510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

I. Background Information

A. 510(k) Number

K260235

B. Applicant

Datar Cancer Genetics Pvt Ltd

C. Proprietary and Established Names

CellDx - Tissue

D. Regulatory Information

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

II. Submission/Device Overview

A. Purpose for submission

New Device

B. Measurand

Somatic single nucleotide variants (SNVs), insertions and deletions (Indels), Copy Number Variant (CNV), and select gene fusions, in genomic DNA and RNA isolated from formalin-fixed paraffine-embedded (FFPE) tumor tissue. A complete list of genes included in the assay can be found in Appendix A.

C. Type of Test

Next-generation sequencing tumor profiling test

III. Intended Use/Indications for Use

A. Intended Use(s)

The CellDx-Tissue is a qualitative in vitro diagnostic (IVD) test that uses next-generation sequencing of DNA and RNA isolated from formalin-fixed paraffine-embedded (FFPE) tumor tissue from patients previously diagnosed with solid malignant neoplasm to detect tumor gene

alterations in a broad multi-gene panel. The test is intended to provide tumor mutation profiling information on somatic mutations, including single nucleotide variants (SNVs), insertions and deletions (indels), one gene amplification, and three fusions.

Information provided by the CellDx-Tissue test is intended for use by qualified healthcare professionals in accordance with professional guidelines in oncology. Results from CellDx-Tissue are not intended to be conclusive or prescriptive for the labeled use of any specific therapeutic product. CellDx-Tissue is a single-site assay performed at Datar Cancer Genetics (DCG).

B. Indication(s) for Use:

Same as above

C. Special Instrument Requirements

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

D. Special Instrument Requirements

Thermo Fisher Ion GeneStudio S5 Prime NGS Systems (qualified by Datar Cancer Genetics)

IV. Device/System Characteristics

A. Device Description

The CellDx-Tissue utilizes an amplicon-based sequencing approach to detect a broad range of clinically significant somatic alterations, including SNVs, InDels, and a gene amplification in tumor tissue DNA (ttDNA), and three fusions in tumor tissue RNA (ttRNA). The test requires FFPE tumor tissue specimen having a surface area of 125 mm^2 or more, a thickness of at least 3 mm, with at least 20% tumor content. Specimens with lower tumor content may be enriched by macro-dissection. A description of required equipment, software, reagents, vendors, and storage conditions is provided in the product labeling (Datar Cancer Genetics Technical Information). The CellDx-Tissue system includes a sequencing instrument, reagents (nucleic acid extraction, library preparation, and sequencing) software (operation of the sequencing instrument and variant calling), and instructions for use (IFU) of the system. All instruments and reagents are qualified by Datar Cancer Genetics.

1. <u>Sample Preparation</u>

The CellDx – Tissue requires genomic DNA and RNA isolated from formalin-fixed paraffin-embedded (FFPE) tissue specimens. The tumor volume and minimum tumor content needed to obtain sufficient DNA and RNA for testing to achieve the stated performance are listed in Table 1. The tumor content of the FFPE specimen is determined by hematoxylin-eosin (HE) staining. If the histopathologist assessed tumor content is < 20%, the tissue should be macro-dissected to select as much viable tumor as possible and minimize the amount of adjacent non-tumor tissue.

Table 1. Specimen Handling and Processing for Validated Specimen Type

Tissue Type	Formalin-fixed paraffin-embedded (FFPE) tumor tissue blocks
Specimen Volume	≥ 3 mm thick; >125 mm^2 total surface area
Tumor Content	≥ 20%
Macrodissection Requirements	If the minimum tumor content is less than 20%, macrodissection will be done to enrich neoplastic content
Limitations	Low (<20%) tumor content, Inadequate tissue volume, Long-term storage degradation.
Storage	Room Temperature

2. Nucleic Acid Extraction

DNA extraction is performed using the QIAamp DNA FFPE Advanced UNG Kit (qualified by DCG) as per the manufacturer's instructions for use. The procedure includes Uracil-N-Glycosylase (UNG) treatment to remove deaminated cytosine residues and an RNase A digestion to enhance the purity of the extracted DNA. The yield of extracted DNA is evaluated by real-time PCR. At least 20 ng DNA is required for library synthesis. RNA extraction procedure includes a DNase digestion step to eliminate contaminating DNA. The yield of extracted RNA is determined using the fluorometric assay, based on a standard curve generated from calibration standards. At least 20 ng RNA is required for reverse transcription (cDNA synthesis) and library synthesis. The assay has been validated with extracted DNA and RNA stored at -20°C and -80°C, respectively for up to 28 days.

3. Library Synthesis

DNA libraries are synthesized using the Oncomine DNA kit (qualified by DCG). The assay employs an amplicon-based targeted enrichment workflow, in which predefined genomic regions are amplified and converted into sequencing-ready libraries through adapter ligation and barcode incorporation. This approach enables multiplexed sequencing across the 517-gene panel. Library preparation entails the targeted amplification of genomic regions, partial digestion of primer sequences, ligation of barcode adapters, and subsequent clean-up and amplification to produce sequencing-competent libraries.

RNA libraries are synthesized using the Oncomine RNA Kit (qualified by DCG). The assay employs an amplicon-based targeted RNA sequencing workflow, in which input RNA is reverse-transcribed to cDNA and targeted regions are selectively amplified to generate sequencing-ready libraries. The workflow includes cDNA synthesis, targeted amplification, adapter ligation with unique barcodes, and subsequent clean-up steps to prepare multiplexed libraries suitable for NGS. DNA and RNA libraries are quantified using the kit which employs a real-time PCR–based method to measure the concentration of adapter-ligated libraries prior to sequencing. Library concentration results are reviewed against predefined quality acceptance criteria, and all quantitation outputs are retained as part of routine quality assurance and traceability.

4. Template Preparation and Chip Loading

Template preparation and chip loading are performed on the Ion Chef™ System (qualified by DCG) using the manufacturer's automated workflow for emulsion PCR (ePCR), enrichment, and chip loading. Quantified libraries meeting the predefined quality criteria are pooled and loaded onto the instrument along with the required reagents and consumables supplied in the template preparation kit. The Ion Chef System (qualified by DCG) performs clonal amplification of library molecules on beads through ePCR, followed by automated enrichment to isolate beads containing amplified templates. The instrument then loads the enriched, template-positive beads onto an Ion 550™ sequencing chip (qualified by DCG), producing a sequencing-ready chip for downstream processing on the compatible semiconductor sequencing platform qualified by Datar Cancer Genetics. Instrument checks and onboard controls ensure workflow integrity, and template-prepared chips proceed directly to sequencing.

5. Sequencing

Sequencing is performed on the Ion GeneStudio™ S5 Prime System (qualified by DCG) using the manufacturer's semiconductor sequencing workflow. The sequencing chip generated during template preparation is loaded into the instrument along with the required sequencing reagents once all automated system checks confirm acceptable setup. A predefined run plan specifies the assay configuration, sample assignments, and chip parameters. After sequencing is initiated, the system performs automated nucleotide flows and signal detection to generate base-level data across all loaded libraries. Sequencing data are accepted only when run-level QC metrics and control results meet validated acceptance criteria.

6. Sequencing Data Analysis

a. Data Management

Sample tracking, data processing, and archival of sequencing data for the CellDx-Tissue assay are managed through a Laboratory Information Management System (LIMS) integrated with DCG's validated bioinformatics pipeline operating on a high-performance server environment. The system tracks and archives run-associated metadata including barcode identifiers, sequencing run identifiers, sample accession numbers, specimen source, library batch identifiers, and assay modality (DNA or RNA). Key functions include tracking sample status across all stages of data analysis, logging analysis iterations applied to each sample, recording software, algorithm, and database versions used for analysis, and archiving pipeline output files (BAM and VCF) together with sequencing run statistics (e.g., total reads generated, mean depth of coverage, uniformity, and on-target rates). All annotation and population databases are maintained as access-restricted, version-locked static copies in a controlled repository. Any update to software, databases, or internal scripts is managed under a formal change-control process requiring documented impact assessment, regression testing using predefined golden datasets, QA/RA approval, and release prior to clinical use.

b. Signal process, Base Calling, Read Alignment, BAM Generation, and Coverage Statistics

Primary data processing is performed using Torrent Suite Software (qualified by DCG). During sequencing, raw semiconductor flow signals are converted into nucleotide bases through signal processing and base calling algorithms. Reads are assigned to individual samples through barcode classification, followed by trimming of barcode and adapter sequences and quality filtering. Filtered reads are aligned to the human reference genome (hg19 / GRCh37.p5) using platform-validated alignment algorithms. Aligned reads are written to Binary Alignment Map (BAM) files, which serve as the input for downstream variant calling and copy number analysis. The software also generates coverage statistics including mean depth of coverage, base coverage uniformity, and percentage of reads on target for each sample.

c. Read Alignment Quality Check

Read alignment metrics are used to assess sequencing and library quality. Reads are aligned to hg19, and mismatches arising from biological variation (true variants) or technical sequencing errors are recorded. Alignment performance is summarized using the Alignment Quality (AQ) score derived from a Phred-scaled $-10\log10$ transformation. The manufacturer default setting of AQ17, corresponding to a base accuracy of 98% (2% error rate), is applied. AQ17 allows longer effective read lengths while accommodating mismatches expected in variant-rich tumor samples. Decreases in AQ are expected in samples with higher mutational burden and are evaluated in the context of overall run and sample QC metrics.

d. Run - Level and Sample - Level Quality Control Checks

i. Run - Level QC

A sequencing run is considered valid only if all of the following criteria are met:

(1) The total sequencing output is ≥ 40 million reads per run.

(2) Acceptable alignment quality (AQ17).

Runs failing any of the above criteria are invalid, investigated, and repeated following troubleshooting and corrective actions.

ii. Sample – Level QC

The sequencing performance is evaluated using the following criteria:

(1) For DNA libraries, the mean target depth is ≥ 500x and ≥ 90% of target amplification with ≥ 100 reads.

(2) For RNA Libraries, the total fusion-mapped reads per run is ≥ 500,000 and reads per RNA pool is ≥ 100,000.

(3) The Contamination Score is < 0.120

Samples that fail QC due to specimen quality, extraction, or library preparation may be re-sequenced, re-prepared, or re-extracted according to the predefined decision rules.

e. Mutation Calling: Single Nucleotide Variants (SNVs) and Insertions / Deletions (indels)

i. Analysis of Positive and Negative Controls

Positive and negative controls are analyzed using the same coverage requirements as patient samples (mean depth ≥ 500× and ≥ 100× coverage across ≥ 90% of target regions). Expected variants must be detected in positive controls within validated variant allele frequency (VAF) ranges, and no reportable variants may be detected in negative controls.

ii. Filters on Sample Coverage

Tumor samples must achieve a minimum mean sequencing depth of ≥ 500× to be eligible for variant calling and reporting.

iii. Filtering for High-Confidence Mutations

Raw SNV and indel calls are subjected to locked filtering thresholds to ensure only high-confidence somatic variants are reported. Variants are classified as:

(1) Hotspot variants: VAF ≥ 2%, total depth ≥ 40, mutant reads ≥ 8, strand bias ≤ 0.9

(2) Non-hotspot variants: VAF ≥ 5%, total depth ≥ 40, mutant reads ≥ 10, strand bias ≤ 0.85

Variants are annotated using AMP/ASCO/CAP somatic guidelines, HGVS nomenclature, and major public databases (e.g., ClinVar, gnomAD, dbSNP, 1000 Genomes). Population variants with a frequency ≥ 5% in any gnomAD or 1000 Genomes subpopulation are excluded. Filtering thresholds are fixed for clinical use, and any modification requires formal change-control and re-validation.

f. Mutation Annotation

Variants with functional consequences including missense, nonsense, frameshift, in-frame insertions/deletions, splice-site, and splice-region alterations are retained. Annotation incorporates gene context, predicted functional impact, and clinical relevance. Common polymorphisms present in population databases or internal normal datasets are excluded from somatic reporting.

g. Tumor Purity Analysis

Tumor purity of the sequenced specimen is estimated bioinformatically using allele frequencies of informative variants and sequencing read distributions. This estimate may differ from the pathologist-reported tumor percentage due to macrodissection or sampling effects. When discrepancies or software limitations are identified, tumor purity is manually reviewed using pathologist estimates and driver mutation VAFs to ensure accurate interpretation of variant calls.

h. Copy Number Analysis

Copy number variation analysis is performed for *ERBB2* using a variability-corrected informatics baseline derived from normal samples. Amplicon coverage log2 ratios are normalized and adjusted for tumor cellularity to estimate copy number and confidence intervals. *ERBB2* amplification is reported as Positive when the observed copy number is ≥ 8.5. Copy numbers ≥ 4 and < 8.5 are reported as Indeterminate / Low-Level Amplification (below the LoD).

i. Fusion Analysis

RNA-based fusion detection is validated for *ALK*, *RET*, and *ROS1*. Fusions are identified using split-read and discordant read-pair evidence. Clinically reportable fusions are reported only when ≥ 500 fusion-supporting reads are detected and validated against internal positive controls. Fusion calls rely exclusively on RNA-level evidence.

7. Controls

a. Positive Control (PC): An external control that consist of a mixture of well-characterized cell line DNA with known somatic variants at defined variant allele frequency (VAF), and RNA with defined fusions (Table 2) and is included in each assay run (batch level).

b. ***Negative Control (NC):*** An external control that is derived from genomic DNA and RNA of a known wild-type (WT) material (Table 2) and is included in each assay run (batch level).

Table 2. Positive and Negative Controls Used in the CellDx-Tissue Assay

Control Type	Qualification
Positive Control DNA	Qualified by DCG
Positive Control RNA	
Negative Control DNA	
Negative Control RNA	

c. No-Template Control (NTC): DNAse, RNAse free water is used as the no template control and will be included in each assay run (batch level).

8. Results Reporting

The CellDx-Tissue assay report provides structured information on detected genomic alterations, level of clinical significance and their clinical implications. The detected genomic alterations are categorized into Level 2 (Variants with Evidence of Clinical Significance) or Level 3 (Variants with Potential Clinical Significance). The CellDx-Tissue does not report mutations in 217 regions among 13473 interrogated target regions due to low coverage and high GC content. A list of all 517 genes is provided in Appendix A; and a list of excluded regions in the genes or genes with variants that are excluded due

to challenging regions (e.g., low complexity / repeats) is provided in Appendix B and Appendix C, respectively.

9. Quality Metrics

Quality metrics are evaluated across the following categories:

- Run Level: Metrics that evaluate the overall sequencing performance, including instrument functions, reagent performance, data yield, and controls.
- Sample Level: Metrics that evaluate the sequencing quality, library performance, and coverage sufficiency.
- Variant (Analyte) Level: Metrics that are quantified for locus-level coverage and read support. Variants passing analyte-level QC are reported.

Table 3. Quality Control Metrics and Assay Cutoff

Quality Metric	Frequency	Acceptance Criteria
Specimen	Sample	Labels intact and Legible, Test Requisition Form(TRF) completely filled, consent signed, No loss of or damage to specimen, Number of FFPE blocks matches TRF, FFPE specimen identifiers match TRF and, LIMS entry
Specimen	Sample	>125mm^2 surface area; ≥3mm thick Tumor content ≥20% (Macro-dissect if <20%)
DNA Yield	Sample	>20 ng
RNA Yield	Sample	>20 ng
Library Yield	Sample	>100 pM
Total Data Output	Run	≥ 40 million reads
Positive Control (PC)	Run (Batch)	DNA: All variants detected within expected VAF ranges RNA: All 6 fusions detected.

Quality Metric	Frequency	Acceptance Criteria
Negative Control (NC)	Run (Batch)	DNA: None of the 890 hotspot mutations should be detected RNA: No fusions detected.
Base Quality (AQ Score)	Run	AQ17 (98% accuracy)
Mean Target Coverage (DNA)	Sample	≥ 500x.
Coverage Uniformity	Sample	≥ 90% of target regions with ≥ 100x coverage
Contamination Score	Sample	≤ 0.120
Mean Target Coverage (RNA)	Sample	Total mapped fusion reads ≥ 500,000 Pool 1, Pool 2 (each): >100,000 reads
SNVs and Indels Calling	Variant	Mutation coverage (total depth) ≥ 40 Number of mutant reads (variant read count) ≥ 8 hotspot variants / ≥ 10 non-hotspot variants Variant Allele Fraction (VAF) ≥ 0.02 hotspot variants / ≥ 0.05 non-hotspot variants Strand bias < 0.9 hotspot variants / < 0.85 non-hotspot variants
ERBB2 Amplification	Variant	CN ≥8.5 is Positive CN ≥4 to <8.5 is Indeterminate / Low Level Amplification CN <4 is Negative
ALK, RET, and *ROS1* Fusions	Variant	Positive where supporting read counts are ≥500

Quality Metric	Frequency	Acceptance Criteria
Test Failure Criteria	Sample	DNA mean coverage < 500x, or Coverage uniformity < 90%, or Contamination score >0.120, or Fusion mapped reads <500,000, or Pool reads <100,000
Reprocessed Specimen	Sample	All above QC criteria met
Report	Sample	Patient ID matches LIMS entry Accreditation Logos match standard template Signing authority signatures present

B. Principles of Operation

The CellDx-Tissue utilizes an amplicon-based sequencing approach to detect a broad range of clinically significant somatic alterations, including small nucleotide variants (SNVs), insertions and deletions (Indels), and *ERBB2* gene amplification in tumor tissue DNA (ttDNA); and *ALK, RET*, and *ROS1* fusions in tumor tissue RNA (ttRNA). The test requires FFPE tumor tissue specimen having a surface area of more than 125 mm^2, a thickness of at least 3 mm, with at least 20% tumor content. Specimens with lower tumor content may be enriched by macro-dissection. The assay involves target enrichment and deep sequencing of specific regions across a comprehensive panel of 517 genes, including oncogenes, tumor suppressor genes, and other clinically relevant genes. DNA and RNA primers are designed to target all coding exons, selected introns and targeted RNA sequences including chimeric fusion reads. Sequence libraries are prepared using DNA and RNA through a multiplex polymerase chain reaction (PCR) amplification step to enrich the target sequences. The target sequences are tagged with unique barcode oligonucleotides for individual sample identification and adaptor oligonucleotides that facilitate anchoring to the sequencing platform. These target sequences are then clonally amplified on microscopic beads using emulsion PCR before sequencing. Multiple barcoded sequence libraries are subsequently pooled and loaded onto a semiconductor sequencing chip. The resulting sequence reads are then aligned to the reference human genome (hg19), to identify gene variants.

C. Determination of Assay Thresholds

1. Requirements on Exon Coverage

A power analysis was conducted to estimate the minimum sequencing depth (total number of reads) needed to detect a mutation with a true underlying variant allele fraction (VAF) of 0.02 or greater, for varying levels of statistical power (0.8 to 0.99), assuming a fixed alpha (Type I error rate) of 0.05. The 95% confidence interval (CI) ranges of observed VAF as a function of sequencing depth were also calculated. This study showed that when

a mutation is present at 0.1 VAF, the 95% CI with a sequencing depth of 500X is expected to fall between 0.075 and 0.13. When the overall coverage is 100X, the 95% CI for a mutation at 10% VAF is estimated to fall between 0.05 and 0.176.

To confirm these estimates, empirical data was obtained to measure the range of observed VAF for expected VAF, utilizing DNA from 20 formalin-fixed, paraffin-embedded (FFPE) normal tissue specimens from unrelated individuals. Equimolar parts of the DNA from these specimens were mixed to create five secondary specimen pools with a range of genetic variants (SNPs) having expected frequencies as low as 2%. A total of 863 common SNPs were considered for this study.

The observed variant frequencies for these common SNPs genotyped in the pooled normal samples, binned by their true underlying variant frequency is shown in Figure 1. The empirical data demonstrated a strong correlation between expected and observed VAF (Pearson's r = 0.99), with a slope of 0.99 and an intercept of 0.013. For SNPs with a true underlying variant fraction of 0.1, the observed variant fraction ranged from 0.069 to 0.138 when the mean coverage of the specimen was 1151X. This range is consistent with the theoretical statistical assessment for a depth of 500X (0.075 to 0.13). This data supports using 0.05 as the lower limit for reporting mutations detected with a true underlying frequency of 10%.

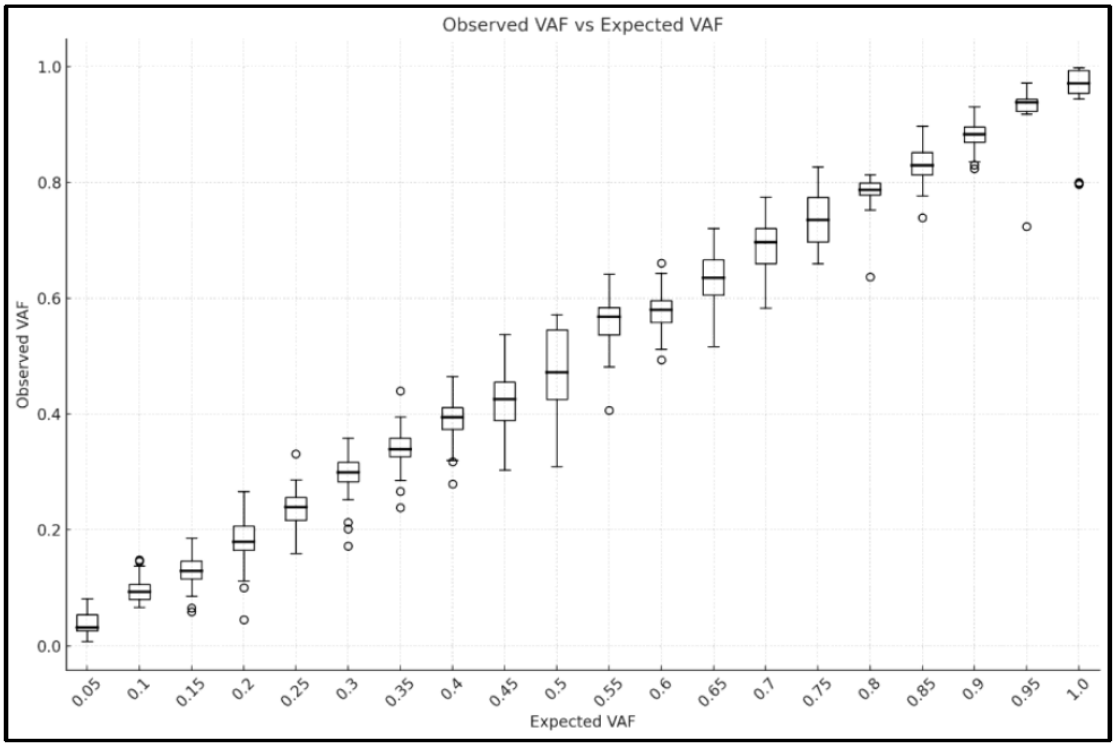
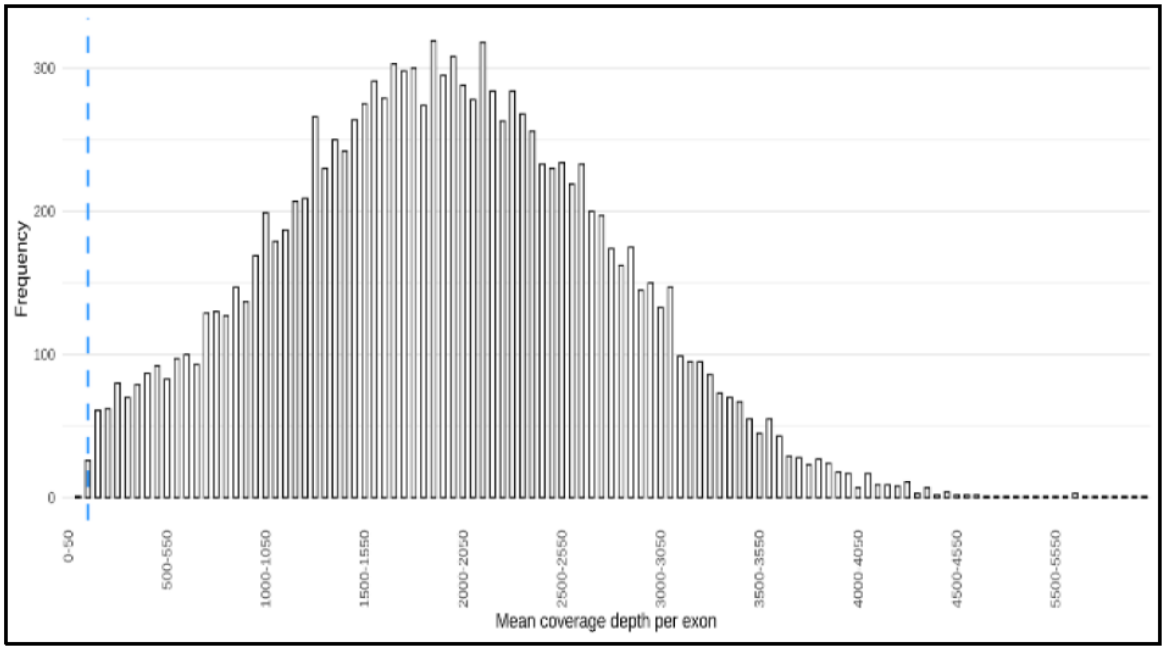


Figure 1. Observed vs. Expected Variant Allele Frequency for the CellDx-Tissue Assay

2. Requirements on Sample Coverage

Twenty FFPE normal (diploid) specimens were profiled using CellDx-Tissue to generate summary statistics across all targeted exons. The mean coverage across all amplicons at optimal depth was 2087X (Range: 1420X-3369X, SD = 481X). The percentage of amplicons with >100X coverage was 99.3% (Range: 98.0-99.9%, SD = 0.5%). When mapped to the exon level, the mean coverage across all targeted exons was 2087X (Range: 1420X-3371X, SD = 482X). Summary statistics were also computed on coverage values per exon normalized by per-sample coverage. Exons with consistently low coverage (median normalized coverage < 0.05) were excluded from SNV/indel reporting, primarily due to high GC content (Appendix B), with the exception of two *TERT* promoter amplicons where variant calling parameters were relaxed with medical justification.

Sequence coverage was further evaluated to establish minimum criteria for the analysis and reporting of variants. Based on the power calculations, a minimum 100X coverage threshold per exon is required to call mutations with a true underlying mutation frequency of 10% or greater, with 95% power at an alpha level of 0.05 (Figure 2).



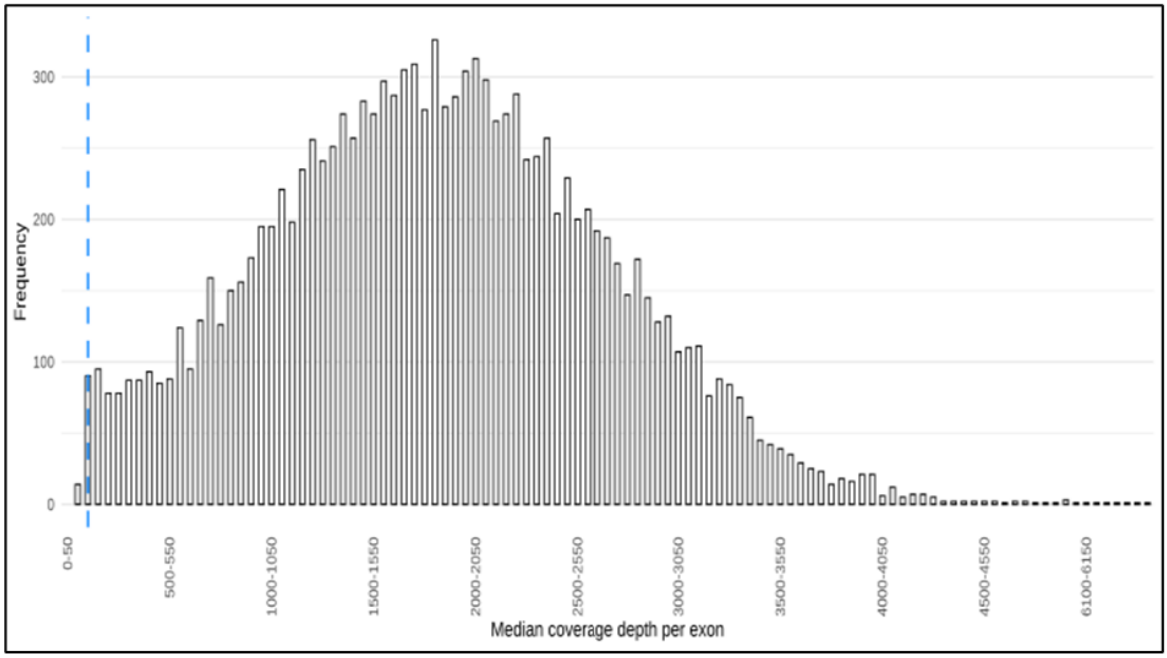


Figure 2. Distribution of Mean and Medium Coverage Values for the Targeted Regions of the CellDx-Tissue Assay using High Coverage Samples. The dashed blue line indicates 100X coverage.

A second set of 20 samples were evaluated at lower sequencing depth. The mean coverage across all amplicons was 471X (Range: 284X-663X, SD = 101X). The percentage of amplicons with >100X coverage was 92.6% (Range: 86.6-96.2%, SD = 2.4%). When mapped to the exon level, the mean coverage across all targeted exons was 471X (Range: 284X-662X, SD = 101X). With the same remaining exons across all genes, 94.1% (Range: 88.0-97.7%, SD = 2.42%) were sequenced to a depth of 100X or greater. The distribution of these mean and medium coverage values for the targeted exons is shown in Figure 3.

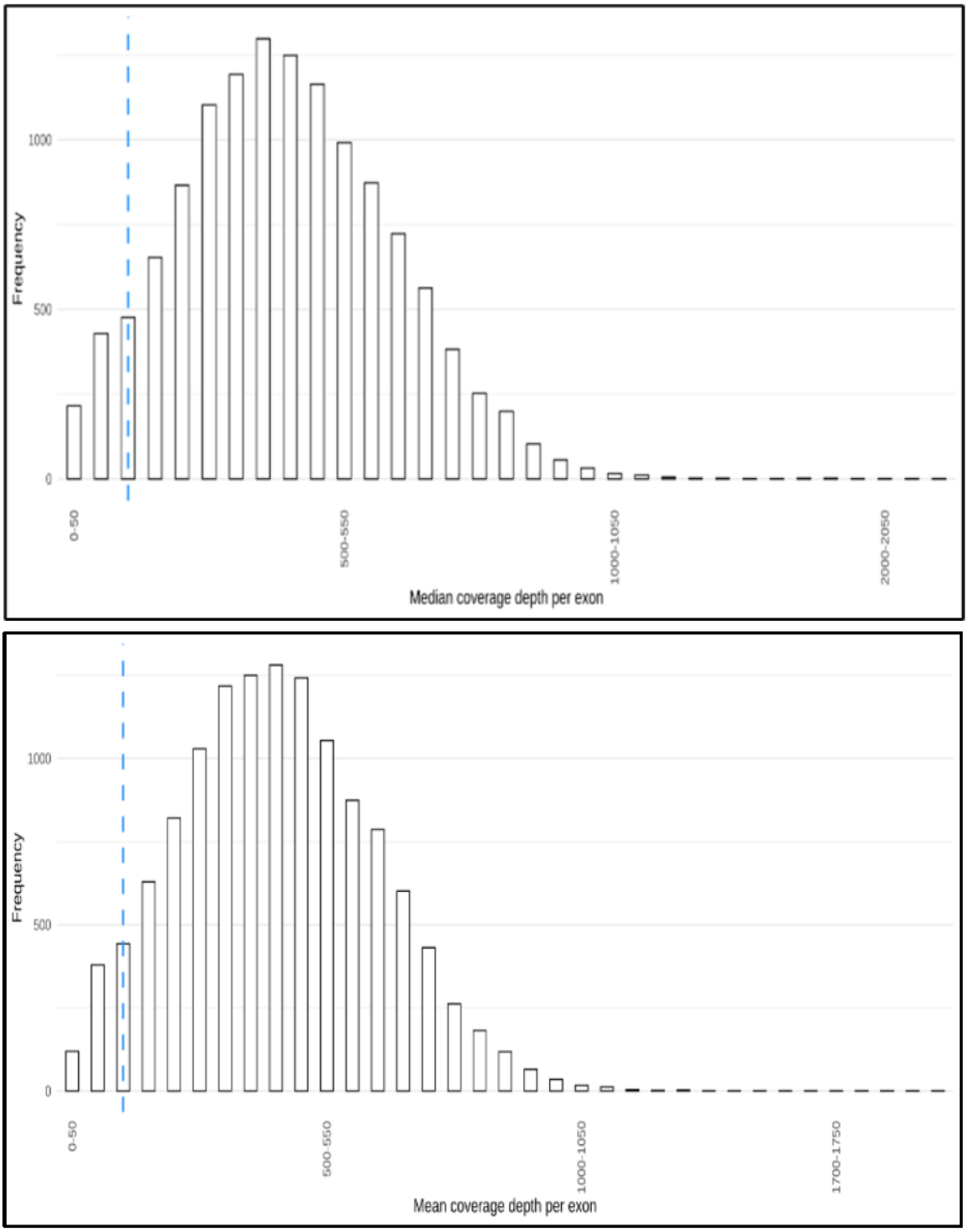


Figure 3. Distribution of Mean and Medium Coverage Values for Targeted Regions of the CellDx – Tissue Assay with Low Coverage Samples. The dashed blue line indicates 100X coverage.

Based on these two studies, the threshold of ≥90% of amplicons achieving 100X coverage was established as a critical quality metric for the CellDx-Tissue assay.

3. **Requirements on Variant Calling Thresholds**

Variant calling parameters such as sequence coverage (Alternate Allele Observation, AO), variant coverage (Coverage Depth, DP) and strand bias (SB) 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 VAF levels for reporting, particularly at 0.02 and 0.05 for hotspot and non-hotspot categories, respectively.

The cutoffs for AO, DP, and SB for hotspot variants and non-hotspot variants were established using two key development studies. The first study was designed to select the optimal parameter cutoff combination utilizing a dataset of 20 CellDx-Tissue runs using a Reference Standard. This involved evaluating multiple cutoffs for each of the three parameters, generating a total of 7,650 combinations. A list of 3,723 positive variants and 4,630 negative variants was used to evaluate the performance of each parameter combination, with the goal of achieving a Positive Predictive Value (PPV) greater than 96%. The relationship between these parameters and PPV, highlighting the selected cutoff combination: AO = 8, DP = 40, SB = 0.90 for hotspot variants; and AO = 10, DP = 40, SB = 0.85 for non-hotspot variants is illustrated in Figure 3.

The second study aimed to determine the optimal cutoff for Coverage Depth (DP) and minimum variant read count. This study used ten non-cancer FFPE samples and evaluated a total of 902 hotspot variants and 6,418 non-hotspot variants at different Coverage Depth cutoffs. The lowest Coverage Depth (DP) that effectively filtered out >99.7% of noise variants were selected, corresponding to a minimum of 8 reads for hotspot variants and 10 reads for non-hotspot variants.

4. **Tumor-Only vs. Matched-Normal Germline Filtering**

A study was conducted to demonstrate analytical equivalence between the CellDx-Tissue tumor-only (T/O) germline filtering approach and a matched tumor–normal (T/N) reference method, as required under 21 CFR 866.6080(b)(1)(iv). The study utilized 52 prospectively selected matched peripheral blood and tumor specimens representing 16 solid tumor types (including bladder / urinary tract (n = 1), bowel (n = 10), breast (n = 13), cervix (n = 2), esophagus / stomach (n = 1), head and neck (n = 3), liver (n = 1), lung (n = 6), ovary / fallopian tube (n = 5), pancreas (n = 3), peritoneum (n = 1), prostate (n = 1), testis (n = 1), thymus (n = 1), uterus (n = 2), and unknown primary (n = 1), and diverse ethnic representation (65% Asian, 27% White/Caucasian, 6% African American and 2% unspecified ethnicity from the US.

The T/O pipeline employed an in-silico filtering strategy that excluded variants with population frequency ≥5% in gnomAD (v2.1.1) or 1000 genome (v Phase 3v5), while the reference method used variants called using torrent variant caller (TVC, v5.18) with total white blood cell (WBC) genomic DNA (gDNA) as the germline control. Clinically actionable variants were defined as those with ClinVar (v 20250323) pathogenic/likely pathogenic classification or OncoKB (v 4.27) Level 1/2 oncogenic annotation with population frequency ≤0.1%. A total of 345 clinically actionable variants were evaluated, including germline pathogenic alterations in *BRCA2, CHEK2, ATM, MSH2, PALB2*, and *RAD51D*.

The study demonstrated 100% PPA (345/345; 95% CI: 98.9% - 100%) and 100% NPA (2,745/2,745; 95% CI: 99.9% - 100%), with zero false negatives (FN) for actionable germline variants. Twenty-one (6.1%) actionable variants had VAFs between 0.02 -0.05, confirming assay sensitivity near the LoD. This analysis concluded the T/O filtering strategy is analytically equivalent to matched T/N subtraction.

D. Substantial Equivalence Information

1. Predicate Device Name(s)

MSK-IMPACT

2. Predicate 510(k) Number(s)

DEN170058

3. Comparison with Predicate(s)

Characteristics	Predicate Device: MSK-IMPACT	Subject Device: CellDx-Tissue
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 CellDx-Tissue is a qualitative in vitro diagnostic (IVD) test that uses next-generation sequencing of DNA and RNA isolated from formalin-fixed paraffine-embedded (FFPE) tumor tissue from patients previously diagnosed with solid malignant neoplasm to detect tumor gene alterations in a broad multi-gene panel. The test is intended to provide tumor mutation profiling information on somatic mutations, including single nucleotide variants (SNVs), insertions and deletions (indels), one gene amplification, and three fusions.

Characteristics	Predicate Device: MSK-IMPACT	Subject Device: CellDx-Tissue
Similarities		
Indications for Use	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.	Information provided by the CellDx-Tissue test is intended for use by qualified healthcare professionals in accordance with professional guidelines in oncology. Results from CellDx-Tissue are not intended to be conclusive or prescriptive for the labeled use of any specific therapeutic product. CellDx-Tissue is a single-site assay performed at Datar Cancer Genetics (DCG).
Specimen Type	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
Test Environment	Single Site Assay	Same
Assay Cut-Off	Does not report mutations below 2% for known hotspot mutations and 5% for non-hotspot mutations	Same
Report Format	Results are reported under one of the following categories: • Variants with Evidence of Clinical Significance • Variants with Potential Clinical Significance	Same

Characteristics	Predicate Device: MSK-IMPACT	Subject Device: CellDx-Tissue
Similarities		
Clinical Evidence Curation	• Uses OncoKB, knowledge base • Classification criteria were developed by MSK to communicate the level of clinical evidence available for individual mutations in the test report.	Variant calls are organized into Variant with Evidence of Clinical Significance or Variant with Potential Clinical Significance depending on the designated cancer type.
Differences		
Genes on Panel	468 (6,357 exons)	517 (6,597 exons)
Variant Types	Somatic Variants including point mutations and small insertions and deletions, and microsatellite instability	Somatic Variants including point mutations and small insertions and deletions, *ERBB2* amplification, and three fusions (*ALK, RET*, and *ROS1*)
Black List	73 exons	157 exons
Analyte	DNA	DNA and RNA
Minimum Tumor Content	10% (20% preferred; 25% for MSI)	> 20%
Nucleic Acid Input	50 ng – 250 ng	DNA: 20 ng – 50 ng RNA: 20 ng – 50 ng
Sequencing Instrument	Illumina HiSeq 2500 Sequencer	Thermo Fisher Ion GeneStudio S5 Prime System
Technology	Hybrid Capture	Amplicon
Sequencing Chemistry	Sequencing by Synthesis (Fluorescent Detection)	Sequencing by Synthesis (Hydrogen Ion Detection)
Controls	• Positive Control • Negative Control • No Template Control (NTC) • Matched Normal	• Positive Control • Negative Control • No Template Control (NTC)

Characteristics	Predicate Device: MSK-IMPACT	Subject Device: CellDx-Tissue
Differences		
Germline Filtering	Matched normal specimen analysis	Computational, population database filtering
Coverage Requirements	≥ 200x mean; 100x for ≥ 98% target regions	≥ 500x; 100x for ≥ 90% target regions
Variant Calling Thresholds	<u>Hotspot:</u> • Mutant reads (AD) ≥ 8 • Mutation coverage (DP) ≥ 20 • Mutation frequency (VAF) ≥ 0.02 <u>Non-hotspot</u> • Mutant reads (AD) ≥ 10, • Mutation coverage (DP) ≥ 20 • Mutation frequency (VAF) ≥ 0.05	<u>Hotspot:</u> • Mutant reads (AD) ≥ 8, • Mutation coverage (DP) ≥ 40 • Strand bias (SB) < 0.9 • Mutation frequency (VAF) ≥ 0.02 <u>Non-hotspot</u> • Mutant reads (AD) ≥ 10, • Mutation coverage (DP) ≥ 40 • Strand bias (SB) < 0.85 • Mutation frequency (VAF) ≥ 0.05
Contamination QC	Percent heterozygous sites at fingerprint SNPs < 55%; Average MAF at homozygous fingerprint SNPs < 2%	Estimated contamination score < 0.120, Contamination score estimated from the signal derived from reference reads at homozygous alternate sites.
Criteria for Calling "Failed" Samples	If a sample presents with mean coverage across all exons < 50x and no mutations are detected due to the low overall coverage, the test is deemed "failed" for the sample	If a sample presents with mean coverage across all exons < 500x for DNA and <500,000 reads for RNA, the test is deemed "failed" for the sample.

E. Standards/Guidance Documents Referenced

The following FDA guidance documents and standards were consulted:

1. Medical Devices – Quality Management Systems: Requirements for Regulatory Purposes. ISO 13485: 2016
2. Medical Devices – Application of Risk Management to Medical Devices. ISO 14971: 2019
3. Medical Laboratories – Requirements for Quality and Competence. ISO 15189: 2022
4. Medical Devices – Symbols to be used with Information to be Supplies by the Manufacturer – Part 1: General Requirements. ISO 15223-1: 2021
5. Medical Device Software – Software Life Cycle Processes. ISO / IEC 62304: 2006
6. Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use – Part 1: General Reequipments. IEC 61010-1: 2010
7. Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements. EN 61326-1: 2013
8. Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline - CLSI EP25-A
9. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition. CLSI EP17-A2
10. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition. CLSI EP05-A3
11. User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline - Second Edition. CLSI EP12-A2

F. Performance Characteristics

1. Analytical Performance – General

The CellDx – Tissue assay is a targeted NGS panel with 517 genes. The targeted regions of interest in CellDx – Tissue are designed to detect SNVs, insertions up to 20 bp and deletions up to 30 bp in length of the targeted genes, as well as *ERBB2* amplifications, *ALK, RET*, and *ROS1* fusions. For SNVs and indels, a representative approach to validation of the targeted genes in the panel was submitted with data representing variant types for SNVs and indels, and at the gene levels for amplifications and fusions 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 the 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, the CellDx – Tissue assay supports repeat samples through the workflow once. Performance throughout the 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 in

Table 4. Data were aggregated for clinical cases from >25 tumor types. Resulting pass rates by tumor type across the workflow are shown in Table 5.

Table 4. Acceptability Rates of CellDx – Tissue Assay

Clinical FFPE Samples	Acceptability Rate (n/N) (95% CI)
First Pass	81.8% (2190/2676) (80.33%–83.25%)
After Repeat Test	93.1% (2492/2676) (92.10%–94.02%)

Table 5. Comparability of Tumor Invalid Rates for the CellDx – Tissue Assay

Organ	Number of Samples Failed				Invalid Rate (n/N) (%)
	Pre-analytical	Pre-run	Post-Run		
	Low Tumor Purity[1]	Low Nucleic Acid Yield[2]	Library Failure[3]	Data QC Failure	
Adrenal Gland	0	0	0	0	(0/2) 0%
Ampulla of Vater	1	0	0	0	(1/33) 3%
Biliary Tract	0	0	0	0	(0/7) 0%
Bladder/Urinary Tract	0	0	0	2	(2/20) 10%
Bone	0	0	0	0	(0/2) 0%
Bowel	6	4	2	35	(47/479) 10%
Breast	3	5	2	3	(13/317) 4%
Cervix	0	0	0	0	(0/41) 0%
CNS/Brain	0	1	1	3	(5/67) 7%
Esophagus/Stomach	0	0	0	9	(9/115) 8%
Head and Neck	12	3	0	17	(32/220) 15%
Kidney	0	0	0	0	(0/18) 0%
Liver	0	0	0	0	(0/12) 0%
Lung	2	3	0	38	(43/695) 6%
Ovary/Fallopian Tube	0	1	0	3	(4/73) 5%
Ovary/Fallopian Tube, Uterus	0	0	0	0	(0/3) 0%
Pancreas	2	0	0	2	(4/27) 15%
Peritoneum	0	0	0	0	(0/2) 0%

Organ	Number of Samples Failed				Invalid Rate (n/N) (%)
	Pre-analytical	Pre-run	Post-Run		
	Low Tumor Purity[1]	Low Nucleic Acid Yield[2]	Library Failure[3]	Data QC Failure	
Pleura	0	1	0	1	(2/2) 100%
Prostate	0	0	0	6	(6/217) 3%
Skin	0	0	5	7	(12/51) 24%
Soft Tissue	1	0	0	0	(1/18) 6%
Testis	0	0	0	1	(1/2) 50%
Thymus	0	0	0	0	(0/4) 0%
Thyroid	0	0	0	0	(0/88) 0%
Unknown Primary	0	0	0	0	(0/2) 0%
Uterus	0	0	0	2	(2/155) 1%
Vulva/Vagina	0	0	0	0	(0/4) 0%
Total	**27**	**18**	**10**	**129**	**(184/2676) 6.9%**

[1] < 20 %
[2] < 20 ng
[3] No amplification

b. Invalid Rates Observed in Method Comparison

Invalid rates were infrequent across all tested biomarkers and were consistently within the acceptance criteria for the CellDx-Tissue assay workflow. A summary of the invalid rates observed during the method comparison study is shown in Table 6.

Table 6. Invalid Rates Observed in the Method Comparison Study.

Category	DNA	RNA	Total
1st Pass Valid	233	221	454
After Repeat Test Valid	10	23	33
Low Nucleic Acid Yield (<20 ng)	14	6	17
Library Failure	1	0	1
Invalid (Sequencing QC Failure)	7	5	12
Total Invalid	22	11	33
Total Attempts	265	252	517
Total Invalid (%)	**8.30%**	**4.4%**	**6.38%**

2. Precision

a. Precision Panel

The within lab precision (between-run and within-run) of the CellDx – Tissue assay was assessed using 17 FFPE clinical samples originating from eight primary tumor types (Bowel (n = 5, 29.4%), Lung (n = 4, 23.5%), Prostate (n = 2, 11.8%), CNS (n = 1, 5.9%), Breast (n = 1, 5.9%), Ovary (n = 2, 11.8%), Esophagus (n=1, 5.9%) and Thyroid (n = 1, 5.9%) to represent different variants and a range of frequencies. Extracted DNA and RNA from each of the 17 samples was tested in duplicate by two different operators on multiple sequencing instruments across ≥3 non-consecutive days using two reagent lots, yielding 24-48 replicates per sample. For each replicate tested, all observed variants which passed the CellDx – Tissue assay QC metrics were reported and assessed for precision.

b. Panel-Wide Precision

Precision was accessed for each variant across all replicates. The positive call rates (PCR) and negative call rates (NCR) were calculated along with the two-sided 95% confidence intervals.

Table 7 summarizes the positive call rate (PCR) and negative call rate (NCR) stratified by mutation type (SNV, insertions, and deletions) and variant allele frequency (VAF). An overall PCR of 98.69% across all samples and replicates (2871/2909; 95% CI: 98.21% – 99.05%; Average VAF range: 2.0 – 85.52), with an increase in PCR at higher VAFs observed, and an overall NCR of 100% (533539/533539; 95% CI: 99.99, 100.00).

The positive call rates for selected individual sequence mutations assessed in the precision study, along with the VAF range, mean SD, and percent CV per variant are presented in Appendix D. A total of 48 SNVs and 15 indels (3 insertions, 15 deletions) are provided.

Table 7. Precision Positive (PCR) and Negative (NCR) Call Rates

Variant Type	VAF Level (%)	Unique Mutations	PCR (%) (n/N)	NCR (%) (n/N)	AF Range (%)	Mean Allele Depth/ FSR Range	Mean Loci Depth Rang
All	AF ≥ 0	63	98.69 (2871/2909)	100 (533539/533539)	2.00 - 85.52	46 - 1455	440 - 1984
	AF ≥ 2.0	63	98.69 (2871/2909)	100 (533539/533539)	2.00 - 85.52	46 - 1455	440 - 1984
	AF ≥ 5.0	60	98.99 (2758/2786)	100 (533539/533539)	3.20 - 85.52	46 - 1455	440 - 1978
	AF ≥ 10.0	53	99.23 (2451/2470)	100 (450769/450769)	7.43 - 85.52	82 - 1455	440 - 1978
	AF ≥ 15.0	48	99.15 (2211/2230)	100 (450769/450769)	15.44 - 85.52	142 - 1455	440 - 1978
Variants with Evidence of Clinical Significance	AF ≥ 0	10	99.56 (455/457)	100 (365377/365377)	2.26 - 79.57	74 - 1455	604 - 1978
	AF ≥ 2.0	10	99.56 (455/457)	100 (365377/365377)	2.26 - 79.57	74 - 1455	604 - 1978
	AF ≥ 5.0	9	100 (411/411)	100 (324483/324483)	20.07 - 79.57	290 - 1455	604 - 1978
	AF ≥ 10.0	9	100 (411/411)	100 (324483/324483)	20.07 - 79.57	290 - 1455	604 - 1978
	AF ≥ 15.0	9	100 (411/411)	100 (324483/324483)	20.07 - 79.57	290 - 1455	604 - 1978
Hotspot Variants	AF ≥ 0	24	97.99 (1073/1095)	100 (409829/409829)	2.00 - 60.77	46 - 1092	737 - 1984
	AF ≥ 2.0	24	97.99 (1073/1095)	100 (409829/409829)	2.00 - 60.77	46 - 1092	737 - 1984
	AF ≥ 5.0	21	98.77 (960/972)	100 (409829/409829)	3.20 - 60.77	46 - 1092	737 - 1978
	AF ≥ 10.0	17	99.25 (791/797)	100 (409829/409829)	15.44 - 60.77	214 - 1092	810 - 1978
	AF ≥ 15.0	17	99.25 (791/797)	100 (409829/409829)	15.44 - 60.77	214 - 1092	810 - 1978

N/A: Not Applicable; FSR: Fusion Supporting Reads

Variant Type	VAF Level (%)	Unique Mutations	PCR (%) (n/N)	NCR (%) (n/N)	AF Range (%)	Mean Allele Depth/ FSR Range	Mean Loci Depth Rang
Non-hotspot Variants	AF ≥ 0	39	99.12 (1798/1814)	100 (407205/407205)	5.04 - 85.52	63 - 1455	440 - 1977
	AF ≥ 2.0	39	99.12 (1798/1814)	100 (407205/407205)	5.04 - 85.52	63 - 1455	440 - 1977
	AF ≥ 5.0	39	99.12 (1798/1814)	100 (407205/407205)	5.04 - 85.52	63 - 1455	440 - 1977
	AF ≥ 10.0	36	99.22 (1660/1673)	100 (324435/324435)	7.43 - 85.52	82 - 1455	440 - 1977
	AF ≥ 15.0	31	99.09 (1420/1433)	100 (324435/324435)	17.53 - 85.52	142 - 1455	440 - 1977
SNVs	AF ≥ 0	48	99.13 (2172/2191)	100 (449879/449879)	2.00 - 79.57	46 - 1455	449 - 1984
	AF ≥ 2.0	48	99.13 (2172/2191)	100 (449879/449879)	2.00 - 79.57	46 - 1455	449 - 1984
	AF ≥ 5.0	45	99.56 (2059/2068)	100 (449879/449879)	3.20 - 79.57	46 - 1455	449 - 1978
	AF ≥ 10.0	41	99.84 (1891/1894)	100 (408049/408049)	10.33 - 79.57	101 - 1455	449 - 1978
	AF ≥ 15.0	39	99.83 (1795/1798)	100 (408049/408049)	15.44 - 79.57	142 - 1455	449 - 1978
Insertions	AF ≥ 0	3	100 (144/144)	100 (85248/85248)	7.43 - 46.99	82 - 571	758 - 1430
	AF ≥ 2.0	3	100 (144/144)	100 (85248/85248)	7.43 - 46.99	82 - 571	758 - 1430
	AF ≥ 5.0	3	100 (144/144)	100 (85248/85248)	7.43 - 46.99	82 - 571	758 - 1430
	AF ≥ 10.0	3	100 (144/144)	100 (85248/85248)	7.43 - 46.99	82 - 571	758 - 1430
	AF ≥ 15.0	1	100 (48/48)	100 (42720/42720)	35.92 - 46.99	571 - 571	1375 - 1375
Deletions	AF ≥ 0	12	96.69 (555/574)	100 (254204/254204)	3.54 - 85.52	69 - 578	440 - 1699
	AF ≥ 2.0	12	96.69 (555/574)	100 (254204/254204)	3.54 - 85.52	69 - 578	440 - 1699
	AF ≥ 5.0	12	96.69 (555/574)	100 (254204/254204)	3.54 - 85.52	69 - 578	440 - 1699
	AF ≥ 10.0	9	96.30 (416/432)	100 (170736/170736)	9.28 - 85.52	108 - 578	440 - 1699
	AF ≥ 15.0	8	95.83 (368/384)	100 (170736/170736)	15.64 - 85.52	269 - 578	440 - 1699

N/A: Not Applicable; FSR: Fusion Supporting Reads

Variant Type	VAF Level (%)	Unique Mutations	PCR (%) (n/N)	NCR (%) (n/N)	AF Range (%)	Mean Allele Depth/ FSR Range
ERBB2 Amplification	1	100 (120/120)	-	N/A	N/A	N/A
ALK Fusion	1	100 (45/45)	-	N/A	7121-30568	N/A
ROS1 Fusion	1	95.0 (38/40)	-	N/A	504-2328	N/A
RET Fusion	1	100 (46/46)	-	N/A	740-175619	N/A

N/A: Not Applicable; FSR: Fusion Supporting Reads

c. Per-Specimen Precision for SNVs and Indels

The precision was calculated for each individual specimen as shown in Table 8. Results from the precision studies were combined across all reportable genes for each specimen. The positive and negative call rates for sequenced mutations (SNVs and indels) in each sample were calculated based on the total number of mutations along with the two-sided 95% CI.

Table 8. Positive and Negative Call Rates per Sample

Sample ID	Cancer Type	Unique Mutations Detected*	PCR Per Mutation	PCR (%) (n/N) (95% CI)	NCR (%) (n/N) (95% CI)
Sample 1	Ovary/ Fallopian Tube	4	45/48 for 1***	98.43 189/192 (95.5, 99.5)	100 42672/42672 (99.99, 100)
			48/48 for 3		
Sample 2	Thyroid	1	48/48 for 1	100 48/48 (92.6, 100.0)	100 42672/42672 (99.99, 100)
Sample 3	Prostate	1	48/48 for 1	100 48/48 (92.6, 100.0)	100 42672/42672 (99.99, 100)
Sample 4	Bowel	6	25/31 for 1**	94.09, 175/186, (88.0, 95.5)	100, 42672/42672, (99.99,100)
			26/31 for 1**		
			31/31 for 4**		

*Across the 48 Replicates
**Reduction in the number of replicates due to QC failure
***Variant with VAF at LoD

Sample ID	Cancer Type	Unique Mutations Detected*	PCR Per Mutation	PCR (%) (n/N) (95% CI)	NCR (%) (n/N) (95% CI)
Sample 5	Lung	1	46/48 for 1***	95.83, 46/48, (85.8, 99.5)	100, 42720/42720, (100, 100)
Sample 6	Bowel	16	42/48 for 2*** 45/48 for 1*** 48/48 for 13	98.04, 753/768, (96.8, 98.8)	99.99, 42522/42528, (99.97,99.99)
Sample 7	Lung	1	47/48 for 1	97.91, 47/48, (89.1, 99.6)	99.91, 42634/42672, (99.88,99.94)
Sample 8	Prostate	2	46/46 for 2**	100, 92/92, (96.0, 100)	100, 40940/40940, (99.99,100)
Sample 9	Lung	6	48/48 for 6	100, 288/288, (98.7, 100)	100, 42623/42624, (99.99,100)
Sample 10	Bowel	12	45/48 for 1*** 47/48 for 1*** 48/48 for 10	99.3, 572/576, (98.7, 100)	100, 42432/42432, (99.99,100)
Sample 11	CNS/ Brain	4	48/48 for 4	100, 192/192, (98.0, 100)	99.94, 42646/42672, (99.91,99.96)
Sample 12	Bowel	3	44/46 for 1**,*** 44/46 for 1** 46/46 for 1**	97.1, 134/138, (98.0, 100)	100, 40801/40802, (99.99,100)
Sample 13	Breast	6	47/48 for 1*** 48/48 for 5	99.65, 287/288, (98.1, 99.9)	100 42623/42624, (99.99, 100)

*Across the 48 Replicates
**Reduction in the number of replicates due to QC failure
***Variant with VAF at LoD

d. Precision for ERBB2 Amplification

Precision of *ERBB2* amplification was evaluated using 15 samples. Three out of the 15 samples were tested at two DNA input levels using two different operators on two instruments across three non-consecutive days using two kit lots, yielding 12 replicates per sample per input level. The remaining samples were tested in duplicate by two different operators on six instruments across four non-consecutive days using two kit lots, yielding 24 replicates per sample per input level. Of the 15 samples, four contained *ERBB2* amplifications and the remaining samples contained no *ERBB2* amplifications. The CellDx-Tissue reports an *ERBB2* amplification when the observed copy number for the gene is determined by the test to be 8.5 copies or more. The tumor purity, input, mean observed copy number (CN), coefficient of variation (%CV), positive and negative call rates along with the 95% CIs for each sample are summarized in Table 9. The call rates were 100% for both amplification and no amplification groups.

Table 9. Summary of the *ERBB2* Gene Amplification Precision

Sample ID	Cancer Type	*ERBB2* Status	Tumor Purity (%)	Input (ng)	Mean CN	CN CV (%)	PCR % (n/N) (95% CI)	NCR % (n/N) (95% CI)
Sample 1	Breast	Positive	58	10	16.3	11.0	100 (24/24) (85.7, 100)	-
				20	16.4	7.0	100 (24/24) (85.7, 100)	-
Sample 2	Bowel	Positive	21	10	8.06	6.3	100 (12/12) (75.8, 100)	-
				20	7.82	4.9	100 (12/12) (75.8, 100)	-
Sample 3	Esophagus	Positive	49	10	15.57	10.2	100 (12/12) (75.8, 100)	-
				20	15.34	7.7	100 (12/12) (75.8, 100)	-
Sample 4	Ovarian / Fallopian Tube	Positive	55	10	14.54	7.1	100 (12/12) (75.8, 100)	-
				20	14.02	7.0	100 (12/12) (75.8, 100)	-

Sample ID	Cancer Type	*ERBB2* Status	Tumor Purity (%)	Input (ng)	Mean CN	CN CV (%)	PCR % (n/N) (95% CI)	NCR % (n/N) (95% CI)
Sample 5	CNS	Negative	92	10	1.98	5.1	-	100 (24/24) (86.2, 100)
				20	1.98	4.6	-	100 (24/24) (86.2, 100)
Sample 6	Bowel	Negative	37	10	1.34	15.2	-	100 (24/24) (86.2, 100)
				20	1.32	14.9	-	100 (24/24) (86.2, 100)
Sample 7	Lung	Negative	28	10	1.97	8.8	-	100 (24/24) (86.2, 100)
				20	1.93	8.8	-	100 (24/24) (86.2, 100)
Sample 8	Prostate	Negative	22	10	1.79	18.6	-	100 (24/24) (86.2, 100)
				20	1.75	13.7	-	100 (22/22) (85.1, 100)
Sample 9	Lung	Negative	20	10	1.28	15.1	-	100 (24/24) (86.2, 100)
				20	1.28	16.4	-	100 (24/24) (86.2, 100)
Sample 10	Bowel	Negative	29	10	1.54	5.7	-	100 (24/24) (86.2, 100)
				20	1.54	10.3	-	100 (24/24) (86.2, 100)
Sample 11	Lung	Negative	45	10	1.58	11.8	-	100 (24/24) (86.2, 100)
				20	1.62	9.2	-	100 (24/24) (86.2, 100)

Sample ID	Cancer Type	*ERBB2* Status	Tumor Purity (%)	Input (ng)	Mean CN	CN CV (%)	PCR % (n/N) (95% CI)	NCR % (n/N) (95% CI)
Sample 12	Bowel	Negative	39	10	2.87	24.2	-	100 (7/7) (59.0, 100)
				20	2.70	8.0	-	100 (24/24) (86.2, 100)
Sample 13	Prostate	Negative	36	10	1.98	6.6	-	100 (24/24) (86.2, 100)
				20	2.08	5.7	-	100 (24/24) (86.2, 100)
Sample 14	Thyroid	Negative	22	10	1.99	2.9	-	100 (24/24) (86.2, 100)
				20	1.99	3.3	-	100 (24/24) (86.2, 100)
Sample 15	Ovary	Negative	56	10	1.52	13.7	-	100 (24/24) (86.2, 100)
				20	1.58	9.1	-	100 (24/24) (86.2, 100)

e. Precision for ALK, RET, and ROS1 Fusions

The precision of RNA fusions was evaluated across different samples with varying tumor purities. The results are summarized in Table 10.

Table 10. Summary of *ALK, RET*, and *ROS1* Fusions

Fusion	Tumor Purity (%)	LoD Level	Mean Supporting Reads (Range)	PCR (%) n/N (95% CI)	NCR (%) n/N (95% CI)
ALK	20	1x*	15062 (7121-30568)	100 45/45 (92.1, 100)	100 609/609 (99.4, 100)

Fusion	Tumor Purity (%)	LoD Level	Mean Supporting Reads (Range)	PCR (%) n/N (95% CI)	NCR (%) n/N (95% CI)
RET	25	1.8x*	24438 (740-175619)	100 46/46 (92.3, 100)	100 608/608 (99.4, 100)
ROS1	45	0.8x**	1273 (504-2328)	95 38/40 (90.8, 100)	100 614/614 (99.4, 100)

f. Analysis of Source of Variance

The Average Positive Agreement (APA) and Average Negative Agreement (ANA) was assessed to analyze the imprecision caused by different sources of variance. Data analysis is presented stratified by variant type and present for 1) overall, 2) instrument to instrument, 3) operator to operator, and 4) day to day (Table 11).

Table 11. Reproducibility of the CellDx – Tissue Assay

Alteration Type	Metric	Overall (%) (95% CI)	Inter-Instrument (%) (95% CI)	Inter-Operator (%) (95% CI)	Inter-Day (%) (95% CI)
Variants with Evidence of Clinical Significance	APA	99.56 (98.42, 99.88)	99.56 (95.19, 100)	99.57 (98.34, 100)	99.57 (96.74, 100)
	ANA	100 (100, 100)	100 (100, 100)	100 (100, 100)	100 (100, 100)
Hotspot Variants	APA	97.99 (96.98, 98.67)	97.94 (95.27, 99.44)	97.95 (96.21, 98.74)	97.99 (95.31, 98.99)
	ANA	100 (100, 100)	100 (100, 100)	100 (100, 100)	100 (100, 100)
Non-hotspot Variants	APA	99.12 (98.57, 99.46)	99.11 (97.62, 99.82)	99.11 (98.27, 99.55)	99.12 (97.76, 99.66)
	ANA	100 (100, 100)	100 (100, 100)	100 (100, 100)	100 (100, 100)
SNVs	APA	99.13 (98.65, 99.44)	99.11 (97.61, 99.72)	99.12 (98.33, 99.50)	99.13 (97.88, 99.61)
	ANA	100 (100, 100)	100 (100, 100)	100 (100, 100)	100 (100, 100)

Alteration Type	Metric	Overall (%) (95% CI)	Inter-Instrument (%) (95% CI)	Inter-Operator (%) (95% CI)	Inter-Day (%) (95% CI)
Insertions (All)	APA	100 (97.40, 100)	100 (86.20, 100)	100 (94.93, 100)	100 (90.36, 100)
	ANA	100 (100, 100)	100 (100, 100)	100 (100, 100)	100 (100, 100)
Insertions (1 – 5 bp)	APA	100 (97.40, 100)	100 (86.20, 100)	100 (94.93, 100)	100 (90.36, 100)
	ANA	100 (100, 100)	100 (100, 100)	100 (100, 100)	100 (100, 100)
Insertions (5 – 10 bp)	APA	N/A	N/A	N/A	N/A
	ANA	100 (100, 100)	100 (100, 100)	100 (100, 100)	100 (100, 100)
Insertions (11 – 30 bp)	APA	N/A	N/A	N/A	N/A
	ANA	100 (100, 100)	100 (100, 100)	100 (100, 100)	100 (100, 100)
Deletions (All)	APA	96.69 (94.89, 97.87)	96.69 (89.77, 98.37)	96.69 (93.71, 98.10)	96.69 (92.13, 98.51)
	ANA	100 (100, 100)	100 (100, 100)	100 (100, 100)	100 (100, 100)
Deletions (1 – 5 bp)	APA	96.39 (94.43, 97.68)	96.38 (88.89, 98.22)	96.38 (93.14, 97.92)	96.39 (91.44, 98.37)
	ANA	100 (100, 100)	100 (100, 100)	100 (100, 100)	100 (100, 100)
Deletions (5 – 10 bp)	APA	N/A	N/A	N/A	N/A
	ANA	100 (100, 100)	100 (100, 100)	100 (100, 100)	100 (100, 100)
Deletions (11 – 30 bp)	APA	100 (92.59, 100)	100 (67.56, 100)	100 (86.20, 100)	100 (75.75, 100)
	ANA	100 (100, 100)	100 (100, 100)	100 (100, 100)	100 (100, 100)

N/A: Not Available / Applicable

Alteration Type	Metric	Overall (%) (95% CI)	Inter-Instrument (%) (95% CI)	Inter-Operator (%) (95% CI)	Inter-Day (%) (95% CI)
ERBB2 Amplification	APA	100 (92.59, 100)	100 (67.56, 100)	100 (86.20, 100)	100 (75.75, 100)
	ANA	100 (99.25, 100)	100 (95.68, 100)	100 (98.51, 100)	100 (97.06, 100)
ALK Fusion	APA	100 (92.13, 100)	100 (60.97, 100)	100 (79.61, 100)	100 (74.12, 100)
	ANA	100 (99.20, 100)	100 (94.65, 100)	100 (97.64, 100)	100 (96.87, 100)
RET Fusion	APA	100 (92.29, 100)	100 (64.57, 100)	100 (79.61, 100)	100 (75.75, 100)
	ANA	100 (99.20, 100)	100 (94.65, 100)	100 (97.64, 100)	100 (96.87, 100)
ROS1 Fusion	APA	95.00 (83.50, 98.62)	91.67 (43.65, 96.99)	95.83 (77.19, 100)	95.00 (72.25, 100)
	ANA	100 (99.20, 100)	100 (94.65, 100)	100 (97.64, 100)	100 (96.87, 100)

N/A: Not Available / Applicable

g. Lot-to-Lot Precision

The performance of the CellDx – Tissue assay was assessed across two unique kit lots by determining the concordance of variant calls in 17 FFPE tissue samples. The two unique lots were used to process 372 test cases in duplicate for a total of 744 observations. All batches were sequenced on the same instrument. Table 12. lists the APA and ANA used to assess lot to lot performance. The overall panel-wide variants (SNVs, Insertions and Deletions) APA is 98.68% and the ANA is 100%. *ERBB2* amplification, *ALK*, and *RET* fusions had a concordance of 100%; and *ROS*1 fusions demonstrated a concordance of 93.75%.

Table 12. Lot-to-Lot Precision of CellDx – Tissue Assay

Variant Type	Performance	Between Lot 1& Lot 2 (%) (95% CI)
Variants with Evidence of Clinical Significance	APA	99.57 (98.34, 100)
	ANA	100 (100, 100)
Panel-Wide (SNVs + Insertions + Deletions)	APA	98.68 (97.97, 99.16)
	ANA	100 (100, 100)
Hotspot SNVs (Including Clinically Significant Variants)	APA	97.95 (96.21, 98.74)
	ANA	100 (100, 100)
Non-hotpot SNVs (Including Clinically Significant Variants)	APA	99.11 (98.27, 99.55)
	ANA	100 (100, 100)
SNVs (Hotspot + Non-hotspot)	APA	99.12 (98.33, 99.50)
	ANA	100 (100, 100)
Insertions	APA	100 (94.93, 100)
	ANA	100 (100, 100)
Deletions	APA	96.69 (93.71, 98.10)
	ANA	100 (100, 100)
ERBB2 Amplification	APA	100 (86.20, 100)
	ANA	100 (98.51, 100)

Variant Type	Performance	Between Lot 1& Lot 2 (%) (95% CI)
ALK Fusion	APA	100 (85.13, 100)
	ANA	100 (98.41, 100)
RET Fusion	APA	100 (85.69, 100)
	ANA	100 (98.41, 100)
ROS1 Fusion	APA	93.75 (76.39, 99.11)
	ANA	100 (98.41, 100)

3. Analytical Sensitivity – Limit of Detection (LoD)

The LoD of the CellDx – Tissue assay for SNVs and indels is defined as the lowest concentration with ≥ 95% of replicates for a variant are reliably detected. The LoD of the CellDx – Tissue assay for *ERBB2* amplification is determined as the minimum tumor purity required for robust reporting of amplification status and incorporated the clinical reporting framework where CN ≥ 8.5 is Positive, CN ≥ 4 to < 8.5 is Indeterminate / Low-Level Amplification (below LoD), and CN < 4 is Negative. The LoD of the CellDx – tissue assay for *ALK, RET,* and *ROS1* fusions is determined as the minimum tumor purity required for robust reporting of fusion status. The recommended nucleic acid input for the CellDx – Tissue assay is 20 ng of DNA and 20 ng of RNA recovered from tissue with a minimum of 20% viable tumor nuclei. Details of the data are discussed and shown below.

a. LoD – SNVs, Insertions, and Deletions

The analytical sensitivity of the CellDx – Tissue assay for SNVs, insertions, and deletions was evaluated by assessing 10 clinical FFPE specimens from seven different cancer types with 29 SNVs, 1 insertion, and 2 deletions. Each sample was diluted to at least five dilution levels and tested in 10 replicates per level using two reagent lots. The call rate was determined for each variant and the LoD was approximated between the call rate that was below 95% and the highest call rate (100%). A summary of the estimation of the LoD range for a set of representative variants is shown in Table 13. The established VAF range for each variant type (Hotspot SNVs, Non-hotspot SNVs, insertions, and deletions) is shown in Table 14.

Table 13. Estimation of LoD Range for Representative Variants

Tumor Type	Variant Type	Level	Gene	AA Change	Avg DP	Avg AD	VAF Range	Mean VAF (%)	PCR (%)
Lung	SNV	3	*TP53*	p.R175H	1869	68	0.026-0.048	3.7	100
Lung	SNV	5	*TET2*	c.3954+3T>G	1426	63	0.024-0.098	4.3	100
Lung	DEL	5	*EGFR*	p.E746_A750del	1939	69	0.026-0.058	3.5	100
Breast	SNV	2	*GPS2*	c.318-6C>T	1886	123	0.056-0.078	6.5	100
Breast	SNV	4	*PIK3CA*	p.H1047R	1678	58	0.022-0.049	3.5	100
Uterus	SNV	2	*TP53*	p.R273C	1955	75	0.024-0.053	3.8	100
Uterus	SNV	2	*CTLA4*	p.A54T	2000	132	0.054-0.078	6.6	100
Uterus	SNV	3	*CTNNB1*	p.G34E	1959	74	0.026-0.045	3.7	100
Head and Neck	SNV	2	*NRAS*	p.A59T	2000	107	0.044-0.071	5.4	100
Head and Neck	SNV	3	*DICER1*	c.3269+6C>T	1921	128	0.045-0.13	6.8	100
Head and Neck	SNV	4	*TP53*	p.G262V	1885	75	0.026-0.047	3.9	100
Head and Neck	SNV	3	*NF2*	p.R196*	659	48	0.059-0.104	6.7	100
Head and Neck	SNV	4	*EP300*	c.3728+5G>A	1271	56	0.035-0.052	4.0	100
Head and Neck	SNV	4	*FAT1*	p.R1205*	1919	118	0.05-0.078	6.1	100

Tumor Type	Variant Type	Level	Gene	AA Change	Avg DP	Avg AD	VAF Range	Mean VAF (%)	PCR (%)
Head and Neck	SNV	4	*LATS1*	p.Q953*	1603	56	0.028-0.047	3.4	100
Head and Neck	SNV	3	*PHF6*	p.Y301*	938	52	0.041-0.077	5.7	100
Bowel	SNV	2	*TP53*	p.R273H	1925	127	0.052-0.077	6.6	100
Esophagus / Stomach	INS	4	*ARID1A*	p.A1136Gfs*57	1503	92	0.042-0.088	6.2	100
Esophagus / Stomach	SNV	3	*TP53*	p.R209*	1924	165	0.064-0.16	8.5	100
Esophagus / Stomach	SNV	3	*POT1*	p.E456*	1281	71	0.032-0.107	5.5	100
Lung	SNV	1	*TP53*	p.R248W	1797	124	0.043-0.094	6.9	100
Uterus	SNV	2	*FANCA*	c.2015-8C>T	1765	101	0.04-0.066	5.7	100
Uterus	SNV	2	*TP53*	p.Y220C	1287	85	0.055-0.083	6.6	100
Uterus	SNV	2	*PPP2R1A*	p.P179R	1708	57	0.022-0.05	3.4	100
Prostate	SNV	5	*MLH1*	p.Q346H	1781	82	0.033-0.056	4.1	100
Prostate	SNV	5	*AR*	p.L702H	1579	83	0.044-0.072	5.4	100
Lung	INS	2	*ARID1A*	p.P1468Lfs*13	938	88	0.076-0.122	9.5	100
Lung	SNV	5	*TCF7L2*	c.1162-12C>T	1720	134	0.039-0.144	8.0	100
Lung	SNV	3	*TP53*	p.Y126*	1911	151	0.029-0.154	7.2	100
Lung	SNV	4	*FANCE*	c.855+6T>A	1029	64	0.026-0.095	5.2	100
Lung	SNV	2	*KMT2C*	p.E1333*	891	105	0.045-0.214	11.5	100
Lung	SNV	3	*KMT2D*	c.840-10G>A	1861	147	0.025-0.153	7.3	100

Table 14. Analysis Sensitivity (LoD VAF) for Representative SNVs and Indels

Variant	Established VAF Range (%)	Tumor Purity (%)	Average Allele Depth	Number of Variants in Clinical Cases in the Established Range
Hotspot SNVs	2.2 – 9.4	7	57 – 124	10
Non-hotspot SNVs	2.4 – 21.4	4 – 24	63 – 105	19
Insertions	4.2 – 8.8	6	92	1
Deletions	2.6 – 12.2	4 – 24	69 – 88	2
Homopolymer Context	4.2 – 8.8	6	92	1
Non-Homopolymer Context	2.6 – 12.2	4 – 24	69 – 88	2

b. LoD – Amplifications and Fusions (Tumor Purity)

i. ERBB2 Amplification

The analytical sensitivity of *ERBB2* was assessed by testing one clinical breast cancer sample diluted with normal FFPE DNA to five detection levels. Each dilution level was run in 10 replicates per level per reagent lot (two lots), resulting in a total of 50 observations. The cutoff for *ERBB2* detection is an observed copy number ≥ 8.5. The data summarized in Table 15 showed 100% call rates for samples with a tumor purity of 15%.

Table 15. Analysis Sensitivity for *ERBB2* Amplification by the CellDx-Tissue Assay

Dilution Level	Tumor Purity (%)	Mean Observed Copy Number (Mean ± SD)	Observed Copy Number Range	Positive Call Rate (%) (n/N)
1	60	30.2 ± 0.8	29–32	100 (10/10)
2	30	15.0 ± 0.7	14–16	100 (10/10)
3	15	8.5 ± 0.2	8–11	100 (10/10)

Dilution Level	Tumor Purity (%)	Mean Observed Copy Number (Mean ± SD)	Observed Copy Number Range	Positive Call Rate (%) (n/N)
4	8	5.1 ± 0.9	4–7	20 (2/10)
5	4	3.15 ± 0.1	3–3	0 (0/10)

ii. *ALK, RET*, and *ROS1* RNA Fusions

The analytical sensitivity of *ALK, RET*, and *ROS1* was assessed by testing four clinical (three lung and one bowel) cancer samples. *ALK* and *RET* clinical samples were diluted with normal FFPE DNA to five dilution levels. *ROS1* clinical samples were diluted to nine dilution levels. Each dilution level was run in 10 replicates per level (across two lots), resulting in a total of 50 observations for *ALK* and *RET* and 90 observations for *ROS1*. The data is summarized in Table 16 – Table 19.

Table 16. Analysis Sensitivity for *ALK* Amplification by the CellDx-Tissue Assay

Dilution Level	Tumor Purity (%)	Fusion Read Counts	Mean Fusion Read Range	Positive Call Rate (%) (n/N)
1	20	4868	1640-8989	100 (10/10)
2	15	1048	603-1544	80 (8/10)
3	11	1104	734-1506	30 (3/10)
4	8	1014	642-1409	30 (3/10)
5	6	931	740-1049	30 (3/10)

Table 17. Analysis Sensitivity for *RET* Amplification by the CellDx-Tissue Assay

Dilution Level	Tumor Purity (%)	Fusion Read Counts	Mean Fusion Read Range	Positive Call Rate (%) (n/N)
1	25	11534	5788-19474	100 (10/10)
2	19	7321	3996-10730	100 (7/7)
3	14	4282	1366-8416	100 (10/10)
4	11	7604	2120-16136	100 (7/7)

Dilution Level	Tumor Purity (%)	Fusion Read Counts	Mean Fusion Read Range	Positive Call Rate (%) (n/N)
5	8	6095	600-18193	86 (6/7)

Table 18. Analysis Sensitivity for *ROS1* Amplification by the CellDx-Tissue Assay

Dilution Level	Tumor Purity (%)	Fusion Read Counts	Mean Fusion Read Range	Positive Call Rate (%) (n/N)
1	70	80775	28692-130814	100 (10/10)
2	53	41636	28054-58784	100 (10/10)
3	39	24685	5883-39701	100 (10/10)
4	30	46067	9422-123494	100 (10/10)
5	22	9000	5861-14743	100 (10/10)
6	12	11498	4925-33280	100 (9/9)
7	9	5692	1785-13236	100 (10/10)
8	7	4393	1015-13653	100 (10/10)
9	5	4346	1605-7058	100 (10/10)
10*	20	39,875	34,176 - 49,210	100 (5/5)
11*	10	30,333	22,285 - 42,599	100 (5/10)
12*	7	1,853	1,158 - 2,641	100 (5/5)
13*	5	1,599	892 - 3,587	100 (5/5)
14*	4	942	715 - 1,431	70 (7/10)
15*	3	883	554 - 1,141	30 (3/10)
16*	2	-	-	0 (0/0)

*Samples and dilution levels are from a Supplemental Study to determine the tumor purity LoD for *ROS1*.

Table 19. Analysis Sensitivity Summary of RNA Fusion

Gene	Tumor Purity Range (%)	Fusion Read Count Range	LoD Tumor Purity (%)	LoD Supporting Reads
ALK	20 – 6	4868-931	20	>1640
RET	25 – 8	11533–6095	14	>1366
*ROS1**	20 – 2	39875-883	5	>892

*Supplemental LoD Study

4. Linearity/Assay Reportable Range

Not applicable

5. Traceability (Controls, Calibrators, or Methods)

a. Traceability

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

b. Stability

Reagent stability is based on the manufacturer expiration dating, verified by Datar Cancer Genetics. Stability of the reagents is monitored through the use of consistent controls.

c. Expected Values (Controls, Calibrators, or Methods)

The external positive control (commercial vendor qualified by Datar Cancer Genetics) contains different confirmed mutations, representing a range of mutation allele frequencies. A negative external control (commercial vendor qualified by Datar Cancer Genetics) contains a non-cancerous cell line derived DNA /RNA with no variants of interest. Both external controls are processed from library preparation through sequencing to serve as an end-to-end control to demonstrate assay performance. Failure of either external control to meet the quality control metrics will result in all test samples on the run be flagged and re-sequenced.

6. Analytical Specificity

a. Cut-Off / False Positive Range (Limit of Blank)

Non-cancerous FFPE tissues were evaluated for analytical specificity to access the risk of false positives in normal tissue when detecting SNVs, indels, amplifications, and fusions using the CellDx – Tissue assay. A total of 37 normal or benign-adjacent tissue were processed across two reagent lots, multiple operators, and days. Ninety (90) false positive events were detected for SNVs and indels for a false positive rate (FPR) of 0.000054 (95 CI: 0.000043 - 0.000066). None of the detected variants were classified as Variants with Evidence of Clinical Significance (Level 2), while four were classified as

Variants with Potential Evidence of Clinical Significance (Level3). The FPR was determined to be 0 (95 CI: 0 - 3.09) for *ERBB2* amplifications, and *ALK, RET,* and *ROS1* fusion genes.

b. Necrotic Tissue and Tumor Block Age

The impact of necrosis on the performance of the CellDx – Tissue assay was evaluated by assessing the invalid rates for both DNA and RNA libraries, defined as the proportion of libraries that failed to meet the predefined quality control thresholds. All 378 FFPE DNA and 377 FFPE RNA samples were evaluated for necrosis over a range of 0 - 80 % and invalid rates were examined. The assay performance was assessed and compared to the predicate device. The DNA and RNA invalid rates ranged from 0 to 10.52% and 0 to 1.78%, respectively (Table 20). The PPA for overall variant (SNVs, insertions, and deletions) detected ranged from 97.8% to 100%, while an NPA of 99.5% was observed (Table 21).

Table 20. Necrotic Tissue Invalid Rate for the CellDx-Tissue Assay

Cancer Types	Sample Count	Necrotic Tissue Content (%)	Invalid Rate (%) (n/N)
DNA			
23	281	0 – 5	1.78 (5/281)
15	72	5 – 20	0 (0/72)
12	19	21 – 40	10.52 (2/19)
4	6	41 – 80	0 (0/6)
RNA			
23	281	0 – 5	1.42 (4/281)
15	71	5 – 20	1.40 (1/71)
12	19	21 – 40	0 (0/19)
4	6	41 – 80	0 (0/6)

Table 21. Concordance of Overall Variant (SNV + Insertions + Deletions) Detection

Necrotic Tissue Content (%)	Sample Count	PPA (%) (95 CI) (n/N)	NPA (%) (95 CI) (n/N)
0 – 5	86	97.8 (93.8, 99.3) (135/138)	99.6 (99.4, 99.7) (13140/13197)

Necrotic Tissue Content (%)	Sample Count	PPA (%) (95 CI) (n/N)	NPA (%) (95 CI) (n/N)
>5 – 20	28	100 (91.0, 100) (39/39)	99.2 (98.9, 99.4) (4268/4303)
21 – 40	8	100 (77.2, 100) (13/13)	99.8 (99.3, 99.9) (1224/1227)
41 – 53	1	100 (34.2, 100) (2/2)	99.3 (96.4, 99.9) (153/153)
Overall	123	98.4 (95.5, 99.5) (189/192)	99.5 (99.4, 99.6) (18784,18880)

c. Interfering Substances

The impact of interfering substances on the performance of the CellDx – Tissue assay was assessed by processing DNA and RNA from FFPE samples tested in the presence of each interfering substance at varying amounts (Table 22). The samples were evaluated for concordance of variant call when compared to samples processed without the interfering substances. Replicates for six test cases were analyzed for six experimental and one baseline condition. Performance was evaluated across nine samples x seven conditions x five replicates. Analysis of all variant types tested (SNVs, indels, amplifications, and fusions showed no effect of exogenous and endogenous interferent for all conditions (Table 23 – Table 25).

Table 22. Endogenous and Exogenous Interfering Substances Tested

Type	Substance	Concentration Spiked	
		At Extraction	At Library Preparation
Exogenous	Ethanol	3x	5%
Exogenous	Index Adaptor	-	3x
Exogenous	Proteinase K	3x	0.04 mg/mL
Exogenous	Wash Buffer	3x	5%
Endogenous	Melanin	0.2 mg/mL	-
Endogenous	Hemoglobin	2 mg/mL	-

Table 23. Interfering Substances Concordance by Test Condition for SNVs and Indels

Test Condition	PCR (%) (n/N) (95% CI)	NCR (%) (n/N) (95% CI)
Ethanol	99.2 (119/120) (95.4, 99.9)	100 (106080/106080) (99.99, 100)
Adaptor	98.8 (115/120) (90.6, 98.2)	100 (106080/106080) (99.99, 100)
Proteinase K	94.9 (112/118) (89.3, 97.6)	100 (104312/104312) (99.99, 100)
Wash Buffer	98.3 (118/120) (94.1, 99.5)	100 (106080/106080) (99.99, 100)
Melanin	100 (110/110) (96.6, 100.0)	100 (97240/97240) (99.99, 100)
Hemoglobin	98.3 (118/120) (94.1, 99.5)	100 (106080/106080) (99.99, 100)

Table 24. Interfering Substances Concordance by Test Condition for *ERBB2*

Test Condition	PCR (%) (n/N) (95% CI)	NCR (%) (n/N) (95% CI)
Ethanol	100 (10/10) (69.2, 100.0)	100 (35/35) (90.1, 100.0)
Adaptor	100 (10/10) (69.2, 100.0)	100 (35/35) (90.1, 100.0)
Proteinase K	100 (10/10) (69.2, 100.0)	100 (35/35) (90.1, 100.0)
Wash Buffer	100 (10/10) (69.2, 100.0)	100 (35/35) (90.1, 100.0)
Melanin	100 (10/10) (69.2, 100.0)	100 (30/30) (88.6, 100.0)

Test Condition	PCR (%) (n/N) (95% CI)	NCR (%) (n/N) (95% CI)
Hemoglobin	100 (10/10) (69.2, 100.0)	100 (35/35) (90.1, 100.0)

Table 25. Interfering Substances Concordance by Test Condition for RNA Fusions (*ALK, RET*, and *ROS1*)

Test Condition	PCR (%) (n/N) (95% CI)	NCR (%) (n/N) (95% CI)
Ethanol	100 (15/15) (78.2, 100)	100 (30/30) (88.4, 100)
Adaptor	100 (15/15) (78.2, 100)	100 (30/30) (88.4, 100)
Proteinase K	100 (15/15) (78.2, 100)	100 (29/29) (88.1, 100)
Wash Buffer	100 (15/15) (78.2, 100)	100 (30/30) (88.4, 100)
Melanin	100 (15/15) (78.2, 100)	100 (30/30) (88.4, 100)
Hemoglobin	100 (15/15) (78.2, 100)	100 (30/30) (88.4, 100)

d. Sample Carryover and Cross-Contamination

Cross-contamination (contamination from one sample to another within the same batch) and sample carryover (contamination from a previous sequencing run when using the same instrument) were assessed by evaluating the false positive rate in 24 FFPE samples. Twelve of the 24 FFPE samples had known positive variants, and the remaining samples were known negative samples. All FFPE samples were assessed across two batched to test for contamination within and between runs. The samples were processed in a checkerboard layout during library synthesis. In the first batch, 24 libraries and 12 libraries underwent a single DNA and RNA sequencing, respectively. The second batch contained 12 DNA libraries and six RNA libraires with known negative samples were processed after the first batch. No positive variant results were observed in the known negative samples tested.

7. Robustness Study

a. Sample Stability

The stability of tissue curls sectioned from FFPE blocks were established using six FFPE tumor specimens stored under appropriate conditions (17.8°C – 26.8°C), for 35 days by comparing the detection of the intended variants within these samples to the baseline timepoint (T0). Three sets of curls from each block were prepared on Day 0. The first set of curls was immediately processed for DNA/RNA extraction and subsequent analysis to establish the T0 baseline. On Day 35 (T35), the remaining stored curls were used for DNA/RNA extraction and analysis for each claimed variant. FFPE curls stored for up to 35 days show concordant variant calls for all variants (Table 26) demonstrating a stability of 28 days for FFPE blocks stored under appropriate conditions.

Table 26. FFPE Tissue Curl Stability Concordance by Variant

Variant Type	PPA (%) (n/N) (95% CI)		NPA (%) (n/N) (95% CI)	
	T0	T35	T0	T35
SNVs + Indels	99.32 (147/148) (96.27, 99.88)	96.96 (278/296) (94.14, 98.47)	100 (884/884) (99.58, 100)	99.55 (880/884) (98.78, 99.85)
ERBB2 Amplification	100 (4/4) (51.01, 100)	100 (8/8) (67.56, 100)	100 (20/20) (83.89, 100)	100 (40/40) (91.24, 100)
ALK and *ROS1* Fusions	100 (8/8) (67.56, 100)	85.7 (12/14) (60.1, 96.0)	100 (64/64) (94.3, 100)	98.4 (126/128) (94.5, 99.6)
RET Fusion	100 (8/8) (67.56, 100)	100 (16/16) (80.6, 100)	100 (8/8) (67.56, 100)	100 (16/16) (80.6, 100)

b. DNA Input

The recommended amount of input DNA for the CellDx-Tissue assay is 20 ng. A DNA input study was performed to demonstrate the analytical performance of the CellDx-Tissue assay across a range of DNA inputs (2.5, 5, 10, 20, 30,40, and 50 ng) using three FFPE tumor samples panning multiple variant types including SNVs, insertions, deletions, and *ERBB2* amplification. Each sample was tested across 12 replicates, two operators, two lot kits, and seven instruments across multiple non-consecutive days. The QC metrics and variant reporting for each sample was evaluated per DNA input level. Table 27 and Table 28 list the PCR and NCR for each input level were analyzed, including SNVs, indels, and *ERBB2* amplifications.

Table 27. Panel-wide (SNVs + Indels) Call Rates at each DNA Input Level

DNA Input (ng)	PCR (%) (n/N) (95% CI)	NCR (%) (n/N) (95% CI)
2.5	85.8 (151/176) (77.8, 88.5)	99.9 (45812769/45813184) (99.9, 100)
5	80.1 (137/171) (69.4, 81.8)	99.9 (38654612/38654874) (99.9, 100)
10	97.8 (175/179) (93.7, 98.8)	99.9 (50108021/51539832) (99.9, 100)
20	97.2 (147/179) (92.9, 98.5)	99.9 (50108137/50108170) (99.9, 100)
30	97.8 (175/179) (93.7, 98.8)	99.9 (50108155/50108170) (99.9, 100)
40	98.3 (176/179) (94.4, 99.1)	99.9 (50108167/50108170) (99.9, 100)
50	100 (180/180) (97.9, 100)	99.9 (51539823/51539832) (99.9, 100)

Table 28. *ERBB2* Call Rates at each DNA Input Level

DNA Input (ng)	Tumor Purity (%)	Mean CN (CN Range)	SD (±)	CV (%)	PCR (%) (n/N) (95% CI)
2.5	21	9.1 (8–11)	1.0	11.5	92 (11/12) (61.5, 99.8)
5	21	9.0 (8–10)	0.7	7.4	83 (10/12) (51.6, 97.9)
10	21	7.4 (6–9)	0.8	10.7	100 (12/12) (73.5, 100)
20	21	7.5 (6–9)	0.9	12.1	100 (12/12) (73.5, 100)
30	21	8.3 (7–10)	1.1	13.7	100 (12/12) (73.5, 100)

DNA Input (ng)	Tumor Purity (%)	Mean CN (CN Range)	SD (±)	CV (%)	PCR (%) (n/N) (95% CI)
40	21	7.1 (6–8)	0.5	7.6	92 (11/12) (61.5, 99.8)
50	21	7.6 (7–9)	0.9	11.8	100 (12/12) (73.5, 100)

c. RNA Input

The recommended amount of input RNA for the CellDx-Tissue assay is 20 ng. A RNA input study was performed to demonstrate the analytical performance of the CellDx-Tissue assay across a range of RNA inputs (2.5, 5, 10, 20, 30,40, and 50 ng) using three FFPE tumor samples including *ALK, RET*, and *ROS1* gene fusions. Each sample was tested across 12 replicates, two operators, two lot kits, and seven instruments across multiple non-consecutive days. The QC metrics and variant reporting for each sample was evaluated per RNA input level. Table 29 - Table 31 list the PCR for each input level were analyzed.

Table 29. *ALK* Call Rates at each RNA Input Level

RNA Input (ng)	Tumor Purity (%)	Mean Read Counts	PCR (%) (n/N) (95% CI)
2.5	29	7358	33 (4/12) (13.8-60.9%)
5	29	9711	100 (12/12) (75.7-100%)
10	29	12428	100 (12/12) (75.7-100%)
20	29	14842	100 (12/12) (75.7-100%)
30	29	16876	100 (12/12) (75.7-100%)
40	29	19954	100 (12/12) (75.7-100%)
50	29	29524	100 (12/12) (75.7-100%)

Table 30. *RET* Call Rates at each RNA Input Level

RNA Input (ng)	Tumor Purity (%)	Mean Read Counts	PCR (%) (n/N) (95% CI)
2.5	73	0	0 (0/12) (0-24.3%)
5	73	527	33.3 (4/12) (13.8-60.9%)
10	73	636	100 (12/12) (75.7-100%)
20	73	943	100 (12/12) (75.7-100%)
30	73	1421	100 (12/12) (75.7-100%)
40	73	2115	100 (12/12) (75.7-100%)
50	73	4771	100 (12/12) (75.7-100%)

Table 31. *ROS1* Call Rates at each RNA Input Level

RNA Input (ng)	Tumor Purity (%)	Mean Read Counts	PCR (%) (n/N) (95% CI)
2.5	29	0	0 (0/12) (0-24.3%)
5	29	549	75 (9/12) (46.8-91.1%)
10	29	776	100 (12/12) (75.7-100%)
20	29	1491	100 (12/12) (75.7-100%)
30	29	2665	100 (12/12) (75.7-100%)

RNA Input (ng)	Tumor Purity (%)	Mean Read Counts	PCR (%) (n/N) (95% CI)
40	29	4569	100 (12/12) (75.7-100%)
50	29	137465	100 (12/12) (75.7-100%)

8. Comparison Studies

a. Method Comparison (Accuracy)

The analytical accuracy of the CellDx-Tissue assay as a tumor profiling assay was evaluated using 252 FFPE tumor tissue samples from patients diagnosed with various solid tumor types. The clinical FFPE samples were selected using an orthogonal method based on the clinical history (known biomarker status) and the availability of tissue for testing. Data was aggregated at the variant level for SNVs and indels and at the gene level for amplification and gene fusions. The accuracy is summarized for the entire cohort of 252 samples for each of the assessed types (SNVs, indels, *ERBB2* amplification, *ALK*, *RET*, and *ROS1* gene fusions). Orthogonal methos used consisted of validated Next Generation Sequencing (NGS), and Fluorescence In Situ Hybridization (FISH). For all analyses, the PPA and NPA were calculated by comparing the concordance between the CellDx-Tissue assay to the appropriate comparator to evaluate the degree of concordance between the assays. (Table 32).

Table 32. Accuracy of the CellDx-Tissue Assay

Variant Category	Orthogonal Method	Analysis Category	PPA (95% CI)	NPA (95% CI)
Overall	NGS	All	98.4 (95.5, 99.5)	99.5 (99.4, 99.6)
		SNV	98.8 (95.6, 99.7)	99.7 (99.6, 99.8)
		Insertion	85.7 (48.7, 97.4)	99.9 (99.8, 99.9)
		Deletion	100 (86.2, 100)	99.9 (99.9, 99.9)

Variant Category	Orthogonal Method	Analysis Category	PPA (95% CI)	NPA (95% CI)
Variants with Evidence of Clinical Significance	NGS	All	98.2 (90.6, 99.7)	99.8 (99.6, 99.9)
		SNV	100 (92.6, 100)	99.8 (99.7, 99.9)
		Insertion	66.7 (20.8, 93.9)	100 (99.9, 100)
		Deletion	100 (56.6, 100)	100 (99.9, 100)
Variants with Potential Clinical Significance	NGS	All	98.5 (94.8, 99.6)	99.4 (99.3, 99.5)
		SNV	98.2 (93.8, 99.5)	99.6 (99.5, 99.7)
		Insertion	100 (51.0, 100)	99.9 (99.8, 99.9)
		Deletion	100 (83.2, 100)	99.9 (99.8, 99.9)
Hotspot	NGS	All	97.8 (92.3, 99.4)	99.9 (99.8, 99.9)
		SNV	97.8 (92.3, 99.4)	99.9 (99.8, 99.9)
		Insertion	-	100 (99.8, 100)
		Deletion	-	100 (99.8, 100)
Non-Hotspot	NGS	All	99 (94.6, 99.8)	99.6 (99.5, 99.7)
		SNV	100 (94.8, 100)	99.8 (99.7, 99.9)
		Insertion	85.7 (48.7, 97.4)	99.9 (99.8, 99.9)
		Deletion	100 (86.2, 100)	99.9 (99.9, 99.9)
ERBB2	FISH	Amplification	91.18 (77.04, 96.95)	100 (92.13, 100)
ALK	FISH	Fusion	94.10 (73.0, 99.0)	100 (89.8, 100)
RET	FISH	Fusion	100 (43.9, 100)	100 (92.7, 100)
ROS1	FISH	Fusion	91.70 (64.6, 98.5)	100 (91.0, 100)

b. Accuracy – SNVs and Indels

The CellDx-tissue accuracy study included a total of 189 unique variants in 88 exons over 41 genes. Variants included 159 SNVs, six insertions, and 24 deletions. Performance was stratified by mutation type and gene for positive percent agreement (PPA) and negative percent agreement (NPA) with two-sided 95% confidence interval (CI). Overall, the CellDx-Tissue assay yielded concordant analytical performance for variant calls across the SNVs and Indels with a PPA of 98.4% and an NPA ≥ 99.5%. The concordance between detected mutations for each category of variants (Variants with Evidence of Clinical Significance, Variants with Potential Clinical Significance, Hotspot, and Non-Hotspot) are shown in Table 33. The complete listing of data can be found in Appendix E.

Table 33. SNV and Indel Concordance between CellDx-Tissue Assay and Orthogonal NGS Comparator

Variant Category	Analysis Category	PPA[1] (n/N) (95% CI)	NPA[1] (n/N) (95% CI)
Overall	All	98.40% (189/192) (95.5%, 99.5%)	99.50% (18784/18880) (99.4%, 99.6%)
	SNV	98.80% (159/161) (95.6%, 99.7%)	99.70% (18851/18911) (99.6%, 99.8%)
	Insertions	85.70% (6/7) (48.7%, 97.4%)	99.90% (19046/19065) (99.8%, 99.9%)
	Deletions	100% (24/24) (86.2%, 100%)	99.90% (19031/19048) (99.9%, 99.9%)
Variants with Evidence of Clinical Significance	All	98.20% (55/56) (90.6%, 99.7%)	99.80% (4405/4414) (99.6%, 99.9%)
	SNV	100% (48/48) (92.6%, 100%)	99.80% (4415/4422) (99.7%, 99.9%)
	Insertions	66.70% (2/3) (20.8%, 93.9%)	100% (4466/4467) (99.9%, 100%)
	Deletions	100% (5/5) (56.6%, 100%)	100% (4464/4465) (99.9%, 100%)

Variant Category	Analysis Category	PPA[1] (n/N) (95% CI)	NPA[1] (n/N) (95% CI)
Variants with Potential Clinical Significance	All	98.50% (134/136) (94.8%, 99.6%)	99.40% (14379/14466) (99.3%, 99.5%)
	SNV	98.20% (111/113) (93.8%, 99.5%)	99.60% (14436/14489) (99.5%, 99.7%)
	Insertions	100% (4/4) (51.0%, 100%)	99.90% (14580/14598) (99.8%, 99.9%)
	Deletions	100% (19/19) (83.2%, 100%)	99.90% (14567/14583) (99.8%, 99.9%)
Hotspot	All	97.80% (89/91) (92.3%, 99.4%)	99.90% (18959/18981) (99.8%, 99.9%)
	SNV	97.80% (89/91) (92.3%, 99.4%)	99.90% (18959/18981) (99.8%, 99.9%)
	Insertions	-	100% (19072/19072) (100%, 100%)
	Deletions	-	100% (19072/19072) (100%, 100%)
Non-Hotspot	All	99.00% (100/101) (94.6%, 99.8%)	99.60% (18897/18971) (99.5%, 99.7%)
	SNV	100% (70/70) (94.8%, 100%)	99.80% (18964/19002) (99.7%, 99.9%)
	Insertions	85.70% (6/7) (48.7%, 97.4%)	99.90% (19046/19065) (99.8%, 99.9%)
	Deletions	100% (24/24) (86.2%, 100%)	99.90% (19031/19048) (99.9%, 99.9%)

Variant Category	Analysis Category	PPA[1] (n/N) (95% CI)	NPA[1] (n/N) (95% CI)
Insertions	1 – 5 bp	80.00% (4/5) (37.6%, 96.4%)	99.90% (19049/19067) (99.9%, 99.9%)
	6 – 10 bp	100% (1/1) (20.7%, 100%)	100% (19070/19071) (100%, 100%)
	11 – 20 bp	100% (1/1) (20.7%, 100%)	100% (19071/19071) (100%, 100%)
Deletions	1 – 5 bp	100% (19/19) (83.2%, 100%)	99.90% (19038/19053) (99.9%, 100%)
	6 – 10 bp	-	100% (19071/19072) (100%, 100%)
	11 – 20 bp	100% (3/3) (43.9%, 100%)	100% (19069/19069) (100%, 100%)
	21 – 30 bp	100% (2/2) (34.2%, 100%)	100% (19069/19070) (100%, 100%)

[1]Two-sided CIs were estimated using the Wilson method.

c. Accuracy – Amplification and Translocations

i. *ERBB2* Amplification Concordance

In total, 83 different FFPE samples representing 11 different tumor types, including Breast, Bowel, Esophagus/Stomach, Lung, Head and Neck, Ovary/Fallopian Tube, Bladder/Urinary Tract, Uterus, Biliary Tract, Bone and Prostate were analyzed for concordance between FISH status and CellDx-Tissue *ERBB2* status. Four samples that had a positive *ERBB2* FISH result were classified as Indeterminate by the CellDx-Tissue (below assay's limit of detection for ERBB2) and were excluded from analysis (see Table 34). Three samples had a positive *ERBB2* FISH result but were negative for *ERBB2* in the CellDx-Tissue assay. Of these three samples, one had a FISH score of 2.02 (borderline) and history of *HER2*-negtive IHC findings. The remaining two discordant samples had a FISH score of 2.84 and 2.94, with a history of *HER2*-negative IHC reported. The PPA and NPA values for *ERBB2* amplification reflected the across borderline and non-borderline samples (Table 34). In non-borderline cases (excluding all cases of a FISH ratio 1.5 – 2.5), a PPA of 93.55% (95% CI: 79.28%, 98.21%) and an NPA of 100% (95% CI: 89.85%, 100%) (Table 35) was observed.

Table 34. *ERBB2* Amplification Concordance between CellDx-Tissue Assay and FISH

CellDx-Tissue	FISH		
	***ERBB2* (+)**	***ERBB2* (-)**	**Total**
***ERBB2* (+) (CN ≥ 8.5)**	31	0	31
***ERBB2* Indeterminate* (CN ≥ 4 to < 8.5)**	4[#]	0	4
***ERBB2* (-) (CN < 4)**	3	45	48
Total	38	45	83
PPA (2-sided 95% CI)	91.18% (77.04, 96.95)		
NPA (2-sided 95% CI)	100% (92.13, 100)		

*Samples with CN ≥ 4 to <8.5 are classified as " Indeterminate / Low-Level Amplification (below LoD)" and require reflex testing with another FDA-cleared test. Per the tiered CellDx – Tissue device *ERBB2* reporting framework, these specimens are excluded from the primary PPA calculation as they are not definitive positive or negative calls by the assay.

[#]Four FISH-positive samples yielded Indeterminate / Low-Level Amplification (Below LOD, CN ≥4 to <8.5) by CellDx-Tissue.

Table 35. *ERBB2* Amplification Concordance between CellDx-Tissue Assay and FISH Borderline Samples

Category	Total Cases	TP	FN	TN	FN	I/LL*	PPA (%) (2-sided 95% CI)	NPA (%) (2-sided 95% CI)
All	83	31	0	45	3	4	91.18 (77.04, 96.95)	100 (92.13, 100)
Excluding FISH 1.8-2.2	78	31	0	42	2	3	93.94 (80.39, 98.32)	100 (91.62, 100)
Only FISH 1.8-2.2	5	0	0	3	1	1	0 (0, 79.35)	100 (43.85, 100)
Excluding FISH 1.5-2.5	68	29	0	34	2	3	93.55 (79.28, 98.21)	100 (89.85, 100)
Only FISH 1.5-2.5	15	2	0	11	1	1	66.67 (20.77, 93.85)	100 (74.12, 100)

*Indeterminate / Low-Level Amplification (below LoD).

ii. *ALK* Fusion Concordance

A total of 51 FFPE samples from six tumor types, including Lung, Thyroid, Soft Tissue, Breast, Bowel and Prostate, were included in the analysis. Out of the 17 *ALK* positive samples, 16 were detected for *ALK* gene fusions using both FISH and CellDx-Tissue assay. One *ALK* positive sample by FISH was not reported as positive

by the CellDx-Tissue assay due to borderline tumor purity. The concordance between the CellDx-Tissue assay and FISH is shown in Table 36.

Table 36. *ALK* Fusion Concordance between CellDx-Tissue Assay and FISH

CellDx-Tissue	FISH		
	***ALK* (+)**	***ALK* (-)**	**Total**
***ALK* (+)**	16	0	16
***ALK* (-)**	1	34	35
Total	17	34	51
PPA (2-sided 95% CI)	94.10% (73.0, 99.0)		
NPA (2-sided 95% CI)	100% (89.8, 100)		

iii. *RET* Fusion Concordance

A total of 52 FFPE samples from six tumor types, including Lung, Thyroid, Bowel, Soft Tissue, Breast and Prostate, were included in the analysis. Out of the three *RET* positive samples, three were detected for *RET* gene fusions using both FISH and CellDx-Tissue assay. The concordance between the CellDx-Tissue assay and FISH is shown in Table 37.

Table 37. *RET* Fusion Concordance between CellDx-Tissue Assay and FISH

CellDx-Tissue	FISH		
	***RET* (+)**	***RET* (-)**	**Total**
***RET* (+)**	3	0	3
***RET* (-)**	0	49	49
Total	3	49	52
PPA (2-sided 95% CI)	100% (43.9, 100)		
NPA (2-sided 95% CI)	100% (92.7, 100)		

iv. *ROS1* Fusion Concordance

A total of 51 FFPE samples from six tumor types, including Lung, Thyroid, Bowel, Soft Tissue, Breast and Prostate, were included in the analysis. Out of the 12 *ROS1* positive samples, 11 were detected for *ROS1* gene fusions using both FISH and CellDx-Tissue assay. One *ROS1* positive sample by FISH was not reported as positive by the CellDx-Tissue assay; this discordant result was confirmed by an independent NGS test and classified as a false negative. The concordance between the CellDx-Tissue assay and FISH is shown in Table 38.

Table 38. *ROS1* Fusion Concordance between CellDx-Tissue Assay and FISH

CellDx-Tissue	FISH		
	***ROS1* (+)**	***ROS1* (-)**	**Total**
***ROS1* (+)**	11	0	11
***ROS1* (-)**	1	39	40
Total	12	39	51
PPA (2-sided 95% CI)	91.70% (64.6, 98.5)		
NPA (2-sided 95% CI)	100% (91.0, 100)		

d. Method Comparison – Wild-Type Calls

The accuracy of wild-type calls was evaluated by observing the NPA of relevant positions of Variants with Evidence of Clinical Significance (Table 39).

Table 39. CellDx-Tissue Wild-Type Accuracy for Variants with Evidence of Clinical Significance.

Variant Category	NPA (%) (n/N) (95% CI Wilson Score)
SNVs	99.80 (4415/4422) (99.7, 99.9)
Insertions	99.98 (4466/4467) (99.9, 100)
Deletions	99.98 (4464/4465) (99.9, 100)

9. Tissue Comparability

A tissue comparability study was performed to evaluate the CellDx-Tissue assay's analytical performance across a diverse range of solid tumor tissue types. The study assessed the CellDx-Tissue assay's performance across more than 20 tumor types for all reportable biomarkers (SNVs, Indels, *ERBB2* gene amplification, and *ALK*, *RET*, and *ROS1* gene fusions) from FFPE samples. The analytical performance of the CellDx-Tissue assay is consistent across the full spectrum of tumor types within the intended use population. The number of false negative (FN) variant calls, false positive (FP) variant calls, true positive (TP) variant calls, and the positive percent agreement (PPA) and false negative rate (FNR) for each tumor type are shown for all reportable biomarkers in Table 40 – Table 44.

Table 40. CellDx-Tissue Assay Performance in SNVs, Insertions, and Deletions

Tissue	Sample Number	Variant Calls				PPA (%) (95% CI)	NPA (%) (95% CI)
		FN	FP	TN	TP		
Adrenal Gland	1			1410148	1	100 (20.65, 100)	100 (99.99, 100)
Biliary Tract	2		3	2820294	1	100 (20.65, 100)	99.99 (99.99, 100)
Bladder/Urinary Tract	2			2820295	3	100 (43.85,100)	100 (99.99, 100)
Bowel	25	1	29	35253643	52	98.11 (90.06, 99.67)	99.99 (99.99, 100)
Breast	26		10	36663825	39	100 (91.03, 100)	100 (99.99, 100)
Cervix	3		3	4230442	2	100 (34.24, 100)	100 (99.99, 100)
CNS/Brain	5			7050735	10	100 (72.25, 100)	100 (99.99, 100)
Esophagus/Stomach	3		2	4230442	3	100 (43.85, 100)	100 (99.99, 100)
Head and neck	6		3	8460884	7	100 (64.57, 100)	100 (99.99, 100)
Lung	15		9	21152197	29	100 (88.30, 100)	100 (99.99, 100)
Ovary/ Fallopian Tube	8	1	9	11281177	5	83.33 (43.65, 96.99)	100 (99.99, 100)
Pancreas	5		9	7050731	5	100 (56.55, 100)	100 (99.99, 100)
Peritoneum	1		1	1410148		NA	100 (99.99, 100)
Prostate	3		3	4230440	4	100 (51.01, 100)	100 (99.99, 100)
Skin	3	1	1	4230443	2	66.67 (20.77, 93.85)	100 (99.99, 100)
Soft Tissue	3			4230440	7	100 (64.57, 100)	100 (99.99, 100)
Testis	1			1410147	2	100 (34.24, 100)	100 (99.99, 100)
Thymus	2		4	2820292	2	100 (34.24, 100)	100 (99.99, 100)
Thyroid	3			4230444	3	100 (43.85, 100)	100 (99.99, 100)
Unknown Primary	1			1410148	1	100 (20.65, 100)	100 (99.99, 100)
Uterus	5		10	7050724	11	100 (74.12, 100)	100 (99.99, 100)
Overall	**123**	**3**	**96**	**173448039**	**189**	**98.44 (95.51, 99.47)**	**100 (99.99, 100)**

Table 41. CellDx-Tissue Assay Performance in *ERBB2* Amplification

Tissue (N)	Variant Calls					PPA (%) (95% CI)	NPA (%) (95% CI)
	FN	FP	TN	TP	I/LL*		
Breast (4)	1		23	18		94.74 (75.36, 99.06)	100 (85.69, 100)
Bowl (32)	1		21	8	2	88.89 (56.50, 98.01)	100 (84.54, 100)
Lung (11)			4	6	1	100 (60.97, 100)	100 (51.01, 100)
Head and Neck (7)	1		4	1	1	100 (34.24, 100)	100 (51.01, 100)
Esophagus/ Stomach (6)			2	4		100 (34.24, 100)	100 (51.01, 100)
Ovary/ Fallopian Tube (6)			4	2		100 (51.01, 100)	100 (34.24, 100)
Pancreas (4)			3	1		100 (34.24, 100)	100 (34.24, 100)
Uterus (4)			2	2		100 (20.65, 100)	100 (43.85, 100)
Bladder/ Urinary Track (3)			2	1		NA	100 (43.85, 100)
Prostate (3)			3			100 (20.65, 100)	100 (34.24, 100)
Biliary Tract (2)				2		NA	100 (34.24, 100)
Cervix (2)			2			100 (34.24, 100)	NA
Bone (1)			1			NA	100 (20.65, 100)
CNS/ Brain (1)			1			NA	100 (20.65, 100)
Liver (1)			1			NA	100 (20.65, 100)

Tissue (N)	Variant Calls					PPA (%) (95% CI)	NPA (%) (95% CI)
	FN	FP	TN	TP	I/LL*		
Thymus (1)			1			NA	100 (20.65, 100)
Overall (126)	**3**	**0**	**74**	**45**	**4**	**93.75 (83.16, 97.85)**	**100 (95.06, 100)**

*Indeterminate / Low-Level Amplification (below LoD)

Table 42. CellDx-Tissue Assay Performance in *ALK* Fusion

Tissue (N)	Variant Calls				PPA (%) (95% CI)	NPA (%) (95% CI)
	FN	FP	TN	TP		
Lung (64)			36	28	100 (84.94, 100)	100 (90.36, 100)
Thyroid (13)			13		NA	100 (77.19, 100)
Head and Neck (6)			6		NA	100 (60.97, 100)
Breast (5)			4	1	100 (20.65, 100)	100 (51.01, 100)
Bowel (4)			4		NA	100 (51.01, 100)
Cervix (3)			3		NA	100 (43.85, 100)
Ovary/ Fallopian Tube (3)			3		NA	100 (43.85, 100)
Pancreas (3)			3		NA	100 (43.85, 100)
Prostate (3)			3		NA	100 (43.85, 100)
Soft Tissue (3)	1		2		0 (0, 79.35)	100 (34.24, 100)
Uterus (3)			3		NA	100 (43.85, 100)
Esophagus/ Stomach (2)			2		NA	100 (34.24, 100)
Bladder/ Urinary Tract (1)			1		NA	100 (20.65, 100)

Tissue (N)	Variant Calls				PPA (%) (95% CI)	NPA (%) (95% CI)
	FN	FP	TN	TP		
Liver (1)			1		NA	100 (20.65, 100)
Thymus (1)			1		NA	100 (20.65, 100)
Overall (115)	**1**		**85**	**29**	**96.67 (83.33, 99.41)**	**100 (95.68, 100)**

Table 43. CellDx-Tissue Assay Performance in *RET* Fusion

Tissue (N)	Variant Calls				PPA (%) (95% CI)	NPA (%) (95% CI)
	FN	FP	TN	TP		
Lung (51)			45	6	100 (54.07, 100)	100 (92.13, 100)
Thyroid (15)			14	1	100 (20.65, 100)	100 (78.47, 100)
Head and Neck (6)			6		NA	100 (60.97, 100)
Breast (5)			5		NA	100 (56.55, 100)
Bowel (4)			4		NA	100 (51.01, 100)
Cervix (3)			3		NA	100 (43.85, 100)
Ovary/ Fallopian Tube (3)			3		NA	100 (43.85, 100)
Pancreas (3)			3		NA	100 (43.85, 100)
Prostate (3)			3		NA	100 (43.85, 100)
Soft Tissue (3)			3		NA	100 (43.85, 100)
Uterus (3)			3		NA	100 (43.85, 100)
Esophagus/ Stomach (2)			2		NA	100 (32.24, 100)
Bladder/ Urinary Tract (1)			1		NA	100 (20.65, 100)

Tissue (N)	Variant Calls				PPA (%) (95% CI)	NPA (%) (95% CI)
	FN	FP	TN	TP		
Liver (1)			1		NA	100 (20.65, 100)
Thymus (1)			1		NA	100 (20.65, 100)
Overall (104)			**97**	**7**	**100 (59.04, 100)**	**100 (96.26, 100)**

Table 44. CellDx-Tissue Assay Performance in *ROS1* Fusion

Tissue (N)	Variant Calls				PPA (%) (95% CI)	NPA (%) (95% CI)
	FN	FP	TN	TP		
Lung (59)	1		38	20	95.24 (77.33, 99.15)	100 (90.77, 100)
Thyroid (13)			13		NA	100 (77.19, 100)
Head and Neck (6)			6		NA	100 (60.97, 100)
Breast (5)			5		NA	100 (56.55, 100)
Bowel (4)			4		NA	100 (51.01, 100)
Cervix (3)			3		NA	100 (43.85, 100)
Ovary/ Fallopian Tube (3)			3		NA	100 (43.85, 100)
Pancreas (3)			3		NA	100 (43.85, 100)
Prostate			2	1	NA	66.67 (20.77, 93.85)
Soft Tissue (3)			3		NA	100 (43.85, 100)
Uterus (3)			3		NA	100 (43.85, 100)
Esophagus/ Stomach (2)			2		NA	100 (34.24, 100)

Tissue (N)	Variant Calls				PPA (%) (95% CI)	NPA (%) (95% CI)
	FN	FP	TN	TP		
Bladder/ Urinary Tract (1)			1		NA	100 (20.65, 100)
Liver (1)			1		NA	100 (20.65, 100)
Thymus (1)			1		NA	100 (20.65, 100)
Overall (110)	**1**		**88**	**21**	**95.45 (72.20, 99.19)**	**100 (95.82, 100)**

V. Instrument Name

Thermo Fisher Ion GeneStudio S5 Prime NGS Systems (qualified by Datar Cancer Genetics)

VI. System Description

1. Modes of Operations:
   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?
   Yes: ✓ Or No:

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?
   Yes: Or No: ✓

2. Software:
   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:
   Yes: ✓ Or No:

3. Level of Concern:
   Moderate

4. Specimen Handing:
   Refer to Device Description section above

5. Calibration and Quality Controls
   Refer to Device Description section above

VII. Other Supportive Instrument Performance Characteristics

Not applicable

VIII. Proposed Labeling

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

IX. Conclusion

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

X. Appendix

Appendix A. List of Genes on the CellDx-Tissue Assay Panel

Gene	Transcript ID	Full-Length Coverage
A1CF	NM_138932.2	-
ABCB1	NM_000927.4	-
ABL1	NM_005157.6	-
ABL2	NM_005158.5	-
ABRAXAS1	NM_139076.3	(Full-length)
ACSM2B	NM_001105069.2	-
ACVR1	NM_001111067.4	-
ACVR1B	NM_020328.4	(Full-length)
ACVR2A	NM_001616.5	(Full-length)
ADAM18	NM_014237.3	-
ADAMTS12	NM_030955.4	(Full-length)
ADAMTS2	NM_014244.5	(Full-length)
AKT1	NM_001014431.2	-
AKT2	NM_001626.6	-
AKT3	NM_005465.7	-
ALK	NM_004304.5	-
AMER1	NM_152424.4	(Full-length)
ANO4	NM_178826.4	-
APC	NM_000038.6	(Full-length)
AR	NM_000044.6	-
ARAF	NM_001654.5	-
ARHGAP35	NM_004491.5	(Full-length)
ARID1A	NM_006015.6	(Full-length)
ARID1B	NM_001371656.1	(Full-length)
ARID2	NM_152641.4	(Full-length)
ARID5B	NM_032199.3	(Full-length)
ARMC4	NM_018076.5	-
ASXL1	NM_015338.6	(Full-length)
ASXL2	NM_018263.6	(Full-length)
ATM	NM_000051.3	(Full-length)
ATP1A1	NM_000701.8	-
ATR	NM_001184.4	(Full-length)
ATRX	NM_000489.5	(Full-length)
AURKA	NM_003600.4	-
AURKB	NM_004217.4	-
AURKC	NM_001015878.2	-
AXIN1	NM_003502.4	(Full-length)
AXIN2	NM_004655.4	(Full-length)
AXL	NM_021913.5	-
B2M	NM_004048.3	(Full-length)

Gene	Transcript ID	Full-Length Coverage
BAP1	NM_004656.4	(Full-length)
BARD1	NM_000465.4	(Full-length)
BCL2	NM_000633.2	-
BCL2L12	NM_138639.1	-
BCL6	NM_001706.5	-
BCOR	NM_001123385.2	(Full-length)
BCR	NM_004327.4	-
BLM	NM_000057.4	(Full-length)
BMP5	NM_021073.4	-
BMPR2	NM_001204.7	(Full-length)
BRAF	NM_004333.6	-
BRCA1	NM_007294.4	(Full-length)
BRCA2	NM_000059.3	(Full-length)
BRINP3	NM_199051.3	-
BRIP1	NM_032043.3	(Full-length)
BTK	NM_000061.3	-
C6	NM_000065.4	-
C8A	NM_000562.3	-
C8B	NM_000066.4	-
CACNA1D	NM_000720.4	-
CALR	NM_004343.4	(Full-length)
CANX	NM_001024649.2	-
CARD11	NM_032415.6	-
CASP8	NM_001080125.2	(Full-length)
CASR	NM_001178065.2	-
CBFB	NM_022845.3	(Full-length)
CBL	NM_005188.4	-
CCND1	NM_053056.3	-
CCND2	NM_001759.4	-
CCND3	NM_001760.5	-
CCNE1	NM_001238.4	-
CD163	NM_004244.5	-
CD274	NM_014143.4	(Full-length)
CD276	NM_001024736.2	(Full-length)
CD79B	NM_001039933.3	-
CDC73	NM_024529.5	(Full-length)
CDH1	NM_004360.5	(Full-length)
CDH10	NM_006727.5	(Full-length)
CDK12	NM_016507.4	(Full-length)
CDK4	NM_000075.4	-
CDK6	NM_001145306.2	-
CDKN1A	NM_078467.3	(Full-length)
CDKN1B	NM_004064.4	(Full-length)

Gene	Transcript ID	Full-Length Coverage
CDKN2A	NM_001195132.1	(Full-length)
CDKN2B	NM_004936.4	(Full-length)
CDKN2C	NM_078626.3	(Full-length)
CHD4	NM_001273.5	-
CHEK1	NM_001274.5	(Full-length)
CHEK2	NM_007194.4	(Full-length)
CIC	NM_015125.4	(Full-length)
CIITA	NM_000246.3	(Full-length)
CNTN6	NM_014461.4	-
CNTNAP4	NM_138994.5	-
CNTNAP5	NM_130773.4	-
COL11A1	NM_001854.4	-
CREBBP	NM_004380.3	(Full-length)
CSF1R	NM_005211.3	-
CSMD3	NM_198123.2	(Full-length)
CTCF	NM_006565.4	(Full-length)
CTLA4	NM_005214.5	(Full-length)
CTNNB1	NM_001904.4	-
CTNND2	NM_001332.4	-
CUL1	NM_003592.3	-
CUL3	NM_003590.5	(Full-length)
CUL4A	NM_001008895.4	(Full-length)
CUL4B	NM_003588.3	(Full-length)
CYLD	NM_001042355.2	(Full-length)
CYP2C9	NM_000771.4	(Full-length)
CYP2D6	NM_000106.6	(Full-length)
CYSLTR2	NM_020377.5	-
DAXX	NM_001141970.2	(Full-length)
DCAF4L2	NM_152418.4	-
DCDC1	NM_001367979.1	-
DDR1	NM_001954.4	-
DDR2	NM_006182.4	-
DDX3X	NM_001356.5	(Full-length)
DGCR8	NM_022720.7	-
DICER1	NM_030621.4	(Full-length)
DNMT3A	NM_022552.4	(Full-length)
DOCK3	NM_004947.5	(Full-length)
DPYD	NM_000110.4	(Full-length)
DROSHA	NM_013235.5	-
DSC1	NM_024421.2	(Full-length)
DSC3	NM_001941.5	(Full-length)
E2F1	NM_005225.3	-
EGFR	NM_005228.5	-

Gene	Transcript ID	Full-Length Coverage
EIF1AX	NM_001412.4	-
ELF3	NM_004433.5	(Full-length)
EMSY	NM_020193.4	-
ENO1	NM_001428.5	(Full-length)
EP300	NM_001429.4	(Full-length)
EPAS1	NM_001430.5	-
EPCAM	NM_002354.3	(Full-length)
EPHA2	NM_004431.5	(Full-length)
ERAP1	NM_016442.4	(Full-length)
ERAP2	NM_001130140.2	(Full-length)
ERBB2	NM_004448.3	-
ERBB3	NM_001982.4	-
ERBB4	NM_005235.3	-
ERCC2	NM_000400.4	(Full-length)
ERCC4	NM_005236.3	(Full-length)
ERCC5	NM_000123.4	(Full-length)
ERG	NM_182918.4	-
ERRFI1	NM_018948.4	(Full-length)
ESR1	NM_001122740.1	-
ETV1	NM_001163147.1	-
ETV4	NM_001986.4	-
ETV5	NM_004454.3	-
ETV6	NM_001987.5	(Full-length)
EZH2	NM_004456.5	-
FAM135B	NM_015912.4	-
FANCA	NM_000135.4	(Full-length)
FANCC	NM_000136.3	(Full-length)
FANCD2	NM_033084.6	(Full-length)
FANCE	NM_021922.3	(Full-length)
FANCF	NM_022725.4	(Full-length)
FANCG	NM_004629.2	(Full-length)
FANCI	NM_001113378.2	(Full-length)
FANCL	NM_001114636.1	(Full-length)
FANCM	NM_020937.4	(Full-length)
FAS	NM_000043.6	(Full-length)
FAT1	NM_005245.4	(Full-length)
FBXW7	NM_033632.3	(Full-length)
FGF19	NM_005117.3	-
FGF23	NM_020638.3	-
FGF3	NM_005247.4	-
FGF4	NM_002007.4	-
FGF7	NM_002009.4	-
FGF9	NM_002010.3	-

Gene	Transcript ID	Full-Length Coverage
FGFR1	NM_001174067.1	-
FGFR2	NM_000141.5	-
FGFR3	NM_000142.4	-
FGFR4	NM_213647.3	-
FLT3	NM_004119.3	-
FLT4	NM_182925.5	-
FOXA1	NM_004496.5	-
FOXL2	NM_023067.4	-
FOXO1	NM_002015.4	-
FUBP1	NM_003902.4	(Full-length)
FYN	NM_153047.4	-
GALNT17	NM_022479.3	-
GATA2	NM_032638.5	-
GATA3	NM_001002295.2	(Full-length)
GLI1	NM_005269.3	-
GLI3	NM_000168.6	-
GNA11	NM_002067.5	-
GNA13	NM_006572.6	(Full-length)
GNAQ	NM_002072.5	-
GNAS	NM_000516.6	-
GPR158	NM_020752.3	-
GPS2	NM_004489.5	(Full-length)
GRID2	NM_001510.4	-
H1-4	NM_005321.3	-
H2BC5	NM_021063.4	-
H3-3A	NM_002107.7	-
H3-3B	NM_005324.5	-
H3C2	NM_003537.4	-
HCN1	NM_021072.4	-
HDAC2	NM_001527.4	(Full-length)
HDAC9	NM_178425.3	(Full-length)
HIF1A	NM_001530.4	-
HLA-A	NM_001242758.1	(Full-length)
HLA-B	NM_005514.8	(Full-length)
HLA-C	NM_001243042.1	-
HNF1A	NM_000545.8	(Full-length)
HRAS	NM_001130442.2	-
ID3	NM_002167.5	(Full-length)
IDH1	NM_005896.3	-
IDH2	NM_002168.4	-
IGF1R	NM_000875.5	-
IKBKB	NM_001556.3	-
IL6ST	NM_002184.4	-

Gene	Transcript ID	Full-Length Coverage
IL7R	NM_002185.5	-
INPP4B	NM_001101669.3	(Full-length)
IRF4	NM_002460.4	-
IRS4	NM_003604.2	-
JAK1	NM_002227.4	(Full-length)
JAK2	NM_004972.4	(Full-length)
JAK3	NM_000215.4	(Full-length)
KCND2	NM_012281.3	-
KCNH7	NM_033272.4	-
KCNJ5	NM_000890.5	-
KDM5C	NM_004187.5	(Full-length)
KDM6A	NM_021140.3	(Full-length)
KDR	NM_002253.3	-
KEAP1	NM_203500.2	(Full-length)
KEL	NM_000420.3	-
KIR3DL1	NM_001322168.1	-
KIT	NM_000222.3	-
KLF4	NM_004235.6	-
KLF5	NM_001730.5	-
KLHL13	NM_001168299.1	(Full-length)
KMT2A	NM_001197104.2	(Full-length)
KMT2B	NM_014727.3	(Full-length)
KMT2C	NM_170606.3	(Full-length)
KMT2D	NM_003482.4	(Full-length)
KNSTRN	NM_033286.3	-
KRAS	NM_033360.4	-
KRTAP21-1	NM_181619.2	-
KRTAP6-2	NM_181604.1	-
LARP4B	NM_015155.3	(Full-length)
LATS1	NM_004690.4	(Full-length)
LATS2	NM_014572.3	(Full-length)
LRRC7	NM_001370785.2	-
MAGOH	NM_002370.4	-
MAP2K1	NM_002755.4	-
MAP2K2	NM_030662.4	-
MAP2K4	NM_003010.4	(Full-length)
MAP2K7	NM_145185.4	(Full-length)
MAP3K1	NM_005921.2	(Full-length)
MAP3K4	NM_005922.4	(Full-length)
MAP3K8	NM_001244134.1	-
MAPK1	NM_002745.5	-
MAPK8	NM_139049.4	(Full-length)
MARCO	NM_006770.3	-

Gene	Transcript ID	Full-Length Coverage
MAX	NM_002382.5	-
MCL1	NM_021960.5	-
MDM2	NM_002392.5	-
MDM4	NM_002393.5	-
MECOM	NM_004991.4	-
MED12	NM_005120.3	-
MEF2B	NM_001145785.2	-
MEN1	NM_000244.3	(Full-length)
MET	NM_001127500.3	-
MGA	NM_001164273.1	(Full-length)
MITF	NM_198159.3	-
MLH1	NM_000249.4	(Full-length)
MLH3	NM_001040108.2	(Full-length)
MPL	NM_005373.3	-
MRE11	NM_005591.4	(Full-length)
MSH2	NM_000251.3	(Full-length)
MSH3	NM_002439.5	(Full-length)
MSH6	NM_000179.3	(Full-length)
MTAP	NM_002451.4	(Full-length)
MTOR	NM_004958.4	-
MTUS2	NM_001033602.3	(Full-length)
MUTYH	NM_001128425.2	(Full-length)
MYB	NM_001130173.2	-
MYBL1	NM_001080416.4	-
MYC	NM_002467.6	-
MYCL	NM_001033082.3	-
MYCN	NM_005378.6	-
MYD88	NM_001172567.2	-
MYOD1	NM_002478.5	-
NBN	NM_002485.5	(Full-length)
NCOR1	NM_006311.4	(Full-length)
NF1	NM_001042492.3	(Full-length)
NF2	NM_000268.4	(Full-length)
NFE2L2	NM_006164.5	-
NLRC5	NM_032206.4	-
NOL4	NM_003787.4	-
NOTCH1	NM_017617.5	(Full-length)
NOTCH2	NM_024408.4	(Full-length)
NOTCH3	NM_000435.3	(Full-length)
NOTCH4	NM_004557.4	(Full-length)
NRAS	NM_002524.5	-
NRG1	NM_013956.5	-
NRXN1	NM_004801.5	-

Gene	Transcript ID	Full-Length Coverage
NSD2	NM_001042424.3	-
NT5C2	NM_001134373.3	-
NTRK1	NM_002529.3	-
NTRK2	NM_006180.5	-
NTRK3	NM_001012338.2	-
NUP93	NM_014669.5	-
NUTM1	NM_175741.2	-
NYAP2	NM_020864.1	-
OR10G8	NM_001004464.1	-
OR2G6	NM_001013355.1	-
OR2L13	NM_175911.3	-
OR2L2	NM_001004686.2	-
OR2L8	NM_001001963.1	-
OR2M3	NM_001004689.1	-
OR2T3	NM_001005495.1	-
OR2T33	NM_001004695.1	-
OR2T4	NM_001004696.1	-
OR2W3	NM_001001957.2	-
OR4A15	NM_001005275.1	-
OR4C15	NM_001001920.2	-
OR4C6	NM_001004704.1	-
OR4M1	NM_001005500.1	-
OR4M2	NM_001004719.2	-
OR5D18	NM_001001952.1	-
OR5F1	NM_003697.1	-
OR5L1	NM_001004738.2	-
OR5L2	NM_001004739.1	-
OR6F1	NM_001005286.1	-
OR8H2	NM_001005200.1	-
OR8I2	NM_001003750.1	-
OR8U1	NM_001005204.1	-
ORC4	NM_181742.3	-
PAK5	NM_177990.3	-
PALB2	NM_024675.4	(Full-length)
PARP1	NM_001618.4	(Full-length)
PARP2	NM_005484.3	(Full-length)
PARP3	NM_001003931.3	(Full-length)
PARP4	NM_006437.4	(Full-length)
PAX5	NM_016734.3	-
PBRM1	NM_018313.5	(Full-length)
PCBP1	NM_006196.4	-
PCDH17	NM_001040429.3	-
PDCD1	NM_005018.3	(Full-length)

Gene	Transcript ID	Full-Length Coverage
PDCD1LG2	NM_025239.4	(Full-length)
PDE1A	NM_001258312.1	-
PDE1C	NM_001191058.4	-
PDGFRA	NM_006206.6	-
PDGFRB	NM_002609.4	-
PDIA3	NM_005313.5	(Full-length)
PGD	NM_002631.4	(Full-length)
PHF6	NM_032458.3	(Full-length)
PIK3C2B	NM_002646.4	-
PIK3CA	NM_006218.4	-
PIK3CB	NM_006219.3	-
PIK3CD	NM_005026.5	-
PIK3CG	NM_002649.3	-
PIK3R1	NM_181523.3	(Full-length)
PIK3R2	NM_005027.4	-
PIM1	NM_002648.4	-
PLCG1	NM_002660.3	-
PLXDC2	NM_032812.9	-
PMS1	NM_000534.5	(Full-length)
PMS2	NM_000535.7	(Full-length)
POLD1	NM_001256849.1	(Full-length)
POLE	NM_006231.4	(Full-length)
POM121L12	NM_182595.4	-
POT1	NM_015450.3	(Full-length)
PPARG	NM_015869.5	-
PPFIA2	NM_003625.5	-
PPM1D	NM_003620.4	(Full-length)
PPP2R1A	NM_014225.6	-
PPP2R2A	NM_002717.4	(Full-length)
PPP6C	NM_002721.5	-
PRDM1	NM_001198.4	(Full-length)
PRDM9	NM_020227.4	(Full-length)
PRKACA	NM_002730.4	-
PRKACB	NM_182948.4	-
PRKAR1A	NM_212471.2	(Full-length)
PSMB10	NM_002801.4	(Full-length)
PSMB8	NM_148919.4	(Full-length)
PSMB9	NM_002800.5	(Full-length)
PTCH1	NM_000264.5	(Full-length)
PTEN	NM_000314.8	(Full-length)
PTPN11	NM_002834.5	-
PTPRD	NM_002839.4	-
PTPRT	NM_133170.4	(Full-length)

Gene	Transcript ID	Full-Length Coverage
PXDNL	NM_144651.5	-
RAC1	NM_018890.4	-
RAD50	NM_005732.4	(Full-length)
RAD51	NM_133487.4	(Full-length)
RAD51B	NM_133509.4	(Full-length)
RAD51C	NM_058216.3	(Full-length)
RAD51D	NM_133629.3	(Full-length)
RAD52	NM_134424.4	(Full-length)
RAD54L	NM_001142548.1	(Full-length)
RAF1	NM_002880.3	-
RARA	NM_000964.4	-
RASA1	NM_002890.3	(Full-length)
RASA2	NM_006506.5	(Full-length)
RB1	NM_000321.2	(Full-length)
RBM10	NM_001204468.1	(Full-length)
RBP3	NM_002900.3	-
RECQL4	NM_004260.4	(Full-length)
REG1A	NM_002909.5	-
REG1B	NM_006507.4	-
REG3A	NM_138937.3	-
REG3G	NM_001008387.3	-
RELA	NM_021975.4	-
RET	NM_020975.6	-
RGS7	NM_002924.6	-
RHEB	NM_005614.4	-
RHOA	NM_001664.4	-
RICTOR	NM_152756.5	-
RIT1	NM_001256821.2	-
RNASEH2A	NM_006397.3	(Full-length)
RNASEH2B	NM_024570.4	(Full-length)
RNASEH2C	NM_032193.4	(Full-length)
RNF43	NM_017763.5	(Full-length)
ROS1	NM_002944.2	-
RPA1	NM_002945.5	(Full-length)
RPL10	NM_006013.5	-
RPL22	NM_000983.4	(Full-length)
RPL5	NM_000969.5	(Full-length)
RPS6KB1	NM_003161.4	-
RPTN	NM_001122965.1	-
RPTOR	NM_020761.3	-
RSPO2	NM_178565.5	-
RSPO3	NM_032784.5	-
RUNDC3B	NM_138290.3	-

Gene	Transcript ID	Full-Length Coverage
RUNX1	NM_001754.4	(Full-length)
RUNX1T1	NM_001198634.2	(Full-length)
SDHA	NM_004168.4	(Full-length)
SDHB	NM_003000.3	(Full-length)
SDHC	NM_003001.5	(Full-length)
SDHD	NM_003002.4	(Full-length)
SETBP1	NM_015559.3	-
SETD2	NM_014159.6	(Full-length)
SF3B1	NM_012433.4	-
SH3RF2	NM_152550.4	-
SIX1	NM_005982.4	-
SIX2	NM_016932.5	-
SLC15A2	NM_021082.4	-
SLC8A1	NM_021097.4	-
SLCO1B3	NM_019844.4	-
SLX4	NM_032444.4	(Full-length)
SMAD2	NM_001003652.4	(Full-length)
SMAD4	NM_005359.6	(Full-length)
SMARCA4	NM_001128849.3	(Full-length)
SMARCB1	NM_003073.5	(Full-length)
SMC1A	NM_006306.4	-
SMO	NM_005631.5	-
SNCAIP	NM_005460.4	-
SOCS1	NM_003745.1	(Full-length)
SOS1	NM_005633.3	-
SOX2	NM_003106.4	-
SOX9	NM_000346.4	(Full-length)
SPEN	NM_015001.3	(Full-length)
SPOP	NM_001007228.2	-
SRC	NM_198291.2	-
SRSF2	NM_003016.4	-
STAG2	NM_001042749.2	(Full-length)
STAT1	NM_007315.4	(Full-length)
STAT3	NM_139276.2	-
STAT5B	NM_012448.4	-
STAT6	NM_003153.5	-
STK11	NM_000455.5	(Full-length)
SUFU	NM_016169.4	(Full-length)
SYT10	NM_198992.4	-
SYT16	NM_001367661.1	-
TAF1	NM_004606.5	-
TAP1	NM_000593.5	(Full-length)
TAP2	NM_018833.2	(Full-length)

Gene	Transcript ID	Full-Length Coverage
TAPBP	NM_172208.2	-
TBX3	NM_016569.4	(Full-length)
TCF7L2	NM_001146274.2	(Full-length)
TERT	NM_198253.3	-
TET2	NM_001127208.2	(Full-length)
TFE3	NM_006521.6	-
TFEB	NM_001167827.3	-
TGFBR1	NM_004612.4	-
TGFBR2	NM_001024847.2	(Full-length)
TMEM132D	NM_133448.3	(Full-length)
TNFAIP3	NM_001270507.2	(Full-length)
TNFRSF14	NM_003820.3	(Full-length)
TOP1	NM_003286.4	-
TOP2A	NM_001067.4	-
TP53	NM_000546.5	(Full-length)
TP63	NM_003722.5	(Full-length)
TPMT	NM_000367.4	-
TPP2	NM_003291.4	(Full-length)
TPTE	NM_199261.3	-
TRHDE	NM_013381.2	-
TRIM48	NM_024114.5	-
TRIM51	NM_032681.4	-
TRRAP	NM_001244580.1	-
TSC1	NM_000368.5	(Full-length)
TSC2	NM_000548.5	(Full-length)
TSHR	NM_000369.4	-
U2AF1	NM_006758.2	-
UGT1A1	NM_000463.3	(Full-length)
USP8	NM_005154.5	-
USP9X	NM_001039590.3	(Full-length)
VHL	NM_000551.4	(Full-length)
WAS	NM_000377.3	-
WT1	NM_024426.6	(Full-length)
XPO1	NM_003400.4	-
XRCC2	NM_005431.2	(Full-length)
XRCC3	NM_001100118.2	(Full-length)
YAP1	NM_001130145.3	-
YES1	NM_005433.4	-
ZBTB20	NM_001164342.2	(Full-length)
ZFHX3	NM_006885.4	(Full-length)
ZIM3	NM_052882.1	-
ZMYM3	NM_201599.3	(Full-length)
ZNF217	NM_006526.2	-

Gene	Transcript ID	Full-Length Coverage
ZNF429	NM_001001415.4	-
ZNF479	NM_033273.2	-
ZNF536	NM_014717.3	-
ZRSR2	NM_005089.3	(Full-length)

Appendix B. List of Excluded Regions in the CellDx-Tissue Assay Panel

Gene	Transcript ID	Chromosome Coordinates	Exon	Amino Acid
A1CF	NM_138932.2	10:52570909-52571036	11	451_460
ACVR1B	NM_020328.4	12:52378938-52379062	7	368_397
ADAMTS2	NM_014244.5	5:178580490-178580630	9	461_505
APC	NM_000038.6	5:112111416-112111495	5	171_177
APC	NM_000038.6	5:112175027-112175161	16	1245_1290
APC	NM_000038.6	5:112176400-112176535	16	1703_1748
ARID1A	NM_006015.6	1:27023349-27023486	1	152_197
ARID1A	NM_006015.6	1:27023804-27023940	1	304_349
ARID1A	NM_006015.6	1:27023554-27023693	1	221_266
ARID1A	NM_006015.6	1:27023113-27023274	1	74_127
ARID1A	NM_006015.6	1:27023248-27023395	1	119_167
ARID1A	NM_006015.6	1:27023895-27024032	1	334_379
ARID1A	NM_006015.6	1:27022975-27023123	1	28_76
ARID1B	NM_001371656.1	6:157099473-157099631	2	220_272
ARID1B	NM_001371656.1	6:157099605-157099761	2	264_316
ARID1B	NM_001371656.1	6:157099056-157099193	2	81_126
ARID1B	NM_001371656.1	6:157100200-157100359	2	463_515
ARID1B	NM_001371656.1	6:157100339-157100475	2	509_554
ARID1B	NM_001371656.1	6:157100427-157100540	2	538_575
ARID2	NM_152641.4	12:46215139-46215251	6	213_229
ARID2	NM_152641.4	12:46123677-46123796	1	19_31
ARMC4	NM_018076.5	10:28142255-28142356	13	534_547
ASXL1	NM_015338.6	20:31017638-31017723	8	189_195
ASXL2	NM_018263.6	2:26068393-26068530	2	20_33
ATM	NM_000051.3	11:108188033-108188127	43	2067_2076
ATM	NM_000051.3	11:108098390-108098524	3	25_32
ATR	NM_001184.4	3:142215112-142215235	34	1955_1966
ATRX	NM_000489.5	X:76891365-76891489	16	1539_1567
ATRX	NM_000489.5	X:76944398-76944496	7	162_169
AXIN1	NM_003502.4	16:338020-338157	11	851_862
BLM	NM_000057.4	15:91295168-91295270	4	317_320
BRIP1	NM_032043.3	17:59763375-59763500	19	868_910
C6	NM_000065.4	5:41201590-41201712	3	84_101

Gene	Transcript ID	Chromosome Coordinates	Exon	Amino Acid
C8B	NM_000066.4	1:57395076-57395210	12	549_592
CBFB	NM_022845.3	16:67063653-67063773	2	34_55
CCND1	NM_053056.3	11:69457730-69457842	2	67_81
CD276	NM_001024736.2	15:73994649-73994786	3	45_90
CDH10	NM_006727.5	5:24491838-24491965	11	542_575
CDH10	NM_006727.5	5:24535170-24535295	5	247_272
CDKN2A	NM_001195132.1	9:21994162-21994298	intronic	intronic
CHD4	NM_001273.5	12:6697015-6697117	24	1156_1189
CHEK2	NM_007194.4	22:29126414-29126556	intronic	intronic
CIC	NM_015125.4	19:42797804-42797935	16	1286_1329
CIC	NM_015125.4	19:42799149-42799314	20	1545_1599
CIC	NM_015125.4	19:42798952-42799102	20	1487_1529
CIC	NM_015125.4	19:42778280-42778419	intronic	intronic
CNTNAP4	NM_138994.5	16:76523547-76523669	12	556_585
CNTNAP4	NM_138994.5	16:76513305-76513422	11	516_551
CSMD3	NM_198123.2	8:113516097-113516215	30	1632_1669
CSMD3	NM_198123.2	8:113668349-113668473	18	972_1002
CSMD3	NM_198123.2	8:113841909-113842029	12	585_620
CUL3	NM_003590.5	2:225449580-225449705	1	7_22
CUL4A	NM_001008895.4	13:113863944-113864054	1	2_38
CUL4A	NM_001008895.4	13:113863976-113864079	1	12_46
CYLD	NM_001042355.2	16:50788298-50788400	5	292_305
CYLD	NM_001042355.2	16:50785802-50785947	4	264_269
DDX3X	NM_001356.5	X:41206876-41206991	17	637_662
DNMT3A	NM_022552.4	2:25459815-25459907	21	803_823
DOCK3	NM_004947.5	3:51394323-51394443	44	1502_1519
DSC1	NM_024421.2	18:28710584-28710709	16	830_860
EPCAM	NM_002354.3	2:47596574-47596708	1	1_22
EPHA2	NM_004431.5	1:16482351-16482467	1	1_27
EPHA2	NM_004431.5	1:16464547-16464685	5	327_372
FANCA	NM_000135.4	16:89882944-89883082	1	1_27
FANCA	NM_000135.4	16:89882874-89883013	1	4_27
FANCM	NM_020937.4	14:45624508-45624620	8	437_452
FANCM	NM_020937.4	14:45654539-45654654	18	1545_1558

Gene	Transcript ID	Chromosome Coordinates	Exon	Amino Acid
FANCM	NM_020937.4	14:45665689-45665788	21	1885_1906
FGF19	NM_005117.3	11:69517956-69518095	2	91_113
FGF19	NM_005117.3	11:69518462-69518558	1	30_62
FGF3	NM_005247.4	11:69633479-69633593	1	37_74
FGF4	NM_002007.4	11:69589456-69589591	1	88_114
FGF4	NM_002007.4	11:69589747-69589885	1	1_36
FGF4	NM_002007.4	11:69589661-69589802	1	18_65
FGFR3	NM_000142.4	4:1803463-1803603	7	248_262
FGFR3	NM_000142.4	4:1803463-1803603	7	247_261
FLT4	NM_182925.5	5:180039486-180039619	26	1144_1179
FOXA1	NM_004496.5	14:38060946-38061083	2	303_348
GLI3	NM_000168.6	7:42005740-42005858	15	939_978
GNA13	NM_006572.6	17:63052330-63052447	1	89_95
HCN1	NM_021072.4	5:45262354-45262493	8	736_782
HCN1	NM_021072.4	5:45262187-45262327	8	791_837
HCN1	NM_021072.4	5:45695914-45696022	1	59_94
HCN1	NM_021072.4	5:45696048-45696189	1	3_50
HDAC2	NM_001527.4	6:114291926-114292056	1	6_18
HDAC2	NM_001527.4	6:114262826-114262939	13	460_479
INPP4B	NM_001101669.3	4:143129669-143129753	14	323_328
JAK1	NM_002227.4	1:65306894-65307021	19	852_883
KDM6A	NM_021140.3	X:44921920-44922026	15	485_509
KDM6A	NM_021140.3	X:44910947-44911016	9	219_240
KEL	NM_000420.3	7:142651264-142651401	8	266_309
KLF5	NM_001730.5	13:73633434-73633575	1	1_37
KLF5	NM_001730.5	13:73633567-73633707	1	35_81
KMT2A	NM_001197104.2	11:118342828-118342961	3	318_362
KMT2A	NM_001197104.2	11:118307485-118307648	1	87_140
KMT2B	NM_014727.3	19:36209127-36209285	1	69_121
KMT2B	NM_014727.3	19:36211486-36211594	3	413_448
KMT2B	NM_014727.3	19:36212068-36212218	3	607_656
KMT2C	NM_170606.3	7:152132733-152132891	1	1_47
KMT2C	NM_170606.3	7:151899993-151900085	26	1342_1364
KMT2D	NM_003482.4	12:49439932-49440058	18	1528_1537

Gene	Transcript ID	Chromosome Coordinates	Exon	Amino Acid
LATS2	NM_014572.3	13:21562584-21562722	4	399_445
LATS2	NM_014572.3	13:21562457-21562596	4	441_487
MAP2K4	NM_003010.4	17:11924214-11924350	1	4_39
MAP2K4	NM_003010.4	17:11924163-11924301	1	1_33
MAP3K1	NM_005921.2	5:56111532-56111687	1	45_96
MAP3K1	NM_005921.2	5:56111787-56111917	1	129_161
MAP3K1	NM_005921.2	5:56111472-56111587	1	25_62
MAP3K1	NM_005921.2	5:56111682-56111823	1	95_141
MAP3K4	NM_005922.4	6:161412930-161413070	1	1_36
MAP3K4	NM_005922.4	6:161412974-161413104	1	4_47
MAP3K4	NM_005922.4	6:161523738-161523852	19	1261_1286
MCL1	NM_021960.5	1:150551662-150551785	1	75_116
MCL1	NM_021960.5	1:150551871-150552002	1	2_46
MEN1	NM_000244.3	11:64572116-64572254	10	467_513
MGA	NM_001164273.1	15:41991035-41991118	4	672_691
MLH1	NM_000249.4	3:37067067-37067175	12	347_363
MLH3	NM_001040108.2	14:75506521-75506638	5	1182_1190
MSH6	NM_000179.3	2:48023229-48023327	intronic	intronic
MSH6	NM_000179.3	2:48032173-48032292	intronic	intronic
MSH6	NM_000179.3	2:48010430-48010570	1	20_66
MYCN	NM_005378.6	2:16082871-16082980	2	228_264
NOTCH1	NM_017617.5	9:139417228-139417354	4	231_248
NOTCH1	NM_017617.5	9:139401067-139401203	24	1301_1309
NOTCH1	NM_017617.5	9:139440064-139440226	1	5_21
NOTCH1	NM_017617.5	9:139396907-139397042	28	1723_1734
NOTCH3	NM_000435.3	19:15281367-15281501	26	1625_1631
NOTCH3	NM_000435.3	19:15271813-15271950	33	2164_2209
NOTCH3	NM_000435.3	19:15288737-15288876	24	1289_1334
NOTCH3	NM_000435.3	19:15288502-15288662	24	1360_1413
NOTCH3	NM_000435.3	19:15288601-15288767	24	1325_1380
NOTCH3	NM_000435.3	19:15311504-15311669	1	17_40
NOTCH3	NM_000435.3	19:15281148-15281279	27	1660_1703
NRXN1	NM_004801.5	2:50318374-50318501	19	1228_1241
OR2G6	NM_001013355.1	1:248685252-248685391	2	102_148

Gene	Transcript ID	Chromosome Coordinates	Exon	Amino Acid
OR2L13	NM_175911.3	1:248262843-248262981	3	56_101
OR2L8	NM_001001963.1	1:248113049-248113177	1	297_312
OR2T4	NM_001004696.1	1:248525216-248525351	1	112_516
OR4A15	NM_001005275.1	11:55136184-55136276	1	276_306
OR4C15	NM_001001920.2	11:55322096-55322225	1	1_94
OR8H2	NM_001005200.1	11:55872770-55872877	2	85_120
OR8H2	NM_001005200.1	11:55873244-55873352	2	243_278
PARP4	NM_006437.4	13:25009001-25009088	31	1398_1426
PARP4	NM_006437.4	13:25008757-25008887	31	1465_1507
PARP4	NM_006437.4	13:25033254-25033346	20	782_784
PARP4	NM_006437.4	13:25016031-25016129	30	1182_1207
PARP4	NM_006437.4	13:25020802-25020919	27	1095_1122
PGD	NM_002631.4	1:10464224-10464351	5	112_150
PLCG1	NM_002660.3	20:39766235-39766366	1	1_29
POLD1	NM_001256849.1	19:50918899-50919029	22	906_922
POLE	NM_006231.4	12:133263801-133263936	1	1_21
POT1	NM_015450.3	7:124493139-124493201	10	235_253
PPP2R2A	NM_002717.4	8:26149297-26149379	1	1_4
PPP2R2A	NM_002717.4	8:26150699-26150821	intronic	intronic
PTCH1	NM_000264.5	9:98270633-98270780	1	1_4
PTCH1	NM_000264.5	9:98270481-98270635	1	4_54
PTEN	NM_000314.8	10:89623876-89624006	intronic	intronic
PTEN	NM_000314.8	10:89623658-89623799	intronic	intronic
RAD52	NM_134424.4	12:1040414-1040536	3	29_53
RAD52	NM_134424.4	12:1036026-1036137	intronic	intronic
RASA1	NM_002890.3	5:86637114-86637222	6	342_350
RASA2	NM_006506.5	3:141205871-141206008	1	1_28
RASA2	NM_006506.5	3:141292020-141292098	13	439_453
RASA2	NM_006506.5	3:141300012-141300124	17	582_584
RASA2	NM_006506.5	3:141299927-141300017	17	559_584
RB1	NM_000321.2	13:48947486-48947588	12	376_392
RB1	NM_000321.2	13:48878051-48878191	1	1_46
RBM10	NM_001204468.1	X:47004746-47004881	1	1_13
RBM10	NM_001204468.1	X:47004871-47005006	1	9_24

Gene	Transcript ID	Chromosome Coordinates	Exon	Amino Acid
RBM10	NM_001204468.1	X:47006725-47006861	2	24_59
RNASEH2B	NM_024570.4	13:51517410-51517498	6	146_160
RNASEH2B	NM_024570.4	13:51484144-51484276	1	1_22
RNASEH2B	NM_024570.4	13:51484233-51484361	1	7_22
rs1052325	NM_001206997.1	4:81884679-81884751	7	223_246
rs11009107	NM_001026383.3	10:33123687-33123810	intronic	intronic
rs11551095	NM_020170.4	19:3186076-3186170	1	17_48
rs12876018	NM_020121.4	13:96540151-96540223	26	989_1012
rs16864976	NM_003469.5	2:224463781-224463901	2	34_73
rs2229482	NM_005529.7	1:22199162-22199284	32	1298_1328
rs2295997	NM_003738.5	1:45293652-45293762	14	605_641
rs7044405	NM_001004487.1	9:35869933-35870039	1	121_155
rs8024779	NM_178232.4	15:89436216-89436352	intronic	intronic
RUNX1	NM_001754.4	21:36164356-36164476	9	466_480
SDHA	NM_004168.4	5:218411-218535	1	1_22
SETD2	NM_014159.6	3:47205247-47205408	1	3_24
SMARCA4	NM_001128849.3	19:11098403-11098543	6	307_354
SMARCB1	NM_003073.5	22:24129272-24129377	1	1_8
SMARCB1	NM_003073.5	22:24129375-24129509	1	6_31
SOCS1	NM_003745.1	16:11348772-11348913	2	142_188
SOCS1	NM_003745.1	16:11349150-11349288	2	17_62
SOX9	NM_000346.4	17:70120011-70120150	3	338_384
SPEN	NM_015001.3	1:16262462-16262599	11	3242_3288
STAG2	NM_001042749.2	X:123202371-123202482	24	756_779
SUFU	NM_016169.4	10:104263902-104264024	1	1_39
TBX3	NM_016569.4	12:115112290-115112427	7	438_483
TBX3	NM_016569.4	12:115112075-115112231	7	503_555
TCF7L2	NM_001146274.2	10:114711012-114711156	2	79_86
TMEM132D	NM_133448.3	12:129822116-129822256	4	407_433
TP63	NM_003722.5	3:189455542-189455660	2	25_64
TPP2	NM_003291.4	13:103328654-103328765	28	1183_1220
TSC1	NM_000368.5	9:135772955-135773050	21	876_890
TSC2	NM_000548.5	16:2106675-2106815	8	226_258
USP9X	NM_001039590.3	X:41055607-41055677	27	1360_1362

Gene	Transcript ID	Chromosome Coordinates	Exon	Amino Acid
USP9X	NM_001039590.3	X:41077608-41077731	37	2070_2105
USP9X	NM_001039590.3	X:41026738-41026852	17	777_808
USP9X	NM_001039590.3	X:41057979-41058081	30	1526_1535
WT1	NM_024426.6	11:32456482-32456627	1	94_142
WT1	NM_024426.6	11:32456860-32456965	1	1_16
XRCC3	NM_001100118.2	14:104173409-104173542	5	69_113
YAP1	NM_001130145.3	11:101981593-101981759	1	5_60
ZFHX3	NM_006885.4	16:72821562-72821701	10	3492_3538
ZMYM3	NM_201599.3	X:70466231-70466357	15	807_849
ZMYM3	NM_201599.3	X:70469263-70469340	7	480_490
ZMYM3	NM_201599.3	X:70472622-70472733	2	126_162
ZMYM3	NM_201599.3	X:70472683-70472778	2	111_142
ZNF217	NM_006526.2	20:52193028-52193147	4	720_759
ZRSR2	NM_005089.3	X:15841002-15841112	11	362_399

Appendix C. List of Excluded Genes in the CellDx-Tissue Assay Panel

Gene	Transcript ID	Chromosome Coordinates	Exon	Amino Acid
A1CF	NM_138932.2	10:52570909-52571036	11	451_460
ACVR1B	NM_020328.4	12:52378938-52379062	7	368_397
ADAMTS2	NM_014244.5	5:178580490-178580630	9	461_505
APC	NM_000038.6	5:112111416-112111495	5	171_177
APC	NM_000038.6	5:112175027-112175161	16	1245_1290
APC	NM_000038.6	5:112176400-112176535	16	1703_1748
ARID1A	NM_006015.6	1:27023349-27023486	1	152_197
ARID1A	NM_006015.6	1:27023804-27023940	1	304_349
ARID1A	NM_006015.6	1:27023554-27023693	1	221_266
ARID1A	NM_006015.6	1:27023113-27023274	1	74_127
ARID1A	NM_006015.6	1:27023248-27023395	1	119_167
ARID1A	NM_006015.6	1:27023895-27024032	1	334_379
ARID1A	NM_006015.6	1:27022975-27023123	1	28_76
ARID1B	NM_001371656.1	6:157099473-157099631	2	220_272
ARID1B	NM_001371656.1	6:157099605-157099761	2	264_316
ARID1B	NM_001371656.1	6:157099056-157099193	2	81_126
ARID1B	NM_001371656.1	6:157100200-157100359	2	463_515
ARID1B	NM_001371656.1	6:157100339-157100475	2	509_554
ARID1B	NM_001371656.1	6:157100427-157100540	2	538_575
ARID2	NM_152641.4	12:46215139-46215251	6	213_229
ARID2	NM_152641.4	12:46123677-46123796	1	19_31
ARMC4	NM_018076.5	10:28142255-28142356	13	534_547
ASXL1	NM_015338.6	20:31017638-31017723	8	189_195
ASXL2	NM_018263.6	2:26068393-26068530	2	20_33
ATM	NM_000051.3	11:108188033-108188127	43	2067_2076
ATM	NM_000051.3	11:108098390-108098524	3	25_32
ATR	NM_001184.4	3:142215112-142215235	34	1955_1966
ATRX	NM_000489.5	X:76891365-76891489	16	1539_1567
ATRX	NM_000489.5	X:76944398-76944496	7	162_169
AXIN1	NM_003502.4	16:338020-338157	11	851_862
BLM	NM_000057.4	15:91295168-91295270	4	317_320
BRIP1	NM_032043.3	17:59763375-59763500	19	868_910
C6	NM_000065.4	5:41201590-41201712	3	84_101
C8B	NM_000066.4	1:57395076-57395210	12	549_592
CBFB	NM_022845.3	16:67063653-67063773	2	34_55
CCND1	NM_053056.3	11:69457730-69457842	2	67_81
CD276	NM_001024736.2	15:73994649-73994786	3	45_90
CDH10	NM_006727.5	5:24491838-24491965	11	542_575
CDH10	NM_006727.5	5:24535170-24535295	5	247_272
CDKN2A	NM_001195132.1	9:21994162-21994298	intronic	intronic
CHD4	NM_001273.5	12:6697015-6697117	24	1156_1189
CHEK2	NM_007194.4	22:29126414-29126556	intronic	intronic

Gene	Transcript ID	Chromosome Coordinates	Exon	Amino Acid
CIC	NM_015125.4	19:42797804-42797935	16	1286_1329
CIC	NM_015125.4	19:42799149-42799314	20	1545_1599
CIC	NM_015125.4	19:42798952-42799102	20	1487_1529
CIC	NM_015125.4	19:42778280-42778419	intronic	intronic
CNTNAP4	NM_138994.5	16:76523547-76523669	12	556_585
CNTNAP4	NM_138994.5	16:76513305-76513422	11	516_551
CSMD3	NM_198123.2	8:113516097-113516215	30	1632_1669
CSMD3	NM_198123.2	8:113668349-113668473	18	972_1002
CSMD3	NM_198123.2	8:113841909-113842029	12	585_620
CUL3	NM_003590.5	2:225449580-225449705	1	7_22
CUL4A	NM_001008895.4	13:113863944-113864054	1	2_38
CUL4A	NM_001008895.4	13:113863976-113864079	1	12_46
CYLD	NM_001042355.2	16:50788298-50788400	5	292_305
CYLD	NM_001042355.2	16:50785802-50785947	4	264_269
DDX3X	NM_001356.5	X:41206876-41206991	17	637_662
DNMT3A	NM_022552.4	2:25459815-25459907	21	803_823
DOCK3	NM_004947.5	3:51394323-51394443	44	1502_1519
DSC1	NM_024421.2	18:28710584-28710709	16	830_860
EPCAM	NM_002354.3	2:47596574-47596708	1	1_22
EPHA2	NM_004431.5	1:16482351-16482467	1	1_27
EPHA2	NM_004431.5	1:16464547-16464685	5	327_372
FANCA	NM_000135.4	16:89882944-89883082	1	1_27
FANCA	NM_000135.4	16:89882874-89883013	1	4_27
FANCM	NM_020937.4	14:45624508-45624620	8	437_452
FANCM	NM_020937.4	14:45654539-45654654	18	1545_1558
FANCM	NM_020937.4	14:45665689-45665788	21	1885_1906
FGF19	NM_005117.3	11:69517956-69518095	2	91_113
FGF19	NM_005117.3	11:69518462-69518558	1	30_62
FGF3	NM_005247.4	11:69633479-69633593	1	37_74
FGF4	NM_002007.4	11:69589456-69589591	1	88_114
FGF4	NM_002007.4	11:69589747-69589885	1	1_36
FGF4	NM_002007.4	11:69589661-69589802	1	18_65
FGFR3	NM_000142.4	4:1803463-1803603	7	248_262
FGFR3	NM_000142.4	4:1803463-1803603	7	247_261
FLT4	NM_182925.5	5:180039486-180039619	26	1144_1179
FOXA1	NM_004496.5	14:38060946-38061083	2	303_348
GLI3	NM_000168.6	7:42005740-42005858	15	939_978
GNA13	NM_006572.6	17:63052330-63052447	1	89_95
HCN1	NM_021072.4	5:45262354-45262493	8	736_782
HCN1	NM_021072.4	5:45262187-45262327	8	791_837
HCN1	NM_021072.4	5:45695914-45696022	1	59_94
HCN1	NM_021072.4	5:45696048-45696189	1	3_50
HDAC2	NM_001527.4	6:114291926-114292056	1	6_18

Gene	Transcript ID	Chromosome Coordinates	Exon	Amino Acid
HDAC2	NM_001527.4	6:114262826-114262939	13	460_479
INPP4B	NM_001101669.3	4:143129669-143129753	14	323_328
JAK1	NM_002227.4	1:65306894-65307021	19	852_883
KDM6A	NM_021140.3	X:44921920-44922026	15	485_509
KDM6A	NM_021140.3	X:44910947-44911016	9	219_240
KEL	NM_000420.3	7:142651264-142651401	8	266_309
KLF5	NM_001730.5	13:73633434-73633575	1	1_37
KLF5	NM_001730.5	13:73633567-73633707	1	35_81
KMT2A	NM_001197104.2	11:118342828-118342961	3	318_362
KMT2A	NM_001197104.2	11:118307485-118307648	1	87_140
KMT2B	NM_014727.3	19:36209127-36209285	1	69_121
KMT2B	NM_014727.3	19:36211486-36211594	3	413_448
KMT2B	NM_014727.3	19:36212068-36212218	3	607_656
KMT2C	NM_170606.3	7:152132733-152132891	1	1_47
KMT2C	NM_170606.3	7:151899993-151900085	26	1342_1364
KMT2D	NM_003482.4	12:49439932-49440058	18	1528_1537
LATS2	NM_014572.3	13:21562584-21562722	4	399_445
LATS2	NM_014572.3	13:21562457-21562596	4	441_487
MAP2K4	NM_003010.4	17:11924214-11924350	1	4_39
MAP2K4	NM_003010.4	17:11924163-11924301	1	1_33
MAP3K1	NM_005921.2	5:56111532-56111687	1	45_96
MAP3K1	NM_005921.2	5:56111787-56111917	1	129_161
MAP3K1	NM_005921.2	5:56111472-56111587	1	25_62
MAP3K1	NM_005921.2	5:56111682-56111823	1	95_141
MAP3K4	NM_005922.4	6:161412930-161413070	1	1_36
MAP3K4	NM_005922.4	6:161412974-161413104	1	4_47
MAP3K4	NM_005922.4	6:161523738-161523852	19	1261_1286
MCL1	NM_021960.5	1:150551662-150551785	1	75_116
MCL1	NM_021960.5	1:150551871-150552002	1	2_46
MEN1	NM_000244.3	11:64572116-64572254	10	467_513
MGA	NM_001164273.1	15:41991035-41991118	4	672_691
MLH1	NM_000249.4	3:37067067-37067175	12	347_363
MLH3	NM_001040108.2	14:75506521-75506638	5	1182_1190
MSH6	NM_000179.3	2:48023229-48023327	intronic	intronic
MSH6	NM_000179.3	2:48032173-48032292	intronic	intronic
MSH6	NM_000179.3	2:48010430-48010570	1	20_66
MYCN	NM_005378.6	2:16082871-16082980	2	228_264
NOTCH1	NM_017617.5	9:139417228-139417354	4	231_248
NOTCH1	NM_017617.5	9:139401067-139401203	24	1301_1309
NOTCH1	NM_017617.5	9:139440064-139440226	1	5_21
NOTCH1	NM_017617.5	9:139396907-139397042	28	1723_1734
NOTCH3	NM_000435.3	19:15281367-15281501	26	1625_1631
NOTCH3	NM_000435.3	19:15271813-15271950	33	2164_2209

Gene	Transcript ID	Chromosome Coordinates	Exon	Amino Acid
NOTCH3	NM_000435.3	19:15288737-15288876	24	1289_1334
NOTCH3	NM_000435.3	19:15288502-15288662	24	1360_1413
NOTCH3	NM_000435.3	19:15288601-15288767	24	1325_1380
NOTCH3	NM_000435.3	19:15311504-15311669	1	17_40
NOTCH3	NM_000435.3	19:15281148-15281279	27	1660_1703
NRXN1	NM_004801.5	2:50318374-50318501	19	1228_1241
OR2G6	NM_001013355.1	1:248685252-248685391	2	102_148
OR2L13	NM_175911.3	1:248262843-248262981	3	56_101
OR2L8	NM_001001963.1	1:248113049-248113177	1	297_312
OR2T4	NM_001004696.1	1:248525216-248525351	1	112_516
OR4A15	NM_001005275.1	11:55136184-55136276	1	276_306
OR4C15	NM_001001920.2	11:55322096-55322225	1	1_94
OR8H2	NM_001005200.1	11:55872770-55872877	2	85_120
OR8H2	NM_001005200.1	11:55873244-55873352	2	243_278
PARP4	NM_006437.4	13:25009001-25009088	31	1398_1426
PARP4	NM_006437.4	13:25008757-25008887	31	1465_1507
PARP4	NM_006437.4	13:25033254-25033346	20	782_784
PARP4	NM_006437.4	13:25016031-25016129	30	1182_1207
PARP4	NM_006437.4	13:25020802-25020919	27	1095_1122
PGD	NM_002631.4	1:10464224-10464351	5	112_150
PLCG1	NM_002660.3	20:39766235-39766366	1	1_29
POLD1	NM_001256849.1	19:50918899-50919029	22	906_922
POLE	NM_006231.4	12:133263801-133263936	1	1_21
POT1	NM_015450.3	7:124493139-124493201	10	235_253
PPP2R2A	NM_002717.4	8:26149297-26149379	1	1_4
PPP2R2A	NM_002717.4	8:26150699-26150821	intronic	intronic
PTCH1	NM_000264.5	9:98270633-98270780	1	1_4
PTCH1	NM_000264.5	9:98270481-98270635	1	4_54
PTEN	NM_000314.8	10:89623876-89624006	intronic	intronic
PTEN	NM_000314.8	10:89623658-89623799	intronic	intronic
RAD52	NM_134424.4	12:1040414-1040536	3	29_53
RAD52	NM_134424.4	12:1036026-1036137	intronic	intronic
RASA1	NM_002890.3	5:86637114-86637222	6	342_350
RASA2	NM_006506.5	3:141205871-141206008	1	1_28
RASA2	NM_006506.5	3:141292020-141292098	13	439_453
RASA2	NM_006506.5	3:141300012-141300124	17	582_584
RASA2	NM_006506.5	3:141299927-141300017	17	559_584
RB1	NM_000321.2	13:48947486-48947588	12	376_392
RB1	NM_000321.2	13:48878051-48878191	1	1_46
RBM10	NM_001204468.1	X:47004746-47004881	1	1_13
RBM10	NM_001204468.1	X:47004871-47005006	1	9_24
RBM10	NM_001204468.1	X:47006725-47006861	2	24_59
RNASEH2B	NM_024570.4	13:51517410-51517498	6	146_160

Gene	Transcript ID	Chromosome Coordinates	Exon	Amino Acid
RNASEH2B	NM_024570.4	13:51484144-51484276	1	1_22
RNASEH2B	NM_024570.4	13:51484233-51484361	1	7_22
rs1052325	NM_001206997.1	4:81884679-81884751	7	223_246
rs11009107	NM_001026383.3	10:33123687-33123810	intronic	intronic
rs11551095	NM_020170.4	19:3186076-3186170	1	17_48
rs12876018	NM_020121.4	13:96540151-96540223	26	989_1012
rs16864976	NM_003469.5	2:224463781-224463901	2	34_73
rs2229482	NM_005529.7	1:22199162-22199284	32	1298_1328
rs2295997	NM_003738.5	1:45293652-45293762	14	605_641
rs7044405	NM_001004487.1	9:35869933-35870039	1	121_155
rs8024779	NM_178232.4	15:89436216-89436352	intronic	intronic
RUNX1	NM_001754.4	21:36164356-36164476	9	466_480
SDHA	NM_004168.4	5:218411-218535	1	1_22
SETD2	NM_014159.6	3:47205247-47205408	1	3_24
SMARCA4	NM_001128849.3	19:11098403-11098543	6	307_354
SMARCB1	NM_003073.5	22:24129272-24129377	1	1_8
SMARCB1	NM_003073.5	22:24129375-24129509	1	6_31
SOCS1	NM_003745.1	16:11348772-11348913	2	142_188
SOCS1	NM_003745.1	16:11349150-11349288	2	17_62
SOX9	NM_000346.4	17:70120011-70120150	3	338_384
SPEN	NM_015001.3	1:16262462-16262599	11	3242_3288
STAG2	NM_001042749.2	X:123202371-123202482	24	756_779
SUFU	NM_016169.4	10:104263902-104264024	1	1_39
TBX3	NM_016569.4	12:115112290-115112427	7	438_483
TBX3	NM_016569.4	12:115112075-115112231	7	503_555
TCF7L2	NM_001146274.2	10:114711012-114711156	2	79_86
TMEM132D	NM_133448.3	12:129822116-129822256	4	407_433
TP63	NM_003722.5	3:189455542-189455660	2	25_64
TPP2	NM_003291.4	13:103328654-103328765	28	1183_1220
TSC1	NM_000368.5	9:135772955-135773050	21	876_890
TSC2	NM_000548.5	16:2106675-2106815	8	226_258
USP9X	NM_001039590.3	X:41055607-41055677	27	1360_1362
USP9X	NM_001039590.3	X:41077608-41077731	37	2070_2105
USP9X	NM_001039590.3	X:41026738-41026852	17	777_808
USP9X	NM_001039590.3	X:41057979-41058081	30	1526_1535
WT1	NM_024426.6	11:32456482-32456627	1	94_142
WT1	NM_024426.6	11:32456860-32456965	1	1_16
XRCC3	NM_001100118.2	14:104173409-104173542	5	69_113
YAP1	NM_001130145.3	11:101981593-101981759	1	5_60
ZFHX3	NM_006885.4	16:72821562-72821701	10	3492_3538
ZMYM3	NM_201599.3	X:70466231-70466357	15	807_849
ZMYM3	NM_201599.3	X:70469263-70469340	7	480_490

Gene	Transcript ID	Chromosome Coordinates	Exon	Amino Acid
ZMYM3	NM_201599.3	X:70472622-70472733	2	126_162
ZMYM3	NM_201599.3	X:70472683-70472778	2	111_142
ZNF217	NM_006526.2	20:52193028-52193147	4	720_759
ZRSR2	NM_005089.3	X:15841002-15841112	11	362_399

Appendix D. Precision Summary of CellDx – Tissue Assay for Selected SNVs, Insertions, and Deletions

Gene Exon	Mutation (cDNA/AA Change)	Normalized Coverage Rance	VAF Range	VAF Mean	SD	%CV	n/N	PCR (%) (95% CI)
AMER1 Exon 2	c.2185G>T p.E729*	0.48-0.77	0.554-0.773	0.641	0.037	5.8	48/48	100 (92.6, 100)
APC Exon 16	c.2138C>G p.S713*	0.66-1.09	0.109-0.196	0.148	0.020	13.8	48/48	100 (92.6, 100)
APC Exon 16	c.4348C>T p.R1450*	0.94-1.68	0.186-0.286	0.233	0.023	10.1	48/48	100 (92.6, 100)
APC Exon 9	c.847C>T p.R283*	0.66-1.41	0.154-0.277	0.234	0.023	9.9	48/48	100 (92.6, 100)
ASXL2 Exon 3	c.143G>A p.S48N	0.82-1.51	0.274-0.351	0.313	0.016	5.2	48/48	100 (92.6, 100)
ATM Exon 47	c.6908delA p.K2303Rfs*7	0.84-2.28	0.287-0.381	0.324	0.020	6.3	42/48	88 (75.3, 94.1)
ATM Exon 8	c.1009C>T p.R337C	0.55-1.19	0.256-0.392	0.326	0.028	8.6	48/48	100 (92.6, 100)
AXIN1 Exon 11	c.2463-14C>T	1.23-1.93	0.436-0.533	0.491	0.017	3.6	48/48	100 (92.6, 100)
B2M Exon 1	c.3G>A	0.79-1.56	0.034-0.086	0.057	0.012	21.3	47/48	98 (89.1, 99.6)
B2M Exon 1	c.43_44delCT p.L15Ffs*41	0.69-1.45	0.035-0.102	0.063	0.012	19.8	48/48	100 (92.6, 100)
B2M Exon 2	c.244_247delTTCT p.F82Ifs*20	0.69-1.45	0.052-0.107	0.068	0.011	16.0	45/48	94 (83.2, 97.9)
B2M Exon 2	c.142_145delTCTG p.S48Gfs*12	0.94-1.73	0.274-0.363	0.322	0.017	5.4	48/48	100 (92.6, 100)
B2M Exon 2	c.68-1G>A	0.94-1.73	0.281-0.361	0.323	0.020	6.1	48/48	100 (92.6, 100)
BRAF Exon 15	c.1799_1800delTGinsAA p.V600E	0.39-0.83	0.359-0.47	0.415	0.023	5.5	48/48	100 (92.6, 100)
BRCA2 Exon 11	c.4588A>T p.K1530*	0.36-0.78	0.413-0.546	0.481	0.030	6.3	46/46	100 (92.3, 100)

Gene Exon	Mutation (cDNA/AA Change)	Normalized Coverage Rance	VAF Range	VAF Mean	SD	%CV	n/N	PCR (%) (95% CI)
BRCA2 Exon 20	c.8581A>T p.R2861*	1.20-2.00	0.738-0.796	0.772	0.013	1.7	48/48	100 (92.6, 100)
CIC Exon 14	c.3216_3217delGT p.S1073Tfs*77	0.27-0.776	0.702-0.855	0.773	0.035	4.6	48/48	100 (92.6, 100)
CUL3 Exon 8	c.1162_1163delCT p.S389Ifs*4	0.37-1.35	0.093-0.182	0.132	0.020	15.2	48/48	100 (92.6, 100)
DNMT3A Exon 23	c.2701delC p.L901Sfs*5	0.78-1.25	0.05-0.13	0.072	0.014,	19.4	46/48	96 (86.0, 98.8)
DNMT3A Exon 6	c.541C>T p.R181C	0.05-0.68	0.227-0.55	0.331	0.086	26.0	31/31	100 (89.0, 100)
EGFR Exon 19	c.2236_2250delGAATTAAGAGAAGCA p.E746_A750del	0.62-1.55	0.201-0.333	0.237	0.023	9.6	48/48	100 (92.6, 100)
ERBB2 Exon 19	c.2305G>T p.D769Y	0.76-1.64	0.265-0.407	0.342	0.032	9.4	46/46	100 (92.3, 100)
ERBB3 Exon 23	c.2783A>G p.E928G	1.07-2.55	0.024-0.042	0.03	0.004	12.9	44/46	96 (84.6, 99.0)
ERCC2 Exon 21	c.1975C>A p.H659N	0.08-0.47	0.262-0.412	0.352	0.030	8.6	48/48	100 (92.6, 100)
ERCC4 Exon 8	c.1811+1G>A	0.86-1.36	0.269-0.382	0.331	0.024	7.3	48/48	100 (92.6, 100)
ETV6 Exon 4	c.427_428delCA p.Q143Afs*10	0.48-1.27	0.27-0.367	0.322	0.023	7.3	48/48	100 (92.6, 100)
FANCA Exon 24	c.2222+7G>A	0.32-0.83	0.387-0.585	0.49	0.041	8.4	48/48	100 (92.6, 100)
FANCA Exon 38	c.3828+1G>C	0.25-0.75	0.686-0.795	0.75	0.020	2.7	48/48	100 (92.6, 100)
FAT1 Exon 26	c.13002A>C p.T4334T	1.16-1.92	0.175-0.26	0.208	0.018	8.7	48/48	100 (92.6, 100)
FUBP1 Exon 14	c.1213C>T p.Q405*	0.49-1.25	0.265-0.351	0.31	0.023	7.3	48/48	100 (92.6, 100)
IDH1 Exon 4	c.395G>A p.R132H	1.60-2.19	0.414-0.503	0.458	0.019,	4.2	48/48	100 (92.6, 100)

Gene Exon	Mutation (cDNA/AA Change)	Normalized Coverage Rance	VAF Range	VAF Mean	SD	%CV	n/N	PCR (%) (95% CI)
KEAP1 Exon 4	c.1531+8G>C	0.88-1.52	0.454-0.565	0.514	0.026,	5.1	48/48	100 (92.6, 100)
KMT2A Exon 3	c.1142delA p.K381Rfs*19	1.34-5.37	0.296-0.384	0.34	0.018	5.4	42/48	88 (75.3, 94.1)
KMT2D Exon 32	c.7061delC p.P2354Lfs*30	0.78-1.55	0.156-0.243	0.208	0.019	9.0	45/48	94 (83.2, 97.9)
KMT2D Exon 49	c.14710C>T p.R4904*	0.85-1.71	0.3-0.373	0.328	0.016	4.9	48/48	100 (92.6, 100)
KRAS Exon 2	c.35G>C p.G12A	1.03-2.52	0.023-0.061	0.039	0.008	20.4	44/46	96 (85.5, 98.8)
KRAS Exon 2	c.34G>T p.G12C	1.32-2.88	0.028-0.069	0.044	0.011	25.8	25/31	81 (63.7, 90.8)
KRAS Exon 2	c.38G>A p.G13D	1.32-2.88	0.281-0.354	0.307	0.018	6.0	31/31	100 (89.0, 100)
KRAS Exon 2	c.35G>T p.G12V	0.96-1.86	0.327-0.404	0.364	0.017	4.6	48/48	100 (92.6, 100)
MAX Exon 4	c.179G>A p.R60Q	0.86-2.06	0.286-0.369	0.325	0.021	6.4	48/48	100 (92.6, 100)
MLH3 Exon 3	c.3367C>T p.Q1123*	1.34-2.07	0.415-0.573	0.489	0.039	8.0	31/31	100 (89.0, 100)
MRE11 Exon 14	c.1563+1G>A	0.64-1.76	0.427-0.546	0.488	0.026	5.4	48/48	100 (92.6, 100)
NBN Exon 2	c.171+4T>C	0.76-1.44	0.419-0.538	0.474	0.025	5.3	46/46	100 (92.3, 100)
NOTCH1 Exon 26	c.4777C>A p.L1593M	0.10-2.37	0.295-0.484	0.361	0.037	10.4	48/48	100 (92.6, 100)
NOTCH3 Exon 4	c.348_349delCT p.D116Efs*45	0.71-1.90	0.328-0.46	0.388	0.028	7.3	47/48	98 (89.1, 99.6)
PIK3CA Exon 21	c.3140A>G p.H1047R	0.92-1.80	0.038-0.082	0.062	0.012	19.5	26/31	84 (67.4, 92.9)

Gene Exon	Mutation (cDNA/AA Change)	Normalized Coverage Rance	VAF Range	VAF Mean	SD	%CV	n/N	PCR (%) (95% CI)
PIK3CA Exon 21	c.3073A>G p.T1025A	0.93-1.58	0.194-0.273	0.233	0.016	6.7	48/48	100 (92.6, 100)
PIK3CA Exon 10	c.1624G>A p.E542K	0.25-1.60	0.502-0.608	0.564	0.018	3.2	48/48	100 (92.6, 100)
POLD1 Exon 17	c.2066G>A p.R689Q	0.10-1.01	0.253-0.356	0.297	0.028	9.5	48/48	100 (92.6, 100)
PPM1D Exon 6	c.1281G>A p.W427*	0.62-1.02	0.245-0.359	0.303	0.026	8.4	48/48	100 (92.6, 100)
PTEN Exon 5	c.389G>A p.R130Q	0.71-1.09	0.032-0.115	0.063	0.014	22.7	48/48	100 (92.6, 100)
PTEN Exon 7	c.741_742insA p.P248Tfs*5	0.48-0.77	0.074-0.149	0.109	0.017	15.9	48/48	100 (92.6, 100)
RAD51B Exon 6	c.453-7C>T	0.65-1.29	0.514-0.645	0.573	0.027	4.7	48/48	100 (92.6, 100)
RBM10 Exon 13	c.1444-2A>C	0.69-1.26	0.193-0.351	0.297	0.036	12.0	31/31	100 (89.0, 100)
RECQL4 Exon 10	c.1704+6G>T	2.00-3.67	0.274-0.364	0.318	0.020	6.4	48/48	100 (92.6, 100)
SETD2 Exon 20	c.7432-10C>T	0.92-1.31	0.103-0.205	0.144	0.020	14.1	48/48	100 (92.6, 100)
SOX9 Exon 3	c.1280_1281dup p.Y428Tfs*43	0.40-1.27	0.096-0.145	0.117	0.010	8.8	48/48	100 (92.6, 100)
TET2 Exon 3	c.1771C>T p.Q591*	0.42-1.16	0.051-0.084	0.07	0.008	11.7	47/48	98 (89.1, 99.6)
TGFBR2 Exon 6	c.1411G>A p.D471N	0.62-1.33	0.297-0.366	0.334	0.014	4.3	48/48	100 (92.6, 100)
TP53 Exon 6	c.614A>G p.Y205C	0.40-1.08	0.209-0.3	0.253	0.019	7.5	48/48	100 (92.6, 100)
TP53 Exon 7	c.742C>T p.R248W	0.59-1.14	0.437-0.537	0.492	0.022	4.5	48/48	100 (92.6, 100)
TP53 Exon 5	c.536A>G p.H179R	1.10-2.45	0.301-0.563	0.507	0.037	7.3	45/48	94 (83.2, 97.9)

Gene Exon	Mutation (cDNA/AA Change)	Normalized Coverage Rance	VAF Range	VAF Mean	SD	%CV	n/N	PCR (%) (95% CI)
TSC2 Exon 27	c.3131+2T>A	0.68-2.98	0.299-0.394	0.344	0.023	6.6	48/48	100 (92.6, 100

Appendix E. Accuracy

Appendix E.1 Concordance for SNV by Gene

Gene	Total Bases	Total Number of Variant per Gene	PPA (2-sided 95% CI)	NPA (2-sided 95% CI)
ABRAXAS1	1831	0	-	100% (100%, 100%)
ACVR2A	2610	0	-	100% (100%, 100%)
AKT1	1023	4	100% (51.01%, 100%)	100% (100%, 100%)
AMER1	3440	1	100% (20.65%, 100%)	100% (100%, 100%)
APC	10461	22	100% (81.57%, 100%)	100% (100%, 100%)
AR	1003	1	-	100% (100%, 100%)
ARHGAP35	4709	0	-	100% (100%, 100%)
ARID1A	8463	11	80% (37.55%, 96.38%)	100% (100%, 100%)
ARID1B	8590	2	100% (20.65%, 100%)	100% (100%, 100%)
ARID2	7469	10	100% (64.57%, 100%)	100% (100%, 100%)
ARID5B	4391	2	100% (34.24%, 100%)	100% (100%, 100%)
ASXL1	5582	0	-	100% (100%, 100%)
ASXL2	5476	1	-	100% (100%, 100%)
ATM	15017	19	85.71% (48.69%, 97.43%)	100% (100%, 100%)
ATR	12585	1	100% (20.65%, 100%)	100% (100%, 100%)
ATRX	11246	1	-	100% (100%, 100%)
AXIN1	3615	1	-	100% (100%, 100%)
AXL	1177	1	-	100% (100%, 100%)
B2M	769	4	100% (43.85%, 100%)	100% (100%, 100%)
BAP1	3550	1	100% (20.65%, 100%)	100% (100%, 100%)
BARD1	3430	0	-	100% (100%, 100%)
BCL6	1911	1	-	100% (100%, 100%)
BCOR	6319	1	-	100% (100%, 100%)
BRAF	953	4	100% (43.85%, 100%)	100% (100%, 100%)
BRCA1	9233	3	100% (34.24%, 100%)	100% (100%, 100%)
BRCA2	13279	6	100% (56.55%, 100%)	100% (100%, 100%)
BRIP1	5546	1	100% (20.65%, 100%)	100% (100%, 100%)
CARD11	1920	1	100% (20.65%, 100%)	100% (100%, 100%)
CASP8	2487	3	100% (34.24%, 100%)	100% (100%, 100%)
CDC73	3019	0	-	100% (100%, 100%)
CDH1	4202	4	100% (34.24%, 100%)	100% (100%, 100%)
CDK12	5966	1	-	100% (100%, 100%)
CDK4	1251	1	100% (20.65%, 100%)	100% (100%, 100%)
CDKN1A	1101	1	100% (20.65%, 100%)	100% (100%, 100%)
CDKN1B	770	2	100% (34.24%, 100%)	100% (100%, 100%)
CDKN2A	1416	6	80% (37.55%, 96.38%)	100% (100%, 100%)
CDKN2B	932	1	-	100% (100%, 100%)
CHEK1	2457	1	-	100% (100%, 100%)
CHEK2	3053	7	100% (43.85%, 100%)	100% (100%, 100%)
CIC	9290	4	100% (20.65%, 100%)	100% (100%, 100%)

Gene	Total Bases	Total Number of Variant per Gene	PPA (2-sided 95% CI)	NPA (2-sided 95% CI)
CREBBP	9902	4	100% (34.24%, 100%)	100% (100%, 100%)
CTCF	3086	3	100% (34.24%, 100%)	100% (100%, 100%)
CTNNB1	500	3	100% (43.85%, 100%)	100% (99.99%, 100%)
CUL3	4404	5	100% (34.24%, 100%)	100% (100%, 100%)
CYLD	4307	0	-	100% (100%, 100%)
DAXX	2919	1	-	100% (100%, 100%)
DDR2	893	2	-	100% (99.99%, 100%)
DDX3X	3080	3	100% (34.24%, 100%)	100% (100%, 100%)
DICER1	7883	4	100% (34.24%, 100%)	100% (100%, 100%)
DNMT3A	5341	6	100% (20.65%, 100%)	100% (100%, 100%)
EGFR	2022	9	100% (60.97%, 100%)	100% (100%, 100%)
ELF3	1686	0	-	100% (100%, 100%)
EP300	9915	5	100% (56.55%, 100%)	100% (100%, 100%)
ERBB2	3025	6	100% (51.01%, 100%)	100% (100%, 100%)
ERBB3	965	2	100% (34.24%, 100%)	100% (100%, 100%)
ERCC2	4456	7	100% (56.55%, 100%)	100% (100%, 100%)
ERCC4	3838	2	100% (20.65%, 100%)	100% (100%, 100%)
ETV6	1999	0	-	100% (100%, 100%)
FANCA	8471	6	100% (51.01%, 100%)	100% (100%, 100%)
FANCC	2965	0	-	100% (100%, 100%)
FANCD2	7785	2	100% (34.24%, 100%)	100% (100%, 100%)
FANCE	2595	2	100% (34.24%, 100%)	100% (100%, 100%)
FANCF	1239	0	-	100% (100%, 100%)
FANCG	3089	2	100% (34.24%, 100%)	100% (100%, 100%)
FANCI	7067	1	100% (20.65%, 100%)	100% (100%, 100%)
FANCL	2170	1	100% (20.65%, 100%)	100% (100%, 100%)
FAT1	16016	5	100% (56.55%, 100%)	100% (100%, 100%)
FBXW7	3767	7	100% (43.85%, 100%)	100% (100%, 100%)
FGF23	1080	1	-	100% (100%, 100%)
FGF3	955	1	100% (20.65%, 100%)	100% (100%, 100%)
FGFR3	1656	1	100% (20.65%, 100%)	100% (100%, 100%)
FGFR4	1463	1	100% (20.65%, 100%)	100% (100%, 100%)
FOXA1	920	2	100% (34.24%, 100%)	100% (100%, 100%)
FUBP1	3648	1	-	100% (100%, 100%)
GATA2	1018	1	100% (20.65%, 100%)	100% (100%, 100%)
GATA3	1902	1	100% (20.65%, 100%)	100% (100%, 100%)
GNAS	1121	4	100% (43.85%, 100%)	100% (100%, 100%)
HLA-A	1588	3	100% (43.85%, 100%)	100% (100%, 100%)
HLA-B	1567	3	-	100% (100%, 100%)
HNF1A	2744	2	100% (20.65%, 100%)	100% (100%, 100%)
HRAS	681	4	100% (43.85%, 100%)	100% (99.99%, 100%)
IDH1	125	3	100% (43.85%, 100%)	100% (99.98%, 100%)
INPP4B	4917	1	0% (0.0%, 79.35%)	100% (100%, 100%)

Gene	Total Bases	Total Number of Variant per Gene	PPA (2-sided 95% CI)	NPA (2-sided 95% CI)
JAK1	5570	2	100% (20.65%, 100%)	100% (100%, 100%)
JAK3	5374	1	-	100% (100%, 100%)
KDM5C	7047	1	100% (20.65%, 100%)	100% (100%, 100%)
KDM6A	7324	3	100% (20.65%, 100%)	100% (100%, 100%)
KDR	933	1	-	100% (100%, 100%)
KEAP1	2396	2	100% (20.65%, 100%)	100% (100%, 100%)
KEL	1963	1	0% (0.0%, 79.35%)	100% (100%, 100%)
KIT	2012	3	100% (43.85%, 100%)	100% (100%, 100%)
KMT2A	14698	2	100% (20.65%, 100%)	100% (100%, 100%)
KMT2B	11058	6	100% (56.55%, 100%)	100% (100%, 100%)
KMT2C	19770	3	100% (34.24%, 100%)	100% (100%, 100%)
KMT2D	20922	9	100% (67.56%, 100%)	100% (100%, 100%)
KRAS	929	30	96.43% (82.29%, 99.37%)	100% (99.99%, 100%)
LATS1	4133	1	100% (20.65%, 100%)	100% (100%, 100%)
LATS2	4047	1	100% (20.65%, 100%)	100% (100%, 100%)
MAP2K1	632	2	100% (34.24%, 100%)	100% (100%, 100%)
MAP3K1	6425	3	100% (34.24%, 100%)	100% (100%, 100%)
MAPK1	1000	1	100% (20.65%, 100%)	100% (100%, 100%)
MAX	1227	1	100% (20.65%, 100%)	100% (100%, 100%)
MEN1	2606	2	100% (20.65%, 100%)	100% (100%, 100%)
MET	1504	7	75.00% (30.06%, 95.44%)	100% (100%, 100%)
MLH1	5020	3	100% (20.65%, 100%)	100% (100%, 100%)
MLH3	5599	1	100% (20.65%, 100%)	100% (100%, 100%)
MPL	946	1	100% (20.65%, 100%)	100% (100%, 100%)
MRE11	3963	2	100% (20.65%, 100%)	100% (100%, 100%)
MSH2	4576	3	100% (20.65%, 100%)	100% (100%, 100%)
MSH3	5529	2	100% (34.24%, 100%)	100% (100%, 100%)
MSH6	5188	0	-	100% (100%, 100%)
MTAP	1674	0	-	100% (100%, 100%)
MUTYH	3016	1	-	100% (100%, 100%)
MYC	1154	1	100% (20.65%, 100%)	100% (100%, 100%)
NBN	3558	1	100% (20.65%, 100%)	100% (100%, 100%)
NCOR1	11641	0	-	100% (100%, 100%)
NF1	12882	11	100% (56.55%, 100%)	100% (100%, 100%)
NF2	3582	3	100% (34.24%, 100%)	100% (100%, 100%)
NFE2L2	936	3	100% (43.85%, 100%)	100% (100%, 100%)
NOTCH1	11747	5	100% (34.24%, 100%)	100% (100%, 100%)
NOTCH2	10611	3	100% (43.85%, 100%)	100% (100%, 100%)
NOTCH3	9364	5	100% (20.65%, 100%)	100% (100%, 100%)
NOTCH4	8205	2	100% (20.65%, 100%)	100% (100%, 100%)
NRAS	829	5	100% (56.55%, 100%)	100% (100%, 100%)
NTRK1	1442	3	100% (20.65%, 100%)	100% (100%, 100%)
PALB2	4460	3	100% (43.85%, 100%)	100% (100%, 100%)

Gene	Total Bases	Total Number of Variant per Gene	PPA (2-sided 95% CI)	NPA (2-sided 95% CI)
PARP1	5183	1	100% (20.65%, 100%)	100% (100%, 100%)
PBRM1	7314	2	100% (34.24%, 100%)	100% (100%, 100%)
PHF6	1804	1	100% (20.65%, 100%)	100% (100%, 100%)
PIK3CA	1750	29	100% (86.68%, 100%)	100% (100%, 100%)
PIK3R1	3282	2	100% (20.65%, 100%)	100% (100%, 100%)
PIK3R2	1441	0	-	100% (100%, 100%)
PMS1	2271	0	-	100% (100%, 100%)
PMS2	4100	10	100% (34.24%, 100%)	100% (100%, 100%)
POLD1	5875	1	-	100% (100%, 100%)
POLE	11804	0	-	100% (100%, 100%)
POT1	3363	0	-	100% (100%, 100%)
PPM1D	2382	1	-	100% (100%, 100%)
PPP2R1A	1277	2	100% (20.65%, 100%)	100% (100%, 100%)
PPP6C	974	1	100% (20.65%, 100%)	100% (100%, 100%)
PRDM1	3323	1	100% (20.65%, 100%)	100% (100%, 100%)
PTCH1	7082	0	-	100% (100%, 100%)
PTEN	2594	10	100% (70.09%, 100%)	100% (100%, 100%)
PTPN11	940	1	-	100% (100%, 100%)
RAD50	6073	2	100% (34.24%, 100%)	100% (100%, 100%)
RAD51B	2324	2	100% (34.24%, 100%)	100% (100%, 100%)
RAD51C	2038	1	100% (20.65%, 100%)	100% (100%, 100%)
RAD51D	1997	1	100% (20.65%, 100%)	100% (100%, 100%)
RAF1	1120	2	100% (20.65%, 100%)	100% (100%, 100%)
RASA1	5763	2	100% (34.24%, 100%)	100% (100%, 100%)
RB1	5388	2	100% (20.65%, 100%)	100% (100%, 100%)
RBM10	5150	5	100% (43.85%, 100%)	100% (100%, 100%)
RECQL4	2856	4	100% (43.85%, 100%)	100% (100%, 100%)
RET	1424	2	100% (34.24%, 100%)	100% (100%, 100%)
RHOA	162	5	100% (43.85%, 100%)	99.99% (99.97%, 100%)
RNF43	3231	0	-	100% (100%, 100%)
SDHA	3393	1	100% (20.65%, 100%)	100% (100%, 100%)
SDHC	981	0	-	100% (100%, 100%)
SETD2	9510	2	100% (34.24%, 100%)	100% (100%, 100%)
SF3B1	1083	2	-	100% (100%, 100%)
SLX4	6660	4	100% (34.24%, 100%)	100% (100%, 100%)
SMAD4	2846	4	100% (43.85%, 100%)	100% (100%, 100%)
SMARCA4	8148	6	100% (51.01%, 100%)	100% (100%, 100%)
SMARCB1	2129	2	-	100% (100%, 100%)
SOX9	1859	3	100% (34.24%, 100%)	100% (100%, 100%)
SPEN	12166	1	100% (20.65%, 100%)	100% (100%, 100%)
SPOP	916	1	-	100% (100%, 100%)
STAG2	6507	8	100% (20.65%, 100%)	100% (100%, 100%)
STAT3	1023	1	100% (20.65%, 100%)	100% (100%, 100%)

Gene	Total Bases	Total Number of Variant per Gene	PPA (2-sided 95% CI)	NPA (2-sided 95% CI)
STK11	2331	6	100% (43.85%, 100%)	100% (100%, 100%)
TBX3	2732	0	-	100% (100%, 100%)
TCF7L2	3618	1	100% (20.65%, 100%)	100% (100%, 100%)
TERT	1206	17	80% (54.81%, 92.95%)	100% (100%, 100%)
TET2	7164	1	-	100% (100%, 100%)
TGFBR2	2501	3	100% (34.24%, 100%)	100% (100%, 100%)
TNFAIP3	2903	1	100% (20.65%, 100%)	100% (100%, 100%)
TNFRSF14	1637	1	-	100% (100%, 100%)
TP53	2344	74	97.01% (89.75%, 99.18%)	100% (100%, 100%)
TP63	1397	1	100% (20.65%, 100%)	100% (100%, 100%)
TSC1	5349	2	100% (20.65%, 100%)	100% (100%, 100%)
TSC2	10092	13	100% (72.25%, 100%)	100% (100%, 100%)
U2AF1	1008	1	100% (20.65%, 100%)	100% (100%, 100%)
UGT1A1	146	4	100% (51.01%, 100%)	100% (99.98%, 100%)
VHL	889	1	-	100% (100%, 100%)
WT1	2792	2	100% (20.65%, 100%)	100% (100%, 100%)
XRCC2	1157	1	100% (20.65%, 100%)	100% (100%, 100%)
ZFHX3	10946	4	100% (43.85%, 100%)	100% (100%, 100%)

Appendix E.2. Concordance for Insertions by Gene

Gene	Total Bases	Total Number of Variant per Gene	PPA (2-sided 95% CI)	NPA (2-sided 95% CI)
ABRAXAS1	1831	0	-	100% (100%, 100%)
ACVR2A	2610	0	-	100% (100%, 100%)
AKT1	1023	0	-	100% (100%, 100%)
AMER1	3440	0	-	100% (100%, 100%)
APC	10461	6	80% (37.55%, 96.38%)	100% (100%, 100%)
AR	1003	0	-	100% (100%, 100%)
ARHGAP35	4709	1	-	100% (100%, 100%)
ARID1A	8463	1	-	100% (100%, 100%)
ARID1B	8590	0	-	100% (100%, 100%)
ARID2	7469	0	-	100% (100%, 100%)
ARID5B	4391	1	-	100% (100%, 100%)
ASXL1	5582	2	0% (0.0%, 65.76%)	100% (100%, 100%)
ASXL2	5476	0	-	100% (100%, 100%)
ATM	15017	10	-	100% (100%, 100%)
ATR	12585	0	-	100% (100%, 100%)
ATRX	11246	0	-	100% (100%, 100%)
AXIN1	3615	0	-	100% (100%, 100%)
AXL	1177	0	-	100% (100%, 100%)
B2M	769	0	-	100% (100%, 100%)
BAP1	3550	0	-	100% (100%, 100%)
BARD1	3430	1	0% (0.0%, 79.35%)	100% (100%, 100%)
BCL6	1911	0	-	100% (100%, 100%)
BCOR	6319	0	-	100% (100%, 100%)
BRAF	953	0	-	100% (100%, 100%)
BRCA1	9233	5	50% (9.45%, 90.55%)	100% (100%, 100%)
BRCA2	13279	2	100% (20.65%, 100%)	100% (100%, 100%)
BRIP1	5546	0	-	100% (100%, 100%)
CARD11	1920	0	-	100% (100%, 100%)
CASP8	2487	0	-	100% (100%, 100%)
CDC73	3019	1	-	100% (100%, 100%)
CDH1	4202	1	0% (0.0%, 79.35%)	100% (100%, 100%)
CDK12	5966	0	-	100% (100%, 100%)
CDK4	1251	0	-	100% (100%, 100%)
CDKN1A	1101	0	-	100% (100%, 100%)
CDKN1B	770	0	-	100% (100%, 100%)
CDKN2A	1416	0	-	100% (100%, 100%)
CDKN2B	932	0	-	100% (100%, 100%)
CHEK1	2457	0	-	100% (100%, 100%)
CHEK2	3053	0	-	100% (100%, 100%)
CIC	9290	1	-	100% (100%, 100%)
CREBBP	9902	0	-	100% (100%, 100%)
CTCF	3086	1	0% (0.0%, 79.35%)	100% (100%, 100%)

Gene	Total Bases	Total Number of Variant per Gene	PPA (2-sided 95% CI)	NPA (2-sided 95% CI)
CTNNB1	500	0	-	100% (99.99%, 100%)
CUL3	4404	0	-	100% (100%, 100%)
CYLD	4307	1	-	100% (100%, 100%)
DAXX	2919	0	-	100% (100%, 100%)
DDR2	893	0	-	100% (100%, 100%)
DDX3X	3080	0	-	100% (100%, 100%)
DICER1	7883	1	-	100% (100%, 100%)
DNMT3A	5341	2	-	100% (100%, 100%)
EGFR	2022	1	100% (20.65%, 100%)	100% (100%, 100%)
ELF3	1686	1	100% (20.65%, 100%)	100% (100%, 100%)
EP300	9915	0	-	100% (100%, 100%)
ERBB2	3025	1	100% (20.65%, 100%)	100% (100%, 100%)
ERBB3	965	0	-	100% (100%, 100%)
ERCC2	4456	0	-	100% (100%, 100%)
ERCC4	3838	0	-	100% (100%, 100%)
ETV6	1999	0	-	100% (100%, 100%)
FANCA	8471	0	-	100% (100%, 100%)
FANCC	2965	1	100% (20.65%, 100%)	100% (100%, 100%)
FANCD2	7785	0	-	100% (100%, 100%)
FANCE	2595	0	-	100% (100%, 100%)
FANCF	1239	0	-	100% (100%, 100%)
FANCG	3089	0	-	100% (100%, 100%)
FANCI	7067	0	-	100% (100%, 100%)
FANCL	2170	0	-	100% (100%, 100%)
FAT1	16016	1	100% (20.65%, 100%)	100% (100%, 100%)
FBXW7	3767	0	-	100% (100%, 100%)
FGF23	1080	0	-	100% (100%, 100%)
FGF3	955	0	-	100% (100%, 100%)
FGFR3	1656	0	-	100% (100%, 100%)
FGFR4	1463	0	-	100% (100%, 100%)
FOXA1	920	0	-	100% (100%, 100%)
FUBP1	3648	1	-	100% (100%, 100%)
GATA2	1018	0	-	100% (100%, 100%)
GATA3	1902	1	100% (20.65%, 100%)	100% (100%, 100%)
GNAS	1121	0	-	100% (100%, 100%)
HLA-A	1588	6	100% (20.65%, 100%)	100% (100%, 100%)
HLA-B	1567	1	-	100% (100%, 100%)
HNF1A	2744	1	-	100% (100%, 100%)
HRAS	681	0	-	100% (100%, 100%)
IDH1	125	0	-	100% (99.98%, 100%)
INPP4B	4917	1	-	100% (100%, 100%)
JAK1	5570	0	-	100% (100%, 100%)
JAK3	5374	0	-	100% (100%, 100%)

Gene	Total Bases	Total Number of Variant per Gene	PPA (2-sided 95% CI)	NPA (2-sided 95% CI)
KDM5C	7047	3	-	100% (100%, 100%)
KDM6A	7324	1	-	100% (100%, 100%)
KDR	933	0	-	100% (100%, 100%)
KEAP1	2396	0	-	100% (100%, 100%)
KEL	1963	0	-	100% (100%, 100%)
KIT	2012	0	-	100% (100%, 100%)
KMT2A	14698	1	100% (20.65%, 100%)	100% (100%, 100%)
KMT2B	11058	0	-	100% (100%, 100%)
KMT2C	19770	2	100% (20.65%, 100%)	100% (100%, 100%)
KMT2D	20922	1	100% (20.65%, 100%)	100% (100%, 100%)
KRAS	929	0	-	100% (100%, 100%)
LATS1	4133	2	-	100% (100%, 100%)
LATS2	4047	0	-	100% (100%, 100%)
MAP2K1	632	0	-	100% (100%, 100%)
MAP3K1	6425	5	100% (51.01%, 100%)	100% (100%, 100%)
MAPK1	1000	0	-	100% (100%, 100%)
MAX	1227	0	-	100% (100%, 100%)
MEN1	2606	0	-	100% (100%, 100%)
MET	1504	1	-	100% (100%, 100%)
MLH1	5020	0	-	100% (100%, 100%)
MLH3	5599	0	-	100% (100%, 100%)
MPL	946	0	-	100% (100%, 100%)
MRE11	3963	0	-	100% (100%, 100%)
MSH2	4576	1	-	100% (100%, 100%)
MSH3	5529	0	-	100% (100%, 100%)
MSH6	5188	1	-	100% (100%, 100%)
MTAP	1674	1	-	100% (100%, 100%)
MUTYH	3016	0	-	100% (100%, 100%)
MYC	1154	0	-	100% (100%, 100%)
NBN	3558	1	-	100% (100%, 100%)
NCOR1	11641	1	-	100% (100%, 100%)
NF1	12882	0	-	100% (100%, 100%)
NF2	3582	0	-	100% (100%, 100%)
NFE2L2	936	0	-	100% (100%, 100%)
NOTCH1	11747	1	100% (20.65%, 100%)	100% (100%, 100%)
NOTCH2	10611	1	-	100% (100%, 100%)
NOTCH3	9364	0	-	100% (100%, 100%)
NOTCH4	8205	1	100% (20.65%, 100%)	100% (100%, 100%)
NRAS	829	0	-	100% (100%, 100%)
NTRK1	1442	0	-	100% (100%, 100%)
PALB2	4460	3	0% (0.0%, 79.35%)	100% (100%, 100%)
PARP1	5183	1	-	100% (100%, 100%)
PBRM1	7314	1	100% (20.65%, 100%)	100% (100%, 100%)

Gene	Total Bases	Total Number of Variant per Gene	PPA (2-sided 95% CI)	NPA (2-sided 95% CI)
PHF6	1804	0	-	100% (100%, 100%)
PIK3CA	1750	0	-	100% (100%, 100%)
PIK3R1	3282	1	-	100% (100%, 100%)
PIK3R2	1441	0	-	100% (100%, 100%)
PMS1	2271	0	-	100% (100%, 100%)
PMS2	4100	1	0% (0.0%, 79.35%)	100% (100%, 100%)
POLD1	5875	0	-	100% (100%, 100%)
POLE	11804	0	-	100% (100%, 100%)
POT1	3363	2	-	100% (100%, 100%)
PPM1D	2382	0	-	100% (100%, 100%)
PPP2R1A	1277	0	-	100% (100%, 100%)
PPP6C	974	0	-	100% (100%, 100%)
PRDM1	3323	0	-	100% (100%, 100%)
PTCH1	7082	0	-	100% (100%, 100%)
PTEN	2594	2	100% (34.24%, 100%)	100% (100%, 100%)
PTPN11	940	0	-	100% (100%, 100%)
RAD50	6073	0	-	100% (100%, 100%)
RAD51B	2324	0	-	100% (100%, 100%)
RAD51C	2038	0	-	100% (100%, 100%)
RAD51D	1997	0	-	100% (100%, 100%)
RAF1	1120	0	-	100% (100%, 100%)
RASA1	5763	0	-	100% (100%, 100%)
RB1	5388	1	-	100% (100%, 100%)
RBM10	5150	0	-	100% (100%, 100%)
RECQL4	2856	0	-	100% (100%, 100%)
RET	1424	0	-	100% (100%, 100%)
RHOA	162	0	-	100% (99.98%, 100%)
RNF43	3231	0	-	100% (100%, 100%)
SDHA	3393	1	-	100% (100%, 100%)
SDHC	981	1	-	100% (100%, 100%)
SETD2	9510	1	-	100% (100%, 100%)
SF3B1	1083	0	-	100% (100%, 100%)
SLX4	6660	2	-	100% (100%, 100%)
SMAD4	2846	0	-	100% (100%, 100%)
SMARCA4	8148	0	-	100% (100%, 100%)
SMARCB1	2129	0	-	100% (100%, 100%)
SOX9	1859	1	100% (20.65%, 100%)	100% (100%, 100%)
SPEN	12166	0	-	100% (100%, 100%)
SPOP	916	0	-	100% (100%, 100%)
STAG2	6507	2	-	100% (100%, 100%)
STAT3	1023	0	-	100% (100%, 100%)
STK11	2331	0	-	100% (100%, 100%)
TBX3	2732	1	100% (20.65%, 100%)	100% (100%, 100%)

Gene	Total Bases	Total Number of Variant per Gene	PPA (2-sided 95% CI)	NPA (2-sided 95% CI)
TCF7L2	3618	0	-	100% (100%, 100%)
TERT	1206	0	-	100% (100%, 100%)
TET2	7164	1	-	100% (100%, 100%)
TGFBR2	2501	1	100% (20.65%, 100%)	100% (100%, 100%)
TNFAIP3	2903	0	-	100% (100%, 100%)
TNFRSF14	1637	0	-	100% (100%, 100%)
TP53	2344	4	100% (43.85%, 100%)	100% (100%, 100%)
TP63	1397	0	-	100% (100%, 100%)
TSC1	5349	1	-	100% (100%, 100%)
TSC2	10092	0	-	100% (100%, 100%)
U2AF1	1008	0	-	100% (100%, 100%)
UGT1A1	146	0	-	100% (99.98%, 100%)
VHL	889	0	-	100% (100%, 100%)
WT1	2792	1	-	100% (100%, 100%)
XRCC2	1157	0	-	100% (100%, 100%)
ZFHX3	10946	1	-	100% (100%, 100%)

Appendix E.3. Concordance for Deletions by Gene

Gene	Total Bases	Total Number of Variant per Gene	PPA (2-sided 95% CI)	NPA (2-sided 95% CI)
ABRAXAS1	1831	1	-	100% (100%, 100%)
ACVR2A	2610	2	-	100% (100%, 100%)
AKT1	1023	0	-	100% (100%, 100%)
AMER1	3440	0	-	100% (100%, 100%)
APC	10461	8	80% (37.55%, 96.38%)	100% (100%, 100%)
AR	1003	0	-	100% (100%, 100%)
ARHGAP35	4709	1	-	100% (100%, 100%)
ARID1A	8463	2	100% (34.24%, 100%)	100% (100%, 100%)
ARID1B	8590	1	100% (20.65%, 100%)	100% (100%, 100%)
ARID2	7469	2	100% (34.24%, 100%)	100% (100%, 100%)
ARID5B	4391	2	100% (20.65%, 100%)	100% (100%, 100%)
ASXL1	5582	0	-	100% (100%, 100%)
ASXL2	5476	2	100% (20.65%, 100%)	100% (100%, 100%)
ATM	15017	4	100% (20.65%, 100%)	100% (100%, 100%)
ATR	12585	0	-	100% (100%, 100%)
ATRX	11246	1	100% (20.65%, 100%)	100% (100%, 100%)
AXIN1	3615	2	-	100% (100%, 100%)
AXL	1177	0	-	100% (100%, 100%)
B2M	769	6	100% (51.01%, 100%)	100% (99.99%, 100%)
BAP1	3550	0	-	100% (100%, 100%)
BARD1	3430	2	100% (20.65%, 100%)	100% (100%, 100%)
BCL6	1911	0	-	100% (100%, 100%)
BCOR	6319	3	0% (0.0%, 79.35%)	100% (100%, 100%)
BRAF	953	0	-	100% (100%, 100%)
BRCA1	9233	1	100% (20.65%, 100%)	100% (100%, 100%)
BRCA2	13279	1	100% (20.65%, 100%)	100% (100%, 100%)
BRIP1	5546	0	-	100% (100%, 100%)
CARD11	1920	0	-	100% (100%, 100%)
CASP8	2487	0	-	100% (100%, 100%)
CDC73	3019	0	-	100% (100%, 100%)
CDH1	4202	2	100% (34.24%, 100%)	100% (100%, 100%)
CDK12	5966	1	100% (20.65%, 100%)	100% (100%, 100%)
CDK4	1251	0	-	100% (100%, 100%)
CDKN1A	1101	0	-	100% (100%, 100%)
CDKN1B	770	0	-	100% (100%, 100%)
CDKN2A	1416	3	100% (34.24%, 100%)	100% (100%, 100%)
CDKN2B	932	0	-	100% (100%, 100%)
CHEK1	2457	0	-	100% (100%, 100%)
CHEK2	3053	0	-	100% (100%, 100%)
CIC	9290	3	100% (43.85%, 100%)	100% (100%, 100%)
CREBBP	9902	1	100% (20.65%, 100%)	100% (100%, 100%)
CTCF	3086	0	-	100% (100%, 100%)

Gene	Total Bases	Total Number of Variant per Gene	PPA (2-sided 95% CI)	NPA (2-sided 95% CI)
CTNNB1	500	0	-	100% (99.99%, 100%)
CUL3	4404	1	-	100% (100%, 100%)
CYLD	4307	0	-	100% (100%, 100%)
DAXX	2919	0	-	100% (100%, 100%)
DDR2	893	0	-	100% (100%, 100%)
DDX3X	3080	2	-	100% (100%, 100%)
DICER1	7883	0	-	100% (100%, 100%)
DNMT3A	5341	0	-	100% (100%, 100%)
EGFR	2022	2	100% (34.24%, 100%)	100% (100%, 100%)
ELF3	1686	0	-	100% (100%, 100%)
EP300	9915	1	-	100% (100%, 100%)
ERBB2	3025	0	-	100% (100%, 100%)
ERBB3	965	0	-	100% (100%, 100%)
ERCC2	4456	0	-	100% (100%, 100%)
ERCC4	3838	0	-	100% (100%, 100%)
ETV6	1999	1	-	100% (100%, 100%)
FANCA	8471	1	-	100% (100%, 100%)
FANCC	2965	0	-	100% (100%, 100%)
FANCD2	7785	0	-	100% (100%, 100%)
FANCE	2595	0	-	100% (100%, 100%)
FANCF	1239	1	100% (20.65%, 100%)	100% (100%, 100%)
FANCG	3089	0	-	100% (100%, 100%)
FANCI	7067	0	-	100% (100%, 100%)
FANCL	2170	0	-	100% (100%, 100%)
FAT1	16016	0	-	100% (100%, 100%)
FBXW7	3767	0	-	100% (100%, 100%)
FGF23	1080	0	-	100% (100%, 100%)
FGF3	955	0	-	100% (100%, 100%)
FGFR3	1656	0	-	100% (100%, 100%)
FGFR4	1463	0	-	100% (100%, 100%)
FOXA1	920	0	-	100% (100%, 100%)
FUBP1	3648	1	-	100% (100%, 100%)
GATA2	1018	0	-	100% (100%, 100%)
GATA3	1902	1	-	100% (100%, 100%)
GNAS	1121	0	-	100% (100%, 100%)
HLA-A	1588	0	-	100% (100%, 100%)
HLA-B	1567	2	-	100% (100%, 100%)
HNF1A	2744	3	0% (0.0%, 79.35%)	100% (100%, 100%)
HRAS	681	0	-	100% (100%, 100%)
IDH1	125	0	-	100% (99.98%, 100%)
INPP4B	4917	1	-	100% (100%, 100%)
JAK1	5570	0	-	100% (100%, 100%)
JAK3	5374	0	-	100% (100%, 100%)

Gene	Total Bases	Total Number of Variant per Gene	PPA (2-sided 95% CI)	NPA (2-sided 95% CI)
KDM5C	7047	0	-	100% (100%, 100%)
KDM6A	7324	2	100% (20.65%, 100%)	100% (100%, 100%)
KDR	933	0	-	100% (100%, 100%)
KEAP1	2396	0	-	100% (100%, 100%)
KEL	1963	0	-	100% (100%, 100%)
KIT	2012	3	100% (43.85%, 100%)	100% (100%, 100%)
KMT2A	14698	1	-	100% (100%, 100%)
KMT2B	11058	0	-	100% (100%, 100%)
KMT2C	19770	4	100% (43.85%, 100%)	100% (100%, 100%)
KMT2D	20922	3	-	100% (100%, 100%)
KRAS	929	0	-	100% (100%, 100%)
LATS1	4133	0	-	100% (100%, 100%)
LATS2	4047	0	-	100% (100%, 100%)
MAP2K1	632	0	-	100% (100%, 100%)
MAP3K1	6425	1	-	100% (100%, 100%)
MAPK1	1000	0	-	100% (100%, 100%)
MAX	1227	0	-	100% (100%, 100%)
MEN1	2606	0	-	100% (100%, 100%)
MET	1504	0	-	100% (100%, 100%)
MLH1	5020	0	-	100% (100%, 100%)
MLH3	5599	1	-	100% (100%, 100%)
MPL	946	0	-	100% (100%, 100%)
MRE11	3963	0	-	100% (100%, 100%)
MSH2	4576	0	-	100% (100%, 100%)
MSH3	5529	2	100% (20.65%, 100%)	100% (100%, 100%)
MSH6	5188	3	0% (0.0%, 79.35%)	100% (100%, 100%)
MTAP	1674	0	-	100% (100%, 100%)
MUTYH	3016	0	-	100% (100%, 100%)
MYC	1154	0	-	100% (100%, 100%)
NBN	3558	0	-	100% (100%, 100%)
NCOR1	11641	1	100% (20.65%, 100%)	100% (100%, 100%)
NF1	12882	12	100% (43.85%, 100%)	100% (100%, 100%)
NF2	3582	0	-	100% (100%, 100%)
NFE2L2	936	0	-	100% (100%, 100%)
NOTCH1	11747	8	100% (34.24%, 100%)	100% (100%, 100%)
NOTCH2	10611	1	-	100% (100%, 100%)
NOTCH3	9364	1	100% (20.65%, 100%)	100% (100%, 100%)
NOTCH4	8205	2	-	100% (100%, 100%)
NRAS	829	0	-	100% (100%, 100%)
NTRK1	1442	0	-	100% (100%, 100%)
PALB2	4460	2	50% (9.45%, 90.55%)	100% (100%, 100%)
PARP1	5183	0	-	100% (100%, 100%)
PBRM1	7314	1	-	100% (100%, 100%)

Gene	Total Bases	Total Number of Variant per Gene	PPA (2-sided 95% CI)	NPA (2-sided 95% CI)
PHF6	1804	1	100% (20.65%, 100%)	100% (100%, 100%)
PIK3CA	1750	3	100% (34.24%, 100%)	100% (100%, 100%)
PIK3R1	3282	4	100% (51.01%, 100%)	100% (100%, 100%)
PIK3R2	1441	1	100% (20.65%, 100%)	100% (100%, 100%)
PMS1	2271	1	0% (0.0%, 79.35%)	100% (100%, 100%)
PMS2	4100	0	-	100% (100%, 100%)
POLD1	5875	0	-	100% (100%, 100%)
POLE	11804	1	-	100% (100%, 100%)
POT1	3363	2	-	100% (100%, 100%)
PPM1D	2382	0	-	100% (100%, 100%)
PPP2R1A	1277	0	-	100% (100%, 100%)
PPP6C	974	0	-	100% (100%, 100%)
PRDM1	3323	0	-	100% (100%, 100%)
PTCH1	7082	2	-	100% (100%, 100%)
PTEN	2594	3	100% (43.85%, 100%)	100% (100%, 100%)
PTPN11	940	0	-	100% (100%, 100%)
RAD50	6073	3	100% (20.65%, 100%)	100% (100%, 100%)
RAD51B	2324	0	-	100% (100%, 100%)
RAD51C	2038	0	-	100% (100%, 100%)
RAD51D	1997	0	-	100% (100%, 100%)
RAF1	1120	0	-	100% (100%, 100%)
RASA1	5763	0	-	100% (100%, 100%)
RB1	5388	2	100% (20.65%, 100%)	100% (100%, 100%)
RBM10	5150	0	-	100% (100%, 100%)
RECQL4	2856	0	-	100% (100%, 100%)
RET	1424	0	-	100% (100%, 100%)
RHOA	162	0	-	100% (99.98%, 100%)
RNF43	3231	2	-	100% (100%, 100%)
SDHA	3393	0	-	100% (100%, 100%)
SDHC	981	0	-	100% (100%, 100%)
SETD2	9510	2	100% (34.24%, 100%)	100% (100%, 100%)
SF3B1	1083	0	-	100% (100%, 100%)
SLX4	6660	2	-	100% (100%, 100%)
SMAD4	2846	0	-	100% (100%, 100%)
SMARCA4	8148	1	100% (20.65%, 100%)	100% (100%, 100%)
SMARCB1	2129	0	-	100% (100%, 100%)
SOX9	1859	4	100% (20.65%, 100%)	100% (100%, 100%)
SPEN	12166	2	-	100% (100%, 100%)
SPOP	916	0	-	100% (100%, 100%)
STAG2	6507	2	-	100% (100%, 100%)
STAT3	1023	0	-	100% (100%, 100%)
STK11	2331	2	100% (34.24%, 100%)	100% (100%, 100%)
TBX3	2732	0	-	100% (100%, 100%)

Gene	Total Bases	Total Number of Variant per Gene	PPA (2-sided 95% CI)	NPA (2-sided 95% CI)
TCF7L2	3618	0	-	100% (100%, 100%)
TERT	1206	1	-	100% (100%, 100%)
TET2	7164	2	100% (20.65%, 100%)	100% (100%, 100%)
TGFBR2	2501	0	-	100% (100%, 100%)
TNFAIP3	2903	0	-	100% (100%, 100%)
TNFRSF14	1637	0	-	100% (100%, 100%)
TP53	2344	11	100% (70.09%, 100%)	100% (100%, 100%)
TP63	1397	0	-	100% (100%, 100%)
TSC1	5349	0	-	100% (100%, 100%)
TSC2	10092	0	-	100% (100%, 100%)
U2AF1	1008	0	-	100% (100%, 100%)
UGT1A1	146	0	-	100% (99.98%, 100%)
VHL	889	1	-	100% (100%, 100%)
WT1	2792	0	-	100% (100%, 100%)
XRCC2	1157	0	-	100% (100%, 100%)
ZFHX3	10946	2	-	100% (100%, 100%)