August 29, 2025

Geneseeq Technology Inc.
Xue Wu
Chief Executive Officer
Suite 1802, 393 University Ave
Toronto, ON M5G 1E6
Canada

Re: K250003
Trade/Device Name: GENESEEQPRIME NGS Tumor Profiling Assay (FFPE) (GS6005)
Regulation Number: 21 CFR 866.6080
Regulation Name: Next Generation Sequencing Based Tumor Profiling
Regulatory Class: Class II
Product Code: PZM
Dated: December 30, 2024
Received: January 2, 2025

Dear Xue Wu:

We have reviewed your section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (the Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device"

(https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR Part 803) for devices or postmarketing safety reporting (21 CFR Part 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

All medical devices, including Class I and unclassified devices and combination product device constituent parts are required to be in compliance with the final Unique Device Identification System rule ("UDI Rule"). The UDI Rule requires, among other things, that a device bear a unique device identifier (UDI) on its label and package (21 CFR 801.20(a)) unless an exception or alternative applies (21 CFR 801.20(b)) and that the dates on the device label be formatted in accordance with 21 CFR 801.18. The UDI Rule (21 CFR 830.300(a) and 830.320(b)) also requires that certain information be submitted to the Global Unique Device Identification Database (GUDID) (21 CFR Part 830 Subpart E). For additional information on these requirements, please see the UDI System webpage at https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/unique-device-identification-system-udi-system.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Zivana Tezak -S

Zivana Tezak, Ph.D.
Branch Chief
Division of Molecular Genetics and Pathology
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 07/31/2026
See PRA Statement below.

510(k) Number *(if known)*
K250003

Device Name
GENESEEQPRIME NGS Tumor Profiling Assay (FFPE)

Indications for Use *(Describe)*
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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary

I. Introduction:

A. Applicant:
Geneseeq Technology Inc.

B. Proprietary and Established Names:
GENESEEQPRIME NGS Tumor Profiling Assay (FFPE)

C. 510(k) Number
K250003

D. Regulatory Information:

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

II. Submission/Device Overview:

A. Purpose for Submission:
New Device

B. Measurand (quantity intended to be measured):
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. For a complete list of genes, please refer to the device user manual for details.

C. Type of Test:
Next-generation sequencing tumor profiling test

III. Intended Use/Indications for Use:

A. Intended Use:
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. Indications for Use:
Same as above

C. Special Conditions for Use Statement(s):
For Prescription Use

For *in vitro* diagnostic use.

D. Special Instrument Requirements:
Illumina NextSeq® 550Dx Sequencer

IV. Device/System Characteristics:

A. Device Description:

1. Targeted Genes of Interest List

The complete list of 425 targeted genes for the GENESEEQPRIME NGS Tumor Profiling Assay (FFPE) is provided in the device user manual.

2. Reagents

The GENESEEQPRIME NGS Tumor Profiling Assay (FFPE) (Ref. No. GS6005, hereafter referred to as "GENESEEQPRIME") includes the following components as listed in Table 1.

Table 1. GENESEEQPRIME kit: Reagent Components and Storage Condition.

Library Preparation and Target Enrichment Kit, Box 1 of 3 (Store at -25°C to -15°C)			
Cap Label	**Component Name**	**Cap Color**	**Volume (µL)**
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
UDI Adaptor for Illumina, Box 2 of 3 (Store at -25℃ to -15℃)			
Cap Label	**Component Name**	**Volume**	**Concentration**
DA01-DA30	UDI Adaptor 1-30	6.5 μL/tube	15 μM
Purification and Capture Beads, Box 3 of 3 (Store at 2 to 8℃)			
Cap Label	**Component Name**		**Volume**
CB	Capture Beads		300 μL
PB	Purification Beads		9.0 mL

3. Material Required but Not Provided:

A list of materials required for upstream preparation of samples for sequencing is but not included as part of the GENESEEQPRIME assay is shown in Table 2.

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 (qPCR method)
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, 20028871
Disposable pipet basin	User's choice
Areosol 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

4. GENESIS Software:

The GENESIS by GENESEEQ™ (hereafter referred to as "GENESIS") software necessary for the GENESEEQPRIME 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. If users need to reduce the amount of storage that raw data occupies, they need super admin privilege to perform raw data deletion via the system interface, and can only do so after successful report generation, with a confirmation prompt. Improper use will result in no report being generated.

5. Instrument:

Sequencing libraries prepared from the GENESEEQPRIME assay must be sequenced on Illumina NextSeq 550Dx sequencing platform. Other instruments that are required for use but not included in the GENESEEQPRIME assay are listed in Table 3.

Table 3. Instruments required, not provided

Equipment	Notes
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.
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.

6. Sample Preparation:

The GENESEEQPRIME assay requires genomic DNA isolated from formalin- fixed, paraffin-embedded (FFPE) tissue sample using a validated commercially available DNA extraction method (column-based or beads-based). The concentration of the extracted genomic DNA can be measured by using a fluorescence quantification method. The total DNA yield of FFPE sample should be no less than 50ng The GENESEEQPRIME assay has been validated with FFPE sample stored at room temperature (15-25 °C) for up to 5 years and extracted genomic DNA samples stored at -25 to -15 °C for up to one year. Longer storage time may compromise the testing results. FFPE tissue specimen should be processed and stored using standardized anatomical pathology protocol (Table 4).

At least 20% of the nucleated cell within the specimen should be represented in tumor tissue for GENESEQPRIME assay. Any sample containing less than 20% tumor content can be macro-dissected before use. Using samples with less than 20% tumor content may compromise the testing results. The tumor volume and minimum tumor content needed to obtain sufficient DNA for testing to achieve the necessary quality performance are shown in the Table 4 below. The recommended number of FFPE sections of each sample for DNA extraction varies depending on the tissue sample size, please see Table 5 for suggestions.

Genomic DNA is extracted from tissue specimens according to the extraction kit protocol. DNA is quantified and concentrated if necessary. The amount of DNA required to perform the test is 50-500 ng. DNA shearing is conducted per protocol and a quality control check is performed. Average fragment size should be ~300bp.

Sheared DNA is stored at -20°C if not proceeding directly to library preparation. The DNA can be stored at 37°C for 10-20 minutes, stored at 2–8°C for 24 hours, or at –20°C for longer periods.

Table 4. Specimen Handling and Processing for Validated Specimen Types

Tissue Type	Volume	Minimum Tumor Proportion	Macrodissection requirements	Limitations	Storage
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

Table 5. Tissue sample size and FFPE section specification for GENESEEQPRIME assay

Surface area of tissue sample (A)	Unstained FFPE slides or curls
A ≥ 1.0 cm * 1.0 cm	2-5 slides/curls at 5-10 μm
0.5 cm * 0.5 cm ≤ A < 1.0 cm * 1.0 cm	5-10 slides/curls at 5-10 μm
A < 0.5 cm * 0.5 cm	10-15 slides/curls at 5-10 μm

7. DNA Extraction:

The kit does not provide reagents for genomic DNA extraction and fragmentation. However, both magnetic beads-based and spin column-based DNA extraction protocols have been validated for the kit. Genomic DNA is isolated from FFPE sections prepared from FFPE tumor tissue samples. The amount and the purity of DNA samples are measured to meet the minimum required standards of the test. The obtained genomic DNA is then mechanically sheared into short DNA fragments with desired length using ultrasonic devices to be read on Illumina sequencing platforms.

8. Library Preparation:

The GENESEEQPRIME assay workflow begins with fragmentation and purification of the genomic DNA. Genomic DNA is first quantified using a fluorometric method. DNA is then fragmented to a desired size of ~300bp base pairs using sonication and purified using magnetic beads. DNA fragments then undergo end repair, A tailing, and adapter ligation, followed by another round of purification.

For the Illumina sequencer to capture DNA fragments for clonal amplification and sequencing reactions, Illumina compatible DNA adaptors need to be added onto the end of fragmented DNA. Each adaptor contains unique index composed of 8 nucleotides to distinguish one sample from another, which enables multiplexing and sequencing multiple libraries in one sequencing run. Excessive adaptors that are not

ligated to DNA will be cleaned by size selection using the magnetic beads. The number of PCR cycles is commensurate with the DNA input amount. Each sample library must be ≥10 ng/µl (≥200 ng total amount) in order to proceed to the following Target Enrichment steps.

9. Hybrid Capture NGS:

DNA libraries with different indexes are pooled together, are denatured by heating and subjected to hybridization with single-stranded DNA oligonucleotides (also called 'probes') in a length of ~120 bp. Probes are biotinylated to allow their binding to streptavidin-coated magnetic beads and washing out of unbound DNA. Capture libraries are enriched using PCR amplification. Target enrichment libraries must be quantified using the KAPA Library Quantification Kit. Successful target enrichment is indicated by a total output volume > 9 ng at a concentration of ≥0.5 ng/µl.

10. Sequencing

Sequencing libraries prepared from this kit are sequenced on Illumina NextSeq 550Dx sequencing platforms. Sample libraries are quantified and normalized into library enrichment pools of up to 8 samples each. A maximum of 28 samples with one positive control and one negative control can be run in one sequencing batch. Pooled sample libraries should be quantified using a qPCR method prior to sequencing.

11. Data Analysis

a) **Data Management System (DMS):** Sequencing data is automatically processed 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 & FASTQ Generation:** Demultiplexing of BCL files is done by adapter sequences indicated on the samples sheet. Paired FASTQ files are generated which contains sequence and base quality score information. The FASTQ formatted data files are used for subsequent processing of samples.

c) **Run QC check:** Quality control for each sequencing run is determined by cluster density and Q30 read proportion. Cluster density measures the number of clusters on a flow cell, with a sequencer cluster density ≥ 135 being the passing threshold. For each run, the proportion of total reads with Q30 must be greater than or equal to 80%.

d) **Read Alignment & BAM Generation:** To map sequence reads for each sample to the human reference genome (hg19/GRCh37), genome alignment is carried out. The resulting alignments are stored as Binary Alignment Map (BAM) files, presenting information about read placement in relation to the reference genome along with quality scores. Subsequently, the aligned BAM files undergo additional processing

within a pipeline to pinpoint genomic alterations.

e) **Sample QC checks:** A bioinformatic analysis of genome haplotypes is conducted on samples to screen for potential contamination. This involves examining pre-defined SNP sites representative of populations and individuals. Samples displaying multiple haplotypes are deemed potentially contaminated and samples with higher than 4% contamination is flagged as failed. Additionally, sequence coverage is evaluated across the panel, necessitating that at least 90% of targeted regions have a minimum coverage exceeding 100x.

f) **Mutation calling:** A fully automated pipeline for bioinformatic analysis is used to identify genomic alterations, including SNVs, indels, *ERBB2* amplifications, *ALK* translocations, *RET* translocations, *ROS1* translocations, *NTRK1* translocations, MSI status, and TMB score.

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 SNVs and indels, at least 2% allele frequency and 5 mutant reads is required. For a hotspot variant, at least a 1% VAF and 4 supporting mutant reads is required. Clinically significant variants must have an allele frequency of greater than 0.4% and at least 4 supporting mutant reads.

ii. ***Amplification:*** GENESEEQPRIME assay is only validated for reporting *ERBB2* amplifications. Only amplifications that have equal to or more than a 1.8-fold change will be reported.

iii. ***Translocation:*** This assay only reports the presence or absence of translocations involving four hotspot genes (*ALK*, *RET*, *ROS1*, and *NTRK1*). For each translocation with a hotspot gene and its canonical partner, a minimum of 6 fusion supporting reads is required. Alternatively, if the partner gene is a non-canonical partner, a minimum of 12 fusion supporting reads is required.

iv. ***Microsatellite Status:*** Microsatellite status for each sample is evaluated based on the mutation status of 61 microsatellite sites from within the regions 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.

v. ***Tumor Mutation Burden (TMB):*** TMB is calculated based on detected sequence mutations and indels. Filtering of sequence mutations is performed to exclude low mutant allele fraction mutations (<2% VAF), 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).

12. Controls:

The GENESEEQPRIME assay kit includes a negative control (NC) and a positive control (PC) to monitor the performance of the instruments and the reagents. All external control samples should be processed the same as the testing samples and should pass all quality control criteria, and all expected mutations should be detected in the PC sample, but not the NC sample.

a) Negative Control:
A non-cancerous cell line (NA18535) is included in the reagent kit and must be included on the sequencing run. The purpose of the negative control is to validate the quality of the sequencing run. No variants of interest should be detected in the negative control sample for sequencing run to pass quality control thresholds.

b) Positive Control:
The positive control sample is included in each kit. The positive control contains 7 alterations of interest (*BRAF* V600E, *EGFR* L858R, *EGFR* Exon 19 Deletion, *KRAS* G13D, *TPM3~NTRK1* translocation, *CD74~ROS1* translocation, *ERBB2* amplification) and must be included on every sequencing run. All 7 alterations must be detected in the positive control sample for a sequencing run to pass quality control.

13. Result reporting:

a) Somatic single nucleotide variants and short insertion/deletion mutations are reported under one of two categories: "Variants with evidence of clinical significance" or "Variants with potential of clinical significance". The two categories are based on the supporting level of clinical evidence listed on the document "CDRH's Approach to Tumor Profiling Next Generation Sequencing Tests". Common germline mutations and polymorphisms are masked from the final report if they appear in 1000g (version 201508), ExAC (version 0.3nontcga), or gnomAD (version r2.0.1). Variants determined to be germline based on the Tumor-Only Sequencing module are also excluded from reporting.

b) Reporting of microsatellite status for samples is either high microsatellite instability (MSI-H), microsatellite stable (MSS), or uncertain status (Indeterminate).

c) Tumor mutational burden (TMB) is reported in terms of mutations per megabase (mut/mb). Driver mutations are not included in the count of mutations. Both synonymous and non-synonymous mutations are included in the count of mutations.

d) Presence of copy number variations on *ERBB2* and gene translocations on four genes (*ALK, RET, ROS1, NTRK1*) are reported. These alterations are reported under one of two categories: "Variants with evidence of clinical significance" or "Variants with potential of clinical significance". The two categories are based on the supporting level of clinical evidence listed on the document "CDRH's Approach to Tumor Profiling Next Generation Sequencing Tests".

e) The turnaround time for the GENESEEQPRIME assay from DNA to final clinical report is typically 5 business days. This includes all steps in the workflow: library preparation, sequencing, bioinformatics analysis, and report generation.

14. 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 GENESEEQPRIME Post-Sequencing Quality Control Metrics

Quality Metric	Level of Qualification	Passing Criteria
Cluster Density	Batch-level	Sequencer Cluster Density ≥ 130
Q30 Reads	Batch-level	%Q30 (Total) ≥ 80%
External Control	Batch-level	7 known mutations in positive control are detected; No level 2 or hotspot mutations detected in negative control
Percent Regions Covered	Sample-level	≥ 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 ≥ 4; VAF ≥ 0.4%
Hotspot SNVs and Indels	Analyte-level	Mutant reads ≥ 4; VAF ≥ 1%
Non-hotspot SNVs and Indels	Analyte-level	Mutant reads ≥ 5; VAF ≥ 2%
MSI Detection	Analyte-level	MSI Score ≥ 16
ERBB2 Amplification	Analyte-level	Fold change ≥ 1.8
Translocations (*ALK*, *NTRK1*, *RET*, and *ROS1*)	Analyte-level	Fusion reads ≥ 6 for canonical partner genes; Fusion reads ≥ 12 for non-canonical partner genes

B. Principle of Operation:

The GENESEEQPRIME assay kit is comprised of reagents for sequencing library preparation and target enrichment for 425 cancer-related genes. The starting material for this kit is extracted genomic DNA from FFPE specimen, which will be subjected to fragmentation, end repair, adaptor ligation, polymerase chain reaction (PCR) amplification and DNA probe hybridization capture of human genomic regions of interest. The regions of interest include the exonic regions, select intronic regions, and specific microsatellite regions of 425 genes within the human genome. The targeted DNA fragments are then enriched using magnetic beads followed by PCR amplification. Library quantification and quality control will be performed on the enriched library before library sequencing.

C. Determination of assay thresholds:

1. Exon coverage

To determine the minimum exon coverage necessary for downstream review, a power analysis was conducted on 11 FFPE samples. We found that mutations with a true frequency of 2% (95% CI: 1.02%-3.90%) is detected with 95% statistical power at >400X coverage. Mutations with a true frequency of > 5% (95% CI: 3.05%-8.10%.) can be reliably detected at >300X (Figure 1).

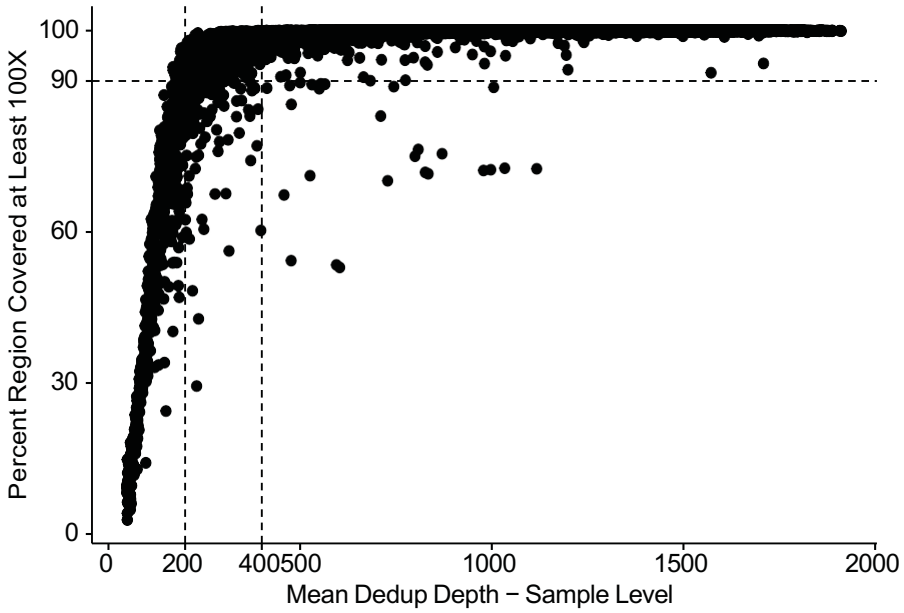
Figure 1. Power analysis of mutation detection based on exon coverage. Mutations were separated into 4 types based on variant allele frequency (True VAF = 20%, 10%, 5%, and 2%). Lower dotted line indicates a 90% sensitivity in detecting variants and the upper dotted line indicates the 95% sensitivity threshold.

Summary metrics were computed for individual exons within a sample cohort of 3000 normal tissue samples to pinpoint regions prone to sequencing artifacts. These specific regions were subsequently eliminated from the GENESEEQPRIME assay, rendering

them ineligible for variant analysis or inclusion in reports. No variants with explicit evidence of clinical significance or somatic hotspot mutations are withheld from the report.

Sequence coverage requirement was evaluated in the remaining regions across a cohort of 200 FFPE samples, and 90% of targeted regions (~4,756 of 5,286 regions) were sequenced to a depth of 100X or greater if the deduplicated sample level coverage is 200X. If the deduplicated sample level coverage is 400X, 90% of regions of interest achieved 200X coverage or greater (Figure 2).

A



B

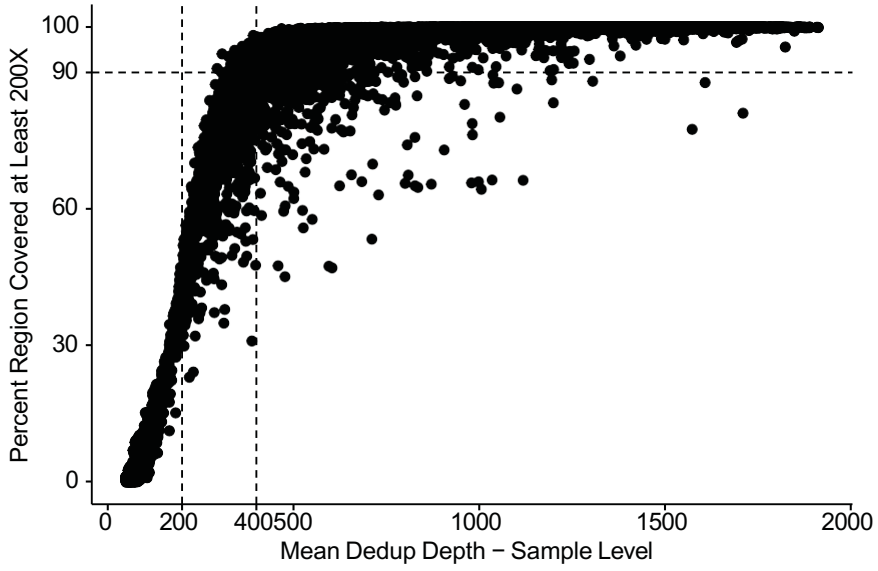


Figure 2. Percent of regions of interest achieving A) 100X or B) 200X coverage in correlation to sample level depth. Horizontal dotted line indicates 90 percent of regions. The leftmost vertical dotted line in each figure indicates the sample level average deduplicated depth of 200X. The rightmost vertical dotted line in each figure indicates the sample level average deduplicated depth of 400X.

2. Sample coverage

Sample level summary metrics of 200 FFPE samples from across 10 tumor types were evaluated to determine the required sample coverage for report generation and analysis. Overall exonic coverage was high in the regions of interest with a high percentage of on target reads. The mean coverage across all targeted regions for the 200 FFPE samples is 978X (± 245X standard deviation).

The assessment of sequence coverage was extended to determine the minimum requirements for the analysis and reporting of variants. A power analysis indicated that a minimum sequence coverage of 100x is essential for accurately detecting mutations with an underlying mutation frequency of 2% or higher. To establish a per-sample threshold, the analysis considered the number of exons in individual samples meeting this coverage criterion. The samples under evaluation encompassed a spectrum 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 robust coverage affirms the capacity to tolerate occasional dips in coverage that might arise due to fluctuations in sample quality. Based on this analysis, the threshold sample coverage for analysis and report generation is set at 100X in at least 90% of evaluated regions. This criterion is employed to assess whether a sample has been sequenced deeply enough to warrant analysis and inclusion in the reporting process.

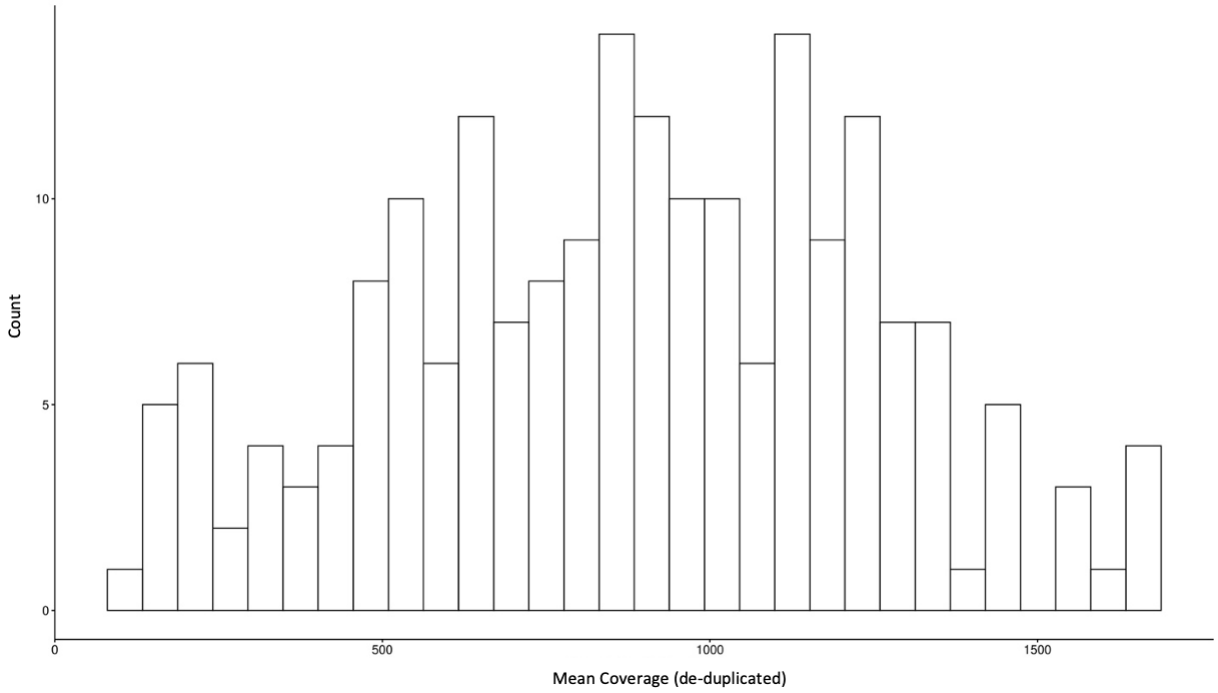


Figure 3. Distribution of mean distinct coverage per sample in GENESEEQPRIME across 200 FFPE samples.

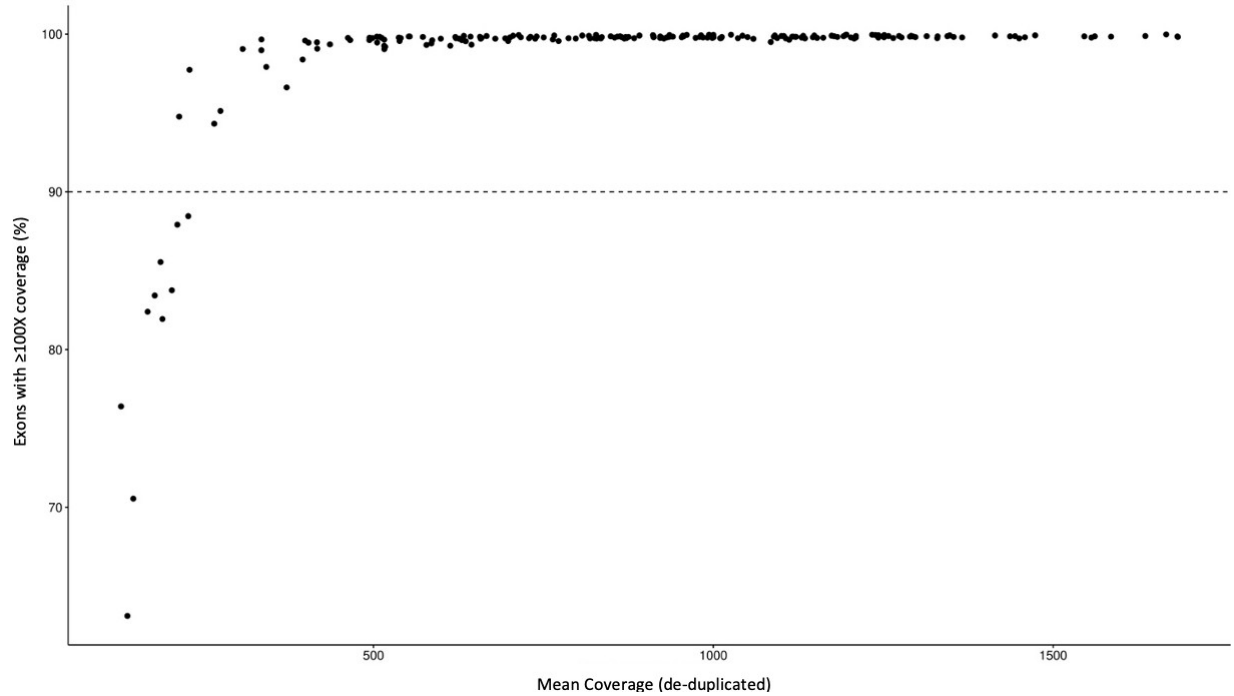


Figure 4. Distribution of mean deduplicated depth per sample and percentage of regions with more than 100X coverage. Dotted line indicates the cut-off of 90% regions with more than 100X coverage.

3. Determination of assay cutoff

To ensure high specificity in mutation detection, 30 normal FFPE samples were analyzed under varying stringency levels, initially yielding 273 false positives (209 non- hotspot, 58 hotspot, 6 clinically significant variants). Applying mutation-specific filters—VAF and supporting read thresholds tailored to each category—eliminated all false positives, achieving a 100% rejection rate. Clinically significant variants are reported by GENESEEQPRIME when VAF is ≥0.4% with ≥4 supporting reads, hotspot variants (from COSMIC v96 and internal databases) at ≥1% VAF with ≥4 reads, and non-hotspot variants at ≥2% VAF with ≥5 reads. For *ERBB2* copy number variation, the assay reports amplification only when fold change exceeds 1.8x relative to diploid state, based on a cut-off set at 4 standard deviations above the mean from 46 *ERBB2*-negative samples. Microsatellite instability (MSI) status is determined by the proportion of somatic sites among total tracked sites, with a validated cut-off set at 16% based on ROC analysis of 100 samples. For gene translocations (*ALK, RET, ROS1,*and *NTRK1*), baseline fusion read counts from 39 FFPE-negative samples were used to establish detection thresholds: ≥6 reads for canonical partners and ≥12 reads for non-canonical partners.

4. Somatic Mutation Detection by Tumor only Sequencing (ToSeq) Module

The GENESIS software includes a machine learning module, ToSeq, which distinguishes somatic from germline SNVs and indels without matched normal samples by evaluating features related to variant location, frequency, pathogenicity, and database presence. Trained on 3,189 tumor-normal pairs and tested on 4,781 independent pairs (7,970 total), ToSeq uses a Distributed Random Forest model that achieved high performance (SNV AUC = 0.9867; indel AUC = 0.9818). In total, 132,734 SNVs and 11,086 indels were assessed, with the ToSeq module demonstrating 95.59% (95% CI: 95.40% - 95.78%) sensitivity and 95.63% (95% CI: 95.43% - 95.82%) positive predictive value against reference classifications.

D. Substantial Equivalence Information

1. Predicate Device Name(s):

PGDx elio Tissue Complete

2. Predicate Device 510(k) Number

K192063

3. Comparison of technological characteristics with the predicate device

Characteristics	**Predicate device:** **PGDx elio tissue complete**	**Subject Device:** **GENESEEQPRIME NGS tumor profiling assay (FFPE)**
Similarities		

Indications for Use	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. 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.	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.
Technology	Hybrid Capture	Same
Specimen Type	Formalin-fixed, paraffin-embedded (FFPE) tumor tissue from patients with solid malignant neoplasms	Same
Target Population	Patients with malignant solid neoplasms	Same
Instrument	Illumina NextSeq 550Dx (qualified by PGDx)	Illumina NextSeq 550Dx
Test Environment	Kit	Same
Differences		
Genes on Panel	505 genes	425 genes
Variant Types	Somatic Variants including point mutations, small insertions, and small deletions, *ERBB2* amplification, 4	Somatic Variants including point mutations, small insertions, and small deletions, *ERBB2* amplification, 4 gene

	gene translocations (*ALK, RET, NTRK2*, and *NTRK3*), MSI and TMB information.	translocations (*ALK, RET, NTRK1*, and *ROS1*), MSI and TMB information.
Black List	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)
Determination of Pipeline Threshold	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%).
Assay cut-off	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. SNVs with lower bound 95% Confidence Interval <5% MAF based on sequence coverage are excluded from reporting. Common germline mutations present in dbSNP, ExAC, and gnomAD are identified and excluded from reporting. Additional germline mutations with ≥ 3 matches in ExAC and MAF ≥ 20% are also excluded from reporting.	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. SNVs and Indels <2% VAF based on sequence coverage are excluded from reporting. Common germline mutations present in 1000g, ExAC, and gnomAD are identified and excluded from reporting. GENESIS software includes a self-developed machine learning model used to distinguish germline SNVs and Indels from somatic SNVs and Indels without the need for a matched normal sample.
Controls	• Positive Control • No template control (NTC) • Normalized to database of common germline SNPs	• Positive Control • Negative Control • Normalized to database of common germline SNPs
Samples per Run (controls excluded)	15	28

Clinical Evidence Curation	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. 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.	GENESEEQPRIME assay reports variants as either a Variant with Evidence of Clinical Significance or a Variant with Potential Clinical Significance depending on the designated cancer type. 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.

E. Guidance Documents Referenced

1. 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)
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. Introduction

This document provides a comprehensive summary of the accuracy, reproducibility, and analytical performance of the GENESEEQPPRIME assay. The outlined performance

studies evaluate the assay's ability to deliver consistent and accurate results across diverse clinical and laboratory settings.

2. Accuracy Study: Method Comparison

Concordance of variant calls, translocation and *ERBB2* detection, microsatellite status, and TMB were compared between appropriate comparator methods and GENESEEQPRIME. SNVs, insertions, and deletions are compared to an externally validated orthogonal NGS method. Gene translocations, CNV, and MSI are compared to the corresponding FISH or IHC test as well as an orthogonal NGS method. TMB is compared to whole exome sequencing (WES) and an orthogonal NGS method. In total, there were 503 FFPE samples in accuracy studies. 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. Ethnic origin of sample included in the method comparison study is summarized in Table 7. Samples originated from multiple sites. Samples cover 40 distinct tumor types. Detailed sample invalid rate for each cancer type post-sequencing is presented in Table 8. Five samples failed pre-sequencing quality control for GENESEEQPRIME. No samples were pre-screened using GENESEEQPRIME results.

Table 7. Distribution of ethnic background of all samples used in the method comparison study between GENESEEQPRIME and the orthogonal NGS device.

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%
Sum	503	100.00%

Table 8. Overall post-sequencing sample pass rate for each assay used in the accuracy study sorted by cancer type.

Cancer Type	GENESEEQPRIME		
	Samples passed QC	Total samples tested	Invalid rate
Bladder Cancer	16	17	5.88%
Breast Cancer	83	91	8.79%
Cervical Cancer	10	11	9.09%
Cholangiocarcinoma	3	3	0.00%
Colorectal Cancer	71	72	1.39%

Endometrial Cancer	28	30	6.67%
Esophageal Cancer	5	6	16.67%
Gastric Cancer	14	15	6.67%
Gastrointestinal Stromal Tumor	3	4	25.00%
Head and Neck Cancer	11	13	15.38%
Liver Cancer	11	11	0.00%
Lung Adenocarcinoma	8	8	0.00%
Melanoma	13	15	13.33%
Non-small cell lung cancer	64	66	3.03%
Others*	28	30	6.67%
Lung adenosquamous carcinoma	3	3	0.00%
Ovarian Cancer	10	11	9.09%
Pancreatic Cancer	6	6	0.00%
Prostate Cancer	32	32	0.00%
Renal Cancer	17	18	5.56%
Skin Cancer	12	14	14.29%
Soft Tissue Sarcoma	7	8	12.50%
Thyroid Cancer	13	14	7.14%
Total	**468**	**498**	**6.02%**

*Other cancer types include central nervous system cancers, fallopian tube, mediastinum, medulloblastoma, penis, testis, vulva, breast cancer (solid tumor), thymus tumor, biliary tract cancer, glioma, lung squamous cell cancer, neuroendocrine tumor, carcinosarcoma, lung-NOS**, lung-SCLC, small intestine cancer, and head and neck cancer.
**NOS: Not otherwise specified

a) Accuracy for SNVs, Indels, CNV, gene translocations, TMB, MSI

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 and the appropriate comparator to evaluate the degree of concordance between the assays (Table 9).

Table 9. Accuracy of GENESEEQPRIME

Variant Category	Orthogonal Method	Analysis Category	PPA (2-sided 95% CI)	NPA (2-sided 95% CI)
Overall (SNVs and indels)	Predicate Device (NGS)	All	92.44% (91.45%, 93.33%)	99.99% (99.99%, 99.99%)
		SNVs	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%)
		SNVs	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%)
		SNVs	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%)
		SNVs	97.89% (96.16%, 98.85%)	99.98% (99.97%, 99.98%)
		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%)
		SNVs	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%)
ERBB2	FISH	Amplification	93.75% (83.16%, 97.85%)	100% (88.30%, 100%)

ALK	FISH	Translocation	88.89% (56.50%, 99.43%)	90.91% (62.26%, 99.53%)
RET	FISH	Translocation	100% (56.55%, 100%)	66.67% (41.71%, 84.82%)
ROS1	FISH	Translocation	100% (67.56%, 100%)	100% (72.24%, 100%)
NTRK1	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

The objective of this analysis is to compare the efficacy of GENESEEQPRIME at filtering out germline mutations in comparison to using databases alone. For comparison, we also obtained data from an orthogonal NGS method. Samples used in the method comparison study were filtered for matching normal sample (buffy coat of adjacent FFPE). 11 FFPE samples with matching normal samples were selected to undergo this analysis. Samples originate from 6 different cancer types and includes colorectal cancer, gastric cancer, head and neck cancer, non-small cell lung cancer, breast cancer, and endometrial cancer (Table 10). The mutation results of each tumor-normal sample pair are compared to identify the reference list of germline and somatic mutations. In these 11 samples, 292 somatic variants were reported in total from the GENESEEQPRIME assay. 8 variants were found to be germline variants based on comparison to the normal control sample (2.74% false positive rate). The orthogonal device reported 276 somatic mutations that were also covered on the GENESEEQPRIME panel. Of those, 38 were determined to be germline variants (13.77% false positive rate). For each sample, 2 to 7 variants were reported by the orthogonal device and were detected but not reported by the GENESEEQPRIME assay. These variants were all found to be present in the germline control sample, indicating that the GENESEEQPRIME assay successfully filtered out germline variants using only tumor samples. Based on the findings from this analysis, we determine that the GENESEEQPRIME is efficient at filtering out germline variants and has germline variants reporting rate 2.74%. In the samples tested, 100% of variants reported by the orthogonal device and filtered out by the GENESEEQPRIME were germline mutations.

Table 10. Summary of germline variants found in each tumor or germline control sample using GENESEEQPRIME.

Sample ID	Germline control type	Cancer type	GENESEEQ PRIME reported variants	Orthogonal reported variants on GENESEE Q PRIME targetable range	Number of variants found in germline control
Sample 1	Buffy Coat	Colorectal Cancer	38	29	3
Sample 2	Buffy Coat	Gastric Cancer	6	11	7
Sample 3	Buffy Coat	Head and Neck Cancer	8	12	3
Sample 4	Tumor adjacent tissue	Colorectal Cancer	150	137	2
Sample 5	Tumor adjacent tissue	Non-Small Cell Lung Cancer	27	19	3
Sample 6	Buffy Coat	Breast Cancer	4	6	2
Sample 7	Tumor adjacent tissue	Breast Cancer	5	6	2
Sample 8	Buffy Coat	Endometrial Cancer	37	33	3
Sample 9	Tumor adjacent tissue	Colorectal Cancer	2	5	3
Sample 10	Buffy Coat	Breast Cancer	8	12	2
Sample 11	Tumor adjacent tissue	Non-Small Cell Lung Cancer	7	6	2

ii. Accuracy - SNVs and Indels

Accuracy of variant calls (variants with evidence of clinical significance, hotspot SNVs, non-hotspot SNVs, insertions, deletions) was obtained using a comparison between the reported results of the GENESEEQPRIME and the predicate (NGS based assay) and was determined using positive percent agreement (PPA) and negative prevent agreement (NPA) at a variant level. The 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 GENESEEQPRIME results. Overall, the GENESEEQPRIME assay yielded concordance analytical performance for variant calls across the SNVs and Indels with a PPA ≥ 91.53% and an NPA ≥ 99.00%. Table 11 shows the concordance between detected mutations for each category of variants (Variants with evidence of clinical significance, variants with potential clinical significance, hotspot, non-hotspot, and overall).

Table 11. Concordance for different categories of variants between GENESEEQPRIME and the predicate device

Variant category	Analysis category	PPA (n/N) (95%CI)	NPA (n/N) (95%CI)
Variants with evidence of clinical significance	All	96.53% (167/173) (92.64%, 98.4%)	99.94% (21811/21823) (99.90%, 99.97%)
	SNVs	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%)
Hotspot variants	All	98.08% (512/522) (96.51%, 98.96%)	99.98% (780573/780758) (99.97%, 99.98%)
	SNVs	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%)
	SNVs	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%)
Variants with potential clinical significance	All	92.19% (2645/2869) (91.15%, 93.12%)	99.99% (1122425051/1122426777) (99.99%, 99.99%)
	SNVs	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%)
Overall	All	92.44% (2812/3042) (91.45%, 93.33%)	99.99% (1120688450/1120690189) (99.99%, 99.99%)
	SNVs	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%)
Insertion	1~5bp	96.43% (108/112) (91.18%,98.60%)	99.99% (374079866/374079935) (99.99%, 99.99%)
	6~10bp	100% (5/5) (56.55%,100%)	99.99% (374080038/374080042) (99.99%, 99.99%)
	11~20bp	100% (2/2) (34.24%,100%)	99.99% (374080044/374080045) (99.99%, 99.99%)
	21~30bp	N/A% (0/0) (N/A, N/A)	99.99% (374080044/374080047) (99.99%, 99.99%)
Deletion	1~5bp	97.60% (325/333) (95.33%,98.78%)	99.98% (373959827/373960003) (99.97%, 99.98%)
	6~10bp	90.91% (10/11) (62.26%,99.53%)	99.99% (373960319/373960325) (99.99%, 99.99%)
	11~20bp	95.83% (23/24) (79.76%,99.79%)	99.99% (373960301/373960312) (99.99%, 99.99%)
	21~30bp	100% (4/4) (51.01%,100%)	99.99% (373960327/373960332) (99.99%, 99.99%)

iii. Accuracy - ERBB2 amplification

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 12). 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 13) was observed.

When borderline FISH values (1.5–2.5) were excluded, the PPA slightly improved to 95.56%, with NPA remaining at 100%. Notably, in cases limited to borderline FISH values (1.5–2.5), the PPA decreased (66.66%), while NPA stayed at 100%, indicating the assay performs more reliably outside borderline ranges.

Table 12. Summary of concordance between GENESEEQPRIME assay and *ERBB2* FISH assay results.

ERBB2 amplification*		FISH		
		Detected	Not detected	Total
GENESEEQPRIME	Detected	45	0	45
	Not detected	3	29	32
	Total	48	29	77
PPA		93.75% (83.16%, 97.85%)		
NPA		100% (88.30%, 100%)		
OPA		96.10% (89.16%, 98.67%)		

*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 13. Summary of concordance between GENESEEQPRIME assay and *ERBB2* FISH borderline assay results.

Category	Total cases	TP	FP	FN	TN	PPA (95% CI)	NPA (95% CI)	PPV (95% CI)	NPV (95% CI)
All Cases	77	45	0	3	29	93.75% (83.16%, 97.85%)	100% (88.30%, 100%)	100% (92.13%, 100%)	90.63% (75.78%, 96.76%)
Excluding FISH 1.5-2.5	67	43	0	2	22	95.56% (85.17%, 98.77%)	100% (85.13%, 100%)	100% (91.80%, 100%)	91.67% (74.15%, 97.68%)
Excluding FISH 1.8-2.2	75	45	0	3	27	93.75% (83.16%, 97.85%)	100% (87.54%, 100%)	100% (92.13%, 100%)	90% (74.38%, 96.54%)
Only FISH 1.5-2.5	10	2	0	1	7	66.66% (20.77%, 98.29%)	100% (64.57%, 100%)	100% (32.23%, 100%)	87.5% (52.91%, 99.36%)
Only FISH 1.8-2.2	2	0	0	0	2	NA (NA)	100% (32.24%, 100%)	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 14 below.

Table 14. Comparison of *ERBB2* amplification for GENESEEQPRIME and orthogonal NGS device

ERBB2 Amplification*		Orthogonal device		
		ERBB2 (+)	*ERBB2* (-)	Total
GENESEEQPRIME	*ERBB2* (+)	32	2	34
	ERBB2 (-)	0	389	389
	Total	32	391	423
PPA (2-sided 95% CI)		100% (89.28%, 100%)		
NPA (2-sided 95% CI)		99.49% (98.15%, 99.86%)		
OPA (2-sided 95% CI)		99.53% (98.29%, 99.87%)		

*Cancer type for samples determined as positive for *ERBB2* amplification by either assay includes breast (n=27), colorectal (n=2), endometrial (n=1), ovary (n=1), esophagus (n=1), lung-NSCLC (n=1), and gallbladder(n=1).

iv. Accuracy - Gene translocation concordance (ALK)

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 GENSEEQPRIME assay and FISH is shown in Table 15.

Table 15. Summary of concordance between GENESEEQPRIME assay and *ALK* FISH assay results.

ALK Translocation*		FISH		
		ALK (+)	*ALK* (-)	Total
	ALK (+)	8	1	9

GENESEEQPRIME	*ALK* (-)	1	10	11
	Total	9	11	20
PPA		88.89% (56.50%, 99.43%)		
NPA		90.91% (62.26%, 99.53%)		
OPA		90.00% (69.9%, 97.21%)		

*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, renal cancer, and colorectal cancer, were used to assess the concordance of the GENESEEQPRIME assay to NGS based predicate device to detect *ALK* translocations. *ALK* translocations were detected in nine out of 10 samples in both the GENESEEQPRIME and the orthogonal comparator. One sample had an *ALK* translocation that was unreported by GENESEEQPRIME due to assay cutoff below the 6 reads required for reporting in GENESEEQPRIME. Further investigation into the analysis pipeline shows that the translocation event was detected by GENESEEQPRIME at only 5 alternate reads. The concordance results are shown in Table 16.

Table 16. Comparison of *ALK* translocation for GENESEEQPRIME and orthogonal device

ALK Translocation*		Orthogonal device		
		ALK (+)	*ALK* (-)	Total
GENESEEQPRIME	*ALK* (+)	9	0	9
	ALK (-)	1	413	414
	Total	10	413	423
PPA		90.00% (59.59%, 99.49%)		
NPA		100% (99.08%, 100%)		
OPA		99.76% (98.67%, 99.99%)		

*Cancer type for samples tested positive for gene translocations by either assay includes lung-NSCLC (n=8), renal (n=1), and colorectal (n=1).

v. Accuracy - Gene translocation concordance (RET)

Twenty (20) FFPE samples from 4 tumor types (non-small cell lung cancer, thyroid cancer, colorectal cancer, and sarcoma) were selected for this analysis. All samples that were found to be positive for *RET* rearrangement using the FISH assay were also detected by GENESEEQPRIME (5/5). NPA for the detection of *RET* rearrangement was 66.67% (10/15). Table 17 shows the results of the analysis in a 2 by 2 matrix.

It is likely that the false positive detection rate for *RET* translocation is due to the difference in sensitivity in the assays; 3 of the 5 false positive *RET* translocations were detected using three separate NGS methods (GENESEEQPRIME, orthogonal NGS

method, biosample repository report) (Table 18). One discordant sample was detected by both GEENSEEQPRIME and the biosample repository, but failed QC for the orthogonal method. Another discordant sample was detected by both GENESEEQPRIME and FISH but was not reported as positive by the biosample repository method. These additional results suggest that the false positive rate for *RET* translocation might be due to the differences in sensitivity between the GENESEEQPRIME assay and FISH, with fewer supporting reads compared to traditional FISH assays.

Table 17. Summary of concordance between GENESEEQPRIME assay and *RET* FISH assay results.

RET Translocation*		FISH		
		RET (+)	*RET* (-)	Total
GENESEEQPRIME	*RET* (+)	5	5	10
	RET (-)	0	10	10
	Total	5	15	20
PPA		100% (56.55%, 100%)		
NPA		66.67% (41.71%, 84.82%)		
OPA		75.00% (53.13%, 88.81%)		

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

Table 18. Detection status of *RET* translocations in 5 discordant FFPE samples using 3 NGS methods

Sample ID	**Cancer Type**	***RET* FISH Test Result**	**GENESEEQPRIME gene translocation Call**	**Orthogonal method gene translocation Call**	**Biorepository gene translocation call**
Sample 1	Lung-NSCLC	Negative	Detected *RET*: exon6~*RET*: exon12 (23 supporting reads)	Detected *RET-RET* (21 supporting reads)	Not detected
Sample 2	Lung-NSCLC	Negative	Detected *CCDC6*: exon1~*RET*: exon12 (84 supporting reads)	Failed QC	Detected *CCDC6*:exon1 ~*RET*:exon12
Sample 3	Thyroid	Negative	Detected *NCOA4*: exon7~*RET*: exon12 (239 supporting reads)	Detected *NCOA4- RET* (186 supporting reads)	Detected *NCOA4*:exon7 ~*RET*:exon12

Sample 4	Colorectal	Negative	Detected *NCOA4*: exon7~*RET*: exon12 (549 supporting reads)	Detected *NCOA4-RET* (60 supporting reads)	Detected *NCOA4*:exon9 ~*RET*:exon12
Sample 5	Colorectal	Negative	Detected NCOA4: exon7~*RET*: exon12 (172 supporting reads)	Detected NCOA4-*RET* (21 supporting reads)	Detected NCOA4:exon9 ~*RET*:exon12

In addition, a total of 10 *RET* positive samples representing five different tumor types including, non-small cell lung cancer, thyroid cancer, colorectal cancer, and neuroendocrine tumor, 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 (Table 19). Three samples with *RET* translocations reported by GENESEEQPRIME were negative for *RET* gene translocation in the orthogonal device report.

Table 19. Comparison of *RET* translocation for GENESEEQPRIME and orthogonal device

RET Translocation*		Orthogonal device		
		Gene translocation	No gene translocation	Total
GENESEEQPRIME	Gene translocation	7	3	10
	No gene translocation	0	413	413
	Total	7	416	423
PPA		100% (64.57%, 100%)		
NPA		99.28% (97.90%, 99.75%)		
OPA		99.29% (97.94%, 99.76%)		

*Cancer type for samples tested positive for gene translocations by either assay includes lung-NSCLC (n =4), thyroid (n =2), colorectal (n =2), neuroendocrine tumor (n =1), and sarcoma (n =1).

vi. Accuracy - Gene translocation concordance (ROS1)

The accuracy of the GENESEEQPRIME assay for calling *ROS1* translocation of samples is evaluated in comparison to the orthogonal method (*ROS1* FISH). Eight (8) out of 8 *ROS1* positive samples and 10 out of 10 negative *RET* samples were concordant between the GENESEEQPRIME and FISH assay. No samples failed for either assay. PPA of *ROS1* gene translocation is found to be 100% (8/8). Negative variant call concordance is 100% (10/10). Table 20 shows the results of the analysis in a 2 by 2 matrix. The results from the above assessment show that the GENESEEQPRIME is highly concordant to

FISH results for detecting *ROS1* translocations.

Table 20. Summary of concordance between GENESEEQPRIME assay and *ROS1* FISH assay results.

ROS1 Translocation*		FISH		
		ROS1 (+)	*ROS1* (-)	Total
GENESEEQPRIME	*ROS1* (+)	8	0	8
	ROS1 (-)	0	10	10
	Total	8	10	18
PPA		100% (67.56%, 100%)		
NPA		100% (72.24%, 100%)		
OPA		100% (82.41%, 100%)		

*Cancer type include NSCLC (n=18).

In addition, *ROS1* positive samples representing two different tumor types including, non-small lung cancer and breast cancer, were used to evaluate concordance between the GENESEEQPRIME assay and the orthogonal validated NGS comparator assay to detect *ROS1* translocations. For *ROS1* translocations, comparisons between the assays show a PPA of 100% for *ROS1* (6/6) translocations, as well as an NPA of 99.28% (414/417) (Table 21). *ROS1* positive FISH results were obtained for two of the three samples with discordant results.

Table 21. Comparison of *ROS1* translocation for GENESEEQPRIME and orthogonal device

ROS1 Translocation		Orthogonal NGS Comparator		
		Gene translocation	No gene translocation	Total
GENESEEQPRIME	Gene translocation	6	3	9
	No gene translocation	0	414	414
	Total	6	417	423
PPA		100% (60.97%, 100%)		
NPA		99.28% (97.91%, 99.76%)		
OPA		99.29% (97.94%, 99.76%)		

*Cancer type for samples tested positive for gene translocations by either assay includes lung-NSCLC (n=8) and breast cancer (n=1)

vii. Gene translocation concordance (NTRK1)

A total of four (4) *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. GENESEEQPRIME assay results were compared to validated orthogonal NGS assay. For *NTRK1* translocations, comparisons between the assays show a PPA of 100% for *NTRK1* (3/3) translocations, as well as an NPA 99.76% (419/420) (Table 21). One sample was reported positive by GENESEEQPRIME that was reported negative for *NTRK1* translocation by the orthogonal comparator. The concordance between the detected *NTRK1* translocations is shown in Table 22.

Table 22. Comparison of *NTRK1* translocation for GENESEEQPRIME and orthogonal device

NTRK1 Translocation		Orthogonal NGS Comparator		
		Gene translocation	No gene translocation	Total
GENESEEQPRIME	Gene translocation	3	1	4
	No gene translocation	0	419	419
	Total	3	420	423
PPA		100% (43.85%, 100%)		
NPA		99.76% (98.66%, 99.99%)		
OPA		99.76% (98.67%, 99.99%)		

*Cancer type for samples tested positive for gene translocations by GENESEEQPRIME include colorectal cancer (n=3) and sarcoma (n=1).

viii. Accuracy - MSI status concordance

The accuracy of the GENESEEQPRIME assay for calling MSI status of samples is evaluated in comparison to the orthogonal method, validated IHC test. A total of 92 samples were used for this analysis, of which 44 were colorectal, 22 of endometrial, 6 of stomach cancer, 2 of cervix, melanoma, skin, bladder, and thyroid, 1 of prostrate, kidney, pancreas, NSCLC, ovary, breast, hand and neck, and small intestine cancer. No samples failed QC for either assay. The positive agreement of GENESEEQPRIME in detecting microsatellite instability in comparison to IHC results is 97.50% (39/40). Negative agreement for the two assays is 90.38% (47/52). Table 23 shows the results of the analysis in a 2 by 2 matrix.

Furthermore, analysis was performed to examine the concordance to IHC of GENESEEQPRIME for specifically colorectal cancer and endometrial cancer samples, as these two tumor types tend to be over- represented in the MSI high population. Separating the samples based on cancer type reveals that CRC and endometrial cancer types had an over agreement of 98.48%, while other cancer types had an overall agreement of 80.77%. One case of false positive MSI was detected in the CRC/endometrial samples. This sample had a score of 19.64%, which is close to the cutoff of 16%.

Table 23. Summary of concordance between GENESEEQPRIME assay and IHC/dMMR assay results.

MSI status*		IHC		
		dMMR	Not detected	Total
GENESEEQPRIME	MSI-H	39	5	44
	MSS	1	47	48
	Total	40	52	92
PPA		97.50% (87.12%,99.87%)		
NPA		90.38% (79.39%, 95.82%)		
OPA		93.48% (86.49%, 96.98%)		
PPA (Only CRC +Endometrial)		100% (89.85%, 100%)		
NPA (Only CRC +Endometrial)		96.88% (84.26%, 99.84%)		
OPA (Only CRC +Endometrial)		98.48% (91.90%, 99.92%)		

*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. To ensure only samples with reliable MSI calls were included in this analysis, samples with an "Indeterminate" result for MSI status were removed. Comparing between the MSI status of all samples using GENESEEQPRIME and the orthogonal assay results showed a PPA of 96.77% (30/31) and an NPA of 97.67% (377/386) (Table 24).

Furthermore, the samples were characterized based on whether or not the tumor type was colorectal cancer (CRC) or endometrial cancer. Table 25 shows the concordance of MSI calls for GENESEEQPRIME and the orthogonal assay for all samples that belong to the CRC or endometrial tumor type. While the PPA achieved 96.15%, 5 discordant cases were observed. Out of a total of 5 discordant samples, 4 of the samples (3 MSI-H and one MSS) had IHC results matching the GENESEEQPRIME MSI call. The remaining discordant sample had an MSI score of 19.64% (near the threshold of 16%).

Table 24. Comparison of MSI status for GENESEEQPRIME and orthogonal device

MSI		Orthogonal device			
		MSI-H	MSS	Indeterminate	Total
	MSI-H	30	9	1	40
	MSS	1	377	1	379

GENESEEQPRIME	Indeterminate	0	4	0	4
	Total	31	390	2	423
PPA (without indeterminate samples)		96.77% (83.81%, 99.83%)			
NPA (without indeterminate samples)		97.67% (95.63%, 98.77%)			
OPA (without indeterminate samples)		97.60% (95.64%, 98.69%)			
PPA (All)		96.77% (83.81%, 99.83%)			
NPA (All)		96.67% (94.38%, 98.04%)			
OPA (All)		96.22% (93.94%, 97.66%)			

*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 25. Comparison of MSI status for CRC or endometrial cancer samples using GENESEEQPRIME and orthogonal device

MSI (CRC or Endometrial cancer)		Orthogonal device			
		MSI-H	MSS	Indeterminate	Total
GENESEEQPRIME	MSI-H	25	4	1	30
	MSS	1	54	1	56
	Indeterminate	0	0	0	0
	Total	26	58	2	86
PPA (without indeterminate samples)		96.15% (81.11%, 99.8%)			
NPA (without indeterminate samples)		93.10% (83.57%, 97.29%)			
OPA (without indeterminate samples)		94.05% (86.81%, 97.43%)			
PPA (All)		92.59% (76.63%, 97.94%)			
NPA (All)		91.53% (81.65%, 96.33%)			
OPA (All)		91.86% (84.14%, 96.00%)			

* 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).

ix. 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. Tumor mutational burden scores as determined by GENESEEQPRIME and an alternative NGS method were compared for 423 samples. 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 above 2% variant allele frequency are included in this count. The TMB score is then reported as the normalized variant count per megabase of coding region. The concordance of the scores is measured using the Pearson correlation (Pearson = 0.9428, Figure 5). A linear regression analysis shows that the samples show a very high linear

correlation (slope = 1.1) between the GENESEEQPRIME and the orthogonal assay when evaluating TMB for the same sample.

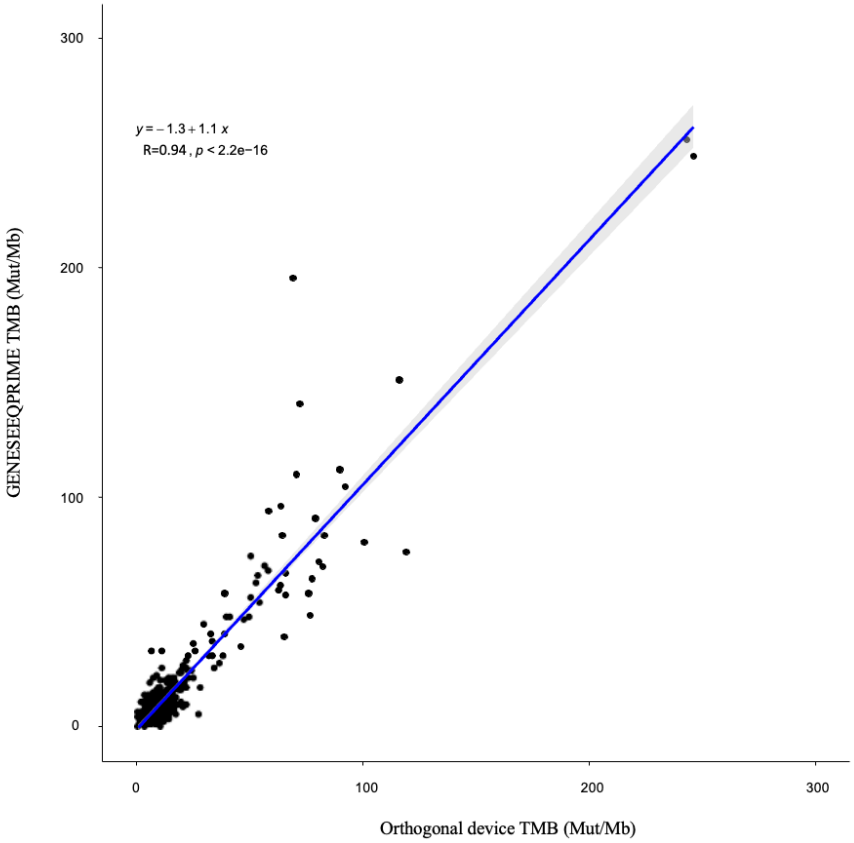


Figure 5. Scatterplot displaying the correlation between the estimated TMB score from GENESEEQPRIME and the orthogonal method. Units for both assays are in mutations per megabase of targetable region for that panel.

TMB Accuracy - Comparison to WES

TMB score concordance between GENESEEQPRIME and the WES orthogonal method are measured through the Pearson correlation coefficient. A total of 208 FFPE specimen were selected to undergo both GENESEEQPRIME sequencing and whole exome sequencing for TMB score determination, with matched FFPE specimens from 3 tumor types, including lung adenocarcinoma (n = 117), lung squamous cell carcinoma (n = 12), and colorectal cancer (n = 79). Both assays were run with tumor and matched normal samples to eliminate germline variants from being included in the TMB score. Figure 6 shows the relationship of TMB scores estimated by GENESEEQPRIME and WES. Correlation between TMB scores determined using the GENESEEQPRIME and WES shows Pearson correlation coefficient of 0.9298. Linear regression analysis shows that the TMB scores produced by GENESEEQPRIME displays correlation with WES analysis with slope = 1.2.

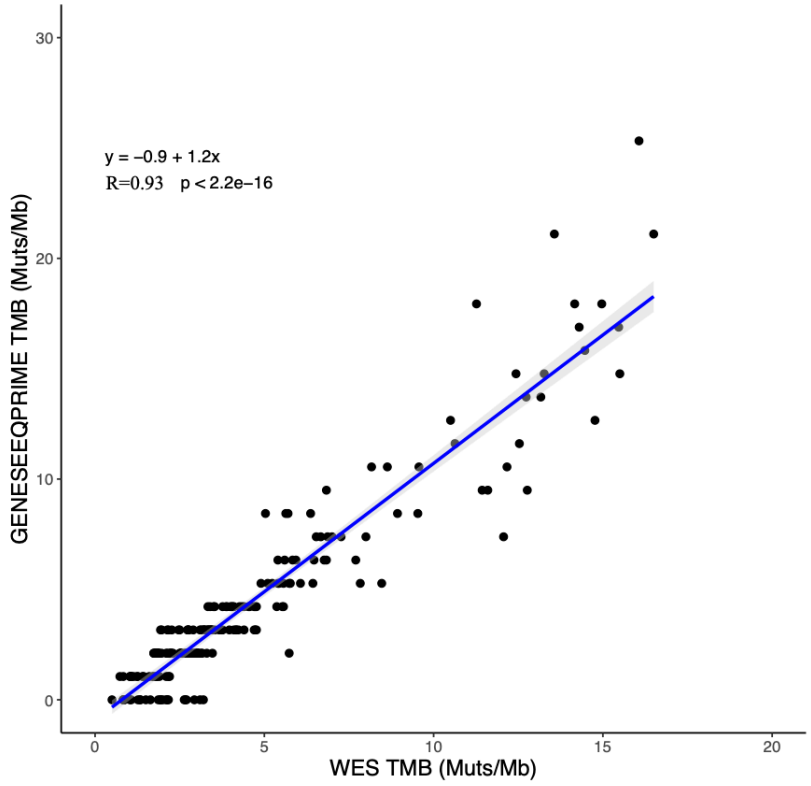


Figure 6. Tumor mutation burden scores measured by GENESEEQPRIME compared to whole exome sequencing with matched normal samples.

b) Wild-type variant calls

In this analysis, the GENESEEQPRIME assay was evaluated for its ability to detect wild-type variants across 15 genes and 52 clinically significant loci, using results from a predicate device as the reference standard. Across 167 clinical samples, GENESEEQPRIME demonstrated a PPA of 96.53% and an NPA of 99.93% for wild-type variant calls, with an OPA of 99.79% (Table 26). These high concordance metrics underscore the assay's analytical robustness in distinguishing both variant and wild-type calls.

Table 26. Performance metrics of wild type variant calls using GENESEEQPRIME

Orthogonal Method	Total cases sample	True Positive	False Positive	False Negative	True Negative	PPA (95% CI)	NPA (95% CI)	OPA (95% CI)
Predicate Assay	167	167	12	6	8499	96.53% (92.64%, 98.40%)	99.93% (99.85%, 99.97%)	99.79% (99.67%, 99.87%)

3. Reproducibility Study

a) Interlaboratory reproducibility study

Three molecular diagnostic testing laboratories in the United States were enrolled in the interlaboratory reproducibility validation of GENESEEQPRIME. Tumor types considered in this analysis include: 1) non-small cell lung cancer, 2) breast cancer, 3) colorectal cancer, 4) melanoma, 5) skin cancer, 6) thyroid cancer, 7) head & neck cancer, 8) esophageal cancer, 9) endometrial cancer, 10) prostate cancer, 11) ovarian cancer, 12) bladder cancer, and 13) stomach cancer. A total of 28 samples were selected. These selected samples represent a cohort of clinically relevant variants, translocations, *ERBB2* amplification, and both MSI-high and MSS samples. Samples were selected to span a range of variant allele frequencies, including those at 1X–1.5X the established LOD. A total of 671 SNVs, 23 insertions, and 121 deletions were found in the samples. At least one sample was selected for presence of each of the translocations of interest (*ALK* translocation*, RET* translocation*, ROS1* translocation*, NTRK1* translocation) or *ERBB2* amplification. Samples were distributed to three laboratories across the USA.

Test conditions involved in determining the reproducibility of the GENESEEQPRIME assay are 1) testing site, 2) test operator, 3) variability between replicates, and 4) variability between testing days. Each sample will be tested in duplicate by 2 different operators across 3 non-consecutive days at each of the 3 independent laboratory sites using a single kit lot (2x2x3x3=36 replicates). Reproducibility was assessed in 5 ways; 1) average positive and negative agreement of between all possible replicates within each test condition listed above is used to analyze sources of variance; 2) coefficient of variance of TMB score between all replicates within each test condition listed above is used to analyze sources of variance; 3) concordance of MSI status calls between all replicates of each samples will be used ; 4) positive call rate for variants that are detected in over 50% of replicates for each sample (Modal PCR) will be used to determine per specimen reproducibility; 5) modal PCR and NCR of variants stratified by allele frequency and variant type (SNV, INS, DEL) were determined to show reproducibility of GENESEEQPRIME performance across all levels and types of variants.

i. Specimen-level reproducibility

First, the degree of variability within each FFPE specimen was evaluated. Detection of sequence variations (SNVs and indels) within each replicate of the 28 specimens was tallied using the modal positive and negative call rate.

Results for sample level call rate for all replicates of 28 clinical specimen that passed QC are shown in Table 27. A total of 671 unique SNVs, 23 unique insertions, 121 unique deletions, and 144 indels were identified as part of this analysis. Overall modal PCR is 97.46% (16137/16558, range: 92.94% – 100%). Modal NCR is 94.27% (10506/11144, range: 83.82% – 96.64%). One replicate of Sample 2 was

excluded from the analysis because it did not meet the sample-level QC metrics. 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. In conclusion, the average reproducibility of alterations within sample replicates when using GENESEEQPRIME assay is 97.35% for modal positive call rate and 93.39% for modal negative call rate.

Table 27. Specimen-level variant call concordance for the GENESEEQPRIME assay

Sample	Unique mutations*	Modal Positive Call Rate (n/N) (95% CI)	Modal Negative Call Rate (n/N) (95% CI)
R01	39	95.35% (1005/1054) (93.91%,96.47%)	91.54% (249/272) (87.63%,94.30%)
R02	8	-	94.32% (249/264) (90.84%,96.53%)
R03	8	100% (34/34) (89.85%,100%)	96.22% (229/238) (92.97%,98.00%)
R04	32	100% (374/374) (98.98%,100%)	96.64% (690/714) (95.05%,97.73%)
R05	50	99.59% (1219/1224) (99.05%,99.83%)	91.81% (437/476) (89.00%,93.95%)
R06	9	100% (68/68) (94.65%,100%)	92.44% (220/238) (88.36%,95.16%)
R07	16	96.73% (296/306) (94.09%,98.22%)	92.02% (219/238) (87.87%,94.83%)
R08	43	94.41% (321/340) (91.44%,96.39%)	95.10% (1067/1122) (93.67%,96.21%)
R09	35	98.47% (837/850) (97.4%,99.1%)	96.18% (327/340) (93.57%,97.75%)
R10	17	100% (68/68) (94.65%,100%)	96.08% (490/510) (94.02%,97.45%)
R11	13	95.8% (228/238) (92.44%,97.7%)	90.20% (184/204) (85.34%,93.56%)
R12	16	100% (102/102) (96.37%,100%)	96.61% (427/442) (94.48%,97.93%)
R13	72	95.86% (1923/2006) (94.9%,96.65%)	96.38% (426/442) (94.20%,97.76%)
R14	10	94.12% (160/170) (89.51%,96.77%)	94.12% (160/170) (89.51%,96.77%)
R15	34	83.82% (57/68) (73.31%,90.72%)	96.60% (1051/1088) (95.35%,97.52%)
R16	8	-	87.50% (238/272) (83.04%,90.92%)

R17	4	100% (68/68) (94.65%,100%)	83.82% (57/68) (73.31%,90.72%)
R18	18	99.51% (203/204) (97.28%,99.97%)	96.57% (394/408) (94.32%,97.95%)
R19	10	95.59% (195/204) (91.83%,97.66%)	95.59% (130/136) (90.71%,97.96%)
R20	14	100% (306/306) (98.76%,100%)	95.29% (162/170) (90.99%,97.60%)
R21	13	92.94% (158/170) (88.07%,95.92%)	95.96% (261/272) (92.90%,97.73%)
R22	98	97.63% (2888/2958) (97.02%,98.12%)	89.57% (335/374) (86.06%,92.28%)
R23	49	98.67% (1409/1428) (97.93%,99.15%)	94.12% (224/238) (90.37%,96.46%)
R24	37	99.26% (540/544) (98.12%,99.71%)	95.94% (685/714) (94.23%,97.16%)
R25	44	99.92% (1223/1224) (99.54%,100%)	89.71% (244/272) (85.52%,92.78%)
R26	19	100% (340/340) (98.88%,100%)	95.75% (293/306) (92.87%,97.50%)
R27	31	98.04% (200/204) (95.07%,99.23%)	93.88% (798/850) (92.07%,95.30%)
R28	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

ii. Panel-wide variant calls

Reproducibility of variant calls was assessed for all detected variants. In total, 815 unique mutations were reported by GENESEEQPRIME for the 28 specimens selected. Of the detected variants, 25 were found to have a mean allele frequency within 1x to 1.5x the limit of detection (3 hotspot variants, 22 non-hotspot variants). 26 of the hotspot variants were deemed to be clinically significant variants based on the cancer type of the sample of origin. The detailed detection rate of variants in the reproducibility study is provided in the device user manual.

Table 28 shows the results for modal PCR and NCR for variants when considering variant allele frequency. Variants found to have a mean allele frequency above the cut off of either 0%, 2%, 5%, 10% or 15% VAF were included in the group. Overall modal PCR is 97.46% (16137/16558, detected average VAF range: 0.4% - 77.06%). Modal NCR is 94.27% (10506/11144). Predictably, modal PCR increase for higher variant allele frequency cut offs. In short, variants with allele frequencies above the LOD of the GENESEEQPRIME assay are reliably detected. Results for call rate of gene translocation, CNV, and MSI are also shown in Table 27. 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 lack of clinical evidence supporting targeted drug use. Considering the total number of variants, GENESEEQPRIME reliably identified 97.56% of hotspot variants in this sample (40/41). Overall, GENESEEQPRIME shows concordance of > 97% for detection of all positive variants above the reported LOD. In addition, detection of gene translocations, CNVs, and microsatellite status are 100% consistent in this sample set.

Table 28. Consistency of detection for alterations of interest in the GENESEEQPRIME assay

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≥0%	815	97.46% (16137/16558)	94.27% (10506/11144)	0.4%-77.06%	4-1527	113-13236
	AF≥2%	590	97.51% (16080/16490)	90.36% (3224/3568)	2%-77.06%	5-1527	113-13236
	AF≥5%	457	98.9% (14425/14586)	81.58% (775/950)	5.16%-77.06%	14-1527	113-11822
	AF≥10%	389	99.24% (12349/12444)	81.15% (633/780)	10.01%-77.06%	24-1527	113-2710
	AF≥15%	288	99.13% (8999/9078)	83.43% (594/712)	15.06%-77.06%	24-1527	113-2710
Variants with Evidence of Clinical Significance	AF≥0%	26	100% (306/306)	95.14% (548/576)	0.4%-52.65%	4-1425	250-2710
	AF≥2%	9	100% (306/306)	-	10.19%-52.65%	76-1425	453-2710
	AF≥5%	9	100% (306/306)	-	10.19%-52.65%	76-1425	453-2710
	AF≥10%	9	100% (306/306)	-	10.19%-52.65%	76-1425	453-2710
	AF≥15%	8	100% (272/272)	-	16.03%-52.65%	81-1425	453-2710
Hotspot variants	AF≥0%	266	99.23% (1417/1428)	95.99% (7305/7610)	0.4%-75.84%	4-1425	206-2710
	AF≥2%	41	100% (1360/1360)	67.65% (23/34)	3.23%-75.84%	17-1425	267-2710
	AF≥5%	39	100% (1292/1292)	67.65% (23/34)	6.1%-75.84%	52-1425	267-2710
	AF≥10%	37	100% (1224/1224)	67.65% (23/34)	10.19%-75.84%	52-1425	267-2710
	AF≥15%	32	100% (1054/1054)	67.65% (23/34)	15.07%-75.84%	52-1425	267-2710
Non-hotspot variants	AF≥0%	549	97.29% (14720/15130)	90.58% (3201/3534)	2%-77.06%	5-1527	113-13236
	AF≥2%	549	97.29% (14720/15130)	90.58% (3201/3534)	2%-77.06%	5-1527	113-13236
	AF≥5%	418	98.79% (13133/13294)	82.1% (752/916)	5.16%-77.06%	14-1527	113-11822

	AF≥10%	352	99.15% (11125/11220)	81.77% (610/746)	10.01%-77.06%	24-1527	113-2437
	AF≥15%	256	99.02% (7945/8024)	84.22% (571/678)	15.06%-77.06%	24-1527	113-2345
Single nucleotide variations	AF≥0%	671	97.52% (11936/12240)	94.65% (10001/10566)	0.4%-75.84%	4-978	113-13236
	AF≥2%	454	97.59% (11879/12172)	91.45% (2983/3262)	2%-75.84%	5-978	113-13236
	AF≥5%	326	99.28% (10329/10404)	82.89% (562/678)	5.16%-75.84%	18-978	113-11822
	AF≥10%	287	99.29% (9014/9078)	82.89% (562/678)	10.19%-75.84%	24-978	113-2498
	AF≥15%	218	99.2% (6678/6732)	82.89% (562/678)	15.06%-75.84%	24-978	113-2498
Insertions	AF≥0%	23	97.19% (760/782)	-	3.6%-77.06%	20-1527	399-2026
	AF≥2%	23	97.19% (760/782)	-	3.6%-77.06%	20-1527	399-2026
	AF≥5%	21	96.92% (692/714)	-	5.24%-77.06%	27-1527	399-2026
	AF≥10%	16	96.88% (527/544)	-	11.77%-77.06%	69-1527	399-2026
	AF≥15%	12	96.81% (395/408)	-	15.99%-77.06%	69-1527	399-2026
Deletions	AF≥0%	121	97.31% (3441/3536)	87.37% (505/578)	1.11%-52.65%	4-1425	267-2710
	AF≥2%	113	97.31% (3441/3536)	78.76% (241/306)	2.11%-52.65%	12-1425	267-2710
	AF≥5%	110	98.15% (3404/3468)	78.31% (213/272)	5.18%-52.65%	14-1425	267-2710
	AF≥10%	86	99.5% (2808/2822)	69.61% (71/102)	10.01%-52.65%	37-1425	285-2710
	AF≥15%	58	99.38% (1926/1938)	94.12% (32/34)	15.13%-52.65%	48-1425	299-2710
ERBB2 amplification		5	100% (170/170)	-	N/A	N/A	N/A
ALK gene translocation		1	100% (33/33)	-	N/A	67-269	403-1114
RET gene translocation		1	100% (34/34)	-	N/A	14-285	896-1942
ROS1 gene translocation		1	100% (34/34)	-	N/A	422-1458	75-212
NTRK1 gene translocation		1	100% (34/34)	-	N/A	28-129	533-1270

iii. Potential sources of variance

To evaluate how different sources of variance may affect the variant calling capabilities of GENESEEQPRIME, precision study samples were tested under various testing conditions. At each of the three testing sites, two operators performed

duplicate tests for each sample over three non-consecutive sequencing dates. In total, 34 observations were generated for each of 27 samples and 33 observations were generated for one sample. Average positive agreement (APA) and average negative agreement (ANA) were used to measure the degree of sameness for each source of variance. TMB concordance is measured using % coefficient of variation (CV).

Table 29 shows the analysis results for each source of variance. Overall agreement for SNVs, insertions, and deletions is high for variants above the LoD of GENESEEQPRIME (APA = 97.53% ~ 98.55%, ANA ≥ 99.99%, %CV < 8.18%). Larger scale variants such as CNV, gene translocation and sample level metrics such as MSI status showed 100% concordance in all pairwise comparisons The residual component was found to be the largest contributor to variance among the conditions tested.

Table 29. Interlaboratory reproducibility results for the GENESEEQPRIME assay

Analysis Type	**Measurement**	**Overall**	**Between sites**	**Between operators**	**Between days**	**Between replicates**
		Value (95% CI)	**Value (95% CI)**	**Value (95% CI)**	**Value (95% CI)**	**Value (95% CI)**
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%)
Variants with evidence of clinical significance≥ 1X LOD	APA	100% (99.96%, 100%)	100% (99.94%, 100%)	100% (99.78%, 100%)	100% (99.67%, 100%)	100% (98.68%, 100%)
	ANA	100% (99.99%, 100%)	100% (99.99%, 100%)	100% (99.99%, 100%)	100% (99.99%, 100%)	100% (99.99%, 100%)
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%)
Hotspot variants ≥ 1X LOD	APA	99.44% (99.37%, 99.51%)	99.43% (99.34%, 99.51%)	99.50% (99.32%, 99.64%)	99.44% (99.19%, 99.61%)	99.30% (98.68%, 99.63%)
	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%)
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%)

Non-hotspot variants ≥ 1X LOD	APA	98.14% (98.10%, 98.17%)	98.12% (98.07%, 98.16%)	98.17% (98.08%, 98.27%)	98.15% (98.03%, 98.27%)	98.25% (98.01%, 98.46%)
	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%)
Single nucleotide variants	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%)
Insertion	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%)
Deletion	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%)
Insertions 1~5bp	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 5~10bp	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%)
Insertions 11~20bp	APA	NaN% (NaN%,NaN%)	NaN% (NaN%,NaN%)	NaN% (NaN%,NaN%)	NaN% (NaN%,NaN%)	NaN% (NaN%,NaN%)
	ANA	NaN% (NaN%,NaN%)	NaN% (NaN%,NaN%)	NaN% (NaN%,NaN%)	NaN% (NaN%,NaN%)	NaN% (NaN%,NaN%)
Insertions 21~30bp	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 1~5bp	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 5~10bp	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 11~20bp	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%)
Deletions 21~30bp	APA	NaN% (NaN%,NaN%)	NaN% (NaN%,NaN%)	NaN% (NaN%,NaN%)	NaN% (NaN%,NaN%)	NaN% (NaN%,NaN%)
	ANA	NaN% (NaN%,NaN%)	NaN% (NaN%,NaN%)	NaN% (NaN%,NaN%)	NaN% (NaN%,NaN%)	NaN% (NaN%,NaN%)
SNVs ≥ 1X LOD	APA	98.55% (98.51%, 98.59%)	98.53% (98.49%, 98.58%)	98.57% (98.47%, 98.66%)	98.59% (98.47%, 98.7%)	98.68% (98.44%, 98.89%)
	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%)
Insertion ≥ 1X LOD	APA	97.25% (97.04%, 97.45%)	97.28% (97.02%, 97.52%)	97.29% (96.75%, 97.75%)	97.03% (96.33%, 97.61%)	97.22% (95.7%, 98.21%)
	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%)
Deletion ≥ 1X LOD	APA	97.53% (97.44%, 97.62%)	97.51% (97.39%, 97.61%)	97.66% (97.43%, 97.86%)	97.54% (97.26%, 97.79%)	97.51% (96.92%, 97.99%)
	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%)
MSI status	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%)

ERBB2 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%)
ALK gene 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%)
RET gene 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%)
ROS1 gene 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%)
NTRK1 gene 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%)
TMB	%CV	8.18%	1.16%	0.54%	0.17%	7.17%

iv. Precision of TMB score across replicates

TMB score was estimated for each of the 28 FFPE samples by dividing the total count of non-driver somatic mutations by the total evaluable exon length. Coefficient of variance (%CV) was used to measure the degree of variation across replicates for each sample, and median %CV across all samples was used to quantify the overall variation across this sample set. Mean TMB for the 28 FFPE specimens ranged from 0.31 mut/Mb to 109.54 mut/Mb, including samples with TMB scores near the analytical borderline at the LOB (1.1mut/Mb). Overall variance in TMB across all sample is 8.18%, which is 7.66% when excluding samples with mean TMB below LOB. Table 30 shows the detailed results from all replicates of the FPFE specimens.

Table 30. TMB score consistency across all repeats

Sample ID	No. of Replicates	SD of TMB	Mean TMB	Range of TMB	% CV
R01	34	1.24	45.97	43.4-48.6	2.7
R02	33	0.72	1.44	0-3.2	50.10
R03*	34	0.67	0.31	0-2.1	212.10
R04	34	1.02	16.62	15.9-19	6.16
R05	34	1.43	44.52	42.3-47.6	3.22
R06	34	0.68	2.29	1.1-4.2	29.56
R07	34	1.15	13.72	11.6-15.9	8.35
R08	34	1.28	10.63	8.5-13.7	12.01
R09	34	0.92	29.47	28.6-31.7	3.14
R10	34	0.63	3.5	3.2-5.3	18.07
R11	34	0.78	9.77	8.5-10.6	8
R12	34	0.75	3.51	3.2-5.3	21.29
R13	34	1.68	75.32	70.9-78.3	2.24
R14	34	1.33	7.24	4.2-9.5	18.42
R15	34	1.35	5.93	4.2-9.5	22.73
R16*	34	0.82	0.95	0-3.2	85.65
R17	34	0.48	2.26	2.1-3.2	21.31
R18	34	0.56	7.69	7.4-8.5	7.32
R19	34	0.7	7.15	5.3-8.5	9.77
R20	34	0.59	11.73	10.6-13.7	5.03
R21	34	1.12	9.59	7.4-11.6	11.7
R22	34	1.93	109.54	106.8-113.1	1.76
R23	34	1.95	54.08	48.6-57.1	3.61
R24	34	1.29	18.78	16.9-22.2	6.85
R25	34	1.33	56.78	55-60.3	2.35
R26	34	0.35	10.72	10.6-11.6	3.3
R27	34	2.7	10.99	8.5-23.3	24.62
R28	34	2.04	74.38	70.9-79.3	2.74
TMB Median CV% (All samples)					8.18
TMB Mean CV% (All samples)					21.58
TMB Median CV% (All samples with the mean TMB greater than LOB)					7.66
TMB mean CV% (All samples with the mean TMB greater than LOB)					11.78

*Samples R03 and R16 have a mean TMB below the LOB (1.1 Mut/Mb) of GENESEEQPRIME assay.

v. Precision of MSI status across replicates

Concordance of sample-level MSI status across samples is used to determine the overall reproducibility of microsatellite status reported by GENESEEQPRIME. Reproducibility of both microsatellite status and score was evaluated independently for each of the 28 samples. Median %CV of all samples was used to quantify the overall variation across this sample set. 6 samples were determined to be microsatellite instability-high (MSI-H), and the remaining 22 samples were microsatellite stable (MSS). The range of scores for MSI-H samples is 25.58 – 75.09. Median overall variance in MSI score across sample replicates is 38.11% (Range: 3.59% - 59.60%). In terms of MSI status, samples showed 100% concordance across all replicates. Table 31 shows the detailed MSI results from all replicates of the FPFE specimens. Only MSI status is given on the final report.

Table 31. MSI Performance in Interlaboratory Reproducibility Study

Sample	Number of Replicates	MSS Count	MSI Count	Average MSI Score	Standard Deviation	CV (%)	Call rate (95%CI)
R01	34	0	34	25.58%	2.60	10.17%	100% (89.85%, 100%)
R02	33	33	0	6.05%	1.91	31.62%	100% (89.57%, 100%)
R03	34	34	0	5.35%	2.18	40.75%	100% (89.85%, 100%)
R04	34	34	0	5.84%	2.17	37.15%	100% (89.85%, 100%)
R05	34	0	34	56.96%	2.68	4.70%	100% (89.85%, 100%)
R06	34	34	0	6.05%	2.53	41.85%	100% (89.85%, 100%)
R07	34	34	0	7.03%	1.71	24.38%	100% (89.85%, 100%)
R08	34	34	0	3.55%	2.02	56.84%	100% (89.85%, 100%)
R09	34	34	0	4.04%	2.41	59.60%	100% (89.85%, 100%)

R10	34	34	0	5.21%	2.06	39.47%	100% (89.85%, 100%)
R11	34	34	0	4.56%	1.91	41.92%	100% (89.85%, 100%)
R12	34	34	0	5.18%	1.94	37.37%	100% (89.85%, 100%)
R13	34	0	34	77.10%	3.40	4.41%	100% (89.85%, 100%)
R14	34	34	0	5.13%	2.00	38.91%	100% (89.85%, 100%)
R15	34	34	0	6.80%	2.74	40.27%	100% (89.85%, 100%)
R16	34	34	0	3.11%	1.85	59.47%	100% (89.85%, 100%)
R17	34	34	0	4.50%	2.05	45.64%	100% (89.85%, 100%)
R18	34	34	0	7.09%	2.59	36.55%	100% (89.85%, 100%)
R19	34	34	0	4.56%	1.71	37.48%	100% (89.85%, 100%)
R20	34	34	0	5.71%	1.85	32.38%	100% (89.85%, 100%)
R21	34	34	0	9.11%	2.35	25.83%	100% (89.85%, 100%)
R22	34	0	34	76.40%	2.94	3.85%	100% (89.85%, 100%)
R23	34	0	34	55.81%	3.15	5.64%	100% (89.85%, 100%)
R24	34	34	0	6.35%	2.46	38.74%	100% (89.85%, 100%)

R25	34	34	0	5.20%	2.16	41.64%	100% (89.85%, 100%)
R26	34	34	0	5.22%	2.25	43.17%	100% (89.85%, 100%)
R27	34	34	0	3.76%	2.00	53.31%	100% (89.85%, 100%)
R28	34	0	34	75.09%	2.70	3.59%	100% (89.85%, 100%)

b) Lot to Lot Precision

Three lots in total were prepared for this comparison. Five FFPE samples with mutations near the established limit of detection were evaluated. The tumor mutation burden for samples ranged from 1.1 to 332 mut/mb. The selected samples have a variety of point mutations, insertions, deletions, *ERBB2* amplification status, microsatellite status, and *RET* translocation. Each sample was tested in triplicate using all three kit lots (5 sample * 3 replicates * 3 kit lots = 45 replicates). Pairwise comparisons were made between all replicates of each individual samples by comparing two kit lots at a time. *ERBB2* amplification and *RET* translocations are classified as concordant if both lots being compared made the same call. All samples were sequenced on the same instrument by the same operator.

The overall APA of panel wide (SNVs and indels) variants was ≥ 98.11% (Table 32). The overall ANA did not fall below 99.99%. Concordance of *ERBB2* amplification, MSI status, and *RET* translocation was 100% across all lot comparisons. For TMB, the range of %CV from each pairwise comparison was found to be 1.55~2.46% (Table 33).

Table 32. Agreement for panel wide variants (SNVs + Insertions + Deletions)

Lot comparison	APA	95% CI	n/N	ANA	95% CI	n/N
L1 v L2	98.48%	(98.15%, 98.75%)	6414/6513	99.99%	(99.99%, 99.99%)	394572468/394572567
L2 v L3	98.77%	(98.47%, 99.01%)	6436/6516	99.99%	(99.99%, 99.99%)	394572484/394572564
L3 v L1	98.11%	(97.75%, 98.41%)	6384/6507	99.99%	(99.99%, 99.99%)	394572450/394572573

Table 33. Coefficient of variance for TMB

Lot comparison	Average %CV
L1 v L2	2.46%
L2 v L3	1.55%
L3 v L1	2.46%

4. Detection Limits: Limit of Detection

a) SNVs, insertions and deletions

The limit of detection of SNVs, insertions, and deletions were defined by the lowest measured median allele frequency at which 95% of repeats detected the variant in question. The mean allele frequency is obtained from the AFs of all the repeats where the variant of interest is detected. Three cell line standards were used to establish the limit of detection and validated with 7 FFPE samples. Target levels for detection were first established using 3 cell line samples. Samples were serially diluted into 5 tiers and 20 replicates.

A total of 461 unique variants were called across 4 categories (Hotspot SNVs, Non-hotspot SNVs, Insertions, and Deletions) (Table 34). The established LOD for hotspot single nucleotide variants is at 2.33% allele frequency. The established LOD for non-hotspot single nucleotide variants is at 3.60% allele frequency. The established LOD for insertions and deletions are at 2.82% and 5.32% allele frequency, respectively. Insertions and deletions were also categorized according to genomic context. Indels within a homopolymer region show an average VAF of 6.98% (range: 2.70% ~ 15.18%), while detected indels outside of these regions show a mean VAF of 3.15% (range: 0.89% ~ 6.36%). Clinical FFPE samples were used to confirm the results under the established LOD. Table 35 shows the limit of detection for representative variants found in the FFPE samples.

Table 34. Established LoD VAF of representative SNV, INS, and DEL variants

Variant type	Established mean VAF Range	Mean of VAF range	Unique cell line variants	Mean of VAF range (FFPE)	Unique clinical cases variants in the established range
HS SNVs	1.15% - 6.73%	2.33%	26	2.14%	22
NHS SNVs	2.23% - 10.13%	3.60%	384	2.95%	265
Insertions	0.93% - 6.37%	2.82%	9	2.93%	4
Deletions	0.89% - 15.18%	5.32%	42	5.44%	9
Indels at homopolymer context*	2.70% - 15.18%	6.98%	25	7.94%	5
Indels not at homopolymer context	0.89% - 6.36%	3.15%	26	2.62%	8

*Homopolymer context - a region containing ≥ 5 consecutive identical nucleotides.

Table 35. Analytical sensitivity of representative SNV, insertion, and deletion variants in FFPE samples

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

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 36 below.

Table 36. 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) *ERBB2*, *ALK*, *RET, ROS1*, *NTRK1*, and MSI
The established analytical sensitivity confirmed by testing clinical FFPE samples for specific translocations, amplifications, and MSI is summarized in Table 37 below.

Table 37. 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
ERBB2 Amplification	5.00	20/20 (100)	739 - 990
ALK Translocation	7.50	20/20 (100)	723 - 990
RET Translocation	10.00	20/20 (100)	679 - 1519
ROS1 Translocation	7.50	20/20 (100)	721 - 2044
NTRK1 Translocation	15.00	20/20 (100)	594 - 1957

i. Translocations
The limit of detection for four categories of structural variants (*ALK* translocation, *RET* translocation, *ROS1* translocation, and *NTRK1* translocation) (Tables 38 ~ 41) was investigated. In this section, 4 FFPE samples were diluted to 5 or 6 levels according to tumor purity. The 95% detection rate of translocations across 20 replicates for each dilution level was used to determine the LOD. The limit of detection for each of the gene translocations investigated in GENESEEQPRIME (*ALK*, *RET*, *ROS1*, and *NTRK1*) are, respectively, 7.5%, 10%, 7.5%, and 15% tumor purity.

Table 38. *ALK* translocation limit of detection

Gene	Tumor Purity	Detected mean variant reads	Call rate (%)
ALK	50.00%	196.5	20/20 (100)
ALK	20.00%	55.5	20/20 (100)
ALK	10.00%	17.3	20/20 (100)
ALK	7.50%	18.9	20/20 (100)
ALK	5.00%	11.8	15/20 (75)

Table 39. *RET* translocation limit of detection

Gene	Tumor Purity	Detected mean variant reads	Call rate (%)
RET	50.00%	132.7	20/20 (100)
RET	20.00%	27	20/20 (100)
RET	15.00%	23.5	20/20 (100)
RET	10.00%	13.7	20/20 (100)
RET	7.50%	12.5	15/20 (75)
RET	5.00%	n.d.	0/20 (0)

n.d. – Not Detected

Table 40. *ROS1* translocation limit of detection

Gene	Tumor Purity	Detected mean variant reads	Call rate (%)
ROS1	50.00%	178.7	20/20 (100)
ROS1	20.00%	41.9	20/20 (100)
ROS1	10.00%	20	20/20 (100)
ROS1	7.50%	16.9	20/20 (100)
ROS1	5.00%	10.3	6/20 (30)

Table 41. *NTRK1* translocation limit of detection

Gene	Tumor Purity	Detected mean variant reads	Call rate (%)
NTRK1	50.00%	71.1	20/20 (100)
NTRK1	30.00%	50.5	20/20 (100)
NTRK1	20.00%	32.6	20/20 (100)
NTRK1	15.00%	26.3	20/20 (100)
NTRK1	12.50%	10.1	16/20 (80)
NTRK1	10.00%	n.d.	0/20 (0)

n.d. – Not Detected

ii. *ERBB2* Amplification

Similar to the method for gene translocations, the limit of detection for CNV is established at the lowest dilution level with >= 95% call rate for *ERBB2* amplification. One clinical sample was diluted to 6 dilutions tiers based on pathology-defined tumor purity. Each tier contains 20 replicates for a total of 120 samples. The observed the limit of detection for *ERBB2* amplification is 5% tumor purity (Table 42).

Table 42. *ERBB2* amplification limit of detection

Gene	Tumor Purity	Detected mean fold change	Call rate (%)
ERBB2	50.00%	11.97	20/20 (100)
ERBB2	20.00%	5.54	20/20 (100)
ERBB2	10.00%	3.01	20/20 (100)
ERBB2	5.00%	1.89	20/20 (100)
ERBB2	4.00%	1.83	18/20 (90)
ERBB2	2.50%	n.d.	0/20 (0)

n.d. – Not Detected

iii. Microsatellite Status

Microsatellite instability LOD was established using three clinical FFPE samples (one colorectal cancer, one endometrial cancer, one gastric cancer). The original samples were determined to have microsatellite instability, and LOD was determined at the lowest tumor purity in which MSI was correctly called in at least 19 out of 20 replicates (>=95% call rate). The observed LOD for MSI calls using the GENESEEQPRIME assay is 20% tumor purity (Table 43).

Table 43. MSI limit of detection

Variant Type	Tumor Purity	Detected mean MSI score	Call rate (%)
MSI-F1	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-F2	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)
MSI-F3	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) Tumor Mutation Burden (TMB)

This portion of analysis used 8 FFPE samples to establish the lowest tumor purity at which TMB calls have minimal fluctuation. Each sample was diluted either 4 or 5 times and contained at least three repeats in each dilution level. Samples 1, 2, 3, and 8 all had five dilutions with five repeats each (n = 25). Sample 4 had four dilutions levels and three repeats each (n = 12). Sample 5 had four dilution tiers with five replicates each (n = 20). Samples 6 and 7 were both diluted five times each, with three replicates in each dilution level (n = 15). In total, 162 replicates were utilized for this portion of analysis. Table 44 shows the characteristic summary of the samples used in this analysis. From this analysis, we conclude that the lowest recommended tumor purity for consistent and robust reporting of TMB scores in the assay for FFPE samples is 12% (Figure 7). This falls within the minimum sample tumor purity of 20% as recommended by the GENESEEQPRIME assay.

Table 44. TMB calls for FFPE sample replicates ≥12% tumor purity

Sample Number	Undiluted TMB score	%CV of replicates ≥12% Tumor purity	Number of replicates ≥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
5	13.4	3.50%	20
6	26.8	6.22%	15
7	22.7	13.90%	12
8	11.3	5.00%	20

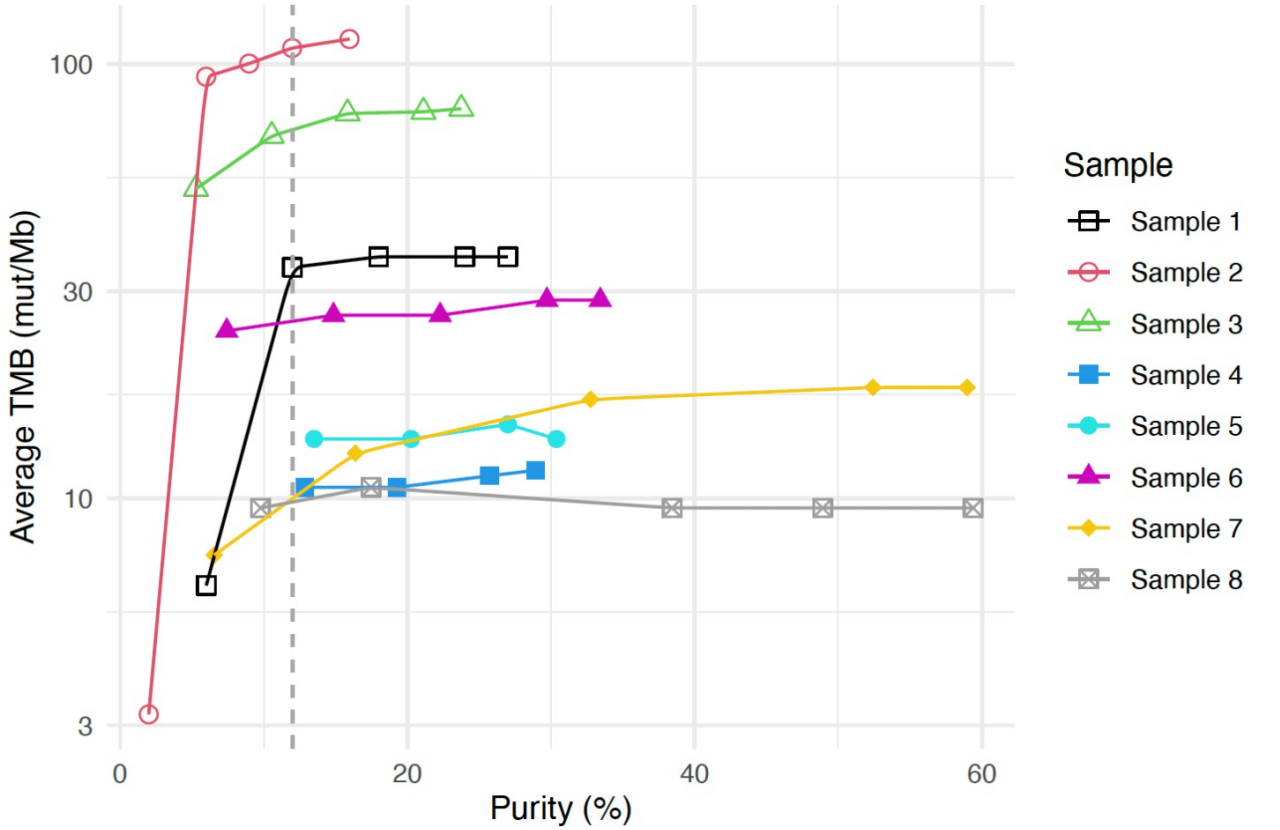


Figure 7. Fluctuation of average TMB for samples with decreasing tumor purity. The x axis shows the tumor purity for each replicate in percentage. The y axis shows the average TMB for the replicates, measured in mutations per megabase. The dotted line denotes the lowest recommended tumor purity of 12% for consistent TMB scores.

5. Detection Limits: Limit of Blank

To establish a limit of blank for the GENESEEQPRIME assay, we analyzed the specificity of variant calls of 42 non-cancerous FFPE samples and 1 reference sample (NA18535). The reference cell line was repeatedly sampled 15 times using the GENESEEQPRIME assay. FFPE samples were each sequenced using two kit lots to produce two replicates. Sample input for FFPE samples is 500ng. A total of 84 replicates for non-cancerous samples were evaluated for the presence of SNVs, Indels, *ERBB2* amplifications, gene translocations, or MSI. No variants were reported in any of the replicates, resulting in a specificity of 100%. TMB calls for the cell line samples ranged between 0 and 1.1 mut/mb. All replicates reported cell line microsatellite status as MSS. In total, 14 variant was detected across 84 FFPE samples (14/84, 16.7%). The detected variants were all classified as non-hotspot variants. For both hotspot variants and variants with evidence of clinical significance, the false positive rate was determined to be < 0.01% (0/84). The false positive rate for insertion and deletion is <0.01% (0/84). All FFPE samples were correctly classified as MSS. For non-cancerous FFPE samples, TMB scores ranged from 0 to 2.1 (mean = 0.21). Two samples were deemed to be false positive based on the established TMB LOB

>1.1 mut/mb using FFPE samples (2/84, 2.38%).

6. Analytical Specificity:

a) Interfering Substance

In this evaluation, the performance of the GENESEEQPRIME assay on variant calling in the presence of endogenous or exogenous substances was evaluated. To simulate extreme experimental conditions, 4 interfering substances (Proteinase K, Melanin, Ethanol, Adapter) were introduced in excess of normal levels (Table 45). with two levels of interfering substance for each for a total of 8 conditions for each sample. A control was also established in which no excess interfering substance was added. The analysis encompassed triplicate measurements for each combination, resulting in a total of 108 samples (4 samples x 4 substances x 2 dilutions x 3 replicates + 4 samples x 1 reference level x 3 replicates). The reference mutation list only included variants which are present in all three replicates of the control sample (sample without any excess substance added). Variant calls were compared between the reference mutation list and each replicate of the sample with interfering substance added. TMB for each sample will be evaluated according to the deviation from the reference TMB using mean absolute percent error. The results demonstrated positive percent agreement (PPA) ≥ 95.14% and a negative percent agreement (NPA) ≥ 99.99% (Table 46). The mean absolute percentage error (MAPE) ranged from 0% to 9.7%, indicating high accuracy for TMB calls across all replicates tested (Table 47). The results show minimal risk to assay performance from interfering substances.

Table 45. List of substances assessed

Substance (abbr.)	Amount in added in excess
Proteinase K 2X (P1)	0.04 mg/mL
Proteinase K 3X (P2)	0.06 mg/mL
Ethanol 2.5% (E1)	2.50%
Ethanol 5% (E2)	5.00%
Adapter 15% (A1)	15%
Adapter 30% (A2)	30%
Melanin 0.2 µg/mL (M1)	0.2 µg/mL
Melanin 1.6 µg/mL (M2)	1.6 µg/mL

Table 46. Panel wide variants – analyte level

Substance	PPA	95% CI	n/N	NPA	95% CI
P1	96.08%	(94.74%, 97.09%)	1029/1071	99.99%	(99.99%, 99.99%)
P2	96.08%	(94.74%, 97.09%)	1029/1071	99.99%	(99.99%, 99.99%)
E1	95.99%	(94.64%, 97.01%)	1028/1071	99.99%	(99.99%, 99.99%)
E2	95.14%	(93.69%, 96.28%)	1019/1071	99.99%	(99.99%, 99.99%)
A1	96.55%	(95.27%, 97.48%)	1034/1071	99.99%	(99.99%, 99.99%)
A2	95.70%	(94.32%, 96.76%)	1025/1071	99.99%	(99.99%, 99.99%)

M1	95.89%	(94.53%, 96.93%)	1027/1071	99.99%	(99.99%, 99.99%)
M2	95.24%	(93.79%, 96.36%)	1020/1071	99.99%	(99.99%, 99.99%)

Table 47. TMB score for each sample under different testing conditions

Sample	Condition	Observed TMB score	Absolute Percent Error	Sample level average % error
F1 (Ref TMB:24.3 mut/Mb)	Proteinase K 2X	23.3	4.1%	4.1%
		23.3	4.1%	
		23.3	4.1%	
	Proteinase K 3X	21.1	13.2%	7.1%
		23.3	4.1%	
		23.3	4.1%	
	Ethanol 2.5%	23.3	4.1%	4.1%
		23.3	4.1%	
		23.3	4.1%	
	Ethanol 5%	24.3	0.0%	2.7%
		23.3	4.1%	
		23.3	4.1%	
	Adapter 15%	23.3	4.1%	4.1%
		23.3	4.1%	
		23.3	4.1%	
	Adapter 30%	22.2	8.6%	8.6%
		22.2	8.6%	
		22.2	8.6%	
	Melanin 0.2 µg/mL	21.1	13.2%	7.1%
		23.3	4.1%	
		23.3	4.1%	
	Melanin 1.6 µg/mL	21.1	13.2%	7.1%
		23.3	4.1%	
		23.3	4.1%	
	Proteinase K 2X	77.2	4.0%	4.4%
		77.2	4.0%	
		76.1	5.3%	
	Proteinase K 3X	77.2	4.0%	4.9%
		76.1	5.3%	
		76.1	5.3%	
		76.1	5.3%	

F2 (Ref TMB: 80.4 mut/Mb)	Ethanol 2.5%	76.1	5.3%	5.3%
		76.1	5.3%	
	Ethanol 5%	74	8.0%	9.7%
		73	9.2%	
		70.9	11.8%	
	Adapter 15%	77.2	4.0%	4.4%
		77.2	4.0%	
		76.1	5.3%	
	Adapter 30%	76.1	5.3%	5.3%
		76.1	5.3%	
		76.1	5.3%	
	Melanin 0.2 µg/mL	76.1	5.3%	5.3%
		76.1	5.3%	
		76.1	5.3%	
	Melanin 1.6 µg/mL	70.9	11.8%	7.9%
		75.1	6.6%	
		76.1	5.3%	
F3 (Ref TMB:345.0 mut/Mb)	Proteinase K 2X	358.5	3.9%	4.8%
		363.8	5.4%	
		362.7	5.1%	
	Proteinase K 3X	358.5	3.9%	4.6%
		361.7	4.8%	
		362.7	5.1%	
	Ethanol 2.5%	365.9	6.0%	5.4%
		361.7	4.8%	
		363.8	5.4%	
	Ethanol 5%	361.7	4.8%	4.8%
		361.7	4.8%	
		361.7	4.8%	
	Adapter 15%	361.7	4.8%	5.0%
		361.7	4.8%	
		363.8	5.4%	
	Adapter 30%	355.3	3.0%	3.5%
		355.3	3.0%	
		360.6	4.5%	
	Melanin 0.2 µg/mL	359.5	4.2%	4.6%
		360.6	4.5%	
		362.7	5.1%	
	Melanin 1.6 µg/mL	362.7	5.1%	4.3%
		359.5	4.2%	

		357.4	3.6%	
F4 (Ref TMB:1.1 mut/Mb)	Proteinase K 2X	1.1	0.0%	0.0%
		1.1	0.0%	
		1.1	0.0%	
	Proteinase K 3X	1.1	0.0%	0.0%
		1.1	0.0%	
		1.1	0.0%	
	Ethanol 2.5%	1.1	0.0%	0.0%
		1.1	0.0%	
		1.1	0.0%	
	Ethanol 5%	1.1	0.0%	0.0%
		1.1	0.0%	
		1.1	0.0%	
	Adapter 15%	1.1	0.0%	0.0%
		1.1	0.0%	
		1.1	0.0%	
	Adapter 30%	1.1	0.0%	0.0%
		1.1	0.0%	
		1.1	0.0%	
	Melanin 0.2 µg/mL	1.1	0.0%	0.0%
		1.1	0.0%	
		1.1	0.0%	
	Melanin 1.6 µg/mL	1.1	0.0%	0.0%
		1.1	0.0%	
		1.1	0.0%	

b) Cross-contamination and carry over

To examine the presence of cross contamination and sample carry over, DNA was extracted from FFPE samples and sequenced in two batches. Tracked mutation positive ("positive") and tracked mutation negative ("negative") samples were prepared in a 96-well plate in a checkerboard pattern. Tracked mutations were defined through droplet digital PCR quantification. Cross contamination of samples sequenced in the same batch was analyzed using the 24 positive samples and 3 negative samples from batch 1. The study screened for presence of known mutations in the results of the negative samples. Sample carry-over was analyzed using 24 positive samples from batch 1 and 19 negative samples from batch 2. Samples from batch 2 were loaded directly after sequencing batch 1 and following recommended cleaning procedures. Similar to the cross-contamination analysis, the study screened for presence of known mutations in the negative samples. No tracked mutations were observed in the known negative samples in either the same sequencing batch or in the subsequent sequencing batch. This shows that the GENESEEQPRIME assay has minimal risk of being influenced by cross contamination or sample carryover.

c) Necrotic tissue

A total of 503 sample were collected from a biobank without prior knowledge of the necrotic tissue content. Necrosis level was provided by the biobank. To evaluate the impact of necrotic tissue content on sample quality, we stratified samples into four categories based on their estimated necrosis levels: 0–5%, 5–20%, 21–40%, and 41–53%. The proportion of samples in each category that passed QC for GENESEEQPRIME testing was assessed. Among the 370 samples with necrosis levels between 0% and 5%, 92.16% (341/370) passed QC. For samples with 5–20% necrosis (n = 115), a slightly higher pass rate of 94.78% (109/115) was observed. Notably, all samples with higher levels of necrosis (21%~40% & 41%~53%) passed QC, yielding a 100% success rate in both groups.

d) Index hopping

To assess index hopping in the GENESEEQPRIME assay, a controlled series of sequencing runs incorporating a synthetic DNA library with a unique dual-index combination, alongside clinical samples and assay controls, was performed. The synthetic DNA library contained a non-biological insert flanked by one of five pairs of randomly selected indices and was spiked into each run using a distinct index identifier to track misassignment events across runs. Detection of synthetic reads in a sample indexed with a different identifier than that used in the corresponding run was considered evidence of index hopping. 5 sequencing runs were tested, using 27 clinical samples and one contrived synthetic sample in each run. Proportion of synthetic reads detected are measured per 10000 sequencing reads generated. Low-level synthetic read contamination was observed in a subset of unrelated samples across all runs, consistent with low-frequency index hopping. These off-target synthetic reads were typically present at levels < 0.001% of total reads.

7. Sample Stability

a) FFPE tissue block

In this study, 21 FFPE clinical samples were utilized, with 50ng of input material taken for testing.

The FFPE samples were stored under appropriate conditions—ambient room temperature (15–25 °C), relative humidity below 60%, protected from direct sunlight, and in sealed cassettes within temperature-stable cabinets—for varying durations of 1 (T1), 3 (T2), 5 (T3), or 7 (T4) years. The pass rate of samples tested was 95.24% (20/21). One FFPE sample passed all quality metrics at T0, but failed library preparation QC after being stored for 7 years. The objective was to analyze and compare the detection of intended variants within these samples after storage to the baseline timepoint (T0). At each time point, 3 FFPE blocks were assessed as biological replicates, and previously assessed samples were removed from the selection pool due to limited residual material. A positive percent agreement (PPA) over 98%% between baseline and samples was observed at 1, 3, and 5 years, and a negative percent agreement (NPA) exceeding 99.99% (Table 48). Of the 20 samples tested, FFPE block samples stored up to 5 years showed highly concordant variant calls (PPA > 95%). These findings demonstrate that the GENESEEQPRIME assay consistently yields highly concordant results across all timepoints and for all alterations.

Based on these results, the GENESEEQPRIME assay can be used with FFPE samples stored for less than 5 years to ensure optimal precision and performance.

Table 48. Panel wide variants – analyte level concordance

Time Point	PPA	95% CI	n/N	NPA	95% CI	n/N
T1 (Year 1)	98.44%	(91.67%, 99.92%)	63/64	99.99%	(99.99%, 99.99%)	21928583/ 21928584
T2 (Year 3)	98.73%	(93.17%, 99.94%)	78/79	99.99%	(99.99%, 99.99%)	21928570/ 21928573
T3 (Year 5)	98.48%	(91.90%, 99.92%)	65/66	100%	(99.99%, 100%)	21928593/ 21928593
T4 (Year 7)	94.44%	(84.89%, 98.09%)	51/54	99.99%	(99.99%, 99.99%)	21928594/ 21928595

b) Extracted Nucleic Acid

DNA was extracted from 10 FFPE samples and sequenced immediately after extraction (T0) and after storage for 6, 12 and 14 months. Samples were stored at ≤-20°C before being processed through GENESEEEQPRIME. T0 baseline samples were used as a reference point for detected variants, *ALK* translocation, TMB score, and MSI status. All samples were tested using kits from a single lot. At 6 months and 12 months post extraction, we observed a 100% concordance rate for detected variants (Table 49; PPA = 100%, NPA = 100%). TMB scores for each sample after storage for 6 months, 12 months or 14 months all show a coefficient of variance of ≤0.79%. *EML4~ALK* translocations were detected by GENESEEQPRIME in all tested repeats of samples 2 and 4. MSI status was 100% concordant to baseline status for all samples tested across all timepoints. Positive percent agreement for each category of alteration (Panel-wide variants, *ALK* gene translocation, MSI status) remains above 95% throughout the time frame tested. Based on these observations, DNA sample storage time of 12 months does not affect the variant calling capabilities of GENESEEQPRIME.

Table 49. Panel wide variants (SNVs + Insertions + Deletions) for extracted DNA stability

Variant Category	Time point	PPA (95% CI)	NPA (95% CI)
Overall	6 Month	100% (95.07%, 100%)	100% (99.99%, 100%)
	12 Month	100% (95.42%, 100%)	99.99% (99.99%, 99.99%)
	14 Month	98.91% (94.10%, 99.94%)	100% (99.99%, 100%)

8. Guard Banding

a) DNA input

Varying amounts of DNA are extracted from FFPE blocks to be used for sequencing. Analysis sensitivity was determined by testing 7 FFPE samples in triplicate, resulting in a total of 126 samples across 6 input levels: 25 ng, 50 ng, 100 ng, 250 ng, 500 ng, and 1000

ng. Mutation calls and mutational burden of each input levels was compared to the reference level of 100 ng. Due to the number of replicates, TMB for each replicate is compared to mean TMB score of the reference.

The analysis revealed that PPA across all detected variants is >95% positive percent agreement for DNA input level ≥ 50ng with a minimum NPA of 99.99% (Table 50). Additionally, hotspot SNVs were found with 100% concordance for 50 ng, 250 ng, and 1000 ng input levels (Table 51). In general, PPA increased as the input DNA amount increased across all variant categories tested. The mean absolute percent error for TMB was found to be less than 8.91% (Table 52). An exception was Case 6, where the MAPE ranged from 16.67%-24.07%, likely due to its low absolute TMB values (1.1-2.1 mut/Mb), which can magnify percent error from small absolute differences. Based on these findings, a minimum DNA input amount of 50 ng is recommended for the GENESEEQPRIME assay. Conversely, input levels higher than 500 ng do not significantly improve performance.

Table 50. Panel wide variants (SNVs + Insertions + Deletions) – analyte level concordance

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

Table 51. HS SNVs – analyte level concordance

Input Level	PPA	95% CI	n/N	NPA	95% CI	n/N
25	90.48%	(71.09%, 97.35%)	19/21	99.99%	(99.99%, 99.99%)	39815/39816
50	100%	(84.54%, 100%)	21/21	99.99%	(99.97%, 99.99%)	39811/39816
250	100%	(84.54%, 100%)	21/21	99.98%	(99.97%, 99.98%)	39810/39816
500	95.23%	(77.33%, 99.76%)	20/21	99.99%	(99.99%, 99.99%)	39815/39816
1000	100%	(84.54%, 100%)	21/21	99.98%	(99.97%, 99.98%)	39810/39816

Table 52. Mean absolute percent error in TMB at various DNA input

Case number	Mean expected TMB score	DNA input level	Observed TMB score	Absolute percent error	Mean absolute percent error
		25 ng	2.1	0.00%	0.00%
			2.1	0.00%	
			2.1	0.00%	
		50 ng	2.1	0.00%	0.00%
			2.1	0.00%	
			2.1	0.00%	
			2.1	0.00%	

Case 1	2.1	250 ng	2.1	0.00%	0.00%
			2.1	0.00%	
		500 ng	2.1	0.00%	0.00%
			2.1	0.00%	
			2.1	0.00%	
		1000 ng	2.1	0.00%	0.00%
			2.1	0.00%	
			2.1	0.00%	
Case 2	11.6	25 ng	10.6	8.62%	8.91%
			11.6	0.00%	
			9.5	18.10%	
		50 ng	11.6	0.00%	0.00%
			11.6	0.00%	
			11.6	0.00%	
		250 ng	11.6	0.00%	0.00%
			11.6	0.00%	
			11.6	0.00%	
		500 ng	11.6	0.00%	0.00%
			11.6	0.00%	
			11.6	0.00%	
		1000 ng	11.6	0.00%	0.00%
			11.6	0.00%	
			11.6	0.00%	
Case 3	99.1	25 ng	99.4	0.34%	2.07%
			97.3	1.82%	
			95.2	4.06%	
		50 ng	98.3	0.78%	0.98%
			99.4	0.34%	
			97.3	1.82%	
		250 ng	97.3	1.82%	1.19%
			99.4	0.34%	
			100.5	1.43%	
		500 ng	97.3	1.82%	2.20%
			97.3	1.82%	
			96.2	2.98%	
		1000ng	97.3	1.82%	2.20%
			97.3	1.82%	
			96.2	2.98%	
Case 4	1.1	25 ng	1.1	0%	0.00%
			1.1	0%	
			1.1	0%	
		50 ng	1.1	0%	0.00%
			1.1	0%	
			1.1	0%	
		250 ng	1.1	0%	0.00%
			1.1	0%	
			1.1	0%	
			1.1	0%	

		500 ng	1.1	0%	0.00%
			1.1	0%	
		1000ng	1.1	0%	0.00%
			1.1	0%	
			1.1	0%	
Case 5	0	25 ng	0	0%	0.00%
			0	0%	
			0	0%	
		50 ng	0	0%	0.00%
			0	0%	
			0	0%	
		250 ng	0	0%	0.00%
			0	0%	
			0	0%	
		500 ng	0	0%	0.00%
			0	0%	
			0	0%	
		1000ng	0	0%	0.00%
			0	0%	
			0	0%	
Case 6	1.8	25 ng	2.1	16.67%	16.67%
			2.1	16.67%	
			2.1	16.67%	
		50 ng	1.1	38.89%	24.07%
			2.1	16.67%	
			2.1	16.67%	
		250 ng	2.1	16.67%	24.07%
			1.1	38.89%	
			2.1	16.67%	
		500 ng	2.1	16.67%	24.07%
			2.1	16.67%	
			1.1	38.89%	
		1000ng	1.1	38.89%	24.07%
			2.1	16.67%	
			2.1	16.67%	
Case 7	1.1	25 ng	1.1	0%	0.00%
			1.1	0%	
			1.1	0%	
		50 ng	1.1	0%	0.00%
			1.1	0%	
			1.1	0%	
		250 ng	1.1	0%	0.00%
			1.1	0%	
			1.1	0%	
		500 ng	1.1	0%	0.00%
			1.1	0%	
			1.1	0%	
			1.1	0%	
			1.1	0%	

		1000ng	1.1	0%	0.00%

b) DNA extraction method

In this analysis, the effect that DNA extraction method may have on the performance of the GENESEEQPRIME assay was examined. A total of four commonly used, commercially available DNA extraction methods were evaluated. FFPE DNA extraction kits include two column-based extraction methods, one column-based FDA registered FFPE DNA extraction kit and one magnetic bead-based extraction method DNA extraction method concordance was evaluated in 8 FFPE solid tumor tissue samples selected to cover all variant types assessed by GENESEEQPRIME, including borderline variants near the limit of detection. The 168 DNA samples were processed with GENESEEQPRIME assay (5 samples x 2 extraction kit lots x 2 operators x 3 extraction methods x 2 assay replicates) + (3 samples x 2 extraction kit lots x 2 operators x 2 extraction methods x 2 assay replicates). Method 2 (M2), Method 3 (M3), and Method 4 (M4) were compared to the reference Method 1 (M1).

The positive percent agreement for Method 2 and Method 1 is 99.16% (95% CI: 98.54%, 99.52%), while the PPA for Method 3 and Method 1 is 99.02% (95% CI: 98.37% - 99.42%) (Table 53). For the Method 4 compared to M1, the PPA was 99.78%. The NPA for all comparisons are 99.99%. PPA remained above 99% for all three comparisons (Method 2 vs M1, Method 3 vs M1, M4 vs M1) across non-hotspot SNVs, deletions, *ALK* translocations, and MSI status. The coefficient of variation for mutational burden of the samples analyzed in this portion is ≤6.13% (Table 54).

The results show that the performance of the GENESEEQPRIME assay is not significantly affected by the method of extraction for FFPE samples. Thus, we believe that the GENESEEQPRIME assay can be applied to samples extracted with any suitable commercially available FFPE DNA extraction kit on the market, provided that the appropriate protocols are followed.

Table 53. Overall variants (SNVs + Insertions + Deletions) – analyte level concordance

Extraction method	PPA	95% CI	n/N	NPA	95% CI	n/N
M1 v M2	99.16%	(98.54%, 99.52%)	1420/1432	99.99%	(99.99%, 99.99%)	175367008/175367048
M1 v M3	99.02%	(98.37%, 99.42%)	1418/1432	99.99%	(99.99%, 99.99%)	175367009/175367048
M1 v M4	99.78%	(99.22%, 99.94%)	926/928	99.99%	(99.99%, 99.99%)	105220096/105220160

Table 54. Coefficient of variation for TMB

Comparison	Sample	Mean TMB	TMB standard deviation	CV (%)
	F1	7.5	0.311	4.15
	F2	76.7	2.036	2.66

M1 v M2 v M3	F3	93.9	1.532	1.72
	F4	56.1	0.879	1.57
	F5	3.2	0	0
M1 vs M4	F6	53.7	0.447	0.83
	F7	87.0	1.194	1.37
	F8	16.5	1.011	6.13

9. Reagent Stability

Analysis of the product stability shows the recommended shelf life and in use stability of the kit. 3 cell line samples and 4 clinical FFPE samples were used to validate the product stability. The real-time stability study demonstrated that the assay's performance meets all acceptance criteria for 10 months post-manufacture. However, at the 12-month timepoint, the overall mutation detection rate fell to 88.89%, which is below the required ≥95% threshold for qualification. The in-use stability study concluded that the kit reagents are stable for up to four freeze-thaw cycles, maintaining a mutation detection rate of 96.83%. Performance degraded significantly after the fifth cycle, with the detection rate dropping to 85.71%. As a result, the validated shelf life for the GENESEEQPRIME assay kit is established at 10 months, with a recommended limit of four freeze-thaw cycles for key reagent boxes to ensure data integrity and optimal performance.

G. Instrument Name:

Illumina NextSeq® 550Dx

H. System Descriptions:

i. Modes of Operation:

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

Yes _____✓_____ No _____

ii. Software:

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

Yes _____ No _____✓_____

I. Other Supportive Instrument Performance Characteristics Data Not Covered in The "Performance Characteristics" Section above:

Not applicable

J. Proposed Labeling:

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

K. Conclusion:

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