Food and Drug Administration
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Silver Spring, MD 20993-0002
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510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

I Background Information:

A 510(k) Number

K192063

B Applicant

Personal Genome Diagnostics

C Proprietary and Established Names

PGDx elio™ tissue complete

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
PZM	Class II	21 CFR 866.6080 - Next Generation Sequencing Based Tumor Profiling Test	Pathology

II Submission/Device Overview:

A Purpose for Submission:

New device

B Measurand:

Somatic single nucleotide variants, insertions and deletions, select amplifications and translocations, microsatellite instability (MSI) and tumor mutation burden (TMB) in human genomic DNA obtained from formalin-fixed, paraffin-embedded tumor tissue. Refer to Appendix A for a list of the genes covered by the assay.

C Type of Test:

Next-generation sequencing tumor profiling test

III Intended Use/Indications for Use:

A Intended Use(s):

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.

B Indication(s) for Use:

Same as above

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

For *in vitro* diagnostic use.

D Special Instrument Requirements:

Illumina NextSeq® 550Dx (qualified by PGDx)

IV Device/System Characteristics:

A Device Description:

1. Reagents

The PGDx elio tissue complete assay is for use as part of a test system with the NextSeq 550Dx and sequencing reagents. Components of the PGDx elio tissue complete assay are listed in Table 1. PGDx provided components include reagent kits, software for data analysis, and a server. The assay contains reagents for 2 full sequencing runs (i.e.,30 samples plus 2 external controls 2 NTC runs). Materials required but not provided are described in the text below Table 1. A detailed list of required instruments, software, reagents, consumables and storage conditions is described in the product labeling (PGDx elio tissue complete User Manual).

Table 1. Reagent Components of the PGDx elio tissue complete assay

Storage Temp. (°C)	Component Name	Volume	Cap Label
Library Preparation Kit, Box 1 of 2			
-25 to -15	ER/AT Buffer	302 µL	ER/AT Buffer
-25 to -15	ER/AT Enzyme	147 µL	ER/AT Enzyme
-25 to -15	Ligation Buffer	1.3 mL	Lig Buffer
-25 to -15	DNA Ligase	431 µL	DNA Ligase
-25 to -15	Hot Start PCR Mix (2x)	1.0 mL	PCR Mix
-25 to -15	Primer Mix (10x)	204 µL	Primer Mix
-25 to -15	Nuclease-Free Water	4.9 mL	None
-25 to -15	MB_Reagents (Adapters; multiple)	20 µL each	MB
Library Preparation Kit, Box 2 of 2 (A0220300)			
2 to 8	Pre-PCR Beads	11.8 mL	None
Capture Kit, Box 1 of 4 (A0220400)			
-25 to -15	Hyb Blocker 1	47 µL	Hyb Blocker 1
-25 to -15	100 µM Primer 1	27 µL	100 µM Primer 1
-25 to -15	100 µM Primer 2	27 µL	100 µM Primer 2
-25 to -15	Hyb Blocker 2	208 µL	Hyb Blocker 2
-25 to -15	RNase Block	23 µL	RNase Block
-25 to -15	Hybridization Buffer	248 µL	Hyb Buffer
-25 to -15	DNA Pol Buffer	431 µL	PCR Buffer
-25 to -15	DNA Pol Enzyme	46 µL	PCR Enzyme
-25 to -15	dNTP Mix	23 µL	dNTP Mix
-25 to -15	Nuclease-Free Water	4.9 mL	None
Capture Kit, Box 2 of 4 (A0220500)			
15 to 30	Binding Buffer (0220501)	37.3 mL	None
15 to 30	Wash Buffer 1	8.8 mL	None
15 to 30	Wash Buffer 2	56.4 mL	None
Capture Kit, Box 3 of 4 (A0220600)			
2 to 8	Post-PCR Beads	6.8 mL	None
2 to 8	Capture Beads	2.4 mL	Capture Beads
Capture Kit, Box 4 of 4 (A0220700)			
-85 to -65	Capture Baits	71 µL	Capture Baits
External Control (A0220900)			
2 to 8	External Control	5 µL	Ext Control

2. Materials Required but Not Provided

For a detailed list of required, but not provided reagents and consumables refer to the product labeling (PGDx elio tissue complete User Manual).

- DNA extraction Kits for FFPE Tissue
- DNA Fragment analyzer reagents
- Sequencing Reagent Kit: The PGDx elio tissue complete is validated for use with the NextSeq 550Dx High Output Reagent Kits (300 Cycle). If using additional NextSeq 550 reagents, PGDx elio IVD assay requires that only PGDx qualified lots of NextSeq 550 reagents be used with the device. A list of NextSeq reagent lots that have been qualified by PGDx for use with PGDx elio IVD assays is available on the PGDx elio Portal. Reagents must only be used with the instructions for use contained in the package insert. The PGDx software is designed to prevent the use of unqualified lots with the software.

3. PGDx elio Server and Software

The proprietary PGDx elioTM server contains analysis and reporting software necessary for the PGDx elio tissue complete assay (software versions are displayed within the PGDx elio platform user interface and on reports). The software is compatible with NextSeq® 550Dx instruments. A list of compatible versions of NextSeq software is available through the PGDx elio Portal. The PGDx elio server saves reports only and does not provide storage or backup of raw sequencing data. PGDx

4. Instrument

The PGDx elio tissue complete is validated for use on the NextSeq 550Dx instrument as part of a test system. NextSeq 550Dx instruments must be qualified by a PGDx representative before use with the PGDx elio platform software. Qualification establishes the instrument as IVD for use with the PDx elio tissue complete assay only. Qualification is performed upon server installation and prior to use. The PGDx elioTM diversiPhi is used to qualify and maintain the instrument.

Other required equipment and the specifications for the specific equipment for use with the PGDx elio tissue complete assay are described in Table 2.

Table 1. Other Required Equipment, Not Provided

Equipment	Notes
DNA shearing instrument	Mechanically shears DNA to the appropriate size.
DNA fragment analyzer	Automated sample processing determines size, quantity and purity for quick library QC.
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.

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.
Mini-centrifuge or micro-centrifuge	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.
Thermal cycler	One 96-well dual-block thermal cycler (or two 96-well single block thermal cyclers) is required in the post-PCR areas.
Tabletop 96-well plate centrifuge	Any plate centrifuge capable of maintaining 280 x g for at least 1 minute is sufficient. Separate plate centrifuges are required for 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.
Tabletop vortex mixer	Separate vortex mixers are required in pre-PCR and post-PCR areas.
Single-channel pipettors (P-2, P-10, P-20, P-200, P-1000)	Separate sets of pipettors are required in pre-PCR and post-PCR areas. Pipettors should be calibrated regularly and verified accurate within 5% of stated volume.
Multi-channel pipettor (P-20, P-200)	Separate sets of pipettors are required in pre-PCR and post-PCR areas. Pipettors should be calibrated regularly and verified accurate within 5% of stated volume.

5. Sample Preparation:

The PGDx elio tissue complete assay requires genomic DNA isolated from FFPE tissue specimens. The tumor volume and minimum tumor content needed to obtain sufficient DNA for testing to achieve stated performance are shown in Table 3. If less than 100% of the tissue section contains ≥20% tumor purity, the tissue should be macro-dissected to select as much viable tumor as possible and minimize the amount of adjacent non-tumor tissue.

Table 3. Specimen Handling and Processing for Validated Specimen Types

Tissue Type	Volume	Minimum Tumor Proportion	Macrodissection Requirements (based on tumor proportion)	Limitations	Storage
FFPE sections	The assay may require up to 10 slides at a minimum 5 microns thick	≥ 20% of viable nuclei in the selected tumor area should consist of tumor cell nuclei	Samples less than 100% tumor nuclei should be macrodissected	Archival FFPE material >14.5 years post-resection is not suitable for analysis	Room temperature

6. DNA Extraction:

PGDx elio tissue complete assay requires genomic DNA isolated from FFPE tissue using an appropriate commercially available DNA extraction method. DNA extraction kits should be able to yield 50 ng of DNA with a minimum concentration of 1 ng/µL. The recommended DNA input for PGDx elio tissue complete is 100 ng of total DNA recovered from tissue with a minimum 20% viable tumor nuclei. While recommended DNA input for the assay is 100ng, results can be obtained with DNA inputs down to 50 ng. The assay has been validated with extracted DNA stored at ≤ -20 °C for up to 9 months.

7. Library Preparation:

The PGDx elio tissue complete assay workflow begins with genomic DNA. Genomic DNA is quantified using a fluorometer. DNA molecules are mechanically sheared to a target size of 200 bp and subjected to a magnetic bead purification step to remove smaller fragments and perform an exchange of buffer. Fragmented DNA is end-repaired, phosphorylated, and adenylated. Indexed adapters are then ligated to the A-tailed DNA molecules. Unincorporated adapters and reagents are removed by magnetic bead purification. Adapter-ligated DNA is enriched by PCR amplification. Primer dimers and residual reagents are removed by magnetic bead purification. Library quality is assessed using a DNA fragment analyzer prior to hybrid capture. Sample libraries must be ≥ 15 ng/µL within the 180-800 bp range with the average peak size ≥ 250 bp in length, and external control (EC) for the batch must be ≥ 15 ng/µL within the 180-800 bp range with the average peak size ≥ 250 bp in length, prior to proceeding to hybridization / target enrichment.

8. Hybrid Capture NGS:

The adapter-ligated library is hybridized with biotinylated RNA library baits and targeted regions are captured using magnetic streptavidin coated beads. Captured DNA libraries are purified to remove baits and incompletely hybridized DNA fragments. Captured libraries are enriched by PCR amplification. Primer dimers and residual reagents are removed by magnetic bead purification. Final library quality is assessed using a DNA fragment analyzer prior to sequencing. Samples and external control must be ≥ 10 nM within the 180-800 bp range with the average size ≥ 250 bp in length. If the level of primer/adapter dimers in sample library lanes (100-180 bp region) is > 5% of the library yield, the library has failed QC and steps starting from library preparation must be repeated.

9. Sequencing:

Sample libraries are quantified and normalized into a sequencing pool of up to 15 samples and an external control. Partial batches are supported using a filler of diverse material, such as PGDx elio diversiPhi, or previously captured libraries. Pooled sample libraries are fluorometrically quantified, loaded on a sequencing flow cell, and sequenced.

10. Data Analysis:

a) **Data Management System (DMS)**: Sequence data is automatically processed using the PGDx elio platform 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 are available in run and case reports, including sample and sequencing quality.

b) **Demultiplexing and FASTQ Generation**: Demultiplexing software generates FASTQ files containing sequence reads and quality scores for each of the samples on a sequencing run. The FASTQ formatted data files are used for subsequent processing of samples.

c) **Indexing QC check**: Samples are checked for an expected yield of sequence reads identified to detect mistakes in pooling samples. Samples outside the expected range are marked as failed.

d) **Read Alignment and BAM Generation**: Genome alignment is performed to map sequence reads for each sample to the human reference genome (hg19/GRCh37). Alignments are saved as Binary Alignment Map (BAM) formatted files, which contain read placement information relative to the reference genome with quality scores. Aligned BAM files are further processed in a pipeline to identify genomic alterations.

e) **Sample QC checks**: Samples are checked for possible contamination through a bioinformatic analysis of genome haplotypes, based on an analysis of pre-defined SNP sites that are characteristic of populations and individuals. Samples containing more than one haplotype are considered potentially contaminated and are marked as failed. Sequence coverage is assessed across the panel requiring 90% of targeted regions with a minimum >100x coverage.

f) **Mutation calling**: A fully automated pipeline for bioinformatic analysis is used to identify genomic alterations, including SNVs, indels, select amplifications and translocations, and MSI, and TMB.

   i. ***SNVs and Indels***: Candidate mutations are evaluated and filtered for characteristics of high confidence somatic variants, including mutant allele frequency, sequence coverage and quality, genomic context, functional annotation, germline status, and prevalence in a database of normal controls. 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.

   ii. ***Amplifications***: The assay is validated to detect ERBB2 amplification. The amplification is identified based on comparing normalized sequence coverage against a collection of normal controls run by PGDx elio tissue complete. A fold change from diploid is estimated from the observed change in coverage combined with an in-silico prediction of tumor purity. ERBB2 gene amplifications are

reported when predicted fold >2.5x are observed in >25% of evaluated regions of interest for the gene. The test is validated for reporting only amplifications in the ERBB2 gene.

iii. ***Translocations:*** The assay is validated to report 4 translocations only, ALK, RET and NTRK2, NTRK3. Translocations are identified based on observations of reads supporting gene fusions in genomic alignments of discordantly mapped or split read pairs.

iv. ***Microsatellite instability***: Microsatellite instability is assessed from select mononucleotide tracts and signatures of genomic context from sequence mutations. A linear classifier determines an overall case status of microsatellite instability-high (MSI-H), microsatellite stable (MSS), or indeterminate by combining the frequency of unstable tracts and signatures of observed mutations.

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 (<5% MAF), common somatic driver mutations, and common germline mutations. Both synonymous and non-synonymous alterations are considered for the mutation load. TMB is reported as the number of mutations per megabase (Muts/Mb)

11. Controls:

a) Negative Control: A no template control (NTC) can be processed to serve as a negative control to validate the acceptability of all the test samples processed through library preparation and capture steps by testing for sample or reagent contamination. The NTC is not included on the sequencing run.

b) Positive Control: An external control that is provided in the PGDx elio tissue complete assay reagent kit consists of cell line derived-DNA with multiple verified sequence mutations. The external control is processed from library preparation through sequencing to serve as an end to end control to demonstrate assay performance. The external control is checked for quality during library preparation and after sequencing. Failure of the external control to meet the pre-defined quality metrics will result in all test samples on the run being reported as “No result.”

12. Result Reporting:

PGDx elio tissue complete reports SNVs and indels in protein coding regions across all genes in the panel. In addition, amplifications are reported for ERBB2 as well as translocations for ALK, RET, NTRK2, and NTRK3. Germline mutations, including common polymorphisms in the population, present in dbSNP v150, ExAC v0.3.1, and gnomAD v2.0.2, are filtered and excluded from reports. SNVs and indels that are not Variants with Evidence of Clinical Significance or hotspots will also be removed from reporting if they have ≥ 3 ExAC hits and have a MAF ≥ 20%. The assay also reports on two genomic signatures, MSI and TMB.

Variants are reported in one of two levels of evidence[1]: Variants with Evidence of Clinical Significance and Variants with Potential Clinical Significance. Variants reported as having evidence of clinical significance are defined by AMP/ASCO/CAP guidelines (Li et al., 2017), specifically, variants meeting Tier 1A evidence. The variants listed in the section Variants with Evidence of Clinical Significance are determined based on the selected tumor type. Only variants clinically associated with the tested tumor type will appear in the Variants with Evidence of Clinical Significance section. Any remaining detected variants will appear as the Variants with Potential Clinical Significance. Any variants clinically associated with tumor types other than the one selected will be reported in the section labeled 'Variants with Potential Clinical Significance. A list of all 505 genes is provided in Appendix A and a list of excluded exons in the genes or excluded regions due to challenging regions (e.g., low complexity/repeats) is provided in Appendix B and Appendix C, respectively .

Reporting software was designed to mask results that have low confidence allele frequencies levels near the calling threshold. The PGDx elio tissue complete analytical pipeline calculates a 95% CI around the estimated MAF for all sequence mutations. PGDx has applied a reporting filter to mask Level 3 non-hotspot SNV calls that have a lower bound 95% CI <5% MAF. By taking this approach, unreliable results at low MAF are filtered out of reporting, while high confidence calls in this range will still be reported.

Indeterminates: For select genes and regions, quality metrics are assessed to check for low coverage or incomplete data needed to identify an alteration. Indeterminate status is reported when 1) no evidence of the alteration was found, but minimum coverage was not met to support the verified limit of detection, or 2) insufficient evidence of the alteration was observed, but minimum coverage thresholds were not met to report the variant. Supporting evidence of detected alterations and coverage in read data is available in the Complete Case Record (CCR). Indeterminate status is reported when evidence of a sequence mutation is observed in regions of low coverage below < 80x. Indeterminate status is also reported for select genes and codons when low coverage is observed and there is no evidence of an alteration. The minimum coverage threshold range is 116x - 248x in cases where select genes and codons are called negative.

13. Quality Metrics

Reporting takes in account the quality metrics outlined in Table 4. Quality metrics are assessed across the following categories:

- Batch-level: Metrics that are quantified per sequencing run; failing batch-level metrics generates “No result” reports samples failing these criteria. If the external control fails these criteria, “No result” is reported for the entire batch of samples.
- Sample-level: Metrics that are quantified per sample; generates “No result” report for a sample failing QC.
- Analyte-level: Metrics that are quantified for individual alteration types and positions, such as sequence coverage. Variants passing analyte-level QC are reported.

[1] Refer to https://www.fda.gov/media/109050/download

Table 4. Summary of PGDx elio tissue complete 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 (Read1 and Read4) ≥ 80% %Q30 (Read2 and Read3) ≥ 85%
External Control	Batch-level	All expected sequence mutations are detected and passes all other quality criteria
Percent Regions Covered	Sample-level	≥ 90% exons with > 100x Median Distinct Coverage
Percent Reads Identified	Sample-level	Percent Reads Identified 15%-35%
Contamination QC	Sample-level	Estimated contamination levels < 2%
Select SNVs and Indels with Evidence of Clinical Significance	Analyte-level	Mutant reads ≥ 4 MAF ≥ 0.4%
Hotspot SNVs and Indels	Analyte-level	Mutant reads ≥ 4 MAF ≥ 2%
Non-hotspot SNVs	Analyte-level	Mutant reads ≥ 6 MAF with lower bound 95% CI ≥ 5%
Non-hotspot Indels	Analyte-level	Mutant reads ≥ 6 MAF ≥ 5%
Homopolymer Indels	Analyte-level	Homopolymer regions < 5 bp or Homopolymer regions ≥ 5 bp with MAF ≥ 12%
ERBB2 Amplification	Analyte-level	Fold change ≥ 2.5 in ≥ 25% regions covered
Translocations (ALK, NTRK2, NTRK3 and RET)	Analyte-level	Fusion reads ≥ 3

B Principle of Operation:

PGDx elio™ tissue complete is an in vitro diagnostic assay that uses targeted next generation sequencing to detect tumor gene alterations in genomic DNA isolated from formalin- fixed paraffin-embedded (FFPE) tumor tissue in a 505 gene panel. PGDx elio

tissue complete targets cancer-associated genes that are enriched from genomic libraries using a hybrid capture-based chemistry. Genomic libraries are prepared and captured. Samples are pooled for sequencing. After sequencing, automated software executes a bioinformatics analysis pipeline to identify genomic alterations in sequence data. The PGDx elio tissue complete assay workflow does not use a patient-matched normal sample but filters polymorphisms using databases. A summary of the alterations found, including a PDF case report, are reported in output files and provided in a user interface as part of the PGDx elio platform software.

C Determination of assay thresholds:

1. Requirements on exon coverage:

A power analysis was conducted to determine the sequence coverage necessary to detect mutations with true underlying MAFs as low as 2%. Statistical power was estimated based on a requirement of 4 mutant observations to make a positive call. 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).

Summary statistics were calculated for individual exons across a cohort of samples to identify exons with consistent below-target coverage. These regions were removed from PGDx elio tissue complete and are not included in variant analysis or reporting. Additional repeat and low complexity regions are also excluded from reporting. The excluded regions are listed in Appendix B and Appendix C. No Variants with Evidence of Clinical Significance or somatic hotspot mutations are masked from reports.

Sequence coverage was evaluated in the remaining regions across a cohort of 175 FFPE samples, and 99.5% of targeted regions (6,991 of 7,026 regions) were sequenced to a depth of 100x or greater with >98% of all regions sequenced to a depth of 250x or greater. Regions with somatic hotspot mutations exhibited sequence coverage >250x. Prediction of tumor mutation burden was maintained in low coverage samples, as demonstrated by low variability across replicates in simulations with coverage loss of up to 10% of exons. Overall, coefficient of variation (CV) estimates are below 25.6%, with an average CV of 11.7%. Excluding TMB scores below limit of blank (LoB), CV estimates are below 20%.

2. Requirements on sample coverage:

Sequence coverage was evaluated across a range of FFPE samples (n=175 across 8 different tissue types) to obtain sample summary statistics. Overall sample coverage was high in targeted regions of interest (Figure 1; Figure 1 shows a bar graph demonstrating the mutant frequency relative to the mean read depth) with high percentage of targeted exons covered (Figure 2). The mean coverage across all targeted regions for the FFPE samples was 915x (SD=375).
Sequence coverage was further evaluated to establish minimum criteria for the analysis and reporting of variants. Based on a power analysis, a minimum sequence coverage of 100x is necessary to call mutations with true underlying mutation frequency of 8% or greater. The number of exons for an individual sample meeting this coverage threshold was evaluated to established a per sample threshold. The samples evaluated included a range of DNA quality

estimates based on DNA fragment analysis. Of 175 samples evaluated, >97% of samples (171 of 175 samples) demonstrated ≥ 100x coverage across at least 90% of targeted regions of interest (Figure 3 and Figure 4). The consistently high coverage supports tolerance of occasional low coverage regions that may be seen with varying sample quality. A threshold of 90% of Regions of Interest (ROIs) with at least 100x coverage was selected and is used to determine if a sample is sequenced to sufficient depth for analysis and reporting.

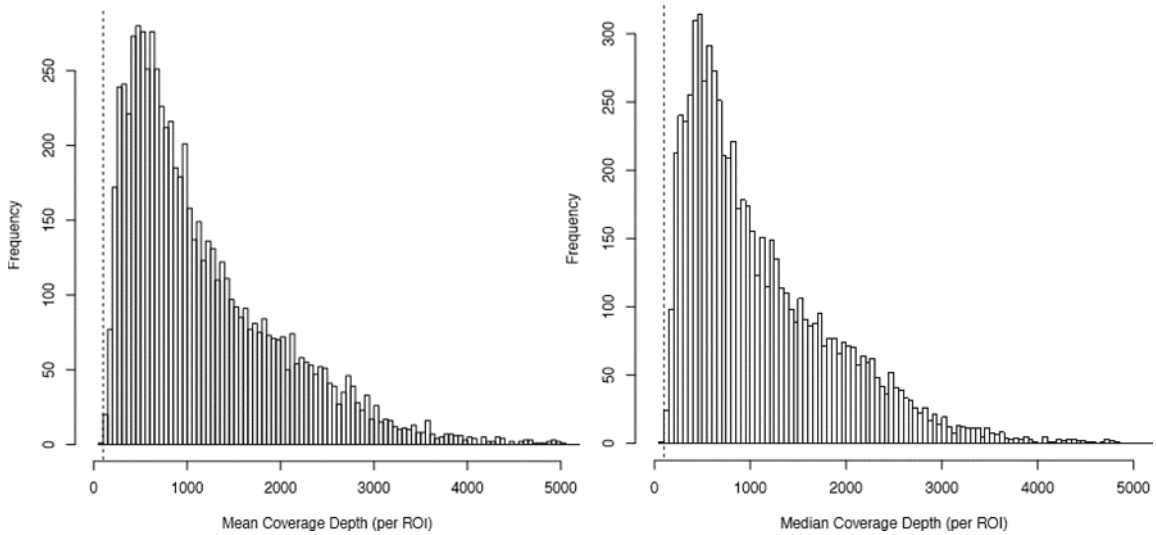

Figure 1. Distribution of mean and median coverage values for targeted regions of PGDx elio tissue complete. Dashed line indicates coverage at 100x.

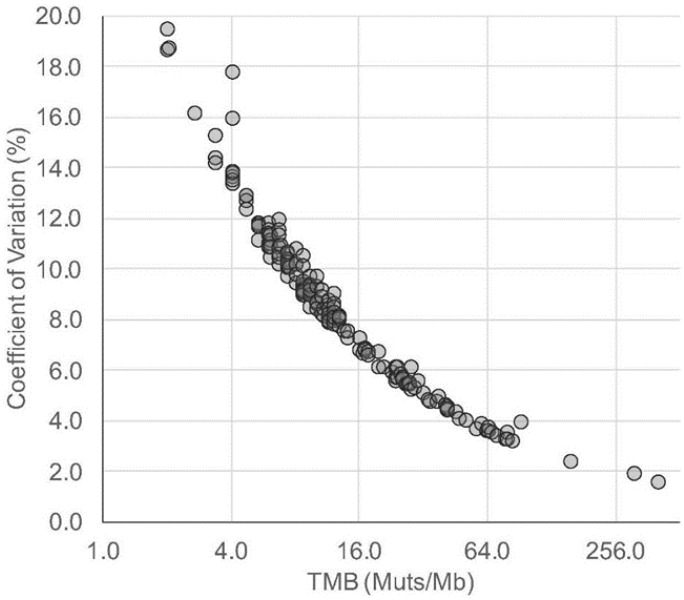

Figure 2. Evaluation of ROI loss and impact on TMB estimation. For each sample with at least 90% ROIs with ≥ 100x coverage (n=175), loss of 10% of ROIs was simulated (n=10,000 simulations per sample) to evaluate the effect on TMB estimates.

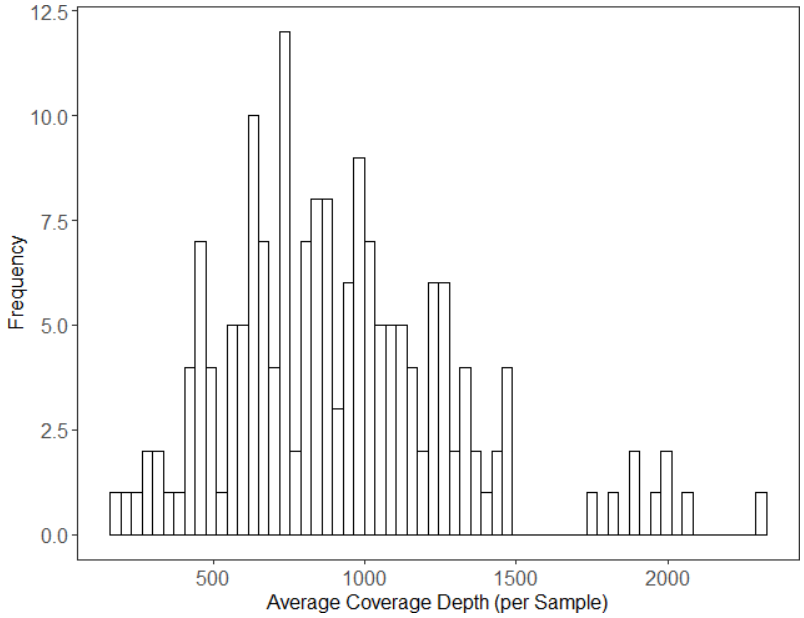

Figure 3. Distribution of mean distinct coverage per sample in PGDx elio tissue complete across 175 FFPE samples.

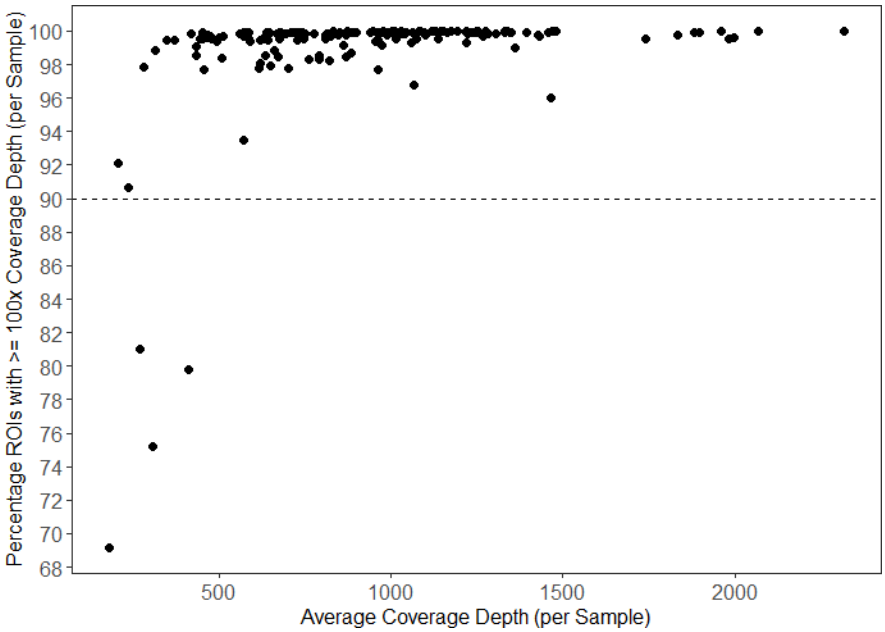

Figure 4. Distribution of mean coverage values per sample (x-axis) and percentage of ROIs in PGDx elio tissue complete with ≥ 100x coverage per sample (y-axis). Dashed line indicates 90% ROIs with ≥ 100x coverage.

3. Requirements on mutation coverage, allele depth and frequency for positive calls:

Variant calling parameters such as sequence coverage, mutation coverage, and mutation frequency were assessed as filters for specificity while maintaining the ability to detect

true positive calls. Thresholds were established to ensure specificity is maintained at targeted MAF levels for reporting. Mutation frequency thresholds were established at 0.4%, 2%, and 5% for sequence mutations based on a categorization of Variants with Evidence of Clinical Significance, somatic hotspots, and non-hotspots positions.

Additional filtering of Variants with Potential Clinical Significance excludes reporting of insertions and deletions in homopolymer regions (5 bp or greater) below 12% MAF as well as sequence mutations with lower bound 95% Confidence Interval <5% MAF based on sequence coverage. Additional quality metrics, such as base quality and strand bias were also incorporated in assessments of confidence for pipeline filters. A cohort of normal FFPE tissue (n=36) was used to provide empirical evidence that specificity was maintained using the pipeline thresholds and filters.

D Substantial Equivalence Information:

1. Predicate Device Name(s):

MSK-IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets):a Hybridization-Capture Based Next Generation Sequencing Assay

2. Predicate 510(k) Number(s):

DEN170058

3. Comparison with Predicate(s):

Characteristics	Predicate Device: MSK-IMPACT (DEN170058)	Subject Device: elio Tissue Complete
Similarities		
Indications for Use	The MSK-IMPACT assay is a qualitative in vitro diagnostic test that uses targeted next generation sequencing of formalin-fixed paraffin-embedded tumor tissue matched with normal specimens from patients with solid malignant neoplasms to detect tumor gene alterations in a broad multi gene panel. The test is intended to provide information on somatic mutations (point mutations and small insertions and deletions) and microsatellite instability for use by qualified health care professionals in accordance with professional guidelines and is not conclusive or prescriptive for labeled use of any specific therapeutic product. MSK-IMPACT is a single-site assay performed at Memorial Sloan Kettering Cancer Center.	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.

Technology	Hybrid Capture	Same
Specimen Types	Formalin-fixed, paraffin-embedded (FFPE) tumor tissue matched with normal specimens from patients with solid malignant neoplasms	Formalin-fixed, paraffin-embedded (FFPE) tumor tissue from patients with solid malignant neoplasms
Target Population	Patients with solid malignant neoplasms	Same
Characteristics	**Predicate Device:** **MSK-IMPACT (DEN170058)**	**Subject Device:** **elio Tissue Complete**
Differences		
Test Environment	Single-site assay (performed at Memorial Sloan Kettering Cancer Center)	Kit
Genes on Panel	468	505
Black List	73 exons	58 genes/exons excluded from reporting due to consistently low coverage and low complexity and repeat genomic regions in 254 genes
Variant types	Intended to provide information on somatic mutations (point mutations and small insertions and deletions), and microsatellite instability	Same except elio Tissue complete includes 1 amplification and 4 fusions and provides information on tumor mutational burden (TMB)
Instrument	Illumina HiSeq® 2500 Sequencing System (qualified by MSK)	Illumina NextSeq 550Dx (qualified by PGDx)
Determination of Pipeline Thresholds	• Based on >200X target coverage, • 100X for ≥ 98% target exons, • hotspot mutation calling threshold (mutation coverage (DP) ≥ 20, mutant reads (AD) ≥ 8, mutation frequency (VF) ≥ 2%, and non-hotspot mutation threshold (DP ≥ 20, AD ≥ 10, VF ≥ 5%)	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).
Assay cut-off	MSK-IMPACT does not report mutations below 2% for known hotspot mutations and 5% for non-hotspot mutations.	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.

Controls	• Matched normal • Positive control • Negative control No template control (NTC)	• Positive control • No template control (NTC) • Normalized to database of common germline SNPs
Clinical Evidence Curation Oncopanel results are reported under one of these two categories: • "Cancer Mutations with Evidence of Clinical Significance" or • "Cancer Mutations with Potential Clinical Significance."	Uses OncoKB, knowledge base that includes biologic, clinical and therapeutic information curated from professional guidelines and recommendations, therapeutic labeling, disease specific expert and advocacy group recommendations, and medical literature. Classification criteria were developed by MSK to communicate the level of clinical evidence available for individual mutations in the test report. OncoKB undergoes periodic updates through the review of new information by a panel of experts	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.

E Standards/Guidance Documents Referenced:

The following FDA guidance documents were consulted:

1. Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable; Guidance for Sponsors, Institutional Review Boards, Clinical Investigators, and Food and Drug Administration Staff (April 25, 2006);
2. eCopy Program for Medical Device Submissions; Guidance for Industry and Food and Drug Administration Staff (December 3, 2015);
3. Refuse to Accept Policy for 510(k)s; Guidance for Industry and Food and Drug Administration Staff (February 21, 2019);
4. Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices; Guidance for Industry and FDA Staff (May 11, 2005);
5. Content of Premarket Submissions for Management of Cybersecurity in Medical Devices; Guidance for Industry and Food and Drug Administration Staff (Draft, October 18, 2018);
6. Medical Device Accessories – Describing Accessories and Classification Pathways; Guidance for Industry and FDA Staff (December 20, 2017);
7. Format for Traditional and Abbreviated 510(k)s – Guidance for Industry and FDA Staff (August 12, 2005);
8. Off-The-Shelf Software Use in Medical Devices; Guidance for Industry, FDA Reviewers, and Compliance (September 9, 1999);
9. Information to Support a Claim of Electromagnetic Compatibility (EMC) of Electrically-Powered Medical Devices; Guidance for Industry and Food and Drug Administration Staff (July 11, 2016); and

10. Benefit-Risk Factors to Consider When Determining Substantial Equivalence in Premarket Notifications (510(k)) with Different Technological Characteristics; Guidance for Industry and Food and Drug Administration Staff (September 25, 2018).

F Performance Characteristics:

1. Analytical Performance- General:

The PGDx elio tissue complete is a targeted NGS panel with 505 genes. The targeted regions of interest in PGDx elio tissue complete are designed to detect single nucleotide variants (SNVs) as well as small insertions and deletions (indels) < 30bp in length in the coding exons of the targeted genes, as well as ERBB2 amplifications, ALK, RET, NTRK2, and NTRK3 translocations, MSI, and TMB. For SNVs and indels, A representative approach to validation of the targeted genes in the panel was submitted with data representing variant types for SNVs and indels, and at the gene level for amplification and translocations indicated with this assay. In addition, the assay was evaluated for performance regarding the panel wide quality metrics.

a) Invalid Rates

Multiple factors can influence overall robustness and performance of complex molecular tests, including pre-analytical factors and overall sample quality. If key in-process or automated data quality metrics are not met, PGDx elio tissue complete supports repeating samples through the workflow. Performance throughout verification and validation of the device was tracked and a summary of the rates for first pass (no repeat) and overall pass (allowing for a single repeat) are presented below. Data were aggregated for clinical cases from >40 tumor types. Resulting pass rates for all samples (clinical samples and cell lines) are presented in Table 5, while Table 6 shows invalid rate by tumor type across the workflow. The data shows that there the performance across tumor types is supportive of a pan tumor profiling.

Table 5. Acceptability Rates of PGDx elio tissue complete

All Samples	**Acceptability Rate (n/N) (2-sided 95% CI)**
First Pass	83.4% (3481/4173) (82.3%, 84.5)
After Repeat Test	94.2% (3931/4173) (93.5%, 94.9)
Clinical FFPE Samples	**Acceptability Rate (n/N) (2-sided 95% CI)**
First Pass	81.8% (2352/2874) (80.4%, 83.2)
After Repeat Test	92.9% (2671/2874) (91.9%, 93.8)

Table 6. Comparability of Tumor Pass Rates for the PGDx elio tissue complete

Tumor Type	Totals Samples	Total Failures	Total Passes	Failed Tumor Purity	Failed Pre-Library Prep	Failed Post-Library Prep	Pass Rate
Adenocarcinoma, NOS	160	37	123	14	4	19	0.77
Bladder Cancer	154	28	126	13	5	10	0.82
Brain Cancer	54	3	51	-	2	1	0.94
Breast Cancer	77	15	62	2	5	8	0.81
Cholangiocarcinoma	41	5	36	1	3	1	0.88
Colorectal Cancer (CRC)	744	122	622	34	19	69	0.84
Esophageal Cancer	105	14	91	9	-	5	0.87
Gastric Cancer	64	12	52	4	3	5	0.81
Gastrointestinal Stromal Tumor (GIST)	17	1	16	-	1	-	0.94
Head And Neck Cancer	72	9	63	6	-	3	0.88
Kidney Cancer	70	8	62	4	2	2	0.89
Liver Cancer	69	11	58	4	2	5	0.84
Lung Cancer, Non-Small Cell (NSCLC)	99	13	86	6	1	6	0.87
Lung Cancer, NOS	1025	197	828	83	46	68	0.81
Lung Cancer, Squamous Cell Carcinoma (SCC)	94	16	78	2	3	11	0.83
Melanoma	131	20	111	2	4	14	0.85
Mesothelioma	11	-	11	-	-	-	1
Pancreatic Cancer	107	21	86	10	5	6	0.8
Prostate Cancer	613	169	444	33	37	99	0.72
Sarcoma, NOS	26	4	22	-	1	3	0.85
Small Cell Lung Cancer	16	3	13	2	-	1	0.81
Thyroid Cancer	40	13	27	3	3	7	0.68

2. Precision/Reproducibility:

a) Interlaboratory Reproducibility

Interlaboratory reproducibility of the PGDx elio tissue complete assay was assessed across 3 different sites, using DNA extracted from 13 FFPE tissue specimens and 1 cell line. Together these 14 samples represented a range of SNVs, indels, ERBB2 amplifications, ALK, RET, and NTRK3 translocations, MSI, and TMB. Each of the

14 samples were tested in duplicate by 2 different operators on 12 sequencing runs across 3 non- consecutive days at each of the 3 independent laboratory sites using a single kit lot (36 total sequencing runs and 504 total replicates). Allele frequencies for the variants in the specimens spanned all ranges. Each replicate began with the workflow post-DNA extraction. The samples used in the multi-site reproducibility study, along with their expected variants, are presented in Table 7 below.

Table 7. Samples used in the multi-site reproducibility study

Tissue Type	Expected SNVs with Evidence of Clinical Significance	Number of Variants with Potential Clinical Significance	Translocation (trans) or Amplification (amp)	Mean TMB score (Muts/Mb)	MSI Status
Cell Line	0	9	NTRK3 trans	7.1	MSS
Mediastinum	0	0	ALK trans	2.3	MSS
Colorectal	0	9	RET trans	8.7	MSS
Sarcoma	0	7	RET trans	9.0	MSS
Colorectal	0	43	ALK trans	50.1	MSI-H
Lung – NOS[1]	KRAS G12A	19	0	18.8	MSS
Lung NSCLC[1]	0	26	ERBB2 amp	22.6	MSS
Colorectal	BRAF V600E	77	0	64.5	MSI-H
Colorectal	BRAF V600E	87	0	97.9	MSI-H
Endometrial	KRAS G12C	28	0	28.0	MSI-H
Colorectal	BRAF V600E	93	0	81.3	MSI-H
Melanoma	BRAF V600K	31	0	41.8	MSS
Appendix	NRAS G13D	8	0	6.2	MSS
Endometrial	KRAS G12C & BRCA2 W2574*	22	0	23.9	MSI-H

[1]NOS: not otherwise specified; NSCLC: non-small cell lung cancer.

b) Panel-wide Reproducibility

Reproducibility was assessed for each positive variant detected across all 36 replicates (Positive call rate) – The positive call rate was calculated based on the total number of mutations along with the two-sided 95% confidence interval.

Table 8 summarizes the positive call rates stratified by mutation type (SNV, insertions, and deletions) and mutant allele frequency (MAF). Overall call rate 86.2% across all samples and replicates (14493/16813, 85.7%-86.7% CI) with increased positive call rate at higher mutant allele frequency (MAFs). In terms of invalid rate, the first pass rate was 90.3% (455/504) and the overall pass rate of the study after repeat testing was 98.2% (495/504) allowing a maximum of 1 round of repeat testing.

The positive call rates for individual sequence mutations assessed in the Interlaboratory Reproducibility study, along with the MAF range, mean, SD, and CV are presented in **Appendix D**. A total of 337 SNVs and 137 indels (22 insertions, 115 deletions) are

provided. Variants are listed by specimen; each specimen is separated by a dark gray line. Discordant cases are denoted in light grey.

Table 8. Interlaboratory Reproducibility Positive Call Rates

Mutation Type	MAF Threshold	Positive Call Rate Among All Observed Mutations	Total Unique Variants	Mean MAF Ranges	Mean AD Range	Mean DP Range
All	MAF ≥ 0	86.2% (14493/16813)	474	0.8-99.6	4-4881	74.3-6569.5
	MAF ≥ 5	88.0% (14483/16458)	464	5.9-99.6	9-4881	74.3-6569.5
	MAF ≥ 8	91.9% (13921/15146)	427	8.1-99.6	9-4881	74.3-6569.5
	MAF ≥ 10	93.1% (13404/14400)	406	10.1-99.6	9-4881	74.3-6569.5
	MAF ≥ 15	96.4% (12387/12846)	362	15.1-99.6	25.8-4881	74.3-6569.5
All SNVs	MAF ≥ 0	88.4% (10549/11937)	337	0.8-99.6	4-4881	109-6569.5
	MAF ≥ 5	91.0% (10539/11582)	327	6.1-99.6	13.5-4881	109-6569.5
	MAF ≥ 8	95.7% (10070/10519)	297	8.1-99.6	13.5-4881	109-6569.5
	MAF ≥ 10	97.7% (9618/9845)	278	10.1-99.6	13.5-4881	109-6569.5
	MAF ≥ 15	97.8% (8773/8966)	253	15.1-99.6	39.2-4881	172.4-6569.5
All Insertions	MAF ≥ 0	82.8% (649/784)	22	6.9-39	20.7-1094.4	153.5-2976.5
	MAF ≥ 5	82.8% (649/784)	22	6.9-39	20.7-1094.4	153.5-2976.5
	MAF ≥ 8	86.9% (619/712)	20	10.4-39	34-1094.4	153.5-2976.5
	MAF ≥ 10	86.9% (619/712)	20	10.4-39	34-1094.4	153.5-2976.5
	MAF ≥ 15	95.9% (614/640)	18	15.7-39	37.6-1094.4	153.5-2976.5
All Deletions	MAF ≥ 0	80.5% (3295/4092)	115	5.9-82	9-2093.4	74.3-4615.3
	MAF ≥ 5	80.5% (3295/4092)	115	5.9-82	9-2093.4	74.3-4615.3
	MAF ≥ 8	82.6% (3232/3915)	110	8.1-82	9-2093.4	74.3-4615.3
	MAF ≥ 10	82.4% (3167/3843)	108	11.8-82	9-2093.4	74.3-4615.3
	MAF ≥ 15	92.6% (3000/3240)	91	15.1-82	25.8-2093.4	74.3-4615.3
ALK	N/A	100% (70/70)	2	N/A	6-155	337-5162.5
NTRK2	N/A	100% (8/8)	1	N/A	24-44	200-1707.5
NTRK3	N/A	100% (36/36)	1	N/A	321-911	1855-6721.5
RET	N/A	100% (71/71)	2	N/A	55-396	963-6208

*≥ refers to all variants greater than the designated MAF; Mean AD: Average allele depth across replicates per variant; Mean DP: Average distinct coverage across replicates per variant

c) Per Specimen:

The modal positive and negative call rates for sequence mutations (SNVs and indels) in each specimen are summarized in Table 9. A modal analysis yielded a 97.8% positive call rate among all positives (410 SNVs and indels).

Table 9. Interlaboratory Reproducibility Modal Call Rates per Specimen

Specimen	Total Unique Mutations Detected Across All Replicates	Modal Positive Call Rate[1] (n/N) (two-sided 95% CI)	Modal Negative Call Rate[2] (n/N) (two-sided 95% CI)
1	10	99.6% (251/252) (97.8%, 99.9%)	97.2% (105/108) (92.2%, 99.1%)
2[3]	0	-	-
3	9	100% (315/315) (98.8%, 100%)	-
4	7	100% (216/216) (98.3%, 100%)	97.2% (35/36) (85.8%, 99.5%)
5	43	99.5% (1462/1470) (98.9%, 99.7%)	91.4% (32/35) (77.6%, 97.0%)
6	20	98.9% (639/646) (97.8%, 99.5%)	88.2% (30/34) (73.4%, 95.3%)
7	26	97.8% (678/693) (96.5%, 98.7%)	95.2% (157/165) (90.7%, 97.5%)
8	81	96.4% (1991/2065) (95.5%, 97.1%)	88.7% (683/770) (86.3%, 90.8%)
9	88	97.8% (2710/2772) (97.1%, 98.3%)	80.3% (318/396) (76.1%, 83.9%)
10	30	99.0% (998/1008) (98.2%, 99.5%)	97.2%)70/72) (90.4%, 99.2%)
11	94	96.1% (2907/3024) (95.4%, 96.8%)	83.1% (299/360) (78.8%, 86.6%)
12	33	99.4% (1109/1116) (98.7%, 99.7%)	97.2% (70/72) (90.4%, 99.2%)
13	9	100% (216/216) (98.3%, 100%)	93.5% (101/108) (87.2%, 96.8%)
14	24	96.8% (732/756) (95.3%, 97.9%)	88.0% (95/108) (80.5%, 92.8%)

[1] Positive call rate was calculated based on variants with majority call detected as positive.
[2] Negative call rate was calculated based on variants detected at least once, but with majority or equal call as negative. For all other locations, the negative call rates are 100%.
[3] Specimen 2 was selected for presence of ALK translocation and had no detected SNVs or indels.

d) Analysis of Source of Variance

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

TMB was assessed using %CV of the TMB score across test sample replicates for samples with a reference TMB above LoB (7.2 Muts/Mb). The results are shown in **(**Table 10).

Table 10. Interlaboratory Reproducibility of PGDx elio tissue complete

Alteration Type	Metric	Overall (95% CI)	Inter-Site (95% CI)	Inter-Operator (95% CI)	Inter-Day (95% CI)	Repeatability (Within-Run) (95% CI)
SNVs	APA	97.8% (97.7%, 97.9%)	97.8% (97.7%, 97.9%)	97.9% (97.7%, 98.0%)	97.9% (97.7%, 98.1%)	97.8% (97.5%, 98.1%)
	ANA	99.9% (99.9%, 100%)	99.9% (99.9%, 100%)	99.9% (99.9%, 100%)	99.9% (99.9%, 100%)	99.9% (99.9%, 100%)
Insertions	APA	95.6% (95.2%, 96.0%)	95.7% (95.2%, 96.2%)	95.4% (94.3%, 96.2%)	95.5% (94.2%, 96.5%)	96.4% (94.6%, 97.6%)
	ANA	99.9% (99.9%, 100%)	99.9% (99.9%, 100%)	99.9% (99.9%, 100%)	99.9% (99.9%, 100%)	99.9% (99.9%, 100%)
Deletions	APA	94.4% (94.2%, 94.6%)	94.2% (93.9%, 94.4%)	94.9% (94.4%, 95.3%)	95.0% (94.4%, 95.5%)	95.5% (94.7%, 96.2%)
	ANA	99.9% (99.9%, 100%)	99.9% (99.9%, 100%)	99.9% (99.9%, 100%)	99.9% (99.9%, 100%)	99.9% (99.9%, 100%)
MSI	APA	99.1% (98.7%, 99.4%)	99.1% (98.7%, 99.4%)	99.0% (97.9%, 99.6%)	99.0% (97.6%, 99.6%)	99.0% (96.6%, 99.7%)
	ANA	99.3% (99.0%, 99.5%)	99.3% (99.0%, 99.6%)	99.3% (98.4%, 99.7%)	99.3% (98.1%, 99.7%)	99.3% (97.4%, 99.8%)
ERBB2 Amplification	APA	100% (99.3%, 100%)	100% (98.9%, 100%)	100% (95.9%, 100%)	100% (94.0%, 100%)	100% (88.6%, 100%)
	ANA	100% (100%, 100%)	100% (99.9%, 100%)	100% (99.7%, 100%)	100% (99.6%, 100%)	100% (99.2%, 100%)
ALK translocation	APA	98.6% (97.7%, 99.1%)	98.6% (97.5%, 99.2%)	98.6% (95.8%, 99.5%)	98.6% (94.8%, 99.6%)	98.6% (92.2%, 99.7%)
	ANA	99.8% (99.6%, 99.9%)	99.8% (99.6%, 99.9%)	99.8% (99.3%, 99.9%)	99.8% (99.1%, 99.9%)	99.8% (98.7%, 100%)
NTRK3 translocation	APA	92.7% (90.4%, 94.5%)	92.5% (89.6%, 94.6%)	93.1% (86.7%, 96.5%)	93.5% (85.4%, 97.3%)	92.3% (79.1%, 97.4%)
	ANA	99.4% (99.2%, 99.5%)	99.3% (99.1%, 99.5%)	99.4% (98.8%, 99.7%)	99.4% (98.7%, 99.8%)	99.3% (98.0%, 99.8%)
RET translocation	APA	98.7% (97.8%, 99.2%)	98.7% (97.7%, 99.3%)	98.6% (95.9%, 99.5%)	98.6% (95.0%, 99.6%)	98.6% (92.4%, 99.8%)
	ANA	99.8% (99.6%, 99.9%)	99.8% (99.6%, 99.9%)	99.8% (99.3%, 99.9%)	99.8% (99.1%, 99.9%)	99.8% (98.7%, 100%)
TMB	CV	3.5%	0.9%	0.4%	0.8%	3.0%

Independently ERBB2 amplification and ALK, NTRK2 and NTRK3, RET translocations were evaluated (one specimen each) and analyzed by ANA and APA. The overall APA for these variants was 97.7% and ANA was 99.9%. For BRCA1 deleterious variants, the APA was 11.8% because 3 replicates from a single case showed detection of a mutation not present in the other replicates. The MAF values for these 3 observations were 1.2%, 0.9%, and 0.8%, respectively (data not shown).

e) Precision for MSI:

Precision of MSI was evaluated across 8 MSS and 6 MSI-H samples with a range of MSI scores. The mean MSI score, MSI range, SD and % CV for the score along with positive call rates are provided for results with 36 replicates obtained in the 3-site reproducibility study. The results demonstrate that precision of MSI values is supported by the PGDx elio tissue complete. Data is shown in Table 11.

Table 11. MSI Performance in the Interlaboratory Reproducibility Study

Case No.	Modal Status	Total Replicates	Mean MSI Score	MSI Score Range	SD	%CV	Positive Call Rate (95% CI)
1	MSS	36	10.5	(4.4, 19.1)	3.7	35.1	100% (90.4%,100%)
2	MSS	35	13.6	(5.8, 20.4)	3.6	26.8	100% (90.4%,100%)
3	MSS	35	13.8	(7.2, 20.2)	3.7	26.8	100% (90.4%,100%)
4	MSS	36	10.5	(3.9, 19.8)	3.2	30.4	100% (90.4%,100%)
5	MSI-H	35	209.7	(203.7, 216.5)	3.6	1.7	100% (90.4%,100%)
6	MSS	34	9.6	(4.7, 16.0)	3.0	31.3	100% (90.4%,100%)
7	MSS	33	-21.9	(-29.9, -13.2)	5.0	-22.8	100% (90.4%,100%)
8	MSI-H	35	223.5	(213.1, 236.3)	6.0	2.7	100% (90.4%,100%)
9	MSI-H	36	271.6	(261.6, 287.2)	5.9	2.2	100% (90.4%,100%)
10	MSI-H	36	77.5	(62.6, 102.5)	6.6	8.5	100% (90.4%,100%)
11	MSI-H	36	219.0	(212.6, 224.0)	2.9	1.3	100% (90.4%,100%)
12	MSS	36	-56.1	(-61.5, -41.5)	4.1	-7.3	100% (90.4%,100%)
13	MSS	36	16.4	(10.4, 25.1)	3.9	23.7	100% (90.4%,100%)
14	MSI-H	36	49.3	(36.7, 61.7)	6.3	12.8	94.4% (81.9%, 98.5%)

f) Precision for Tumor Mutational Burden (TMB):

Precision of TMB was evaluated across 11 samples (with TMB scores near the analytical borderline value of TMB LoB of 7.2 Muts/Mb) in the 3-site reproducibility site. The distribution of replicates by site, operator, and day is across site, operator, and day per samples and score are provided in Figure 5 (Figure 5 shows the distribution of replicates per site and operator for each of representative specimens by TMB score and test day). The data demonstrates high precision for TMB scores.

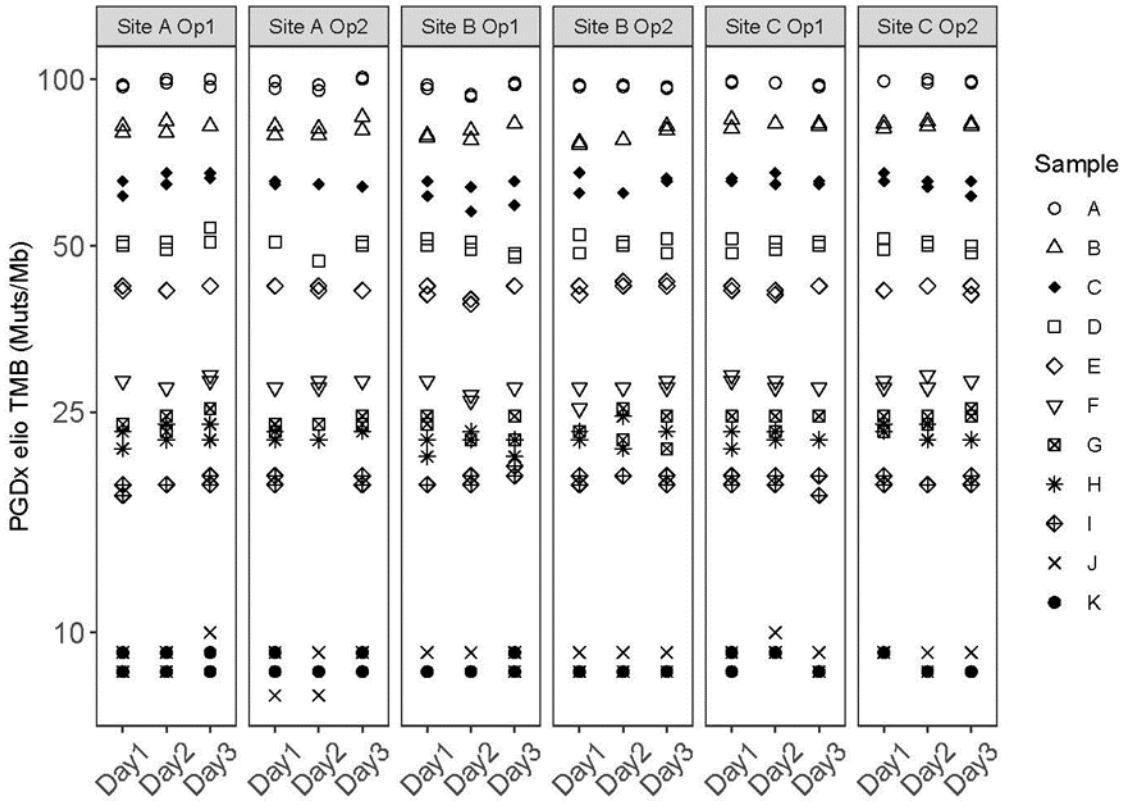

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Figure 5: TMB Performance in the Interlaboratory Reproducibility Study by Site, Operator, and Day.

g) Precision – cell lines:

Prior to performing the 3-site reproducibility study with clinical specimens, a study with DNA extracted from 6 blended cell line samples and a single colorectal FFPE sample that was MSI-H were evaluated. Together these samples represented a variety of DNA alterations, including over 600 unique alterations across a range of mutant allele fractions (MAFs). Each of the 7 samples was tested in duplicate by 2 different operators on 6 distinct sequencing runs at each of the 3 independent laboratory sites using a single kit lot. The positive call rate observed for the distinct variant types was consistent with the data observed with the clinical specimens (Table 12) though the cell line data had a larger number of variants with low MAFs

due to a dilution effect. Cell lines were blended at low concentrations reducing the MAF of insertions below thresholds and left only 20 total insertions (and only 3 at ≥15% MAF) for assessment.

Table 12. Multi-Site Reproducibility Study with Cell Lines:

Mutation Type	Positive Call Rate	Variants
All	83.8% (24497/29232)	812
SNVs	85.6% (21543/25164)	699
Insertions	81.6% (248/304)	19
Deletions	76.8% (2571/3348)	93

h) Lot-to-lot Precision

Performance of PGDx elio tissue complete was assessed across 3 unique kit lots by determining concordance of variant calls in FFPE tissue samples. The 3 unique kit lots were utilized to process 5 test cases in triplicate for a total of 45 observations. All batches were sequenced on the same instrument. Table 13 lists the Average Positive Agreement (APA) and Average Negative Agreement (ANA) used to assess lot to lot performance. APA for all variants is > 86%, and %CV for TMB analyses is < 10%. The performance is consistent with that of the reproducibility study.

Table 13. Lot-to-Lot Precision of PGDx elio tissue complete

Variant Type	Performance	Between Lot 1 & Lot 2	Between Lot 1 & Lot 3	Between Lot 2 & Lot 3
Variants with Evidence of Clinical Significance	APA	98.7% (93.0%, 99.8%)	96.1% (89.2%, 98.7%)	97.4% (91.1%, 99.3%)
	ANA	99.9% (99.6%, 100%)	99.8% (99.4%, 99.9%)	99.9% (99.5%, 100%)
MSI	APA	100% (75.8%, 100%)	100% (75.8%, 100%)	100% (75.8%, 100%)
	ANA	100% (82.4%, 100%)	100% (82.4%, 100%)	100% (82.4%, 100%)
SNVs	APA	92.1% (90.8%, 93.2%)	91.9% (90.6%, 93.0%)	91.9% (90.7%, 93.0%)
	ANA	99.9% (99.9%, 100%)	99.9% (99.9%, 100%)	99.9% (99.9%, 100%)
Insertions	APA	88.9% (80.2%, 94.0%)	88.9% (80.2%, 94.0%)	87.2% (78.0%, 92.9%)
	ANA	99.9% (99.9%, 100%)	99.9% (99.9%, 100%)	99.9% (99.9%, 100%)
Deletions	APA	86.2% (82.6%, 89.1%)	89.8% (86.7%, 92.2%)	87.3% (83.9%, 90.0%)
	ANA	99.9% (99.9%, 100%)	99.9% (99.9%, 100%)	99.9% (99.9%, 100%)

ERBB2 Amplification	APA	100% (61.0%, 100%)	100% (61.0%, 100%)	100% (61.0%, 100%)
	ANA	100% (86.2%, 100%)	100% (86.2%, 100%)	100% (86.2%, 100%)
ALK Translocation	APA	100% (61.0%, 100%)	100% (61.0%, 100%)	100% (61.0%, 100%)
	ANA	100% (96.7%, 100%)	100% (96.7%, 100%)	100% (96.7%, 100%)
TMB	CV	9.5%	7.9%	7.1%

3. Analytical Sensitivity - Limit of Detection (LoD):

The recommended DNA input for PGDx elio tissue complete is 100 ng of total DNA with a minimum 20% tumor purity. The LoD of the PGDx elio tissue complete assay is defined as the mutant allele fraction (MAF) at which 95% of replicates for a variant type are reliably detected. The LoD study was comprised of two steps: LoD establishment using cell lines and LoD confirmation with 10 FFPE clinical tumor samples from clinical cases across a diverse set of cancers (4 SNVs, 4 insertions and 4 deletions). Select specimens were used to evaluate specific mutations with evidence of clinical significance. Specimens were selected for allele frequencies near the claimed cut-offs. Details of the data are discussed and shown below.

a) LoD - SNVs, Insertions, and Deletions:

b)

Target levels for detection were first established in a dilution series from cell lines with up to 5 target MAF levels. The analytical sensitivity and LOD95 was then confirmed in clinical FFPE specimens. Data was aggregated across 2 reagent kit lots when possible, otherwise the lot with the higher MAF was used. Cell lines were used to establish the LoD MAF range for 451 SNVs and 31 indels across the panel. A total of 150 observations were generated (3 samples with 10 replicates at 5 dilution levels). Positive call status and MAF was evaluated for select variants identified in 10 FFPE clinical specimens diluted with normal DNA derived from FFPE tissue. Each specimen was processed with 2 kit lots of PGDx elio tissue complete across 10 replicates for a total of 200 observations (Table 14). The established analytical sensitivity ranges were confirmed at ≥ 95% call rate with FFPE clinical cases on a per variant level (Table 14) and using all somatic variants identified in FFPE clinical cases representing a range of MAFs for hotspot and non-hotspot positions (Table 15). A summary of the LoD mean mutant allele frequency and range as well as the positive call rate are displayed for each variant type by variant type for the entire panel across all replicates is shown in the Table 25 below. A range of 5.9-12.6% MAF was observed using the lowest average MAF where the positive call rates was ≥ 95%. The observed sequencing depth (DP), allele depth (AD), mutation allele frequency (MAF) range and average MAF are included.

Table 14. Analytical Sensitivity (LoD MAF) for SNVs and Indels in FFPE Tumor Tissue

Mut Type	Gene	AA Change	DP Range	AD Range	MAF Range	Mean MAF	Positive Call Rate
SNV	BRAF	V600E	491-804	6-31	1.0-5.3%	3.1	100%
SNV	EGFR	L858R	1257-2460	19-80	1.5-4.7%	3.3	100%
SNV	BRCA2	Splice Site Acceptor	934-1600	27-61	2.3-5.2%	3.4	100%
SNV	TP53	Q331*	537-696	15-43	2.4-6.2%	4.3	100%
SNV	KRAS	G12V	280-396	6-24	2.1-8.4%	5.0	100%
SNV	NRAS	G13D	530-1635	25-85	2.7-9.3%	6.6	100%
SNV	TERT	Promoter	255-431	13-33	3.9-8.5%	5.9	100%
INS	TSC1	Q654Tfs*34	547-1443	28-164	5.1-11.4%	8.1	100%
INS	BRCA2	S3366Nfs*5	731-973	58-113	6.9-12.7%	8.9	100%
INS	TSC2	D1690Gfs*27	463-877	51-114	8.7-16.9%	12.0	95.0%
INS	BBC3	R243Qfs*7	781-1530	95-254	12.2-19.2%	14.3	95.0%
DEL	EGFR	L747_E749del	479-969	22-48	3.5-6.7%	4.6	100%
DEL	SMARCA4	E525Afs*8	1312-2150	99-195	6.4-9.7%	8.2	95.0%
DEL	SOX9	S484Wfs*?	2133-3350	209-360	9.2-12.8%	10.7	95.0%
DEL	KDM6A	S700Lfs*29	2062-2697	209-315	10.1-12.2%	11.3	100%

Table 15. Analytical Sensitivity (LoD MAF) for Representative SNVs and Indels

Variant	Established MAF Range	Cell Line Variants	Number of Variants in Clinical Cases in the Established Range
Hotspot SNVs	3.1% to 5.4%	8	2
Non-hotspot SNVs	6.3% to 17.8%	443	176
Indels at homopolymer context[1]	13.7% to 17.5%	10	9
Indels at non-homopolymer context	6.1% to 10.9%	19	4
Insertions	6.1% to 15.8%	4	6
Deletions	6.5% to 17.5%	25	13

[1] Greater than or equal to 5 bp repeat

Additional evaluations of analytical sensitivity performance used dilution series of FFPE clinical specimens. The positive call rates, sequence coverage, and mutant allele fraction are provided for a total of 11 SNVs, 3 insertions, and 5 deletions from 5 clinical FFPE specimens with 5 replicates per dilution level. A range of 5.9-12.6% MAF was observed using the lowest average MAF where the positive call rates was ≥ 95%. (Table 16 - Table 34).

An in-depth variant analysis of cell-line samples from LoD establishment studies were assessed to further demonstrate analytical sensitivity. The positive call rates, sequence coverage (DP), allele depth (AD), and mutant allele fraction (MAF) are provided for a total of 13 SNVs, 4 insertions, and 4 deletions from a cell-line based dilution series of 3 samples with 10 replicates at 5 dilution levels (Table 35-Table 55).

Table 16: KRAS G12D SNV

KRAS SNV (Clinical Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
40%	G12D	487-565	87-133	20.4	100% (5/5)
30%		519-580	79-92	15.6	100% (5/5)
20%		554-593	35-73	10.2	100% (5/5)
15%		583-624	33-39	5.9	100% (3/3)

Table 17: APC R213* SNV

APC SNV (Clinical Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
40%	R213*	264-343	59-85	23.1	100% (5/5)
30%		309-374	56-98	21.7	100% (5/5)
20%		302-327	33-42	12.0	100% (5/5)
15%		305-326	16-23	6.1	100% (3/3)

Table 18: PIK3CA Y1021C SNV

PIK3CA SNV (Clinical Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
40%	Y1021C	609-790	137-192	25.0	100% (5/5)
30%		620-791	91-115	15.1	100% (5/5)
20%		684-835	74-87	10.8	100% (5/5)
15%		727-817	43-61	6.5	100% (3/3)

Table 19: MEN1 R206H SNV

MEN1 SNV (Clinical Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
40%	R206H	1476-1802	332-431	22.8	100% (5/5)
30%		1585-1746	230-286	15.6	100% (5/5)
20%		1586-1911	170-207	11.0	100% (5/5)
15%		1939-2230	130-148	6.6	100% (3/3)

Table 20: ACVR1 R160*SNV

ACVR1 SNV (Clinical Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
40%	R160*	1192-1501	274-351	22.8	100% (5/5)
30%		1266-1691	251-358	19.9	100% (5/5)
20%		1329-1561	163-207	12.4	100% (5/5)
15%		1432-1572	103-107	6.9	100% (3/3)

Table 21: PDCD1 R272Q SNV

PDCD1 SNV (Clinical Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
40%	R272Q	1116-1360	211-276	20.0	100% (5/5)
30%		1271-1523	193-310	17.6	100% (5/5)
20%		1147-1447	88-173	10.5	100% (5/5)
15%		1360-1507	95-103	7.0	100% (3/3)

Table 22: SMAD3 V294M SNV

SMAD3 SNV (Clinical Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
40%	V294M	1091-1487	280-321	23.6	100% (5/5)
30%		1384-1618	240-301	18.4	100% (5/5)
20%		1425-1523	147-169	10.6	100% (5/5)
15%		1552-1673	104-129	7.0	100% (3/3)

Table 23: CREBBP P885H SNV

CREBBP SNV (Clinical Dilution Series 5)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
40%	P885H	1853-2160	455-542	24.6	100% (5/5)
30%		1888-2670	366-502	19.6	100% (5/5)
20%		2009-2420	202-289	11.2	100% (5/5)
15%		2156-2564	160-181	7.1	100% (3/3)

Table 24: PIK3CG T128M SNV

PIK3CG SNV (Clinical Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
40%	T128M	2194-2894	431-618	21.2	100% (5/5)
30%		2545-2866	376-458	15.4	100% (5/5)
20%		2707-3333	224-356	9.5	100% (5/5)
15%		3083-3423	215-255	7.1	100% (3/3)

Table 25: NOTCH1 R4904* SNV

NOTCH1 SNV (Clinical Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
40%	R4904*	1451-1657	307-393	23.0	100% (5/5)
30%		1547-1924	274-359	18.4	100% (5/5)
20%		1633-1791	179-213	11.3	100% (5/5)
15%		1591-1826	109-147	7.3	100% (3/3)

Table 26: KMT2D R4904* SNV

KMT2D SNV (Clinical Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
40%	R4904*	1451-1657	307-393	23.0	100% (5/5)
30%		1547-1924	274-359	18.4	100% (5/5)
20%		1633-1791	179-213	11.3	100% (5/5)
15%		1591-1826	109-147	7.3	100% (3/3)

Table 27: APC Insertion

APC INS (Clinical Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
40%	T1556Nfs*3	331-383	69-90	21.8	100% (5/5)
30%		305-479	52-93	15.8	100% (5/5)
20%		424-461	30-55	10.1	100% (5/5)
15%		385-457	21-32	6.4	100% (3/3)

Table 28: TLR9 Insertion

TLR9 INS (Clinical Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
40%	R389Pfs*76	2007-2500	470-606	23.9	100% (5/5)
30%		2044-2341	342-371	15.9	100% (5/5)
20%		2376-2652	249-303	11.0	100% (5/5)
15%		2597-2815	179-187	6.8	100% (3/3)

Table 29: ARID1A Insertion

ARID1A Insertion (Clinical Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
40%	H688Sfs*129	1228-1571	304-348	23.3	100% (5/5)
30%		1395-1634	256-402	21.2	100% (5/5)
20%		1374-1484	164-187	12.6	100% (5/5)
15%		1513-1661	98-126	7.1	100% (3/3)

Table 30: ARID1A Deletion

ARID1A DEL (Clinical Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
40%	Q1493Hfs*6	1362-1631	219-303	18.0	100% (5/5)
30%		1284-1692	135-229	12.6	100% (5/5)
20%		1658-1658	149-149	9.0	20.0% (1/5)
15%		1678-1811	128-131	7.4	66.7% (2/3)

Table 31: RAD51C Deletion

RAD51C Deletion (Clinical Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
40%	D202Ifs*37	213-272	22-44	13.0	100% (5/5)
30%		237-319	20-36	9.8	100% (5/5)
20%		220-270	16-20	7.5	80.0% (4/5)
15%		265-327	17-25	7.4	100% (3/3)

Table 32: TET2 Deletion

TET2 DEL (Clinical Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
40%	K117Rfs*11	515-593	108-137	21.9	100% (5/5)
30%		521-657	91-109	16.7	100% (5/5)
20%		609-741	57-85	11.1	100% (5/5)
15%		656-726	51-62	8.3	100% (3/3)

Table 33: NKX3-1 Deletion

NKX3-1 DEL (Clinical Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
40%	P58_E59del	939-1190	161-202	17.4	100% (5/5)
30%		953-1281	92-185	11.5	100% (5/5)
20%		1093-1112	75-109	8.4	40.0% (2/5)
15%		963-963	82-82	8.5	33.3% (1/3)

Table 34: B2M Deletion

B2M DEL (Clinical Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
40%	L15Ffs*41	2175-2731	475-592	22.0	100% (5/5)
30%		2322-2866	432-626	19.3	100% (5/5)
20%		2120-2439	239-348	12.6	100% (5/5)
15%		2252-2479	196-220	8.7	100% (3/3)

Table 35: BRAF V600E SNV

BRAF SNV (Cell Line Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
Level 1	V600E	879-1167	71-110	9.0	100% (10/10)
Level 2		948-1126	51-107	6.6	100% (10/10)
Level 3		860-1114	17-38	2.8	100% (10/10)
Level 4		878-1147	20-36	2.5	100% (10/10)
Level 5		1017-1257	10-29	1.6	90.0% (9/10)

Table 36: EGFR L858R SNV

EGFR SNV (Cell Line Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
Level 1	L858R	1819-2593	210-288	11.4	100% (10/10)
Level 2		1942-2383	149-204	8.1	100% (10/10)
Level 3		1344-1763	35-77	3.5	100% (10/10)
Level 4		1601-2131	46-70	2.9	100% (10/10)
Level 5		1844-2333	27-42	1.7	100% (10/10)

Table 37: KRAS SNV

KRAS SNV (Cell Line Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
Level 1	G13D	897-1046	71-107	9.5	100% (10/10)
Level 2		1059-1320	56-115	7.1	100% (10/10)
Level 3		951-1349	32-58	4.2	100% (10/10)
Level 4		930-1123	24-43	3.2	100% (10/10)
Level 5		982-1147	18-30	2.1	100% (10/10)

Table 38: EGFR SNV

EGFR SNV (Cell Line Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
Level 1	T790M	2600-3602	294-497	12.5	100% (10/10)
Level 2		2551-3501	230-308	8.6	100% (10/10)
Level 3		1808-2231	79-110	4.4	100% (10/10)
Level 4		1991-2980	45-93	2.8	100% (10/10)
Level 5		2443-3117	41-69	2.1	100% (10/10)

Table 39: NRAS SNV

NRAS SNV (Cell Line Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
Level 1	Q61K	776-996	64-105	10.0	100% (10/10)
Level 2		953-1112	50-88	7.1	100% (10/10)
Level 3		743-1136	34-67	5.0	100% (10/10)
Level 4		679-1006	19-44	3.4	100% (10/10)
Level 5		860-963	16-33	2.4	100% (10/10)

Table 40: NRAS SNV

NRAS SNV (Cell Line Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
Level 1	A146T	1018-1208	102-154	12.0	100% (10/10)
Level 2		1089-1453	81-117	7.7	100% (10/10)
Level 3		1363-1564	66-102	5.5	100% (10/10)
Level 4		1025-1572	30-53	3.3	100% (10/10)
Level 5		1040-1307	21-40	2.4	100% (10/10)

Table 41: PIK3CA SNV

PIK3CA SNV (Cell Line Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
Level 1	G118D	152-252	7-23	8.1	100% (10/10)
Level 2		199-268	10-16	5.4	100% (10/10)
Level 3		200-313	5-11	2.7	60.0% (6/10)
Level 4		251-296	6-7	2.4	30.0% (3/10)
Level 5		259-329	7-7	2.4	20.0% (2/10)

Table 42: TP53 SNV

TP53 SNV (Cell Line Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
Level 1	R273H	902-1326	126-202	14.6	100% (10/10)
Level 2		971-1296	89-126	10.1	100% (10/10)
Level 3		832-1242	33-63	4.5	100% (10/10)
Level 4		1008-1240	27-38	3.0	90.0% (9/10)
Level 5		1123-1242	27-29	2.3	30.0% (3/10)

Table 43: CTNNB1 SNV

CTNNB1 SNV (Cell Line Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
Level 1	S33Y	1075-1269	115-189	13.7	100% (10/10)
Level 2		1276-1599	95-145	8.2	100% (10/10)
Level 3		1493-1794	68-122	5.2	100% (10/10)
Level 4		1250-1705	37-64	3.1	100% (10/10)
Level 5		1101-1534	28-48	2.5	70.0% (7/10)

Table 44: EGFR SNV

EGFR SNV (Cell Line Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
Level 1	G719S	941-1145	66-100	8.0	100% (10/10)
Level 2		1022-1168	42-67	5.2	100% (10/10)
Level 3		1068-1276	29-58	3.8	100% (10/10)
Level 4		872-1196	22-36	2.8	90.0% (9/10)
Level 5		937-957	22-27	2.6	20.0% (2/10)

Table 45: TP53 SNV

TP53 SNV (Cell Line Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
Level 1	S241F	1601-2004	196-254	12.2	100% (10/10)
Level 2		1907-2331	148-203	8.7	100% (10/10)
Level 3		1730-2136	87-154	5.9	100% (10/10)
Level 4		1634-1993	57-104	4.2	100% (10/10)
Level 5		1946-2266	42-77	2.8	100% (10/10)

Table 46: BRCA2 SNV

BRCA2 SNV (Cell Line Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
Level 1	S3094*	1614-1985	225-315	15.7	100% (10/10)
Level 2		1822-2188	149-217	9.4	100% (10/10)
Level 3		2038-2382	123-154	6.3	100% (10/10)
Level 4		1713-2279	66-107	4.3	100% (10/10)
Level 5		1973-2086	61-63	3.1	30.0% (3/10)

Table 47: BRCA1 SNV

BRCA1 SNV (Cell Line Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
Level 1	R1443*	3555-4041	357-486	11.1	100% (10/10)
Level 2		3887-4935	187-319	6.1	100% (10/10)
Level 3		4168-5324	154-256	4.1	100% (10/10)
Level 4		4588-5017	140-160	3.2	20.0% (2/10)
Level 5		N/A			0% (0/10)

Table 48: SOX9 Insertion

SOX9 INS (Cell Line Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
Level 1	P415Rfs*56	3508-3986	271-417	9.1	100% (10/10)
Level 2		3071-3873	171-243	6.1	100% (10/10)
Level 3		2623-3298	143-187	5.6	60.0% (6/10)
Level 4		N/A			0% (0/10)
Level 5		N/A			0% (0/10)

Table 49: MAML1 Insertion

MAML1 INS (Cell Line Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
Level 1	R476Sfs*22	2536-2958	219-277	9.0	100% (10/10)
Level 2		2884-3303	147-225	5.7	80.0% (8/10)
Level 3		N/A			0% (0/10)
Level 4		N/A			0% (0/10)
Level 5		N/A			0% (0/10)

Table 50: CTNNA1 Insertion

CTNNA1 INS (Cell Line Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
Level 1	A798Gfs*80	2462-2799	228-278	9.7	100% (10/10)
Level 2		2637-3223	161-203	6.3	90.0% (9/10)
Level 3		N/A			0% (0/10)
Level 4		N/A			0% (0/10)
Level 5		N/A			0% (0/10)

Table 51: RASA1 Insertion

RASA1 INS (Cell Line Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
Level 1	N492Kfs*9	225-294	27-54	15.8	100% (10/10)
Level 2		N/A			0% (0/10)
Level 3		N/A			0% (0/10)
Level 4		N/A			0% (0/10)
Level 5		N/A			0% (0/10)

Table 52: EGFR Deletion

EGFR Deletion (Cell Line Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
Level 1	E746_A750 del	759-964	98-138	13.5	100% (10/10)
Level 2		808-1012	64-101	9.4	100% (10/10)
Level 3		723-980	48-71	6.9	100% (10/10)
Level 4		639-891	29-50	4.8	100% (10/10)
Level 5		799-903	21-35	3.2	100% (10/10)

Table 53: MSH6 Deletion

MSH6 Deletion (Cell Line Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
Level 1	L290*	1574-2065	346-480	22.8	100% (10/10)
Level 2		1769-2119	285-345	16.7	100% (10/10)
Level 3		1553-2060	177-246	11.6	100% (10/10)
Level 4		1473-1865	125-193	9.0	100% (10/10)
Level 5		1723-1966	98-127	6.3	100% (10/10)

Table 54: RNF43 Deletion

RNF43 DEL (Cell Line Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
Level 1	G659Vfs*41	2670-3804	407-532	14.5	100% (10/10)
Level 2		2770-3451	256-353	10.6	100% (10/10)
Level 3		2033-2554	110-145	5.8	60.0% (6/10)
Level 4		2136-3134	79-126	3.6	50.0% (5/10)
Level 5		2628-3210	87-105	3.3	30.0% (3/10)

Table 55: MED12 Deletion

MED12 DEL (Cell Line Dilution Series)					
Dilution	**AA Change**	**DP Range**	**AD Range**	**Average MAF**	**Positive Call Rate**
Level 1	Splice Site Del	680-982	100-206	17.6	100% (10/10)
Level 2		872-1040	91-134	11.8	100% (10/10)
Level 3		864-1150	60-82	7.6	100% (10/10)
Level 4		984-1141	52-61	5.3	40.0% (4/10)
Level 5		1126-1126	66-66	5.9	10.0% (1/10)

c) LoD - MSI, Translocations, and Amplifications (Tumor Purity)

Analytical sensitivity of ERBB2, ALK, RET, NTRK3, and MSI was confirmed by testing 7 clinical FFPE cases (NSCLC, breast and CRC) diluted with normal FFPE DNA to achieve targeted detection levels (variants at low tumor purity). Each unique case was confirmed at ≥ 95% call rate at 1 tumor purity level, with 10 replicates per kit lot, across 2 unique lots for translocations and amplifications. For MSI-H, 3 FFPE clinical specimen cases were confirmed at 1 tumor purity level with 10 replicates each. Analytical sensitivity for specific translocations, amplifications, and MSI-H are summarized in Table 56.

Table 56. Analytical Sensitivity (LoD Tumor Purity) of PGDx elio tissue complete –Translocations, Amplifications and MSI

Variant	Confirmed LoD Tumor Purity	Positive Call Rate (n/N) (2-sided 95% CI)	Mean Coverage Range
MSI-H	18.1%	100% (30/30)	

		(88.6%, 100%)	
ERBB2 amplifications	4.4%	95.0% (19/20) (76.4%, 99.1%)	881-1271
ALK translocations[1]	5.6%	100% (17/17) (81.6%, 100%)	786-1282
NTRK3 translocations	11.5%	100% (20/20) (83.9%, 100%)	674 - 1156
NTRK2 Translocation	30%	100% (20/20) (83.9%, 100%)	unavailable
RET translocations	12.8%	100% (20/20) (83.9%, 100%)	762-1143

[1]The enrolled ALK case was evaluated with only 17 total replicates due to insufficient DNA quantity.

i. *ALK:*

The ALK Limit of Detection Clinical Confirmation study was examined to determine the number of fusion reads observed by PGDx elio tissue complete in these samples. Call rates of 100% across 2 lots were observed in dilutions with 5.6% tumor content with decreasing call rates at lower levels, 3.2% and 1% (Table 57)

Table 57. Limit of Detection Confirmation for ALK Translocations with Clinical FFPE Specimens

	Dilution	Mean and Standard Deviation Observed Fusion Read Counts	Mean Observed Tumor Purity	ALK Gene Distinct Coverage	Positive Call Rate (n/N) (2-sided 95% CI)
ALK Translocations (Gene Partner: EML4)	Dilution 1	6.8 ± 2.1	2.1%	974 to 1644	75% (15/20) (53.1%, 88.8%)
	Dilution 2	6.5 ± 2.8	3.6%	804 to 1576	90% (18/20) (69.9%, 97.2%)
	Dilution 3	15.5 ± 3.5	5.6%	786 to 1282	100% (17/17) (64%, 94.8%)

ii. *Microsatellite Instability (MSI):*

Three (3) additional, independent FFPE cases were used to confirm the LoD with respect to tumor purity for MSI, two of these cases were CRC and one was endometrial cancer. These 3 independent FFPE cases were used in the confirmation of LoD with 10 replicates per kit lot across 2 lots for a total of 60 observations (20 per sample). (Figure 6 shows three boxplots; the boxplots represent different tumor proportions relative to a red line that is a presumptive cut off for MSI-H. The boxplots show the distribution of replicate results.) The FFPE clinical case used for the establishment of LoD was CRC.

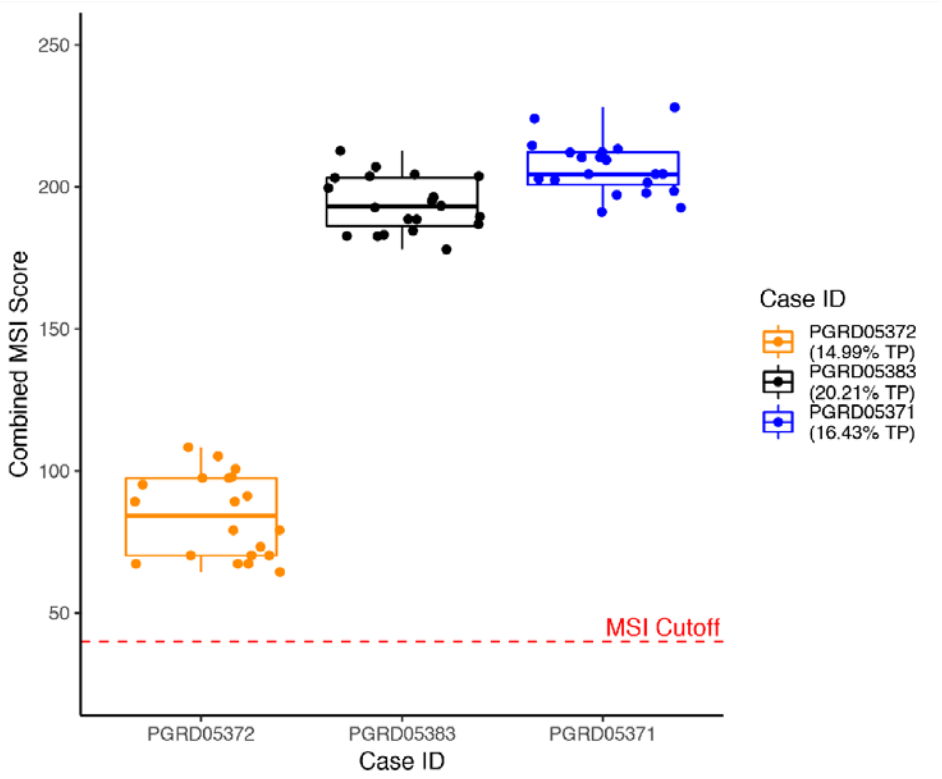

Figure 6. Combined MSI score across three LOD confirmation cases with 20 replicates each. TP = tumor purity.

These data were used to confirm a tumor purity LoD for MSI of 18%, which is above the recommended tumor purity input for PGDx elio tissue complete. As shown in the graphs above, the precision of MSI calling near the LoD of the assay is robust across the range of MSI scores. The data demonstrated that the MSI-H result was maintained at approximately 20% tumor purity.

d) *LoD – Tumor Mutation Burden (TMB) and DNA input*

The minimum tumor purity requirement for input into PGDx elio tissue complete is 20%. The minimum tumor purity required for robust reporting of TMB scores by PGDx elio tissue complete was confirmed using 8 clinical FFPE cases. Samples 1-5 were serially diluted across 3 levels with 5 replicates per level and 1 level with 3 replicates (18 total), sample 6 was serially diluted across 5 levels at 10 replicates per level (50 total), and samples 7-8 were serially diluted across 5 levels with 5 replicates per level (25 total with 10 ≥15% tumor purity). The total number of replicates per sample and the %CV of all replicates with ≥ 15% tumor purity are shown below in Table 58. Together these data show PGDx elio tissue complete has consistent TMB performance across tumor purities at or above 15%. The claim for TMB score is 20% tumor purity (Figure 7; the figure shows 8 lines representative of 8 samples across multiple tumor purities and the expected consistency in scores as a function of tumor purity).

Table 58. TMB Robustness for Samples ≥15% Tumor Purity

Sample	Reference Undiluted TMB Score	%CV of Replicates ≥ 15% Tumor	Number of Replicates ≥ 15% Tumor

		Purity	Purity
1	33.4	12.5%	18
2	24.8	10.5%	18
3	50.8	6.3%	18
4	64.7	15.9%	18
5	31.5	8.0%	18
6	455.4	3.3%	50
7	10.0	14.8%	10
8	14.6	6.0%	10

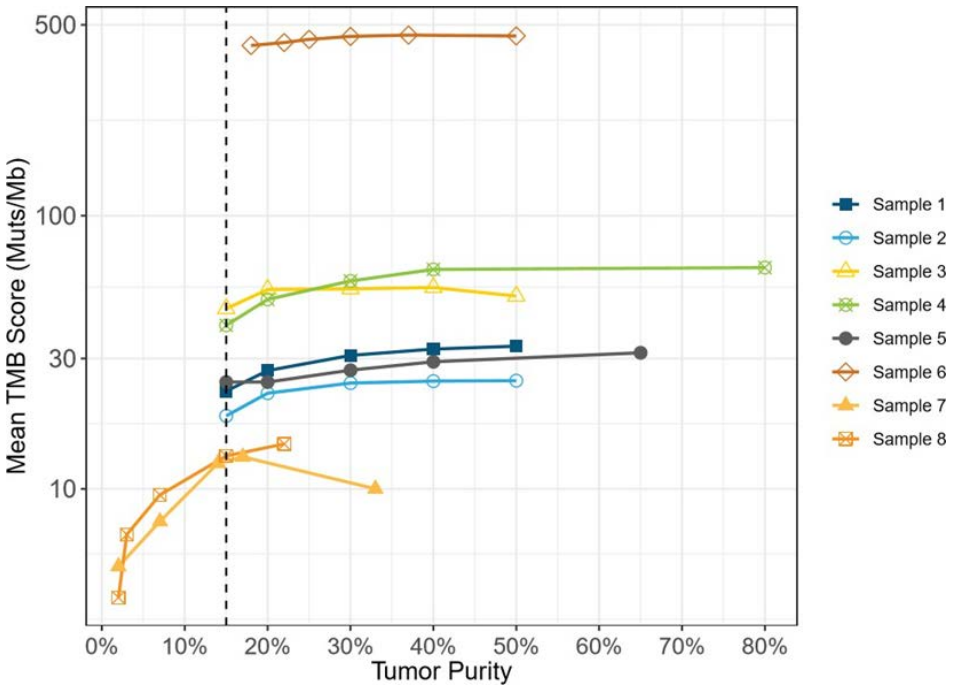

Figure 7. Linearity of TMB score with tumor purity in PGDx elio tissue complete. The tumor purity is shown on the x-axis and the mean TMB score of the replicates at a specific tumor purity is shown on the y-axis

4. Linearity/assay reportable range

Not applicable

5. Traceability, Stability, Expected Values (controls, calibrators, or methods)

a) Traceability:

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

b) *Stability/Shelf life*:

Product expiration dating is based on testing at multiple time points with specimens representative of variant types with 3 lots of the PGDx elio tissue complete assay reagent kit. The current shelf life for the PGDx elio tissue complete assay reagent kit is 6 months when stored according to the temperatures indicated on the label.

PGDx elio tissue complete can be used commercially for up to 4 freeze/thaw cycles.

c) *Transport Conditions*:

PGDx elio tissue complete kits are shipped using an insulated container with dry ice (all -80 °C and -20 °C reagents) and a Controlled Room Temperature container (with predetermined configuration of gel (cold and/or frozen) packs, meant for 4 °C and room temperature reagents) to maintain the product for up to 72 hours when stored at ambient
temperature.

d) Expected values (controls, calibrators or methods)

An external control that is provided in the PGDx elio tissue complete assay reagent kit consists of cell line derived-DNA with multiple verified sequence mutations. The external control is processed from library preparation through sequencing to serve as an end to end control to demonstrate assay performance. The external control is checked for quality during library preparation and after sequencing. Failure of the external control to meet the pre-defined quality metrics will result in all test samples on the run being reported as "No result." In addition, several quality metrics are established as thresholds for reporting results to provide for high confidence data.

6. Analytical Specificity:

a) Cut-off/False positive rate (Limit of Blank in DNA NGS)

Non-cancerous FFPE tissues and reference materials were evaluated for analytical specificity to assessed risk of false positives in normal tissues when detecting SNVs, indels, translocations, amplifications, MSI and TMB using PGDx elio tissue complete.

i. *Reference standards*: Two reference standards from National Institute of Standards and Technology (NIST), NA24531 and NA24385 were evaluated by PGDx elio tissue complete for variants reported. Specificity was observed at 100% with no unverified mutations reported across 5 replicates for each standard. Four (4) sequence mutations previously characterized in the standards were also reported by PGDx elio tissue complete and determined to be germline mutations that are of rare prevalence in the general population.

ii. *FFPE Clinical Specimens – 100ng input:* Analytical specificity of SNVs, indels, RET, ALK, NTRK translocations, ERBB2 amplifications, MSI and

TMB was further assessed with non-cancerous FFPE tissues. Unique test cases from 34 normal FFPE samples were processed with the recommended 100 ng DNA input across 2 different lots of the PGDx elio tissue complete assay kit. For Variants with Evidence of Clinical Significance, the rate of false positives is < 0.1% while the false positive rate for hotspot SNVs is < 3.2% (n=2/63). For MSI-H (qualitative call), the false positive rate was 0 (n = 26). For TMB, the false positive rate (defined as scores >7.3 Muts/Mb was 4.8%.

iii. *FFPE Clinical Specimens – 50ng input:* In addition to the assessment at the recommended DNA input of 100 ng, test cases from normal FFPE tissue types were prepared to assess specificity of the assay at 50 ng DNA input. For Variants with Evidence of Clinical Significance, the rate of false positives is < 0.1% while the false positive rate for hotspot SNVs is < 3.8% (n=1/26). For MSI-H (qualitative call), the false positive rate is < 1.6%. For TMB, the false positive rate (defined as scores >7.2 Muts/Mb was 11.5% (3/26).

b) Index Cross-Contamination

To demonstrate the ability of PGDx elio tissue complete in detection of contaminating samples, this study assessed whether samples artificially mixed in silico to a known degree were detected as contaminated. Five FFPE clinical samples of each African, East Asian, and European genetic ancestry (15 FFPE samples total) that had been previously sequenced and shown not to be contaminated were used. In silico, the data from these 5 samples from each ancestry group were mixed with all others of the same ancestry and 1 sample from both other ancestries. According to the results, this device will not detect all instances of contamination when both sample and contaminant are from individuals of Asian ancestry. A limitation in the package insert was established to caution that care should be taken to avoid cross-contamination of such samples.

c) Necrotic Tissue

The impact of necrosis on the performance of PGDx elio tissue complete was evaluated by assessing the first pass and overall pass rates (after one repeat test) of samples processed in the accuracy study (see Clinical Performance section below). Of 521 samples enrolled for accuracy, 448 were evaluated for necrosis over a range of 0-75%. The data indicated there is no correlation between necrosis and pass/fail rate.

d) Interfering Substances

The impact of interfering substances on the performance of the PGDx elio tissue complete assay was assessed by processing DNA from FPPE samples tested in the presence of each interfering substance at varying amounts (Table 59). The samples were evaluated for concordance of variant calls when compared to samples processed without the interfering substances. Replicates for 5 test cases were analyzed for 8 experimental and 2 baseline conditions. Performance was evaluated across 5 samples X 10 conditions X 15 replicates using multiple operators and

instruments. Samples were selected to be near the LoD. Analysis of all variant types tested (SNVs, indels, translocations, amplifications and MSI) showed no effect of exogenous interferent for all conditions: PPA (≥ 97.2%) and NPA (≥ 99.9%) (Table 60). The TMB mean absolute percent error (MAPE) ranged from 0% to 6.0% across conditions (Table 61). The results show minimal risk to assay performance from interfering substances.

Table 59. Exogeneous Interfering Substances Tested

Substance	Amount in Excess of Standard Conditions
Proteinase K	2X and 3X
Adapter	15% and 30%
Melanin	0.2 µg/mL and 1.6 µg/mL
Ethanol	2.5% and 5%

Table 60. Exogenous Interfering Substances Concordance by Test Condition

Test Condition	PPA % (n/N) (2-sided 95% CI)	NPA % (n/N) (2-sided 95% CI)
ProK 2x	98.3% (569/579) (96.9%, 99.1%)	99.9% (33532222/33532287) (99.9%, 100%)
ProK 3x	97.2% (563/579) (95.6%, 98.3%)	99.9% (33532206/33532287) (99.9%, 100%)
Adapter 15%	98.2% (218/222) (95.5%, 99.3%)	99.9% (33532275/33532284) (99.9%, 100%)
Adapter 30%	99.5% (221/222) (97.5%, 99.9%)	99.9% (33532273/33532284) (99.9%, 100%
Melanin 0.2 µg/mL	99.1% (220/222) (96.8%, 99.8%)	99.9% (33532272/33532284) (99.9%, 100%)
Melanin 1.6 µg/mL	100% (222/222) (98.3%, 100%)	99.9% (33532272/33532284) (99.9%, 100%)
Ethanol 2.5%	100% (222/222) (98.3%, 100%)	99.9% (33532274/33532284) (99.9%, 100%)
Ethanol 5%	100% (222/222) (98.3%, 100%)	99.9% (33532275/33532284) (99.9%, 100%)

Table 61. Exogenous Interfering Substances Concordance of TMB Mean Absolute Percent Error reported

Condition	Observed TMB Score	Absolute Percent Error	Mean Absolute Percent Error
Adapter 15%	10.8	0.0%	0%
	10.8	0.0%	
	10.8	0.0%	
Adapter 30%	11.5	6.5%	2.2%
	10.8	0.0%	
	10.8	0.0%	

Melanin 0.2 µg/mL	10.8	0.0%	0%
	10.8	0.0%	
	10.8	0.0%	
Melanin 1.6 µg/mL	10.8	0.0%	0%
	10.8	0.0%	
	10.8	0.0%	
Ethanol 2.5%	10.8	0.0%	0%
	10.8	0.0%	
	10.8	0.0%	
Ethanol 5%	10.8	0.0%	0%
	10.8	0.0%	
	10.8	0.0%	
Adapter 15%	41.5	2.6%	2.0%
	41.5	2.6%	
	42.3	0.7%	
Adapter 30%	43.9	3.1%	2.5%
	43.9	3.1%	
	43.1	1.2%	
Melanin 0.2 µg/mL	42.3	0.7%	3.5%
	43.1	1.2%	
	46.2	8.5%	
Melanin 1.6 µg/mL	43.1	1.2%	2.3%
	41.5	2.6%	
	43.9	3.1%	
Ethanol 2.5%	42.3	0.7%	3.2%
	44.6	4.7%	
	40.8	4.2%	
Ethanol 5%	44.6	4.7%	6.0%
	46.2	8.5%	
	44.6	4.7%	
Adapter 15%	14.6	2.1%	2.6%
	13.8	3.5%	
	14.6	2.1%	
Adapter 30%	14.6	2.1%	2.1%
	14.6	2.1%	
	14.6	2.1%	
Melanin 0.2 µg/mL	14.6	2.1%	2.1%
	14.6	2.1%	
	14.6	2.1%	
Melanin 1.6 µg/mL	14.6	2.1%	2.1%
	14.6	2.1%	
	14.6	2.1%	
Ethanol 2.5%	14.6	2.1%	2.1%
	14.6	2.1%	
	14.6	2.1%	
Ethanol 5%	13.8	3.5%	2.6%
	14.6	2.1%	

	14.6	2.1%	
Adapter 15%	8.5	0.0%	0%
	8.5	0.0%	
	8.5	0.0%	
Adapter 30%	8.5	0.0%	0%
	8.5	0.0%	
	8.5	0.0%	
Melanin 0.2 µg/mL	8.5	0.0%	0%
	8.5	0.0%	
	8.5	0.0%	
Melanin 1.6 µg/mL	8.5	0.0%	0%
	8.5	0.0%	
	8.5	0.0%	
Ethanol 2.5%	8.5	0.0%	3.1%
	8.5	0.0%	
	7.7	9.4%	
Ethanol 5%	8.5	0.0%	0%
	8.5	0.0%	
	8.5	0.0%	
ProK 2x	126.2	1.3%	1.0%
	126.9	1.8%	
	124.6	0.0%	
ProK 3x	123.9	0.6%	1.6%
	126.9	1.8%	
	127.7	2.5%	
ProK 2x	22.3	1.3%	3.8%
	21.5	4.9%	
	23.8	5.3%	
ProK 3x	21.5	4.9%	2.5%
	22.3	1.3%	
	22.3	1.3%	
ProK 2x	50	6.7%	5.3%
	50.8	5.2%	
	51.5	3.9%	
ProK 3x	52.3	2.4%	3.1%
	56.2	4.9%	
	54.6	1.9%	
ProK 2x	12.3	0.0%	4.3%
	13.1	6.5%	
	13.1	6.5%	
ProK 3x	12.3	0.0%	4.3%
	13.1	6.5%	
	13.1	6.5%	

e) Sample Carryover and Cross-Contamination:

Cross-contamination (contamination from one sample to another within the same batch) and sample carryover (contamination from a previous sequencing run when using the same instrument) were assessed by evaluating false positive and false negative variant calls in 29 FFPE samples. Seven (7) of the 29 cases had known positive variants, the remaining samples were known negative samples. All FFPE samples were assessed across 2 batches to test for contamination within and between runs. In batch 1, a checkerboard pattern within a 96-well plate was created by alternating the samples with representative positive variants and known negative samples. Batch 2 contained known negative samples and was pooled and sequenced directly after completion of batch 1 sequencing, following standard instrument cleaning procedures. No positive variant results were observed in known negative samples tested. Sample carryover and cross-contamination were not observed in any of the conditions evaluated.

7. Robustness Studies

a) Sample Stability:

DNA stability was assessed for extracted DNA stored at ≤ -20 °C prior to processing through PGDx elio tissue complete. A total of 45 unique clinical samples from 11 different tissue types were tested. The duration of DNA storage at the time of the evaluation in this study ranged from 97 to 377 days, with a median of 330 days (~10.6 months) and a mean of 295 days (~9.5 months).
Samples were sequenced at the time of initial extraction to determine a reference status of the variants, which was labeled as T0. PGDx elio tissue complete demonstrated robust analytical performance and concordant results for all variants assessed (MSI, amplifications, translocations, and sequence mutations) using DNA specimens stored for various times. Performance was maintained across all of the DNA storage times with PPA ≥ 93.2% and NPA > 99.9% (Table 62).

Table 62. DNA Stability Variant Concordance for PGDx elio tissue complete

	0-6 months PPA % (n/N) (2-sided 95% CI)	**6-12 months PPA % (n/N) (2-sided 95% CI)**	**>12 months PPA % (n/N) (2-sided 95% CI)**
Variants Aggregated	97.6% (161/165) (93.9%, 99.1%)	93.2% (772/828) (91.3%, 94.8%)	96.5% (223/231) (93.3%, 98.2%)

b) DNA Extraction

PGDx elio tissue complete may be used with an appropriate commercially available DNA extraction method. Three DNA extraction methods were evaluated. Three commonly used, commercially available DNA extraction kits included a column-based extraction method and a bead-based extraction method. DNA extraction method concordance was evaluated in 2 studies, including 1 cell line

and 10 FFPE solid tumor tissue samples selected to contain all variant types assessed by PGDx elio tissue complete, including borderline variants near the LoD. Each of the samples were extracted in duplicate by 2 operators for each of the 3 DNA extraction kits. The 48 DNA samples were processed with PGDx elio tissue complete in duplicate resulting in 96 total sequencing reactions (4 samples x 2 extractions x 2 operators x 3 extraction methods x 2 assay replicates). Method 2 (bead-based) and Method 3 (automated) were compared to the reference Method 1 (column-based). The overall pass rate for FFPE samples was 93.1% (67/72). PGDx elio tissue complete yielded concordant analytical performance for variant calls across the DNA extraction methods positive percent agreement (PPA) was >97% negative percent agreement (NPA) was >99.9% between methods. The TMB CV for all assessed cases was <12.5%. These data demonstrate that an appropriate commercially available FFPE DNA extraction method may be used to extract DNA for the PGDx elio tissue complete assay.

c) DNA Input

The optimal and recommended amount of input DNA for the assay is 100 ng. Minimum (50 ng) and recommended (100 ng) DNA input requirements were established by measuring assay performance with different inputs from FFPE tumor tissues (25-200 ng). To evaluate assay performance across a range of DNA inputs, 4 unique FFPE samples with known variants were prepared in triplicate at 10, 25, 50, 100 and 200 ng DNA input levels. The 4 FFPE cases assessed contained representative SNVs, indels, amplifications, translocations, MSI and TMB. The first pass acceptability rate for PGDx elio tissue complete was 100% across all DNA inputs for all DNA inputs except 10ng DNA which was 75% (9/12). After repeat testing, all 12 specimens yielded results.

The variant calls for these samples were compared to the respective reference DNA input of 100 ng for each case to assess concordance. Table 63 describes PPA and NPA for each input level where aggregated variants were analyzed, including SNVs, indels, amplifications, translocations, and MSI. For TMB, the mean absolute percent error rate at each DNA input level was compared to 100 ng (Table 64). These data indicate the assay is robust around the recommended 100 ng DNA input.

Table 63. Variant concordance of DNA inputs compared to Results with 100 ng Reference DNA Input

DNA Input	Variant Call Concordance (n/N) (2-sided 95% CI)
10 ng	PPA - 92.2% (177/192) (87.5%, 95.2%)
	NPA - 99.9% (26825815/26825826) (99.9%, 100%)
25 ng	PPA - 94.8% (182/192) (90.7%, 97.1%)
	NPA - 99.9% (26825815/26825826) (99.9%, 100%)
50 ng	PPA - 96.9% (186/192) (93.4%, 98.6%)
	NPA - 99.9% (26825822/26825826) (99.9%, 100%)
200 ng	PPA - 97.4% (187/192) (94.0%, 98.9%)
	NPA - 99.9% (26825818/26825826) (99.9%, 100%)

Table 64. Concordance of TMB Mean Absolute Percent Error Above LoB for PGDx elio tissue complete For DNA Input Range

Case No.	Mean Expected TMB Score	DNA Input	Observed TMB Score	Absolute Percent Error	Mean Absolute Percent Error
Case 1	19.2	10 ng	22.3	16.1%	18.7%
			23.8	24.0%	
			22.3	16.1%	
		25 ng	17.7	7.8%	5.2%
			17.7	7.8%	
			19.2	0%	
		50 ng	17.7	7.8%	5.2%
			20.0	4.2%	
			18.5	3.6%	
		200 ng	19.2	0%	1.2%
			19.2	0%	
			18.5	3.6%	
Case 2	43.1	10 ng	43.9	1.9%	4.8%
			40.8	5.3%	
			40.0	7.2%	
		25 ng	42.3	1.9%	1.3%
			43.9	1.9%	
			43.1	0%	
		50 ng	41.5	3.7%	3.6%
			43.9	1.9%	
			45.4	5.3%	
		200 ng	42.3	1.9%	2.4%
			44.6	3.5%	
			43.9	1.9%	

8. Comparison Studies:

a) Method Comparison (Accuracy)

The analytical accuracy of PGDx elio tissue complete as a tumor profiling device was evaluated using 582 clinical FFPE samples, obtained from patients with a variety of tumor types (n=35). Due to the rarity of specific genetic variants in solid tumor FFPE samples, most samples selected for this study were pre-screened, resulting in an enrichment of certain variants relative to real-world clinical prevalence. Data was aggregated at the variant level for SNVs and indels, gene level for amplifications and translocations, and case level for MSI and TMB. Out of 521 FFPE tumor specimens, 455 had both predicate and PGDx elio tissue complete results, over 35 tumor types, with 763 unique true positive variants observed in 578 exons over 272 genes. Among those variants, there were 620 SNVs, 44 insertions and 99 deletions.

Accuracy is summarized for the entire cohort of 582 samples for each of the assessed variants types (SNVs, indels, MSI, TMB, and structural variants). The Orthogonal

Method consisted of validated NGS and PCR methods. For the translocations, amplification, and sequence mutation results, PPV and NPV were first calculated for each variant for which PGDx elio results were known before the orthogonal results. Additionally, PPA/NPA were calculated by adjusting for the proportion of variant positive samples by test device. (Table 65)

Table 65. Accuracy of PGDx elio tissue complete

Variant	Orthogonal Method	Performance (n/N) (2-sided 95% CI)
SNVs with Evidence of Clinical Significance	2 NGS targeted panels	PPA – 97.2% (35/36) (85.8%, 99.5%)
		NPA – 99.9% (3994/3996) (99.8%, 99.9%)
SNVs with Potential Clinical Significance	2 NGS targeted panels	PPA – 86.4% (591/684) (83.6%, 88.8%)
		NPA – 99.9% (179614528/179614696) (99.9%, 99.9%)
Hotspot SNVs	2 NGS targeted panels and PCR	PPA – 97.1% (132/136) (92.7%, 98.9%)
		NPA – 99.9% (35845/35850) (99.9%, 99.9%)
Non-hotspot SNVs	2 NGS targeted panels	PPA – 85.1% (516/606) (82.1%, 87.8%)
		NPA – 99.9% (178513452/178513618) (99.9%, 99.9%)
Hotspot indels	2 NGS targeted panels and PCR	PPA – 100% (21/21) (84.5%, 100%)
		NPA – 99.9% (4115/4118) (99.8%, 99.9%)
Non-hotspot indels	NGS targeted panel	PPA – 81.4% (79/97) (72.6%, 87.9%)
		NPA – 99.9% (67104842/67104857) (99.9%, 99.9%)
Insertions with Potential Clinical Significance	NGS targeted panel	PPA – 80.8% (21/26) (62.1%, 91.5%)
		NPA – 99.9% (67497962/67497964) (99.9%, 99.9%)
Deletions with Potential Clinical Significance	NGS targeted panel	PPA – 82.7% (62/75) (72.6%, 89.6%) NPA – 99.9% (67497902/67497915) (99.9%, 99.9%)

Variant	Orthogonal Method	Performance (n/N) (2-sided 95% CI)
MSI (18 tumor types)[1]	PCR	PPA – 98.8% (79/80) (93.3%, 99.8%)
		NPA – 99.3% (142/143) (96.1%, 99.9%)
MSI (CRC/endometrial)	PCR	PPA – 100.0% (51/51) (93.0%, 100.0%)
		NPA – 100.0% (33/33) (89.6%, 100.0%)
		PPA – 96.6% (28/29) (82.8%, 99.4%)

Variant	Orthogonal Method	Performance (n/N) (2-sided 95% CI)
MSI (Non-CRC/non-endometrial)	PCR	NPA – 99.1% (109/110) (95.0%, 99.8%)
ERBB2 amplifications (All Cases)	FISH	PPA – 75.0% (42/56) (62.3%, 84.5%)
		NPA – 96.7% (88/91) (90.8%, 98.9%)
ERBB2 amplifications (Excluding Borderlines)[4]	FISH	PPA – 87.0% (40/46) (74.3%, 93.9%)
		NPA – 95.9% (71/74) (88.7%, 98.6%)
ALK translocations	FISH	PPA – 92.9% (13/14) (68.5%, 98.7%)
		NPA – 98.2% (56/57) (90.7%, 99.7%)
NTRK2 translocations	NGS targeted panel	PPA – 1 (1/1) (20.7%, 100%)
		NPA – 100% (69/69) (94.7%, 100%)
NTRK3 translocations	NGS translocation panel	PPA - 66% (2/3) (0.0%, 79.3%)
		NPA – 100% (12/12) (75.8%, 100%)
RET translocations	FISH	PPA – 55.6% (5/9) (26.7%, 81.1%)[5]
		NPA – 100% (18/18) (82.4%, 100%)

[1] MSI accuracy was assessed in 18 tumor types: ampulla (1), bladder (7), breast (21), colorectal (66), endometrial (18), esophagus (1), fallopian tube (1), gall bladder (1), gastric (40), lung (39), kidney (3), omentum (1), ovarian (2), prostate (4), sarcoma (3), skin (8), thyroid (2), and cancer of unknown primary (5).

[2] An EGFR T790M mutation reported by PGDx elio tissue complete was not confirmed by PCR. A third orthogonal method (ddPCR) confirmed the presence of this EGFR T790M mutation at 2.1% MAF.

[4] Borderlines are defined by a HER2/CEP17 ratio between 1.5 and 2.5 by FISH.

[5] No read data supporting RET translocations was found in the raw data for the discrepant cases. In addition to the clinical samples processed to assess RET translocations, 3 RET translocation-positive cell lines were also tested with PGDx elio tissue complete. All 3 cell lines were positive for a fusion either by a validated assay performed by the cell line provider, or via literature. PGDx elio tissue complete detected all 3 fusions in these cell lines.

i. Accuracy – SNVs

The PGDx elio tissue complete accuracy study included 923 unique SNVs from 307 genes with 84 unique insertions from 60 genes with 159 unique deletions from 101 genes. Performance was stratified by mutation type and gene for positive percent agreement (PPA) and negative percent agreement (NPA) with 95% confidence interval (CI). Results are shown fin tables below. Orthogonal data was derived from 2 competitor NGS panels from which the complete sequencing ROI was not available. Additionally, differences in sensitivity were not accounted for in this data set. Differences not due to low allelic fraction were limited to variants of unknown significance and are expected based on differences in filtering employed by PGDx elio tissue complete and comparator methods. Therefore, agreement may be underrepresented.

Following tables show the results of accuracy study by gene: the percent positive agreement for SNVs by gene, and the percent positive agreement for insertions and deletions by gene. Since the orthogonal data was tested prior to knowing the PGDx elio tissue complete results, the PPA could be calculated. The complete listing of Data in in **Appendix E (1-3).**

ii. Accuracy –Concordance to FISH for ERBB2 amplification:

In total 147 different tumor tissues representing 20 different tumors types of which 70 breast and 29 gastric cancer cases) were analyzed for concordance between FISH status and PGDx elio tissue complete ERBB2 status. The ability of the assay to detect ERBB2 amplifications was assessed in 2 studies: the first study contained the expected prevalence of FISH borderline samples and the second study enriched the population for ERBB2 borderline cases. The PPA and NPA values for ERBB2 amplification reflect the totals across both studies and is an over representation of borderline cases. The data shows high concordance in the non-borderline cases (excluding all cases of FISH ratio 1.5-2.5), with PPA of 87%, and NPA, PPV and NPV at or above 92% (Table 66).

The PGDx elio tissue complete reported the majority of borderline positive fish tests as negative. The PGDx elio tissue complete threshold for reporting an ERBB2 positive result is 2.5-fold change. Consequently, the data presented in this table show that the 2.0 to 2.5 positive borderline range had reduced agreement. This 2.5-fold threshold was established during feasibility to ensure 100% specificity (no false positives). Therefore, cases that fall into the FISH positive borderline range with FISH ratios of 2.0-2.5 are generally not reported by the PGDx elio tissue complete assay due to the assay's 2.5-fold change threshold for reporting an amplified ERBB2 status. Specifics of the borderline performance are shown in Table 66. The estimate of frequency of ERBB2 amplification with an ERBB2/Chromosome 17 FISH ratio of 2-2.5 is estimated to be 2.2%. Although the test is not authorized for companion diagnostic testing and a statement in the instructions for use cautions that testing with an FDA-approved companion diagnostic should be performed to assess patients for therapy selection.

Table 66. ERBB2 Amplification Concordance compared to PathVysion Her-2 Probe Kit

Category	Total Cases	TP	FP	FN	TN	PPA (95% CI)	NPA (95% CI)	PPV (95% CI)	NPV (95% CI)
All Cases*	147	42	3	14	88	75.0% (62.3%, 84.5%)	96.7% (90.8%, 98.9%)	93.3% (82.1%, 97.7%)	86.3% (78.3%, 91.6%)
Excluding FISH 1.5-3.0, IHC 2+	97	38	2	1	56	97.4% (86.8%, 99.5%)	96.6% (88.3%, 99.0%)	95.0% (83.5%, 98.6%)	98.2% (90.7%, 99.7%)
Excluding FISH 1.5-2.5	120	40	3	6	71	87.0% (74.3%, 93.9%)	95.9% (88.7%, 98.6%)	93.0% (81.4%, 97.6%)	92.2% (84.0%, 96.4%)
Only FISH 1.5-2.5	27	2	0	8	17	20.0% (5.7%, 51.0%)	100.0% (81.6%, 100.0%)	100.0% (34.2%, 100.0%)	68.0% (48.4%, 82.8%)

* Breast, gastric, colorectal, lung, skin, endometrial, esophagus, renal, bladder, fallopian tube, gallbladder, ovarian, peritoneal, and sarcoma

[1]FISH borderline is defined as a FISH ERBB2/Chr17 ratio of 1.5-2.5.

Table 67. Summary of Concordance between PGDx elio tissue complete and ERBB2 FISH Including Borderline Cases

	ERBB2 FISH		
PGDx elio tissue complete	ERBB2 Positive	ERBB2 Negative	Total
ERBB2 Positive	42	3	45
ERBB2 Negative	14	88	102
Total	56	91	147

iii. Accuracy – Concordance to FISH for RET:

RET translocations were compared to a commercial RET Break Apart FISH probe Kit. Following table presents results for RET translocation concordance without regard to sample selection from either orthogonal method or test device for RET translocations. The calculations are not adjusted for the specimens that were first determined to be positive by the FISH assay. Seven (7) of the specimens deemed positive by the PGDx were 100% positive with the orthogonal comparator. For the twenty (20) specimens for which the orthogonal information was known for these samples prior to running them through the PGDx elio tissue complete assay, the PGDx had four false negatives (Table 68).

Table 68. RET translocation concordance

Orthogonal Method	Total Cases	Total Variants	True Positive	False Positives	False Negatives	True Negatives	PPA (95% CI)	NPA (95% CI)
RET Break Apart FISH Probe Kit	27	27	5	0	4	18	55.6% (26.7%, 81.1%)	100% (82.41%, 100%)

iv. Accuracy – Concordance to FISH for ALK Translocation:

The ability of the PGDx elio tissue complete assay to detect ALK was evaluated using 71 specimens (14 FISH positive and 57 FISH negative). The results demonstrated high concordance to FISH (Tables 60 and 70).

ALK borderline performance in FFPE specimens as demonstrated from Limit of Detection:

The lowest % nuclei scored specimen was 50% and lacked specimens at the borderline equivocal zone (15%). ALK FISH borderline samples at the cut-off of 15% cells positive are rare, with expected prevalence of 1% or less. Since PGDx was unable to obtain clinical FFPE samples in the ALK FISH equivocal zone, consideration was given for performance with ALK borderline using two two sets of data: assessment of performance with low tumor proportions (refer to data in Analytical sensitivity section) and a supplemental in silico study was performed that assessed analytical accuracy for ALK at decreased positive FISH signal. This study used the sequencing data from ALK positive clinical FFPE specimens that were diluted bioinformatically in order to assess the

expected positive call rate at varying FISH levels. ALK translocations were assessed in silico due to limited availability of clinical cases close to the ALK FISH equivocal zone (10%-50% rearrangement positive nuclei). A total of 410 observations were generated for ALK by downsampling 10 clinical samples from analytical accuracy to 4 tumor purity dilution levels with 10 replicates per level, to mimic samples in the FISH equivocal zone. For example, if the undiluted sample had a FISH score of 50% from analytical accuracy, the sample was diluted with wild type reads by a factor of 0.8 to get to a 40% positive nuclei FISH score. These data demonstrate an LoD of 30% positive nuclei by FISH, when identifying the lowest level with a ≥95% positive call rate (Table 71). The FISH level, average positive call rate, in addition to the range of call rates across all cases per level, and the range of fusion reads across all replicates per level is provided Table 91 below. These data demonstrate an 88% positive call rate at 20% positive nuclei by FISH

Table 69. Summary of Concordance between PGDx elio tissue complete and ALK FISH

	ALK FISH		
PGDx elio tissue complete	ALK Positive	ALK Negative	Total
ALK Positive	13	1	14
ALK Negative	1	56	57
Total	14	57	71

Table 70. Concordance for ALK Translocations

Orthogonal Method	Total Cases	Total Variant Observations	TP	FP	FN	TN	PPA (%), 95% CI (%)	NPA (%), 95% CI (%)
ALK Break Apart FISH Probe Kit	71	71	13	1	1	56	92.9% (68.5%, 98.7%)	98.2% (90.7%, 99.7%)

Table 71. In silico Analytical Sensitivity for ALK Translocations

FISH (%Positive Nuclei)	**FISH Call**	**PGDx elio tissue complete Positive Call Rate (%) (n/N) (95% CI)**
50 - 88	+	100% (10/10) (72%, 100%)
40	+	98% (98/100) (93%, 99%)
30	+	95% (95/100) (89%, 98%)
20	+	88% (88/100) (80%, 93%)
10	-	80% (80/100) (71%, 87%)

v. Method Comparison Study for Wild-Type Calls

A study was conducted to assess accuracy for 75 selected hotspots within 20 genes. The selected hotspot variants included mutations that were coding hotspot mutations (missense or frameshift). A total of 112 specimens were tested, and the accuracy of PGDx elio tissue complete results at all 75 positions was compared to results obtained with two orthogonal methods (42 samples using 1 method, and 70 using a second method). Within the 112 specimens, there were 112 mutations across samples and 8,283 wild-type calls. Overall variant-level concordance (PPA and NPA) was 96.4% and 99.9% respectively with two-sided 95% confidence intervals of (91.1%, 99.0%) for mutations (PPA), and (99.9%, 99.9%) for wild-type locations (NPA). Table 72 shows summarized results for these hotspot variants.

Table 72. Summarized results for hotspot variants

Orthogonal Method	Total Cases	True Positives	False Positives	False Negatives	True Negatives	PPA (%), 95% CI (%)	NPA (%), 95% CI (%)	OPA (%), 95% CI (%)
Comparator 1	42	31	2	2	3115	93.9% (79.8%, 99.3%)	99.93% (99.76%, 99.99%)	99.9% (99.7%, 99.99%)
Comparator 2	70	77	3	2	5168	97.5% (91.2%, 99.7%)	99.94% (99.83%, 99.98%)	99.9% (99.77%, 99.96%)
Aggregated	112	108	5	4	8283	96.4% (91.1%, 99.0%)	99.93% (99.85%, 99.98%)	99.9% (99.8%, 99.99%)

vi. TMB Accuracy

The PGDx elio tissue complete assay reports a TMB score comprised of sequence mutations detected across the entire coding region of interest per sample. The ability of PGDx elio tissue complete to accurately identify TMB in multiple solid tissue FFPE tumor types was assessed by comparing to matched tumor-normal whole exome sequencing results. Across 8 tumor types (non-small cell lung carcinoma (NSCLC)), melanoma, renal, bladder, endometrial, triple negative breast, head and neck, lung-NOS (not otherwise specified), 118 cases were enrolled covering a dynamic range of 1.5-118.5 Muts/Mb. The Spearman correlation coefficient was used to determine the relationship between the 2 assays. Assessment of all 118 cases resulted in a Spearman correlation coefficient of 0.903. The results in Figure 8 show concordance between PGDx elio tissue complete TMB scores and tumor-normal whole exome sequencing.

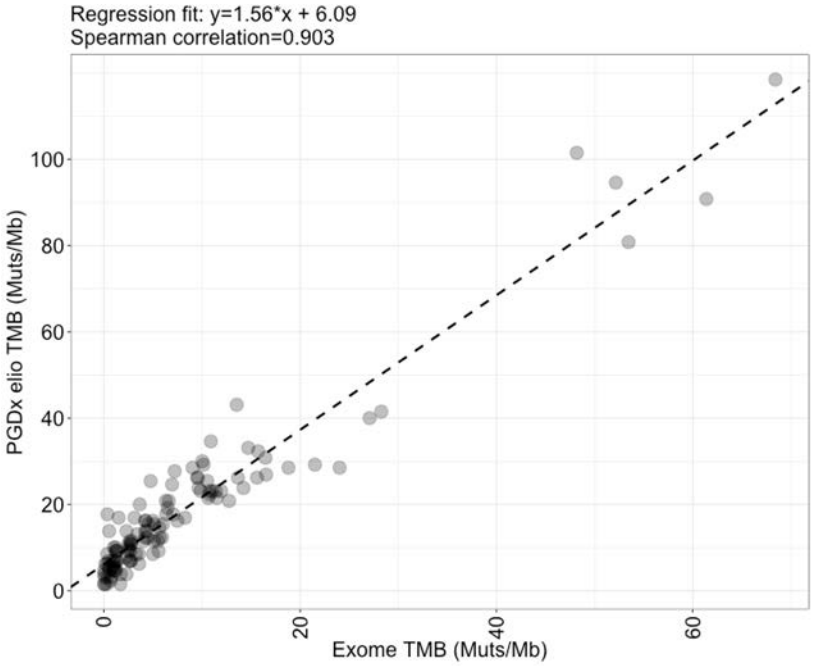

Figure 8. PGDx elio tissue complete TMB score vs. Matched Tumor-Normal Exome Sequencing.

Below is the calculated PPA for sequence mutations, broken down by insertions vs. deletions as well as by indel length. Differences in calls were detected at a higher proportion amongst deletions, however, the overall proportion of deletions to all mutations in the mutation load is relatively minor compared to the contribution of the SNVs in the mutation load.

vii. Accuracy – MSI

The accuracy of PGDX elio tissue test calling of MSI status in tumor tissue was evaluated in a method comparison study against a validated PCR based MSI test. This study evaluated 115 cases of which 66 cases were of colorectal cancer (CRC), 18 of endometrial cancer (EC) and 21 non-CRC/EC cases. An additional 168 non-CRC/non-EC cases were tested in a supplemental study to ascertain accuracy of MSI calls in range of tumor types. All samples were randomized, and study participants blinded to MSI status from orthogonal test results for these cases. Of the 283 samples tested 54 samples failed to produce results with the PGDx elio Tissue test of which 10 also failed to produce a result with the PCR test. Of specimens that produced a result with both PGDx elio Tissue and MIS PCR test, the PPA is 98.8% (79/80) with 95% CI 93.3%-99.8% for MSI-H status and NPA 99.3% (142/143) with 95% CI 96.1%-99.9% for MSS status. When including specimens with failed and indeterminate test results the PPA is 94.0% (79/84) with 95% CI 86.8%-97.4% for MSI-H status and NPA is 77.6% (142/183) with 95% CI 71.0%-83.0% for MSS status. Study Results are summarized below in Table 73.

Table 73. PGDx elio Tissue MSI Performance for All Cases

		MSI PCR				Total
		MSI	MSS	Failed	Indeterminate	
PGDx elio tissue complete	MSI	79	1	0	1[1]	81
	MSS	1	142	0	5[2]	148
	Failed	4	40	10	0	54
Total		84	183	10	6	283
Excluding failed/ indeterminate specimens with 95% CI	PPA	98.8% (79/80) (93.3%, 99.8%)				
	NPA	99.3% (142/143) (96.1%, 99.9%)				
	PPV	98.8% (79/80) (93.3%, 99.8%)				
	NPV	99.3% (142/143) (96.1%, 99.9%)				
Accounting for failed/ indeterminate specimens with 95% CI	PPA	94.0% (79/84) (86.8%, 97.4%)				
	NPA	77.6% (142/183) (71.0%, 83.0%)				
	PPV	97.5% (79/81) (91.4%, 99.3%)				
	NPV	95.9% (142/148) (91.4%, 98.1%)				

[1]This case was MSI-H by PGDx elio, and a commercial PCR assay gave an "Indeterminate" result.
[2]These 5 cases did not have matching normal DNA to test via commercial PCR.

The accuracy of PGDx elio Tissue test evaluable samples by tumor types with high MSI prevalence (CRC, EC and Gastric cancer) and aggregate of other tumors types is summarized in Tables 74, 75 and 76 below. PPV/ NPV values in the following tables do not account for prevalence, they are purely technical / analytically calculated values to account for concordance conditional on the PGDx elio result or conditional on the comparator).

Table 74. Concordance for MSI Status by Tumor Type

Tumor Type	PPA (n/N) (95% CI)	NPA (n/N) (95% CI)
CRC	100% (35/35) (90.1%, 100.0%)	100% (31/31) (88.97%, 100.0%)
Endometrial	100% (16/16) (80.63%, 100.0%)	100% (2/2) (34.23%, 100.0%)
Gastric	100% (16/16) (80.63%, 100.0%)	100% (24/24) (86.2%, 100.0%)
Other[1]	92.3% (12/13) (66.7%, 98.6%)	98.8% (85/86) (93.7%, 99.8%)

[1]Other tumor types include 15 tumor types: ampulla (1), bladder (7), breast (21), esophagus (1), fallopian tube (1), gall bladder (1), lung (39), kidney (3), omentum (1), ovarian (2), prostate (4), sarcoma (3), skin (8), thyroid (2), and carcinoma of unknown primary (5). A thyroid cancer case was the lone discrepant case in this cohort.

Table 75. MSI Performance for CRC and Endometrial Cases

		MSI PCR				Total
		MSI	MSS	Failed	Indeterminate	
PGDx elio tissue complete	MSI	51	0	0	0	51
	MSS	0	33	0	0	33
	Failed	0	0	0	0	0
Total		51	33	0	0	84
	PPA	100% (51/51) (93.0%, 100%)				
	NPA	100% (33/33) (89.6%, 100%				
	PPV	100% (51/51) (93.0%, 100%)				
	NPV	100% (33/33) (89.6%, 100%				

Table 76. MSI Performance for Non-CRC and Non-Endometrial Cases

		MSI PCR				Total
		MSI	MSS	Failed	Indeterminate	
PGDx elio tissue complete	MSI	28	1	0	1[1]	30
	MSS	1	109	0	5[2]	115
	Failed	4	40	10	0	54
Total		33	150	10	6	199
Excluding failed/ indeterminate specimens with 95% CI	PPA	96.6% (28/29) (82.8%, 99.4%)				
	NPA	99.1% (109/110) (95.0%, 99.8%)				
	PPV	96.6% (28/29) (82.8%, 99.4%)				
	NPV	99.1% (109/110) (95.0%, 99.8%)				
Accounting for failed/ indeterminate specimens with 95% CI	PPA	84.8% (28/33) (69.1%, 93.4%)				
	NPA	72.7% (109/150) (65.0%, 79.2%)				
	PPV	93.3% (28/30) (78.7%, 98.2%)				
	NPV	94.8% (109/115) (89.1%, 97.6%)				

viii. ***Accuracy - Other:*** The effect of GC content and exon length were explored on coverage for the assay. It was determined that ff the 7026 total exons in PGDx elio tissue complete, there are 59 challenging exons demonstrating ≤100x coverage in 30% of samples or greater from Analytical Accuracy. The exons that tend to be shorter in length such that short exons with low GC content are the most vulnerable to reduced coverage. Overall the the Pass rate for specimens was assessed for the PGDx elio tissue complete and determined to be similar to the pass rate observed

with real world evidence indicating that the accuracy of the PGDx elio tissue complete is representative performance across tumor types with coverage that exceeded the quality metrics established for the assay. Data is shown in Table 77.

Table 77. Overall Pass/Invalid Rate for the Specimens from the Accuracy Study

Tumor Type	Passing Samples	Total Samples	Invalid Rate (%)
Bladder	6	7	14.3%
Brain	10	10	0%
Breast	60	72	16.7%
Colorectal	91	97	6.2%
Endometrial	27	27	0%
Gastric	25	31	19.4%
Glioma	4	4	0%
Head and Neck	5	6	16.7%
Lung – NOS[1]	64	68	5.9%
Melanoma	34	36	5.6%
NOS[1]	8	8	0%
NSCLC[1]	85	92	7.6%
Other[2]	21	22	4.5%
Ovarian	8	9	11.1%
Pediatric Glioma	9	9	0%
Prostate	7	8	12.5%
Skin	4	4	0%
Triple Negative Breast	11	11	0%
TOTAL	479	521	8.1%

[1]NOS: not otherwise specified; NSCLC: non-small cell lung cancer.
[2]Other ($n \leq 3$ cases per tumor type): cervical, cholangiocarcinoma, gallbladder, pancreatic, rhabdomyosarcoma, trachea, esophageal, fallopian tube, liver, mediastinum, peritoneal, renal, and thyroid.

N. Instrument Name:

Illumina NextSeq® 550Dx (qualified by PGDx)

O. System Descriptions:

1. Modes of Operation:

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

   Yes ____X____ or No _____

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

Yes _____ or No ____X____

2. Software:

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

Yes ____X____ or No _____

5. Calibration & Quality Controls:

PGDx uses DiversiPhi to qualify the instrument and monitor instrument performance. The instrument and assay employ both in-process QC Checks and physical controls. See description in traceability section for calibrator value assignments.

PGDx elio tissue complete requires 90% of exons with sequence coverage >100x for reporting of sequence alterations in a sample, otherwise the sample is marked failed. The device uses automated QC metrics that align to the following categories:

1) "Batch-level" = metrics quantified per sequencing run; failing batch-level metrics generates "No result" reports samples failing these criteria. If the external control fails these criteria, "No result" is reported for the entire batch of samples.
2) "Sample-level" = metrics quantified per sample; generates "No result" report for that sample failing QC.
3) "Analyte-level" = metrics quantified on an analyte (variant) call-level, whereby multiple different passing statuses can exist within the same sample. All samples receive reports for all variants passing analyte-level QC.

When a report is generated, all metrics are assessed dynamically on a per-sample basis.

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

Not applicable

Q. Proposed Labeling:

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

R. Conclusion:

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

S. Other Supportive Instrument Performance Characteristics Data:

Instrument concordance: A study was performed to demonstrate agreement between NExtSeq 550 Dx instruments and NextSeq 550 Dx reagents and instruments and reagents qualified by PGDx. PPA and NPA were assessed for each variant out put (SNVs, indels, translocations, amplifications, MSI and TMB). The study demonstrated no discordance attributable to instrument or reagent.

diversiPhi validation: Studies were conducted to validate the analytical performance of the PGDx elio tissue complete using PGDx elio diversiPhi to fill partial sequencing run batches. Testing was conducted by 1 operator on a total of 4 different NextSeq instruments, using 1 lot of PGDx elio tissue complete kit and 1 lot of PGDx elio diversiPhi. Two unique blends were assessed across 3 prospective batches containing diversiPhi to reprent different lane ratios sequenced: 6.25% (14 samples + 1 external control), 50% (7 samples plus one external control) and 87.5% (1 sample + external control). These runs were then compared back to the reference data whichincluded 0.0% diversiPhi (15 samples + 1 EC). The data demonstrated that diversiPhi could be used to fill partial sequencing batches based on observation that the overall concordance consistent with the analytical validation data.

Database: As a distributed kit, the software includes database information regarding the variants and their assignment to either Variants with Evidence of Clinical Significance or Variants with Potential Clinical Significance. A description of the assignment and curation process was provided. Report Generation in PGDx Elio Tissue Complete:
The PGDx elio tissue complete software generates reports for each sequencing run and sample processed. The software does not include annotation regarding the individual variants. The report includes tumor type, allele frequency, and functional information.

There are three reported outputs, 1) Case Report, 2) Complete Case Record (CCR), and 3) Complete Run Record (CRR).

The Case Report is the primary report of identified alterations for a sample. The Case Report divides variants into 2 sections: ‘Variants with Evidence of Clinical Significance’ on page 1 only and ‘Variants with Potential Clinical Significance’ on subsequent pages. The variants listed in the section ‘Variants with Evidence of Clinical Significance’ are determined based on the selected tumor type. Only variants clinically associated with the selected tumor type will appear on page 1 of the Case Report in the ‘Variants with Evidence of Clinical Significance’ section. Any remaining variants will appear in the ‘Variants with Potential Clinical Significance’ section starting on the second page. A qualified healthcare professional
selects the appropriate tumor type to ensure the corresponding variants of clinical significance appear on page 1. Any variants clinically associated with tumor types other than the one selected will be reported in the section labeled 'Variants with Potential Clinical Significance' and will appear on page 2 and subsequent pages.

VIII. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable, and the special controls for this device type.

IX. Patient Perspectives

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

X. Conclusion:

The submitted information in this 510(k) notification supports the Indications For Use for elioTissue Complete and demonstrates that the elio Tissue Complete assay is as safe and effective as the predicate device and therefore supports a substantial equivalence conclusion.

Appendix A: PGDx elio tissue complete Targeted regions of Interest

Gene Name	Chr	Ensembl Gene ID	HGNC ID	Gene Name	Chr	Ensembl Gene ID	HGNC ID
ABL1	chr9	ENSG00000097007	76	ABL2	chr1	ENSG00000143322	77
ACVR1	chr2	ENSG00000115170	171	ACVR1B	chr12	ENSG00000135503	172
ADORA2A	chr22	ENSG00000128271	263	AKT1	chr14	ENSG00000142208	391
AKT2	chr19	ENSG00000105221	392	AKT3	chr1	ENSG00000117020	393
ALK	chr2	ENSG00000171094	427	ALOX12B	chr17	ENSG00000179477	430
AMER1	chrX	ENSG00000184675	26837	APC	chr5	ENSG00000134982	583
AR	chrX	ENSG00000169083	644	ARAF	chrX	ENSG00000078061	646
ARFRP1	chr20	ENSG00000101246	662	ARID1A	chr1	ENSG00000117713	11110
ARID1B	chr6	ENSG00000049618	18040	ARID2	chr12	ENSG00000189079	18037
ARID5B	chr10	ENSG00000150347	17362	ASXL1	chr20	ENSG00000171456	18318
ASXL2	chr2	ENSG00000143970	23805	ATM	chr11	ENSG00000149311	795
ATR	chr3	ENSG00000175054	882	ATRX	chrX	ENSG00000085224	886
AURKA	chr20	ENSG00000087586	11393	AURKB	chr17	ENSG00000178999	11390
AXIN1	chr16	ENSG00000103126	903	AXIN2	chr17	ENSG00000168646	904
AXL	chr19	ENSG00000167601	905	B2M	chr15	ENSG00000166710	914
BAP1	chr3	ENSG00000163930	950	BARD1	chr2	ENSG00000138376	952
BBC3	chr19	ENSG00000105327	17868	BCL2	chr18	ENSG00000171791	990
BCL2L1	chr20	ENSG00000171552	992	BCL2L11	chr2	ENSG00000153094	994
BCL2L2	chr14	ENSG00000129473	995	BCL6	chr3	ENSG00000113916	1001
BCOR	chrX	ENSG00000183337	20893	BCORL1	chrX	ENSG00000085185	25657
BCR	chr22	ENSG00000186716	1014	BIRC2	chr11	ENSG00000110330	590
BLM	chr15	ENSG00000197299	1058	BMPR1A	chr10	ENSG00000107779	1076
BRAF	chr7	ENSG00000157764	1097	BRCA1	chr17	ENSG00000012048	1100
BRCA2	chr13	ENSG00000139618	1101	BRD4	chr19	ENSG00000141867	13575
BRIP1	chr17	ENSG00000136492	20473	BTG1	chr12	ENSG00000133639	1130
BTG2	chr1	ENSG00000159388	1131	BTK	chrX	ENSG00000010671	1133
BUB1B	chr15	ENSG00000156970	1149	C11orf30	chr11	ENSG00000158636	18071
CALR	chr19	ENSG00000179218	1455	CARD11	chr7	ENSG00000198286	16393
CASP8	chr2	ENSG00000064012	1509	CBFB	chr16	ENSG00000067955	1539
CBL	chr11	ENSG00000110395	1541	CCND1	chr11	ENSG00000110092	1582
CCND2	chr12	ENSG00000118971	1583	CCND3	chr6	ENSG00000112576	1585
CCNE1	chr19	ENSG00000105173	1589	CD22	chr19	ENSG00000012124	1643
CD274	chr9	ENSG00000120217	17635	CD276	chr15	ENSG00000103855	19137
CD70	chr19	ENSG00000125726	11937	CD79A	chr19	ENSG00000105369	1698
CD79B	chr17	ENSG00000007312	1699	CDC73	chr1	ENSG00000134371	16783
CDH1	chr16	ENSG00000039068	1748	CDK12	chr17	ENSG00000167258	24224
CDK4	chr12	ENSG00000135446	1773	CDK6	chr7	ENSG00000105810	1777
CDK8	chr13	ENSG00000132964	1779	CDKN1A	chr6	ENSG00000124762	1784
CDKN1B	chr12	ENSG00000111276	1785	CDKN1C	chr11	ENSG00000129757	1786
CDKN2A	chr9	ENSG00000147889	1787	CDKN2B	chr9	ENSG00000147883	1788
CDKN2C	chr1	ENSG00000123080	1789	CEBPA	chr19	ENSG00000230259	1833

Gene Name	Chr	Ensembl Gene ID	HGNC ID	Gene Name	Chr	Ensembl Gene ID	HGNC ID
CHD2	chr15	ENSG00000173575	1917	CHD4	chr12	ENSG00000111642	1919
CHEK1	chr11	ENSG00000149554	1925	CHEK2	chr22	ENSG00000183765	16627
CIC	chr19	ENSG00000079432	14214	CREBBP	chr16	ENSG00000005339	2348
CRKL	chr22	ENSG00000099942	2363	CSF1	chr1	ENSG00000184371	2432
CSF1R	chr5	ENSG00000182578	2433	CSF2	chr5	ENSG00000164400	2434
CSF3	chr17	ENSG00000108342	2438	CSF3R	chr1	ENSG00000119535	2439
CTCF	chr16	ENSG00000102974	13723	CTLA4	chr2	ENSG00000163599	2505
CTNNA1	chr5	ENSG00000044115	2509	CTNNB1	chr3	ENSG00000168036	2514
CUL3	chr2	ENSG00000036257	2553	CUL4A	chr13	ENSG00000139842	2554
CXCR2	chr2	ENSG00000180871	6027	CXCR4	chr2	ENSG00000121966	2561
CYLD	chr16	ENSG00000083799	2584	CYP17A1	chr10	ENSG00000148795	2593
DAXX	chr6	ENSG00000204209	2681	DCUN1D1	chr3	ENSG00000043093	18184
DDB2	chr11	ENSG00000134574	2718	DDR1	chr6	ENSG00000204580	2730
DDR2	chr1	ENSG00000162733	2731	DICER1	chr14	ENSG00000100697	17098
DIS3	chr13	ENSG00000083520	20604	DNMT1	chr19	ENSG00000130816	2976
DNMT3A	chr2	ENSG00000119772	2978	DNMT3B	chr20	ENSG00000088305	2979
DOT1L	chr19	ENSG00000104885	24948	E2F3	chr6	ENSG00000112242	3115
EED	chr11	ENSG00000074266	3188	EGFL7	chr9	ENSG00000172889	20594
EGFR	chr7	ENSG00000146648	3236	EIF1AX	chrX	ENSG00000173674	3250
EP300	chr22	ENSG00000100393	3373	EPAS1	chr2	ENSG00000116016	3374
EPCAM	chr2	ENSG00000119888	11529	EPHA2	chr1	ENSG00000142627	3386
EPHA3	chr3	ENSG00000044524	3387	EPHA5	chr4	ENSG00000145242	3389
EPHA7	chr6	ENSG00000135333	3390	EPHB1	chr3	ENSG00000154928	3392
EPHB4	chr7	ENSG00000196411	3395	ERBB2	chr17	ENSG00000141736	3430
ERBB3	chr12	ENSG00000065361	3431	ERBB4	chr2	ENSG00000178568	3432
ERCC1	chr19	ENSG00000012061	3433	ERCC2	chr19	ENSG00000104884	3434
ERCC3	chr2	ENSG00000163161	3435	ERCC4	chr16	ENSG00000175595	3436
ERCC5	chr13	ENSG00000134899	3437	ERCC6	chr10	ENSG00000225830	3438
ERCC8	chr5	ENSG00000049167	3439	ERG	chr21	ENSG00000157554	3446
ERRFI1	chr1	ENSG00000116285	18185	ESR1	chr6	ENSG00000091831	3467
ETV1	chr7	ENSG00000006468	3490	ETV4	chr17	ENSG00000175832	3493
ETV5	chr3	ENSG00000244405	3494	ETV6	chr12	ENSG00000139083	3495
EWSR1	chr22	ENSG00000182944	3508	EXT1	chr8	ENSG00000182197	3512
EXT2	chr11	ENSG00000151348	3513	EZH2	chr7	ENSG00000106462	3527
FAM175A	chr4	ENSG00000163322	25829	FAM46C	chr1	ENSG00000183508	24712
FANCA	chr16	ENSG00000187741	3582	FANCB	chrX	ENSG00000181544	3583
FANCC	chr9	ENSG00000158169	3584	FANCD2	chr3	ENSG00000144554	3585
FANCE	chr6	ENSG00000112039	3586	FANCF	chr11	ENSG00000183161	3587
FANCG	chr9	ENSG00000221829	3588	FANCI	chr15	ENSG00000140525	25568
FANCL	chr2	ENSG00000115392	20748	FANCM	chr14	ENSG00000187790	23168
FAS	chr10	ENSG00000026103	11920	FAT1	chr4	ENSG00000083857	3595
FBXW7	chr4	ENSG00000109670	16712	FGF10	chr5	ENSG00000070193	3666

Gene Name	Chr	Ensembl Gene ID	HGNC ID	Gene Name	Chr	Ensembl Gene ID	HGNC ID
FGF12	chr3	ENSG00000114279	3668	FGF14	chr13	ENSG00000102466	3671
FGF19	chr11	ENSG00000162344	3675	FGF23	chr12	ENSG00000118972	3680
FGF3	chr11	ENSG00000186895	3681	FGF4	chr11	ENSG00000075388	3682
FGF6	chr12	ENSG00000111241	3684	FGFR1	chr8	ENSG00000077782	3688
FGFR2	chr10	ENSG00000066468	3689	FGFR3	chr4	ENSG00000068078	3690
FGFR4	chr5	ENSG00000160867	3691	FH	chr1	ENSG00000091483	3700
FLCN	chr17	ENSG00000154803	27310	FLT1	chr13	ENSG00000102755	3763
FLT3	chr13	ENSG00000122025	3765	FLT4	chr5	ENSG00000037280	3767
FOXA1	chr14	ENSG00000129514	5021	FOXL2	chr3	ENSG00000183770	1092
FOXP1	chr3	ENSG00000114861	3823	FRS2	chr12	ENSG00000166225	16971
FUBP1	chr1	ENSG00000162613	4004	GABRA6	chr5	ENSG00000145863	4080
GATA1	chrX	ENSG00000102145	4170	GATA2	chr3	ENSG00000179348	4171
GATA3	chr10	ENSG00000107485	4172	GATA4	chr8	ENSG00000136574	4173
GATA6	chr18	ENSG00000141448	4174	GID4	chr17	ENSG00000141034	28453
GLI1	chr12	ENSG00000111087	4317	GNA11	chr19	ENSG00000088256	4379
GNA13	chr17	ENSG00000120063	4381	GNAQ	chr9	ENSG00000156052	4390
GNAS	chr20	ENSG00000087460	4392	GPC3	chrX	ENSG00000147257	4451
GPR124	chr8	ENSG00000020181	17849	GREM1	chr15	ENSG00000166923	2001
GRIN2A	chr16	ENSG00000183454	4585	GRM3	chr7	ENSG00000198822	4595
GSK3B	chr3	ENSG00000082701	4617	H3F3A	chr1	ENSG00000163041	4764
H3F3B	chr17	ENSG00000132475	4765	H3F3C	chr12	ENSG00000188375	33164
HDAC1	chr1	ENSG00000116478	4852	HDAC2	chr6	ENSG00000196591	4853
HDAC6	chrX	ENSG00000094631	14064	HGF	chr7	ENSG00000019991	4893
HIST1H1C	chr6	ENSG00000187837	4716	HIST1H2BD	chr6	ENSG00000158373	4747
HIST1H3B	chr6	ENSG00000124693	4776	HNF1A	chr12	ENSG00000135100	11621
HRAS	chr11	ENSG00000174775	5173	HSD3B1	chr1	ENSG00000203857	5217
HSP90AA1	chr14	ENSG00000080824	5253	HSP90AB1	chr6	ENSG00000096384	5258
ICOSLG	chr21	ENSG00000160223	17087	ID3	chr1	ENSG00000117318	5362
IDH1	chr2	ENSG00000138413	5382	IDH2	chr15	ENSG00000182054	5383
IFNGR1	chr6	ENSG00000027697	5439	IGF1	chr12	ENSG00000017427	5464
IGF1R	chr15	ENSG00000140443	5465	IGF2	chr11	ENSG00000167244	5466
IGF2R	chr6	ENSG00000197081	5467	IKBKE	chr1	ENSG00000143466	14552
IKZF1	chr7	ENSG00000185811	13176	IL10	chr1	ENSG00000136634	5962
IL7R	chr5	ENSG00000168685	6024	INHBA	chr7	ENSG00000122641	6066
INPP4A	chr2	ENSG00000040933	6074	INPP4B	chr4	ENSG00000109452	6075
INSR	chr19	ENSG00000171105	6091	IRF2	chr4	ENSG00000168310	6117
IRF4	chr6	ENSG00000137265	6119	IRS1	chr2	ENSG00000169047	6125
IRS2	chr13	ENSG00000185950	6126	JAK1	chr1	ENSG00000162434	6190
JAK2	chr9	ENSG00000096968	6192	JAK3	chr19	ENSG00000105639	6193
JUN	chr1	ENSG00000177606	6204	KAT6A	chr8	ENSG00000083168	13013
KDM5A	chr12	ENSG00000073614	9886	KDM5C	chrX	ENSG00000126012	11114
KDM6A	chrX	ENSG00000147050	12637	KDR	chr4	ENSG00000128052	6307

Gene Name	Chr	Ensembl Gene ID	HGNC ID	Gene Name	Chr	Ensembl Gene ID	HGNC ID
KEAP1	chr19	ENSG00000079999	23177	KEL	chr7	ENSG00000197993	6308
KIT	chr4	ENSG00000157404	6342	KLF4	chr9	ENSG00000136826	6348
KLHL6	chr3	ENSG00000172578	18653	KMT2A	chr11	ENSG00000118058	7132
KMT2C	chr7	ENSG00000055609	13726	KMT2D	chr12	ENSG00000167548	7133
KRAS	chr12	ENSG00000133703	6407	LATS1	chr6	ENSG00000131023	6514
LATS2	chr13	ENSG00000150457	6515	LMO1	chr11	ENSG00000166407	6641
LRP1B	chr2	ENSG00000168702	6693	LTK	chr15	ENSG00000062524	6721
LYN	chr8	ENSG00000254087	6735	LZTR1	chr22	ENSG00000099949	6742
MAF	chr16	ENSG00000178573	6776	MAGI2	chr7	ENSG00000187391	18957
MAML1	chr5	ENSG00000161021	13632	MAP2K1	chr15	ENSG00000169032	6840
MAP2K2	chr19	ENSG00000126934	6842	MAP2K4	chr17	ENSG00000065559	6844
MAP3K1	chr5	ENSG00000095015	6848	MAP3K13	chr3	ENSG00000073803	6852
MAPK1	chr22	ENSG00000100030	6871	MAX	chr14	ENSG00000125952	6913
MCL1	chr1	ENSG00000143384	6943	MDC1	chr6	ENSG00000137337	21163
MDM2	chr12	ENSG00000135679	6973	MDM4	chr1	ENSG00000198625	6974
MED12	chrX	ENSG00000184634	11957	MEF2B	chr19	ENSG00000213999	6995
MEN1	chr11	ENSG00000133895	7010	MERTK	chr2	ENSG00000153208	7027
MET	chr7	ENSG00000105976	7029	MITF	chr3	ENSG00000187098	7105
MKNK1	chr1	ENSG00000079277	7110	MLH1	chr3	ENSG00000076242	7127
MLH3	chr14	ENSG00000119684	7128	MPL	chr1	ENSG00000117400	7217
MRE11A	chr11	ENSG00000020922	7230	MSH2	chr2	ENSG00000095002	7325
MSH3	chr5	ENSG00000113318	7326	MSH6	chr2	ENSG00000116062	7329
MST1R	chr3	ENSG00000164078	7381	MTAP	chr9	ENSG00000099810	7413
MTOR	chr1	ENSG00000198793	3942	MUTYH	chr1	ENSG00000132781	7527
MYB	chr6	ENSG00000118513	7545	MYC	chr8	ENSG00000136997	7553
MYCL	chr1	ENSG00000116990	7555	MYCN	chr2	ENSG00000134323	7559
MYD88	chr3	ENSG00000172936	7562	MYOD1	chr11	ENSG00000129152	7611
NBN	chr8	ENSG00000104320	7652	NCOA3	chr20	ENSG00000124151	7670
NCOR1	chr17	ENSG00000141027	7672	NF1	chr17	ENSG00000196712	7765
NF2	chr22	ENSG00000186575	7773	NFE2L2	chr2	ENSG00000116044	7782
NFKBIA	chr14	ENSG00000100906	7797	NKX2-1	chr14	ENSG00000136352	11825
NKX3-1	chr8	ENSG00000167034	7838	NOTCH1	chr9	ENSG00000148400	7881
NOTCH2	chr1	ENSG00000134250	7882	NOTCH3	chr19	ENSG00000074181	7883
NOTCH4	chr6	ENSG00000204301	7884	NPM1	chr5	ENSG00000181163	7910
NRAS	chr1	ENSG00000213281	7989	NSD1	chr5	ENSG00000165671	14234
NT5C2	chr10	ENSG00000076685	8022	NTRK1	chr1	ENSG00000198400	8031
NTRK2	chr9	ENSG00000148053	8032	NTRK3	chr15	ENSG00000140538	8033
NUP93	chr16	ENSG00000102900	28958	NUTM1	chr15	ENSG00000184507	29919
PAK1	chr11	ENSG00000149269	8590	PAK3	chrX	ENSG00000077264	8592
PAK7	chr20	ENSG00000101349	15916	PALB2	chr16	ENSG00000083093	26144
PARK2	chr6	ENSG00000185345	8607	PARP1	chr1	ENSG00000143799	270
PARP2	chr14	ENSG00000129484	272	PARP3	chr3	ENSG00000041880	273

Gene Name	Chr	Ensembl Gene ID	HGNC ID	Gene Name	Chr	Ensembl Gene ID	HGNC ID
PAX5	chr9	ENSG00000196092	8619	PAX8	chr2	ENSG00000125618	8622
PBRM1	chr3	ENSG00000163939	30064	PDCD1	chr2	ENSG00000188389	8760
PDCD1LG2	chr9	ENSG00000197646	18731	PDGFRA	chr4	ENSG00000134853	8803
PDGFRB	chr5	ENSG00000113721	8804	PDK1	chr2	ENSG00000152256	8809
PDPK1	chr16	ENSG00000140992	8816	PHOX2B	chr4	ENSG00000109132	9143
PIK3C2B	chr1	ENSG00000133056	8972	PIK3C2G	chr12	ENSG00000139144	8973
PIK3C3	chr18	ENSG00000078142	8974	PIK3CA	chr3	ENSG00000121879	8975
PIK3CB	chr3	ENSG00000051382	8976	PIK3CD	chr1	ENSG00000171608	8977
PIK3CG	chr7	ENSG00000105851	8978	PIK3R1	chr5	ENSG00000145675	8979
PIK3R2	chr19	ENSG00000105647	8980	PIK3R3	chr1	ENSG00000117461	8981
PIM1	chr6	ENSG00000137193	8986	PLCG2	chr16	ENSG00000197943	9066
PLK2	chr5	ENSG00000145632	19699	PMAIP1	chr18	ENSG00000141682	9108
PMS1	chr2	ENSG00000064933	9121	PMS2	chr7	ENSG00000122512	9122
PNRC1	chr6	ENSG00000146278	17278	POLD1	chr19	ENSG00000062822	9175
POLE	chr12	ENSG00000177084	9177	POLH	chr6	ENSG00000170734	9181
POT1	chr7	ENSG00000128513	17284	PPARG	chr3	ENSG00000132170	9236
PPP2R1A	chr19	ENSG00000105568	9302	PPP2R2A	chr8	ENSG00000221914	9304
PRDM1	chr6	ENSG00000057657	9346	PREX2	chr8	ENSG00000046889	22950
PRKAR1A	chr17	ENSG00000108946	9388	PRKCI	chr3	ENSG00000163558	9404
PRKDC	chr8	ENSG00000253729	9413	PRSS1	chr7	ENSG00000204983	9475
PRSS8	chr16	ENSG00000052344	9491	PTCH1	chr9	ENSG00000185920	9585
PTEN	chr10	ENSG00000171862	9588	PTK2	chr8	ENSG00000169398	9611
PTPN11	chr12	ENSG00000179295	9644	PTPRD	chr9	ENSG00000153707	9668
PTPRO	chr12	ENSG00000151490	9678	PTPRS	chr19	ENSG00000105426	9681
PTPRT	chr20	ENSG00000196090	9682	QKI	chr6	ENSG00000112531	21100
RAC1	chr7	ENSG00000136238	9801	RAD21	chr8	ENSG00000164754	9811
RAD50	chr5	ENSG00000113522	9816	RAD51	chr15	ENSG00000051180	9817
RAD51B	chr14	ENSG00000182185	9822	RAD51C	chr17	ENSG00000108384	9820
RAD51D	chr17	ENSG00000185379	9823	RAD52	chr12	ENSG00000002016	9824
RAD54B	chr8	ENSG00000197275	17228	RAD54L	chr1	ENSG00000085999	9826
RAF1	chr3	ENSG00000132155	9829	RANBP2	chr2	ENSG00000153201	9848
RARA	chr17	ENSG00000131759	9864	RASA1	chr5	ENSG00000145715	9871
RB1	chr13	ENSG00000139687	9884	RBM10	chrX	ENSG00000182872	9896
RECQL4	chr8	ENSG00000160957	9949	REL	chr2	ENSG00000162924	9954
RET	chr10	ENSG00000165731	9967	RFWD2	chr1	ENSG00000143207	17440
RHOA	chr3	ENSG00000067560	667	RICTOR	chr5	ENSG00000164327	28611
RIT1	chr1	ENSG00000143622	10023	RNF43	chr17	ENSG00000108375	18505
ROS1	chr6	ENSG00000047936	10261	RPA1	chr17	ENSG00000132383	10289
RPS6KA4	chr11	ENSG00000162302	10433	RPS6KB2	chr11	ENSG00000175634	10437
RPTOR	chr17	ENSG00000141564	30287	RUNX1	chr21	ENSG00000159216	10471
RUNX1T1	chr8	ENSG00000079102	1535	RYBP	chr3	ENSG00000163602	10480
SBDS	chr7	ENSG00000126524	19440	SDHA	chr5	ENSG00000073578	10680

Gene Name	Chr	Ensembl Gene ID	HGNC ID	Gene Name	Chr	Ensembl Gene ID	HGNC ID
SDHAF2	chr11	ENSG00000167985	26034	SDHB	chr1	ENSG00000117118	10681
SDHC	chr1	ENSG00000143252	10682	SDHD	chr11	ENSG00000204370	10683
SETD2	chr3	ENSG00000181555	18420	SF3B1	chr2	ENSG00000115524	10768
SGK1	chr6	ENSG00000118515	10810	SH2D1A	chrX	ENSG00000183918	10820
SHQ1	chr3	ENSG00000144736	25543	SLIT2	chr4	ENSG00000145147	11086
SLX4	chr16	ENSG00000188827	23845	SMAD2	chr18	ENSG00000175387	6768
SMAD3	chr15	ENSG00000166949	6769	SMAD4	chr18	ENSG00000141646	6770
SMARCA4	chr19	ENSG00000127616	11100	SMARCB1	chr22	ENSG00000099956	11103
SMARCD1	chr12	ENSG00000066117	11106	SMO	chr7	ENSG00000128602	11119
SNCAIP	chr5	ENSG00000064692	11139	SOCS1	chr16	ENSG00000185338	19383
SOX10	chr22	ENSG00000100146	11190	SOX17	chr8	ENSG00000164736	18122
SOX2	chr3	ENSG00000181449	11195	SOX9	chr17	ENSG00000125398	11204
SPEN	chr1	ENSG00000065526	17575	SPOP	chr17	ENSG00000121067	11254
SPTA1	chr1	ENSG00000163554	11272	SRC	chr20	ENSG00000197122	11283
STAG2	chrX	ENSG00000101972	11355	STAT3	chr17	ENSG00000168610	11364
STAT4	chr2	ENSG00000138378	11365	STK11	chr19	ENSG00000118046	11389
STK40	chr1	ENSG00000196182	21373	SUFU	chr10	ENSG00000107882	16466
SUZ12	chr17	ENSG00000178691	17101	SYK	chr9	ENSG00000165025	11491
TAF1	chrX	ENSG00000147133	11535	TBX3	chr12	ENSG00000135111	11602
TEK	chr9	ENSG00000120156	11724	TERC	chr3	ENSG00000270141	11727
TERT	chr5	ENSG00000164362	11730	TET1	chr10	ENSG00000138336	29484
TET2	chr4	ENSG00000168769	25941	TGFBR1	chr9	ENSG00000106799	11772
TGFBR2	chr3	ENSG00000163513	11773	TIPARP	chr3	ENSG00000163659	23696
TLR4	chr9	ENSG00000136869	11850	TLR7	chrX	ENSG00000196664	15631
TLR8	chrX	ENSG00000101916	15632	TLR9	chr3	ENSG00000239732	15633
TMEM127	chr2	ENSG00000135956	26038	TMPRSS2	chr21	ENSG00000184012	11876
TNFAIP3	chr6	ENSG00000118503	11896	TNFRSF14	chr1	ENSG00000157873	11912
TOP1	chr20	ENSG00000198900	11986	TOP2A	chr17	ENSG00000131747	11989
TP53	chr17	ENSG00000141510	11998	TP53BP1	chr15	ENSG00000067369	11999
TP63	chr3	ENSG00000073282	15979	TRAF7	chr16	ENSG00000131653	20456
TSC1	chr9	ENSG00000165699	12362	TSC2	chr16	ENSG00000103197	12363
TSHR	chr14	ENSG00000165409	12373	TYRO3	chr15	ENSG00000092445	12446
U2AF1	chr21	ENSG00000160201	12453	VEGFA	chr6	ENSG00000112715	12680
VHL	chr3	ENSG00000134086	12687	VTCN1	chr1	ENSG00000134258	28873
WAS	chrX	ENSG00000015285	12731	WEE1	chr11	ENSG00000166483	12761
WHSC1	chr4	ENSG00000109685	12766	WHSC1L1	chr8	ENSG00000147548	12767
WISP3	chr6	ENSG00000112761	12771	WRN	chr8	ENSG00000165392	12791
WT1	chr11	ENSG00000184937	12796	XIAP	chrX	ENSG00000101966	592
XPA	chr9	ENSG00000136936	12814	XPC	chr3	ENSG00000154767	12816
XPO1	chr2	ENSG00000082898	12825	XRCC1	chr19	ENSG00000073050	12828
XRCC2	chr7	ENSG00000196584	12829	XRCC3	chr14	ENSG00000126215	12830
YAP1	chr11	ENSG00000137693	16262	YES1	chr18	ENSG00000176105	12841

Gene Name	Chr	Ensembl Gene ID	HGNC ID	Gene Name	Chr	Ensembl Gene ID	HGNC ID
ZBTB2	chr6	ENSG00000181472	20868	ZNF217	chr20	ENSG00000171940	13009
ZNF703	chr8	ENSG00000183779	25883				

Appendix B: List of Genes/Exons Excluded from Reporting in PGDx elio tissue complete due to Consistently Low Coverage

Gene	Transcript	Exon	Gene	Transcript	Exon
ABL2	CCDS30947.1	2	MRE11A	CCDS8299.1	18, 19
APC	CCDS4107.1	4, 13	MSH2	CCDS1834.1	4, 16
ARID2	CCDS31783.1	7	MYB	CCDS47481.1	1
ATM	CCDS31669.1	1, 15, 28, 60	NBN	CCDS6249.1	6, 12, 15, 16
ATR	CCDS3124.1	37	NCOR1	CCDS11175.1	8, 14
ATRX	CCDS14434.1	2, 3, 5, 8, 10, 12, 13, 14, 15, 20, 22, 33	NPM1	CCDS4376.1	7
AURKA	CCDS13451.1	3	NT5C2	CCDS7544.1	2, 5, 8, 10, 14
BIRC2	CCDS58169.1	7	NTRK3	CCDS10340.1	9
BMPR1A	CCDS7378.1	1	PAK1	CCDS44687.1	14
BRIP1	CCDS11631.1	9, 12	PAK3	CCDS14554.1	4, 7, 9
BTK	CCDS14482.1	3, 8	PBRM1	CCDS43099.1	8
BUB1B	CCDS10053.1	13	PIK3C2G	CCDS44839.1	6, 9
CD274	CCDS6464.1	5	PIK3C3	CCDS11920.1	25
CD79A	CCDS12589.1	4	PIK3CB	CCDS3104.1	6
CDK8	CCDS9317.1	2, 8, 9	PMS1	CCDS2302.1	7
CHD2	CCDS10374.2	32	PMS2	CCDS5343.1	13
CHEK2	CCDS13843.1	4, 6, 7	POT1	CCDS5793.1	1
CREBBP	CCDS10509.1	21	PRKCI	CCDS3212.2	15
CSF1	CCDS30797.1	1	PRKDC	NM_006904	2, 4, 5, 14, 19, 75
CYLD	CCDS42164.1	13	PRSS8	CCDS45469.1	2
DCUN1D1	CCDS3240.1	2	PTEN	CCDS31238.1	3, 8
DNMT1	CCDS12228.1	7, 11, 13	PTK2	CCDS56557.1	3, 28
DOT1L	CCDS42460.1	12	PTPN11	CCDS9163.1	1
EIF1AX	CCDS14196.1	4, 5, 7	PTPRD	CCDS43786.1	5, 6, 15
EP300	CCDS14010.1	23	PTPRO	CCDS44837.1	5
EPCAM	CCDS1833.1	4, 8, 9	RAD50	CCDS34233.1	9, 16, 17, 18, 19, 20
ERCC3	CCDS2144.1	4	RAD51C	CCDS11611.1	6
ERCC5	CCDS32004.1	5	RAF1	CCDS2612.1	12
ERCC8	CCDS3978.1	2, 5, 12	RANBP2	CCDS2079.1	2, 8
ETV1	CCDS55088.1	4	RASA1	CCDS34200.1	6, 15, 19

FAM175A	CCDS3605.2	3, 4, 7	RB1	CCDS31973.1	6, 9, 11, 14, 15, 16, 17
FANCA	CCDS32515.1	9	REL	CCDS1864.1	8, 9, 10
FANCB	CCDS14161.1	3, 4, 5	RFWD2	CCDS30944.1	6, 20
FANCC	CCDS35071.1	3	RICTOR	CCDS34148.1	9, 12, 19, 22
FANCD2	CCDS2595.1	13, 19	ROS1	CCDS5116.1	3
FANCI	CCDS10349.2	10	SETD2	CCDS2749.2	2
FANCL	CCDS1860.1	14	SF3B1	CCDS33356.1	11
FANCM	CCDS32070.1	8, 18, 19	SH2D1A	CCDS14608.1	4
FAS	CCDS7394.1	6, 7	SLIT2	CCDS3426.1	7, 12
FUBP1	CCDS683.1	4, 5	SPTA1	CCDS41423.1	1, 46, 49
GNA13	CCDS11661.1	3	STAG2	CCDS43990.1	1, 2, 4, 5, 6, 8, 10, 11, 13, 18, 19, 20, 22, 25, 33
GPC3	CCDS14638.1	4	STAT3	CCDS32656.1	10
HDAC2	CCDS43493.2	13	STAT4	CCDS2310.1	5, 10, 11, 13, 23
HGF	CCDS47626.1	12	SUZ12	CCDS11270.1	4, 5, 6, 11, 15
INPP4B	CCDS3757.1	2	TAF1	CCDS14412.1	23, 26, 31, 33
IRS2	CCDS9510.1	2	TET1	CCDS7281.1	8
JAK1	CCDS41346.1	1	TMPRSS2	CCDS33564.1	13
JAK2	CCDS6457.1	13	TOP2A	CCDS45672.1	32
JAK3	CCDS12366.1	12	TP53	CCDS11118.1	2
KDM6A	CCDS14265.1	4, 5, 7, 8, 9, 14, 21	TSHR	CCDS9872.1	8
KMT2C	CCDS5931.1	9, 22, 30	TYRO3	CCDS10080.1	1
KRAS	NM_033360	6	WEE1	CCDS44536.1	2
LRP1B	CCDS2182.1	6, 70	WRN	CCDS6082.1	6, 13, 26
MAGI2	CCDS5594.1	7, 11	XIAP	CCDS14606.1	2, 3, 4, 5
MAX	CCDS9771.1	2	XPO1	CCDS33205.1	5, 7, 21
MED12	CCDS43970.1	43	XRCC2	CCDS5933.1	2
MERTK	CCDS2094.1	12	YES1	CCDS11824.1	2
MLH1	CCDS2663.1	15			

Appendix C: Low Complexity and Repeat Genomic Regions Excluded from Reporting of Non-hotspot SNVs and Indels Classified as Variants with Potential Significance

Gene	Transcript	Exons	Masked region coordinates
ABL2	CCDS44283.1	10	chr1:179079938-179079982
ACVR1B	NM_004302	1	chr12:52345518-52345523
AMER1	CCDS14377.2	1	chrX:63411959-63412030
APC	CCDS4107.1	15	chr5:112177303-112177356
AR	CCDS14387.1	1	chrX:66765159-66765262, chrX:66766339-66766409

ARID1A	CCDS285.1	1; 16	chr1:27022978-27023029, chr1:27023138-27023173, chr1:27023257-27023297, chr1:27023908-27023938; chr1:27100182-27100206
ARID1B	CCDS5251.2	1	chr6:157099075-157099100, chr6:157099166-157099187, chr6:157099303-157099376, chr6:157099403-157099459, chr6:157099483-157099507, chr6:157099699-157099743, chr6:157099785-157099807, chr6:157099872-157099898, chr6:157099975-157100144, chr6:157100203-157100228, chr6:157100239-157100267, chr6:157100431-157100457
ASXL2	NM_018263	6	chr2:25991700-25991731
ATM	CCDS31669.1	5; 30	chr11:108114678-108114679; chr11:108164038-108164039
ATRX	CCDS14434.1	9	chrX:76938248-76938317
AURKB	NM_004217	2	chr17:8113547-8113552
BAP1	CCDS2853.1	12	chr3:52438494-52438531
BBC3	CCDS12697.1	1	chr19:47731476-47731557
BCL2	CCDS11981.1	1	chr18:60985649-60985680, chr18:60985766-60985796
BCL2L11	CCDS2092.1	3	chr2:111886202-111886257, chr2:111886264-111886330
BCL6	CCDS3289.1	6	chr3:187443285-187443290
BCORL1	CCDS14616.1	3; 6	chrX:129147643-129147991; chrX:129159222-129159250
BRAF	CCDS5863.1	1	chr7:140624399-140624427
BRD4	CCDS12328.1	8; 12; 13; 18	chr19:15366894-15367032; chr19:15355275-15355365; chr19:15353892-15354030; chr19:15349669-15349728
BTG1	CCDS9043.1	1	chr12:92539312-92539313
C12orf5	NM_020375	6	chr12:4462895-4462986
CALR	CCDS12288.1	9	chr19:13054605-13054699
CASP8	NM_001228	10	chr2:202151326-202151327
CBL	CCDS8418.1	1	chr11:119077233-119077255
CCND1	CCDS8191.1	5	chr11:69465976-69466008
CCND3	CCDS4863.1	1	chr6:41909327-41909356
CDK12	CCDS11337.1	5	chr17:37650872-37650941
CDKN1C	CCDS7738.1	1	chr11:2906071-2906255
CEBPA	CCDS54243.1	1	chr19:33792575-33792645, chr19:33792755-33792776, chr19:33792966-33792998, chr19:33793008-33793046, chr19:33793200-33793222
CECR5	CCDS33595.1	1	chr22:17640043-17640090
CHD4	CCDS8552.1	3	chr12:6711145-6711167
CIC	CCDS12601.1	20	chr19:42799128-42799253
CPM	NM_001874	9	chr12:69246532-69246641

CREBBP	CCDS10509.1	29; 31	chr16:3781847-3781928; chr16:3778401-3778464
CSF1R	CCDS4302.1	10; 21	chr5:149447797-149447822; chr5:149433732-149433770
CUL4A	CCDS41908.1	1	chr13:113864010-113864059
DAXX	CCDS4776.1	4	chr6:33287797-33287870, chr6:33287881-33287921
DCTN5	NM_032486	6	chr16:23681264-23681290
DHFR	NM_000791	1	chr5:79950700-79950733
DICER1	CCDS9931.1	22	chr14:95562982-95563006
DNMT1	CCDS45958.1	5	chr19:10290861-10290912
DNMT3B	CCDS13205.1	9	chr20:31381340-31381403
E2F3	CCDS4545.1	1	chr6:20402595-20402623, chr6:20402825-20402851
EGFR	CCDS5514.1	28	chr7:55272947-55272950
EPCAM	CCDS1833.1	7	chr2:47607046-47607105
EPHA5	CCDS3513.1	1	chr4:66535417-66535459
EPM2AIP1	NM_014805	1	chr3:37027991-37028191
ERCC1	CCDS12663.1	8	chr19:45916804-45916834
ERCC2	CCDS46112.1	12	chr19:45862116-45862172
ERCC6	CCDS7229.1	4	chr10:50732295-50732345
ERCC6-PGBD3	NM_001277058	5	chr10:50732295-50732345
ETV4	NM_001986	1	chr17:41623303-41623307
EWSR1	NM_005243	1	chr22:29664316-29664323
EXT1	CCDS6324.1	1	chr8:119122697-119122718
EZH2	CCDS5891.1	5	chr7:148525889-148525916
FANCB	CCDS14161.1	8	chrX:14861687-14861699
FANCI	CCDS10349.2	20; 28	chr15:89835986-89836016; chr15:89848570-89848576
FANCM	CCDS32070.1	15	chr14:45650631-45650632
FAT1	CCDS47177.1	2	chr4:187584500-187584573
FBXW7	CCDS3777.1	1	chr4:153332605-153332629
FGF12	NM_004113	6	chr3:191861788-191861791
FGF3	CCDS8195.1	1	chr11:69633564-69633585
FGFR1	CCDS6107.2	3	chr8:38285914-38285934
FGFR3	CCDS3353.1	1; 12	chr4:1795660-1795662; chr4:1807532-1807555
FH	CCDS1617.1	7	chr1:241667340-241667373
FLCN	CCDS32580.1	5	chr17:17124691-17124698
FOXA1	CCDS9665.1	2	chr14:38060999-38061027, chr14:38061130-38061175, chr14:38061517-38061546
FOXL2	CCDS3105.1	1	chr3:138664555-138664728, chr3:138664862-138664906, chr3:138665015-138665065
FUBP1	CCDS683.1	1	chr1:78444615-78444645
GATA4	CCDS5983.1	1	chr8:11566172-11566204

GATA6	CCDS11872.1	1	chr18:19751622-19751654, chr18:19751750-19751778, chr18:19751820-19751861, chr18:19752073-19752104
GID4	CCDS11190.1	1	chr17:17942777-17942786
GNA11	CCDS12103.1	1	chr19:3094648-3094650
GNA13	CCDS11661.1	1	chr17:63052427-63052430
GNAS	CCDS13472.1	1	chr20:57466780-57466782
GPR124	CCDS6097.2	1; 19	chr8:37654829-37654868; chr8:37699121-37699165, chr8:37699300-37699323, chr8:37699468-37699491
HDAC2	CCDS43493.2	12	chr6:114264518-114264579
HSP90AA1	CCDS9967.1	4; 8	chr14:102551150-102551210, chr14:102551247-102551294; chr14:102549393-102549481
HSP90AB1	CCDS4909.1	5	chr6:44218030-44218204
IGF2R	CCDS5273.1	1	chr6:160390277-160390337
IKZF3	NM_012481	8	chr17:37916698-37916877
IL10	CCDS1467.1	2	chr1:206944739-206944762
IL7R	NM_002185	1	chr5:35857070-35857077
INHBA	CCDS5464.1	2	chr7:41729668-41729754
INSR	CCDS12176.1	1; 13	chr19:7293863-7293896; chr19:7141825-7141829
IRS1	CCDS2463.1	1	chr2:227660808-227660829, chr2:227661396-227661419
IRS2	CCDS9510.1	1	chr13:110434567-110434613, chr13:110435242-110435359, chr13:110436297-110436322, chr13:110437266-110437303, chr13:110437860-110437967, chr13:110438196-110438245, chr13:110438313-110438342, chr13:110438362-110438402
KAT6A	CCDS6124.1	16	chr8:41790634-41790788, chr8:41791830-41791934, chr8:41792010-41792078
KCNMB3	NM_171830	1	chr3:178969433-178969647
KDM6A	CCDS14265.1	1	chrX:44732821-44732848
KLHL6	CCDS3245.2	3	chr3:183226057-183226155
KMT2A	CCDS31686.1	1; 3	chr11:118307274-118307393, chr11:118307400-118307430; chr11:118344478-118344563
KMT2C	CCDS5931.1	36; 43	chr7:151879585-151879610; chr7:151859821-151859869
KMT2D	CCDS44873.1	34; 39	chr12:49431291-49431320, chr12:49432661-49432709; chr12:49426230-49426255, chr12:49426566-49426788, chr12:49426888-49426923, chr12:49427251-49427287, chr12:49427650-49427696
LATS2	CCDS9294.1	3	chr13:21562480-21562521
LRP1B	CCDS2182.1	90	chr2:140992353-140992455
LTK	CCDS10077.1	7	chr15:41803369-41803433
LZTR1	CCDS33606.1	7	chr22:21343964-21343973

MAF	CCDS10928.1	1	chr16:79633069-79633128, chr16:79633134-79633214, chr16:79633217-79633263, chr16:79633356-79633387
MAGI2	CCDS5594.1	22	chr7:77648702-77649011
MAML1	CCDS34315.1	1	chr5:179160112-179160114, chr5:179160341-179160388
MAP2K4	CCDS11162.1	1	chr17:11924223-11924261
MAP3K1	CCDS43318.1	14	chr5:56177849-56177875
MAPK1	CCDS13795.1	1	chr22:22221709-22221732
MAX	CCDS9774.1	4	chr14:65550975-65551019
MED12	CCDS43970.1	42	chrX:70360589-70360699
MEF2B	CCDS12394.1	7	chr19:19256719-19256741
MEF2BNB	NM_005919	10	chr19:19256719-19256741
MEF2BNB-MEF2B	CCDS12394.1	7	chr19:19256719-19256741
MEN1	CCDS31600.1	3	chr11:64575022-64575037
MKNK1	CCDS538.1	7	chr1:47037087-47037213
MSH2	CCDS1834.1	3	chr2:47637248-47637290
MSH3	CCDS34195.1	1; 5; 7	chr5:79950700-79950733; chr5:79968061-79968062; chr5:79970800-79970804
MSH6	CCDS1836.1	10	chr2:48033916-48033917
MTOR	CCDS127.1	38	chr1:11190667-11190732
MYCN	CCDS1687.1	1; 2	chr2:16082632-16082719, chr2:16082862-16082914; chr2:16085613-16085653
MYOD1	CCDS7826.1	1; 2	chr11:17741867-17741901; chr11:17742459-17742488
NCOA3	CCDS13406.1	17; 18	chr20:46277842-46277843; chr20:46279815-46279902
NCOR1	CCDS11175.1	11; 34	chr17:16042320-16042321; chr17:15967423-15967467
NKX2-1	CCDS9659.1	2	chr14:36986714-36986744, chr14:36986759-36986780, chr14:36986815-36986931
NKX3-1	CCDS6042.1	1	chr8:23540150-23540171
NOTCH3	CCDS12326.1	1; 18; 24; 33	chr19:15311649-15311698; chr19:15291906-15291975; chr19:15288608-15288691; chr19:15272196-15272233
NOTCH4	CCDS34420.1	1; 24	chr6:32191659-32191691; chr6:32166912-32166924
NPM1	CCDS4376.1	6	chr5:170819938-170819980
NT5C2	CCDS7544.1	17	chr10:104849427-104849471
NUTM1	NM_175741	1	chr15:34635841-34635896
OS9	CCDS31843.1	11	chr12:58112064-58112081
PAK1	CCDS44687.1	5	chr11:77069990-77070015
PAK3	CCDS14554.1	5	chrX:110406187-110406227
PALB2	CCDS32406.1	10	chr16:23632681-23632687
PDK1	CCDS2250.1	1	chr2:173420914-173420948
PGBD3	NM_170753	1	chr10:50732295-50732329

PHOX2B	CCDS3463.1	3	chr4:41747990-41748055, chr4:41748071-41748133
PIK3CB	CCDS3104.1	2	chr3:138474594-138474595
PIK3CG	CCDS5739.1	1	chr7:106509944-106509996
PIK3R2	CCDS12371.1	1; 5; 13; 15	chr19:18266923-18266974; chr19:18272243-18272288; chr19:18279283-18279358; chr19:18280065-18280102
PIM1	NM_002648	1	chr6:37138209-37138267
PLCG2	CCDS42204.1	29	chr16:81973495-81973521
POLD1	CCDS12795.1	12	chr19:50910238-50910239
POLE	CCDS9278.1	1; 43	chr12:133263868-133263889; chr12:133210870-133210938
PPP2R1A	CCDS12849.1	7	chr19:52719061-52719092
PPP2R2A	CCDS34867.1	9	chr8:26223904-26223924
PTK2	NM_005607	2	chr8:141994175-141994220
PTPN11	CCDS9163.1	7	chr12:112910806-112910836
PTPRD	CCDS43786.1	7	chr9:8524923-8524958
PTPRS	CCDS12140.1	11	chr19:5229532-5229560
RAD21	CCDS6321.1	11	chr8:117862876-117862908
RAD51B	CCDS9789.1	10	chr14:69061200-69061322
RAD51D	CCDS45646.1	3	chr17:33443876-33443926
RAD52	CCDS8507.2	8	chr12:1025547-1025614
RARA	CCDS11366.1	8	chr17:38512422-38512447
RASA1	CCDS34200.1	1; 14	chr5:86564538-86564594; chr5:86669978-86669979
RB1	CCDS31973.1	1	chr13:48878076-48878129
RBM10	CCDS14274.1	3	chrX:47030561-47030605
RECQL4	NM_004260	1	chr8:145743169-145743178
RET	CCDS7200.1	1	chr10:43572743-43572772
RPS6KA4	CCDS8073.1	1	chr11:64126706-64126708
RPS6KB2	CCDS41677.1	1; 15	chr11:67196015-67196017; chr11:67202527-67202593
SDHD	NM_001276506	4	chr11:111963802-111963931
SGK1	NM_001143678	1	chr6:134496805-134496809
SLC7A8	NM_012244	1	chr14:23652347-23652368
SMAD3	NM_005902	1	chr15:67358483-67358484
SMARCA4	CCDS12253.1	3; 9; 26; 32	chr19:11097197-11097241; chr19:11107027-11107057; chr19:11144441-11144543; chr19:11170486-11170547
SMO	CCDS5811.1	1; 12	chr7:128829040-128829062; chr7:128851941-128851972
SOCS1	CCDS10546.1	1	chr16:11349182-11349242
SOX10	CCDS13964.1	1	chr22:38379782-38379793
SOX17	CCDS6159.1	2	chr8:55372215-55372241, chr8:55372259-55372288
SOX9	CCDS11689.1	3	chr17:70120020-70120137

SPEN	CCDS164.1	10; 11	chr1:16248775-16248823; chr1:16262459-16262524
SPTA1	CCDS41423.1	2	chr1:158654963-158655029
SUFU	CCDS7537.1	1; 2	chr10:104263929-104263985; chr10:104268969-104269058
SUZ12	CCDS11270.1	1	chr17:30264348-30264373
TAF1	CCDS14412.1	38	chrX:70683756-70683789
TERT	CCDS3861.2	1; 2	chr5:1294898-1294919; chr5:1294664-1294687
TET2	CCDS47120.1	9	chr4:106196233-106196313
TGFBR1	CCDS6738.1	1	chr9:101867538-101867566
TGFBR2	CCDS33727.1	2	chr3:30664689-30664734
TIPARP	CCDS3177.1	5	chr3:156422774-156422854
TMPRSS2	NM_005656	1	chr21:42880030-42880086
TOP1	CCDS13312.1	3; 4	chr20:39690040-39690066; chr20:39704828-39704926
TP53BP1	CCDS10096.1	4	chr15:43773131-43773185
TSC1	CCDS6956.1	21	chr9:135771988-135772008
TSPAN31	NM_005981	6	chr12:58141528-58141727
U2AF1	CCDS13694.1	8	chr21:44513267-44513310
UBFD1	NM_019116	7	chr16:23582262-23582278
VEGFA	CCDS34457.1	1	chr6:43738518-43738568, chr6:43738719-43738756
WAS	CCDS14303.1	10	chrX:48547297-48547329
WEE1	CCDS7800.1	1	chr11:9595504-9595535, chr11:9595580-9595611, chr11:9595666-9595743, chr11:9595941-9595986
WHSC1L1	CCDS43729.1	23	chr8:38133374-38133402
WRN	CCDS6082.1	11; 28	chr8:30945377-30945432; chr8:31004567-31004568
WT1	CCDS7878.2	1	chr11:32456485-32456525
XRCC1	CCDS12624.1	9	chr19:44056379-44056429
ZNF2	NM_021088	5	chr2:95848977-95849096, chr2:95849362-95849668
ZNF703	CCDS6094.1	1; 2	chr8:37553541-37553569; chr8:37554932-37554977, chr8:37555934-37555962, chr8:37555979-37556010

Appendix D. Interlaboratory Reproducibility Summary of PGDx elio tissue complete per Variant, Per Specimen Tested

Gene	Amino Acid Change	Mutation Type	Mean MAF (%)	MAF Range	SD	%CV	Positive Call Rate % (n/N)
ATM	T1871I	SNV	19.1	(13.0, 23.1)	2.3	11.9	100% (35/35)
FGF10	V123I	SNV	46.7	(37.3, 53.6)	3.7	8.0	100% (35/35)

GRIN2A	N1085K	SNV	48.9	(45.8, 53.4)	1.6	3.2	100% (35/35)
PIK3CA	R808W	SNV	33.3	(26.3, 41.3)	3.6	10.8	100% (35/35)
PREX2	D927Y	SNV	20.7	(18.1, 24.9)	1.6	7.6	100% (35/35)
RB1	S612Y	SNV	21.1	(15.5, 25.7)	2.7	12.9	100% (35/35)
RUNX1T1	V163I	SNV	19.4	(16.9, 23.6)	1.6	8.5	100% (35/35)
SNCAIP	A412E	SNV	26.2	(19.1, 34.2)	2.8	10.8	100% (35/35)
TP53	R175H	SNV	56.6	(51.4, 61.7)	2.1	3.7	100% (35/35)
APC	I1307K	SNV	19.7	(19.7, 19.7)	N/A	N/A	2.8% (1/36)
ATM	K1964N	SNV	45.7	(37.0, 51.0)	3.1	6.7	100% (36/36)
MAP3K1	T1082I	SNV	71.9	(64.6, 76.9)	2.6	3.6	100% (36/36)
MYC	S363P	SNV	74.2	(71.1, 76.2)	1.1	1.5	100% (36/36)
POLH	R93Q	SNV	50.8	(43.3, 58.2)	3.3	6.5	100% (36/36)
TERT	Promoter	SNV	72.3	(63.4, 79.5)	2.7	3.7	100% (36/36)
TP53	A159D	SNV	40.5	(36.5, 43.4)	1.7	4.3	100% (36/36)
ARAF	R255Gfs*37	DEL < 15bp	78.2	(71.1, 85.0)	3.3	4.3	100% (35/35)
ARID1A	G314Afs*49	DEL < 15bp	36.2	(29.6, 43.4)	2.9	7.9	100% (35/35)
ARID1B	A415Pfs*15	DEL < 15bp	35.8	(31.0, 41.2)	2.5	7.0	100% (35/35)
B2M	T93Lfs*10	DEL < 15bp	33.8	(26.8, 39.3)	3.2	9.5	100% (35/35)
B2M	D96Mfs*7	DEL < 15bp	37.6	(30.1, 45.0)	3.8	10.1	100% (35/35)
BCOR	Q1208Tfs*8	INS < 15bp	18.8	(15.1, 26.1)	2.0	10.8	100% (35/35)
BCR	M1119I	SNV	22.8	(19.3, 25.3)	1.2	5.3	80.0% (28/35)
CDK12	Q1291Rfs*3	DEL < 15bp	36.4	(32.9, 39.4)	1.7	4.8	100% (35/35)
CDKN1A	R140Q	SNV	34.5	(30.1, 39.1)	1.9	5.5	100% (35/35)
CTNNB1	R661Q	SNV	34.6	(28.2, 43.5)	3.3	9.7	100% (35/35)
CYLD	Y22Tfs*25	DEL < 15bp	35.2	(26.4, 42.8)	3.8	10.9	100% (35/35)
DOT1L	R292H	SNV	32.1	(30.1, 35.0)	1.2	3.9	100% (35/35)
EPAS1	D539G	SNV	34.8	(30.7, 37.8)	1.5	4.3	100% (35/35)

EPHA7	D751V	SNV	7.8	(7.5, 8.1)	0.3	3.8	8.6% (3/35)
EXT1	L490Wfs*9	DEL < 15bp	36.7	(33.4, 42.3)	1.9	5.1	100% (35/35)
FANCM	V1336Lfs*2	DEL < 15bp	37.2	(29.6, 44.8)	3.8	10.2	100% (35/35)
FGFR3	L752I	SNV	32.4	(28.8, 35.0)	1.6	5.0	100% (35/35)
FGFR4	S141N	SNV	37.4	(34.2, 40.6)	1.7	4.5	100% (35/35)
FUBP1	I301Yfs*22	DEL < 15bp	35.0	(26.2, 43.4)	3.8	10.8	100% (35/35)
GATA4	A33V	SNV	34.4	(30.4, 37.4)	1.6	4.7	100% (35/35)
GNAS	R232C	SNV	33.9	(29.7, 37.9)	1.9	5.6	100% (35/35)
GRM3	T725I	SNV	32.9	(29.3, 38.4)	2.0	6.1	100% (35/35)
HDAC1	E468del	DEL < 15bp	30.4	(25.7, 34.4)	2.1	6.8	100% (35/35)
IGF2R	D1317Tfs*27	DEL < 15bp	36.1	(30.1, 41.1)	2.8	7.7	100% (35/35)
JAK3	Q39Pfs*13	INS < 15bp	25.9	(23.4, 29.0)	1.6	6.3	97.1% (34/35)
LRP1B	C3409Y	SNV	18.0	(14.4, 24.8)	2.5	14.0	100% (35/35)
LZTR1	T428M	SNV	32.1	(27.4, 36.2)	1.8	5.6	100% (35/35)
MCL1	L21_G24del	DEL < 15bp	34.8	(31.1, 40.5)	2.3	6.7	100% (35/35)
MDC1	Splice Site	SNV	35.7	(31.9, 39.3)	2.1	5.8	100% (35/35)
MSH3	G301Rfs*3	INS < 15bp	32.7	(26.6, 37.5)	2.6	8.0	100% (35/35)
MSH3	K383Rfs*32	DEL < 15bp	72.4	(63.7, 82.1)	5.2	7.1	100% (35/35)
NOTCH1	H2207Mfs*41	DEL < 15bp	34.3	(31.2, 36.6)	1.4	4.1	100% (35/35)
NOTCH4	T1669R	SNV	32.6	(28.8, 37.7)	1.7	5.4	100% (35/35)
PHOX2B	A118V	SNV	32.6	(28.9, 35.5)	1.6	5.0	100% (35/35)
PRKDC	L3909F	SNV	27.7	(25.0, 31.9)	1.3	4.9	100% (35/35)
PTK2	E821del	DEL < 15bp	37.0	(28.8, 44.2)	3.6	9.8	100% (35/35)
QKI	H152R	SNV	33.2	(27.2, 39.8)	3.2	9.8	100% (35/35)
SLX4	T918A	SNV	34.9	(32.5, 37.6)	1.5	4.4	100% (35/35)
SOX17	R343W	SNV	28.2	(25.8, 31.2)	1.4	5.1	100% (35/35)
SOX2	A288T	SNV	29.7	(26.6, 32.9)	1.6	5.4	100% (35/35)
SOX9	S387Rfs*14	DEL < 15bp	34.9	(29.8, 38.7)	2.5	7.0	100% (35/35)

TGFBR2	R553C	SNV	33.9	(30.8, 37.0)	1.4	4.2	100% (35/35)
TP53	R273C	SNV	23.2	(18.3, 26.2)	1.8	8.0	100% (35/35)
CDH1	N144K	SNV	45.2	(41.5, 48.8)	1.7	3.8	100% (36/36)
GNAS	R201C	SNV	3.4	(3.4, 3.4)	N/A	N/A	2.8% (1/36)
HIST1H1C	A8T	SNV	6.1	(6.1, 6.1)	N/A	N/A	2.8% (1/36)
KRAS	G12A	SNV	2.7	(2.7, 2.7)	N/A	N/A	2.8% (1/36)
PTCH1	W1339_R1345del	DEL ≥ 15bp	40.7	(35.8, 46.0)	2.2	5.5	100% (36/36)
RARA	P407S	SNV	46.8	(42.1, 52.9)	2.7	5.7	100% (36/36)
RNF43	R437Q	SNV	64.9	(61.3, 66.8)	1.0	1.6	100% (36/36)
TP53BP1	G1963R	SNV	16.0	(12.7, 19.5)	1.8	11.0	97.2% (35/36)
TP53BP1	Y1605C	SNV	10.4	(8.0, 14.0)	1.2	11.2	100% (36/36)
TP53BP1	P801S	SNV	16.9	(13.7, 20.9)	1.5	8.7	100% (36/36)
ATM	K1656*	SNV	40.9	(33.9, 50.0)	3.7	9.1	100% (34/34)
BARD1	G264C	SNV	28.8	(23.7, 32.9)	2.4	8.2	100% (34/34)
CBL	Splice Site	SNV	28.6	(23.8, 33.3)	4.4	15.2	11.8% (4/34)
CBL	Splice Site	SNV	33.0	(24.7, 43.9)	4.5	13.7	82.4% (28/34)
DNMT3A	E27D	SNV	31.3	(27.2, 34.5)	1.8	5.6	100% (34/34)
ERCC4	E836K	SNV	48.8	(45.1, 53.1)	1.8	3.7	100% (34/34)
FGF19	A121D	SNV	50.7	(44.9, 56.6)	2.6	5.1	100% (34/34)
GNAS	R201C	SNV	31.5	(27.6, 35.8)	1.8	5.7	100% (34/34)
GRM3	E231*	SNV	17.0	(12.8, 18.9)	1.2	7.2	100% (34/34)
HIST1H1C	A8T	SNV	55.3	(51.4, 59.7)	1.5	2.7	100% (34/34)
JAK3	G659W	SNV	15.3	(12.7, 17.6)	1.2	8.1	100% (34/34)
KRAS	G12A	SNV	23.7	(17.9, 28.0)	2.6	11.1	100% (34/34)
MED12	Splice Site	SNV	29.2	(24.5, 33.6)	2.1	7.3	100% (34/34)
MYD88	R230C	SNV	15.3	(11.4, 17.7)	1.6	10.1	100% (34/34)
NTRK2	Splice Site	SNV	18.0	(12.9, 27.4)	3.0	16.9	100% (34/34)

RFWD2	G85Afs*15	DEL < 15bp	16.7	(13.6, 24.6)	2.1	12.5	100% (34/34)
RUNX1T1	Splice Site	SNV	7.6	(6.0, 9.6)	0.8	11.0	97.1% (33/34)
SLX4	A748V	SNV	18.6	(15.9, 21.7)	1.9	10.0	100% (34/34)
TET1	I1021T	SNV	54.4	(49.1, 60.8)	2.8	5.1	100% (34/34)
TSC2	R901C	SNV	47.0	(42.7, 53.7)	2.3	5.0	100% (34/34)
ARID1B	E1999K	SNV	12.2	(9.7, 14.9)	1.1	9.4	100% (33/33)
ASXL1	R394P	SNV	21.8	(15.7, 25.3)	1.9	9.0	100% (33/33)
CD276	Y436S	SNV	30.6	(27.7, 35)	1.4	4.5	100% (33/33)
CUL3	M1X	SNV	12.4	(11.3, 13.4)	1.5	12.0	6.1% (2/33)
EPHB4	A172P	SNV	22.9	(19.9, 26.3)	1.3	5.6	100% (33/33)
EXT1	R57C	SNV	9.8	(8.5, 12.5)	1.0	10.4	100% (33/33)
FAT1	L599R	SNV	6.6	(6.2, 7.0)	0.6	8.6	6.1% (2/33)
ICOSLG	A254T	SNV	10.2	(8.7, 12.5)	0.9	8.8	100% (33/33)
INPP4A	C540F	SNV	22.7	(19.4, 26.3)	1.5	6.4	100% (33/33)
INPP4B	Splice Site	SNV	20.6	(15.5, 27.4)	2.7	13.2	100% (33/33)
KEAP1	G603W	SNV	28.1	(25.1, 30.8)	1.5	5.5	100% (33/33)
LATS2	R624S	SNV	23.3	(19.3, 27.6)	2.0	8.4	100% (33/33)
LRP1B	C2182F	SNV	10.1	(7.6, 13.4)	1.3	13.2	100% (33/33)
LRP1B	LD391FY	SNV	14.1	(9.3, 16.4)	1.7	11.8	97.0% (32/33)
LRP1B	L391F	SNV	19.6	(19.6, 19.6)	N/A	N/A	3.0% (1/33)
PBRM1	E187*	SNV	23.2	(18.7, 27.6)	2.3	9.7	100% (33/33)
PBRM1	E1175K	SNV	8.7	(7.4, 9.9)	1.8	20.4	6.1% (2/33)
PDGFRA	V367L	SNV	20.7	(17.5, 25.4)	1.8	8.8	100% (33/33)
PTPRT	V1272L	SNV	10.9	(8.1, 13.8)	1.3	12.0	100% (33/33)
RIT1	R200C	SNV	25.0	(21.4, 32.6)	2.2	8.7	100% (33/33)
SPEN	Q1577E	SNV	7.3	(6.6, 8.2)	0.4	6.0	57.6% (19/33)
STAT4	E138K	SNV	15.4	(11.5, 21.9)	2.1	13.5	100% (33/33)
STK40	Q81H	SNV	20.2	(16.8, 23.7)	1.5	7.6	100% (33/33)

SUFU	T13Wfs*29	DEL ≥ 15bp	5.9	(5.9, 5.9)	N/A	N/A	3.0% (1/33)
TET1	A1645S	SNV	22.3	(18.4, 27.2)	2.1	9.6	100% (33/33)
TP53	V157F	SNV	27.0	(24.0, 30.9)	1.6	5.8	100% (33/33)
ALK	A96T	SNV	6.9	(6.8, 7.0)	0.1	2.0	5.7% (2/35)
ARID1A	P65Rfs*36	DEL < 15bp	11.8	(11.8, 11.8)	N/A	N/A	2.9% (1/35)
ARID1B	G530S	SNV	30.4	(23.4, 36.8)	3.0	9.9	100% (35/35)
AXIN1	R712*	SNV	34.8	(29.3, 40.4)	2.6	7.3	97.1% (34/35)
AXIN1	K641Rfs*64	DEL < 15bp	13.8	(8.3, 17.8)	2.0	14.5	100% (35/35)
AXIN2	G665Afs*24	DEL < 15bp	39.2	(34.2, 45.4)	2.4	6.2	100% (35/35)
B2M	Y46Cfs*10	DEL < 15bp	30.3	(22.7, 40.1)	4.5	15.0	100% (35/35)
B2M	V69Wfs*34	DEL < 15bp	33.1	(23.0, 41.0)	3.9	11.7	100% (35/35)
BCOR	S1740del	DEL < 15bp	59.4	(47.0, 74.2)	5.2	8.7	100% (35/35)
BCORL1	P1681Qfs*20	DEL < 15bp	18.6	(14.0, 23.0)	2.6	13.8	97.1% (34/35)
BCR	A1153S	SNV	18.1	(14.0, 20.7)	1.8	9.9	97.1% (34/35)
BRAF	V600E	SNV	30.5	(23.1, 39.7)	4.1	13.4	100% (35/35)
BRCA1	Splice Site	SNV	1.2	(1.2, 1.2)	N/A	N/A	2.9% (1/35)
BRCA1	Splice Site	SNV	0.9	(0.9, 0.9)	N/A	N/A	2.9% (1/35)
BRCA1	Splice Site	SNV	0.8	(0.8, 0.8)	N/A	N/A	2.9% (1/35)
BRCA2	R118C	SNV	18.6	(18.3, 18.8)	0.4	1.9	5.7% (2/35)
CASP8	I392Sfs*4	DEL < 15bp	14.8	(13.3, 20.4)	2.8	18.9	17.1% (6/35)
CCNE1	M16V	SNV	7.5	(6.4, 10.4)	1.1	14.3	71.4% (25/35)
CHD2	E480Gfs*24	INS < 15bp	29.2	(21.2, 37.4)	3.7	12.7	100% (35/35)
CTNNA1	L785I	SNV	8.6	(6.1, 11.7)	1.3	15.2	100% (35/35)
CTNNB1	C439Y	SNV	29.4	(25.3, 32.5)	1.8	6.1	100% (35/35)
DOT1L	G555D	SNV	29.9	(22.4, 34.9)	3.0	9.9	100% (35/35)
EPHA2	T922M	SNV	6.7	(6.2, 7.1)	0.4	5.8	11.4% (4/35)
EPHA3	V543I	SNV	28.7	(19.9, 41.0)	4.4	15.4	100% (35/35)
EPHA5	K978T	SNV	12.4	(8.2, 18.5)	2.6	21.1	97.1% (34/35)

EPHA5	S359Lfs*63	DEL < 15bp	26.0	(20.2, 37.7)	4.0	15.5	97.1% (34/35)
EPHB1	F479S	SNV	29.7	(23.2, 37.9)	3.2	10.7	100% (35/35)
ERCC4	Splice Site	SNV	26.0	(18.5, 35.1)	4.1	15.9	100% (35/35)
ERCC4	M361Wfs*15	DEL < 15bp	13.3	(12.0, 14.9)	1.5	11.1	8.6% (3/35)
ETV5	A458V	SNV	27.9	(24.7, 32.0)	2.1	7.5	100% (35/35)
FAM46C	A75T	SNV	12.8	(10.1, 16.0)	1.4	10.7	100% (35/35)
FGF19	D178G	SNV	7.2	(6.2, 8.9)	0.8	11.4	42.9% (15/35)
GATA3	S237Afs*29	DEL < 15bp	12.2	(12.1, 12.3)	0.1	0.8	8.6% (3/35)
GPR124	R208H	SNV	8.2	(6.2, 12.8)	1.4	17.6	97.1% (34/35)
IGF1R	A263T	SNV	6.9	(6.2, 8.1)	0.6	8.9	51.4% (18/35)
IGF2R	M1486V	SNV	29.5	(25.3, 33.6)	2.0	6.7	100% (35/35)
JAK3	A810V	SNV	30.6	(25.1, 34.4)	2.6	8.5	100% (35/35)
KMT2A	P773Rfs*8	DEL < 15bp	15.6	(13.9, 17.5)	1.8	11.8	11.4% (4/35)
KMT2C	G146*	SNV	28.6	(19.3, 37.3)	4.0	14.0	100% (35/35)
KMT2D	P2354Lfs*30	DEL < 15bp	34.2	(26.9, 40.6)	3.5	10.1	100% (35/35)
LRP1B	T2407S	SNV	25.7	(19.0, 34.3)	3.7	14.3	100% (35/35)
MAP3K13	S941L	SNV	9.0	(8.4, 9.9)	0.8	8.6	8.6% (3/35)
MLH3	N674Ifs*6	DEL < 15bp	18.3	(16.9, 24.4)	2.4	13.2	25.7% (9/35)
MRE11A	R604C	SNV	27.4	(16.5, 35.1)	4.5	16.3	100% (35/35)
MSH3	N385Qfs*19	INS < 15bp	30.8	(23.9, 38.6)	3.9	12.8	100% (35/35)
MSH3	K383Rfs*32	DEL < 15bp	15.1	(9.0, 24)	4.0	26.2	57.1% (20/35)
MTOR	M2327V	SNV	30.1	(21.9, 38.3)	4.3	14.4	100% (35/35)
MTOR	P728H	SNV	7.6	(7.6, 7.6)	N/A	N/A	2.9% (1/35)
NBN	R466Kfs*5	INS < 15bp	27.1	(18.0, 37.3)	4.5	16.7	100% (35/35)
NF1	R2450*	SNV	27.1	(19.2, 36.7)	4.7	17.5	100% (35/35)
PALB2	N280Tfs*8	DEL < 15bp	31.4	(21.3, 40.7)	4.5	14.3	100% (35/35)
PIK3CA	R88Q	SNV	28.3	(17.7, 39.0)	5.3	18.7	100% (35/35)
PIK3CG	V745Sfs*47	DEL < 15bp	12.7	(12.7, 12.7)	N/A	N/A	2.9% (1/35)

PIK3R1	S690Y	SNV	30.2	(23.8, 44.8)	3.7	12.1	100% (35/35)
PRKAR1A	L68P	SNV	7.5	(6.5, 8.3)	0.6	8.5	28.6% (10/35)
PTEN	R173C	SNV	26.1	(16.7, 34.6)	4.0	15.2	100% (35/35)
PTEN	R173H	SNV	31.8	(21.1, 45.4)	4.5	14.1	100% (35/35)
PTPRT	A784T	SNV	28.4	(22.2, 36.6)	2.9	10.3	100% (35/35)
QKI	K134Rfs*14	DEL < 15bp	13.7	(13.4, 14.0)	0.4	3.1	5.7% (2/35)
RANBP2	D1965Rfs*21	INS < 15bp	30.4	(21.6, 41.2)	4.7	15.5	100% (35/35)
RET	R833C	SNV	30.0	(24.7, 37.4)	2.9	9.8	100% (35/35)
RNF43	G659Vfs*41	DEL < 15bp	68.5	(63.1, 79.6)	3.8	5.5	100% (35/35)
RPA1	L547I	SNV	25.3	(19.0, 35.2)	4.2	16.4	100% (35/35)
RPS6KB2	T170M	SNV	46.5	(40.3, 51.2)	2.3	5.0	100% (35/35)
SDHB	E95del	DEL < 15bp	30.8	(21.6, 43.8)	5.1	16.7	97.1% (34/35)
SETD2	T1652Lfs*12	INS < 15bp	26.7	(19.4, 37.6)	4.4	16.6	100% (35/35)
SH2D1A	P38S	SNV	19.5	(15.8, 22.8)	1.9	9.7	100% (35/35)
SMO	P694Lfs*82	DEL < 15bp	33.9	(28.4, 41.7)	4.0	11.9	54.3% (19/35)
SRC	T250M	SNV	6.7	(6.0, 8.4)	0.7	10.3	40.0% (14/35)
TEK	G896D	SNV	29.0	(24.5, 35.2)	2.7	9.2	100% (35/35)
TGFBR2	K153Afs*3	DEL < 15bp	32.4	(24.5, 41.8)	3.9	12.2	100% (35/35)
TLR9	N586D	SNV	29.0	(23.5, 32.5)	2.2	7.5	100% (35/35)
TP53	L252del	DEL < 15bp	13.2	(9.8, 19.5)	1.9	14.2	88.6% (31/35)
TP53	R273C	SNV	2.2	(2.2, 2.2)	N/A	N/A	2.9% (1/35)
TP53	K382Nfs*?	DEL < 15bp	12.4	(12.2, 12.7)	0.4	2.8	5.7% (2/35)
TP53	R175H	SNV	2.2	(2.2, 2.2)	N/A	N/A	2.9% (1/35)
TSC2	G62W	SNV	12.2	(8.2, 16.6)	2.1	17.4	100% (35/35)
TSC2	F1510del	DEL < 15bp	50.6	(42.0, 67.0)	4.2	8.3	100% (35/35)
WHSC1	P1343Qfs*?	DEL < 15bp	34.8	(26.1, 40.4)	3.0	8.7	85.7% (30/35)
XRCC1	V108I	SNV	9.4	(7.3, 12.7)	1.5	16.1	100% (35/35)
YES1	L57F	SNV	29.5	(22.1, 39.5)	3.7	12.4	100% (35/35)

AKT3	L208*	DEL < 15bp	34.6	(23.6, 49.4)	7.1	20.6	100% (36/36)
AKT3	K273E	SNV	9.4	(9.3, 9.5)	0.1	1.5	5.6% (2/36)
ALK	A1553Pfs*5	DEL < 15bp	38.6	(33.7, 42.8)	2.2	5.7	100% (36/36)
ASXL1	H631N	SNV	37.8	(35.0, 40.5)	1.6	4.2	100% (36/36)
ATM	K482Nfs*14	DEL < 15bp	8.1	(5.6, 12.0)	1.6	19.6	83.3% (30/36)
AXIN1	V835Wfs*?	DEL < 15bp	18.8	(15.2, 21.3)	1.7	9.2	94.4% (34/36)
AXIN1	F102S	SNV	7.9	(6.0, 9.5)	1.0	12.1	100% (36/36)
AXIN2	G546Afs*143	DEL < 15bp	12.1	(12.1, 12.1)	N/A	N/A	2.8% (1/36)
BLM	N515Mfs*16	DEL < 15bp	65.3	(55.3, 74.7)	4.7	7.3	100% (36/36)
BRAF	V600E	SNV	34.9	(21.0, 44.8)	4.9	13.9	100% (36/36)
BRD4	T658I	SNV	8.2	(6.9, 10.4)	0.9	10.5	94.4% (34/36)
C11orf30	R68L	SNV	33.1	(23.8, 43.8)	4.5	13.5	100% (36/36)
C11orf30	T1088M	SNV	8.3	(6.4, 10.6)	1.1	13.5	88.9% (32/36)
CALR	D199V	SNV	35.2	(32.8, 41.2)	1.6	4.6	100% (36/36)
CBL	T568I	SNV	6.8	(5.9, 9.2)	0.8	11.5	55.6% (20/36)
CCNE1	E311G	SNV	34.9	(30.2, 38.7)	1.8	5.2	100% (36/36)
CD276	A105T	SNV	34.4	(31.4, 37.6)	1.6	4.8	100% (36/36)
CIC	A1487S	SNV	36.6	(32.3, 41.4)	2.2	5.9	100% (36/36)
CREBBP	P1423Lfs*36	DEL < 15bp	33.6	(29.3, 38.3)	2.1	6.1	100% (36/36)
CREBBP	R413*	SNV	37.3	(29.6, 41.6)	2.6	6.9	100% (36/36)
CREBBP	R1443C	SNV	7.4	(6.8, 9.0)	0.8	11.0	16.7% (6/36)
CSF1	V434M	SNV	35.3	(32.4, 38.7)	1.5	4.3	100% (36/36)
CXCR4	L8Cfs*21	DEL < 15bp	7.2	(5.3, 9.2)	1.3	17.8	41.7% (15/36)
DDR2	V603A	SNV	39.8	(35.0, 48.4)	2.7	6.9	100% (36/36)
DNMT1	G1605D	SNV	35.1	(31.7, 39.4)	1.9	5.3	100% (36/36)
EPHA2	P294H	SNV	7.2	(6.1, 9.4)	0.7	9.3	97.2% (35/36)
ERBB3	H1304Mfs*7	DEL < 15bp	36.0	(31.6, 39.7)	2.0	5.7	100% (36/36)
FANCG	A514V	SNV	6.8	(6.0, 9.5)	0.8	11.3	72.2% (26/36)

FANCI	V383I	SNV	40.5	(32.2, 47.9)	3.9	9.7	100% (36/36)
FAT1	V2616I	SNV	7.2	(6.3, 9.8)	0.8	10.8	61.1% (22/36)
FGFR1	A625T	SNV	34.2	(29.6, 37.6)	1.9	5.7	100% (36/36)
FLT1	G896R	SNV	10.6	(8.3, 12.5)	1.0	9.7	100% (36/36)
GATA4	E147K	SNV	35.9	(27.3, 43.5)	4.1	11.4	94.4% (34/36)
GRIN2A	D137G	SNV	35.7	(32.2, 40.1)	2.0	5.5	100% (36/36)
GSK3B	E379*	SNV	7.9	(7.0, 10.5)	1.5	18.6	13.9% (5/36)
INSR	R819H	SNV	35.5	(32.5, 39.8)	1.6	4.6	100% (36/36)
IRS2	A887V	SNV	34.0	(28.7, 40.2)	2.4	6.9	100% (36/36)
JAK1	E637D	SNV	35.7	(32.2, 39.1)	1.6	4.4	100% (36/36)
KAT6A	Q1600K	SNV	36.3	(30.0, 40.5)	2.0	5.4	100% (36/36)
KAT6A	D1114Rfs*2	INS < 15bp	33.9	(23.6, 42.7)	5.1	15.0	100% (36/36)
KAT6A	R765*	SNV	11.8	(7.9, 14.7)	1.4	12.2	100% (36/36)
KMT2C	R2609Q	SNV	36.2	(32.6, 40.2)	1.8	5.1	100% (36/36)
KMT2D	E5161D	SNV	8.1	(6.3, 9.8)	0.8	9.5	100% (36/36)
MAGI2	M1?	SNV	7.4	(6.7, 9.8)	0.8	11.4	33.3% (12/36)
MCL1	A84T	SNV	50.6	(46.0, 56.9)	2.1	4.1	100% (36/36)
MEF2B	T274Pfs*?	DEL < 15bp	50.3	(43.6, 56.1)	3.6	7.2	36.1% (13/36)
MERTK	F230Sfs*20	DEL < 15bp	7.9	(6.0, 10.8)	1.2	15.1	100% (36/36)
MRE11A	N511Ifs*13	DEL < 15bp	36.7	(28.9, 48.8)	4.9	13.3	100% (36/36)
MSH3	N385Qfs*19	INS < 15bp	15.7	(14.2, 18.5)	1.3	8.3	50.0% (18/36)
MSH6	F1088Sfs*2	DEL < 15bp	45.2	(39.9, 57)	3.4	7.5	91.7% (33/36)
MST1R	Q932H	SNV	59.1	(54.1, 63.1)	2.3	3.9	100% (36/36)
MUTYH	A55V	SNV	37.6	(34.4, 41.3)	1.6	4.3	100% (36/36)
NCOA3	A420T	SNV	7.7	(6.4, 9.6)	0.8	10.7	100% (36/36)
NCOA3	Splice Site	SNV	31.9	(28.3, 37.2)	1.9	5.8	100% (36/36)
NCOR1	P1197H	SNV	35.1	(30.7, 39.2)	2.2	6.2	100% (36/36)
NFKBIA	L148Yfs*16	DEL < 15bp	35.9	(32.3, 40.6)	1.9	5.2	100% (36/36)

NSD1	T725M	SNV	45.7	(34.6, 55.4)	4.1	8.9	100% (36/36)
NTRK3	V21I	SNV	74.5	(69.6, 78.2)	2.1	2.8	100% (36/36)
PARK2	V330Rfs*17	INS < 15bp	36.8	(34.5, 42.0)	1.5	4.0	100% (36/36)
PARP3	Splice Site	SNV	58.8	(55.0, 69.1)	2.9	4.9	100% (36/36)
PAX5	A322Lfs*11	DEL < 15bp	35.6	(30.9, 42.1)	2.8	7.7	97.2% (35/36)
PAX8	V314M	SNV	36.8	(32.7, 42.5)	2.0	5.4	100% (36/36)
PHOX2B	R71G	SNV	35.7	(31.2, 39.2)	1.8	5.0	100% (36/36)
PIK3CB	K886E	SNV	50.2	(44.7, 59.5)	3.2	6.3	100% (36/36)
PRKDC	A2584V	SNV	36.8	(27.8, 44.4)	3.9	10.7	100% (36/36)
PRKDC	A2293V	SNV	33.2	(27.7, 37.7)	2.4	7.3	100% (36/36)
PRKDC	P1262L	SNV	47.6	(44.6, 50.6)	1.6	3.4	100% (36/36)
PTCH1	Y847H	SNV	33.6	(28.7, 39.6)	2.5	7.6	100% (36/36)
RAD54B	S239del	DEL < 15bp	48.2	(36.2, 58.5)	4.9	10.2	100% (36/36)
RANBP2	K2433Sfs*3	INS ≥ 15bp	11.3	(9.5, 12.7)	1.6	14.4	11.1% (4/36)
RANBP2	K2433Sfs*3	INS ≥ 15bp	10.4	(10.4, 10.4)	N/A	N/A	2.8% (1/36)
RNF43	G659Vfs*41	DEL < 15bp	82.0	(76.1, 88.1)	3.1	3.8	100% (36/36)
SMAD2	D300V	SNV	32.6	(26.3, 39.3)	3.3	10.0	100% (36/36)
SOX9	V313Lfs*?	DEL ≥ 15bp	59.5	(53.5, 68.9)	3.2	5.3	100% (36/36)
SPEN	P3120H	SNV	9.6	(7.6, 12.2)	1.1	11.6	100% (36/36)
STAG2	R1133Q	SNV	56.9	(49.5, 65.4)	4.1	7.2	100% (36/36)
SYK	M166Cfs*18	DEL < 15bp	39.0	(34.8, 44.2)	2.6	6.6	100% (36/36)
TERT	A242T	SNV	31.5	(26.7, 35.1)	2.2	7.0	100% (36/36)
TERT	Promoter	SNV	2.1	(2.1, 2.1)	N/A	N/A	2.8% (1/36)
TGFBR2	W10*	SNV	54.8	(45.5, 62.5)	3.5	6.4	100% (36/36)
TLR4	Q562Tfs*9	INS < 15bp	35.8	(31.4, 45.6)	3.4	9.5	100% (36/36)
TP53	Splice Site	SNV	36.9	(32.7, 40.6)	1.6	4.3	100% (36/36)
TP53	Splice Site	SNV	37.8	(34.5, 41.9)	1.9	5.0	100% (36/36)
WRN	C539Y	SNV	30.8	(23.4, 41.7)	4.5	14.7	100% (36/36)

WT1	P110L	SNV	37.2	(31.4, 47.7)	3.4	9.2	97.2% (35/36)
XPC	R579Q	SNV	12.1	(9.4, 15.8)	1.5	12.4	100% (36/36)
XPO1	A41V	SNV	29.6	(20.1, 36.9)	4.0	13.4	100% (36/36)
XRCC2	L90F	SNV	33.0	(22.5, 40.5)	3.8	11.6	100% (36/36)
ARID1A	D1850Tfs*33	DEL < 15bp	33.4	(27.6, 37.9)	2.1	6.4	100% (36/36)
ARID1A	T294Pfs*69	DEL < 15bp	31.8	(28.2, 36.2)	1.9	5.9	83.3% (30/36)
ASXL1	G660D	SNV	29.1	(26.1, 34.5)	1.8	6.1	100% (36/36)
BRAF	V600L	SNV	1.3	(1.3, 1.3)	N/A	N/A	2.8% (1/36)
BRCA1	A942V	SNV	29.9	(22.0, 36.2)	3.1	10.5	100% (36/36)
BRCA2	E2226Sfs*6	DEL < 15bp	47.0	(38.3, 57.8)	4.3	9.1	100% (36/36)
BRIP1	V76I	SNV	23.8	(17.7, 31.5)	3.1	13.1	100% (36/36)
CDH1	R868H	SNV	48.4	(41.5, 53.3)	2.6	5.3	100% (36/36)
CIC	P509Hfs*14	DEL < 15bp	32.0	(26.6, 36.2)	2.2	6.8	100% (36/36)
CTCF	T317Rfs*91	DEL < 15bp	27.2	(21.6, 31.7)	2.5	9.3	100% (36/36)
DICER1	E1705K	SNV	29.6	(25.3, 35.3)	2.2	7.3	100% (36/36)
EPHA2	K343*	SNV	29.7	(25.3, 35.0)	2.0	6.6	100% (36/36)
ERBB3	R81Q	SNV	30.6	(24.4, 37.3)	2.6	8.6	100% (36/36)
ERBB3	D112Y	SNV	28.8	(24.0, 32.4)	1.9	6.6	100% (36/36)
FGFR4	P528Qfs*53	DEL < 15bp	29.9	(25.6, 36.0)	1.9	6.4	100% (36/36)
GLI1	G274Afs*6	DEL < 15bp	27.5	(24.1, 33.3)	2.1	7.8	100% (36/36)
JAK1	K860Nfs*16	DEL < 15bp	27.5	(19.5, 34.3)	3.2	11.7	100% (36/36)
JAK1	P861Tfs*4	INS < 15bp	21.8	(16.7, 28.4)	2.4	11.0	100% (36/36)
KMT2A	P773Rfs*8	DEL < 15bp	13.7	(13.7, 13.7)	N/A	N/A	2.8% (1/36)
KMT2D	V160M	SNV	26.8	(22.5, 31.7)	2.1	7.9	100% (36/36)
KRAS	G12C	SNV	22.6	(17.4, 30.2)	3.5	15.3	100% (36/36)
MAP3K13	S331*	DEL < 15bp	30.6	(23.7, 35.3)	2.6	8.6	100% (36/36)
MED12	V151L	SNV	32.0	(27.5, 38.5)	2.2	7.0	100% (36/36)
MED12	G391S	SNV	20.9	(16.2, 26.4)	2.2	10.6	100% (36/36)

MTOR	A89D	SNV	26.0	(18.6, 33.0)	3.2	12.1	100% (36/36)
NKX3-1	P221Hfs*?	DEL < 15bp	27.1	(22.9, 30.7)	1.9	6.9	100% (36/36)
NSD1	E1853Sfs*2	DEL < 15bp	29.3	(22.4, 37.8)	4.2	14.2	97.2% (35/36)
PIK3R1	T576del	DEL < 15bp	24.9	(19.3, 32.9)	2.9	11.8	100% (36/36)
PNRC1	Q71Sfs*112	DEL < 15bp	35.1	(30.7, 39.2)	2.0	5.6	91.7% (33/36)
PTEN	C124S	SNV	54.7	(48.7, 61.2)	3.4	6.2	100% (36/36)
ABL1	L522F	SNV	35.8	(32.0, 44.2)	2.2	6.2	100% (36/36)
ABL1	R785Gfs*3	DEL < 15bp	21.4	(16.8, 25.2)	2.0	9.5	100% (36/36)
ARAF	R30C	SNV	67.5	(62.0, 73.7)	3.0	4.5	100% (36/36)
ARID1A	G314Afs*49	DEL < 15bp	37.7	(28.1, 42.6)	3.2	8.6	100% (36/36)
ATR	M2324V	SNV	33.6	(22.1, 44.1)	5.3	15.9	100% (36/36)
AXIN2	G665Afs*24	DEL < 15bp	14.0	(12.4, 15.9)	1.1	7.7	86.1% (31/36)
B2M	L15Ffs*41	DEL < 15bp	69.3	(65.7, 75.2)	2.3	3.4	100% (36/36)
BARD1	M768V	SNV	34.6	(29.4, 40.4)	2.3	6.5	100% (36/36)
BCOR	P326L	SNV	99.6	(98.3, 100)	0.4	0.4	100% (36/36)
BCORL1	A74Qfs*42	DEL < 15bp	21.0	(15.1, 33.1)	3.7	17.4	69.4% (25/36)
BLM	A1203V	SNV	33.6	(26.9, 41.0)	3.4	10.2	100% (36/36)
BRAF	V600E	SNV	41.4	(31.8, 47.2)	3.1	7.4	100% (36/36)
BRCA2	L3055I	SNV	30.9	(25.4, 35.7)	2.7	8.7	100% (36/36)
BRD4	P1184S	SNV	31.7	(25.6, 39.1)	2.7	8.4	100% (36/36)
BTG2	A82V	SNV	33.0	(28.9, 36.1)	1.9	5.7	100% (36/36)
CARD11	R555Efs*37	DEL < 15bp	42.0	(32.7, 47.8)	3.6	8.5	94.4% (34/36)
CDH1	P126Rfs*89	DEL < 15bp	38.1	(31.9, 43.1)	2.8	7.5	88.9% (32/36)
CDH1	E243del	DEL < 15bp	19.6	(16.5, 24.2)	2.1	10.8	100% (36/36)
CREBBP	T1688M	SNV	35.8	(30.5, 41.6)	2.2	6.3	100% (36/36)
CREBBP	R1446H	SNV	7.4	(7.0, 7.6)	0.2	3.4	13.9% (5/36)
CTLA4	L28Sfs*32	INS < 15bp	31.0	(27.4, 39.2)	2.3	7.5	100% (36/36)
CTNNA1	L598P	SNV	34.0	(28.3, 40.2)	2.3	6.9	100% (36/36)

DICER1	G1097R	SNV	32.9	(26.7, 37.2)	3.0	9.1	100% (36/36)
DIS3	R396W	SNV	34.3	(27.6, 40.9)	2.9	8.3	100% (36/36)
EP300	Splice Site	SNV	33.2	(26.5, 39.3)	2.6	7.7	100% (36/36)
EPAS1	C479S	SNV	12.3	(8.4, 15.9)	1.6	13.3	100% (36/36)
EPHB1	R682H	SNV	36.0	(31.0, 41.0)	2.5	6.9	100% (36/36)
ERBB3	L917W	SNV	8.3	(6.4, 10.3)	0.9	11.1	97.2% (35/36)
ERBB4	G223R	SNV	33.7	(28.5, 41.1)	2.7	8.2	100% (36/36)
ERCC1	A199T	SNV	8.1	(6.4, 12.4)	1.2	14.9	91.7% (33/36)
FAM175A	C144R	SNV	9.7	(7.6, 13.3)	1.5	15.8	75.0% (27/36)
FANCL	L219F	SNV	11.2	(8.0, 19.3)	2.5	22.7	100% (36/36)
FANCM	V1336Lfs*2	DEL < 15bp	36.1	(28.7, 47.7)	4.1	11.4	100% (36/36)
FAT1	V2927M	SNV	20.1	(14.2, 24.5)	2.3	11.2	100% (36/36)
FBXW7	D440Sfs*55	DEL < 15bp	28.4	(21.8, 34)	2.7	9.5	100% (36/36)
FGFR4	P528Qfs*53	DEL < 15bp	38.5	(31.5, 44.3)	2.8	7.3	100% (36/36)
FLCN	D300V	SNV	33.3	(26.4, 40.0)	2.7	8.1	100% (36/36)
FLT4	P1077T	SNV	33.1	(27.3, 38.0)	2.4	7.2	100% (36/36)
FOXP1	V515Gfs*14	DEL < 15bp	38.1	(32.5, 46.8)	3.6	9.4	69.4% (25/36)
FOXP1	G422C	SNV	21.6	(17.2, 27.2)	1.9	8.9	100% (36/36)
FUBP1	S11Lfs*43	DEL < 15bp	38.3	(32.9, 44.1)	2.9	7.5	41.7% (15/36)
GLI1	Splice Site	SNV	25.7	(22.7, 29.7)	1.8	7.0	100% (36/36)
HGF	G557del	DEL < 15bp	27.4	(20.9, 31.9)	2.4	8.8	100% (36/36)
HNF1A	E79G	SNV	25.5	(20.0, 29.1)	1.8	6.9	100% (36/36)
HRAS	A59T	SNV	33.6	(27.1, 37.3)	2.3	6.7	100% (36/36)
INHBA	L230M	SNV	29.9	(24.3, 36.1)	2.6	8.8	100% (36/36)
INPP4B	G187W	SNV	32.1	(26.3, 39.9)	2.6	8.0	100% (36/36)
KDM6A	A422V	SNV	67.9	(58.8, 78.6)	4.6	6.7	100% (36/36)
KLF4	A472T	SNV	7.4	(6.5, 8.4)	0.7	9.0	63.9% (23/36)
KMT2A	L3131I	SNV	11.4	(9.3, 13.9)	1.4	11.9	97.2% (35/36)

KMT2C	I1344Nfs*11	INS < 15bp	29.5	(23.9, 36.0)	3.1	10.4	100% (36/36)
KMT2D	R5533Q	SNV	25.1	(19.1, 30.7)	2.4	9.6	100% (36/36)
KMT2D	Q2416Sfs*10	DEL < 15bp	7.3	(5.6, 8.9)	1.3	18.1	27.8% (10/36)
LRP1B	N4135Mfs*14	DEL < 15bp	34.6	(26.1, 43.8)	4.1	12.0	100% (36/36)
LRP1B	Y4129Wfs*5	DEL < 15bp	34.3	(24.5, 41.2)	4.1	11.8	97.2% (35/36)
LYN	E110Kfs*15	DEL < 15bp	21.9	(16.0, 27.1)	2.4	10.8	100% (36/36)
LZTR1	G19D	SNV	34.4	(30.3, 38.6)	2.2	6.5	100% (36/36)
MAGI2	E1234K	SNV	17.7	(13.7, 21.5)	1.8	10.4	100% (36/36)
MLH1	K196Nfs*6	DEL < 15bp	14.9	(12.0, 20.9)	3.2	21.5	25.0% (9/36)
MLH3	N800K	SNV	33.8	(26.0, 39.6)	3.2	9.4	100% (36/36)
MSH3	K383Rfs*32	DEL < 15bp	7.1	(7.1, 7.1)	N/A	N/A	2.8% (1/36)
MST1R	T1297I	SNV	22.2	(18.2, 26.2)	1.7	7.8	100% (36/36)
MUTYH	R365Gfs*40	DEL < 15bp	39.6	(33.0, 43.1)	2.6	6.6	100% (36/36)
MYC	E42del	DEL < 15bp	35.3	(29.6, 40.4)	2.4	6.8	100% (36/36)
MYCN	S336Lfs*15	DEL < 15bp	38.4	(33.4, 44.6)	2.6	6.8	100% (36/36)
NCOA3	T357I	SNV	40.1	(32.5, 49.1)	3.5	8.8	100% (36/36)
NFE2L2	Start Variant	DEL < 15bp	33.9	(20.7, 41.7)	3.8	11.3	91.7% (33/36)
NOTCH2	S1419Afs*8	DEL < 15bp	40.6	(33.7, 45.5)	2.6	6.5	100% (36/36)
NUTM1	A8T	SNV	37.8	(32.7, 42.7)	2.4	6.3	100% (36/36)
PARK2	N428Mfs*7	DEL < 15bp	14.1	(12.1, 17.6)	1.4	10.0	61.1% (22/36)
PDCD1LG2	P186Lfs*14	DEL < 15bp	12.4	(12.4, 12.4)	N/A	N/A	2.8% (1/36)
PIK3CD	F912Lfs*30	DEL < 15bp	13.2	(10.3, 16.1)	1.9	14.7	47.2% (17/36)
PIK3R2	K503E	SNV	33.0	(30.5, 37.9)	1.6	4.8	100% (36/36)
PTPRD	P403Lfs*7	DEL < 15bp	35.5	(29, 48.6)	3.3	9.4	100% (36/36)
PTPRD	R139H	SNV	31.2	(26.5, 36.4)	2.4	7.6	100% (36/36)
PTPRS	G1466R	SNV	34.9	(30.2, 44)	2.6	7.5	100% (36/36)
RAC1	T79M	SNV	9.3	(6.8, 13.5)	1.7	17.8	86.1% (31/36)
RET	T742M	SNV	18.1	(15.8, 19.9)	1.2	6.6	69.4% (25/36)

RICTOR	Q657H	SNV	34.5	(25.5, 38.7)	3.0	8.8	100% (36/36)
RNF43	G659Vfs*41	DEL < 15bp	81.1	(70.9, 87.4)	3.5	4.3	100% (36/36)
RNF43	R117Pfs*41	DEL < 15bp	23.1	(19.4, 28.0)	2.1	9.3	66.7% (24/36)
RNF43	R117S	SNV	16.5	(13.6, 20.0)	1.5	9.4	97.2% (35/36)
RNF43	R117Tfs*41	DEL < 15bp	19.7	(19.7, 19.7)	N/A	N/A	2.8% (1/36)
RNF43	C290*	SNV	7.3	(7.3, 7.3)	N/A	N/A	2.8% (1/36)
SMARCB1	D367N	SNV	34.7	(29.1, 39.5)	2.2	6.2	100% (36/36)
SOX2	E78V	SNV	12.0	(9.0, 14.7)	1.3	11.0	100% (36/36)
SOX2	P284L	SNV	35.5	(31.2, 39.4)	2.0	5.8	100% (36/36)
SOX9	Q439*	SNV	75.1	(72.5, 81.6)	1.8	2.4	100% (36/36)
STAT4	C436Lfs*27	INS < 15bp	19.4	(13.2, 27.9)	2.8	14.3	100% (36/36)
TET2	D945N	SNV	33.3	(28.4, 37.0)	2.0	6.0	100% (36/36)
TGFBR2	K153Afs*3	DEL < 15bp	41.8	(36.3, 49.0)	2.9	7.0	100% (36/36)
TLR9	M58V	SNV	7.2	(6.2, 9.0)	0.7	10.3	72.2% (26/36)
TOP1	K558R	SNV	33.2	(24.5, 39.9)	3.2	9.8	100% (36/36)
XPC	A2D	SNV	7.6	(7.6, 7.6)	N/A	N/A	2.8% (1/36)
ADORA2A	V172I	SNV	29.9	(25.3, 33.6)	1.6	5.3	100% (36/36)
ARID1A	L2089P	SNV	29.3	(25.8, 33.2)	1.6	5.5	100% (36/36)
ARID1B	K2088N	SNV	35.6	(30.9, 39.5)	1.7	4.9	100% (36/36)
ARID2	A1729V	SNV	27.5	(20.7, 37.0)	3.6	13.1	100% (36/36)
BARD1	E652Vfs*69	INS ≥ 15bp	7.8	(5.5, 11.5)	1.8	23.2	80.6% (29/36)
BCORL1	G1170E	SNV	30.4	(24.5, 34.4)	2.2	7.1	100% (36/36)
BRAF	V600K	SNV	57.9	(52.2, 64.8)	2.7	4.7	100% (36/36)
BRCA2	Splice Site	SNV	2.2	(2.2, 2.2)	N/A	N/A	2.8% (1/36)
DAXX	R260C	SNV	31.0	(28.2, 35.2)	1.5	5.0	100% (36/36)
DNMT3A	S337*	SNV	10.3	(6.7, 14.5)	1.6	15.5	100% (36/36)
DNMT3B	S20L	SNV	20.5	(17.1, 25)	1.5	7.3	100% (36/36)

DOT1L	K401M	SNV	26.1	(21.3, 30.1)	1.9	7.3	100% (36/36)
EPHA7	R811Q	SNV	43.7	(33.0, 52.0)	4.2	9.6	100% (36/36)
GSK3B	L187F	SNV	28.1	(21.3, 35.0)	3.2	11.4	100% (36/36)
HSD3B1	V224_Y225insH	INS < 15bp	39.0	(34.9, 43.5)	1.9	5.0	100% (36/36)
IGF2R	G356V	SNV	30.3	(26.1, 38.1)	3.1	10.2	100% (36/36)
IKZF1	E16K	SNV	19.4	(16.4, 21.9)	1.4	7.1	100% (36/36)
IRS2	P790L	SNV	14.5	(11.9, 19.2)	1.4	9.7	100% (36/36)
KDR	W570*	SNV	34.1	(27.0, 40.7)	2.8	8.1	100% (36/36)
KMT2D	P4360L	SNV	22.3	(19.3, 25.7)	1.5	6.7	100% (36/36)
MAP3K13	P38S	SNV	30.4	(26.2, 33.9)	2.1	6.9	100% (36/36)
MSH2	F394L	SNV	20.8	(16.6, 26.3)	2.4	11.6	100% (36/36)
MSH3	A61_A62insAPPAPP	INS ≥ 15bp	6.9	(6.9, 6.9)	N/A	N/A	2.8% (1/36)
NOTCH3	G1631R	SNV	28.8	(17.7, 42.7)	3.9	13.4	100% (36/36)
NOTCH3	G974K	SNV	38.1	(34.3, 42.4)	2.2	5.7	100% (36/36)
PDGFRB	D688N	SNV	13.7	(11.3, 17.4)	1.4	10.2	100% (36/36)
PREX2	R1080K	SNV	28.7	(23.7, 34.4)	2.7	9.5	100% (36/36)
PTPRT	M1397I	SNV	19.8	(17.2, 22.6)	1.3	6.5	100% (36/36)
RNF43	C511*	SNV	54.7	(50.1, 56.8)	1.4	2.6	100% (36/36)
ROS1	S1705L	SNV	17.0	(10.5, 28.7)	3.8	22.4	100% (36/36)
RUNX1T1	R42C	SNV	30.7	(24.9, 36.4)	2.5	8.1	100% (36/36)
SETD2	Splice Site	SNV	26.6	(21.2, 34.6)	3.0	11.2	100% (36/36)
SNCAIP	E142K	SNV	15.1	(11.1, 18.4)	1.4	9.6	100% (36/36)
CSF1R	R144H	SNV	19.3	(18.2, 19.8)	0.7	3.4	13.9% (5/36)
FANCI	E247G	SNV	9.2	(9.2, 9.2)	N/A	N/A	2.8% (1/36)
KDM5A	G630E	SNV	51.3	(42.5, 56.3)	2.9	5.6	100% (36/36)
MAGI2	R1084*	SNV	19.0	(14.5, 26.3)	2.3	12.1	100% (36/36)
MRE11A	T426S	SNV	9.2	(9.2, 9.2)	N/A	N/A	2.8% (1/36)

MTOR	R281C	SNV	66.1	(52.9, 72.0)	3.4	5.1	100% (36/36)
NBN	E658G	SNV	45.9	(32.9, 60.4)	5.6	12.3	100% (36/36)
NOTCH1	G1892R	SNV	23.0	(20.0, 25.6)	1.2	5.3	100% (36/36)
NRAS	G13D	SNV	26.2	(19.4, 32.1)	3.0	11.5	100% (36/36)
BARD1	K596Nfs*9	DEL < 15bp	12.7	(12.7, 12.7)	N/A	N/A	2.8% (1/36)
BRCA2	W2574*	SNV	9.4	(4.1, 15.4)	2.3	24.5	100% (36/36)
BRIP1	V720A	SNV	9.4	(7.7, 12.8)	1.8	19.3	16.7% (6/36)
CSF1	L160I	SNV	20.7	(17.4, 28.1)	2.2	10.7	100% (36/36)
CTCF	R339Q	SNV	20.3	(15.3, 23.3)	1.9	9.2	100% (36/36)
DDR2	L229P	SNV	6.5	(6.0, 7.3)	0.5	7.5	16.7% (6/36)
HNF1A	A491T	SNV	10.9	(7.7, 14.1)	1.3	11.9	100% (36/36)
IRS1	M316T	SNV	10.7	(9.3, 12.9)	0.9	8.8	100% (36/36)
IRS2	R1146S	SNV	6.9	(6.1, 8.3)	0.6	8.3	83.3% (30/36)
KDM5A	G302E	SNV	13.7	(9.6, 21.3)	2.8	20.2	100% (36/36)
KMT2D	P4770H	SNV	34.2	(29.9, 38.2)	2.1	6.1	100% (36/36)
KMT2D	G3354W	SNV	37.6	(34.3, 40.8)	1.5	4.0	100% (36/36)
KRAS	G12C	SNV	27.4	(20.3, 35.5)	3.2	11.5	100% (36/36)
MAX	H28R	SNV	19.8	(12.9, 24)	2.2	11.3	100% (36/36)
MSH3	K383Rfs*32	DEL < 15bp	20.9	(15.1, 33.3)	3.9	18.7	86.1% (31/36)
NF2	I264S	SNV	10.5	(8.4, 12.9)	1.3	12.0	100% (36/36)
NOTCH4	P1484S	SNV	10.1	(8.1, 13.5)	1.1	10.6	100% (36/36)
PDGFRA	R718W	SNV	17.8	(8.4, 23.4)	2.7	14.9	100% (36/36)
PIK3R1	R574_L581del	DEL ≥ 15bp	8.6	(5.7, 11.9)	1.7	19.9	97.2 % (35/36)
PTEN	P95L	SNV	16.4	(9.8, 21.6)	3.1	19.1	97.2% (35/36)
PTEN	R130G	SNV	16.7	(11.9, 26.3)	3.1	18.4	100% (36/36)
SETD2	A2201T	SNV	37.0	(30.6, 40.7)	2.3	6.3	100% (36/36)
SPEN	R75H	SNV	7.8	(6.4, 9.8)	0.8	10.9	88.9% (32/36)

TAF1	A668Gfs*31	INS < 15bp	19.4	(16.9, 24)	1.9	9.8	80.6% (29/36)

Appendix E: Accuracy

Appendix E.1: Concordance for SNVs by Gene:

Gene	Number of exons	Number of unique mutations	Number of samples	PPA (%), 95% CI (%)	NPA (%), 95% CI (%)
ABL1	1	1	1	100% (20.65%, 100%)	100% (99.9%, 100%)
ABL2	2	4	7	0.0% (0.0%, 49.0%)	99.9% (99.9%, 99.9%)
ACVR1B	2	2	2	N/A	99.9% (99.8%, 99.9%)
AKT1	1	1	1	100% (20.65%, 100%)	100% (99.7%, 100%)
AKT2	1	1	1	100% (20.65%, 100%)	100% (99.7%, 100%)
AKT3	1	1	1	0.0% (0.0%, 79.4%)	100% (99.7%, 100%)
ALK	4	4	4	100% (51.01%, 100%)	100% (99.9%, 100%)
ALOX12B	1	1	1	N/A	99.9% (99.7%, 99.9%)
AMER1	1	2	2	N/A	99.9% (99.9%, 99.9%)
APC	5	18	15	77.8% (54.8%, 91.0%)	99.9% (99.9%, 99.9%)
AR	4	6	6	100% (51.01%, 100%)	99.9% (99.9%, 99.9%)
ARID1A	3	6	6	100% (60.96%, 100%)	100% (99.9%, 100%)
ARID1B	3	7	7	66.7% (30.0%, 90.3%)	99.9% (99.9%, 99.9%)
ARID2	5	6	6	100% (56.55%, 100%)	99.9% (99.9%, 99.9%)
ARID5B	1	1	1	N/A	99.9% (99.8%, 99.9%)
ASXL1	1	1	1	100% (20.65%, 100%)	100% (99.9%, 100%)
ASXL2	2	2	2	100% (20.65%, 100%)	99.9% (99.9%, 99.9%)
ATM	7	9	8	83.3% (43.6%, 97.0%)	99.9% (99.9%, 99.9%)

ATR	1	1	1	100% (20.65%, 100%)	99.9% (99.9%, 99.9%)
ATRX	2	5	5	75.0% (30.1%, 95.4%)	99.9% (99.9%, 99.9%)
AXIN1	2	2	2	100% (34.23%, 100%)	99.9% (99.9%, 99.9%)
AXL	5	5	5	100% (56.55%, 100%)	100% (99.9%, 100%)
BAP1	1	1	1	N/A	99.9% (99.7%, 99.9%)
BARD1	2	5	4	100% (51.01%, 100%)	99.9% (99.9%, 99.9%)
BBC3	1	1	1	0.0% (0.0%, 79.4%)	100% (99.3%, 100%)
BCL2L1	1	1	1	100% (20.65%, 100%)	100% (99.25%, 100%)
BCOR	1	1	1	100% (20.65%, 100%)	100% (99.9%, 100%)
BCORL1	2	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
BLM	1	1	1	N/A	99.9% (99.9%, 99.9%)
BMPR1A	1	1	1	100% (20.65%, 100%)	100% (99.7%, 100%)
BRAF	5	8	46	95.8% (79.8%, 99.3%)	99.9% (99.9%, 99.9%)
BRCA1	5	9	10	57.1% (25.0%, 84.2%)	99.9% (99.9%, 99.9%)
BRCA2	5	9	9	77.8% (45.3%, 93.7%)	100% (99.9%, 100%)
BRD4	2	2	2	0.0% (0.0%, 79.4%)	99.9% (99.9%, 99.9%)
BRIP1	2	3	3	0.0% (0.0%, 65.8%)	99.9% (99.9%, 99.9%)
C11orf30	0	1	1	0.0% (0.0%, 79.4%)	100% (99.9%, 100%)
CALR	1	1	1	N/A	99.9% (99.5%, 99.9%)
CARD11	1	1	1	100% (20.65%, 100%)	100% (99.9%, 100%)
CBL	3	3	3	100% (43.85%, 100%)	100% (99.9%, 100%)
CCND1	1	1	1	100% (20.65%, 100%)	100% (99.6%, 100%)
CD22	1	1	1	N/A	99.9% (99.8%, 99.9%)
CDH1	5	5	5	100% (51.01%, 100%)	99.9% (99.9%, 99.9%)

CDK12	2	2	2	100% (34.23%, 100%)	99.9% (99.9%, 99.9%)
CDK6	1	1	1	100% (20.65%, 100%)	100% (99.6%, 100%)
CDKN1A	1	1	1	100% (20.65%, 100%)	100% (99.22%, 100%)
CDKN1B	1	1	1	N/A	99.8% (99.1%, 99.9%)
CDKN2A	2	5	4	50.0% (15.0%, 85.0%)	99.9% (99.7%, 99.9%)
CEBPA	1	1	1	N/A	99.9% (99.7%, 99.9%)
CHD2	1	1	1	N/A	99.9% (99.9%, 99.9%)
CHD4	1	1	1	100% (20.65%, 100%)	99.9% (99.9%, 99.9%)
CHEK1	1	1	1	100% (20.65%, 100%)	100% (99.7%, 100%)
CIC	4	4	4	100% (51.01%, 100%)	99.9% (99.9%, 99.9%)
CREBBP	2	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
CRKL	1	1	1	100% (20.65%, 100%)	100% (99.6%, 100%)
CSF1R	1	2	2	0.0% (0.0%, 65.8%)	99.9% (99.9%, 99.9%)
CTCF	1	1	1	100% (20.65%, 100%)	100% (99.8%, 100%)
CTNNB1	3	4	4	100% (43.85%, 100%)	99.9% (99.9%, 99.9%)
CXCR4	1	3	3	100% (20.65%, 100%)	99.9% (99.8%, 99.9%)
CYLD	1	1	1	100% (20.65%, 100%)	100% (99.9%, 100%)
CYP17A1	1	1	1	N/A	99.9% (99.6%, 99.9%)
DAXX	2	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
DDR2	3	3	3	100% (34.23%, 100%)	99.9% (99.9%, 99.9%)
DICER1	2	2	2	100% (34.23%, 100%)	99.9% (99.9%, 99.9%)
DIS3	3	3	3	100% (34.23%, 100%)	99.9% (99.9%, 99.9%)
DNMT1	1	1	1	0.0% (0.0%, 79.4%)	100% (99.9%, 100%)
DNMT3A	3	4	5	50.0% (15.0%, 85.0%)	99.9% (99.9%, 99.9%)

DNMT3B	1	1	1	100% (20.65%, 100%)	99.9% (99.9%, 99.9%)
DOT1L	6	8	6	100% (60.96%, 100%)	99.9% (99.9%, 99.9%)
E2F3	1	1	1	100% (20.65%, 100%)	100% (99.7%, 100%)
EGFL7	1	1	1	100% (20.65%, 100%)	100% (99.5%, 100%)
EGFR	3	5	58	92.3% (66.7%, 98.6%)	99.9% (99.9%, 99.9%)
EP300	1	2	2	50.0% (9.5%, 90.5%)	100% (99.9%, 100%)
EPHA3	3	3	3	100% (34.23%, 100%)	99.9% (99.9%, 99.9%)
EPHA5	5	6	6	100% (60.96%, 100%)	100% (99.9%, 100%)
EPHA7	4	4	4	100% (43.85%, 100%)	99.9% (99.9%, 99.9%)
ERBB2	3	3	2	100% (43.85%, 100%)	100% (99.9%, 100%)
ERBB3	4	5	5	100% (56.55%, 100%)	100% (99.9%, 100%)
ERBB4	6	6	5	100% (56.55%, 100%)	99.9% (99.9%, 99.9%)
ERCC2	1	1	1	100% (20.65%, 100%)	100% (99.8%, 100%)
ERCC3	1	1	1	100% (20.65%, 100%)	100% (99.8%, 100%)
ERCC4	2	4	4	100% (20.65%, 100%)	99.9% (99.9%, 99.9%)
ERCC5	1	1	1	100% (20.65%, 100%)	100% (99.9%, 100%)
ERG	1	1	1	100% (20.65%, 100%)	100% (99.7%, 100%)
ERRFI1	0	1	1	0.0% (0.0%, 79.4%)	100% (99.7%, 100%)
ESR1	3	4	3	75.0% (30.1%, 95.4%)	99.9% (99.9%, 99.9%)
FAM175A	1	1	1	100% (20.65%, 100%)	100% (99.6%, 100%)
FAM46C	1	1	1	100% (20.65%, 100%)	100% (99.7%, 100%)
FANCA	2	2	2	100% (20.65%, 100%)	99.9% (99.9%, 99.9%)
FANCG	1	1	1	100% (20.65%, 100%)	99.9% (99.7%, 99.9%)
FANCL	2	2	2	100% (20.65%, 100%)	99.9% (99.7%, 99.9%)

FAT1	7	10	9	100% (70.08%, 100%)	99.9% (99.9%, 99.9%)
FBXW7	4	8	7	75.0% (40.9%, 92.9%)	100% (99.9%, 100%)
FGF14	0	1	1	0.0% (0.0%, 79.4%)	100% (99.49%, 100%)
FGF19	1	1	1	N/A	99.8% (99.1%, 99.9%)
FGF3	3	3	3	100% (43.85%, 100%)	100% (99.8%, 100%)
FGF6	1	1	1	100% (20.65%, 100%)	100% (99.38%, 100%)
FGFR1	1	1	1	100% (20.65%, 100%)	100% (99.8%, 100%)
FGFR2	2	3	3	100% (34.23%, 100%)	99.9% (99.9%, 99.9%)
FGFR3	1	1	1	0.0% (0.0%, 79.4%)	100% (99.8%, 100%)
FGFR4	2	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
FLCN	2	2	2	100% (20.65%, 100%)	99.9% (99.8%, 99.9%)
FLT1	2	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
FLT3	1	1	1	N/A	99.9% (99.9%, 99.9%)
FLT4	3	3	3	100% (34.23%, 100%)	99.9% (99.9%, 99.9%)
FOXL2	1	1	1	100% (20.65%, 100%)	99.9% (99.7%, 99.9%)
FOXP1	3	3	3	100% (43.85%, 100%)	100% (99.9%, 100%)
GABRA 6	1	1	1	100% (20.65%, 100%)	100% (99.7%, 100%)
GATA2	1	1	1	100% (20.65%, 100%)	100% (99.7%, 100%)
GATA3	2	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
GATA4	2	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
GLI1	2	4	4	100% (51.01%, 100%)	100% (99.9%, 100%)
GNAS	1	4	3	50.0% (15.0%, 85.0%)	100% (99.9%, 100%)
GPR124	1	1	1	100% (20.65%, 100%)	100% (99.9%, 100%)
GRIN2A	4	7	7	100% (60.96%, 100%)	99.9% (99.9%, 99.9%)

GRM3	2	3	3	50% (20.7%, 100%)	99.9% (99.9%, 99.9%)
GSK3B	1	1	1	100% (20.65%, 100%)	100% (99.7%, 100%)
H3F3A	1	1	1	100% (20.65%, 100%)	100% (99.06%, 100%)
HGF	1	1	1	100% (20.65%, 100%)	100% (99.8%, 100%)
HIST1H1C	1	1	1	0.0% (0.0%, 79.4%)	100% (99.4%, 100%)
HNF1A	2	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
HSD3B1	1	3	2	33.3% (6.2%, 79.2%)	99.9% (99.8%, 99.9%)
IDH1	2	2	2	100% (34.23%, 100%)	99.9% (99.8%, 99.9%)
IGF1R	2	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
IKBKE	1	1	1	N/A	99.9% (99.7%, 99.9%)
IKZF1	3	3	3	100% (43.85%, 100%)	100% (99.9%, 100%)
INHBA	2	3	3	66.7% (20.8%, 93.9%)	100% (99.9%, 100%)
INPP4A	1	1	1	100% (20.65%, 100%)	100% (99.9%, 100%)
INPP4B	3	3	3	N/A	99.9% (99.9%, 99.9%)
INSR	3	3	3	100% (43.85%, 100%)	100% (99.9%, 100%)
IRF4	4	4	4	75.0% (30.1%, 95.4%)	100% (99.9%, 100%)
IRS1	2	3	3	100% (34.23%, 100%)	99.9% (99.9%, 99.9%)
IRS2	1	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
JAK1	1	1	1	100% (20.65%, 100%)	100% (99.9%, 100%)
JAK2	0	1	1	0.0% (0.0%, 79.4%)	100% (99.9%, 100%)
JAK3	3	3	3	100% (34.23%, 100%)	99.9% (99.9%, 99.9%)
KDM5A	2	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
KDM5C	4	5	5	100% (51.01%, 100%)	99.9% (99.9%, 99.9%)
KDM6A	3	3	3	100% (43.85%, 100%)	99.9% (99.9%, 99.9%)

KDR	4	4	4	100% (51.01%, 100%)	100% (99.9%, 100%)
KEAP1	1	1	1	100% (20.65%, 100%)	100% (99.8%, 100%)
KIT	3	4	4	66.7% (20.8%, 93.9%)	99.9% (99.9%, 99.9%)
KLHL6	1	1	1	100% (20.65%, 100%)	100% (99.8%, 100%)
KMT2A	1	1	1	100% (20.65%, 100%)	100% (99.9%, 100%)
KMT2C	6	7	7	100% (43.85%, 100%)	99.9% (99.9%, 99.9%)
KMT2D	6	9	7	100% (43.85%, 100%)	99.9% (99.9%, 99.9%)
KRAS	4	11	19	94.7% (75.4%, 99.1%)	100% (99.9%, 100%)
LATS2	1	1	1	100% (20.65%, 100%)	100% (99.9%, 100%)
LRP1B	8	8	8	100% (60.96%, 100%)	99.9% (99.9%, 99.9%)
LTK	2	2	2	N/A	99.9% (99.9%, 99.9%)
MAGI2	4	4	4	100% (34.23%, 100%)	99.9% (99.9%, 99.9%)
MAP2K4	1	1	1	0.0% (0.0%, 79.4%)	100% (99.8%, 100%)
MAP3K1	3	4	4	66.7% (20.8%, 93.9)	99.9% (99.9%, 99.9%)
MAP3K1 3	3	3	3	100% (20.65%, 100%)	99.9% (99.9%, 99.9%)
MCL1	1	2	2	100% (34.23%, 100%)	100% (99.8%, 100%)
MDC1	2	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
MDM2	2	2	2	N/A	99.9% (99.8%, 99.9%)
MDM4	1	1	1	100% (20.65%, 100%)	100% (99.7%, 100%)
MED12	3	4	4	50.0% (9.5%, 90.5%)	99.9% (99.9%, 99.9%)
MEF2B	2	2	1	100% (34.23%, 100%)	100% (99.6%, 100%)
MEN1	1	1	1	100% (20.65%, 100%)	100% (99.8%, 100%)
MERTK	1	1	1	N/A	99.9% (99.8%, 99.9%)
MET	2	2	2	100% (20.65%, 100%)	99.9% (99.9%, 99.9%)

MLH1	1	1	1	0.0% (0.0%, 79.4%)	100% (99.8%, 100%)
MPL	1	1	1	N/A	99.9% (99.7%, 99.9%)
MSH2	3	3	3	100% (34.23%, 100%)	99.9% (99.9%, 99.9%)
MSH3	3	4	3	100% (20.65%, 100%)	99.9% (99.9%, 99.9%)
MSH6	1	1	1	100% (20.65%, 100%)	100% (99.9%, 100%)
MTAP	2	2	2	N/A	99.8% (99.5%, 99.9%)
MTOR	4	4	4	100% (51.01%, 100%)	100% (99.9%, 100%)
MYCL	1	1	1	N/A	99.9% (99.7%, 99.9%)
MYD88	2	2	2	100% (20.65%, 100%)	99.9% (99.7%, 99.9%)
MYOD1	1	1	1	100% (20.65%, 100%)	100% (99.6%, 100%)
NBN	3	4	4	100% (34.23%, 100%)	99.9% (99.9%, 99.9%)
NCOA3	2	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
NCOR1	3	3	3	66.7% (20.8%, 93.9)	100% (99.9%, 100%)
NF1	4	5	5	75.0% (30.1%, 95.4%)	99.9% (99.9%, 99.9%)
NF2	2	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
NKX2-1	1	1	1	100% (20.65%, 100%)	100% (99.7%, 100%)
NOTCH1	4	5	5	100% (43.85%, 100%)	99.9% (99.9%, 99.9%)
NOTCH2	3	5	5	50.0% (15.0%, 85.0%)	99.9% (99.9%, 99.9%)
NOTCH3	6	7	6	75.0% (30.1%, 95.4%)	99.9% (99.9%, 99.9%)
NOTCH4	3	4	4	75.0% (30.1%, 95.4%)	100% (99.9%, 100%)
NRAS	3	6	7	100% (64.56%, 100%)	100% (99.9%, 100%)
NSD1	4	6	6	83.3% (43.6%, 97.0%)	100% (99.9%, 100%)
NTRK1	2	3	3	100% (34.23%, 100%)	99.9% (99.9%, 99.9%)
NTRK2	2	2	2	100% (20.65%, 100%)	99.9% (99.9%, 99.9%)

NTRK3	3	3	3	100% (34.23%, 100%)	99.9% (99.9%, 99.9%)
NUP93	2	3	3	100% (43.85%, 100%)	100% (99.9%, 100%)
PAK3	1	1	1	100% (20.65%, 100%)	100% (99.7%, 100%)
PAK7	1	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
PALB2	2	2	2	0.0% (0.0%, 79.4%)	99.9% (99.9%, 99.9%)
PARK2	2	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
PARP1	1	1	1	0.0% (0.0%, 79.4%)	100% (99.9%, 100%)
PAX5	3	3	3	100% (43.85%, 100%)	100% (99.9%, 100%)
PBRM1	1	1	1	100% (20.65%, 100%)	100% (99.9%, 100%)
PDCD1	1	1	1	100% (20.65%, 100%)	100% (99.6%, 100%)
PDCD1LG2	3	4	4	75.0% (30.1%, 95.4%)	100% (99.9%, 100%)
PDGFRA	2	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
PDGFRB	1	1	1	100% (20.65%, 100%)	99.9% (99.9%, 99.9%)
PDPK1	1	1	1	0.0% (0.0%, 79.4%)	100% (99.8%, 100%)
PIK3C2B	2	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
PIK3C2G	2	2	2	N/A	99.9% (99.9%, 99.9%)
PIK3CA	8	17	24	88.0% (70.0%, 95.8%)	99.9% (99.9%, 99.9%)
PIK3CB	1	1	1	100% (20.65%, 100%)	100% (99.9%, 100%)
PIK3CD	1	1	1	100% (20.65%, 100%)	100% (99.9%, 100%)
PIK3CG	2	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
PIK3R1	4	5	5	100% (51.01%, 100%)	99.9% (99.9%, 99.9%)
PIK3R2	2	3	3	66.7% (20.8%, 93.9%)	100% (99.9%, 100%)
PIM1	1	1	1	100% (20.65%, 100%)	100% (99.6%, 100%)
PLCG2	0	1	1	0.0% (0.0%, 79.4%)	100% (99.9%, 100%)

PMAIP1	1	1	1	100% (20.65%, 100%)	100% (97.68%, 100%)
PMS2	1	2	2	0.0% (0.0%, 79.4%)	99.9% (99.9%, 99.9%)
POLD1	2	2	2	100% (20.65%, 100%)	99.9% (99.9%, 99.9%)
POLE	2	2	2	100% (34.2%, 100%)	99.9% (99.9%, 99.9%)
PRDM1	1	1	1	100% (20.65%, 100%)	100% (99.8%, 100%)
PREX2	7	8	8	83.3% (43.6%, 97.0%)	99.9% (99.9%, 99.9%)
PRKAR1A	3	3	3	100% (43.85%, 100%)	100% (99.9%, 100%)
PRKCI	1	1	1	100% (20.65%, 100%)	100% (99.8%, 100%)
PRKDC	6	11	6	28.6% (8.2%, 64.1%)	99.9% (99.9%, 99.9%)
PTCH1	3	3	3	100% (43.85%, 100%)	100% (99.9%, 100%)
PTEN	5	10	11	100% (72.24%, 100%)	99.9% (99.9%, 99.9%)
PTPN11	3	3	3	100% (43.85%, 100%)	99.9% (99.9%, 99.9%)
PTPRD	4	4	4	100% (51.01%, 100%)	99.9% (99.9%, 99.9%)
PTPRO	3	3	2	N/A	99.9% (99.7%, 99.9%)
PTPRS	1	1	1	100% (20.65%, 100%)	100% (99.9%, 100%)
PTPRT	5	5	5	100% (51.01%, 100%)	99.9% (99.9%, 99.9%)
QKI	1	1	1	100% (20.65%, 100%)	99.9% (99.7%, 99.9%)
RAD50	1	1	1	100% (20.65%, 100%)	100% (99.9%, 100%)
RAD52	1	1	1	N/A	99.9% (99.5%, 99.9%)
RAF1	2	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
RANBP2	1	4	4	33.3% (6.1%, 79.2%)	99.9% (99.9%, 99.9%)
RARA	2	2	2	100% (20.65%, 100%)	99.9% (99.8%, 99.9%)
RB1	3	3	3	100% (34.23%, 100%)	99.9% (99.9%, 99.9%)
RBM10	1	1	1	100% (20.65%, 100%)	100% (99.9%, 100%)

RECQL4	2	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
REL	2	2	2	N/A	99.9% (99.8%, 99.9%)
RET	6	6	6	100% (60.96%, 100%)	100% (99.9%, 100%)
RFWD2	1	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
RHOA	1	1	1	N/A	99.8% (99.0%, 99.9%)
RICTOR	2	3	3	100% (43.85%, 100%)	100% (99.9%, 100%)
RNF43	3	3	3	100% (43.85%, 100%)	100% (99.9%, 100%)
ROS1	5	6	6	100% (51.01%, 100%)	99.9% (99.9%, 99.9%)
RPTOR	1	1	1	100% (20.65%, 100%)	100% (99.9%, 100%)
RUNX1	1	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
RUNX1T 1	2	3	3	100% (20.65%, 100%)	99.9% (99.8%, 99.9%)
RYBP	1	1	1	100% (20.65%, 100%)	100% (99.42%, 100%)
SDHA	1	1	1	N/A	99.9% (99.7%, 99.9%)
SETD2	3	4	4	100% (51.01%, 100%)	100% (99.9%, 100%)
SLIT2	2	2	2	N/A	99.9% (99.9%, 99.9%)
SLX4	3	4	4	100% (43.85%, 100%)	99.9% (99.9%, 99.9%)
SMAD3	2	2	2	100% (34.23%, 100%)	100% (99.8%, 100%)
SMAD4	4	6	6	100% (51.01%, 100%)	99.9% (99.9%, 99.9%)
SMARCA4	7	7	7	85.7% (48.7%, 97.4%)	100% (99.9%, 100%)
SMARCB1	1	1	1	0.0% (0.0%, 79.4%)	100% (99.66%, 100%)
SMARCD1	1	1	1	100% (20.65%, 100%)	100% (99.8%, 100%)
SMO	1	1	1	100% (20.65%, 100%)	100% (99.8%, 100%)
SNCAIP	3	3	3	100% (20.65%, 100%)	99.9% (99.9%, 99.9%)
SOX2	1	1	1	100% (20.65%, 100%)	100% (99.6%, 100%)

SOX9	2	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
SPEN	3	9	9	100% (64.56%, 100%)	99.9% (99.9%, 99.9%)
SPTA1	8	9	7	100% (56.55%, 100%)	99.9% (99.9%, 99.9%)
SRC	1	1	1	100% (20.65%, 100%)	99.9% (99.6%, 99.9%)
STAG2	2	2	2	0.0% (0.0%, 65.8%)	100% (99.9%, 100%)
STAT3	1	1	1	N/A	99.9% (99.7%, 99.9%)
STAT4	1	1	1	100% (20.65%, 100%)	100% (99.8%, 100%)
STK11	3	3	3	100% (34.23%, 100%)	99.9% (99.9%, 99.9%)
STK40	1	1	1	100% (20.65%, 100%)	100% (99.7%, 100%)
SYK	1	1	1	100% (20.65%, 100%)	100% (99.8%, 100%)
TAF1	3	3	3	100% (34.23%, 100%)	99.9% (99.9%, 99.9%)
TBX3	1	2	2	100% (20.65%, 100%)	99.9% (99.9%, 99.9%)
TEK	2	2	2	100% (20.65%, 100%)	99.9% (99.9%, 99.9%)
TERC	1	1	1	N/A	99.8% (98.8%, 99.9%)
TERT	2	2	12	100% (70.1%, 100%)	99.9% (99.9%, 99.9%)
TET2	4	10	9	60.0% (31.3%, 83.2%)	100% (99.9%, 100%)
TGFBR1	2	2	2	100% (34.23%, 100%)	100% (99.8%, 100%)
TGFBR2	2	2	2	100% (34.23%, 100%)	100% (99.9%, 100%)
TNFAIP 3	1	1	1	100% (20.65%, 100%)	100% (99.8%, 100%)
TP53	8	39	48	98.0% (89.3%, 99.6%)	99.9% (99.9%, 99.9%)
TP53BP1	1	1	1	100% (20.65%, 100%)	100% (99.9%, 100%)
TP63	1	1	1	100% (20.65%, 100%)	100% (99.8%, 100%)
TSC1	1	1	1	100% (20.65%, 100%)	100% (99.9%, 100%)
TSC2	3	3	3	50% (20.7%, 100%)	99.9% (99.9%, 99.9%)

TSHR	1	1	1	0.0% (0.0%, 79.4%)	100% (99.8%, 100%)
TYRO3	1	1	1	N/A	99.9% (99.8%, 99.9%)
U2AF1	1	2	2	50% (20.7%, 100%)	100% (99.7%, 100%)
WHSC1	2	2	2	100% (20.65%, 100%)	99.9% (99.9%, 99.9%)
WT1	1	1	1	100% (20.65%, 100%)	100% (99.8%, 100%)
XPO1	1	2	2	50.0% (9.5%, 90.5%)	100% (99.9%, 100%)
YAP1	2	2	2	50.0% (9.5%, 90.5%)	100% (99.9%, 100%)

Appendix E.2. Concordance for Insertions by Gene:

Gene	Number of exons	Number of unique mutations	Number of samples	PPA (%), 95% CI (%)	NPA (%), 95% CI (%)
ALOX12B	1	1	1	100% (20.7%, 100%)	100% (99.8%, 100%)
APC	2	2	2	100% (34.2%, 100%)	100% (99.9%, 100%)
ARID2	1	1	1	0% (0.0%, 79,4%)	100% (99.9%, 100%)
ASXL1	1	1	1	N/A	99.9% (99.9%, 100%)
ASXL2	1	1	1	100% (20.7%, 100%)	100% (99.9%, 100%)
ATR	1	1	1	100% (20.7%, 100%)	100% (99.9%, 100%)
AXIN2	1	1	1	0% (0.0%, 79,4%)	100% (99.9%, 100%)
BARD1	1	1	1	0% (0.0%, 79,4%)	100% (99.8%, 100%)
BBC3	1	1	1	0% (0.0%, 79,4%)	100% (99.3%, 100%)
BCL2L1	1	1	1	0% (0.0%, 79,4%)	100% (99.3%, 100%)
CASP8	1	1	1	100% (20.7%, 100%)	100% (99.7%, 100%)
CDH1	1	2	2	100% (34.2%, 100%)	100% (99.9%, 100%)
CIC	1	1	1	100% (20.7%, 100%)	100% (99.9%, 100%)
CTCF	1	1	1	100% (20.7%, 100%)	100% (99.8%, 100%)

GATA3	1	2	2	100% (34.2%, 100%)	100% (99.9%, 100%)
KMT2D	1	1	1	100% (20.7%, 100%)	100% (99.9%, 100%)
MDM2	1	1	1	100% (20.7%, 100%)	100% (99.7%, 100%)
NF1	1	1	1	100% (20.7%, 100%)	100% (99.9%, 100%)
NKX3-1	1	1	1	N/A	99.8% (98.8%, 99.9%)
PTEN	2	2	2	100% (34.2%, 100%)	100% (99.8%, 100%)
RASA1	1	1	1	100% (20.7%, 100%)	100% (99.9%, 100%)
RB1	1	1	1	100% (20.7%, 100%)	100% (99.8%, 100%)
TBX3	1	1	1	100% (20.7%, 100%)	100% (99.8%, 100%)
TET2	1	1	1	100% (20.7%, 100%)	100% (99.9%, 100%)
TSC2	1	1	1	100% (20.7%, 100%)	100% (99.9%, 100%)

Appendix E.3 Concordance for Deletions by Gene:

Gene	Number of exons	Number of unique mutations	Number of samples	PPA (%), 95% CI (%)	NPA (%), 95% CI (%)
AKT3	1	1	1	100% (20.7%, 100%)	100% (99.7%, 100%)
ALOX12B	1	1	1	0% (0.0%, 79.4%)	100% (99.8%, 100%)
AMER1	2	1	2	100% (34.2%, 100%)	100% (99.9%, 100%)
ARID1A	6	5	4	100% (56.6%, 100%)	100% (99.9%, 100%)
ATM	1	1	1	0% (0.0%, 79.4%)	100% (99.9%, 100%)
ATRX	1	1	1	100% (20.7%, 100%)	100% (99.9%, 100%)
AXIN1	1	1	1	100% (20.7%, 100%)	100% (99.9%, 100%)
B2M	2	2	1	N/A	99.4% (98.0%, 99.9%)
BLM	2	1	2	100% (34.2%, 100%)	100% (99.9%, 100%)
BRCA2	2	2	2	100% (34.2%, 100%)	100% (99.9%, 100%)

CCND2	1	1	1	100% (20.7%, 100%)	100% (99.6%, 100%)
CD79A	1	1	1	100% (20.7%, 100%)	100% (99.4%, 100%)
CDH1	5	5	5	80% (37.6%, 96,4%)	100% (99.9%, 100%)
CDK12	1	1	1	100% (20.7%, 100%)	100% (99.9%, 100%)
CIC	1	1	1	0% (0.0%, 79.4%)	100% (99.9%, 100%)
CREBBP	1	1	1	0% (0.0%, 79.4%)	100% (99.9%, 100%)
DNMT1	1	1	1	100% (20.7%, 100%)	100% (99.9%, 100%)
DNMT3B	2	1	2	100% (20.7%, 100%)	99.9% (99.9%, 100%)
EGFL7	1	1	1	100% (20.7%, 100%)	100% (99.5%, 100%)
EGFR	15	3	15	100% (79.6%, 100%)	100% (99.9%, 100%)
ERCC5	1	1	1	100% (20.7%, 100%)	100% (99.9%, 100%)
FLT4	1	1	1	N/A	99.9% (99.9%, 100%)
HNF1A	1	1	1	100% (20.7%, 100%)	100% (99.8%, 100%)
INHBA	1	1	1	0% (0.0%, 79.4%)	100% (99.7%, 100%)
IRS2	1	1	1	0% (0.0%, 79.4%)	100% (99.9%, 100%)
JAK1	4	4	2	100% (51.0%, 100%)	100% (99.9%, 100%)
KLF4	1	1	1	0% (0.0%, 79.4%)	100% (99.7%, 100%)
KMT2A	1	1	1	100% (20.7%, 100%)	100% (99.9%, 100%)
KMT2C	1	1	1	100% (20.7%, 100%)	100% (99.9%, 100%)
KMT2D	1	1	1	100% (20.7%, 100%)	100% (99.9%, 100%)
MAP2K4	1	1	1	100% (20.7%, 100%)	100% (99.7%, 100%)
MEF2B	1	1	1	100% (20.7%, 100%)	100% (99.6%, 100%)
MEN1	1	1	1	100% (20.7%, 100%)	100% (99.8%, 100%)
MITF	1	1	1	100% (20.7%, 100%)	100% (99.7%, 100%)

MSH3	6	2	6	100% (43.9%, 100%)	99.9% (99.9%, 100%)
NBN	2	2	2	100% (20.7%, 100%)	99.9% (99.9%, 100%)
NCOR1	1	1	1	N/A	99.9% (99.9%, 100%)
NF1	2	2	1	100% (34.2%, 100%)	100% (99.9%, 100%)
NOTCH4	2	2	2	100% (34.2%, 100%)	100% (99.9%, 100%)
PALB2	2	2	2	100% (20.7%, 100%)	99.9% (99.9%, 100%)
PIK3C2G	1	1	1	100% (20.7%, 100%)	100% (99.9%, 100%)
PIK3C3	1	1	1	100% (20.7%, 100%)	100% (99.9%, 100%)
PLK2	1	1	1	N/A	99.9% (99.7%, 99.9%)
POLE	2	1	2	100% (34.2%, 100%)	100% (99.9%, 100%)
PREX2	2	2	2	50% (9.5%, 906%)	100% (99.9%, 100%)
PRKCI	1	1	1	100% (20.7%, 100%)	100% (99.7%, 100%)
PTEN	2	2	2	50% (9.5%, 906%)	100% (99.8%, 100%)
RNF43	3	1	3	100% (43.9%, 100%)	100% (99.9%, 100%)
RUNX1	1	1	1	N/A	99.9% (99.6%, 99.9%)
SMAD4	1	1	1	N/A	99.9% (99.7%, 99.9%)
SOX9	2	2	2	50% (9.5%, 906%)	100% (99.9%, 100%)
SPEN	1	1	1	N/A	99.9% (99.9%, 100%)
SYK	1	1	1	100% (20.7%, 100%)	100% (99.8%, 100%)
TGFBR1	1	1	1	0% (0.0%, 79.4%)	100% (99.7%, 100%)
TGFBR2	1	1	1	N/A	99.9% (99.7%, 99.9%)
TP53	5	5	5	80% (37.6%, 96,4%)	100% (99.9%, 100%)
WHSC1	1	1	1	100% (20.7%, 100%)	100% (99.8%, 100%)