (51.01% ,100%)	99.82% (99.53% ,99.93%)
<i>PMS1</i>	6	16	16	60% (31.27% ,83.18%)	99.78% (99.53% ,99.9%)
<i>PMS2</i>	7	16	14	66.67% (30% ,90.32%)	99.61% (99.29% ,99.79%)
<i>POLD1</i>	15	26	23	100% (79.61% ,100%)	99.67% (99.41% ,99.81%)
<i>POLE</i>	17	32	29	100% (81.57% ,100%)	99.78% (99.64% ,99.87%)
<i>POLH</i>	3	4	8	0% (0% ,94.87%)	99.86% (99.59% ,99.95%)
<i>POT1</i>	4	4	7	100% (43.85% ,100%)	99.93% (99.63% ,100%)
<i>PPP2R1A</i>	8	16	14	100% (75.75% ,100%)	99.77% (99.42% ,99.91%)
<i>PRDM1</i>	2	4	9	100% (43.85% ,100%)	99.96% (99.77% ,100%)
<i>PREX2</i>	14	29	36	65% (43.29% ,81.88%)	99.69% (99.42% ,99.84%)
<i>PRKARIA</i>	4	3	4	100% (34.24% ,100%)	99.91% (99.51% ,100%)
<i>PRKCI</i>	7	10	10	87.5% (52.91% ,99.36%)	99.89% (99.59% ,99.97%)
<i>PRKDC</i>	2	2	2	100% (5.13% ,100%)	99.99% (99.95% ,100%)
<i>PRKN</i>	10	18	17	100% (64.57% ,100%)	99.21% (98.59% ,99.56%)
<i>PRSS1</i>	5	7	10	100% (56.55% ,100%)	99.73% (99.02% ,99.93%)
<i>PTCH1</i>	16	18	22	83.33% (55.2% ,95.3%)	99.86% (99.7% ,99.94%)
<i>PTEN</i>	10	33	26	91.3% (73.2% ,97.58%)	99.16% (98.46% ,99.54%)
<i>PTK2</i>	8	8	8	100% (43.85% ,100%)	99.84% (99.64% ,99.93%)
<i>PTPN11</i>	8	11	13	50% (18.76% ,81.24%)	99.72% (99.35% ,99.88%)

Gene	Number of Exons	Number of Unique Mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>QKI</i>	6	8	8	66.67% (20.77% ,98.29%)	99.51% (98.86% ,99.79%)
<i>RAC1</i>	5	6	6	100% (43.85% ,100%)	99.53% (98.62% ,99.84%)
<i>RAD50</i>	15	17	19	88.89% (56.5% ,99.43%)	99.8% (99.6% ,99.9%)
<i>RAD51</i>	1	3	3	100% (34.24% ,100%)	99.9% (99.45% ,99.99%)
<i>RAD51B</i>	4	9	9	100% (5.13% ,100%)	99.23% (98.49% ,99.61%)
<i>RAD51C</i>	6	8	9	100% (34.24% ,100%)	99.47% (98.85% ,99.76%)
<i>RAD51D</i>	3	3	6	100% (43.85% ,100%)	100% (99.61% ,100%)
<i>RAD54L</i>	5	5	8	NaN% (NaN% ,NaN%)	99.78% (99.48% ,99.9%)
<i>RAF1</i>	7	8	10	100% (34.24% ,100%)	99.69% (99.33% ,99.86%)
<i>RARA</i>	4	3	5	100% (34.24% ,100%)	99.93% (99.59% ,100%)
<i>RB1</i>	19	26	24	77.78% (54.79% ,91%)	99.71% (99.43% ,99.85%)
<i>RECQL4</i>	8	15	26	100% (60.97% ,100%)	99.75% (99.53% ,99.87%)
<i>RET</i>	12	25	22	100% (78.47% ,100%)	99.67% (99.41% ,99.82%)
<i>RHOA</i>	3	3	3	100% (43.85% ,100%)	100% (99.34% ,100%)
<i>RICTOR</i>	11	18	20	78.57% (52.41% ,92.43%)	99.92% (99.8% ,99.97%)
<i>RNF43</i>	10	17	19	100% (70.09% ,100%)	99.66% (99.33% ,99.83%)
<i>ROS1</i>	20	37	59	66.67% (48.78% ,80.77%)	99.9% (99.79% ,99.95%)
<i>RPTOR</i>	8	8	12	100% (60.97% ,100%)	99.95% (99.82% ,99.99%)
<i>RUNX1</i>	6	18	19	100% (60.97% ,100%)	99.12% (98.46% ,99.49%)
<i>RUNX1T1</i>	5	8	10	100% (67.56% ,100%)	100% (99.78% ,100%)
<i>SBDS</i>	3	9	9	100% (34.24% ,100%)	99.07% (98.09% ,99.55%)

Gene	Number of Exons	Number of Unique Mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>SDHA</i>	8	10	10	100% (60.97% ,100%)	99.8% (99.48% ,99.92%)
<i>SDHB</i>	2	1	2	100% (5.13% ,100%)	100% (99.55% ,100%)
<i>SDHD</i>	2	2	2	100% (5.13% ,100%)	99.79% (98.83% ,99.99%)
<i>SETD2</i>	10	28	22	94.44% (74.24% ,99.72%)	99.87% (99.76% ,99.93%)
<i>SF3B1</i>	10	17	19	92.31% (66.69% ,99.61%)	99.9% (99.74% ,99.96%)
<i>SGK1</i>	5	6	8	100% (51.01% ,100%)	99.87% (99.54% ,99.97%)
<i>SMAD2</i>	4	6	6	80% (37.55% ,98.97%)	99.93% (99.6% ,100%)
<i>SMAD3</i>	4	7	6	100% (51.01% ,100%)	99.76% (99.31% ,99.92%)
<i>SMAD4</i>	8	17	17	93.33% (70.18% ,99.66%)	99.88% (99.56% ,99.97%)
<i>SMARCA4</i>	18	30	33	94.74% (75.36% ,99.73%)	99.78% (99.6% ,99.88%)
<i>SMARCB1</i>	4	7	7	100% (56.55% ,100%)	99.83% (99.37% ,99.95%)
<i>SMO</i>	6	10	12	100% (56.55% ,100%)	99.79% (99.5% ,99.91%)
<i>SOCS1</i>	2	1	2	NaN% (NaN% ,NaN%)	99.84% (99.11% ,99.99%)
<i>SOX2</i>	1	10	9	100% (67.56% ,100%)	99.79% (99.23% ,99.94%)
<i>SPOP</i>	6	8	8	83.33% (43.65% ,99.15%)	99.82% (99.35% ,99.95%)
<i>SRC</i>	5	6	7	100% (43.85% ,100%)	99.81% (99.45% ,99.94%)
<i>STAG2</i>	8	10	10	60% (23.07% ,88.24%)	99.86% (99.68% ,99.94%)
<i>STAT3</i>	7	8	10	100% (56.55% ,100%)	99.87% (99.62% ,99.96%)
<i>STK11</i>	10	11	13	100% (72.25% ,100%)	99.92% (99.56% ,100%)
<i>SUFU</i>	5	8	10	100% (51.01% ,100%)	99.72% (99.29% ,99.89%)
<i>TEK</i>	10	17	17	72.73% (43.44% ,90.25%)	99.82% (99.61% ,99.92%)

Gene	Number of Exons	Number of Unique Mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>TERT</i>	8	39	52	53.33% (36.14% ,69.77%)	99.72% (99.46% ,99.85%)
<i>TET2</i>	2	17	28	85.71% (48.69% ,99.27%)	99.71% (99.47% ,99.84%)
<i>TGFBR2</i>	6	7	12	100% (60.97% ,100%)	99.94% (99.68% ,100%)
<i>TMEM127</i>	3	2	3	NaN% (NaN% ,NaN%)	99.72% (98.99% ,99.92%)
<i>TNFAIP3</i>	4	5	7	100% (34.24% ,100%)	99.87% (99.63% ,99.96%)
<i>TNFRSF14</i>	4	4	6	100% (5.13% ,100%)	99.46% (98.42% ,99.82%)
<i>TOP1</i>	4	6	6	100% (43.85% ,100%)	99.87% (99.62% ,99.96%)
<i>TOP2A</i>	11	14	15	75% (40.93% ,92.85%)	99.87% (99.71% ,99.94%)
<i>TP53</i>	16	169	159	100% (97.45% ,100%)	97.87% (96.8% ,98.59%)
<i>TP63</i>	2	9	9	100% (60.97% ,100%)	99.85% (99.57% ,99.95%)
<i>TSC1</i>	9	11	13	87.5% (52.91% ,99.36%)	99.91% (99.75% ,99.97%)
<i>TSC2</i>	16	18	31	88.89% (56.5% ,99.43%)	99.83% (99.68% ,99.91%)
<i>TSHR</i>	4	13	14	71.43% (35.89% ,91.78%)	99.74% (99.43% ,99.88%)
<i>U2AF1</i>	1	4	4	100% (43.85% ,100%)	99.86% (99.22% ,99.99%)
<i>VEGFA</i>	5	4	5	100% (51.01% ,100%)	100% (99.69% ,100%)
<i>VHL</i>	3	7	9	100% (56.55% ,100%)	99.69% (98.86% ,99.91%)
<i>WAS</i>	4	8	8	100% (51.01% ,100%)	99.73% (99.32% ,99.9%)
<i>WRN</i>	17	22	19	80% (49.02% ,94.33%)	99.72% (99.51% ,99.84%)
<i>WT1</i>	8	17	18	100% (75.75% ,100%)	99.67% (99.22% ,99.86%)
<i>XPA</i>	2	1	2	NaN% (NaN% ,NaN%)	99.88% (99.31% ,99.99%)
<i>XPC</i>	8	12	14	80% (37.55% ,98.97%)	99.75% (99.49% ,99.88%)

Gene	Number of Exons	Number of Unique Mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>XRCC2</i>	2	4	6	66.67% (20.77% ,98.29%)	99.88% (99.33% ,99.99%)
<i>YAP1</i>	3	3	4	100% (34.24% ,100%)	99.93% (99.58% ,100%)
<i>ZNF217</i>	3	14	17	100% (56.55% ,100%)	99.71% (99.46% ,99.85%)
<i>ZNF703</i>	3	9	12	80% (37.55% ,98.97%)	99.77% (99.42% ,99.91%)

### Appendix E.2. Concordance for Insertions by Gene

Gene	Number of Exons	Number of Unique mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>AKT2</i>	1	1	1	100% (5.13% ,100%)	100% (99.73% ,100%)
<i>AKT3</i>	1	1	1	100% (5.13% ,100%)	100% (99.73% ,100%)
<i>APC</i>	2	14	14	100% (78.47% ,100%)	100% (99.95% ,100%)
<i>AR</i>	1	1	1	100% (5.13% ,100%)	100% (99.86% ,100%)
<i>ARID1A</i>	3	6	6	100% (34.24% ,100%)	99.94% (99.83% ,99.97%)
<i>ARID1B</i>	1	2	2	100% (5.13% ,100%)	99.99% (99.92% ,100%)
<i>ARID2</i>	1	1	1	100% (5.13% ,100%)	100% (99.93% ,100%)
<i>ASXL1</i>	1	5	5	100% (34.24% ,100%)	99.94% (99.81% ,99.98%)
<i>ATM</i>	1	1	1	100% (5.13% ,100%)	100% (99.96% ,100%)
<i>ATR</i>	1	1	1	100% (5.13% ,100%)	100% (99.95% ,100%)
<i>ATRX</i>	1	3	3	100% (34.24% ,100%)	99.99% (99.92% ,100%)
<i>AURKB</i>	1	1	1	NaN% (NaN% ,NaN%)	99.9% (99.45% ,100%)
<i>AXIN2</i>	2	3	3	NaN% (NaN% ,NaN%)	99.88% (99.65% ,99.96%)
<i>B2M</i>	1	1	1	NaN% (NaN% ,NaN%)	99.72% (98.44% ,99.99%)

Gene	Number of Exons	Number of Unique mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>BAP1</i>	2	3	3	100% (43.85% ,100%)	100% (99.82% ,100%)
<i>BARD1</i>	1	1	1	100% (5.13% ,100%)	100% (99.84% ,100%)
<i>BMPRIA</i>	1	1	1	100% (5.13% ,100%)	100% (99.76% ,100%)
<i>EGFR</i>	1	3	3	100% (34.24% ,100%)	99.97% (99.84% ,100%)
<i>KIT</i>	1	1	1	100% (5.13% ,100%)	100% (99.87% ,100%)
<i>PALB2</i>	1	1	1	100% (5.13% ,100%)	100% (99.89% ,100%)
<i>BRAF</i>	1	2	2	100% (34.24% ,100%)	100% (99.83% ,100%)
<i>BRCA1</i>	2	3	3	100% (43.85% ,100%)	100% (99.93% ,100%)
<i>BRCA2</i>	2	2	2	100% (5.13% ,100%)	99.99% (99.94% ,100%)
<i>CBL</i>	1	1	1	100% (5.13% ,100%)	100% (99.86% ,100%)
<i>CDH1</i>	3	3	3	100% (43.85% ,100%)	100% (99.86% ,100%)
<i>CDK12</i>	1	1	1	100% (5.13% ,100%)	100% (99.91% ,100%)
<i>CDKN1A</i>	1	2	2	100% (34.24% ,100%)	100% (99.23% ,100%)
<i>CDKN1B</i>	1	1	1	100% (5.13% ,100%)	100% (99.36% ,100%)
<i>CEBPA</i>	1	1	1	100% (5.13% ,100%)	100% (99.64% ,100%)
<i>CREBBP</i>	2	3	3	100% (43.85% ,100%)	100% (99.95% ,100%)
<i>CTCF</i>	4	6	6	100% (56.55% ,100%)	99.95% (99.74% ,100%)
<i>CTLA4</i>	1	1	1	100% (5.13% ,100%)	100% (99.43% ,100%)
<i>DNMT3A</i>	1	1	1	NaN% (NaN% ,NaN%)	99.96% (99.79% ,100%)
<i>ERBB2</i>	1	1	1	100% (5.13% ,100%)	100% (99.9% ,100%)
<i>ETV1</i>	1	1	1	NaN% (NaN% ,NaN%)	99.93% (99.61% ,100%)

Gene	Number of Exons	Number of Unique mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>FANCC</i>	1	1	1	100% (5.13% ,100%)	100% (99.77% ,100%)
<i>FANCF</i>	1	2	2	100% (34.24% ,100%)	100% (99.66% ,100%)
<i>FANCL</i>	1	2	2	NaN% (NaN% ,NaN%)	99.82% (99.36% ,99.95%)
<i>FANCM</i>	1	1	1	100% (5.13% ,100%)	100% (99.94% ,100%)
<i>FAT1</i>	3	3	3	100% (5.13% ,100%)	99.99% (99.95% ,100%)
<i>FBXW7</i>	1	1	1	100% (5.13% ,100%)	100% (99.78% ,100%)
<i>FGF19</i>	1	1	1	100% (5.13% ,100%)	100% (99.41% ,100%)
<i>FGFR3</i>	1	7	7	NaN% (NaN% ,NaN%)	99.71% (99.4% ,99.86%)
<i>FGFR4</i>	1	1	1	NaN% (NaN% ,NaN%)	99.95% (99.74% ,100%)
<i>FLCN</i>	1	3	3	NaN% (NaN% ,NaN%)	99.83% (99.49% ,99.94%)
<i>FLT4</i>	2	2	2	100% (5.13% ,100%)	99.97% (99.86% ,100%)
<i>FOXA1</i>	1	1	1	NaN% (NaN% ,NaN%)	99.93% (99.6% ,100%)
<i>GATA1</i>	1	1	1	NaN% (NaN% ,NaN%)	99.92% (99.55% ,100%)
<i>GATA3</i>	1	3	3	100% (34.24% ,100%)	99.92% (99.58% ,100%)
<i>GATA6</i>	1	1	1	NaN% (NaN% ,NaN%)	99.94% (99.68% ,100%)
<i>GRM3</i>	1	1	1	100% (5.13% ,100%)	100% (99.85% ,100%)
<i>HNF1A</i>	1	2	2	100% (5.13% ,100%)	99.95% (99.7% ,100%)
<i>IDH2</i>	1	1	1	NaN% (NaN% ,NaN%)	99.93% (99.58% ,100%)
<i>IFNGR1</i>	1	1	1	100% (5.13% ,100%)	100% (99.74% ,100%)
<i>IGF2</i>	1	1	1	100% (5.13% ,100%)	100% (99.3% ,100%)
<i>JAK1</i>	1	1	1	100% (5.13% ,100%)	100% (99.89% ,100%)

Gene	Number of Exons	Number of Unique mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>KMT2A</i>	1	1	1	NaN% (NaN% ,NaN%)	99.99% (99.95% ,100%)
<i>KMT2C</i>	2	2	2	NaN% (NaN% ,NaN%)	99.99% (99.95% ,100%)
<i>LRP1B</i>	4	4	4	100% (43.85% ,100%)	99.99% (99.96% ,100%)
<i>MAP2K1</i>	1	1	1	100% (5.13% ,100%)	100% (99.68% ,100%)
<i>MAP3K1</i>	1	2	2	100% (5.13% ,100%)	99.98% (99.88% ,100%)
<i>MED12</i>	1	1	1	NaN% (NaN% ,NaN%)	99.98% (99.91% ,100%)
<i>MSH6</i>	2	5	5	100% (43.85% ,100%)	99.95% (99.82% ,99.99%)
<i>MTOR</i>	1	1	1	NaN% (NaN% ,NaN%)	99.99% (99.93% ,100%)
<i>NBN</i>	2	2	2	100% (5.13% ,100%)	99.95% (99.72% ,100%)
<i>NF1</i>	2	2	2	NaN% (NaN% ,NaN%)	99.98% (99.91% ,99.99%)
<i>NOTCH1</i>	1	1	1	100% (5.13% ,100%)	100% (99.95% ,100%)
<i>NOTCH2</i>	2	2	2	100% (34.24% ,100%)	100% (99.95% ,100%)
<i>NOTCH3</i>	1	1	1	100% (5.13% ,100%)	100% (99.94% ,100%)
<i>NSD1</i>	3	3	3	100% (5.13% ,100%)	99.97% (99.9% ,99.99%)
<i>NTRK1</i>	1	1	1	100% (5.13% ,100%)	100% (99.84% ,100%)
<i>PARP1</i>	1	1	1	NaN% (NaN% ,NaN%)	99.97% (99.81% ,100%)
<i>PIK3R1</i>	1	3	3	100% (34.24% ,100%)	99.93% (99.59% ,100%)
<i>PMS2</i>	1	1	1	100% (5.13% ,100%)	100% (99.85% ,100%)
<i>POLH</i>	1	1	1	NaN% (NaN% ,NaN%)	99.95% (99.74% ,100%)
<i>PRDM1</i>	1	1	1	100% (5.13% ,100%)	100% (99.85% ,100%)
<i>PREX2</i>	1	1	1	NaN% (NaN% ,NaN%)	99.97% (99.81% ,100%)

Gene	Number of Exons	Number of Unique mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>PRKCI</i>	1	1	1	100% (5.13% ,100%)	100% (99.79% ,100%)
<i>PRSSI</i>	1	1	1	NaN% (NaN% ,NaN%)	99.87% (99.24% ,99.99%)
<i>PTEN</i>	4	9	8	100% (51.01% ,100%)	99.59% (99.03% ,99.82%)
<i>RAD50</i>	1	1	1	NaN% (NaN% ,NaN%)	99.97% (99.86% ,100%)
<i>RAD51D</i>	1	1	1	100% (5.13% ,100%)	100% (99.61% ,100%)
<i>RBI</i>	2	2	2	100% (5.13% ,100%)	99.96% (99.8% ,100%)
<i>RNF43</i>	1	1	1	100% (5.13% ,100%)	100% (99.84% ,100%)
<i>RPTOR</i>	1	1	1	NaN% (NaN% ,NaN%)	99.98% (99.86% ,100%)
<i>RUNX1</i>	1	1	1	100% (5.13% ,100%)	100% (99.72% ,100%)
<i>SGK1</i>	1	1	1	100% (5.13% ,100%)	100% (99.76% ,100%)
<i>SMAD4</i>	1	1	1	100% (5.13% ,100%)	100% (99.77% ,100%)
<i>SMARCA4</i>	2	2	2	100% (5.13% ,100%)	99.98% (99.89% ,100%)
<i>STAG2</i>	1	1	1	NaN% (NaN% ,NaN%)	99.97% (99.85% ,100%)
<i>STK11</i>	2	2	2	100% (5.13% ,100%)	99.92% (99.57% ,100%)
<i>TNFAIP3</i>	1	1	1	NaN% (NaN% ,NaN%)	99.96% (99.76% ,100%)
<i>TNFRSF14</i>	1	1	1	100% (5.13% ,100%)	100% (99.31% ,100%)
<i>TOP2A</i>	1	1	1	100% (5.13% ,100%)	100% (99.92% ,100%)
<i>TP53</i>	3	3	3	100% (43.85% ,100%)	100% (99.68% ,100%)
<i>TSC2</i>	1	1	1	NaN% (NaN% ,NaN%)	99.98% (99.9% ,100%)
<i>WRN</i>	1	1	1	NaN% (NaN% ,NaN%)	99.98% (99.87% ,100%)
<i>XRCC2</i>	1	1	1	NaN% (NaN% ,NaN%)	99.88% (99.33% ,99.99%)

Gene	Number of Exons	Number of Unique mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>ZNF217</i>	1	1	1	NaN% (NaN% ,NaN%)	99.97% (99.82% ,100%)
<i>ZNF703</i>	1	1	1	NaN% (NaN% ,NaN%)	99.94% (99.68% ,100%)

### Appendix E.3. Concordance for Deletions by Gene

Gene	Number of Exons	Number of Unique Mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>ATM</i>	4	5	5	100% (34.24% ,100%)	99.97% (99.9% ,99.99%)
<i>AMERI</i>	1	7	7	100% (60.97% ,100%)	99.97% (99.83% ,100%)
<i>APC</i>	4	23	22	100% (83.89% ,100%)	99.96% (99.9% ,99.99%)
<i>ARAF</i>	3	7	7	100% (43.85% ,100%)	99.78% (99.44% ,99.91%)
<i>ARID1A</i>	9	28	24	100% (75.75% ,100%)	99.74% (99.58% ,99.84%)
<i>ARID1B</i>	3	4	4	100% (34.24% ,100%)	99.97% (99.89% ,99.99%)
<i>ARID2</i>	2	2	2	100% (34.24% ,100%)	100% (99.93% ,100%)
<i>ASXL1</i>	1	1	1	NaN% (NaN% ,NaN%)	99.98% (99.88% ,100%)
<i>CDK12</i>	2	4	4	100% (43.85% ,100%)	99.98% (99.87% ,100%)
<i>BRCA2</i>	6	10	10	100% (64.57% ,100%)	99.97% (99.91% ,99.99%)
<i>ATR</i>	1	1	1	NaN% (NaN% ,NaN%)	99.99% (99.93% ,100%)
<i>ATRX</i>	1	2	2	NaN% (NaN% ,NaN%)	99.97% (99.9% ,99.99%)
<i>AXIN2</i>	3	11	8	100% (67.56% ,100%)	99.88% (99.65% ,99.96%)
<i>AXL</i>	1	1	1	100% (5.13% ,100%)	100% (99.8% ,100%)
<i>B2M</i>	2	8	6	100% (51.01% ,100%)	98.88% (97.15% ,99.56%)
<i>BAP1</i>	1	1	1	100% (5.13% ,100%)	100% (99.82% ,100%)

Gene	Number of Exons	Number of Unique Mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>BARD1</i>	1	2	2	NaN% (NaN% ,NaN%)	99.91% (99.69% ,99.98%)
<i>BLM</i>	3	14	13	100% (60.97% ,100%)	99.81% (99.63% ,99.9%)
<i>EGFR</i>	1	11	11	100% (74.12% ,100%)	100% (99.89% ,100%)
<i>MET</i>	1	1	1	100% (5.13% ,100%)	100% (99.91% ,100%)
<i>BRCA1</i>	2	3	3	NaN% (NaN% ,NaN%)	99.95% (99.84% ,99.98%)
<i>BRD4</i>	2	3	3	NaN% (NaN% ,NaN%)	99.93% (99.78% ,99.98%)
<i>BTK</i>	1	1	1	100% (5.13% ,100%)	100% (99.81% ,100%)
<i>CASP8</i>	2	2	2	100% (5.13% ,100%)	99.93% (99.62% ,100%)
<i>CBL</i>	1	1	1	100% (5.13% ,100%)	100% (99.86% ,100%)
<i>CCND1</i>	1	1	1	100% (5.13% ,100%)	100% (99.57% ,100%)
<i>CDH1</i>	2	2	2	100% (34.24% ,100%)	100% (99.86% ,100%)
<i>CDKN2A</i>	2	6	5	100% (43.85% ,100%)	99.36% (98.13% ,99.78%)
<i>CDKN2C</i>	1	1	1	100% (5.13% ,100%)	100% (99.25% ,100%)
<i>CHD4</i>	1	2	2	100% (34.24% ,100%)	100% (99.93% ,100%)
<i>CHEK1</i>	2	4	4	100% (51.01% ,100%)	100% (99.73% ,100%)
<i>CHEK2</i>	2	2	2	NaN% (NaN% ,NaN%)	99.89% (99.59% ,99.97%)
<i>CREBBP</i>	5	7	6	100% (43.85% ,100%)	99.95% (99.86% ,99.98%)
<i>CTCF</i>	3	3	3	100% (43.85% ,100%)	100% (99.82% ,100%)
<i>CTLA4</i>	1	1	1	NaN% (NaN% ,NaN%)	99.85% (99.16% ,99.99%)
<i>CTNNB1</i>	2	3	3	100% (43.85% ,100%)	100% (99.84% ,100%)
<i>CYLD</i>	2	2	2	100% (34.24% ,100%)	100% (99.87% ,100%)

Gene	Number of Exons	Number of Unique Mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>DAXX</i>	2	2	2	NaN% (NaN% ,NaN%)	99.91% (99.67% ,99.98%)
<i>DICER1</i>	1	1	1	100% (5.13% ,100%)	100% (99.93% ,100%)
<i>DNMT3A</i>	1	1	1	NaN% (NaN% ,NaN%)	99.96% (99.79% ,100%)
<i>EP300</i>	5	5	4	100% (51.01% ,100%)	99.99% (99.92% ,100%)
<i>EPAS1</i>	1	1	1	100% (5.13% ,100%)	100% (99.85% ,100%)
<i>EPHA2</i>	3	3	3	100% (34.24% ,100%)	99.97% (99.81% ,100%)
<i>EPHA3</i>	3	3	3	100% (34.24% ,100%)	99.97% (99.81% ,100%)
<i>EPHA5</i>	1	2	2	100% (5.13% ,100%)	99.97% (99.82% ,100%)
<i>ERBB3</i>	2	2	2	100% (5.13% ,100%)	99.98% (99.86% ,100%)
<i>ERBB4</i>	3	3	3	100% (5.13% ,100%)	99.95% (99.81% ,99.99%)
<i>ERCC4</i>	2	2	2	100% (34.24% ,100%)	100% (99.86% ,100%)
<i>ERCC5</i>	2	2	2	100% (5.13% ,100%)	99.97% (99.84% ,100%)
<i>ESR1</i>	1	1	1	100% (5.13% ,100%)	100% (99.79% ,100%)
<i>ETV1</i>	1	1	1	NaN% (NaN% ,NaN%)	99.93% (99.61% ,100%)
<i>ETV4</i>	1	1	1	NaN% (NaN% ,NaN%)	99.93% (99.61% ,100%)
<i>ETV6</i>	1	1	1	100% (5.13% ,100%)	100% (99.72% ,100%)
<i>EXT1</i>	1	1	1	100% (5.13% ,100%)	100% (99.83% ,100%)
<i>FANCA</i>	3	3	3	100% (5.13% ,100%)	99.95% (99.83% ,99.99%)
<i>FANCD2</i>	1	1	1	NaN% (NaN% ,NaN%)	99.98% (99.87% ,100%)
<i>FANCE</i>	1	1	1	100% (5.13% ,100%)	100% (99.76% ,100%)
<i>FANCF</i>	1	2	2	100% (5.13% ,100%)	99.91% (99.5% ,100%)

Gene	Number of Exons	Number of Unique Mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>FANCI</i>	1	1	1	100% (5.13% ,100%)	100% (99.9% ,100%)
<i>FANCL</i>	1	1	1	100% (5.13% ,100%)	100% (99.66% ,100%)
<i>FANCM</i>	1	2	2	100% (5.13% ,100%)	99.98% (99.91% ,100%)
<i>FAT1</i>	5	7	7	100% (60.97% ,100%)	99.99% (99.96% ,100%)
<i>FBXW7</i>	3	4	4	100% (43.85% ,100%)	99.94% (99.68% ,100%)
<i>FLCN</i>	1	1	1	NaN% (NaN% ,NaN%)	99.94% (99.68% ,100%)
<i>FLT4</i>	2	2	2	100% (34.24% ,100%)	100% (99.9% ,100%)
<i>FOXA1</i>	1	6	5	100% (43.85% ,100%)	99.79% (99.38% ,99.93%)
<i>FOXL2</i>	1	1	1	NaN% (NaN% ,NaN%)	99.91% (99.5% ,100%)
<i>GATA1</i>	1	1	1	NaN% (NaN% ,NaN%)	99.92% (99.55% ,100%)
<i>GATA2</i>	1	1	1	100% (5.13% ,100%)	100% (99.73% ,100%)
<i>GATA3</i>	3	4	4	100% (43.85% ,100%)	99.92% (99.58% ,100%)
<i>GATA4</i>	1	1	1	NaN% (NaN% ,NaN%)	99.92% (99.58% ,100%)
<i>GRIN2A</i>	1	1	1	100% (5.13% ,100%)	100% (99.91% ,100%)
<i>HNF1A</i>	1	13	11	100% (70.09% ,100%)	99.79% (99.46% ,99.92%)
<i>IDH2</i>	1	1	1	100% (5.13% ,100%)	100% (99.72% ,100%)
<i>IFNGR1</i>	1	1	1	100% (5.13% ,100%)	100% (99.74% ,100%)
<i>IGF1R</i>	1	1	1	100% (5.13% ,100%)	100% (99.91% ,100%)
<i>IL7R</i>	2	2	2	NaN% (NaN% ,NaN%)	99.86% (99.47% ,99.96%)
<i>INPP4B</i>	1	1	1	100% (5.13% ,100%)	100% (99.86% ,100%)
<i>JAK1</i>	2	9	8	100% (60.97% ,100%)	99.91% (99.75% ,99.97%)

Gene	Number of Exons	Number of Unique Mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>JUN</i>	1	1	1	NaN% (NaN% ,NaN%)	99.9% (99.43% ,99.99%)
<i>KDM5A</i>	3	5	5	NaN% (NaN% ,NaN%)	99.9% (99.77% ,99.96%)
<i>KIT</i>	1	0	1	NaN% (NaN% ,NaN%)	100% (99.87% ,100%)
<i>KMT2A</i>	2	5	4	NaN% (NaN% ,NaN%)	99.96% (99.9% ,99.98%)
<i>KMT2C</i>	2	2	2	NaN% (NaN% ,NaN%)	99.99% (99.95% ,100%)
<i>LRP1B</i>	1	1	1	100% (5.13% ,100%)	100% (99.97% ,100%)
<i>LYN</i>	1	1	1	100% (5.13% ,100%)	100% (99.75% ,100%)
<i>LZTR1</i>	2	2	2	100% (5.13% ,100%)	99.96% (99.78% ,100%)
<i>MAP2K1</i>	1	1	1	100% (5.13% ,100%)	100% (99.68% ,100%)
<i>MAP2K4</i>	1	1	1	100% (5.13% ,100%)	100% (99.69% ,100%)
<i>MAP3K1</i>	2	3	3	100% (34.24% ,100%)	99.98% (99.88% ,100%)
<i>MCL1</i>	2	2	2	100% (5.13% ,100%)	99.9% (99.46% ,100%)
<i>MED12</i>	2	3	3	NaN% (NaN% ,NaN%)	99.95% (99.87% ,99.98%)
<i>MEN1</i>	2	4	4	100% (43.85% ,100%)	99.95% (99.69% ,100%)
<i>MLH3</i>	1	3	3	100% (34.24% ,100%)	99.98% (99.87% ,100%)
<i>MPL</i>	1	1	1	100% (5.13% ,100%)	100% (99.8% ,100%)
<i>MSH2</i>	4	4	3	100% (51.01% ,100%)	100% (99.86% ,100%)
<i>MSH6</i>	2	5	5	100% (5.13% ,100%)	99.9% (99.75% ,99.96%)
<i>MUTYH</i>	1	1	1	100% (5.13% ,100%)	100% (99.77% ,100%)
<i>NBN</i>	3	3	3	100% (34.24% ,100%)	99.95% (99.72% ,100%)
<i>NF1</i>	6	7	7	100% (64.57% ,100%)	100% (99.95% ,100%)

Gene	Number of Exons	Number of Unique Mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>NF2</i>	1	1	1	100% (5.13% ,100%)	100% (99.79% ,100%)
<i>NFE2L2</i>	1	1	1	100% (5.13% ,100%)	100% (99.79% ,100%)
<i>NFKBIA</i>	1	1	1	100% (5.13% ,100%)	100% (99.6% ,100%)
<i>NKX2-1</i>	1	1	1	NaN% (NaN% ,NaN%)	99.91% (99.49% ,100%)
<i>NOTCH1</i>	2	3	3	100% (34.24% ,100%)	99.99% (99.93% ,100%)
<i>NSD1</i>	2	4	4	100% (34.24% ,100%)	99.97% (99.9% ,99.99%)
<i>PAK3</i>	1	1	1	NaN% (NaN% ,NaN%)	99.94% (99.65% ,100%)
<i>PALB2</i>	2	3	3	100% (43.85% ,100%)	100% (99.89% ,100%)
<i>PARP1</i>	1	1	1	NaN% (NaN% ,NaN%)	99.97% (99.81% ,100%)
<i>PBRM1</i>	3	3	3	100% (43.85% ,100%)	100% (99.92% ,100%)
<i>PDCD1</i>	1	2	2	NaN% (NaN% ,NaN%)	99.77% (99.16% ,99.94%)
<i>PIK3CA</i>	1	3	3	100% (34.24% ,100%)	99.97% (99.82% ,100%)
<i>PIK3R1</i>	4	6	6	100% (60.97% ,100%)	100% (99.72% ,100%)
<i>PMS1</i>	2	3	3	100% (34.24% ,100%)	99.96% (99.8% ,100%)
<i>PMS2</i>	2	3	3	100% (43.85% ,100%)	100% (99.85% ,100%)
<i>POLD1</i>	2	2	2	100% (5.13% ,100%)	99.97% (99.83% ,100%)
<i>POLE</i>	1	1	1	100% (5.13% ,100%)	100% (99.94% ,100%)
<i>POLH</i>	2	2	2	100% (5.13% ,100%)	99.95% (99.74% ,100%)
<i>PREX2</i>	1	2	2	100% (5.13% ,100%)	99.97% (99.81% ,100%)
<i>PRKCI</i>	1	1	1	100% (5.13% ,100%)	100% (99.79% ,100%)
<i>PRKN</i>	2	2	2	100% (34.24% ,100%)	100% (99.73% ,100%)

Gene	Number of Exons	Number of Unique Mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>PTCH1</i>	4	9	8	100% (51.01% ,100%)	99.88% (99.73% ,99.95%)
<i>PTEN</i>	6	15	13	100% (64.57% ,100%)	99.34% (98.7% ,99.66%)
<i>QKI</i>	1	5	5	100% (43.85% ,100%)	99.8% (99.29% ,99.95%)
<i>RAD50</i>	1	1	1	NaN% (NaN% ,NaN%)	99.97% (99.86% ,100%)
<i>RAD51B</i>	1	2	2	NaN% (NaN% ,NaN%)	99.81% (99.3% ,99.95%)
<i>RAD51C</i>	1	1	1	100% (5.13% ,100%)	100% (99.66% ,100%)
<i>RAF1</i>	1	1	1	100% (5.13% ,100%)	100% (99.8% ,100%)
<i>RARA</i>	2	4	4	100% (34.24% ,100%)	99.86% (99.48% ,99.96%)
<i>RBI</i>	6	7	7	100% (60.97% ,100%)	99.96% (99.8% ,100%)
<i>RECQL4</i>	1	2	2	100% (34.24% ,100%)	100% (99.89% ,100%)
<i>RET</i>	1	1	1	100% (5.13% ,100%)	100% (99.89% ,100%)
<i>RICTOR</i>	1	1	1	NaN% (NaN% ,NaN%)	99.98% (99.89% ,100%)
<i>RNF43</i>	3	20	19	100% (81.57% ,100%)	99.87% (99.62% ,99.96%)
<i>ROSI</i>	1	1	1	100% (5.13% ,100%)	100% (99.95% ,100%)
<i>RUNX1T1</i>	1	1	1	100% (5.13% ,100%)	100% (99.78% ,100%)
<i>SDHB</i>	1	1	1	100% (5.13% ,100%)	100% (99.55% ,100%)
<i>SETD2</i>	4	6	6	100% (51.01% ,100%)	99.97% (99.91% ,99.99%)
<i>SF3B1</i>	3	3	3	100% (43.85% ,100%)	100% (99.9% ,100%)
<i>SMAD3</i>	2	2	2	NaN% (NaN% ,NaN%)	99.84% (99.43% ,99.96%)
<i>SMAD4</i>	2	2	2	100% (5.13% ,100%)	99.94% (99.66% ,100%)
<i>SMARCA4</i>	3	4	4	100% (51.01% ,100%)	100% (99.92% ,100%)

Gene	Number of Exons	Number of Unique Mutations	Number of Samples	PPA 95 CI (%)	NPA 95 CI (%)
<i>SMARCB1</i>	1	1	1	100% (5.13% ,100%)	100% (99.67% ,100%)
<i>SMO</i>	1	1	1	NaN% (NaN% ,NaN%)	99.96% (99.76% ,100%)
<i>SOX2</i>	1	2	2	100% (5.13% ,100%)	99.9% (99.41% ,99.99%)
<i>SPOP</i>	1	1	1	100% (5.13% ,100%)	100% (99.66% ,100%)
<i>STAG2</i>	1	1	1	NaN% (NaN% ,NaN%)	99.97% (99.85% ,100%)
<i>STAT3</i>	1	1	1	100% (5.13% ,100%)	100% (99.83% ,100%)
<i>STK11</i>	4	4	4	100% (43.85% ,100%)	99.92% (99.57% ,100%)
<i>TET2</i>	1	2	2	100% (5.13% ,100%)	99.97% (99.84% ,100%)
<i>TGFBR2</i>	1	4	4	100% (51.01% ,100%)	100% (99.78% ,100%)
<i>TNFAIP3</i>	1	1	1	100% (5.13% ,100%)	100% (99.84% ,100%)
<i>TOP2A</i>	2	4	4	100% (43.85% ,100%)	99.98% (99.88% ,100%)
<i>TP53</i>	9	35	34	100% (86.68% ,100%)	99.14% (98.42% ,99.53%)
<i>TSC1</i>	4	7	6	100% (60.97% ,100%)	99.97% (99.84% ,100%)
<i>TSC2</i>	1	1	1	NaN% (NaN% ,NaN%)	99.98% (99.9% ,100%)
<i>TSHR</i>	1	1	1	100% (5.13% ,100%)	100% (99.83% ,100%)
<i>VHL</i>	3	4	4	100% (51.01% ,100%)	100% (99.4% ,100%)
<i>WAS</i>	2	2	2	100% (5.13% ,100%)	99.93% (99.63% ,100%)
<i>WRN</i>	1	1	1	100% (5.13% ,100%)	100% (99.91% ,100%)
<i>WT1</i>	1	1	1	100% (5.13% ,100%)	100% (99.75% ,100%)
<i>ZNF217</i>	2	2	2	100% (5.13% ,100%)	99.97% (99.82% ,100%)
<i>ZNF703</i>	1	2	2	100% (5.13% ,100%)	99.94% (99.68% ,100%)