SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Next generation sequencing oncology panel, somatic or germline variant detection system
Device Trade Name:	TruSight™ Oncology Comprehensive
Device Procode:	PQP
Applicant's Name and Address:	Illumina Inc., 5200 Illumina Way, San Diego, CA 92122
Date(s) of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P230011
Date of FDA Notice of Approval:	August 21st, 2024

Breakthrough Device: Granted breakthrough device status [previously Expedited Access Pathway (EAP)] on January 17, 2019.

II. INDICATIONS FOR USE

TruSight Oncology Comprehensive is a qualitative in vitro diagnostic test that uses targeted next-generation sequencing to detect variants in 517 genes using nucleic acids extracted from formalin-fixed, paraffin embedded (FFPE) tumor tissue samples from cancer patients with solid malignant neoplasms using the Illumina® NextSeq™ 550Dx instrument. The test can be used to detect single nucleotide variants, multi- nucleotide variants, insertions, and deletions from DNA, and fusions in 24 genes and splice variants in one gene from RNA. The test also reports a Tumor Mutational Burden (TMB) score.

The test is intended to be used as a companion diagnostic to identify cancer patients who may benefit from treatment with the targeted therapies listed in Table 1, in accordance with the approved therapeutic product labeling.

In addition, the test is intended to provide tumor profiling information for use by qualified health care professionals in accordance with professional guidelines in oncology for patients with solid malignant neoplasms. Genomic findings other than those listed in Table 1 of the intended use statement are not conclusive or prescriptive for labeled use of any specific therapeutic product.

Table 1. Companion Diagnostic Indications

Tumor Type	Biomarker(s) Detected	Therapy
Solid Tumors	*NTRK*1/2/3 fusions	VITRAKVI® (larotrectinib)
Non-Small Cell Lung Cancer (NSCLC)	*RET* fusions	RETEVMO® (selpercatinib)

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the TruSight Oncology Comprehensive labeling.

V. DEVICE DESCRIPTION

TruSight Oncology (TSO) Comprehensive is a distributed *in vitro* diagnostic (IVD) test. The assay is for use as part of a test system with the NextSeq 550Dx instrument and sequencing reagents. The TSO Comprehensive assay contains reagents, software, and procedures for testing DNA and RNA extracted from formalin-fixed, paraffin-embedded (FFPE) tumor samples.

The assay employs qualitative next-generation sequencing (NGS) comprehensive genomic profiling (CGP) that assesses genomic variants in a large panel of cancer-related genes listed in Appendix 1. It is an enrichment based targeted NGS test comprised of library preparation and enrichment reagents to enable DNA and/or RNA sequencing from FFPE tissue from solid tumors encompassing multiple cancer types. DNA and/or RNA extracted from FFPE tissue is used to prepare libraries, which are then enriched for cancer-related genes and sequenced on the NextSeq 550Dx instrument. TSO Comprehensive user facing software allows sequencing run set up, secondary analysis, and annotation of detected variants from the sequencing results generated on the NextSeq 550Dx instrument.

TSO Comprehensive can be run with DNA and RNA, DNA only, or RNA only samples. The reported results will be reflective of the input nucleic acid.

The test detects single nucleotide variants (SNVs), multi-nucleotide variants (MNVs) and insertions and deletions from DNA, and gene fusions and a splice variant from RNA. The assay detects small DNA variants in 517 genes, RNA fusions in 24 genes and RNA splice variants in one gene. The TSO Comprehensive assay also reports a Tumor Mutational Burden (TMB) score.

Test Output

The output of the test includes results from the following levels:

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

Genomic findings other than those listed in Table 1 of the Companion diagnostic indications table of the intended use statement (i.e., Levels 2 and 3) are not prescriptive or conclusive for labeled use of any specific therapeutic product.

Test Kit Contents

The TSO Comprehensive assay is for use as part of a test system with the NextSeq 550Dx instrument and associated sequencing reagents. Components of the TSO Comprehensive assay are listed in Table 2. Illumina provides reagent kits and software (Local Run Manager TSO Comprehensive (US) analysis module), which supports both tumor profiling and companion diagnostic (CDx) claims (please refer to the "Software" section below for additional details). The Knowledge Base (updated monthly) is also required to be used with the assay for tumor profiling and is available for download on the Illumina Lighthouse Portal. The assay contains reagents with sufficient volume to generate 24 DNA and 24 RNA libraries inclusive of patient samples and controls (sold separately). Materials required but not provided are described in the text below Table 2. A detailed list of required instruments, software, reagents, consumables, and storage conditions is further described in the product labeling (TSO Comprehensive Package Insert).

Table 2. Reagent Components of the TSO Comprehensive Assay

Storage Temp (ºC)	Component Name	Volume (uL)	Quantity
TSO Comprehensive RNA Library Prep			
-25 to -15	First Strand Synthesis Mix (FSM)	260	1
-25 to -15	Second Strand Mix (SSM)	720	1
-25 to -15	Elution Primer Frag Mix (EPH3)	250	1
-25 to -15	Reverse Transcriptase (RVT)	70	1
TSO Comprehensive Library Prep			
-25 to -15	End Repair A-tailing A (ERA1-A)	85	2
-25 to -15	End Repair A-tailing B (ERA1-B)	210	2
-25 to -15	Adapter Ligation Buffer 1 (ALB1)	1730	2
-25 to -15	DNA Ligase 3 (LIG3)	190	2
-25 to -15	Short Universal Adapters 1 (SUA1)	290	1
-25 to -15	UMI Adapters v1 (UMI)	290	1
-25 to -15	Stop Ligation Buffer (STL)	480	2
-25 to -15	Enhanced PCR Mix (EPM)	550	2
2 to 8	Resuspension Buffer (RSB)	12400	1
2 to 8	Sample Purification Beads (SPB)	6110	2
2 to 8	TE Buffer (TEB)	10000	1
TSO Comprehensive Index Primers			
-25 to -15	UP Index Primers	24	16
-25 to -15	CP Index Primers	20	16

Storage Temp (ºC)	Component Name	Volume (uL)	Quantity
TSO Comprehensive Enrichment			
2 to 8	Target Capture Buffer 1 (TCB1)	870	2
2 to 8	Streptavidin Mag Beads (SMB)	7780	2
2 to 8	2N NaOH (HP3)	400	2
2 to 8	Elute Target Buffer 2 (ET2)	290	2
2 to 8	Library Normalization Beads 1 (LNB1)	1040	1
2 to 8	Library Normalization Wash 1 (LNW1)	4800	2
2 to 8	Library Normalization Storage Buffer 1 (LNS1)	3500	2
2 to 8	Resuspension Buffer (RSB)	12400	1
2 to 8	Sample Purification Beads (SPB)	6110	2
-25 to -15	Target Capture Additives 1 (TCA1)	521	2
-25 to -15	Enhanced Enrichment Wash (EEW)	50400	1
-25 to -15	Enrichment Elution 2 (EE2)	1650	3
-25 to -15	Enhanced PCR Mix (EPM)	550	2
-25 to -15	PCR Primer Cocktail 3 (PPC3)	150	2
-25 to -15	Library Normalization Additives 1 (LNA1)	4600	1
-25 to -15	PhiX Internal Control (PX3 or PhiX)	10	1
TSO Comprehensive Content Set			
-25 to -15	Oncology RNA Probe Pool (OPR1)	290	1
-25 to -15	Oncology DNA Probe Pool 2 (OPD2)	290	1

Reagents/Consumables Required but not Provided

For a detailed list of required, but not provided reagents and consumables, please refer to the product labeling (TSO Comprehensive Package Insert).

- DNA/RNA Extraction and Purification Reagents
- DNA/RNA Quantification Reagents
- TruSight Oncology DNA Control
- TruSight Oncology RNA Control
- Ethanol (EtOH) 100% (200 proof), molecular biology grade
- RNase/DNase-free water
- NextSeq 550Dx High-Output Reagent Kit v2.5 (300 cycles)

Required Equipment/Materials, Not Provided

For a detailed list of required, but not provided equipment, please refer to the product labeling (TSO Comprehensive Package Insert).

- NextSeq 550Dx Instrument
- Ultrasonicator
- Thermal cycler
- Vortexer
- Microsample incubators
- Microcentrifuge

- Plate Centrifuge
- Plate shaker
- Sealing wedge or roller
- Magnetic Stand
- Precision pipettes
- Pipette-aid
- Ice or cold block
- 10 mL serological pipettes
- Adhesive seals for 96-well plates
- Microcentrifuge tubes (1.7mL and 2mL), nuclease-free
- Nuclease-free reagent reservoirs
- 15mL and 50mL conical tubes
- Aerosol-resistant pipette tips
- 96-well storage plates, 0.8 mL
- 96-well PCR plates compatible with thermal cycler, 0.2 ml (polypropylene wells)
- Dry heat block

Software

The Local Run Manager TSO Comprehensive (US) analysis module resides on the NextSeq 550Dx instrument as part of the Local Run Manager software to facilitate TSO Comprehensive assay run setup and to perform the secondary analysis of sequencing results. It contains the following components:

- TSO Comprehensive Claims Packages
- TSO Comprehensive Software Suite
- TSO Comprehensive USB Kit

Knowledge Base: Updated regularly and available for download on the Illumina Lighthouse Portal.

Instruments

TSO Comprehensive assay is validated for use on the Illumina NextSeq 550Dx instrument as part of a test system. An Illumina service representative installs the appropriate version of the TSO Comprehensive (US) analysis module on the Local Run Manager NextSeq 550Dx instrument, including the Knowledge Base, prior to use of the TSO Comprehensive assay and conducts training for the end user. Other required equipment and the specifications for the specific equipment for use with the TSO Comprehensive assay are described in the "Required Equipment/Materials, Not Provided" section.

Principles of Operation

The TSO Comprehensive assay involves the processes as described below.

Specimen Requirements, Collection, and Preparation

The TSO Comprehensive assay requires nucleic acid (DNA and/or RNA) isolated from FFPE tissue specimens. Before performing the TSO Comprehensive assay, tissue samples should be examined by a pathologist to ensure that it is appropriate for this test. Tissue cannot be decalcified and should be fixed using formalin fixative suitable for molecular

analyses (for example, 10% neutral-buffered formalin). Recommended tissue volume is ≥ 1.0 mm^3, which is equivalent to a cumulative viable tissue area (the sum of the viable tissue area in all sections submitted for extraction) of ≥ 200 mm^2 using 5 µm thick sections, or ≥ 100 mm^2 using 10 µm thick sections. Tumor content for RNA variants depends on the extent of expression. A minimum of 20% tumor content (by area) is required to detect somatic driver mutations. A high amount of necrotic tissue (≥25%) can interfere with the TSO Comprehensive assay's ability to detect RNA fusions. If sample sections contain more than 25% necrosis in total tissue area, then the necrotic tissue must be macrodissected.

Nucleic Acid Extraction

The TSO Comprehensive assay requires nucleic acid (DNA and/or RNA) isolated from FFPE tissue using appropriate extraction methods. The required input for the TSO Comprehensive assay is 40 ng RNA and/or 40 ng DNA. For DNA, extraction kits should be able to yield this input amount at a minimum concentration of 3.33 ng/uL. Shearing requires a final volume of 52 µl (0.77 ng/µl) with a minimum of 40 µl TEB (provided) used as the diluent. For RNA, extraction kits should be able to yield this input amount in a final volume of 8.5 uL (minimum concentration of 4.7 ng/uL). Proteinase K or equivalent enzyme should not be increased during extraction from the standard concentration provided (interference was tested using 0.04 mg/mL, as well as 2.6 mg/mL and 5.2 mg/mL, 2 times and 4 times the excess from a concentration of 1.3 mg/mL per assay reaction, respectively). This assay has been validated with extracted DNA stored at -25°C to -15°C for up to 28 days, and extracted RNA stored at -85°C to -65°C for up to 28 days.

Library Preparation

Library preparation is performed using the TSO Comprehensive Library Prep Kit (PN 20031118, PN 20031119) for DNA and the RNA Library Prep Kit (PN 20031127) for RNA. For RNA, 40 ng total is converted to double-stranded complementary DNA (cDNA). For genomic DNA (gDNA), 40 ng of gDNA is sheared into small fragments. Universal adapters for sequencing are ligated onto the cDNA and gDNA fragments. P5 and P7 adapter sequences are incorporated into each library to enable the capture of library fragments onto the surface of the flow cell during sequencing. The adapters include i5 and i7 index sequences to identify each individual sample and, in the case of libraries from gDNA samples, individual molecules with the use of Unique Molecular Identifiers (UMIs).

Enrichment

Resultant libraries are enriched for specific genes of interest using a capture-based method. Biotinylated probe sequences that span gene regions of interest targeted by the assay are hybridized to the libraries. The probes and hybridized targeted libraries are isolated from non- targeted libraries by capture with streptavidin magnetic particles. The targeted enriched libraries are washed and amplified. The quantity of each enriched library is normalized using a bead-based method to ensure equal representation in the pooled libraries for sequencing.

Sequencing

Normalized, enriched libraries are pooled and clustered onto a flow cell, and then sequenced using sequencing by synthesis (SBS) chemistry on the NextSeq 550Dx instrument. SBS chemistry uses a reversible terminator method to detect single, fluorescently labeled deoxynucleotide triphosphate (dNTP) bases as they are incorporated into growing DNA strands. During each sequencing cycle, a single dNTP is added to the nucleic acid chain. The dNTP label serves as a terminator for polymerization. After each dNTP incorporation, the fluorescent dye is imaged to identify the base, and then cleaved to allow incorporation of the next nucleotide. Four reversible terminator-bound dNTPs (A, G, T, and C) are present as single, separate molecules. As a result, natural competition minimizes incorporation bias.

Data Analysis

Primary Analysis
During the primary analysis, base calls are made directly from signal intensity measurements during each sequencing cycle, resulting in base-by-base sequencing. A quality score is assigned to each base call, and the sequencing data is stored in binary base call (BCL) format.

Secondary Analysis
Secondary analysis includes the following sequential steps:

- Validation of run processing and quality control
  Sequencing run quality metrics are evaluated to determine if they are within an acceptable range. Please refer to Table 3 for additional information about run-level quality metrics.

- FASTQ File Generation
  Sequencing data stored in BCL format is demultiplexed using index sequences unique to each sample added during the library preparation step to assign clusters to the library from which they originated. Each cluster contains two indexes (i5 and i7 sequences, one at each end of the library fragment). The combination of those index sequences is used to demultiplex the pooled libraries. After demultiplexing, FASTQ files are generated, which are files that contain the sequencing reads for each individual sample library and the associated quality scores for each base call, excluding reads from any clusters that did not pass filter.

- Alignment and Error Correction
  Sequencing reads derived from DNA sample libraries are aligned to a reference genome using the Burrows-Wheeler Aligner (BWA-MEM) to align DNA sequences to the hg19 reference genome, generating BAM files (*.bam), and BAM index files (*.bam.bai). These files are further processed to remove errors (including errors introduced during PCR amplification or sequencing). Reads derived from the same unique DNA molecule are collapsed into a single representative sequence using their unique molecular identifier (UMI). Indel realignment is performed to recover signal for insertions and deletions that may have been lost during initial alignment.

Simultaneously, overlapping read pairs are bioinformatically combined into a single consensus read. All reads are then output as a final set of BAM files with corresponding BAM index files.

Sequencing reads derived from RNA sample libraries are downsampled to approximately 30 million reads per RNA sample library by randomly selecting reads from the input FASTQ files following a probability distribution. The ends of the RNA sequences are then trimmed to a maximum length of 76 base pairs and are aligned to the hg19 reference genome. Candidate splice junctions are identified, generating BAM files and BAM index files for aligned reads, and a tab-delimited text file for candidate splice junctions, using STAR aligner. Duplicate reads are then marked in the BAM files so they can be excluded from downstream steps.

- Variant Calling

  Small variant calling is performed for DNA sample libraries to detect small variants [single-nucleotide variants (SNVs), multi-nucleotide variants (MNVs) up to 3 base pairs (bp) in length, insertions, and deletions. TSO Comprehensive performance has been demonstrated for insertions up to 24 base pairs and deletions up to 25 base pairs. The error-corrected BAM files (collapsed and insertions and deletions realigned) are used as input by an initial variant calling algorithm to detect small variants. This creates an unfiltered genome Variant Call Format (gVCF) file, which contain reference or variant calls for each targeted locus. Candidate variants are then filtered for assay-specific and sample processing artifacts by using adjusted quality scores calculated by comparing the observed variant frequency against a baseline noise distribution for the same site (assay-specific artifacts) and by comparing the evidence for the candidate variant against the baseline level of noise associated with that base change in the sample (sample-specific artifacts). This results in filtered gVCF files, which then uses a phased variant caller to identify MNVs, indels, and deletions in the EGFR and RET genes that are candidates for phasing. Overlapping reads are clustered in the neighborhood into a minimal set of clusters that contain the same variants. Variants are detected by examining the Concise Idiosyncratic Gapped Alignment Report (CIGAR) strings in the BAM file and comparing read sequences to the reference genome sequence. Finally, MNVs, indels, and deletions detected by the phased variant caller are merged into the filtered gVCF files resulting in merged gVCF files.

  Fusion calling and splice variant calling are performed for RNA sample libraries. For fusion calling, candidate fusions are identified from supplemental alignments and anomalous read pairs (reads aligning to different chromosomes or in unexpected orientations) in the BAM files for the fusion genes targeted by the TSO Comprehensive assay. These reads are assembled into candidate fusion contigs, which are then aligned back to the reference genome (hg19). These candidate fusion contigs are then evaluated against various filters before being reported as detected (please refer to 'RNARNA Fusion Calling' in the Local Run Manager TruSight Oncology Comprehensive (US) analysis module Workflow Guide for additional information). For splice variant calling, candidate splice variants (junctions) from the alignment stage are compared against a database of known transcripts and a splice variant baseline of non-tumor junctions. Any splice variants that match the database or

baseline are filtered out unless they are in a set of junctions with known oncological function. If there is sufficient read support, the candidate splice variant is kept.

Tertiary Analysis

Tertiary analysis, performed by the Local Run Manager TSO Comprehensive (US) analysis module, consists of annotation of the different variant types followed by the calling/score calculation specific to the intended variants.

- Annotation

  Detected small DNA variants are annotated using the Nirvana annotation engine with information from the RefSeq database and various population databases (COSMIC, ClinVar, dbSNP, 1000 Genomes, and gnomAD). For TMB, using the gVCF from the small variant phasing step as input, Nirvana annotates filtered small variant calls with static (not updatable) annotation databases for use by the downstream TMB calculation. For tumor profiling variants, Nirvana annotates filtered small variant calls with an updatable RefSeq database (included as part of the KB and may be updated periodically).

  RNA fusions identified during RNA fusion calling are merged with fusions from proximal genes identified during RNA splice variant calling. The merged fusions are then annotated with gene symbols or names corresponding to a static database of transcripts (GENCODE Release 19).

  Detected RNA splice variants are annotated using the Nirvana annotation engine with information from the RefSeq database. Nirvana annotates detected RNA splice variant calls with a static (not updatable) RefSeq database for use by downstream Companion Diagnostic calling. Splice variants are annotated with transcript-level changes (affected exons in the gene transcript) with respect to RefSeq, the same as the static RefSeq database used by the small variant annotation process. For tumor profiling variants, Nirvana annotates detected RNA splice variant calls with an updatable RefSeq database (included as part of the KB and may be updated periodically). Splice variants are annotated with transcript-level changes (affected exons in the gene transcript) with respect to RefSeq.

- TMB Score Calculation

  Calculation of the TMB score [which includes SNVs, insertion, and deletion variants and is derived from the count of non-driver somatic variants per megabase (evaluable region)] is generated from the gVCF file generated by the Small Variant Filter step and the annotations generated during small variant Annotation by calculating the number of somatic non-hotspot variants with VAF ≥ 5% divided by the evaluable region size. Driver mutations are identified and filtered based on COSMIC count. Variants are flagged as likely germline for calculating the TMB score, applying a combination of population database and post-database filtering strategies. After database filtering, the proximity filter labels variants as germline if they are surrounded by database-labeled germline variants, and variants identified as likely germline are excluded from the TMB score calculation. The evaluable region is the

dynamically adjusted per sample based on sequencing depth, and genomic regions with a high background noise level are excluded from the TMB calculation.

- Companion Diagnostic (CDx) Calling
  For each installed companion diagnostic (CDx) intended use, the TSO Comprehensive (US) analysis module determines the applicability of the CDx intended use for each patient sample based on the tumor type of the patient sample. If the tumor type of the patient is an exact match or a descendant of the tumor type for a CDx intended use, it is considered applicable to that CDx intended use. If the tumor type of the patient is not applicable to a CDx intended use, then the CDx intended use is not evaluated for that sample.

  If a required nucleic acid type for a CDx intended use is not sequenced or fails QC, or required controls in the run fail, then the patient sample is not evaluated for that CDx intended use. If a variant type or biomarker required for a CDx intended use fails QC, then the patient sample is not evaluated for that CDx intended use. When it is determined that a CDx intended use is applicable for a patient sample, the required libraries are sequenced, required QC measures pass, and required controls pass, the companion diagnostic intended use is evaluated for the patient sample. Detected variants and/or biomarkers in the patient sample are evaluated to determine the result for the CDx intended use. The evaluation is done through an algorithm specific to the CDx intended use, which assesses the presence and/or absence of variants/biomarkers that match the CDx intended use.

- Tumor Profiling Variant Calling
  After companion diagnostic results are determined, all passing, detected variants in a patient sample are matched against the installed Knowledge Base Base to determine the cancer mutations that have evidence of clinical significance (Level 2) or have potential clinical significance (Level 3). A genomic finding is either a single variant with evidence of clinical significance or potential clinical significance, or a grouping of variants that, when detected together, have evidence of clinical significance or potential clinical significance.

- When multiple variants are listed together as a genomic finding, it means that there is evidence for clinical significance or potential clinical significance for those variants together, in at least one of the sources listed in the Informatics Details of the report. If there are multiple genomic findings, and a variant is included in more than one of these findings, then that variant may be listed more than one time on a report. A genomic finding with a single variant will only be listed at the highest level, including the "Companion Diagnostics Results" section, where it meets criteria for reporting. Refer to "Positive CDx Results" section in the Local Run Manager TruSight Oncology Comprehensive (US) analysis module Workflow Guide for additional details.

Results Report Generation

The interpreted variant results and the TMB score are summarized in the TSO Comprehensive assay results report generated for the testing laboratory, which consists of

a “Companion Diagnostics Results” section, an “Other Alterations and Biomarkers Identified” section for reporting "" Cancer Mutations with Evidence of Clinical Significance” (Level 2) or “ Cancer Mutations with Potential Clinical Significance” (Level 3), a “Companion Diagnostics QC” section, and a “Companion Diagnostics Intended Use Evaluated” section. Please refer to the LRM TSO Comp Workflow Guide for additional information about the test reports.

Controls

Positive Controls
The TruSight Oncology DNA Control and TruSight Oncology RNA Control are required for use as positive controls. These controls consist of a multiplexed blend of biosynthetic DNA/RNA with multiple verified sequence mutations in a background of genomic DNA/RNA. The controls should be included for each DNA and RNA sequencing run (including combined runs) within a given library preparation event. The controls will be checked for quality of library preparation after sequencing. Failure of the control to meet the pre-defined quality metrics will result in all test samples on the run related to that specific control (DNA Control for DNA samples, RNA Control for RNA samples) to be invalidated.

No Template Control (NTC)
A no template control (NTC) consists of Tris-EDTA Buffer solution (provided in TSO Comprehensive kit as reagent “TEB”) for DNA samples, and DNase/RNase-free water (not included in the TSO Comprehensive kit) for RNA samples to determine if the library preparation is free of contamination by evaluating for the presence of sequencing reads (median exon coverage for DNA, number of genes with median deduplicated coverage for RNA) that map to the genes targeted by the assay. The NTC should be included for each DNA and RNA sequencing run (including combined runs) within a given library preparation event. If a NTC control fails, the assay instructions for use will require that the entire library preparation event is repeated for all samples.

Quality Metrics

Quality metrics (Table 3) are assessed across the following categories:

- Run-level: Metrics that are quantified per sequencing run; if the external control fails these criteria, no results are reported for the entire batch of samples.
- Sample-level: Metrics that are quantified per sample; no device results are generated for samples failing these metrics.
- Analyte-level: Metrics that are quantified for individual alteration types and positions. Variants passing analyte-level metrics are reported.

Table 3. Summary of TSO Comprehensive post-sequencing library quality control metrics

Variant Type	Metric	Run/Sample/ Analyte	Required Value	Description	Impact of Failure
All variants	PCT_RF_READ	Run-Level	≥ 80.0	Percentage of	Sequencing run

Variant Type	Metric	Run/Sample/ Analyte	Required Value	Description	Impact of Failure
	S (%)			reads passing filter (PF).	invalidated. No results reported for any sample in the run.
	PCT_Q30_R1 (%)	Run-Level	≥ 80.0	Average percent of base calls with quality score of Q30 or higher for Read 1.	
	PCT_Q30_R2 (%)	Run-Level	≥ 80.0	Average percent of base calls with quality score of Q30 or higher for Read 2.	
DNA variants	CONTAMINATION_SCORE	Sample-Level	≤ 3106 OR (> 3106 and P_VALUE ≤ 0.049)	A metric assessing the likelihood of contamination using the VAF of common variants. The contamination score is based on VAF distribution of SNPs. The contamination P value is used to assess highly rearranged genomes. It is only applicable when contamination score is above Upper Spec Limit.	No TMB or small variant results called
	MEDIAN_INSERT_SIZE (bp)	Sample-Level	≥ 70	The median fragment length in the sample.	
	MEDIAN_EXON_COVERAGE	Sample-Level	≥ 150	Median exon fragment coverage across all exon bases.	
	PCT_EXON_50	Sample-Level	≥ 90.0	Percent exon	

Variant Type	Metric	Run/Sample/ Analyte	Required Value	Description	Impact of Failure
	X (%)			bases with 50X fragment coverage.	
	Small DNA Variants[6]	Analyte-Level	AQ ≥ 20 for frequent COSMIC mutations[1] AQ ≥ 60 for other mutations[1]	Metric to determine whether a small DNA variant is called	Small DNA variant not called
	Small DNA Variants[6]	Analyte-Level	LQ ≥ 20 for frequent COSMIC mutations[2] LQ ≥ 60 for other mutations[2]	Metric to determine whether a small DNA variant is called	
	TMB	Analyte-Level	N/A[3]	N/A	N/A
RNA variants	Median_CV_Gene_500X	Sample-Level	≤ 0.93	For each gene with at least 500x coverage, the coefficient of variation in coverage across the gene body is computed. This metric is the median of these values. A high value indicates a high level of variation and indicates a problem in library preparation such as low sample input and/or probe pulldown issues. This metric is computed using all reads (including reads marked as duplicates).	No fusions or splice variant results called.
	Total_On_Target	Sample-Level	≥ 9,000,000	The total number	

Variant Type	Metric	Run/Sample/ Analyte	Required Value	Description	Impact of Failure
	_Reads			of reads that map to the target regions. This metric is computed using all reads (including reads marked as duplicates).	
	MEDIAN_INSERT_SIZE (bp)	Sample-Level	≥ 80	The median fragment length in the sample.	
	RNA Fusions	Analyte-Level	Fusion score ≥ 0.45[4] Deduped supporting reads ≥ 5	Metric to determine whether a RNA fusion gene is called	RNA fusion not called
	RNA Splice Variants	Analyte-Level	Splice score = 1[5]	Metric to determine whether a RNA splice variant is called	RNA splice variant not called

[1]Variant quality score (AQ) is calculated as -10*$\log_{10}$(p-value), where p-value is the binomial test by comparing the observed VAF and depth to a baseline of normal FFPE samples.
[2]Likelihood ratio (LQ) is calculated as -10$\log_{10}$ (Likelihood (observed variant is an error l DP error rate/Likelihood (observed variant is a mutation 1 DP, VAF)
[3]Evaluation of TMB does not have a reporting threshold, as the value is quantitative (score), not qualitative (classification)
[4]Fusion score is a weighted sum that takes into account the number of unique supporting reads, fusion contig length, breakpoint homology, and other associated metrics.
[5]Splice score increments by 0.1 for each supporting reads for the splice junction and is capped at 1.
[6]Accuracy of small DNA tumor profiling variants below 5% variant allele frequency (VAF) has not been established.

Table 4. TruSight Oncology Control Metrics

Output Type	Metric	Specification	Impact of Specification Failure
Positive Control	DNA External Control	23 of 24 specified variants detected	No small DNA variant or TMB results reported.
	RNA External Control	12 of 13 specified variants detected	No fusions or splice variant results reported.
Non-Template Control (NTC)	DNA Median Exon Coverage	≤8	No small DNA variant or TMB results reported
	RNA Gene above Median Cutoff	≤1	No fusions or splice variant results reported.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are FDA approved companion diagnostic (CDx) alternatives for the detection of genetic alterations using FFPE tumor specimens as listed in Table 1 of the TSO Comprehensive assay intended use statement (Section II). The approved CDx tests are listed in Table 5 below.

Table 5. Alternative FDA-approved CDx assays for CDx biomarkers identified by the TSO Comprehensive Assay

Indication	Gene	Device (PMA)	Company	Technology	Therapy
Solid Tumors	*NTRK*1/2/3 Fusions	FoundationOne® CDx (P170019/S017)	Foundation Medicine, Inc.	NGS	VITRAKVI® (larotrectinib)
Non-Small Cell Lung Cancer (NSCLC)	RET Fusions	OncomineDx Target Test (P160045/S019 and P160045/S031)	Life Technologies Corporation	NGS	RETEVMO® (selpercatinib)
Solid Tumors	RET Fusions	FoundationOne® CDx (P170019/S043)	Foundation Medicine, Inc.	NGS	RETEVMO® (selpercatinib)

For additional details see the FDA List of Cleared or Approved Companion Diagnostic Devices at: https://www.fda.gov/medical-devices/vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-vitro-and-imaging-tools

VII. MARKETING HISTORY

The TSO Comprehensive assay was originally CE-marked and commercially available March 2022, as 'TruSight™ Oncology Comprehensive (EU)" for use as a tumor profiling test in the European Union.

In May 2022, the *NTRK*1/2//3 companion diagnostic (CDx) claim was added to the TruSight Oncology Comprehensive (EU) Intended Use and it became commercially available in the European Union on May 24, 2022.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect TSO Comprehensive assay results and subsequently improper patient management decisions. Patients with false positive results may undergo treatment with the therapy listed in the intended use statement without clinical benefit and may experience adverse reactions associated with the therapy. Patients with false negative results may not be

considered for treatment with the indicated therapy. There is also a risk of delayed results, which may lead to delay of treatment with the appropriate indicated therapy.

No adverse events were reported in connection with the clinical studies used to support this PMA as the studies were performed retrospectively using banked samples. For the specific adverse events related to the approved therapeutics, please see approved FDA therapeutic product labeling.

IX. SUMMARY OF NONCLINICAL STUDIES

Laboratory Studies

Performance characteristics for the TSO Comprehensive assay were established using DNA and RNA derived from a wide range of FFPE tissue types. FFPE samples were tested for analysis of small DNA variants, fusions, and splice variants, including CDx and Tumor Profiling variants, and a range of TMB scores.

1. Accuracy – Comparison to an Orthogonal Method

i. CDx Accuracy

NTRK1, NTRK2, NTRK3 Fusion Detection in Solid Tumor Samples

The accuracy of the TSO Comprehensive assay for detecting *NTRK* fusions (*NTRK*1, *NTRK*2, or *NTRK*3) in patients with solid tumors was evaluated by assessing the concordance of *NTRK* fusion results between the TSO Comprehensive assay and a validated NGS-based comparator method as one of the studies to support the use of the TSO Comprehensive as a companion diagnostic (CDx) for larotrectinib. A retrospective, noninterventional study was conducted using a total of 516 FFPE samples consisting of both larotrectinib clinical trial samples (See Section X. for study details) and supplemental negative samples. These samples were tested with the TSO Comprehensive assay at one external site and with the NGS-based comparator method at a central laboratory. Accuracy of the TSO Comprehensive *NTRK* fusion calls by the assay was estimated relative to the comparator method. Positive percent agreement (PPA), negative percent agreement (NPA), and the associated two-sided 95% confidence intervals (CIs) were calculated.

Of the 516 samples, 499 were tested by both methods, with seventeen of the samples not tested with one of the assays due to failed extraction, unknown reason (for the comparator method), or protocol deviation. Of the 499 samples tested by both methods, 170 (34.1%) were larotrectinib trial samples and 329 (65.9%) were supplemental samples. In addition, 85 samples (72 supplemental, 13 clinical trial) had invalid TSO Comprehensive assay results (invalid rate is 17%). Of these 85 samples, 53 also had invalid comparator method results, and the comparator method had 7 additional invalid results (32 total samples with invalid TSO Comprehensive results, but valid comparator results). In total, 407 of the 499 samples had valid results by both methods.

The concordance analysis is shown in Table 6 (aggregate) and Table 6 (by gene). Based on valid test results, aggregate PPA was 96.6% (114/118; 95% CI: 91.5%–99.1%) and aggregate NPA was 94.5% (273/289; 95% CI: 91.2%–96.8%). Sixteen samples were negative for *NTRK* fusions by the comparator method but positive by the TSO Comprehensive assay, leading to the current NPA value. Of these 16 samples, 6 were TSO positive due to a putative isolated contamination event that occurred during the study. These 6 samples all contained the same fusion but were in the same library preparation plate as a sample with an identical fusion with high supporting reads. This putative contamination was investigated during an additional Cross-contamination study to understand discordant results described in the paragraph below. Of the remaining 10 samples positive by TSO Comprehensive assay and negative by the comparator method, 7 samples were positive with the local test (LT) used in the original therapeutic clinical trial, and 3 samples were LT negative. Six of these 7 LT positive patients showed clinical trial benefit with larotrectinib.

Table 6. *NTRK* Accuracy Study: Concordance Between the TSO Comprehensive Assay and Comparator Method for Detection of *NTRK* Fusions

		Comparator Method Result		
		***NTRK* Fusion Positive**	***NTRK* Fusion Negative**	**Total**
TSO Comprehensive Assay Result	*NTRK* Fusion Positive	114	16[2]	130
	NTRK Fusion Negative	4	273	277
	Total	118	289	407
Agreement Statistics	PPA% (n/N; 95% CI[1])	96.6% (114/118; 91.5%-99.1%)		
	NPA% (n/N; 95% CI[1])	94.5% (273/289; 91.2%-96.8%)		

[1]CI based on Clopper-Pearson (exact) method

[2]6 of the 16 samples were TSO Comprehensive positive due to an isolated contamination event during study testing. Out of the remaining 10 samples, 7 were positive by the LT, and 6 of those 7 showed clinical benefit with the therapeutic

Table 7. *NTRK* Accuracy Study: Concordance Between the TSO Comprehensive Assay and Comparator Method for Detection of *NTRK* Fusions by Gene

	Orthogonal Positive			Orthogonal Negative			
	TSO Comp Positive	TSO Comp Negative	TSO Comp Positive	TSO Comp Negative			
Gene	**CP**	**DN**	**DP**	**CN**	**Total**	**PPA (n/N), (95% CI)[1]**	**NPA (n/N), (95% CI)[1]**
*NTRK*1	40	3	2	362	407	93.0% (40/43), (80.9%, 98.5%)	99.5% (362/364), (98.0%, 99.0%)
*NTRK*2	7	0	4	396	407	100.0% (7/7), (59.0%, 100.0%)	99.0% (396/400), (97.5%, 99.0%)
*NTRK*3	67	3	10	327	407	95.7% (67/70), (88.0%, 99.1%)	97.0% (327/337), (94.6%, 98.6%)
Total	114	4	16	273	407	96.6% (114/118), (91.5%-99.1%)	94.5% (273/289), (91.2%-96.8%)

Abbreviations: CN = Concordant Negative; CP = Concordant Positive; DN = Discordant Negative; DP = Discordant Positive
[1]CI based on Clopper-Pearson (Exact) method.

Testing of discordant results was performed for samples in both the *NTRK* Accuracy and Clinical Bridging studies due to low NPA values in both studies, possibly due to a suspected contamination event. A total of 142 RNA samples, comprised of 107 concordant samples (53 concordant negative, 54 concordant positive), 26 discordant samples, and 9 samples with unknown *NTRK* status from any of the methods used (TSO Comprehensive, comparator method, and LT), were re-tested with the TSO Comprehensive assay. Of the 142 samples, 133 produced valid results. Of the 6 samples that were suspected to be contaminated, presented as positive in the original Accuracy study in Table 6 above, only 4 were available for re-testing. All 4 of those samples were *NTRK* fusion-negative upon re-testing. Additionally, 6 out of the remaining 126 samples with valid retest results changed *NTRK* fusion status upon retesting. Of those samples, 3 were concordant *NTRK* fusion-positives by original testing, changing to negative upon re-test. None of the concordant *NTRK* fusion-negatives from the original testing changed to positives. Further, a sensitivity analysis was performed for the results from the testing of the previously discussed discordant samples. The PPA in the imputed dataset was estimated to be 93.3% (95% CI: 87.9%, 98.7%) and NPA was estimated at 97.3% (95% CI: 95.4%, 99.3%). The PPA and NPA from the imputed dataset are within the confidence limits of the original estimates (Tables 6 and 7).

RET Fusion Detection in NSCLC Samples

The accuracy of the TSO Comprehensive assay for detecting RET fusions in patients with NSCLC was evaluated by assessing the concordance of RET fusion results between the TSO Comprehensive assay and a validated NGS-based comparator method as one of the studies to support the use of the TSO Comprehensive as a companion diagnostic (CDx)

for selpercatinib. A retrospective, noninterventional study was conducted using a total of 219 FFPE samples consisting of 93 samples obtained from LIBRETTO-001 trial (see Section X. for study details) and 126 supplemental negative samples obtained from commercial sources. These samples were tested with the TSO Comprehensive assay at one external site and with the NGS-based a comparator method at a central laboratory. Accuracy of the TSO Comprehensive assay RET fusion calls by the TSO Comprehensive assay was estimated relative to the comparator method. Positive percent agreement (PPA), negative percent agreement (NPA), and the associated two-sided 95% confidence intervals (CIs) were calculated.

Of the 219 FFPE samples tested, 14 samples had invalid TSO Comprehensive assay results (invalid rate of 6.4%). Of these 14 (13 supplemental and 1 clinical trial sample), 8 also had invalid comparator method results. The comparator method had 15 additional invalid results and 22 samples with no calls. In addition, there were 6 samples (all supplemental) with invalid TSO Comprehensive results and valid comparator method results. In total, 168 of the 219 samples had valid results by both methods (69 RET-fusion positive clinical trial specimens and 99 supplemental RET-fusion negative samples). The concordance analysis is shown in Table 8. Based on valid test results, the Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) between TSO Comprehensive assay and comparator method were 100.0% (69/69, 95% CI: 94.8% to 100.0%), and 97.0% (96/99, 95% CI: 91.4% to 99.4%), respectively.

For the discordant specimens called RET fusion-positive by TSO Comp and RET fusion negative by the orthogonal method, all were clinical trial samples that tested RET fusion-positive by the LT used for enrollment. One sample contained a fusion partner that could not be interrogated by the orthogonal assay, but clinical data showed that the patient had a verified partial response (PR) to therapy, thus suggesting the presence of a RET fusion. For the other two samples, the amount of supporting reads were low, and may have been below the variant calling threshold for the orthogonal assay. These subjects were unevaluable for response assessment.

Table 8. RET Accuracy Study: Concordance Between the TSO Comprehensive Assay and Comparator Method for Detection of RET Fusions in NSCLC

		Comparator Method Result		
		RET Fusion Positive	**RET Fusion Negative**	**Total**
TSO Comprehensive Assay Result	RET Fusion Positive	69	3	72
	RET Fusion Negative	0	96	96
	Total	69	99	168
Agreement Statistics	PPA% (n/N; 95% CI[1])	100.0% (69/69; 94.8% -100.0%)		
	NPA% (n/N; 95% CI[1])	97.0% (96/99; 91.4% - 99.4%)		

[1]CI based on Clopper-Pearson (exact) method

ii. Tumor Profiling Accuracy

Detection of small DNA variants, TMB, RNA fusions, and RNA splice variants was evaluated with FFPE specimens (both characterized and uncharacterized) using appropriate comparator methods to assess the accuracy of the TSO Comprehensive assay.

Small DNA Variants

The detection of small DNA variants was assessed by comparison with results obtained using an externally validated NGS targeted panel assay (evNGS). A total of 508 unique samples were tested by the evNGS, of which 19 were invalidated due to insufficient quality or quantity of DNA samples (n=3) or libraries (n=16), leaving 489 unique samples with valid evNGS results. Those 508 samples were also tested with the TSO Comprehensive assay, of which there were 77 invalidated samples due to failing contamination QC (n=8), failing small DNA variant QC (n=50), and samples mistakenly tested by the evNGS after an invalid result (n=21), leaving 431 with valid TSO Comp results. Of these 431 samples, 6 were invalid by the evNGS, leaving 425 samples, of which 11 were excluded due to a sample swap, leaving 414 samples with valid results for both assays. This set of 414 FFPE samples (representing SNVs, MNVs, insertions, and deletions) from 16 different tissue types that had valid results for both TSO Comprehensive assay and evNGS were the evaluable sample set (Table 9).

Table 9. Number of Evaluable Samples (n=414) by Cancer/Tissue Type

Tissue Type/Cancer Type	Number of DNA Samples included in Final Data Analysis
Bladder	19
Brain	21
Breast	30
CRC	52
Gastric	24
Head and Neck	1
Liver	21
Medullary Thyroid	38
NSCLC	57
Ovary	16
Pancreas	24
Papillary Thyroid	26
Prostate	36
Skin	25

Tissue Type/Cancer Type	Number of DNA Samples included in Final Data Analysis
Soft Tissue	2
Uterus	22
Total	**414**

The reported variants from TSO Comprehensive assay were compared with results of the evNGS method and used to calculate the PPA and NPA. A summary of overall PPA and NPA per clinical significance and variant type for small DNA variants are provided in Table 10 below. The observed PPA for all Level 2 variants was 95.5%, which includes an observed PPA of 96.1% for Level 2 SNVs. In addition, there was a lower PPA of 80.7% for all Level 3 variants. Differences in Level 2 and Level 3 variant calls were attributed to the differences in cutoff between the evNGS method and TSO Comprehensive, since the evNGS method does not report any variants below the 5% VAF filter. A number of Level 3 variants in the study were below the calling threshold of the evNGS assay and the lower PPA for Level 3 variants was attributed to detection of deamination artifacts by the comparator method. A limitation in the device labeling addresses lack of accuracy data for samples >5% VAF, since accuracy of small DNA tumor profiling variants below 5% variant allele frequency (VAF) has not been established.

Table 10. Concordance Summary for Small DNA Variants by Variant Type and Clinical Significance

Variant Category	Clinical Significance[4]	PPA (n/N) (95% CI[1])	NPA (n/N) (95% CI[1])
SNVs	Level 2	96.1% (99/103) (90.4%, 98.5%)	>99.9% (9832/9833) (>99.9%, >99.9%)
	Level 3	76.9% (373/485)[3] (73.0%, 80.4%)	>99.9% (212277/212311) (>99.9%, >99.9%)
	All	80.3% (472/588) (76.9%, 83.3%)	>99.9% (219211/219246) (>99.9%, >99.9%)
MNVs	Level 2	100% (5/5) (56.6%, 100.0%)	100.0% (9931/9931) (>99.9%, 100.0%)
	Level 3	90.0% (9/10) (59.6%, 98.2%)	>99.9% (212785/212786) (>99.9%, >99.9%)
	All	93.3% (14/15) (70.2%, 98.8%)	>99.9% (219818/219819) (>99.9%, >99.9%)
Insertions	Level 2	100.0% (1/1) (20.7%, 100.0%)	100.0% (9935/9935) (>99.9%, 100.0%)
	Level 3	86.0% (49/57) (74.7%, 92.7%)	>99.9% (212738/212739) (>99.9%, >99.9%)
	All	86.2% (50/58) (75,1%, 92.8%)	>99.9% (219775/219776) (>99.9%, >99.9%)
Deletions	Level 2	66.7% (2/3)[2] (20.8% - 93.9%)	>99.9% (9932/9933) (>99.9%, >99.9%)
	Level 3	90.3% (139/154) (84.6% - 94.0%)	>99.9% (212624/212642) (>99.9%, >99.9%)
	All	89.8% (141/157) (84.1%, 93.6%)	>99.9% (219658/219677) (>99.9%, >99.9%)
	Level 2	95.5% (107/112) (90.0% - 98.1%)	>99.9% (9822/9824) (99.9%, >99.9%)

All Variants	Level 3	80.7% (570/706) (77.7%, 83.5%)	>99.9% (212036/212090) (> 99.9%, > 99.9%)
	All	82.8% (677/818) (80.0%, 85.2%)	>99.9% (218960/219016) (>99.9%, >99.9%)

[1]95% 2-sided confidence interval calculated via the Wilson score method.
[2]TruSight Oncology Comprehensive assay detected an EGFR clinically significant deletion but with 1 nucleotide difference in the alternate sequence relative to the clinically significant deletion found by the comparator method, which caused the discordant result.
[3]These were discordant negatives detected only by the comparator assay. Based on root cause analysis, 91% (102/112) of them are due to deamination artifacts, which were either not present in the TruSight Oncology Comprehensive bam file (n=49), or were filtered out as having low LQ score (n=53)
[4]Level 2 = Cancer Mutations with Evidence of Clinical Significance, Level 3 = Cancer Mutations with Evidence of Potential Clinical Significance

Since the evNGS method does not report any variants below the 5% VAF filter, concordance for variants above VAF ≥5% has been also calculated, by variant category. When looking at results ≥ 5% VAF, observed PPA was 98.1% (95% CI: 93.4% - 99.5%) for all Level 2 variants, which includes Level 2 SNVs with an observed PPA of 99.0% (95% CI: 94.5%, 99.8%), Level 2 MNVs with an observed PPA of 100.0% (95% CI: 56.6%, 100.0%), and Level 2 insertions with an observed PPA of 100.0% (95% CI: 20.7%, 100.0%). Further, an observed PPA of 97.5% (95% CI: 95.8% - 98.5%) was seen for all Level 3 variants, including an observed PPA of 98.0% (95% CI: 96.0% - 99.0%) for Level 3 SNVs, an observed PPA of 90.0% for Level 3 MNVs (95% CI: 59.6% - 98.2%), an observed PPA of 96.1% for Level 3 insertions (95% CI: 86.8% - 98.9), and an observed PPA of 97.1% **(95%** CI: 92.7% - 98.9%) for Level 3 deletions.

A summary of accuracy (PPA and NPA) of insertions and deletions binned by size is presented in Table 11 below. Aggregate PPA for both variant types are lower (86.2% for insertions, 89.8% for deletions) due to differences in Level 2 and Level 3 variant calls that were expected based on the differences in cutoff between the evNGS method and TSO Comprehensive. Looking at insertions and deletions ≥5% VAF, all variants had a PPA of >95%, with the lone exception of a single deletion in the 11-15 bp range; however, the concordance in the 16-20 bp range was 100%. In addition, results from the concordance analysis across insertions and deletions of all sizes regardless of clinical validity showed detection of 24 bp insertion (1/1; 95% CI: 20.7%-100.0), as well 24 bp deletions (2/2; 95% CI: 34.2%-100.0%).

Table 11. Concordance Summary of All Levelled (Level 2 and 3) Insertion and Deletion Variants by Size

Variant Category	Indel Bin Size	Total Unique Variants	True Positives	False Positives	False Negatives	True Negatives	PPA (n/N) (95% CI[1])	NPA (n/N) (95% CI[1])
	1-5	46	47	1	7	219779	87.0% (47/54) (75.6%-93.6%)	>99.9% (219779/219780) (>99.9%->99.9%)
	6-10	2	1	0	1	219832	50.0% (1/2) (9.5%-90.5%)	100.0% (219832/219832) (>99.9%-100.0%)
	11-15	2	2	0	0	219832	100.0% (2/2) (34.2%-100.0%)	100.0% (219832/219832) (>99.9%-100.0%)
INS	All	50	50	1	8	219775	86.2% (50/58) (75.1%-92.8%)	>99.9% (219775/219776) (>99.9%->99.9%)
	1-5	129	132	18	15	219669	89.8% (132/147) (83.8%-93.7%)	>99.9% (219669/219687) (>99.9%->99.9%)
	6-10	5	5	0	0	219829	100.0% (5/5) (56.6%-100.0%)	100.0% (219829/219829) (>99.9%-100.0%)
	11-15	2	0	1	1	219832	0.0% (0/1) (0.0%-79.3%)	>99.9% (219832/219833) (>99.9%->99.9%)
	16-20	3	4	0	0	219830	100.0% (4/4) (51.0%-100.0%)	100.0% (219830/219830) (>99.9%->99.9%)
DEL	All	139	141	19	16	219658	89.8% (141/157) (84.1%-93.6%)	>99.9% (219658/219677) (>99.9%->99.9%)

[1]95% 2-sided confidence interval calculated via the Wilson score method.

Accuracy results (PPA and NPA) for Variants with Evidence of Clinical Significance (Level 2) by gene and variant are included in Table 12 below, and a summary of concordance of all small DNA variants by gene is included in Appendix 2.

Table 12. Accuracy of Variants with Evidence of Clinical Significance (Level 2) by Gene and Variant

Gene	Variant	Concordant Positive Calls	Called only in evNGS (Discordant Negative)	Called only in TSO Comp (Discordant Positive)	Concordant Negative Calls	PPA (%) (95% CI)	NPA (%) (95% CI)
BARD1	Trp91Ter	0	1	0	413	0.0 (0.0, 79.3)	100.0 (99.1, 100.0)
BRAF	Val600Glu	41	1	0	372	97.6 (87.7, 95.6)	100.0 (99.0, 100.0)
BRIP1	Arg162Ter	0	1	0	413	0.0 (0.0, 79.3)	100.0 (99.1, 100.0)
CDK12	Leu453IlefsTer9	1	0	0	413	100.0 (20.7, 100.0)	100.0 (99.1, 100.0)
CHEK2	Thr367MetfsTer15	1	0	0	413	100.0 (20.7,100.0	100.0 (99.1, 100.0)
EGFR	Leu747_Ala750del	0	0	1	413	N/A	99.8 (98.6, >99.9)
	Leu747_Thr751delinsSer	0	1	0	413	0.0 (0.0, 79.3)	100.0 (99.1, 100.0)
	Leu858Arg	1	0	0	413	100.0 (20.7, 100.0)	100.0 (99.1, 100.0)
KRAS	Ala146Thr	2	0	0	412	100.0 (34.2, 100.0)	100.0 (99.1, 100.0)
	Gln61Leu	1	0	0	413	100.0 (20.7, 100.0)	100.0 (99.1, 100.0)
	Gly12Ala	12	0	0	402	100.0 (75.8, 100.0)	100.0 (99.1, 100.0)
	Gly12Cys	18	0	1	395	100.0 (82.4, 100.0)	99.7 (98.6, >99.9)
	Gly12Phe	1	0	0	413	100.0 (20.7, 100.0)	100.0 (99.1, 100.0)
	Gly12Ser	1	0	0	413	100.0 (20.7, 100.0)	100.0 (99.1, 100.0)
	Gly12Val	7	0	0	407	100.0 (64.6, 100.0)	100.0 (99.1, 100.0)
	Gly13Ala	4	0	0	410	100.0 (51.0, 100.0)	100.0 (99.1, 100.0)
NRAS	Gly12Ala	1	0	0	413	100.0 (20.7, 100.0)	100.0 (99.1, 100.0)

Gene	Variant	Concordant Positive Calls	Called only in evNGS (Discordant Negative)	Called only in TSO Comp (Discordant Positive)	Concordant Negative Calls	PPA (%) (95% CI)	NPA (%) (95% CI)
PALB2	Gln472Ter	0	1	0	413	0.0 (0.0, 79.3)	100.0 (99.1, 100.0)
PTEN	Leu320Ter	1	0	0	413	100.0 (20.7, 100.0)	100.0 (99.1, 100.0)
RET	Ala883Phe	1	0	0	413	100.0 (20.7, 100.0)	100.0 (99.1, 100.0)
	Cys609Tyr	1	0	0	413	100.0 (20.7, 100.0)	100.0 (99.1, 100.0)
	Cys630Arg	1	0	0	413	100.0 (20.7, 100.0)	100.0 (99.1, 100.0)
	Cys634Arg	2	0	0	412	100.0 (34.2,100.0)	100.0 (99.1, 100.0)
	Cys634Phe	1	0	0	413	100.0 (20.7, 100.0)	100.0 (99.1, 100.0)
	Cys634Ser	3	0	0	411	100.0 (43.9,100.0)	100.0 (99.1, 100.0)
	Cys634Tyr	2	0	0	412	100.0 (34.2, 100.0)	100.0 (99.1, 100.0)
	Met918Thr	3	0	0	411	100.0 (43.9, 100.0)	100.0 (99.1, 100.0)
	Val804Met	1	0	0	413	100.0 (20.7, 100.0)	100.0 (99.1, 100.0)

Wild Type Accuracy

For assessing accuracy of wild-type calls, evaluation of clinically relevant positions was performed by observation of the NPA of Level 2 small DNA variants. Results in Table 14 (by variant type) show an NPA of >99.9% for all relevant Level 2 variant types.

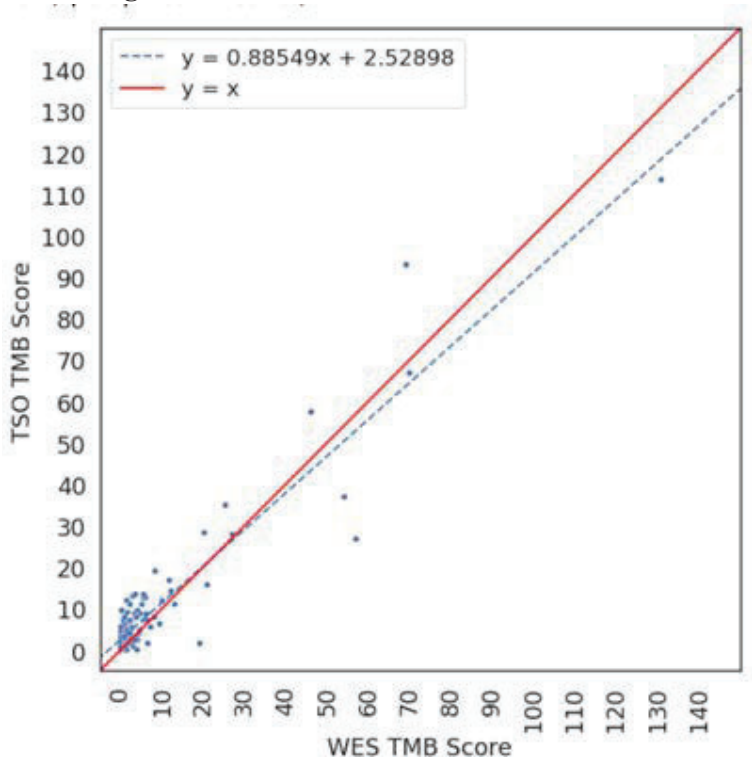
Table 14. Evaluation of Wild-Type Accuracy using NPA of Variants of Clinical Significance (Level 2)

Category	NPA (n/N)	95% CI (Wilson Score)	95% CI (Exact Method)
SNVs	>99.9% (9832/9833)	>99.9, >99.9	99.9, >99.9
MNVs	100.0% (9931/9931)	>99.9, 100.0	>99.9, 100.0
Insertions	100.0% (9935/9935)	>99.9, 100.0	>99.9, 100.0
Deletions	>99.9% (9932/9933)	>99.9, >99.9	99.9, >99.9

Tumor Mutational Burden (TMB) Score

Concordance of TMB score was determined by comparing the TMB scores (somatic mutations/megabase) between a validated Whole Exome Sequencing (WES) comparator method and TSO Comprehensive assay. 246 tumor-normal paired samples were sent for reference testing by the WES, and 29 were excluded due to failure of QC metrics. For the remaining 217 pairs, 33 were determined to be mismatched, and 60 pairs were excluded due to contamination or insufficient coverage. The remaining 124 FFPE samples from 14 different tissue types across a range of TMB scores (0.8-115.7 Mut/Mb) with valid results by both TSO Comprehensive and WES comparator method were analyzed. Linear regression analysis with WES as the predictor and TSO Comprehensive as the outcome had a y-intercept of 2.53, a slope of 0.89, and Pearson's correlation coefficient of 0.94 (Figure 1).

Figure 1. TMB Score Correlation between WES and TSO Comprehensive



RNA Splice Variants

Accuracy for splice variant detection was calculated by comparing TSO Comprehensive assay results to a validated qPCR comparator method for EGFRvIII. Concordance analysis was performed on a total of 16 unique FFPE RNA samples from brain tissue. Table 15 summarizes the concordance study results for EGFRvIII. In summary, EGFR had a PPA and NPA of 100%.

Table 15. Concordance Analysis Between TSO Comprehensive assay and qPCR Assay for EGFRvIII

TSO Comprehensive Results	qPCR Positive	qPCR Negative
Positive	3	0
Negative	0	13
PPA (n/N) (95% CI)	100% (3/3) (44%, 100%)	
NPA (n/N) (95% CI)	100% (13/13) (77%, 100%)	

RNA Fusions - Comparison to a Composite Method

TSO Comprehensive assay fusions were compared to a validated composite method consisting of an RNA whole exome NGS panel (weNGS), a targeted NGS fusion panel

(tNGS), and droplet digital PCR (ddPCR). A breakdown of the composite methodology is shown in Table 16. In short, the NGS exome panel overlapped with all the genes for which TSO Comprehensive assay can detect fusions. However, because the limit of detection of the weNGS assay was 4–8x that of TSO Comprehensive assay (based on the number of supporting reads observed in the overlapping fusion calls), a composite method using two additional methods with greater sensitivity, but less breadth, was used for fusions (NGS Targeted Panel and ddPCR).

Table 16. Composite Reference Method Testing for RNA Fusions

		weNGS	
		Detected	**Not Detected**
TSO Comprehensive	**Detected**	Tested with tNGS if covered by assay; if not, tested with ddPCR	Tested with tNGS if covered by assay; if not, tested with ddPCR
	Not Detected	Tested with tNGS if covered by assay; if not, tested with ddPCR	Randomly selected samples tested with tNGS or ddPCR if fusions are not covered by tNGS

A total of 255 unique RNA samples representing 14 tissue types were tested. All samples passed TSO Comprehensive metrics. Of the 255 samples, 220 samples were not selected based on prior screening (hereafter "uncharacterized"), while 35 samples were selected for the study because they were fusion positive with TSO Comprehensive assay or a predecessor assay (hereafter known as "characterized" samples). Performance for these 35 characterized samples was adjusted using a mean fusion prevalence. Composite concordance results for the characterized and uncharacterized samples (and their respective performance characteristics) are shown in Tables 17 and 18, respectively. The relatively low PPA for the characterized samples is reflective of the prevalence of the included fusions, as PPA and NPA were adjusted for fusion prevalence; therefore, the combined performance across characterized and uncharacterized samples was calculated using an inverse-variance weighted average to account for the adjustment for prevalence in the characterized samples. These concordance results are shown in Table 19. In summary, the PPA of the combined fusions was 87.29.

Table 17. Summary of TSO Comp Versus Composite Method Results for RNA Fusions in 35 Characterized Samples)

		Composite Method		
		Detected	**Not Detected**	**Total**
TSO Comprehensive Assay	**Detected**	25	4	29
	Not Detected	2	809[1]	811
	Total	27	813	840
	PPA (95% CI)[2,3]	60.34% (29.73%, 100%)		
	NPA (95% CI)[2,3]	99.94% (99.86%, 99.98%)		

[1]809 calculated as 35 samples x 24 fusion outcomes/sample - 25 TP - 4 FP - 2 FN
[2]Confidence intervals calculated by bootstrap.
[3]Performance characteristics have been adjusted for prevalence

Table 18. Summary of TSO Comp Versus Composite Method Outcomes for RNA Fusions in 220 Uncharacterized Samples

		Composite Method		
		Detected	**Not Detected**	**Total**
TSO Comprehensive Assay	**Detected**	12	0	12
	Not Detected	1	5267[1]	5268
	Total	13	5267	5280
	PPA (95% CI)[2]	92.31% 66.69% - 99.61%)		
	NPA (95% CI)[2]	100% (99.93% - 100%)		

[1]5,267 calculated as 220 samples x 24 fusion outcomes/sample – 12 TP – 0 FP – 1 FN
[2]Confidence intervals calculated by the Wilson Method.

Table 19. Summary of Performance for RNA Fusions (Combined for Characterized and Uncharacterized)

PPA (95% CI[1])	NPA (95% CI[1])
87.29% (67.83%, 96.35%)	99.99% (99.98%, >99.99%)

[1]Confidence intervals calculated by bootstrap.

A summary of the concordance data by characterization and by gene is also presented in Table 20 below, which shows PPA/NPA for all 24 fusions genes in the TSO Comprehensive panel. Please note that, for the PPA/NPA values, characterized samples were adjusted by prevalence, while uncharacterized samples were not.

Table 20. Concordance for RNA Fusions by Gene and Sample Characterization

Gene	# TP	# FP	# FN	# TN	Characterized	PPA (n/N) (95% CI*)	NPA (n/N) (95% CI*)	PPV (n/N) (95% CI*)	NPV (n/N) (95% CI*)	Adjusted PPA (%)	Adjusted NPA (%)
AXL	2	0	0	33	YES	100.0 (2/2) (34.2-100.0)	100.0 (33/33) (89.6-100.0)	100.0 (2/2) (34.2-100.0)	100.0 (33/33) (89.6-100.0)	100.0	100.0
AXL	1	0	0	219	NO	100.0 (1/1) (20.7-100.0)	100.0 (219/219) (98.3-100.0)	100.0 (1/1) (20.7-100.0)	100.0 (219/219) (98.3-100.0)	-	-
BCL2	1	0	0	34	YES	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0	100.0
BCL2	0	0	0	220	NO	-	100.0 (220/220) (98.3->99.9)	-	100.0 (220/220) (98.3->99.9)	-	-
BRAF	2	0	0	33	YES	100.0 (2/2) (34.2-100.0)	100.0 (33/33) (89.6-100.0)	100.0 (2/2) (34.2-100.0)	100.0 (33/33) (89.6-100.0)	100.0	100.0
BRAF	0	0	0	220	NO	-	100.0 (220/220) (98.3->99.9)	-	100.0 (220/220) (98.3->99.9)	-	-
CDK4	1	0	0	34	YES	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0	100.0
CDK4	0	0	0	220	NO	-	100.0 (220/220) (98.3->99.9)	-	100.0 (220/220) (98.3->99.9)	-	-
EGFR	1	3	0	31	YES	100.0 (1/1) (20.7-100.0)	91.2 (31/34) (77.0-97.0)	25.0 (1/4) (4.6-69.9)	100.0 (31/31) (89.0-100.0)	100.0	100.0
EGFR	0	0	0	220	NO	-	100.0 (220/220) (98.3->99.9)	-	100.0 (220/220) (98.3->99.9)	-	-
EML4	1	0	1	33	YES	50.0 (1/2) (9.5-90.5)	100.0 (33/33) (89.6-100.0)	100.0 (1/1) (20.7-100.0)	97.1 (33/34) (85.1-99.5)	1.3	100.0

Gene	# TP	# FP	# FN	# TN	Characterized	PPA (n/N) (95% CI*)	NPA (n/N) (95% CI*)	PPV (n/N) (95% CI*)	NPV (n/N) (95% CI*)	Adjusted PPA (%)	Adjusted NPA (%)
EML4	1	0	0	219	NO	100.0 (1/1) (20.7-100.0)	100.0 (219/219) (98.3-100.0)	100.0 (1/1) (20.7-100.0)	100.0 (219/219) (98.3-100.0)	-	-
ERG	1	0	0	34	YES	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0	100.0
ERG	6	0	1	213	NO	85.7 (6/7) (48.7-97.4)	100.0 (213/213) (98.2->99.9)	100.0 (6/6) (61.0-100.0)	99.5 (213/214) (97.4-99.9)	-	-
ESR1	2	0	0	33	YES	100.0 (2/2) (34.2-100.0)	100.0 (33/33) (89.6-100.0)	100.0 (2/2) (34.2-100.0)	100.0 (33/33) (89.6-100.0)	100.0	100.0
ESR1	2	0	0	218	NO	100.0 (2/2) (34.2-100.0)	100.0 (218/218) (98.3-100.0)	100.0 (2/2) (34.2-100.0)	100.0 (218/218) (98.3-100.0)	-	-
ETV1	1	0	0	34	YES	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0	100.0
ETV1	0	0	0	220	NO	-	100.0 (220/220) (98.3->99.9)	-	100.0 (220/220) (98.3->99.9)	-	-
ETV4	1	0	0	34	YES	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0	100.0
ETV4	0	0	0	220	NO	-	100.0 (220/220) (98.3->99.9)	-	100.0 (220/220) (98.3->99.9)	-	-
EWSR1	1	0	0	34	YES	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0	100.0
EWSR1	0	0	0	220	NO	-	100.0 (220/220) (98.3->99.9)	-	100.0 (220/220) (98.3->99.9)	-	-

Gene	# TP	# FP	# FN	# TN	Characterized	PPA (n/N) (95% CI*)	NPA (n/N) (95% CI*)	PPV (n/N) (95% CI*)	NPV (n/N) (95% CI*)	Adjusted PPA (%)	Adjusted NPA (%)
FGFR1	1	0	0	34	YES	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0	100.0
FGFR1	0	0	0	220	NO	-	100.0 (220/220) (98.3->99.9)	-	100.0 (220/220) (98.3->99.9)	-	-
FGFR2	1	0	0	34	YES	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0	100.0
FGFR2	0	0	0	220	NO	-	100.0 (220/220) (98.3->99.9)	-	100.0 (220/220) (98.3->99.9)	-	-
FGFR3	1	1	1	32	YES	50.0 (1/2) (9.5-90.5)	97.0 (32/33) (84.7-99.5)	50.0 (1/2) (9.5-90.5)	97.0 (32/33) (84.7-99.5)	0.2	100.0
FGFR3	0	0	0	220	NO	-	100.0 (220/220) (98.3->99.9)	-	100.0 (220/220) (98.3->99.9)	-	-
FLI1	1	0	0	34	YES	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0	100.0
FLI1	0	0	0	220	NO	-	100.0 (220/220) (98.3->99.9)	-	100.0 (220/220) (98.3->99.9)	-	-
KIF5B	2	0	0	33	YES	100.0 (2/2) (34.2-100.0)	100.0 (33/33) (89.6-100.0)	100.0 (2/2) (34.2-100.0)	100.0 (33/33) (89.6-100.0)	100.0	100.0
KIF5B	0	0	0	220	NO	-	100.0 (220/220) (98.3->99.9)	-	100.0 (220/220) (98.3->99.9)	-	-
NRG1	1	0	0	34	YES	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0	100.0

Gene	# TP	# FP	# FN	# TN	Characterized	PPA (n/N) (95% CI*)	NPA (n/N) (95% CI*)	PPV (n/N) (95% CI*)	NPV (n/N) (95% CI*)	Adjusted PPA (%)	Adjusted NPA (%)
NRG1	0	0	0	220	NO	-	100.0 (220/220) (98.3->99.9)	-	100.0 (220/220) (98.3->99.9)	-	-
NTRK1	1	0	0	34	YES	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0	100.0
NTRK1	0	0	0	220	NO	-	100.0 (220/220) (98.3->99.9)	-	100.0 (220/220) (98.3->99.9)	-	-
NTRK2	1	0	0	34	YES	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0	100.0
NTRK2	0	0	0	220	NO	-	100.0 (220/220) (98.3->99.9)	-	100.0 (220/220) (98.3->99.9)	-	-
NTRK3	1	0	0	34	YES	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0	100.0
NTRK3	0	0	0	220	NO	-	100.0 (220/220) (98.3->99.9)	-	100.0 (220/220) (98.3->99.9)	-	-
PAX3	1	0	0	34	YES	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0	100.0
PAX3	0	0	0	220	NO	-	100.0 (220/220) (98.3->99.9)	-	100.0 (220/220) (98.3->99.9)	-	-
RAF1	1	0	0	34	YES	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0	100.0
RAF1	0	0	0	220	NO	-	100.0 (220/220) (98.3->99.9)	-	100.0 (220/220) (98.3->99.9)	-	-

Gene	# TP	# FP	# FN	# TN	Characterized	PPA (n/N) (95% CI*)	NPA (n/N) (95% CI*)	PPV (n/N) (95% CI*)	NPV (n/N) (95% CI*)	Adjusted PPA (%)	Adjusted NPA (%)
RET	2	0	0	33	YES	100.0 (2/2) (34.2-100.0)	100.0 (33/33) (89.6-100.0)	100.0 (2/2) (34.2-100.0)	100.0 (33/33) (89.6-100.0)	100.0	100.0
RET	2	0	0	218	NO	100.0 (2/2) (34.2-100.0)	100.0 (218/218) (98.3-100.0)	100.0 (2/2) (34.2-100.0)	100.0 (218/218) (98.3-100.0)	-	-
TMPRSS2	1	0	0	34	YES	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0 (1/1) (20.7-100.0)	100.0 (34/34) (89.8->99.9)	100.0	100.0
TMPRSS2	6	0	1	213	NO	85.7 (6/7) (48.7-97.4)	100.0 (213/213) (98.2->99.9)	100.0 (6/6) (61.0-100.0)	99.5 (213/214) (97.4-99.9)	-	-

[1]Performance characteristics were adjusted for prevalence for characterized samples, and non-adjusted for uncharacterized samples.

2. Precision and Reproducibility

a. Precision

Two studies were conducted to evaluate within-laboratory precision for the TSO Comprehensive assay. Study 1 evaluated CDx variants (*NTRK* and RET fusions). Study 2 evaluated TMB.

CDx Precision

Within-laboratory precision was evaluated for *NTRK*1, *NTRK*2, and *NTRK*3 fusions in 6 FFPE samples from 5 tumor types (lower grade glioma, glioblastoma multiforme, colorectal cancer, myofibroblastic sarcoma, secretory breast cancer) and RET fusions in 4 samples from 3 tumor types (non-small cell lung cancer, thyroid cancer, and atypical Spitz tumor from skin tissue specimen). Each sample was tested at two variant levels: ~1x LoD (low variant level) and ~2–3x LoD (high variant level) with the exception of the sample harboring CCDC6-RET, which was only tested at the low variant level. Each of the samples at each test level was run in duplicates in each library preparation event across three (3) operators. Each operator started library preparation on three (3) non-consecutive start days and sequenced on three (3) designated NextSeq 550Dx instruments. Three (3) reagents lots were tested, generating 54 observations per sample per level.

Variant calling was evaluated separately for the two variant levels for a given variant from pooled observations across all variables (operators, reagent lots, instruments, days, and replicates). The percent positive calls (PPC) and percent negative calls (PNC) for *NTRK* fusions and associated two-sided 95% confidence interval (Wilson score) are summarized

in Table 21 (overall), and the PPC confidence interval (Wilson score) by operator and by lot are shown in Table 22 and Table 23, respectively. The percent positive calls (PPC) and percent negative calls (PNC) for RET fusions and associated two-sided 95% confidence interval (Wilson score) are summarized in Table 24 (overall), and the PPC confidence interval (Wilson score) by operator and by lot are shown in Table 25 and Table 26, respectively. It should be noted that some levels had fewer than 54 observations; these were due to invalid libraries that did not meet the necessary QC metrics (4 out of 1096 libraries failed, 0.4% invalid rate).

For *NTRK* fusions, at the higher variant level (~2 – 3x LoD), the TSO Comprehensive assay demonstrated 100% PPC for all fusions except one (LMNA-*NTRK*1 had 99.0% PPC). It should be noted that LMNA-*NTRK*1 at the high level had more than 54 observations; this was due to the preparation of two dilution mixtures targeting to the same variant level. Therefore, the observations were combined. At the low variant level (~1x LoD), the PPC for RNA fusions ranged from 94.44% to 100%. For variants with PPC < 95% (BCAN-*NTRK*1), the supporting reads were below the respective Limits of Detection (53.2 supporting reads for BCAN-*NTRK*1). 100% PNC was achieved for all variants at both levels.

Table 21. Overall Qualitative Results for *NTRK* Fusions

Variant Level	Fusion	Mean Supporting Reads	PPC (95% CI)	PNC (95% CI)
~1x LoD	TPM3-*NTRK*1	20.2	100.0 (54/54) (93.4, 100.0)	100.0 (537/537) (99.3, 100.0)
	BCAN-*NTRK*1	22.1	94.4 (51/54) (84.9, 98.1)	100.0 (591/591) (99.4, 100.0)
	LMNA-*NTRK*1	12.2	98.1 (51/52) (89.9, 99.7)	100.0 (539/539) (99.3, 100.0)
	ETV6-*NTRK*2	20.3	100.0 (54/54) (93.4, 100.0)	100.0 (591/591) (99.4, 100.0)
	ETV6-*NTRK*3	16.2	100.0 (54/54) (93.4, 100.0)	100.0 (537/537) (99.3, 100.0)
	ETV6-*NTRK*3 (FFPE cell line)	23.1	98.1 (53/54) (90.2, 99.7)	
	KANK1-*NTRK*3	13.5	100.0 (54/54) (93.4, 100.0)	100.0 (591/591) (99.4,100.0)
~2-3x LoD	TPM3-*NTRK*1	57.1	100.0 (54/54) (93.4, 100.0)	100.0 (481/481) (99.2, 100.0)
	BCAN-*NTRK*1	53.2	100.0 (54/54) (93.4, 100.0)	100.0 (535/535) (99.3, 100.0)
	LMNA-*NTRK*1	35.1	99.0 (103/104) (94.8, 99.8)	100.0 (431/431) (99.1, 100.0)

Variant Level	Fusion	Mean Supporting Reads	PPC (95% CI)	PNC (95% CI)
	ETV6-*NTRK*2	52.0	100.0 (54/54) (93.4, 100.0)	100.0 (535/535) (99.3, 100.0)
	ETV6-*NTRK*3	41.7	100.0 (54/54) (93.4, 100.0)	100.0 (481/481) (99.2, 100.0)
	ETV6-*NTRK*3 (FFPE cell line)	28.3	100.0 (54/54) (93.4, 100.0)	
	KANK1-*NTRK*3	39.2	100.0 (54/54) (93.4, 100.0)	100.0 (535/535) (99.3, 100.0)

Table 22. Qualitative Results for Targeted *NTRK* Fusions by Operator

Variant Level	Targeted Fusions	Operator	N	PPC (%) (n/N)	95% CI[1]
~1x LoD	TPM3-*NTRK*1	1	18	100.0 (18/18)	(82.4, 100.0)
		2	18	100.0 (18/18)	(82.4, 100.0)
		3	18	100.0 (18/18)	(82.4, 100.0)
	BCAN-*NTRK*1	1	18	100.0 (18/18)	(82.4, 100.0)
		2	18	83.3 (15/18)	(60.8, 94.2)
		3	18	100.0 (18/18)	(82.4, 100.0)
	LMNA-*NTRK*1	1	18	100.0 (18/18)	(82.4, 100.0)
		2	18	100.0 (18/18)	(82.4, 100.0)
		3	16	93.8 (15/16)	(71.7, 98.99)
	ETV6-*NTRK*2	1	18	100.0 (18/18)	(82.4, 100.0)
		2	18	100.0 (18/18)	(82.4, 100.0)
		3	18	100.0 (18/18)	(82.4, 100.0)
	ETV6-*NTRK*3	1	18	100.0 (18/18)	(82.4, 100.0)
		2	18	100.0 (18/18)	(82.4, 100.0)
		3	18	100.0 (18/18)	(82.4, 100.0)
	ETV6-*NTRK*3 (FFPE cell Line)	1	18	100.0 (18/18)	(82.4, 100.0)
		2	18	100.0 (18/18)	(82.4, 100.0)
		3	18	94.4 (17/18)	(74.2, 99.0)
	KANK1-*NTRK*3	1	18	100.0 (18/18)	(82.4, 100.0)
		2	18	100.0 (18/18)	(82.4, 100.0)
		3	18	100.0 (18/18)	(82.4, 100.0)
	Aggregate *NTRK* fusions (low level)		376	98.7 (371/376)	(96.9, 99.4)
~2-3x LoD	TPM3-*NTRK*1	1	18	100.0 (18/18)	(82.4, 100.0)
		2	18	100.0 (18/18)	(82.4, 100.0)
		3	18	100.0 (18/18)	(82.4, 100.0)
	BCAN-*NTRK*1	1	18	100.0 (18/18)	(82.4, 100.0)

Variant Level	Targeted Fusions	Operator	N	PPC (%) (n/N)	95% CI[1]
		2	18	100.0 (18/18)	(82.4, 100.0)
		3	18	100.0 (18/18)	(82.4, 100.0)
	LMNA-*NTRK*1[4]	1	36	97.2 (35/36)	(85.8, 99.5)
		2	32	100.0 (32/32)	(89.3, 100.0)
		3	36	100.0 (36/36)	(90.4, 100.0)
	ETV6-*NTRK*2	1	18	100.0 (18/18)	(82.4, 100.0)
		2	18	100.0 (18/18)	(82.4, 100.0)
		3	18	100.0 (18/18)	(82.4, 100.0)
	ETV6-*NTRK*3	1	18	100.0 (18/18)	(82.4, 100.0)
		2	18	100.0 (18/18)	(82.4, 100.0)
		3	18	100.0 (18/18)	(82.4, 100.0)
	ETV6-*NTRK*3 (FFPE cell Line)	1	18	100.0 (18/18)	(82.4, 100.0)
		2	18	100.0 (18/18)	(82.4, 100.0)
		3	18	100.0 (18/18)	(82.4, 100.0)
	KANK1-*NTRK*3	1	18	100.0 (18/18)	(82.4, 100.0)
		2	18	100.0 (18/18)	(82.4, 100.0)
		3	18	100.0 (18/18)	(82.4, 100.0)
	Aggregate *NTRK* fusions (high level)		428	99.8 (427/428)	(98.7, 100.0)

[1]: 2-sided 95% CI with Wilson Score test

.

Table 23. Qualitative Results for *NTRK* Fusions by Lot

Variant Level	Targeted Fusions	Lot[2]	N	PPC (%) (n/N)	95% CI[1]
~1x LoD	TPM3-*NTRK*1	M	18	100.0 (18/18)	(82.4, 100.0)
		PQ2	18	100.0 (18/18)	(82.4, 100.0)
		PQ3	18	100.0 (18/18)	(82.4, 100.0)
	BCAN-*NTRK*1	M	18	100.0 (18/18)	(82.4, 100.0)
		PQ2	18	94.4 (17/18)	(74.2, 99.0)
		PQ3	18	88.9 (16/18)	(67.2, 96.9)
	LMNA-*NTRK*1	IUO5	18	100.0 (18/18)	(82.4,100.0)
		IUO6	18	100.0 (18/18)	(82.4,100.0)
		IUO6.5	16	93.8(15/16)	(71.7, 98.9)
	ETV6-*NTRK*2	M	18	100.0 (18/18)	(82.4, 100.0)
		PQ2	18	100.0 (18/18)	(82.4, 100.0)
		PQ3	18	100.0 (18/18)	(82.4, 100.0)
	ETV6-*NTRK*3	M	18	100.0 (18/18)	(82.4, 100.0)
		PQ2	18	100.0 (18/18)	(82.4, 100.0)

Variant Level	Targeted Fusions	Lot[2]	N	PPC (%) (n/N)	95% CI[1]
		PQ3	18	100.0 (18/18)	(82.4, 100.0)
	ETV6-*NTRK*3 (FFPE cell Line)	M	18	100.0 (18/18)	(82.4, 100.0)
		PQ2	18	100.0 (18/18)	(82.4, 100.0)
		PQ3	18	94.4 (17/18)	(74.2, 99.0)
	KANK1-*NTRK*3	PQ1	18	100.0 (18/18)	(82.4, 100.0)
		PQ2	18	100.0 (18/18)	(82.4, 100.0)
		PQ3	18	100.0 (18/18)	(82.4, 100.0)
	Aggregate *NTRK* fusions (low level)		376	98.7 (371/376)	(96.9, 99.4)
~2-3x LoD	TPM3-*NTRK*1	M	18	100.0 (18/18)	(82.4, 100.0)
		PQ2	18	100.0 (18/18)	(82.4, 100.0)
		PQ3	18	100.0 (18/18)	(82.4, 100.0)
	BCAN-*NTRK*1	M	18	100.0 (18/18)	(82.4, 100.0)
		PQ2	18	100.0 (18/18)	(82.4, 100.0)
		PQ3	18	100.0 (18/18)	(82.4, 100.0)
	LMNA-*NTRK*1[4]	IUO5	36	97.2 (35/36)	(85.8, 99.5)
		IUO6	36	100.0 (36/36)	(90.4, 100.0)
		IUO6.5	32	100.0 (32/32)	(89.3, 100.0)
	ETV6-*NTRK*2	M	18	100.0 (18/18)	(82.4, 100.0)
		PQ2	18	100.0 (18/18)	(82.4, 100.0)
		PQ3	18	100.0 (18/18)	(82.4, 100.0)
	ETV6-*NTRK*3	M	18	100.0 (18/18)	(82.4, 100.0)
		PQ2	18	100.0 (18/18)	(82.4, 100.0)
		PQ3	18	100.0 (18/18)	(82.4, 100.0)
	ETV6-*NTRK*3 (FFPE cell Line)	M	18	100.0 (18/18)	(82.4, 100.0)
		PQ2	18	100.0 (18/18)	(82.4, 100.0)
		PQ3	18	100.0 (18/18)	(82.4, 100.0)
	KANK1-*NTRK*3	PQ1	18	100.0 (18/18)	(82.4, 100.0)
		PQ2	18	100.0 (18/18)	(82.4, 100.0)
		PQ3	18	100.0 (18/18)	(82.4, 100.0)

Variant Level	Targeted Fusions	Lot[2]	N	PPC (%) (n/N)	95% CI[1]
	Aggregate *NTRK* fusions (high level)		428	99.8 (427/428)	(98.7, 100.0)

[1]: 2-sided 95% CI with Wilson Score test
[2]: 3 waves of testing took place over course of study. 3 sets of unique lots were tested per wave (i.e Lots M, PQ2, and PQ3 for wave 1, Lots PQ1, PQ2 and PQ3 for wave 2 and Lots IUO5, IUO6 and IUO 6.5 for wave 3)

For RET fusions, at the higher variant level (~2 – 3x LoD), the TSO Comprehensive assay demonstrated 100% PPC for all fusions. It should be noted that KIF5B-RET at the low level had more than 54 observations; this was due to the preparation of two dilution mixtures targeting to the same variant level. Therefore, the observations were combined. At the low variant level (~1x LoD), the PPC for RET fusions ranged from 90.7% to 98.1%. For variants with PPC < 95% (NCOA4-RET), the supporting reads were below the respective Limits of Detection (15.8 supporting reads for NCOA4-RET). 100% PNC was achieved for all variants at both levels.

Table 24. Overall Qualitative Results for RET Fusions

Variant Level	Targeted Fusions	Mean Supporting Reads	PPC (n/N) (95% CI)	PNC (n/N) (95% CI)
~1x LoD	NCOA4-RET[1]	13.3	90.7 (49/54) (80.1, 96.0)	100.0 (537/537) (99.3, 100.0)
	CCDC6-RET[2]	18.7	98.1 (53/54) (90.2, 99.7)	100.0 (591/591) (99.4, 100.0)
	KIF5B-RET (sample 1)[3]	17.3	95.4 (103/108) (89.6, 98.0)	100.0 (430/430) (99.1, 100.0)
	KIF5B-RET (sample 2)[3]	17.3	96.2 (51/53) (87.2, 99.0)	
~3x LoD	NCOA4-RET[1]	24.8	100.0 (54/54) (93.4, 100.0)	100.0 (481/481) (99.2, 100.0)
	CCDC6-RET[2]	N/A	Not tested[4]	100.0 (589/589) (99.4, 100.0)
	KIF5B-RET (sample 1)[3]	43.8	100.0 (54/54) (93.4, 100.0)	100.0 (428/428) (99.1, 100.0)
	KIF5B-RET (sample 2)[3]	44.6	100.0 (53/53) (93.2, 100.0)	

[1]Thyroid tissue
[2]Atypical Spitz tumor
[3]Lung Tissue from NSCLC
[4]CCDC6-RET was tested at one level only (i.e., ~1x C95) due to insufficient materials.

Table 25. Qualitative Results for RET Fusions by Operator

Variant Level	Targeted Fusions	Operator[2]	N	PPC (n/N)	95% CI[1]
~1x LoD	NCOA4-RET	1	18	94.4 (17/18)	(74.2, 99.0)
		2	18	83.3 (15/18)	(60.8, 94.2)
		3	18	94.4 (17/18)	(74.2, 99.0)
	CCDC6-RET[3]	1	18	100.0 (18/18)	(82.4, 100.0)
		2	18	100.0 (18/18)	(82.4, 100.0)
		3	18	94.4 (17/18)	(74.2, 99.0)
	KIF5B-RET[4] (Sample ID 10755)	1	36	91.7 (33/36)	(78.2, 97.1)
		2	16	93.8 (15/16)	(71.7, 98.9)
		3	36	97.2 (35/36)	(85.8, 99.5)
		4	20	100.0 (20/20)	(83.9, 100.0)
	KIF5B-RET (Sample ID 10756)	1	18	94.4 (17/18)	(74.2, 99.0)
		2	7	100.0 (7/7)	(64.6, 100.0)
		3	18	94.4 (17/18)	(74.2, 99.0)
		4	10	100.0 (10/10)	(72.2, 100.0)
Aggregate RET fusions (low level)			269	95.2 (256/269)	(91.9, 97.2)
~3x LoD	NCOA4-RET	1	18	100.0 (18/18)	(82.4, 100.0)
		2	18	100.0 (18/18)	(82.4, 100.0)
		3	18	100.0 (18/18)	(82.4, 100.0)
	KIF5B-RET (Sample ID 10755)	1	18	100.0 (18/18)	(82.4, 100.0)
		2	8	100.0 (8/8)	(67.6, 100.0)
		3	18	100.0 (18/18)	(82.4, 100.0)
		4	10	100.0 (10/10)	(72.2, 100.0)
	KIF5B-RET (Sample ID 10756)	1	18	100.0 (18/18)	(82.4, 100.0)
		2	7	100.0 (7/7)	(64.6, 100.0)
		3	18	100.0 (18/18)	(82.4, 100.0)
		4	10	100.0 (10/10)	(72.2, 100.0)
Aggregate RET Fusions (high level)			161	100.0 (161/161)	(97.7, 100.0)

[1]: 2-sided 95% CI with Wilson Score test.
[2]: For the RNA fusions KIF5B-RET (Sample ID 10755) and KIF5B-RET (Sample ID 10756) operator 4 replaced operator 2 for 10 of the 18 replicates and 20 of the 36 replicates for high and low levels, respectively for testing with lots IUO6 and IUO6.5.
[3]: CCDC6-RET was tested at one level only (i.e., ~1x C95) due to insufficient materials.
[4]: Two dilution mixtures for KIF5B-RET (sample 10755) "low" level and LMNA-*NTRK*1 (sample 19219) "high" level were tested. The two dilution mixtures having slightly different dilution-fold in the normal diluent were intended to target to the same variant level. Since the observed mean supporting reads for both mixtures were comparable, therefore, the observations from both mixtures were combined for analysis.

Table 26. Qualitative Results for RET Fusions by Lot

Variant Level	Targeted Fusions	Lot[2]	N	PPC (n/N)	95% CI[1]
~1x LoD	NCOA4-RET	M	18	83.3 (15/18)	(60.8, 94.2)
		PQ2	18	94.4 (17/18)	(74.2, 99.0)
		PQ3	18	94.4 (17/18)	(74.2, 99.0)
	CCDC6-RET[3]	M	18	94.4 (17/18)	(74.2, 99.0)
		PQ2	18	100.0 (18/18)	(82.4, 100.0)
		PQ3	18	100.0 (18/18)	(82.4, 100.0)
	KIF5B-RET[4] (Sample ID 10755)	IUO5	36	91.7 (33/36)	(78.2, 97.1)
		IUO6	36	97.2 (35/36)	(85.8, 99.5)
		IUO6.5	36	97.2 (35/36)	(85.8, 99.5)
	KIF5B-RET (Sample ID 10756)	IUO5	17	94.1 (16/17)	(73.0, 99.0)
		IUO6	18	94.4 (17/18)	(74.2, 99.0)
		IUO6.5	18	100.0 (18/18)	(82.4, 100.0)
Aggregate RET fusions (low level)			269	95.2 (256/269)	(91.9, 97.2)
~3x LoD	NCOA4-RET	M	18	100.0 (18/18)	(82.4, 100.0)
		PQ2	18	100.0 (18/18)	(82.4, 100.0)
		PQ3	18	100.0 (18/18)	(82.4, 100.0)
	KIF5B-RET (Sample ID 10755)	IUO5	18	100.0 (18/18)	(82.4, 100.0)
		IUO6	18	100.0 (18/18)	(82.4, 100.0)
		IUO6.5	18	100.0 (18/18)	(82.4, 100.0)
	KIF5B-RET (Sample ID 10756)	IUO5	17	100.0 (17/17)	(81.6, 100.0)
		IUO6	18	100.0 (18/18)	(82.4, 100.0)
		IUO6.5	18	100.0 (18/18)	(82.4, 100.0)
Aggregate RET fusions (low level)			161	100.0 (161/161)	(97.7, 100.0)

[1]: 2-sided 95% CI with Wilson Score test
[2]: 3 waves of testing took place over course of study. 3 sets of unique lots were tested per wave (i.e Lots M, PQ2, and PQ3 for wave 1, Lots PQ1, PQ2 and PQ3 for wave 2 and Lots IUO5, IUO6 and IUO 6.5 for wave 3)
[3]: CCDC6-RET was tested at one level only (i.e., ~1x C95) due to insufficient materials.
[4]: Two dilution mixtures for KIF5B-RET (sample 10755) "low" level and LMNA-*NTRK*1 (sample 19219) "high" level were tested. The two dilution mixtures having slightly different dilution-fold in the normal diluent were intended to target to the same variant level. Since the observed mean supporting reads for both mixtures were comparable, therefore, the observations from both mixtures were combined for analysis.

Restricted maximum likelihood (REML) variance components analysis was performed to evaluate total variation of the underlying continuous variable (supporting reads for RNA fusions) and estimate the components of precision [standard deviation (SD), coefficient of variation (CV)] for each source of variation [operators, instruments, days, reagent lots, residual and total]. The results are presented in Table 27 for *NTRK* fusions and Table 28 for RET fusions. The residual component was the largest contributor to total variance for both small DNA variants and RNA fusions at both levels, supporting the conclusion that

detection of these variants by TSO Comprehensive assay is robust to operators, lots, instruments, and days.

Table 27. SD and CV Results for *NTRK* Fusions

Supporting Reads Level	Fusion	N Valid Attempts	Mean Supporting Reads	Operator SD (%CV)	Instrument SD (%CV)	Lot SD (%CV)	Day SD (%CV)	Residual SD (%CV)	Total SD (%CV)
~1x LoD	TPM3-*NTRK*1	54	20.2	2.33 (12)	0.94 (5)	3.31 (16)	0.83 (4)	5.70 (28)	7.10 (35)
	BCAN-*NTRK*1	54	22.1	3.38 (15)	1.41 (6)	1.78 (8)	0.00 (0)	6.03 (27)	7.28 (33)
	LMNA-*NTRK*1	52	12.2	1.36 (11)	1.25 (10)	1.59 (13)	0.00 (0)	4.74 (39)	5.33 (44)
	ETV6-*NTRK*2	54	20.3	0.00 (0)	3.18 (16)	4.36 (21)	0.00 (0)	8.30 (41)	9.90 (49)
	ETV6-*NTRK*3	54	16.2	2.28 (14)	2.36 (15)	2.17 (13)	0.00 (0)	4.65 (29)	6.10 (38)
	ETV6-*NTRK*3 (cell line)	54	23.1	4.55 (20)	1.18 (5)	0.00 (0)	0.00 (0)	6.73 (29)	8.21 (36)
	KANK1-*NTRK*3	54	13.5	0.74 (5)	0.11 (1)	1.09 (8)	0.00 (0)	4.22 (31)	4.42 (33)
2~3x LoD	TPM3-*NTRK*1	54	57.1	11.21 (20)	1.18 (2)	5.68 (10)	2.03 (4)	11.86 (21)	17.44 (31)
	BCAN-*NTRK*1	54	53.2	8.22 (15)	0.76 (1)	5.59 (11)	2.89 (5)	11.34 (21)	15.37 (29)
	LMNA-*NTRK*1	104	35.1	1.47 (4)	5.92 (17)	8.11 (23)	2.92 (8)	10.69 (30)	15.03 (43)
	ETV6-*NTRK*2	54	52	0.00 (0)	4.07 (8)	7.07 (14)	5.72 (11)	12.91 (25)	16.31 (31)
	ETV6-*NTRK*3	54	41.7	7.16 (17)	0.40 (1)	6.40 (15)	0.00 (0)	10.74 (26)	14.41 (35)
	ETV6-*NTRK*3 (cell line)	54	28.3	7.93 (28)	1.02 (4)	0.00 (0)	0.00 (0)	9.05 (32)	12.08 (43)
	KANK1-*NTRK*3	54	39.2	5.10 (13)	0.00 (0)	4.78 (12)	0.00 (0)	9.44 (24)	11.74 (30)

%CV: Percent coefficient of variation.
SD: Standard deviation.

Table 28. SD and CV Results for Targeted RET Fusions

Supporting Reads Level	Fusion	N Valid Attempts	Mean Supporting	Operator SD (%CV)	Instrument SD (%CV)	Lot SD (%CV)	Day SD (%CV)	Residual SD (%CV)	Total SD (%CV)
~1x LoD	NCOA4-RET	54	13.3	1.67 (13)	0.00 (0)	0.00 (0)	1.67 (13)	5.09 (38)	5.61 (42)
	CCDC6-RET	54	18.7	0.00 (0)	1.14 (6)	5.44 (29)	0.00 (0)	6.17 (33)	8.30 (44)
	KIF5B-RET (Sample 1)	108	17.3	2.11 (12)	2.50 (14)	2.89 (17)	3.52 (20)	7.09 (41)	9.04 (52)
	KIF5B-RET (Sample 2)	53	17.3	2.05 (12)	3.72 (22)	3.65 (21)	2.41 (14)	5.95 (34)	8.52 (49)
~2-3x LoD	NCOA4-RET[1]	54	24.8	3.05 (12)	0.00 (0)	5.92 (24)	0.00 (0)	6.78 (27)	9.50 (38)
	KIF5B-RET (Sample 1)	54	43.8	4.15 (9)	0.96 (2)	12.57 (29)	6.52 (15)	15.23 (35)	21.23 (48)
	KIF5B-RET (Sample 2)	53	44.6	5.37 (12)	4.97 (11)	13.73 (31)	0.00 (0)	12.41 (28)	19.90 (45)

%CV: Percent coefficient of variation.
SD: Standard deviation.

Additionally, to support the precision for small DNA variants, Table 29 below provides a summary of precision data from non-targeted small DNA variants in Study 1. In summary, 2,760 unique small DNA variants, which is made up of 2605 SNVs, 15 MNVs, 68 deletions, and 72 insertions, from 6 unique FFPE samples, were analyzed, with all variant types having a PPA/NPA of >98%.

Table 29. Precision Data from Non-targeted variants

Variant Class		Number of Variant-Level-Sample Sets[1]	PPA (n/N) 95% CI
SNVs	18131	98.93% (754,519/762,705)	(98.9%, 98.95%)
MNVs	94	99.01% (3989/4029)	(98.65%, 99.27%)
Deletions	428	99.10% (14,612/14,744)	(98.94%, 99.24%)
Insertions	19013	98.43% (17,002/17,273)	(98.23%, 98.61%)
Variant Class		**Number of Variant-Level-Sample Sets[1]**	**NPA (n/N) 95% CI**
SNVs	12607	99.08% (504,226/508,915)	(99.05%, 99.1%)
MNVs	80	99.26% (3207/3231)	(98.9%, 99.5%)
Deletions	409	98.63% (15,804/16,023)	_98.44%, 98.8%
Insertions	377	98.55% (13,556/13,755)	98.34%, 98.74%

[1]Variants were counted based on sample-level combinations and not based on unique variants across samples and levels.

TMB Precision

Five (5) NSCLC FFPE DNA samples for TMB were used to evaluate precision at different levels across a range of scores. Each of the samples was run in duplicate across three (3) operators, three (3) days, with three (3) library preparations for three (3) reagent lots using three NextSeq 550Dx instruments generating 54 observations per level. Some levels had fewer than 54 observations due to invalid libraries that did not meet the necessary QC metrics (4 out of 270 libraries failed, invalid rate of 1.5%).

The total variation of TMB score, along with the contribution by source (instruments, operators, lots, days, and residual), was quantified using a variance components model across a range of scores. The standard deviation (SD) and coefficient of variation (CV) are presented in Table 21 for TMB by level. In summary, the residual component remained the largest contributor to total variance for TMB scores.

Table 30. Quantitative TMB Score SD and CV Results

Level	Mean TMB Score	N Valid Attempts	Operator SD (%CV)	Instrument SD (%CV)	Lot SD (%CV)	Day SD (%CV)	Residual SD (%CV)	Total SD (%CV)
L1	0.3	52	0.00 (0)	0.06 (23)	0.00 (0)	0.08 (30)	0.40 (146)	0.41 (151)

Level	Mean TMB Score	N Valid Attempts	Operator SD (%CV)	Instrument SD (%CV)	Lot SD (%CV)	Day SD (%CV)	Residual SD (%CV)	Total SD (%CV)
L2	8.4	53	0.00 (0)	0.14 (2)	0.00 (0)	0.00 (0)	0.71 (8)	0.73 (9)
L3	15.1	54	0.00 (0)	0.00 (0)	0.20 (1)	0.00 (0)	1.16 (8)	1.18 (8)
L4	20.3	53	0.00 (0)	0.00 (0)	0.06 (0)	0.00 (0)	0.56 (3)	0.57 (3)
L5	42.3	54	0.00 (0)	0.00 (0)	0.15 (0)	0.00 (0)	1.37 (3)	1.38 (3)

b. Reproducibility

Two studies were conducted to evaluate Reproducibility for the TSO Comprehensive assay. Study 1 evaluated CDx variants (*NTRK* and RET RNA fusions). Study 2 evaluated tumor profiling variants (small DNA variants, TMB, non-CDx RNA fusions, and RNA splice variants).

CDx Reproducibility

Reproducibility of the TSO Comprehensive assay across 3 testing sites (1 internal, 2 external). At each site, each operator (2) tested the panel members in duplicate over the course of 3 non-consecutive testing days, generating 6 observations per target per panel member. In total, 36 observations were generated per panel member (3 sites/instruments × 2 operators × 3 start days × 2 within-run replicates).

Testing was conducted with a panel that included 7 members from FFPE samples in 3 different tissue types (brain, soft tissue, and breast) and 5 members from 3 cell lines containing *NTRK*1–3 RNA fusion variants, and 4 members from FFPE samples in 3 different tissue types (papillary thyroid, unclassified thyroid, and lung) containing RET RNA fusion variants. Panel members were tested at both low (~1x LoD) and high (2-3x LoD) variant levels.

Percent positive calls (PPC) and percent negative calls (PNC) for targeted RNA fusion variants, at both the high and low levels, were determined. Two-sided 95% confidence intervals (CIs) associated were also calculated using the Wilson score method. Analyses were performed to estimate PPC and PNC (with associated 95% CIs) in the targeted high-level and low-level panel members by combining TSO Comprehensive assay observations for a given target in a group of panel members representing the applicable RNA fusions across sites/instruments, operators, and runs. For each targeted variant, TSO Comprehensive assay observations in other panel members at the high level targeted for the same variant type, but not containing the same variant as determined by the majority rule, were combined to calculated PNC. It should be noted that some levels had fewer than 36 observations; these were due to invalid libraries that did not meet the necessary QC metrics (4 out of 576 libraries failed, invalid rate of 0.7%).

For the *NTRK* fusion panel members at 2-3x LoD, PPC values ranged from 94.4% to 100.0%, with the lowest PPC value being the BCAN-*NTRK*1 panel member (PPC = 94.4% [34/36; 95% CI: 81.9% to 98.5%]), which was below the respective LoD (53.1 supporting reads), causing several discordant samples at both the low and high levels (Table 31). PNC values for the high-level *NTRK* fusion panel members were all 100.0%. For the low-level *NTRK* fusion panel members, PPC values ranged from 80.6% (BCAN-*NTRK*1) to 100.0%, and all PNC values were 100.0%. Further, none of the individual sites showed any additional discordance (Table 32).

Table 31. Overall PPC of TSO Comprehensive Assay for Detection of *NTRK* Fusions in High- and Low-Level Targeted Panel Members

Variant Level	Targeted Fusion	n	Mean Supporting Reads	PPC (%) (n/N)	95% CI[1]
~2-3x LoD	LMNA-*NTRK*1	36	37.9	100.0 (36/36)	(90.4, 100.0)
	BCAN-*NTRK*1	36	33.6	94.4 (34/36)	(81.9, 98.5)
	ETV6-*NTRK*2	36	24.6	100.0 (36/36)	(90.4, 100.0)
	TRIM24-*NTRK*2[2]	36	36.6	100.0 (36/36)	(90.4, 100.0)
	ETV6-*NTRK*3	36	56.4	100.0 (36/36)	(90.4, 100.0)
	BTBD1-*NTRK*3	35	32.9	100.0 (35/35)	(90.1, 100.0)
~1x LoD	LMNA-*NTRK*1	36	13.8	94.4 (34/36)	(81.9, 98.5)
	BCAN-*NTRK*1	36	16.9	80.6 (29/36)	(65.0, 90.2)
	ETV6-*NTRK*2	35	15.2	94.3 (33/35)	(81.4, 98.4)
	STRN-*NTRK*2[2]	36	13.6	100.0 (36/36)	(90.4, 100.0)
	ETV6-*NTRK*3	36	24.8	100.0 (36/36)	(90.4, 100.0)
	BTBD1-*NTRK*3	36	18.1	100.0 (36/36)	(90.4, 100.0)

[1]95% 2-sided confidence interval (CI) calculated via the Wilson Score method.
[2]2 different fusions (STRN-*NTRK*2 and TRIM24-*NTRK*2) were used for the low- and high-level panel members representing *NTRK*2 fusions due to limited *NTRK*2 fusion positive FFPE tissue.

Table 32. PPC of TSO Comprehensive Assay for Detection of *NTRK* Fusions in High- and Low-Level Targeted Panel Members by Site

Variant Level	Targeted Fusions	Site	n	PPC (%) (n/N)	95% CI [1]
~1x LoD	LMNA-*NTRK*1	S1	12	100.0 (12/12)	(75.8, 100.0)
		S2	12	100.0 (12/12)	(75.8, 100.0)
		S3	12	83.3 (10/12)	(55.2, 95.3)
	BCAN-*NTRK*1	S1	12	100.0 (12/12)	(75.8, 100.0)
		S2	12	83.3 (10/12)	(55.2, 95.3)
		S3	12	58.3 (7/12)	(31.9, 80.7)
	ETV6-*NTRK*2	S1	12	100.0 (12/12)	(75.8, 100.0)

Variant Level	Targeted Fusions	Site	n	PPC (%) (n/N)	95% CI [1]
		S2	11	100.0 (11/11)	(74.1, 100.0)
		S3	12	83.3 (10/12)	(55.2, 95.3)
	STRN-*NTRK*2	S1	12	100.0 (12/12)	(75.8, 100.0)
		S2	12	100.0 (12/12)	(75.8, 100.0)
		S3	12	100.0 (12/12)	(75.8, 100.0)
	ETV6-*NTRK*3	S1	12	100.0 (12/12)	(75.8, 100.0)
		S2	12	100.0 (12/12)	(75.8, 100.0)
		S3	12	100.0 (12/12)	(75.8, 100.0)
	BTBD1-*NTRK*3	S1	12	100.0 (12/12)	(75.8, 100.0)
		S2	12	100.0 (12/12)	(75.8, 100.0)
		S3	12	100.0 (12/12)	(75.8, 100.0)
~2-3x LoD	LMNA-*NTRK*1	S1	12	100.0 (12/12)	(75.8, 100.0)
		S2	12	100.0 (12/12)	(75.8, 100.0)
		S3	12	100.0 (12/12)	(75.8, 100.0)
	BCAN-*NTRK*1	S1	12	100.0 (12/12)	(75.8, 100.0)
		S2	12	91.7 (11/12)	(64.6, 98.5)
		S3	12	91.7 (11/12)	(64.6, 98.5)
	ETV6-*NTRK*2	S1	12	100.0 (12/12)	(75.8, 100.0)
		S2	12	100.0 (12/12)	(75.8, 100.0)
		S3	12	100.0 (12/12)	(75.8, 100.0)
	TRIM24-*NTRK*2	S1	12	100.0 (12/12)	(75.8, 100.0)
		S2	12	100.0 (12/12)	(75.8, 100.0)
		S3	12	100.0 (12/12)	(75.8, 100.0)
	ETV6-*NTRK*3	S1	12	100.0 (12/12)	(75.8, 100.0)
		S2	12	100.0 (12/12)	(75.8, 100.0)
		S3	12	100.0 (12/12)	(75.8, 100.0)
	BTBD1-*NTRK*3	S1	12	100.0 (12/12)	(75.8, 100.0)
		S2	11	100.0 (11/11)	(74.1, 100.0)
		S3	12	100.0 (12/12)	(75.8, 100.0)

[1]95% 2-sided confidence interval (CI) calculated via the Wilson Score method

Table 33. Overall PNC of TSO Comprehensive Assay for Detection of *NTRK* Fusions in High- and Low-Level Targeted Panel Members

Variant Level	Targeted Fusions	n[1]	PNC (%) (n/N)	95% CI [2]
~2-3 LoD	LMNA-*NTRK*1	180	100.0 (180/180)	(97.9, 100.0)
	BCAN-*NTRK*1	251	100.0 (251/251)	(98.5, 100.0)
	ETV6-*NTRK*2	251	100.0 (251/251)	(98.5, 100.0)
	TRIM24-*NTRK*2	216	100.0 (216/216)	(98.2, 100.0)
	ETV6-*NTRK*3	144	100.0 (144/144)	(97.4, 100.0)
	BTBD1-*NTRK*3	216	100.0 (216/216)	(98.2, 100.0)
~1x LoD	LMNA-*NTRK*1	213	100.0 (213/213)	(98.2, 100.0)
	BCAN-*NTRK*1	249	100.0 (249/249)	(98.5, 100.0)
	ETV6-*NTRK*2	250	100.0 (250/250)	(98.5, 100.0)
	STRN-*NTRK*2	249	100.0 (249/249)	(98.5, 100.0)
	ETV6-*NTRK*3	177	100.0 (177/177)	(97.9, 100.0)
	BTBD1-*NTRK*3	249	100.0 (249/249)	(98.5, 100.0)

[1]All observations pooled from panel member-variant combinations for which the majority call is negative, ie. targeted variants harboring fusions with less than 50% calls positive.
[2]95% 2-sided confidence interval (CI) calculated via the Wilson Score method.

For the RET fusion panel members at 2-3x LoD, PPC and PNC values were 100.0% (Table 34 and Table 36). For the low-level RET fusion panel members, PPC values were 97.2% and 97.1%, and PNC values were both 100.0%. Further, none of the individual sites showed any additional discordance (Table 35).

Table 34. Overall PPC of TSO Comprehensive Assay for Detection of RET Fusions in High- and Low-Level Targeted Panel Members

Variant Level	Targeted Fusion	n	Mean Supporting Reads	PPC (%) (n/N)	95% CI[1]
~1x LoD	NCOA4-RET[3]	36	15.8	97.2 (35/36)	(85.8, 99.5)
	KIF5B-RET[2,4]	34	16.6	97.1 (33/34)	(85.1, 99.5)
~2-3x LoD	NCOA4-RET[3]	36	36.7	100.0 (36/36)	(90.4, 100.0)
	CCDC6-RET[2,3]	36	33.4	100.0 (36/36)	(90.4, 100.0)

[1]95% 2-sided confidence interval (CI) calculated via the Wilson Score method.
[2]2 different fusions (KIF5B-RET and CCDC6-RET) were used for low- and high-level panel members due to sample insufficiency.
[3]Thyroid tissue
[4]Lung tissue from NSCLC

Table 35. PPC of TSO Comprehensive Assay for Detection of RET Fusions in High- and Low-Level Targeted Panel Members by Site

Variant Level	Targeted Fusions	Site	n	PPC (%) (n/N)	95% CI [1]
~1x LoD	NCOA4-RET	S1	12	100.0 (12/12)	(75.8, 100.0)
		S2	12	100.0 (12/12)	(75.8, 100.0)
		S3	12	91.7 (11/12)	(64.6, 98.5)
	KIF5B-RET	S1	11	100.0 (11/11)	(74.1, 100.0)
		S2	11	90.9 (10/11)	(62.3, 98.4)
		S3	12	100.0 (12/12)	(75.8, 100.0)
~2-3x LoD	NCOA4-RET	S1	12	100.0 (12/12)	(75.8, 100.0)
		S2	12	100.0 (12/12)	(75.8, 100.0)
		S3	12	100.0 (12/12)	(75.8, 100.0)
	CCDC6-RET	S1	12	100.0 (12/12)	(75.8, 100.0)
		S2	12	100.0 (12/12)	(75.8, 100.0)
		S3	12	100.0 (12/12)	(75.8, 100.0)

[1]95% 2-sided confidence interval (CI) calculated via the Wilson Score method.

Table 36. Overall PNC of TSO Comprehensive Assay for Detection of RET Fusions in High- and Low-Level Targeted Panel Members

Variant Level	Targeted Fusions	n[1]	PNC (%) (n/N)	95% CI [2]
~1x LoD	NCOA4-RET	213	100.0% (213/213)	(98.2%, 100.0%)
	KIF5B-RET	251	100.0% (251/251)	(98.5%, 100.0%)
~2-3x LoD	NCOA4-RET	215	100.0% (215/215)	(98.2%, 100.0%)
	CCDC6-RET	251	100.0% (251/251)	(98.5%, 100.0%)

[1]All observations pooled from panel member-variant combinations for which the majority call is negative, i.e., targeted variants harboring fusions with less than 50% calls positive.
[2]95% 2-sided confidence interval (CI) calculated via the Wilson Score method.

Table 37 (*NTRK* Fusions) and Table 38 (RET Fusions) shows the variance components analysis of supporting reads across the approximately 36 observations within each targeted fusion. The SD and %CV (total and for each source) were calculated and presented for each targeted fusion.

Table 37. Variance Components Analysis of Supporting Reads *NTRK* Fusion Panel Members

Variant Level	Fusion	n	Mean Supporting Reads	Site SD (%CV)	Operator SD (%CV)	Day SD (%CV)	Replicate SD (%CV)	Total SD (%CV)
~2-3x LoD	LMNA-*NTRK*1	36	37.9	3.52 (9)	3.37 (9)	6.93 (18)	9.04 (24)	12.39 (33)
	BCAN-*NTRK*1	36	33.6	13.75 (41)	7.87 (23)	5.40 (16)	8.95 (27)	18.98 (57)
	ETV6-*NTRK*2	36	24.6	8.03 (33)	3.50 (14)	4.20 (17)	4.86 (20)	10.86 (44)

Variant Level	Fusion	n	Mean Supporting Reads	Site SD (%CV)	Operator SD (%CV)	Day SD (%CV)	Replicate SD (%CV)	Total SD (%CV)
	TRIM24-*NTRK2*	36	36.6	11.44 (31)	4.24 (12)	6.82 (19)	6.87 (19)	15.57 (43)
	ETV6-*NTRK3*	36	56.4	11.49 (20)	10.20 (18)	9.25 (16)	8.69 (15)	19.93 (35)
	BTBD1-*NTRK3*	35	32.9	1.49 (5)	2.65 (8)	2.16 (7)	10.47 (32)	11.11 (34)
~1x LoD	LMNA-*NTRK1*	36	13.8	1.79 (13)	0.00 (0)	2.74 (20)	4.37 (32)	5.47 (40)
	BCAN-*NTRK1*	36	16.9	2.92 (17)	2.98 (18)	4.61 (27)	5.82 (34)	8.52 (50)
	ETV6-*NTRK2*	35	15.2	0.00 (0)	3.41 (22)	3.83 (25)	4.39 (29)	6.75 (45)
	STRN-*NTRK2*	36	13.6	1.77 (13)	0.61 (5)	2.33 (17)	2.57 (19)	3.95 (29)
	ETV6-*NTRK3*	36	24.8	6.03 (24)	3.46 (14)	0.00 (0)	6.39 (26)	9.44 (38)
	BTBD1-*NTRK3*	36	18.1	0.93 (5)	0.00 (0)	0.00 (0)	6.64 (37)	6.71 (37)

%CV: Percent coefficient of variation.
SD: Standard deviation.

Table 38. Variance Components Analysis of Supporting Reads in Targeted RET Fusion Panel Member

Variant Level	Fusion	n	Mean Supporting Reads	Site SD (%CV)	Operator SD (%CV)	Day SD (%CV)	Replicate SD (%CV)	Total SD (%CV)
~1x LoD	NCOA4-RET	36	15.8	2.08 (13)	1.03 (7)	0.00 (0)	5.11 (32)	5.61 (36)
	KIF5B-RET	34	16.6	2.07 (12)	0.00 (0)	1.58 (10)	5.83 (35)	6.39 (39)
~2-3x LoD	NCOA4-RET	36	36.7	4.64 (13)	4.09 (11)	6.17 (17)	5.20 (14)	10.17 (28)
	CCDC6-RET	36	33.4	7.25 (22)	2.56 (8)	6.53 (20)	5.51 (16)	11.49 (34)

%CV: Percent coefficient of variation.
SD: Standard deviation.

Panel-wide Reproducibility

A second study was performed to assess the reproducibility of the TSO Comprehensive assay across 3 testing sites (2 external and 1 internal) to assess panel wide reproducibility for tumor profiling. At each site, each operator (2) using single instrument tested panel members in duplicate using 4 unique reagent lots over the course of 4 non-consecutive testing days, generating 8 observations per target per panel member. In total, 48 observations were generated per panel member (3 sites x 2 operators/instruments x 4 lots x 2 sequencing runs).

Testing was conducted using extracted DNA and RNA samples from FFPE tissue specimens and 1 FFPE cell line (with 1 FFPE tissue specimen and the FFPE cell line used to create 2 panel members each). Specimens by tumor type are shown in Table 39.

Table 39. Number of Specimens per tumor type in Panel-Wide Study

Tumor Type	Number of Specimens
Bladder	1
Bone	1
Brain	1
Breast	3
Colon	6
FFPE Cell Line	1
Jejunum	1
Kidney	1
Liver	1
Lung-NOS	2
Lung-NSCLC	4
Ovary	2
Prostate	4
Skin	1
Soft Tissue	3
Stomach	2
Thyroid	1
Unknown	4
Uterus	1

A total of 42 panel members were tested including DNA panel members with small DNA variants (SNVs, MNVs, insertions, and deletions), different TMB scores, and RNA panel members with fusions and splice variants that cover the 24 fusion genes and the EGFRvIII splice variant in the tumor profiling claims. Variants for which a sample was selected were targeted to a specified level by mixing the nucleic acid from the sample with nucleic acid from normal tissue. Both targeted and non-targeted variants were included in the analysis. Mean observed variant levels were categorized as <2×LoD, ~2–3×LoD, or >3×LoD.

PPC for small DNA variants and RNA variants were calculated by combining observations across sequencing runs and sites. PNC were similarly calculated for small DNA variants and RNA variants. For each targeted variant, TSO Comprehensive assay observations in panel members of the same variant type but containing other variants, not derived from the same source specimen, nor meeting the majority rule for that variant (< 50% of calls were positive) were combined across sites, operators/instruments, days, reagent lots, and sequencing runs to calculate PNC. Two-sided 95% confidence intervals (CIs) were calculated using the Wilson score method. It should be noted that some levels had fewer than 48 observations; unless otherwise stated, these were due to invalid libraries that did not meet the necessary QC metrics (191 out of 996 DNA libraries failed, invalid rate of 19.2%; 20 out of 1056 RNA libraries failed, invalid rate of 1.9%).

Small DNA Variants

Table 40 (overall) and Table 41 (by site) shows PPCs for targeted small DNA variants. PPCs ranged from 91.3% to 100% for the majority of small DNA variants. PNCs were 100% across small DNA variants. In addition, a summary of panel-wide precision by specimen and variant is shown in Appendix 3.

Table 40. Overall PPC of TSO Comprehensive Assay for Detection of Small DNA Variants in Combined Targeted Panel Members

Observed Variant Level[1]	Variant Type	Targeted Variant (nucleotide)	Targeted Variant (amino acid)	Mean VAF (%)[2]	PPC (%) (n/N)	95% CI[3]
<2xLOD	INSERTION	chr1_27024001_C_CG	ARID1A Q372fs*28	8.4	100.0 (4/4)[4]	(51.0, 100.0)
	INSERTION	chr17_7578470_C_CGGGCGG	TP53 P152_P153dup	15.7	100.0 (2/2)[5]	(34.2, 100.0)
~2-3xLOD	DELETION	chr5_112175751_CT_C	APC L1488fsTer19	18.1	100.0 (28/28)	(87.9, 100.0)
	DELETION	chr5_112175675_AAG_A	APC S1465WfsTer3	16.6	100.0 (40/40)	(91.2, 100.0)
	INSERTION	chr5_112175951_G_GA	APC T1556NfsTer3	22.7	100.0 (32/32)	(89.3, 100.0)
	INSERTION	chr5_112175675_A_AAG	APC S1465fs*9	10.0	100.0 (48/48)	(92.6, 100.0)
	SNV	chr7_140453136_A_T	BRAF V600E	4.5	91.3 (42/46)[6]	(79.7, 96.6)
	DELETION	chr7_55242465_GGAATTAAGAGAAGCA_G	EGFR E746_A750del	11.2	100.0 (46/46)	(92.3, 100.0)
	SNV	chr7_55259515_T_G	EGFR L858R	4.5	100.0 (38/38)	(90.8, 100.0)
	DELETION	chr22_41574678_GC_G	EP300 H2324fs*29	24.5	100.0 (44/44)	(92.0, 100.0)

Observed Variant Level[1]	Variant Type	Targeted Variant (nucleotide)	Targeted Variant (amino acid)	Mean VAF (%) [2]	PPC (%) (n/N)	95% CI[3]
	INSERTION	chr17_37880981_A_AGCATACGTGATG	ERBB2 Y772_A775dup	7.5	100.0 (36/36)	(90.4, 100.0)
	SNV	chr2_209113112_C_T	IDH1 R132H	15.5	100.0 (36/36)	(90.4, 100.0)
	MNV	chr12_25398284_CC_AT	KRAS G12I	11.1	100.0 (38/38)	(90.8, 100.0)
	INSERTION	chr9_139399350_C_CG	NOTCH1 R1598fs*12	14.6	100.0 (48/48)	(92.6, 100.0)
	DELETION	chr10_89720798_GTACT_G	PTEN T319fs*1	15.7	100.0 (44/44)	(92.0, 100.0)
	INSERTION	chr17_7574029_C_CGGAT	TP53 R333HfsTer5	15.4	100.0 (48/48)	(92.6, 100.0)
>3x LoD	MNV	chr7_140453136_AC_TT	BRAF V600K	13.0	100.0 (46/46)	(92.3, 100.0)
	INSERTION	chr4_153332910_C_CAGG	FBXW7 T15_G16insP	13.0	100.0 (44/44)	(92.0, 100.0)
	DELETION	chr5_112175751_CT_C	APC L1488fs*18	15.0	100.0 (46/46)	(92.3, 100.0)
	DELETION	chr17_7574002_CG_C	TP53 R342fs*3	15.8	100.0 (44/44)	(92.0, 100.0)

[1]Variant level calculated from mean observed variant allele frequency.
[2]Mean variant allele frequency calculated from observed assay results.
[3]95% two-sided confidence interval calculated via the Wilson Score Method.
[4]This sample with this variant (TPSAD6) had only 4 valid observations at 1 site due to numerous failed libraries (n=60) due to sample quality; as such, they failed several DNA QC metrics
[5]The sample with this variant (TPSBD1) had only 2 valid observations at 1 site due to numerous failed libraries (n=60) due to sample quality; as such, they failed several DNA QC metrics
[6]BRAF V600E had 4 discordant observations due to a low VAF in those replicates (>0.02)

Table 41. PPC of TSO Comprehensive Assay for Detection of Small DNA Variants in Combined Targeted Panel Members by Site

Observed Variant Level[1]	Variant Type	Gene	Targeted Variant	Site	N	PPC (%) (n/N)	95% CI [2]
~2-3×C95	SNV	BRAF	V600E	S1	14	100.0 (14/14)	(78.5, 100.0)
		BRAF		S2	16	87.5 (14/16)	(64.0, 96.5)
		BRAF		S3	16	87.5 (14/16)	(64.0, 96.5)
		EGFR	L858R	S1	16	100.0 (16/16)	(80.6, 100.0)
		EGFR		S2	14	100.0 (14/14)	(78.5, 100.0)
		EGFR		S3	8	100.0 (8/8)	(67.6, 100.0)
		IDH1	R132H	S1	16	100.0 (16/16)	(80.6, 100.0)

Observed Variant Level[1]	Variant Type	Gene	Targeted Variant	Site	N	PPC (%) (n/N)	95% CI [2]
		IDH1		S2	16	100.0 (16/16)	(80.6, 100.0)
		IDH1		S3	4	100.0 (4/4)	(51.0, 100.0)
	MNV	KRAS	G12I	S1	16	100.0 (16/16)	(80.6, 100.0)
		KRAS		S2	16	100.0 (16/16)	(80.6, 100.0)
		KRAS		S3	6	100.0 (6/6)	(61.0, 100.0)
	INSERTION	APC	T1556Nfs Ter3	S1	12	100.0 (12/12)	(75.8, 100.0)
		APC		S2	14	100.0 (14/14)	(78.5, 100.0)
		APC		S3	6	100.0 (6/6)	(61.0, 100.0)
		APC	S1465fs* 9	S1	16	100.0 (16/16)	(80.6, 100.0)
		APC		S2	16	100.0 (16/16)	(80.6, 100.0)
		APC		S3	16	100.0 (16/16)	(80.6, 100.0)
		ERBB2	Y772_A7 75dup	S1	16	100.0 (16/16)	(80.6, 100.0)
		ERBB2		S2	16	100.0 (16/16)	(80.6, 100.0)
		ERBB2		S3	4	100.0 (4/4)	(51.0, 100.0)
		NOTCH	R1598fs* 12	S1	16	100.0 (16/16)	(80.6, 100.0)
		NOTCH		S2	16	100.0 (16/16)	(80.6, 100.0)
		NOTCH		S3	16	100.0 (16/16)	(80.6, 100.0)
		TP53	R333Hfs Ter5	S1	16	100.0 (16/16)	(80.6, 100.0)
		TP53		S2	16	100.0 (16/16)	(80.6, 100.0)
		TP53		S3	16	100.0 (16/16)	(80.6, 100.0)
	DELETION	APC	L1488fsT er19	S1	16	100.0 (16/16)	(80.6, 100.0)
		APC		S2	6	100.0 (6/6)	(61.0, 100.0)
		APC		S3	6	100.0 (6/6)	(61.0, 100.0)
		APC	S1465Wf sTer3	S1	16	100.0 (16/16)	(80.6, 100.0)
		APC		S2	14	100.0 (14/14)	(78.5, 100.0)
		APC		S3	10	100.0 (10/10)	(72.2, 100.0)
		EGFR	E746_A7 50del	S1	16	100.0 (16/16)	(80.6, 100.0)
		EGFR		S2	14	100.0 (14/14)	(78.5, 100.0)
		EGFR		S3	16	100.0 (16/16)	(80.6, 100.0)
		EP300	H2324fs* 29	S1	16	100.0 (16/16)	(80.6, 100.0)
		EP300		S2	14	100.0 (14/14)	(78.5, 100.0)
		EP300		S3	14	100.0 (14/14)	(78.5, 100.0)
		PTEN	T319fs*1	S1	16	100.0 (16/16)	(80.6, 100.0)
		PTEN		S2	14	100.0 (14/14)	(78.5, 100.0)
		PTEN		S3	14	100.0 (14/14)	(78.5, 100.0)
	All Variant Types	All Genes	All Targeted Variants	S1	218	100.0 (218/218)	(98.3, 100.0)
		All Genes		S2	202	99.0 (200/202)	(96.5, 99.7)

Observed Variant Level[1]	Variant Type	Gene	Targeted Variant	Site	N	PPC (%) (n/N)	95% CI [2]
		All Genes		S3	152	98.7 (150/152)	(95.3, 99.6)
>3×C95	MNV	BRAF	V600K	S1	16	100.0 (16/16)	(80.6, 100.0)
		BRAF		S2	16	100.0 (16/16)	(80.6, 100.0)
		BRAF		S3	14	100.0 (14/14)	(78.5, 100.0)
	INSERTION	FBXW7	T15_G16insP	S1	16	100.0 (16/16)	(80.6, 100.0)
		FBXW7		S2	16	100.0 (16/16)	(80.6, 100.0)
		FBXW7		S3	12	100.0 (12/12)	(75.8, 100.0)
	DELETION	APC	L1488fs*18	S1	16	100.0 (16/16)	(80.6, 100.0)
		APC		S2	16	100.0 (16/16)	(80.6, 100.0)
		APC		S3	14	100.0 (14/14)	(78.5, 100.0)
		TP53	R342fs*3	S1	16	100.0 (16/16)	(80.6, 100.0)
		TP53		S2	14	100.0 (14/14)	(78.5, 100.0)
		TP53		S3	14	100.0 (14/14)	(78.5, 100.0)
	All Variant Types	All Genes	All Targeted Variants	S1	64	100.0 (64/64)	(94.3, 100.0)
		All Genes		S2	62	100.0 (62/62)	(94.2, 100.0)
		All Genes		S3	54	100.0 (54/54)	(93.4, 100.0)
<2×C95	INSERTION	ARID1A	Q372fs*28	S1	4	100.0 (4/4)	(51.0, 100.0)
		TP53	P152_P153dup	S1	2	100.0 (2/2)	(34.2, 100.0)
	All Variant Types	All Genes	All Targeted Variants	S1	6	100.0 (6/6)	(61.0, 100.0)

[1] Variant level calculated from mean observed variant allele frequency.
[2] 95% two-sided confidence interval calculated via the Wilson Score method

Table 43 shows the variance component analysis of VAF results for each source of variation and total variation in all panel members with targeted small DNA variants. There were two small DNA targeted variants for which the number of observations was too small for a variance components model to be fitted. For these two targeted variants, overall SDs were 0.027 for variant chr1_27024001_C_CG and 0.001 for variant chr17_7578470_C_CGGGCGG (N=4 and N=2 respectively).

Table 43. Variance Components Analysis of VAF for Targeted Small DNA Variants

Targeted Variant (nucleotide)	Targeted Variant (amino acid)	N	Mean VAF (%)	Site SD (%CV)	Operator (Site) SD (%CV)	Day (Site, Operator) SD (%CV)	Lot SD (%CV)	Run SD (%CV)	Total SD (%CV)
chr2_209113112_C_T	IDH1 R132H	36	15.5	0.008 (4.9)	0.006 (4.1)	0.034 (22.1)	0.000 (0.0)	0.016 (10.2)	0.039 (25.2)
chr4_153332910_C_CAGG	FBXW7 T15_G16insP	44	13.0	0.000 (0.0)	0.000 (0.0)	0.013 (10.3)	0.014 (11.1)	0.008 (6.1)	0.021 (16.3)
chr5_112175675_A_AAG	APC S1465fs*9	48	10.0	0.000 (0.0)	0.000 (0.0)	0.010 (10.4)	0.003 (2.9)	0.003 (3.3)	0.011 (11.3)
chr5_112175675_AAG_A	APC S1465WfsTer3	40	16.6	0.000 (0.0)	0.000 (0.0)	0.024 (14.2)	0.000 (0.0)	0.011 (6.7)	0.026 (15.7)
chr5_112175751_CT_C	APC L1488fsTer19	28	18.1	0.000 (0.0)	0.000 (0.0)	0.029 (15.8)	0.019 (10.8)	0.008 (4.7)	0.036 (19.7)
chr5_112175751_CTTTA_C	APC L1488fs*SESe18	46	15.5	0.000 (0.0)	0.009 (5.6)	0.023 (14.9)	0.015 (9.7)	0.008 (5.5)	0.030 (19.4)
chr5_112175951_G_GA	APC T1556NfsTer3	32	22.7	0.000 (0.0)	0.006 (2.5)	0.034 (15.1)	0.000 (0.0)	0.011 (4.9)	0.036 (16.1)
chr7_55242465_GGAATTAAGAGAAGCA_G	EGFR E746_A750del	46	11.2	0.000 (0.0)	0.004 (3.8)	0.015 (13.7)	0.005 (4.1)	0.008 (6.9)	0.018 (16.3)
chr7_55259515_T_G	EGFR L858R	38	4.5	0.003 (6.0)	0.000 (0.0)	0.012 (27.3)	0.000 (0.0)	0.003 (6.8)	0.013 (28.8)

chr7_140453136_A_T	BRAF V600E	46	4.5	0.000 (0.0)	0.000 (0.0)	0.016 (34.9)	0.000 (0.0)	0.006 (12.2)	0.017 (36.9)
chr7_140453136_AC_TT	AF V600K	BR46	13.0	0.000 (0.0)	0.004 (2.9)	0.017 (13.4)	0.003 (2.6)	0.006 (4.9)	0.019 (14.8)
chr9_139399350_C_CG	NOTCH1 R1598fs*12	48	14.6	0.015 (10.2)	0.000 (0.0)	0.012 (8.2)	0.000 (0.0)	0.004 (2.8)	0.020 (13.4)
chr10_89720798_GTACT_G	PTEN T319fs*1	44	15.7	0.000 (0.0)	0.003 (2.0)	0.021 (13.6)	0.002 (1.6)	0.010 (6.4)	0.024 (15.3)
chr12_25398284_CC_AT	KRAS G12I	38	11.1	0.000 (0.0)	0.000 (0.0)	0.019 (16.8)	0.003 (2.5)	0.008 (7.3)	0.020 (18.5)
chr17_7574002_CG_C	TP53 R342fs*3	44	15.8	0.007 (4.2)	0.000 (0.0)	0.021 (13.5)	0.013 (8.6)	0.013 (8.2)	0.029 (18.4)
chr17_7574029_C_CGGAT	TP53 R333HfsTer5	48	15.4	0.000 (0.0)	0.000 (0.0)	0.017 (11.0)	0.006 (3.8)	0.010 (6.6)	0.021 (13.4)
chr17_37880981_A_AGCATACGTGATG	ERBB2 Y772_A775dup	36	7.5	0.013 (16.9)	0.006 (8.1)	0.013 (16.7)	0.000 (0.0)	0.004 (4.7)	0.019 (25.5)
chr22_41574678_GC_G	EP300 H2324fs*29	44	24.5	0.006 (2.4)	0.002 (0.6)	0.019 (7.9)	0.000 (0.0)	0.005 (2.1)	0.021 (8.6)

%CV: Percent coefficient of variation.
SD: Standard deviation.
VAF, variant allele frequency

TMB

To evaluate the reproducibility of TMB scores, a quantitative analysis of the score was conducted in 18 targeted TMB panel members, which represented a range of expected TMB scores (0.8-123.1 Mut/Mb). Table 44 shows the variance component analysis of TMB score results for each source of variation and total variation in the TMB panel members. Total SD of TMB score ranged from 0.2 (%CV =29) for panel member

TPSAD10 to 1.9 (%CV = 24) for panel member TPSAD3. There were 2 TMB panel members (TPSAD6 and TPSBD1 – not included in the table below) for which the number of observations was too small (N = 4 and N=2 respectively) for a variance components model to be fitted (TPSAD6 and TPSBD1). For these panel members, overall SD was 0.4 and 1.7, respectively.

Table 44. Variance Components Analysis of TMB Score for Targeted TMB Panel Members

Panel Member	N	Mean TMB Score	Site SD (%CV)	Operator (Site) SD (%CV)	Day (Site, Operator) SD (%CV)	Lot SD (%CV)	Run SD (%CV)	Total SD (%CV)
TPSAD1	38	2.7	0.0 (0)	0.0 (0)	0.6 (22)	0.1 (4)	0.5 (19)	0.8 (29)
TPSAD2	46	3.5	0.0 (0)	0.9 (25)	1.0 (28)	0.0 (0)	0.8 (24)	1.5 (44)
TPSAD3	36	8.2	1.1 (14)	0.0 (0)	1.1 (13)	0.7 (9)	0.9 (11)	1.9 (24)
TPSAD4	46	1.6	0.0 (0)	0.0 (0)	0.5 (33)	0.0 (0)	0.5 (33)	0.7 (46)
TPSAD5	38	4.8	0.2 (5)	0.0 (0)	0.8 (17)	0.3 (6)	0.7 (14)	1.1 (23)
TPSAD7	32	6.0	0.1 (2)	0.1 (1)	0.4 (6)	0.0 (0)	0.4 (6)	0.6 (9)
TPSAD10	44	0.8	0.0 (0)	0.0 (0)	0.0 (0)	0.1 (6)	0.2 (28)	0.2 (29)
TPSAD11	48	1.5	0.0 (0)	0.3 (18)	0.4 (24)	0.0 (0)	0.6 (38)	0.7 (48)
TPSBD2	46	4.0	0.0 (0)	0.0 (0)	0.6 (14)	0.0 (0)	0.5 (12)	0.7 (18)
TPSBD3	28	7.6	0.2 (2)	0.0 (0)	0.8 (10)	0.0 (0)	0.5 (7)	1.0 (13)
TPSBD4	44	63.2	0.3 (1)	0.6 (1)	0.4 (1)	0.0 (0)	0.7 (1)	1.1 (2)
TPSBD5	44	5.4	0.0 (0)	0.3 (5)	0.7 (13)	0.0 (0)	0.6 (11)	1.0 (18)
TPSBD6	40	123.1	0.3 (0)	0.0 (0)	1.0 (1)	0.0 (0)	0.9 (1)	1.4 (1)
TPSBD7	46	12.6	0.0 (0)	0.0 (0)	0.7 (5)	0.0 (0)	1.1 (8)	1.3 (10)
TPSBD8	44	5.6	0.0 (0)	0.0 (0)	0.2 (4)	0.0 (0)	0.1 (2)	0.3 (4)
TPSBD12	47	6.3	0.4 (6)	0.0 (0)	1.1 (17)	0.0 (0)	0.6 (10)	1.3 (20)

%CV: Percent coefficient of variation.
SD: Standard deviation.

RNA Variants

Table 45 (overall) and Table 46 (by site) shows PPCs for targeted RNA variants. PPCs ranged from 91.7% for KIF5B-RET to 100% for most RNA variants. PNC was 100% for each targeted RNA variant, except for the FGFR2-SRPK2 fusion (PNC = 99.60% (984/988; 95% CI: 98.96% to 99.84%).

Table 45. Overall PPC of TSO Comprehensive Assay for Detection of RNA Variants in Combined Targeted Panel Members

Observed Variant Level[1]	Variant Type	Targeted Variant	Mean Supporting Reads[2]	PPC (%) (n/N)	95% CI[3]
<2xLOD	Fusion	KIF5B-RET	11.6	91.7 (44/48)	(80.4, 96.7)
	Fusion	RAF1-VGLL4	15.9	100.0 (46/46)	(92.3, 100.0)

Observed Variant Level[1]	Variant Type	Targeted Variant	Mean Supporting Reads[2]	PPC (%) (n/N)	95% CI[3]
~2-3xLOD	Fusion	DHX8;ETV4-STAT3	48.9	100.0 (46/46)	(92.3, 100.0)
	Fusion	ESR1-CCDC170	45.1	100.0 (46/46)	(92.3, 100.0)
	Fusion	FGFR1-GSR	61.1	100.0 (46/46)	(92.3, 100.0)
	Fusion	FGFR2-SRPK2	53.4	100.0 (48/48)	(92.6, 100.0)
	Fusion	FGFR3-TACC3	53.5	100.0 (48/48)	(92.6, 100.0)
	Fusion	HNRNPUL1-AXL	58.0	100.0 (48/48)	(92.6, 100.0)
	Fusion	MKRN1-BRAF	33.4	100.0 (48/48)	(92.6, 100.0)
	Fusion	TMPRSS2-ERG	43.5	97.9 (47/48)	(89.1, 99.6)
>3xLOD	Fusion	ACPP-ETV1	44.7	100.0 (46/46)	(92.3, 100.0)
	Fusion	BCL2-IGHJ5	124.9	100.0 (46/46)	(92.3, 100.0)
	Fusion	EGFR-GALNT13	49.8	100.0 (46/46)	(92.3, 100.0)
	Fusion	EML4-ALK	49.3	100.0 (48/48)	(92.6, 100.0)
	Fusion	PAX3-FOXO1	70.1	100.0 (48/48)	(92.6, 100.0)
	Fusion	SPIDR-NRG1	51.5	100.0 (48/48)	(92.6, 100.0)
	Splice Variant	EGFRvIII	64.0	100.0 (46/46)	(92.3, 100.0)
	Fusion	CDK4-DPY19L2	55.7	97.8 (45/46)	(88.7, 99.6)
	Fusion	EWSR1-FLI1	31.6	100.0 (48/48)	(92.6, 100.0)

[1]Variant level calculated from mean observed supporting reads.
[2]Mean supporting reads calculated from observed assay results.
[3]95% two-sided confidence interval calculated via the Wilson Score method.

Table 46. PPC of TSO Comprehensive Assay for Detection of RNA Variants in Combined Targeted Panel Members (by Site)

Observed Variant Level[1]	Variant Type	Targeted Variant	Site	N	PPC (%) (n/N)	95% CI [2]
>3×C95	Fusion	ACPP-ETV1	S1	14	100.0 (14/14)	(78.5, 100.0)
			S2	16	100.0 (16/16)	(80.6, 100.0)
			S3	16	100.0 (16/16)	(80.6, 100.0)
		BCL2-IGHJ5	S1	14	100.0 (14/14)	(78.5, 100.0)
			S2	16	100.0 (16/16)	(80.6, 100.0)
			S3	16	100.0 (16/16)	(80.6, 100.0)

Observed Variant Level[1]	Variant Type	Targeted Variant	Site	N	PPC (%) (n/N)	95% CI [2]
		CDK4-DPY19L2	S1	14	100.0 (14/14)	(78.5, 100.0)
			S2	16	93.8 (15/16)	(71.7, 98.9)
			S3	16	100.0 (16/16)	(80.6, 100.0)
		EGFR-GALNT13	S1	16	100.0 (16/16)	(80.6, 100.0)
			S2	16	100.0 (16/16)	(80.6, 100.0)
			S3	14	100.0 (14/14)	(78.5, 100.0)
		EML4-ALK	S1	16	100.0 (16/16)	(80.6, 100.0)
			S2	16	100.0 (16/16)	(80.6, 100.0)
			S3	16	100.0 (16/16)	(80.6, 100.0)
		EWSR1-FLI1	S1	16	100.0 (16/16)	(80.6, 100.0)
			S2	16	100.0 (16/16)	(80.6, 100.0)
			S3	16	100.0 (16/16)	(80.6, 100.0)
		PAX3-FOXO1	S1	16	100.0 (16/16)	(80.6, 100.0)
			S2	16	100.0 (16/16)	(80.6, 100.0)
			S3	16	100.0 (16/16)	(80.6, 100.0)
		SPIDR-NRG1	S1	16	100.0 (16/16)	(80.6, 100.0)
			S2	16	100.0 (16/16)	(80.6, 100.0)
			S3	16	100.0 (16/16)	(80.6, 100.0)
	Splice Variant	EGFRvIII	S1	14	100.0 (14/14)	(78.5, 100.0)
			S2	16	100.0 (16/16)	(80.6, 100.0)
			S3	16	100.0 (16/16)	(80.6, 100.0)
~2-3×C95	Fusion	DHX8;ETV4-STAT3	S1	14	100.0 (14/14)	(78.5, 100.0)
			S2	16	100.0 (16/16)	(80.6, 100.0)
			S3	16	100.0 (16/16)	(80.6, 100.0)
		ESR1-CCDC170	S1	14	100.0 (14/14)	(78.5, 100.0)
			S2	16	100.0 (16/16)	(80.6, 100.0)
			S3	16	100.0 (16/16)	(80.6, 100.0)
		FGFR1-GSR	S1	14	100.0 (14/14)	(78.5, 100.0)
			S2	16	100.0 (16/16)	(80.6, 100.0)
			S3	16	100.0 (16/16)	(80.6, 100.0)
		FGFR2-SRPK2	S1	16	100.0 (16/16)	(80.6, 100.0)
			S2	16	100.0 (16/16)	(80.6, 100.0)
			S3	16	100.0 (16/16)	(80.6, 100.0)
		FGFR3-TACC3	S1	16	100.0 (16/16)	(80.6, 100.0)
			S2	16	100.0 (16/16)	(80.6, 100.0)
			S3	16	100.0 (16/16)	(80.6, 100.0)
		HNRNPUL1-AXL	S1	16	100.0 (16/16)	(80.6, 100.0)
			S2	16	100.0 (16/16)	(80.6, 100.0)
			S3	16	100.0 (16/16)	(80.6, 100.0)
		MKRN1-BRAF	S1	16	100.0 (16/16)	(80.6, 100.0)
			S2	16	100.0 (16/16)	(80.6, 100.0)
			S3	16	100.0 (16/16)	(80.6, 100.0)
			S1	16	93.8 (15/16)	(71.7, 98.9)

Observed Variant Level[1]	Variant Type	Targeted Variant	Site	N	PPC (%) (n/N)	95% CI [2]
		TMPRSS2-ERG	S2	16	100.0 (16/16)	(80.6, 100.0)
			S3	16	100.0 (16/16)	(80.6, 100.0)
<2×C95	Fusion	KIF5B-RET	S1	16	100.0 (16/16)	(80.6, 100.0)
			S2	16	87.5 (14/16)	(64.0, 96.5)
			S3	16	87.5 (14/16)	(64.0, 96.5)
		RAF1-VGLL4	S1	14	100.0 (14/14)	(78.5, 100.0)
			S2	16	100.0 (16/16)	(80.6, 100.0)
			S3	16	100.0 (16/16)	(80.6, 100.0)

Table 47 shows the variance component analysis of supporting read results for each source of variation and total variation in all panel members with targeted RNA variants.

Table 47. Variance Components Analysis of Supporting Reads for Targeted RNA Variants

Targeted Variant	N	Mean Supporting Reads	Site SD (%CV)	Operator (Site) SD (%CV)	Day (Site, Operator) SD (%CV)	Lot SD (%CV)	Run SD (%CV)	Total SD (%CV)
ACPP-ETV1	46	44.7	10.38 (23)	0.00 (0)	13.01 (29)	5.90 (13)	2.28 (5)	17.80 (40)
BCL2-IGHJ5	46	124.9	38.22 (31)	13.24 (11)	29.08 (23)	9.51 (8)	8.30 (7)	51.39 (41)
DHX8;ETV4-STAT3	46	48.9	18.27 (37)	13.42 (27)	17.01 (35)	0.00 (0)	1.50 (3)	28.38 (58)
EGFR-GALNT13	46	49.8	0.00 (0)	6.90 (14)	14.86 (30)	2.08 (4)	2.82 (6)	16.75 (34)
EML4-ALK	48	49.3	0.00 (0)	12.18 (25)	19.10 (39)	8.83 (18)	1.94 (4)	24.39 (49)
ESR1-CCDC170	46	45.1	2.30 (5)	0.00 (0)	12.37 (27)	0.00 (0)	8.08 (18)	14.95 (33)
FGFR1-GSR	46	61.1	8.57 (14)	1.31 (2)	11.15 (18)	9.23 (15)	5.18 (8)	17.65 (29)
FGFR2-SRPK2	48	53.4	3.18 (6)	10.90 (20)	15.85 (30)	15.29 (29)	3.10 (6)	24.97 (47)
FGFR3-TACC3	48	53.5	17.43 (33)	0.00 (0)	12.38 (23)	5.81 (11)	3.46 (6)	22.42 (42)
HNRNPUL1-AXL	48	58.0	0.00 (0)	12.15 (21)	18.22 (31)	0.00 (0)	3.96 (7)	22.26 (38)
KIF5B-RET	48	11.6	0.89 (8)	0.00 (0)	3.97 (34)	1.44 (12)	1.09 (9)	4.45 (38)

Targeted Variant	N	Mean Supporting Reads	Site SD (%CV)	Operator (Site) SD (%CV)	Day (Site, Operator) SD (%CV)	Lot SD (%CV)	Run SD (%CV)	Total SD (%CV)
MKRN1-BRAF	48	33.4	6.98 (21)	8.19 (25)	13.02 (39)	6.63 (20)	4.00 (12)	18.58 (56)
PAX3-FOXO1	48	70.1	12.45 (18)	10.79 (15)	17.91 (26)	3.02 (4)	2.42 (3)	24.65 (35)
RAF1-VGLL4	46	15.9	1.46 (9)	1.52 (10)	3.80 (24)	4.42 (28)	1.23 (8)	6.32 (40)
SPIDR-NRG1	48	51.5	4.78 (9)	0.00 (0)	10.69 (21)	5.94 (12)	3.29 (6)	13.54 (26)
TMPRSS2-ERG	48	43.5	5.63 (13)	8.81 (20)	9.98 (23)	0.00 (0)	6.21 (14)	15.73 (36)
EGFRvIII splice variant	46	64.0	12.70 (20)	0.42 (1)	17.69 (28)	0.00 (0)	2.34 (4)	21.90 (34)
NRG1-EGFR	46	9.4	<0.01 (<0.1)	2.27 (24.1)	3.64 (38.6)	1.92 (20.4)	2.16 (23.0)	5.17 (54.9)
CDK4-DPY19L2	45	55.7	3.14 (6)	0.00 (0)	9.67 (17)	0.00 (0)	11.80 (21)	15.58 (28)
EWSR1-FLI1	48	31.6	7.51 (24)	3.03 (10)	7.40 (23)	0.00 (0)	1.55 (5)	11.08 (35)

%CV: Percent coefficient of variation.
SD: Standard deviation.

Controls Reproducibility

Reproducibility of the TruSight Oncology DNA control and TruSight Oncology RNA control was evaluated in 2 separate studies.

For the TSO DNA Control, reproducibility was evaluated at 3 testing sites, with 2 operators/instruments using 3 reagent lots over 3 non-consecutive days, testing 3 lots of the DNA controls controls in duplicate, for a total of 108 observations across all sites. A total of 112 valid observations were assessed due to repeat testing at one of the sites. Controls were evaluated for library validity based on the QC metric used by the TSO Comprehensive assay (23 out of 24 variant calls required to pass). In summary, 98.2% of the DNA control libraries passed [110/112, 95% CI: (93.7%, 99.5%)] with only 2 failing observations at one of the sites.

For the TSO RNA Control, reproducibility was evaluated at 3 testing sites, with 2 operators/instruments using 4 reagent lots over 4 non-consecutive days, testing 3 lots of the RNA controls in duplicate across 2 sequencing runs for a total of 96 observations across all sites. Controls were evaluated for library validity based on the QC metric used by the TSO Comprehensive assay (12 out of 13 variant calls required to pass). In

summary, 100.0% of the RNA control libraries passed [96/96, 95% CI: (96.2%,100%)], with no failing observations at any of the sites.

3. Analytical Sensitivity: Limit of Blank (LoB)

The percentage of false positives (out of the total expected negatives) were assessed by replicate testing of FFPE normal or benign tumor-adjacent tissue that should not contain somatic variants for small DNA variants, RNA fusions, and RNA splice variants. False positives were not analyzed for TMB as there is no clinical cutoff. Six (6) DNA and six (6) RNA FFPE samples were run in duplicate with 2 operators across 3 days for each of the 2 reagent lots. In total, there were 168 possible observations for DNA and 228 observations for RNA reduced by invalid libraries (0invalid DNA libraries, 1 invalid RNA library for each variant type. The percentage of false positives was calculated at the position level (approximately 1.9 million positions per sample) for small DNA variants and was calculated at the sample level for RNA variants. The percentage of false positives for DNA variant types is shown in Table 48. TSO Comprehensive assay has no CDx (Level 1) claims for small DNA variants, so no small DNA variants false positives are reported for Level 1.

Further analysis of the 271 small DNA variant false positives showed that, when they were leveled by the TSO Comprehensive assay KB, it was shown that none of the false positives were clinically significant (Level 2). In addition, there were 4 false positives in Level 3 arising from 2 variants across 4 (2.4%) observations out of the 168 observations. The percentage of false positives for RNA fusions and splice variants was 0% as shown in Table 49.

Table 48. False Positives by DNA Variant Type

Variant Type	False Positives (%)
Small DNA Variants	0.0001 (271/295,801,567)
TMB	N/A*

*False positives are not applicable because TMB is reported as a score and does not have a qualitative outcome.

Table 49. False Positives by RNA Variant Type

Variant Type	False Positives (%)
Fusion	0 (0/227)
Splice Variant	0 (0/227)

4. Analytical Sensitivity: Limit of Detection (LoD)

Two studies were conducted to assess the Limits of Detection for TSO Comprehensive assay. Study 1 evaluated RET fusions, and *NTRK*1 – 3 Fusions. Study 2 evaluated other tumor profiling variants.

CDx LoD

The Limit of Detection (LoD) of *NTRK*1–3 and RET fusions were determined in Study 1. FFPE cell lines were first used to estimate the LoD values for each fusion gene which were then verified with clinical FFPE samples. 4 FFPE samples in 3 different tissue types (papillary thyroid, skin, and lung) and 1 FFPE-treated cell line with RET fusions, and 7 FFPE samples in 4 different tissue types (brain, soft tissue, breast, and colon) and 2 FFPE-treated cell lines with *NTRK*1–3 fusions. For the verification, there were 18 observations for each test level per lot per variant generated by 3 operators and 3 NextSeq 550Dx instruments initiating library preparation on 3 non-consecutive days with 2 replicates of each sample per test level, and 3 reagent lots (54 total observations per variant). The claimed limits of detection for each fusion (Table 50) are the lowest mean supporting reads that reached a hit rate (point estimate $\geq$ 95%. Invalid rate was 2.7% (26 out of 962 RNA libraries failed).

Table 50. Limit of Detection for *NTRK* and RET Fusions

Gene	Fusion	Tumor Type	Limit of Detection (Supporting Reads)
*NTRK*1	LMNA-*NTRK*1	Colon Cancer	12.2
	TPM3-*NTRK*1	Glioblastoma Multiforme	20.2
	BCAN-*NTRK*1	Low-Grade Glioma	53.2
*NTRK*2	STRN-*NTRK*2	Sarcoma	13.6
	ETV6-*NTRK*2	Myofibroblastic Sarcoma	20.3
*NTRK*3	KANK1-*NTRK*3	Colon Cancer	13.5
	ETV6-*NTRK*3	Secretory Breast Cancer	16.2
RET	NCOA4-RET	Papillary Thyroid	15.8
	KIF5B-RET	Non-Small Cell Lung Cancer	16.6
	CCDC6-RET	Atypical Spitz Tumor	18.7

Tumor Profiling LoD

The Limit of Detection (LoD) of the claimed tumor profiling variants (SNVs, MNVs, insertions, deletions in DNA; fusions and splice variants in RNA) reported by TSO Comprehensive assay were evaluated in Study 2. 23 FFPE samples from 17 tissue types, as well as 2 cell lines, were used to evaluate small DNA variants from different genomic contexts. 2 FFPE samples in 2 different tissues (lung and unknown) were used to assess RNA splice variants, and 21 samples in 21 different tissue types were used to assess RNA fusions variants (1 sample per claimed non-CDx fusion; RET was also included in this study). Samples were diluted to multiple test levels, with six observations generated per level by two operators, each using a different reagent lot and instrument. Invalid rates were 4.2% (17 out of 407 DNA libraries failed) and 6.9% (95 out of 1378 RNA libraries failed).

Small DNA Variants

The LoDs of 10 small DNA variants classes (25 variants in total) were determined and summarized as ranges (Table 37). One of 3 insertions greater than 5 bp had an LoD of 21.5% VAF, which was different that the LoDs for the other 2 insertions in the same category (3.4% and 3.6% VAF). This was due to that insertion being in a low complexity region where the insertion adds additional repeats, impacts alignment, and requires more reads for consistent detection.

Table 51. Limit of Detection for Small DNA Variants

Variant Class / Genomic Context	Number of Variants	Range (% VAF)
SNVs	5	1.6-6.4
MNVs	3	2.2 - 4.8
Insertion (1-2bp) near homopolymer repeats	2	8.6 - 10.4
Insertion (1-2bp) near dinucleotide repeats[1]	2	3.8 - 5.1
Insertion (3-5bp)	2	3.0 - 5.6
Insertion (> 5bp and up to 25bp)	3	3.4 - 21.5
Deletion (1-2bp) near homopolymer repeats	2	9.4 - 10.0
Deletion (1-2bp) near dinucleotide repeats	2	3.3 - 7.0
Deletion (3-5bp)	2	2.8 - 6.4
Deletion (> 5 and up to 25bp)	2	4.7 - 5.5

[1]LoD for this specific variant class was determined using cell lines.

Analysis of non-targeted variants was performed from Study 1 samples that had at least 5 testing levels. Each non-targeted variant was analyzed individually and an LoD estimated only for variants with at least one level >0% and ≤ 95% hit rate, and at least one level ≥ 95% hit rate. Table 38 shows percentiles along with the minimum and maximum LoDs observed by class for the non-targeted variants. The non-targeted variants provide more variants by class than were tested in the targeted variants and are consistent with the LoD ranges from Table 52.

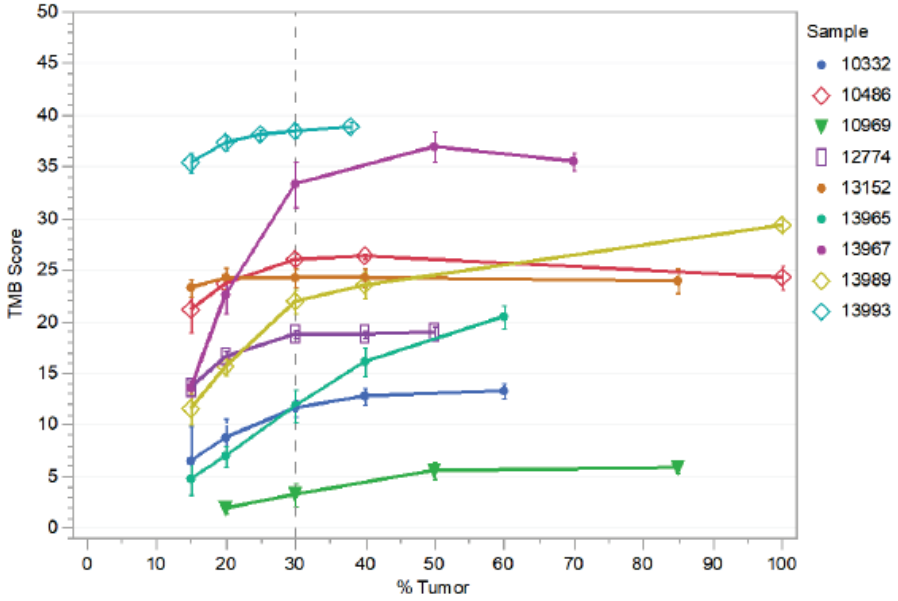
Table 52. Summary Statistics for Limits of Detection by Class of Non-Targeted Variants (from Study 1)

Variant Type	N	Min VAF (%)	25%	50%	75%	90%	Max VAF (%)
SNV	862	2.0	4.7	5.9	7.9	9.7	59.2
MNV	5	3.8	4.0	5.0	8.6	9.5	9.5
Insertion	24	3.9	6.0	8.4	9.7	16.6	26.1
Deletion	24	3.4	6.3	8.1	8.9	12.4	16.7

TMB

Nine samples with a range of TMB scores were evaluated for the effect of tumor content on TMB score. These samples included 4 from bladder tissue, 3 from endometrial tissue, 1 from breast tissue, and 1 from skin. Each sample was tested at 5 dilution levels, with 5 replicates, and were each diluted with normal tissue from the same patient to mimic a range of tumor content for each sample. Figure 2 shows the observed correlation between TMB score versus tumor content, with each data point being the mean across replicates (the grey dashed line represents 30% tumor content for reference). In summary, TMB scores show minimal change after 30% tumor content, with all but the sample with the lowest TMB score showing a reduction in TMB score after 30%. However, as TSO Comprehensive does not have a qualitative cutoff for TMB (ie., Low vs. High), the tumor content required to maintain a TMB-high classification (above cutoff) status cannot be determined.

Figure 2. TMB Score vs. Percent (%) Tumor Content



RNA Fusions

LoDs were determined for 18 fusions, accounting for the 20 non-CDx fusion genes in the TSO Comprehensive assay panel, which ranged from 8.3 to 31.3 supporting reads (Table 53). In addition, the KIF5B and RET genes was tested both here and in Study 1, and the LoD values shown in Table 53 represent the conservative (higher) values obtained from Study 1.

Table 53. Limit of Detection for Fusions

Fusion	Gene A Breakpoint	Gene B Breakpoint	LoD (supporting reads)
EWSR1-FLI1	29683123	128675261	8.3
TMPRSS2-PMFBP1	42866283	72153988	9.0

Fusion	Gene A Breakpoint	Gene B Breakpoint	LoD (supporting reads)
ACPP-**ETV1**	132036419	14028762	9.5
CDK4-DPY19L2	58145954	64041141	10.7
MKRN1-**BRAF**	140158806	140487383	11.0
RAF1-VGLL4	12641189	11606492	11.2
EGFR-GALNT13	55087056	155295102	12.3
EML4-ALK	42553391	29446394	12.8
SPIDR-**NRG1**	48353103	32453345	12.8
TMPRSS2-**ERG**	42880007	39817543	13.2
ESR1-CCDC170	152023138	151914240	13.5
NCOA4-RET[1]	51582937	43612030	15.8
DHX8;**ETV4**-STAT3	41613847	40474300	16.2
KIF5B-RET[1]	32311775	43612032	16.6
FGFR3-TACC3	1801536	1736997	17.5
PAX3-FOXO1	223084859	41134997	19.0
FGFR1-GSR	38274821	30569602	23.7
FGFR2-SRPK2	123353223	104926165	24.7
HNRNPUL1-**AXL**	41782201	41743847	26.3
BCL2-IGHJ5	60793496	106330066	31.3

[1]The RET gene, which is part of the TSO Comp panel, is already accounted for in Study 1 and is not bolded here.

Splice Variants

The EGFRvIII splice variant had a LoD of 16.7 supporting reads.

Tumor Content

The results in the study inform recommendations for tumor content for clinical specimens. In general, the greater the tumor content, the higher the signal (VAF or supporting reads) for variants in the tumor. The minimum tumor content recommendations are due to the fact that LoD values for small DNA variants are no greater than 10.4% VAF (with the exception of the TP53 insertion). Therefore, to detect driver mutations in the tumor (50% VAF), 20% tumor content is recommended, so that these mutations would have a 10.0% VAF and be at or above LoD. At 20% tumor content, fusions with 74 supporting reads would be consistently detected based on a Limit of Detection of 14.7 supporting reads.

5. Reagent Kit Lot Interchangeability

A reagent kit lot interchangeability study was not performed because assay components from different reagent kit master lots are not interchangeable. Reagent kit lots are identified on the reagent kit box label and master lot sheet.

6. Tissue Comparability

Tissue comparability was assessed using samples evaluated in the Accuracy for Tumor Profiling Markers study by assessment of valid DNA or RNA samples after sequencing (evaluation by contamination QC, followed by library QC for DNA; evaluation by library QC for RNA). 274 DNA samples and 181 RNA samples were evaluated by tissue type. Results showed low aggregate library validity rate across all sample types (75% in DNA, 83% in RNA), and specifically in certain tissue types (prostate, ovary, and liver for DNA; skin, brain, and papillary thyroid tissue for RNA). This was mainly attributed to the low quality of input nucleic acids into library preparation caused by sample age (libraries made from FFPE samples ≥5 years of age showed lower validity), FFPE block storage conditions (observed batch effect based on specific vendor), and level of sample degradation before and after fixation. Because of the sourcing strategy for these samples, these conditions were unknown, and a number of presumably lower quality samples did not pass the TSO Comprehensive QC metrics. Intended use clinical specimens for TSO Comp are generally expected to be less than one year old with generally acceptable fixation and storage methods.

7. Interference

The impact of potential endogenous and exogenous substances on the performance of the TSO Comprehensive assay was evaluated in both tumor profiling and CDx variants. Endogenous substances (melanin and hemoglobin) were spiked into the samples during the nucleic acid extraction process. Exogenous substances (ethanol, xylene, Proteinase K, and excess index primers) were also spiked into the purified nucleic acid before library preparation. For all substances except index primers, four replicates of the sample libraries were generated by one or more operators. For index primers, 12 replicates of the sample libraries were generated by one operator. Replicates of each spiked substance, endogenous control, or exogenous control of the same sample were processed at the same time. For all substances except for index primers, 8 FFPE samples from 4 different tissue types (medullary thyroid, breast, lung, colon) were used for DNA analysis and 13 FFPE samples from 9 different tissue types were used for RNA analysis. For index primers, 6 FFPE samples from 3 different tissue types (thyroid, bladder, colon) were used for DNA analysis and 5 FFPE samples from 4 different tissue types (lung, thyroid, colon, breast) were used for RNA analysis. In addition to the evaluated substances, there was also a no-spiked endogenous control and a buffer/water-spiked exogenous control for each unique sample. Invalid rates were 8% (32 out of 398 DNA libraries failed) and 2.4% (15 out of 615 RNA libraries failed).

The effect of necrosis was also assessed on a different set of 8 FFPE samples from 3 different tissue types (brain, colon, and lung) with a wide range of necrotic tissue (3%-70%). There was a macrodissected, “no necrosis” control for each necrosis sample. Four

replicates of DNA and RNA libraries and their controls were processed together. For all interferents, sample replicates per substance were compared to their respective control replicates for detection of small DNA variants, TMB, RNA fusions, and RNA splice variants.

A summary of qualitative results for endogenous and exogenous substances is provided in Table 56. For small DNA variant detection, none of the substances tested interfere with the detection of small DNA variants or with TMB score, and VAF levels were unaffected by the presence of any of the tested variants. In addition, the presence of necrotic tissue up to 70% did not interfere with TMB score or small DNA variant detection. For RNA variant detection, none of the substances tested interfered with the detection of RNA fusion variants or splice variants. Necrosis was shown to interfere with detection of both RNA fusions and splice variants. Additional testing was performed on RNA variants using samples with known necrotic tissue, which showed interference ≥25% (by area) necrotic content for RNA fusions.

Proteinase K (0.04 mg/ml in nucleic acid) was also shown to interfere with both RNA fusions and splice variants. Increased concentrations of Proteinase K during the extraction process were also evaluated with testing at 2.6 mg/ml and 5.2 mg/ml during the extraction process (2x and 4x of the standard concentration, 1.3 mg/mL, per reaction), which showed RNA fusions inhibited (reduced supporting reads) at 4x, but not 2x, and splice variants (reduced supporting reads) were inhibited at 2x.

Table 56. Interference Results per Variant Type

Variant Type	Test Variable	Test Condition	PPC (n/N) (95% CIs)	PNC (Small DNA Variants) or FPR (RNA variants)[1] (n/N) (95% CIs)
Small DNA Variants	Necrosis	Necrosis Control (N)	99.9 (38781/38816) (99.9, 99.9)	> 99.9 (44152731/44152853) (> 99.9, > 99.9)
		Necrosis Test	99.8 (36257/36323) (99.8, 99.9)	> 99.9 (44025893/44026042) (> 99.9, > 99.9)
	Endogenous Substances	Endogenous Control	> 99.9 (38779/38796) (99.9, > 99.9)	> 99.9 (56659285/56659355) (> 99.9, > 99.9)
		Hemoglobin (2 mg/mL)	99.9 (38733/38762) (99.9, 99.9)	> 99.9 (56301763/56301826) (> 99.9, > 99.9)
		Melanin (0.2 µg/ml)	> 99.9 (38763/38779) (99.9, > 99.9)	> 99.9 (56639703/56639777) (> 99.9, > 99.9)
	Exogenous Substances	Exogenous Control	> 99.9 (38524/38536) (99.9, > 99.9)	> 99.9 (50257771/50257837) (> 99.9, > 99.9)

Variant Type	Test Variable	Test Condition	PPC (n/N) (95% CIs)	PNC (Small DNA Variants) or FPR (RNA variants)[1] (n/N) (95% CIs)
		Ethanol (5%)	> 99.9 (38481/38487) (> 99.9, > 99.9)	> 99.9 (50245187/50245298) (> 99.9, > 99.9)
		Proteinase K (0.04 mg/ml)	> 99.9 (38507/38521) (99.9, > 99.9)	> 99.9 (50191323/50191416) (> 99.9, > 99.9)
		Xylene (0.0001%)	99.9 (38466/38489) (99.9, > 99.9)	> 99.9 (49627396/49627449) (> 99.9, > 99.9)
RNA Fusions	Necrosis	Necrosis Control (N)	87.5 (14/16) (64.0, 96.5)	0 (0/30) (0, 11.4)
		Necrosis Test	50.0 (8/16) (28.0, 72.0)	0 (0/30) (0, 11.4)
	Endogenous Substances	Endogenous Control	98.9 (91/92) (94.1, 99.8)	19.2 (10/52) (10.8, 31.9)
		Hemoglobin (2 mg/mL)	96.7 (89/92) (90.8, 98.9)	17.4 (8/46) (9.1, 30.7)
		Melanin (0.2 µg/ml)	96.7 (89/92) (90.8, 98.9)	20.0 (10/50) (11.2, 33.0)
	Exogenous Substances	Exogenous Control	97.9 (92/94) (92.6, 99.4)	7.8 (4/51) (3.1, 18.5)
		Ethanol (5%)	91.7 (88/96) (84.4, 95.7)	12.0 (6/50) (5.6, 23.8)
		Proteinase K (0.04 mg/ml)	92.7 (89/96) (85.7, 96.4)	8.5 (4/47) (3.4, 19.9)
		Xylene (0.0001%)	93.8 (90/96) (87.0, 97.1)	11.8 (6/51) (5.5, 23.4)
	Half Concentration Proteinase K	Control for 0.5x Proteinase K	98.8 (83/84) (93.6, 99.8)	6.3 (2/32) (1.7, 20.1)
		Test for 0.5x Proteinase K	98.8 (83/84) (93.6, 99.8)	3.1 (1/32) (0.6, 15.7)
	Increased Proteinase K Extraction	Control for Increased Proteinase K	98.7 (75/76) (92.9, 99.8)	3.1 (1/32) (0.6, 15.7)
		2x Proteinase K Test	97.4 (74/76) (90.9, 99.3)	3.1 (1/32) (0.6, 15.7)
		4x Proteinase K Test	94.7 (71/75) (87.1, 97.9)	0 (0/31) (0, 11.0)
RNA Splice Variants	Necrosis	Necrosis Control (N)	Not evaluable[2]	6.7 (2/30) (1.8, 21.3)

Variant Type	Test Variable	Test Condition	PPC (n/N) (95% CIs)	PNC (Small DNA Variants) or FPR (RNA variants)[1] (n/N) (95% CIs)
		Necrosis Test	Not evaluable[2]	3.3 (1/30) (0.6, 16.7)
	Endogenous Substances	Endogenous Control	91.7 (11/12) (64.6, 98.5)	5.8 (3/52) (2.0, 15.6)
		Hemoglobin (2 mg/mL)	72.7 (8/11) (43.4, 90.3)	0.0 (0/46) (0.0, 7.7)
		Melanin (0.2 µg/ml)	91.7 (11/12) (64.6, 98.5)	12.0 (6/50) (5.6, 23.8)
	Exogenous Substances	Exogenous Control	90.9 (10/11) (62.3, 98.4)	3.9 (2/51) (1.1, 13.2)
		Ethanol (5%)	100.0 (12/12) (75.8, 100.0)	10.0 (5/50) (4.3, 21.4)
		Proteinase K (0.04 mg/ml)	54.5 (6/11) (28.0, 78.7)	4.3 (2/47) (1.2, 14.2)
		Xylene (0.0001%)	91.7 (11/12) (64.6, 98.5)	11.8 (6/51) (5.5, 23.4)
	Half Concentration Proteinase K	Control for 0.5x Proteinase K	87.5 (7/8) (52.9, 97.8)	9.4 (3/32) (3.2, 24.2)
		0.5x Proteinase K	87.5 (7/8) (52.9, 97.8)	3.1 (1/32) (0.6, 15.7)
	Increased Proteinase K Extraction	Control for Increased Proteinase K	100 (8/8) (67.6, 100)	6.3 (2/32) (1.7, 20.1)
		2x Proteinase K Test	100 (8/8) (67.6, 100)	3.1 (1/32) (0.6, 15.7)
		4x Proteinase K Test	100 (8/8) (67.6, 100)	3.2 (1/31) (0.6, 16.2)

[1]The percentage of false positives among the total observations. FPR is calculated for RNA variants in situations where a true negative count is not defined.

[2]PPC for splice variants and necrosis could not be evaluated as the selected variant was "truth negative" (based on the control condition) due to low supporting reads. This resulted in non-zero FPRs as observations where the splice variant was detected were treated as false positives. However, a quantitative analysis showed a reduction in supporting reads for the splice variant; as such, conservative interpretation is that necrosis interferes with splice variants.

A summary of qualitative results for excess index primers is shown in Table 57. For small DNA variants and TMB, excess index primers did not interfere with the detection of small DNA variants or with TMB score, and VAF levels were unaffected by the presence of any of the tested variants. For RNA fusions and splice variants, splice variants were inhibited by excess index primers (30%).

Table 57. Index Primer Interference Results by Variant Type

Variant Type	Test Condition	PPC (n/N) (95% CIs)	PNC (Small DNA Variants) or FPR (RNA variants)[1] (n/N) (95% CIs)
Small DNA Variants	Control (N)	99.8 (38110/38176) (99.8, 99.9)	> 99.9 (53476564/53477059) (> 99.9, > 99.9)
	Test A (15% index primer)	99.6 (37986/38142) (99.5, 99.7)	> 99.9 (53387835/53388310) (> 99.9, > 99.9)
	Test B (30% index primer)	99.7 (38029/38155) (99.6, 99.7)	> 99.9 (53446387/53446968) (> 99.9, > 99.9)
RNA Fusions	Control (N)	95.7 (22/23) (79.0, 99.2)	0 (0/23) (0, 14.3)
	Test A (15% index primer)	90.9 (20/22) (72.2, 97.5)	0 (0/22) (0, 14.9)
	Test B (30% index primer)	100 (23/23) (85.7, 100)	0 (0/23) (0, 14.3)
RNA Splice Variants	Control (N)	100 (12/12) (75.5, 100)	0 (0/12) (0, 24.2)
	Test A (15% index primer)	100 (12/12) (75.5, 100)	0 (0/12) (0, 24.2)
	Test B (30% index primer)	83.3 (10/12) (55.2, 95.3)	0 (0/12) (0, 24.2)

[1]The percentage of false positives among the total observations. FPR is calculated for RNA variants in situations where a true negative count is not defined.

8. Guardbanding

a. Nucleic Acid Input Titration

TSO Comprehensive assay (TSO Comprehensive assay assay) requires a fixed input of 40 ng of DNA or RNA sample. 6 FFPE samples for DNA extracted from 5 different tissue types, and 6 FFPE samples for RNA extracted from 4 different tissue types, each containing one or more CDx or Tumor Profiling variants to establish tolerances for nucleic acid input variation that could lead to assay failure or negatively impact output of the assay. Each FFPE sample was tested at five (5) input levels (20 ng, 30 ng, 40 ng (nominal), 80 ng, and 200 ng) in triplicate to evaluate assay performance. All variants were evaluated qualitatively (except for TMB) and quantitatively. A summary of results is provided in Table 58. In summary, small DNA variant detection and RNA variant detection were not affected by difference in input amount. For RNA splice variants, 2 replicates at the nominal level were discordant due to a variant below the respective LoD. In addition, RNA fusions (35% decrease in supporting reads) and RNA splice variants (43% decrease in supporting reads) were affected by the 20 ng input condition as compared to the nominal 40 ng input condition, which could lead to inconsistent RNA

variant detection at 20 ng input. Invalid rates were 11.2% (20 out of 180 DNA libraries failed) and 3.9% (7 out of 180 RNA libraries failed).

Table 58. Input Titration Results per Variant Type

Variant Type	Test Condition	PPC (n/N) (95% CIs)	PNC (Small DNA Variants) or FPR (RNA variants)[1] (n/N) (95% CIs)
Small DNA Variants	20 ng	99.7 (19746 / 19800) (99.6, 99.8)	> 99.9 (26376758 / 26376816) (> 99.9, > 99.9)
	30 ng	99.1 (49443 / 49917) (99.0, 99.1)	> 99.9 (61637296 / 61637587) (> 99.9, > 99.9)
	40 ng (Control)	99.6 (49934 / 50124) (99.6, 99.7)	> 99.9 (62915486 / 62915753) (> 99.9, > 99.9)
	80 ng	99.8 (49983 / 50100) (99.7, 99.8)	> 99.9 (63810079 / 63810702) (> 99.9, > 99.9)
	200 ng	99.9 (50020 / 50094) (99.8, 99.9)	> 99.9 (63808104 / 63808860) (> 99.9, > 99.9)
RNA Fusions	20 ng	100 (20/20) (83.9, 100)	3.1 (1/32) (0.6, 15.7)
	30 ng	100 (22/22) (85.1, 100)	0 (0/33) (0, 10.4)
	40 ng (Control)	100 (24/24) (86.2, 100)	0 (0/36) (0, 9.6)
	80 ng	100 (24/24) (86.2, 100)	0 (0/36) (0, 9.6)
	200 ng	100 (24/24) (86.2, 100)	8.3 (3/36)[2] (2.9, 21.8)
RNA Splice Variants	20 ng	41.7 (5/12) (19.3, 68.0)	0 (0/32) (0, 10.7)
	30 ng	100 (12/12) (75.8, 100)	0 (0/32) (0, 10.7)
	40 ng (Control)	83.3 (10/12)3 (55.2, 95.3)	0 (0/32) (0, 10.7)
	80 ng	100 (12/12) (75.8, 100)	33.3 (12/36)[2] (20.2, 49.7)
	200 ng	100 (12/12) (75.8, 100)	44.4 (16/36)[2] (29.5, 60.4)

[1]The percentage of false positives among the total observations. FPR is calculated for RNA variants in situations where a true negative count is not defined.

[2]Minor RNA variant species (same gene, different breakpoints, fewer supporting reads) were a result of increasing supporting reads with increasing input; as such, they were detected at 80 ng and 200 ng inputs and not in the nominal condition.
[3]2 replicates at the nominal level were discordant due to a variant below the respective LoD

QC Metrics

The effects of increasing input amount on the QC metrics used to validate DNA and RNA libraries was evaluated in the same sample set. DNA results demonstrated that median exon coverage (MEDIAN_EXON_COVERAGE) increased with increasing input amount above the nominal input (40 ng) for all samples tested, median insert size (MEDIAN_INSERT_SIZE) increased with increasing input up to 40 ng for some of the samples tested, and percent exon coverage at 50X (PCT_EXON_50X) was not affected by input amounts above 30 ng for all samples tested. RNA results demonstrated that total on target reads (TOTAL_ON_TARGET_READS) showed a positive correlation with increasing input above the nominal amount (40 ng), but MEDIAN_CV_GENE_500X and MEDIAN_INSERT_SIZE were not impacted by increasing input.

b. Workflow/Critical Components

Guardbanding around nominal conditions was performed for steps throughout the assay workflow (Table 59). 6 FFPE samples in 5 different tissue types for DNA, and 6 FFPE samples in 4 different tissue types for RNA were tested to allow for analysis of small DNA variants, TMB, RNA fusions, and RNA splice variants with CDx and Tumor Profiling variants. For DNA small variants, TMB, and RNA fusions, libraries were generated in duplicate per library preparation event by two operators for each workflow testing condition and a nominal condition for a total of 4 observations per sample, per condition. For splice variants, libraries were generated with two replicates per library preparation event by two operators for each of two workflow testing conditions and a nominal condition per test step (8 observations per sample). Variant calling was evaluated both qualitatively (except for TMB) and quantitatively. Invalid rates were 11.4% (37 out of 324 DNA libraries failed) and 3.0% (28 out of 924 RNA libraries failed).

Table 59. Tested Workflow Steps

Workflow Step (Identifier)	Steps to Tested (nominal condition)	Condition 1	Condition 2
Synthesize First Strand cDNA (A)[1]	Pipette Mixing step (Mix 10 times after adding RVT Master Mix)	Mix 5 times after adding RVT Master Mix	Mix 15 times after adding RVT Master Mix
Clean up cDNA (B)[1]	Residual Ethanol volumes (Complete Removal)	Complete Removal then add back 2 uL	Complete removal, then add back 4 uL
Perform End Repair and A-Tailing (C)	Incubation Times (30C for 30 minutes/72C for 20 minutes)	30C for 25 minutes/72C for 15 minutes	30C for 35 minutes/72C for 25 minutes

Workflow Step (Identifier)	Steps to Tested (nominal condition)	Condition 1	Condition 2
Ligate Adapters (D)	Incubation Times for operators to add STL then wait to clean up (30 minutes)	20 minutes	35 minutes
Clean Up Ligation (E)	Residual Ethanol volumes (Complete Removal)	Complete Removal then add back 2 uL	Complete removal, then add back 4 uL
Capture Targets (One and Two) (F)	SMB Bead Mixing Time for 1st and 2nd capture (1 minute vortexing)	45 seconds vortexing	1:15 minutes vortexing
Capture Targets (One and Two) (G)	Time to Transfer SMB to Plate after Mixing (Immediate)	Wait 30 seconds	Wait 1 minute
Capture Targets (One and Two) (H)	EEW Pipette Mixing (Pipette minimum of 10 times, ensure beads are fully resuspended)	Pipette mix 5 times, don't check for full resuspension	Pipette mix 10 times, don't check for full resuspension
Clean Up Amplified Enriched Library (I)	Residual Ethanol volumes (Complete removal)	Complete Removal then add back 2 uL	Complete removal, then add back 4 uL
Clean Up Amplified Enriched Library (J)	Elution volume transfer to normalization (Elute in 32 µl/Transfer 30 µl)	Elute in 32 µl/Transfer 25 µl	Elute in 35 µl/Transfer 32 µl
Normalize Libraries (K)	Mixing time for LNB1 (Vortex 1 minute, make sure beads resuspended)	45 seconds vortexing	1:15 minutes vortexing, make sure beads resuspended

[1]Steps A and B apply to the RNA workflow only

Results are shown for small DNA variants and RNA variants in Table 60 and Table 61, respectively. In summary, small DNA variant calling was not affected by deviations relative to the nominal condition in the DNA workflow steps. There was also no significant effect on VAF scores relative to the nominal condition for small DNA variants. TMB score was affected by undermixing of reagent EEW relative to the nominal condition (step H, Condition 1), but unaffected at any of the other DNA workflow steps relative to the nominal condition. RNA fusion calling was unaffected by deviations in any of the tested steps, but underlying supporting reads were reduced by residual amounts of ethanol after the ligation clean up stage (Step H, Condition 2). Underlying supporting reads for RNA fusions were unaffected at any of the other workflow steps relative to the nominal condition.

For RNA splice variants, the low PPC seen in the nominal condition for many of the workflow steps (A, C, F, H-K) was the result of the FFPE sample used, which contained a MET splice variant below the LoD. Underlying supporting reads for splice variants were reduced by 35% in the presence of 4 µL of residual EtOH after adapter ligation clean up (step E).

Table 60. Workflow Guardbanding Results for Small DNA Variants

Variant Type	Test Workflow	PPC (n/N) (95% CIs)		PNC (n/N) (95% CIs)[1]	
		Condition 1	Condition 2	Condition 1	Condition 2
Small DNA Variants	C	98.8 (17700 / 17910) (98.7, 99.0)	99.1 (17767 / 17938) (98.9, 99.2)	> 99.9 (23261043 / 23261244) (> 99.9, > 99.9)	> 99.9 (24047851 / 24048098) (> 99.9, > 99.9)
	D	99.2 (17177 / 17323) (99.0, 99.3)	99.2 (18644 / 18800) (99.0, 99.3)	> 99.9 (22086218 / 22086447) (> 99.9, > 99.9)	99.9989 (24309232 / 24309494) (> 99.9, > 99.9)
	E	99.2 (18592 / 18736) (99.1, 99.3)	99.1 (18588 / 18751) (99.0, 99.3)	> 99.9 (24247843 / 24248225) (> 99.9, > 99.9)	99.9984 (24274251 / 24274646) (> 99.9, > 99.9)
	F	99.1 (17542 / 17704) (98.9, 99.2)	99.1 (17560 / 17719) (99.0, 99.2)	> 99.9 (22591147 / 22591366) (> 99.9, > 99.9)	99.999 (22783719 / 22783943) (> 99.9, > 99.9)
	G	98.9 (17527 / 17715) (98.8, 99.1)	98.9 (17543 / 17736) (98.7, 99.1)	> 99.9 (22332199 / 22332408) (> 99.9, > 99.9)	99.999 (22501485 / 22501704) (> 99.9, > 99.9)
	H	99.3 (17584 / 17704) (99.2, 99.4)	99.2 (17545 / 17694) (99.0, 99.3)	> 99.9 (23854738 / 23855029) (> 99.9, > 99.9)	99.999 (23805004 / 23805252) (> 99.9, > 99.9)
	I	99.3 (17676 / 17800) (99.2, 99.4)	99.2 (17650 / 17794) (99.0, 99.3)	> 99.9 (24492854 / 24493149) (> 99.9, > 99.9)	99.999 (24381939 / 24382179) (> 99.9, > 99.9)
	J	99.2 (17419 / 17567) (99.0, 99.3)	99.1 (17402 / 17559) (99.0, 99.2)	> 99.9 (22726512 / 22726723) (> 99.9, > 99.9)	99.9992 (22719204 / 22719396) (> 99.9, > 99.9)
	K	99.1 (16250 / 16397) (98.9, 99.2)	99.0 (17408 / 17584) (98.8, 99.1)	> 99.9 (20764832 / 20765039) (> 99.9, > 99.9)	> 99.9 (22202812 / 22203000) (> 99.9, > 99.9)

[1]The percentage of false positives among the total observations. FPR is calculated for RNA variants in situations where a true negative count is not defined.

Table 61. Workflow Guardbanding Results for RNA Variants

Variant Type	Test Workflow	PPC (n/N) (95% CIs)		FPR (n/N) (95% CIs)[1]	
		Condition 1	Condition 2	Condition 1	Condition 2
RNA Fusions	A	100 (12/12) (75.8, 100)	100 (12/12) (75.8, 100)	0 (0/12) (0, 24.2)	0 (0/12) (0, 24.2)
	B	100 (12/12) (75.8, 100)	100 (11/11) (74.1, 100)	0 (0/12) (0, 24.2)	0 (0/11) (0, 25.9)
	C	100 (12/12) (75.8, 100)	100 (12/12) (75.8, 100)	0 (0/12) (0, 24.2)	0 (0/12) (0, 24.2)
	D	100 (10/10) (72.8, 100)	100 (11/11) (74.1, 100)	0 (0/10) (0, 27.8)	0 (0/11) (0, 25.9)
	E	90.9 (10/11) (62.3, 98.4)	75.0 (9/12)[2] (46.8, 91.1)	10 (1/11) (1.6, 37.7)	0 (0/12) (0, 24.2)
	F	100 (12/12) (75.8, 100)	100 (12/12) (75.8, 100)	0 (0/12) (0, 24.2)	0 (0/12) (0, 24.2)
	G	100 (12/12) (75.8, 100)	100 (12/12) (75.8, 100)	0 (0/12) (0, 24.2)	0 (0/12) (0, 24.2)
	H	100 (11/11) (74.1, 100)	91.6 (11/12) (64.6, 98.5)	0 (0/11) (0, 25.9)	0 (0/12) (0, 24.2)
	I	100 (12/12) (75.8, 100)	91.6 (11/12) (64.6, 98.5)	0 (0/12) (0, 24.2)	0 (0/12) (0, 24.2)
	J	100 (12/12) (75.8, 100)	100 (12/12) (75.8, 100)	0 (0/12) (0, 24.2)	0 (0/12) (0, 24.2)
	K	100 (10/10) (72.8, 100)	100 (10/10) (72.8, 100)	0 (0/10) (0, 27.8)	0 (0/10) (0, 27.8)
Splice Variants	A	81.3 (13/16) (57.0, 93.4)	93.8 (15/16) (71.7, 98.9)	0 (0/16) (0, 19.4)	0 (0/15) (0, 20.4)
	B	100 (6/6) (61, 100)	62.5 (5/8) (30.6, 86.3)	33.3 (2/6) (9.7, 70.0)	12.5 (1/8) (2.2, 47.1)
	C	81.3 (13/16) (57.0, 93.4)	64.3 (9/14) (38.8, 83.7	0 (0/16) (0, 19.4)	0 (0/16) (0, 19.4)
	D	100 (7/7) (64.6, 100)	87.5 (7/8) (52.9, 97.8)	57.1 (4/7) (25.0, 84.2)	87.5 (7/8) (52.9, 97.8)
	E	100 (8/8) (67.6, 100)	100 (8/8) (67.6, 100)	62.5 (5/8) (30.6, 86.3)	62.5 (5/8) (30.6, 86.3)
	F	56.3 (9/16) (33.2,76.9)	87.5 (14/16) (64.0, 96.5)	0 (0/16) (0, 19.4)	0 (0/16) (0, 19.4)
	G	93.8 (15/16) (71.7, 98.9)	87.5 (14/16) (64.0, 96.5)	0 (0/16) (0, 19.4)	0 (0/16) (0, 19.4)
	H	81.3 (13/16) (57.0, 93.4)	93.8 (15/16) (71.7, 98.9)	0 (0/16) (0, 19.4)	0 (0/16) (0, 19.4)
	I	87.5 (14/16) (64.0, 96.5)	93.8 (15/16) (71.7, 98.9)	0 (0/16) (0, 19.4)	0 (0/16) (0, 19.4)
	J	71.4 (5/7) (35.9, 91.8)	85.7 (6/7) (48.7, 97.4)	33.3 (2/6) (9.7, 70.0)	57.1 (4/7) (25.0, 84.2)
	K	87.5 (14/16) (64.0, 96.5)	81.3 (13/16) (57.0, 93.4)	0 (0/16) (0, 19.4)	0 (0/16) (0, 19.4)

[1]The percentage of false positives among the total observations. FPR is calculated for RNA variants in situations where a true negative count is not defined.

[2]Low PPC was due to 2 misses with a single fusion (LMNA-*NTRK*1) below the limit of detection, which was also missed twice in the nominal condition, indicating random variation in supporting reads near the calling threshold, rather than the testing condition.

9. Cross-Contamination

The cross-contamination study was conducted to evaluate 1) if false positive results were due to well-to-well contamination during sample library preparation or run-to-run contamination between consecutive sequencing runs, and 2) the sensitivity of the two QC metrics designed to detect contamination in DNA samples (CONTAMINATION_SCORE and P_VALUE). Analysis for 1) was performed both for small DNA variants (including TMB) and RNA variants, and analysis for 2) was performed for just DNA variants.

For 1), libraries were prepared from characterized FFPE samples in a checkerboard layout with alternating samples to evaluate well-to-well contamination, and with alternating indexes to evaluate sequencing run-to-run contamination when sequenced consecutively on the same NextSeq 550Dx instrument. The cross-contamination study showed zero contamination events observed by examining the detected variants in each sample, with no false positives detected.

An additional study was also conducted to test cross contamination with a single "donor" sample with an *NTRK* fusion at a high number of supporting reads (> 3000) relative to the distribution observed in the clinical study for *NTRK* in a "donor-receiver" layout, with the "donor" surrounded by RNA positive controls acting as the "receiver", as they do not contain the specific *NTRK* fusion used by the "donor". No cross contamination was observed.

For 2), FFPE tumor DNA samples were mixed with varying amounts of FFPE normal DNA samples to create purposely contaminated samples. Contamination detection using the above-described metrics was evaluated. In total, 1112 contamination observations were generated, and contamination was detected in 95% (1054) of the observations. The detection rate was increased to 96% (939/976) when the percentage of contamination was between 10% to 90% (mass/mass). Of the 37 observations between 10% to 90% contamination where contamination was not detected, 12 did not meet the coverage specification to call small DNA variants and TMB.

10. Extraction Method Equivalency

Three extraction kits for DNA and RNA were evaluated with the TSO Comprehensive assay. The three extraction kits isolated both DNA and RNA from the same FFPE tissue sections but differed in their deparaffinization agent and nucleic acid binding steps (Table 62). 4 FFPE samples for DNA from 2 different tissue types and 5 FFPE samples for RNA from 5 different tissue types were used. Samples were subjected to nucleic acid extraction (3 kits x 2 operators x 3 days x 2 replicates) to obtain DNA and RNA in the same event (36 total observations). For some FFPE samples with fusion genes, an alternative design was used (samples extracted in triplicate using the three extraction kits and tested in four library prep replicates for a total of 36 total observations). Mean differences between extraction kits were calculated with Kit 1 as the control since Kit 1 was used to extract most of the nucleic acids used for TSO Comprehensive assay analytical studies. The

difference was reported if the mean values (VAF, score, or supporting reads) between the extraction kits were significantly different. The mean difference relative to Kit 1 was reported to illustrate how different extraction kits would affect the other TSO Comprehensive assay analytical studies. Invalid rates were 5% (25 out of 465 DNA libraries failed) and 1% (2 out of 383 RNA libraries failed).

Table 62. Extraction Kit Characteristics

Kit	Deparaffinization Agent	Nucleic Acid Binding
1	Proprietary	Column
2	Xylene	Column
3	Mineral Oil	Magnetic beads

Qualitative analysis showed that there was no discernable difference in variant calling for any of the variant types, as all had PPCs >95%. Table 63 shows the summary of the effects of the extraction kits on variant calling (quantitative analysis). For small DNA variants, the variance between kits was small compared to the residual, and there was no significant difference associated with kits for several levels of VAF analyzed. For TMB, between kit variance was small compared to the residual. For fusions and splice variants, there was a large increase in supporting reads (~51% increase in Kit 2 and 23% increase in Kit 3 for fusions, 48% increase in Kits 2 and 3 for splice variants).

Table 63. Extraction Kit Impacts on Variant Calling

Variant Type (units)	Variant Calling (Mean Difference relative to Kit 1)
Small DNA Variants (VAF)	Not technically significant Targeted variants: between kit variance was small relative to residual Non-targeted variants: No significant differences for the first two VAF bins. No meaningful differences when statistical significance observed.
TMB (mutation per megabase)	Not technically significant, between-kit variance was small relative to residual
Fusions (supporting reads)	Kit 2 had 51% and Kit 3 had 23% increase in supporting reads
Splice Variants (supporting reads)	Kit 2 and Kit 3 had 48% increase in supporting reads

Table 64 shows the summary of the effects of the extraction kits on library validity. In short, Kits 2 and 3 had no significant variation in library QC metrics between in relation to Kit 1.

Table 64. Extraction Kit Impacts on Library Validity

Variant Type	Library QC Metric	Mean difference relative to Kit 1
DNA Small Variants/TMB	Median Exon Coverage(count) PCT Exon50X (%) Median Insert Size (bp)	Kit 2 lower by 56 reads Kit 3 higher by 0.298% Kit 2 and Kit 3 lower by 3 bp

Variant Type	Library QC Metric	Mean difference relative to Kit 1
RNA (Fusions/Splice Variants)	Median Insert Size (bp) Log (Median CV Gene500X) Total on Target Reads	Kit 3 higher by 2 bp Kit 2 higher by 0.029 No significant difference

Combined, these data demonstrate that different appropriate extraction methods may be used to extract nucleic acid for use with the TSO Comprehensive assay.

11. Specimen Stability Studies

a. Slide-Mounted FFPE Tissue

Stability of FFPE slides was evaluated using 8 FFPE samples for DNA from 3 different tissue types and 9 FFPE samples for RNA from 8 different tissue types. FFPE blocks with targeted variants were sectioned (eight sections/time point, 5 µm each), mounted on slides, randomized into two time points (T0 and T1), and stored in a calibrated incubator set at 22°C to represent average room temperature (15°C to 30°C). Nucleic acid was extracted from the samples at T0 (one day) and T1 (five weeks) after sections were cut to support a stability claim of 28 days. At each time point, the tissue from each of the slides was removed, pooled together for a given sample, and the respective nucleic acid was extracted and stored at their respective temperatures (DNA at -25˚C to -15°C, RNA at -85˚C to -65°C).). Libraries were generated by four operators for each of the FFPE samples (four independent library preparation events) with two replicates per sample for a total of eight observations per time point.point Additional testing for CDx variants (RET and *NTRK* fusions) was conducted by two operators (two library preparation events) with two replicates per library preparation each for a total of eight observations per time point. Baseline was compared to T1 for small DNA variants, TMB, RNA fusions, and RNA splice variants with CDx and Tumor Profiling variants for both qualitative and quantitative analysis. Invalid rates were 0% for DNA and 12.0% (20 out of 167 libraries failed) for RNA. Qualitative analysis showed that targeted DNA variants had a 100% PPC in the test condition, with an aggregate PPC of >99% for all variants, and targeted RNA variants had a PPC of 95% in the test condition. RNA fusions did have a higher-than-normal false positive rate in both the baseline and test condition due to one sample (*NTRK*1 fusion) having a low level of supporting reads. Quantitative analysis showed a statistically significant difference associated with time among small DNA variants at high VAF levels (>0.1); however, these were deemed negligible due to the large sample number analyzed. There was also a significant difference associated with time in RNA fusions and splice variants, which saw a decrease in supporting reads (between 25%-29% reduction) but did not affect variant calling. These data indicate that slide mounted FFPE tissue is stable for up to 28 days when stored at room temperature.

b. Nucleic Acid

The stability of nucleic acids (DNA and RNA) and their associated quantitation for use with the TSO Comprehensive assay was evaluated using 8 FFPE samples for DNA from 4 different tissue types, and 9 FFPE samples for RNA from 8 different tissue types. FFPE blocks were sectioned, and all nucleic acids were extracted at once. Extracted nucleic acid was thoroughly mixed, quantified, checked for nucleic acid quality, and aliquoted into two

sets of single-use tubes to be frozen for two time points: T0 (control) and T1 (35 days) to support a stability claim of 28 days. All extracted RNA was stored at -85°C to -65°C, and all extracted DNA was stored at -25°C to -15°C until being tested at T0 or T1 with multiple replicates and operators. Libraries were generated by operators for each of the samples (4 independent library preparation events) with 2 replicates (8 observations per sample per time point). Additional testing for CDx variants (RET and NTRK fusions) was conducted by two operators (2 library preparation events) with 2 replicates per library preparation each (8 observations per timepoint). Baseline was compared to T1 for small DNA variants, TMB, RNA fusions, and RNA splice variants with CDx and Tumor Profiling variants for both qualitative and quantitative analysis. Invalid rates were 3.9% (5 out of 127 libraries failed for DNA) and 0% for RNA. Qualitative analysis showed that both targeted DNA and RNA variants had >90% PPC, and quantitative analysis showed no significant differences across time points for either DNA or RNA variants. These data indicate that nucleic acids and their associated quantitation for use with the TSO Comprehensive assay are stable for up to 28 days when stored at the recommended temperatures (RNA at -85°C to -65°C and DNA at -25°C to -15°C).

c. Library

The stability of libraries utilizing each of the 6 safe stopping points for the assay (refer to Table 65) was evaluated.

Stopping points A-D were evaluated using 8 FFPE samples for DNA from 3 different tissue types, and 6 FFPE samples for RNA in 4 different tissue types. Libraries were generated in duplicate by 3 operators for each of the FFPE samples for each stopping point condition. Operators stored the respective plate at -25°C to -15°C at each stopping point to support the claim for the storage time tested (T1). T1 (variable, refer to Table 65) was compared to T0 (baseline) for small DNA variants, TMB, RNA fusions, and RNA splice variants with CDx and Tumor Profiling variants for all stopping points (A-D). Invalid rates were 0.9% (2 out of 240 DNA libraries failed) and 4.5% (8 out of 120 RNA libraries failed). Qualitative analysis showed that small DNA variants and RNA variants (fusions and splice variants) had >95% PPC; however, RNA fusions had a higher than normal (>10%) false positive rate in conditions A, B, and D. This was due to a sample with an *NTRK*3 fusion that was well below the LoD for that specific fusion (7 supporting reads as compared to 16.2 supporting reads for the LoD). Quantitative analysis showed that DNA and RNA variants showed no significant differences across time points, which supports the stability claims and storage temperatures listed in Table 65.

Stopping point E (normalized libraries) was evaluated using 5 FFPE samples for DNA from 4 different tissue types, and 4 FFPE samples for RNA from 3 different tissue types. Libraries were generated and tested by 3 operators in singlet. Control libraries (T0) were sequenced immediately at the end of the workflow. Aliquots from the same libraries were held at the stopping point at -25°C to -15°C for the time (T1) to support the days listed in Table 6565. T1 (≥ 39 days) was compared to T0 (baseline) for small DNA variants, TMB, RNA fusions, and RNA splice variants with CDx and Tumor Profiling variants. There were no invalid libraries out of 30 DNA and 24 RNA libraries. Qualitative analysis showed that both targeted DNA and RNA variants had >90% PPC, and quantitative analysis showed no significant differences across time points for either DNA or RNA

variants, which supports a claim of 32 days for normalized libraries stored at -25°C to -15°C.

Table 65. Library Storage Times

Library Type	Plate	Number of Days	Storage Temperature
cDNA (A, RNA only)	PCF PCR	≤ 7	-25°C to -15°C
Fragmented gDNA (A, DNA only)	LP PCR	≤ 7	-25°C to -15°C
Pre-enrichment (B)	ALS PCR	≤ 30	-25°C to -15°C
Post-enrichment (C)	ELU2 PCR	≤ 7	-25°C to -15°C
Post-enrichment PCR (D)	PL PCR	≤ 30	-25°C to -15°C
Normalized (E)	NL PCR	≤ 3232	-25°C to -15°C

12. Reagent Stability Studies

a. Reagent Stability – Real Time

Real-time stability of the TSO Comprehensive assay reagents was evaluated using 5 FFPE samples for DNA from 5 different tissue types, and 4 FFPE samples for RNA from 3 different tissue types containing the CDx and Tumor Profiling variants that are part of the TSO Comprehensive assay claim. Three (3) reagent lots were tested across 6 time points (3 months, 6 months, 9 months, 13 months, 19 months, and 25 months) with three operators performing library preparation events for each lot, including one replicate of each sample, for a total of three replicates per lot. The kits were stored in final kitted configuration for the duration of the study at storage conditions defined on the product label (frozen kit components at -15°C to -25°C, refrigerated kit components at 2°C to 8°C). The kits were tested against appearance and functional kit release criteria at specified time points. Invalid rates were 0.9% (3 out of 326 DNA libraries failed) and 4.3% (11 out of 258 RNA libraries failed).

Evaluation of variant calling (qualitative analysis) and trend analysis (quantitative analysis) was assessed. For targeted small DNA variants, RNA fusions, and RNA splice variants, aggregate PPC across all time points was >95%. In addition, there were no significant differences associated with time for TMB score. FFor RNA fusions, RNA splice variants, and small DNA variants, there was a significant difference associated with time in one of the lots tested; however, the significance did not cause the respective values (VAF and supporting reads) to fall below the allowable drift for each of the respective variants.

These data support the stability claim of 24 months for the TSO Comprehensive assay reagents at their appropriate storage temperatures.

b. Reagent Stability – In-Use

Kit in-use stability of the TSO Comprehensive assay kit was evaluated under standard use conditions over the course of the shelf life (24 months). The frozen components of the reagent kit were subjected to multiple freeze/thaws and refrigerated reagents were brought up to room temperature to support up to 4 uses of the kit. Five (5) FFPE samples for DNA in 4 different tissue types, and 6 FFPE samples in for RNA in 5 different tissue types were used to evaluate in-use stability compromising CDx and Tumor profiling variants to be claimed. For each time point, a kit was used to prepare a total of 24 DNA and 24 RNA libraries with a total of three library preparation events for each sample were performed for each time point. Qualitative and quantitative analyses were assessed for all variant types to be claimed. There were no invalid samples in this study for either DNA or RNA libraries.

Evaluation of variant calling (qualitative analysis) and trend analysis (quantitative analysis) was assessed. For small DNA variants, RNA fusions, and RNA splice variants, PPC was 100%. In addition, there were no significant differences in TMB score, RNA splice variants, or RNA fusions associated with time. However, for small DNA variants, several VAF bins were determined to have significant differences associated with time; however, these differences were negligible and pose a minimal risk to the assay.

These data support up to 4 freeze/thaws of the kit with sufficient reagent volume for 24 DNA and 24 RNA libraries.

c. Transport Stability

Kit transport stability of the TSO Comprehensive assay was evaluated using 8 FFPE samples for DNA from 5 different tissue types, and 10 FFPE samples for RNA from 5 different tissue types to show functionality of the reagent kits after exposure to a real-world international shipping and distribution condition. Product packaging was separated into validated shipping containers based on storage temperatures, with one shipping test case (real world shipment from Illumina's San Diego headquarters to Illumina's Singapore (SG) distribution center and shipped back to San Diego) and one control non-shipped case (transportation one building to another within Illumina HQ and back again). Functional testing was performed using the above-named samples harboring CDx and Tumor Profiling variants that are part of the TSO Comprehensive assay claim and consisted of four library preps performed by two operators generating 6 replicates of each sample. The physical attributes (packaging) of the kits were also observed. There were no invalid samples for either DNA or RNA libraries. Functional testing concluded that shipping did not negatively impact variant calling for small DNA variants (PPC = 100%) and RNA fusions (PPC = 97%), relative to the control condition. RNA splice variants did show a negative impact due to shipping (PPC = 75% in the test condition). In addition, both RNA fusions and RNA splice variants showed a significant difference in supporting reads associated with shipping (decrease of between 20%-24%). TMB did not show a significant difference in score associated with shipping, and physical examination of packaging showed no evidence of major or minor damage.

To further investigate the issue with RNA fusions and splice variants, a second set of testing was done consisting of three different library preps by three operators per condition generating 6 replicates of each RNA sample to determine if the effect was real. Both RNA

fusions and RNA splice variants showed no significant difference in supporting reads associated with shipping after the re-test, and RNA splice variant detection was also not affected (PPC = 100%); the original issue was most likely due to between library preparation variability.

1. Animal Studies

No animal studies were conducted using TSO Comprehensive.

2. Additional Studies

No additional studies were conducted using TSO Comprehensive assay.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

Illumina, Inc. conducted 2 separate clinical bridging studies to establish reasonable assurance of safety and effectiveness of the TSO Comprehensive assay for detection of *NTRK*1, *NTRK*2, and *NTRK*3 fusions in solid tumor patients who may benefit from treatment with larotrectinib, and RET fusions in non-small cell lung cancer (NSCLC) patients who may benefit from treatment with selpercatinib. Data from these clinical studies were the basis for the PMA approval decision. A summary of each of the clinical studies is presented below.

1. Clinical Bridging Study to Identify *NTRK*1, *NTRK*2, and *NTRK*3 Fusions in Solid Tumor Patients to be Treated with Larotrectinib

A. **Study Design**

To establish clinical effectiveness of the TSO Comprehensive assay as a companion diagnostic (TSO Comprehensive assay hereafter "CDx") for detecting *NTRK*1, *NTRK*2, and *NTRK*3 fusions in patients with solid tumors who may benefit from treatment with VITRAKVI (larotrectinib), samples from patients enrolled in the larotrectinib clinical trials LOXO-TRK-14001 (NCT02122913), NAVIGATE (NCT02576431, LOXO-TRK-15002), and SCOUT (NCT02637687, LOXO-TRK-15003), hereafter referred to collectively as the "larotrectinib trial samples", using a data cutoff of 15 JUL 2019, and supplemented with commercially sourced *NTRK* fusion negative FFPE tissue specimens, were tested to support a TSO Comprehensive assay Concordance Study and Clinical Bridging Study.

LOXO-TRK-14001 (NCT02122913) was a multicenter, open-label, Phase 1, dose escalation study in adult patients with advanced solid tumors (all-comers) unselected for *NTRK* fusion positive cancer. Following the dose escalation portion of the study, a dose expansion was initiated for patients with documented *NTRK* fusion positive cancer and for patients whom the investigator believed might benefit from a highly selective TRK inhibitor. NAVIGATE (NCT02576431, LOXO-TRK-15002) is an ongoing, multicenter, open-label, Phase 2, basket study in patients 12 years of age and older with recurrent advanced solid tumors with a documented *NTRK* fusion as assessed by an outside laboratory. SCOUT (NCT02637687,

LOXO-TRK-15003) is an ongoing, multicenter, open-label, Phase 1/2 study in pediatric patients aged from birth to 21 years with advanced solid or primary central nervous system (CNS) tumors.

Of the *NTRK* fusion positive patients included in the TSO Comprehensive assay study, the larotrectinib primary analysis set (PAS) comprised of 55 patients, and the larotrectinib extended primary analysis set (ePAS4) comprised of 164 patients.

The clinical validity of the TSO Comprehensive assay for detecting *NTRK*1, *NTRK*2, or *NTRK*3 fusions in patients with solid tumors who may benefit from treatment with larotrectinib was evaluated in a clinical bridging study. The study was conducted to assess the clinical effectiveness of the TSO Comprehensive assay to identify *NTRK*1, *NTRK*2, or *NTRK*3 fusion positive patients for treatment with larotrectinib, and to assess the concordance between the TSO Comprehensive assay and local tests (LTs) used to determine *NTRK* fusion status for the larotrectinib clinical trials.

Key Inclusion Criteria for Clinical Study Samples

- Sample is from a patient who was enrolled in LOXO-TRK-14001 (NCT02122913), NAVIGATE (NCT02576431, LOXO-TRK-15002), SCOUT (NCT02637687, LOXO-TRK-15003) studies with known LT *NTRK* fusion status and was treated with at least 1 dose of larotrectinib on or prior to 15 JUL 2019.
- Sample was collected/retained under informed consent and is available. Sample can be FFPE tumor tissue (in block or slide form) and/or purified RNA (and DNA, if available) extracted from the FFPE tumor tissue.

Key Exclusion Criteria for Clinical Trial Samples

- Sample H&E shows no tumor present.
- Sample does not meet RNA requirements outlined in the TSO Comprehensive assay package insert.
- Sample was withdrawn/rejected or does not have appropriate consent.

Follow-up Schedule

The TSO Comp bridging study involved a retrospective testing of samples; as such, no additional patient follow-up was conducted in regard to the clinical bridging study.

Clinical Endpoints

The primary endpoint for the larotrectinib analysis of efficacy was overall response rate (ORR) according to independent review committee (IRC) assessment in a pooled dataset from the 3 clinical studies. ORR was assessed based on the proportion of patients with best

overall response of confirmed complete response (CR), pathological CR (pCR), or confirmed partial response (PR) based on RECIST, version 1.1 criteria.

The objectives of the TSO Comprehensive assay clinical bridging study were to:

- Demonstrate safety and effectiveness of the TSO Comprehensive assay in identifying *NTRK* fusion positive patients for treatment with larotrectinib.
- Assess concordance of results for the *NTRK* gene fusion status between the TSO Comprehensive assay and the local clinical trial assays.

B. Accountability of PMA Cohort

Full Analysis Set Sample Accountability

Disposition of the patient samples based on *NTRK* gene fusion status, as determined by the LTs, is shown in Table 66 for each of the larotrectinib clinical studies (LOXO-TRK-14001, LOXO-TRK-15002, LOXO-TRK-15003, thereafter Study 14001, 15002, and 15003, respectively). A total of 279 patients were enrolled in the larotrectinib studies as of 15 JUL 2019. Of the 279 patients, 235 patients had been tested for *NTRK* gene fusion status resulting in 208 *NTRK* gene fusion positive patients and 27 *NTRK* gene fusion negative patients as determined by LT results. For 44 patients, the *NTRK* fusion status is unknown, as testing was not required for patient eligibility in the Study 14001 and Study 15003 dose escalation phases.

Table 66. Larotrectinib Clinical Trial Patients by LT *NTRK* Fusion Status (All Clinical Trial Patients)

	Study 14001 N=75 (100%)	Study 15002 N=116 (100%)	Study 15003 N=88 (100%)	Total N=279 (100%)
NTRK fusion positive, n (%)	13 (17.3%)	116 (100.0%)	79 (89.8%)	208 (74.6%)
NTRK fusion negative, n (%)	26 (34.7%)	0	1 (1.1%)	27 (9.7%)
NTRK fusion status unknown, n (%)	36 (48.0%)	0	8 (9.1%)	44 (15.8%)

Abbreviations: *NTRK* = Neurotrophic Tyrosine Receptor Kinase.
All percentages were calculated using N as denominator.
Data cut-off 15 JUL 2019.

TruSight Oncology Comprehensive assay / LT assay agreement analysis was performed using the larotrectinib clinical trial patients enrolled as of 15 JUL 2019 with known *NTRK* status, and the supplemental samples determined to be *NTRK* fusion negative by one of two NGS-based representative LTs.

The disposition of patient samples used in the TSO Comprehensive assay clinical study is shown in Table 67 (clinical trial *NTRK* fusion positive patients), Table 68 (clinical trial *NTRK* fusion negative patients), Table 69 (supplemental *NTRK* fusion negative patients) and Figure 3.

Of the 208 *NTRK* fusion positive patients, 154 had samples available for testing with the TSO Comprehensive assay. Fifteen (15) samples were invalid due to failing sample sequencing quality metrics and one (1) sample was not tested due to a protocol deviation.

The CDx *NTRK* fusion positive CDx evaluable set thus included 138 clinical trial patients (Table 67).

Of the 27 clinical trial *NTRK* fusion negative patients, 24 had samples available for testing with the TSO Comprehensive assay. Two (2) samples were invalid due to failing sample sequencing metrics. The CDx *NTRK* fusion negative CDx evaluable set thus included 22 clinical trial patients (Table 68).

A supplemental set of *NTRK* fusion negative patient samples was obtained by screening procured samples using one of two representative LTs. More than 350 FFPE samples were initially procured and examined for tumor content by pathology assessment. Samples meeting requirements were extracted to RNA, followed by *NTRK* fusion screening. Of the procured FFPE samples, 266 were successfully extracted and confirmed *NTRK* fusion negative by one of two representative LTs. Of the 266 samples, 260 were available for testing with the TSO Comprehensive assay (Table 48). Of the 260 supplemental LT negative samples available for testing by TSO Comprehensive assay, 38 samples were invalid due to failing the sample sequencing metrics (n=25) or failing run sequencing (n=13). The CDx *NTRK* fusion negative CDx evaluable set thus included 222 supplemental LT negative patients (Table 69). When combined with the evaluable *NTRK* fusion negative larotrectinib clinical trial patients (n=22), the total *NTRK* fusion negative CDx evaluable set was made up of 244 patients. In summary, the total device study population (n=501 patients) included 208 LT fusion positive patients, 27 LT fusion negative clinical trial patients, and 266 LT fusion negative supplemental patients. Of the 501 patients, samples were available for 438 patients and 437 were tested by the TSO Comprehensive assay. The 437 patients tested included 153 LT fusion positive patients and 284 LT fusion negative patients. Testing by TSO Comprehensive assay resulted in 382 patients with valid results forming the CDx evaluable population (138 LT fusion positive patients and 244 LT negative patients).

The CDx non-evaluable population (119 total) included 63 patient samples not available for testing by TSO Comprehensive assay (54 LT fusion positive samples, 3 LT fusion negative clinical trial samples, 6 LT fusion negative supplemental sample), 1 sample not tested due to protocol deviation, and 55 samples with invalid results by TSO Comprehensive assay (15 LT fusion positive samples, 2 LT fusion negative sample clinical trial samples, and 38 LT fusion negative supplemental samples) (Figure 3).

Table 67. Accountability of larotrectinib Clinical Trial LT *NTRK* Fusion Positive Patients Evaluable for the CDx Device Study (FPAS)

	Study 14001 N=13 (100%)	Study 15002 N=116 (100%)	Study 15003 N=79 (100%)	Total N=208 (100%)
NTRK fusion positive CDx evaluable set, n (%)	8 (61.5%)	80 (69.0%)	50 (63.3%)	138 (66.3%)
NTRK fusion positive CDx non-evaluable set, n (%)	5 (38.5%)	36 (31.0%)	29 (36.7%)	70 (33.7%)
Reasons for CDx non-evaluable, n (%)				
Sample not available / insufficient for CDx testing	4 (30.8%)	30 (25.9%)	20 (25.3%)	54 (26.0%)
Not tested, protocol deviation	1 (7.7%)	0	0	1 (0.5%)
Failed sample sequencing	0	6 (5.2%)	9 (11.4%)	15 (7.2%)

Abbreviations: CDx = TSO Comprehensive assay Companion Diagnostic; FPAS = Fusion Positive Analysis Set; *NTRK* = Neurotrophic Tyrosine Receptor Kinase.
All percentages were calculated using N as denominator.

Table 68. Accountability of larotrectinib Clinical Trial LT *NTRK* Fusion Negative Patients Evaluable for the CDx Device Study (FNAS-CLIN)

	Study 14001 N=26 (100%)	Study 15002 N=0 (100%)	Study 15003 N=1 (100%)	Total N=27 (100%)
NTRK fusion negative CDx evaluable set from clinical studies, n (%)	21 (80.8%)	0	1 (100.0%)	22 (81.5%)
NTRK fusion negative CDx non-evaluable set from clinical studies, n (%)	5 (19.2%)	0	0	5 (18.5%)
Reasons for CDx non-evaluable, n (%)				
Sample not available / insufficient for CDx testing	3 (11.5%)	0	0	3 (11.1%)
Failed sample sequencing	2 (7.7%)	0	0	2 (7.4%)

Abbreviations: CDx = TSO Comprehensive assay Companion Diagnostic; FNAS-CLIN = *NTRK* Gene Fusion Negative Patients from the Larotrectinib Clinical Studies;; *NTRK* = Neurotrophic Tyrosine Receptor Kinase.
All percentages were calculated using N as denominator.

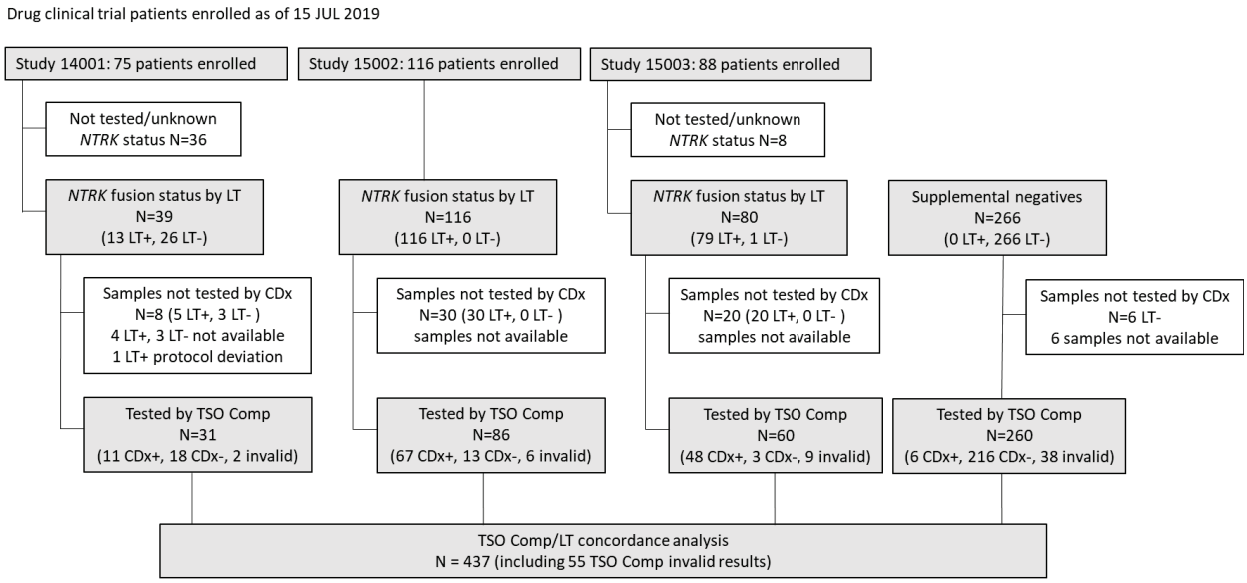
Table 69. Accountability of Supplemental LT *NTRK* Fusion Negative Patients for the CDx Device Study (FNAS-SUPP)

	Total N=266 (100%)
NTRK fusion negative CDx evaluable set from supplemental negative samples, n (%)	222 (83.5%)
NTRK fusion negative CDx non-evaluable set from supplemental negative samples, n (%)	44 (16.5%)
Reasons for CDx non-evaluable, n (%)	

	Total N=266 (100%)
Sample not available / insufficient for CDx testing	6 (2.3%)
Failed sample sequencing	25 (9.4%)
Failed run sequencing	13 (4.9%)

Abbreviations: CDx = TSO Comprehensive assay Companion Diagnostic; FNAS-SUPP = Supplemental *NTRK* Gene Fusion Negative Samples; *NTRK* = Neurotrophic Tyrosine Receptor Kinase.
All percentages were calculated using N as denominator.

Figure 3. TSO Comprehensive assay Clinical Study Sample Accountability – Full Analysis Set



Abbreviations: CDx = TSO Comprehensive assay Companion Diagnostic; *NTRK* = Neurotrophic Tyrosine Receptor Kinase

Primary Efficacy Analysis Sample Accountability

The primary efficacy analysis for the device clinical study uses the same patient set as the larotrectinib NDA primary analysis set (PAS). PAS was comprised of the first 55 patients, both pediatric and adult, enrolled in Studies 14001, 15002, or 15003 meeting the following criteria:

- Documented *NTRK* gene fusion as determined by local testing
- Non-CNS primary tumor with 1 or more measurable lesions at baseline as assessed by RECIST v1.1
- Received 1 or more doses of larotrectinib.

Disposition of the efficacy primary analysis set is shown in Table 70 and summarized in Figure 4. Study 14001 contributed 8 patients, Study 15002 contributed 35, and Study 15003 contributed 12 for a total of 55 patients. Of the 55 *NTRK* fusion positive patients in the efficacy set, 42 had samples available for testing with the TSO Comprehensive assay. Of the

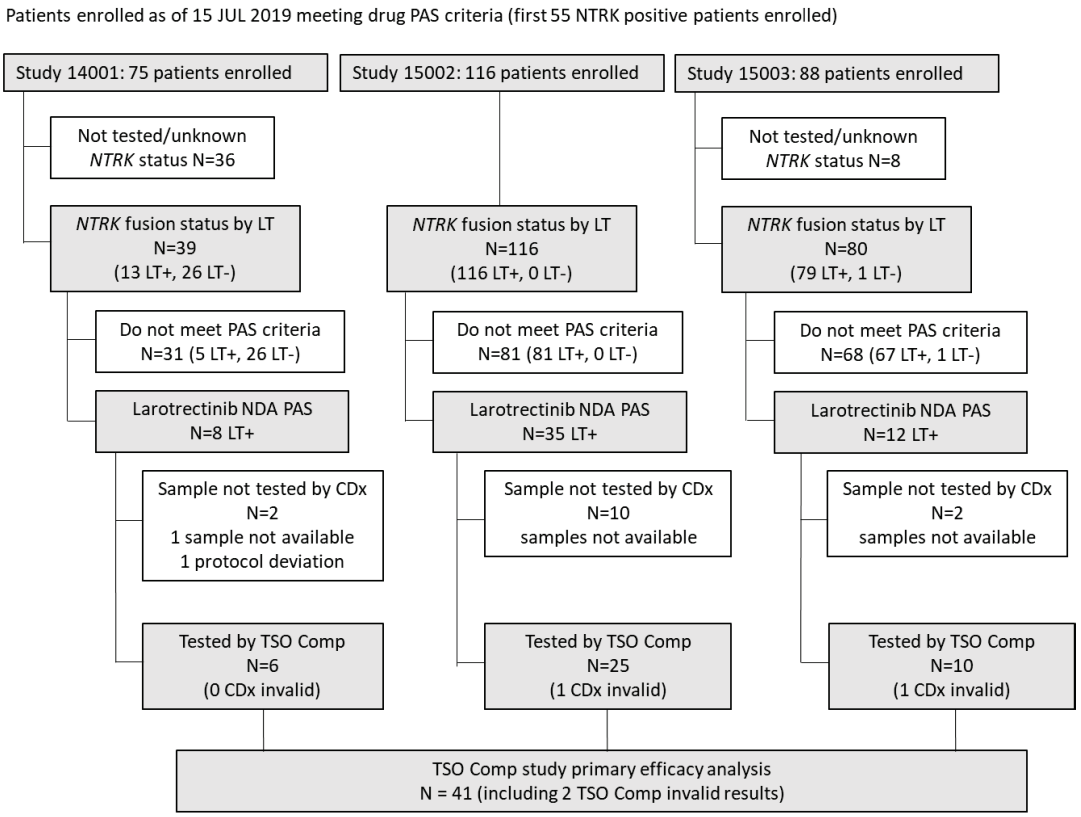
42 samples, 2 samples were invalid due to failing sample sequencing and 1 sample was not tested due to protocol deviation. The CDx-evaluable efficacy primary analysis set was therefore comprised of 39 *NTRK* fusion positive clinical trial patients (Table 70).

Table 70. Accountability of larotrectinib Clinical Trial LT *NTRK* Fusion Positive Patients in the Efficacy Primary Analysis Set (PAS) for the CDx Device Study

	14001 N=8 (100%)	15002 N=35 (100%)	15003 N=12 (100%)	Total N=55 (100%)
NTRK fusion positive CDx evaluable set, n (%)	6 (75.0%)	24 (68.6%)	9 (75.0%)	39 (70.9%)
NTRK fusion positive CDx non-evaluable set, n (%)	2 (25.0%)	11 (31.4%)	3 (25.0%)	16 (29.1%)
Reasons for CDx non-evaluable, n (%)				
Sample not available / insufficient for CDx testing	1 (12.5%)	10 (28.6%)	2 (16.7%)	13 (23.6%)
Not tested, protocol deviation	1 (12.5%)	0	0	1 (1.8%)
Failed sample sequencing	0	1 (2.9%)	1 (8.3%)	2 (3.6%)

Abbreviations: CDx = TSO Comprehensive assay Companion Diagnostic; *NTRK* = Neurotrophic Tyrosine Receptor Kinase; PAS = Primary Analysis Set.
All percentages were calculated using N as denominator.

Figure 4. Device Clinical Study Sample Accountability - Efficacy Analysis Set



C. Study Population Demographics and Baseline Parameters

Baseline Characteristics for the *NTRK* Fusion Positive Population

Baseline characteristics for the *NTRK* fusion positive population in the full CDx analysis set are shown in Table 71 (demographic) and Table 72 (disease).

Table 71. Comparison of Baseline Demographic Characteristics Between the CDx Evaluable and CDx Non-Evaluable LT *NTRK* Fusion Positive Patients (FPAS)

	CDx Evaluable Set N=138 (100%)	CDx Non-Evaluable Set N=70 (100%)	Total N=208 (100%)
Age, years			
n	138	70	208
Mean	34.93	33.29	34.38
SD	26.62	27.72	26.94
Min	0.1	0.3	0.1
Median	38.00	31.00	34.00
Max	84.0	79.0	84.0
Age group, n (%)			
<18 years	52 (37.7%)	31 (44.3%)	83 (39.9%)
18+ years	86 (62.3%)	39 (55.7%)	125 (60.1%)
Gender, n (%)			
Female	64 (46.4%)	42 (60.0%)	106 (51.0%)
Male	74 (53.6%)	28 (40.0%)	102 (49.0%)
Race, n (%)			
White	97 (70.3%)	57 (81.4%)	154 (74.0%)
Asian	16 (11.6%)	3 (4.3%)	19 (9.1%)
Black	7 (5.1%)	1 (1.4%)	8 (3.8%)
Mixed/Other/Unknown	18 (13.0%)	9 (12.9%)	27 (13.0%)
Ethnicity, n (%)			
Not Hispanic or Latino	111 (80.4%)	58 (82.9%)	169 (81.3%)
Hispanic or Latino	11 (8.0%)	5 (7.1%)	16 (7.7%)
Unknown	16 (11.6%)	7 (10.0%)	23 (11.1%)

Abbreviations: CDx = TSO Comprehensive assay Companion Diagnostic; FPAS = Fusion Positive Analysis Set; *NTRK* = Neurotrophic Tyrosine Receptor Kinase; SD = Standard Deviation.
All percentages were calculated using N as denominator.
Data cut-off 15 JUL 2019.

Table 72. Comparison of Baseline Disease Characteristics Between the CDx Evaluable and CDx Non-Evaluable LT *NTRK* Fusion Positive Patients (FPAS)

	CDx Evaluable Set N=138 (100%)	CDx Non-Evaluable Set N=70 (100%)	Total N=208 (100%)
Primary tumor type, n (%)			
Appendix	1 (0.7%)	0	1 (0.5%)
Bone sarcoma	1 (0.7%)	1 (1.4%)	2 (1.0%)
Breast	3 (2.2%)	3 (4.3%)	6 (2.9%)
Cancer of unknown primary	0	1 (1.4%)	1 (0.5%)
Cervix	1 (0.7%)	0	1 (0.5%)
Cholangiocarcinoma	1 (0.7%)	1 (1.4%)	2 (1.0%)
Colon	6 (4.3%)	2 (2.9%)	8 (3.8%)
Congenital mesoblastic nephroma	1 (0.7%)	0	1 (0.5%)
Gastrointestinal stroma	4 (2.9%)	0	4 (1.9%)
Hepatic	1 (0.7%)	0	1 (0.5%)
Infantile fibrosarcoma	23 (16.7%)	11 (15.7%)	34 (16.3%)
Lung	5 (3.6%)	10 (14.3%)	15 (7.2%)
Melanoma	5 (3.6%)	2 (2.9%)	7 (3.4%)
Pancreas	2 (1.4%)	0	2 (1.0%)
Primary CNS	11 (8.0%)	13 (18.6%)	24 (11.5%)
Prostate	1 (0.7%)	0	1 (0.5%)
Salivary gland	17 (12.3%)	6 (8.6%)	23 (11.1%)
Sarcoma	1 (0.7%)	0	1 (0.5%)
Soft tissue sarcoma	30 (21.7%)	9 (12.9%)	39 (18.8%)
Thyroid	24 (17.4%)	11 (15.7%)	35 (16.8%)
Stage of disease at initial diagnosis, n (%)			
I	16 (11.6%)	7 (10.0%)	23 (11.1%)
II	27 (19.6%)	5 (7.1%)	32 (15.4%)
III	25 (18.1%)	18 (25.7%)	43 (20.7%)
IV	33 (23.9%)	20 (28.6%)	53 (25.5%)
Unknown	37 (26.8%)	20 (28.6%)	57 (27.4%)
Extent of disease at enrollment, n (%)			
Locally advanced	29 (21.0%)	18 (25.7%)	47 (22.6%)
Metastatic	98 (71.0%)	39 (55.7%)	137 (65.9%)
Primary CNS	11 (8.0%)	13 (18.6%)	24 (11.5%)
ECOG performance status at baseline, n (%)			
0	73 (52.9%)	32 (45.7%)	105 (50.5%)
1	51 (37.0%)	27 (38.6%)	78 (37.5%)
2	12 (8.7%)	9 (12.9%)	21 (10.1%)
3	1 (0.7%)	2 (2.9%)	3 (1.4%)
Unknown	1 (0.7%)	0	1 (0.5%)

Abbreviations: CDx = TSO Comprehensive assay Companion Diagnostic; CNS = Central Nervous System; ECOG = Eastern Cooperative Oncology Group; FPAS = Fusion Positive Analysis Set; *NTRK* = Neurotrophic Tyrosine Receptor Kinase.
All percentages were calculated using N as denominator.
Data cut-off 15 JUL 2019.

Baseline Characteristics for the *NTRK* Fusion Negative Clinical Trial Patients

Baseline characteristics for the *NTRK* fusion negative clinical trial patients are shown in Table 73 and Table 74.

Table 73. Comparison of Baseline Demographic Characteristics Between the CDx Evaluable and CDx Non-Evaluable larotrectinib Clinical Trial LT *NTRK* Fusion Negative Patients (FNAS-CLIN)

	CDx Evaluable Set N=22 (100%)	CDx Non-Evaluable Set N=5 (100%)	Total N=27 (100%)
Age, years			
n	22	5	27
Mean	58.30	59.60	58.54
SD	12.62	9.71	11.98
Min	17.6	46.0	17.6
Median	59.00	64.00	60.00
Max	76.0	69.0	76.0
Age group, n (%)			
<18 years	1 (4.5%)	0	1 (3.7%)
18+ years	21 (95.5%)	5 (100.0%)	26 (96.3%)
Gender, n (%)			
Female	12 (54.5%)	3 (60.0%)	15 (55.6%)
Male	10 (45.5%)	2 (40.0%)	12 (44.4%)
Race, n (%)			
White	19 (86.4%)	5 (100.0%)	24 (88.9%)
Black	1 (4.5%)	0	1 (3.7%)
Mixed/Other/Unknown	2 (9.1%)	0	2 (7.4%)
Ethnicity, n (%)			
Not Hispanic or Latino	18 (81.8%)	5 (100.0%)	23 (85.2%)
Hispanic or Latino	4 (18.2%)	0	4 (14.8%)

Abbreviations: CDx = TSO Comprehensive assay Companion Diagnostic; FNAS-CLIN = *NTRK* Gene Fusion Negative Patients from Larotrectinib Clinical Studies; *NTRK* = Neurotrophic Tyrosine Receptor Kinase; SD = Standard Deviation.
All percentages were calculated using N as denominator.
Data cut-off 15 JUL 2019.

Table 74. Comparison of Baseline Disease Characteristics Between the CDx Evaluable and CDx Non-Evaluable larotrectinib Clinical Trial LT *NTRK* Fusion Negative Patients (FNAS-CLIN)

	CDx Evaluable set N=22 (100%)	CDx Non-Evaluable Set N=5 (100%)	Total N=27 (100%)
Primary tumor type, n (%)			
Anal	0	1 (20.0%)	1 (3.7%)
Bone sarcoma	0	1 (20.0%)	1 (3.7%)
Breast	1 (4.5%)	0	1 (3.7%)
Cholangiocarcinoma	1 (4.5%)	0	1 (3.7%)
Colon	5 (22.7%)	0	5 (18.5%)
Hepatic	1 (4.5%)	0	1 (3.7%)
Lung	3 (13.6%)	0	3 (11.1%)
Melanoma	1 (4.5%)	0	1 (3.7%)
Oral	1 (4.5%)	0	1 (3.7%)
Ovarian	1 (4.5%)	0	1 (3.7%)
Pancreas	1 (4.5%)	0	1 (3.7%)
Primary CNS	1 (4.5%)	0	1 (3.7%)
Salivary gland	0	1 (20.0%)	1 (3.7%)
Soft tissue sarcoma	3 (13.6%)	2 (40.0%)	5 (18.5%)
Thymus	2 (9.1%)	0	2 (7.4%)
Thyroid	1 (4.5%)	0	1 (3.7%)
Stage of disease at initial diagnosis, n (%)			
I	2 (9.1%)	1 (20.0%)	3 (11.1%)
II	3 (13.6%)	1 (20.0%)	4 (14.8%)
III	7 (31.8%)	0	7 (25.9%)
IV	10 (45.5%)	3 (60.0%)	13 (48.1%)
Extent of disease at enrollment, n (%)			
Metastatic	21 (95.5%)	5 (100.0%)	26 (96.3%)
Primary CNS	1 (4.5%)	0	1 (3.7%)
ECOG performance status at baseline, n (%)			
0	8 (36.4%)	1 (20.0%)	9 (33.3%)
1	13 (59.1%)	4 (80.0%)	17 (63.0%)
2	1 (4.5%)	0	1 (3.7%)

Abbreviations: CDx = TSO Comprehensive assay Companion Diagnostic; CNS = Central Nervous System; ECOG = Eastern Cooperative Oncology Group; FNAS-CLIN = *NTRK* Gene Fusion Negative Patients from the Larotrectinib Clinical Studies; *NTRK* = Neurotrophic Tyrosine Receptor Kinase.
All percentages were calculated using N as denominator.
Data cut-off 15 JUL 2019.

D. Safety and Effectiveness Results

Safety Results

The safety with respect to treatment with larotrectinib was addressed during the review of the NDA. Please refer to fda.gov/drugs for complete safety information on VITRAKVI® (larotrectinib).

Testing for the TSO Comprehensive assay device study was conducted retrospectively on banked study specimens and the test results were not reported to patients. No patient treatment decisions were made as a result of any of the TSO Comprehensive assay test results; and there were no Unanticipated Adverse Device Effect (UADE) events related to the study products or procedures to patients or operators.

Effectiveness Results – Concordance

There were 437 samples tested by TSO Comprehensive assay. Of those, 55 had invalid TSO Comprehensive assay results (invalid rate of 12.6%). Agreement of TSO Comprehensive assay results relative to the LT results among samples with valid results are shown in Table 75.

Table 75. *NTRK* Clinical Bridging Study: Concordance Between the TSO Comprehensive Assay and LT for Detection of *NTRK* Fusions

		LT Result		
		***NTRK* Fusion Positive**	***NTRK* Fusion Negative**	**Total**
TSO Comprehensive Assay Results	*NTRK* Fusion Positive	123	9[a]	132
	NTRK Fusion Negative	15	235	250
	Total	138	244	382
Agreement Statistics	PPA% (n/N; 95% CI[b])	89.1% (123/138; 82.7%-93.8%)		
	NPA% (n/N; 95% CI[b])	96.3% (235/244; 93.1%-98.3%)		

[a]Five of the 9 samples were TSO Comprehensive assay positive due to an isolated contamination event during study testing.
[b]CI based on Clopper-Pearson (exact) method.

Agreements between the TSO Comprehensive assay and LTs by type (for example, RNA NGS, FISH) are shown in Table 76.

Table 76. *NTRK* Clinical Bridging Study: Concordance Between the TSO Comprehensive Assay and LT for Detection of *NTRK* Fusions by LT Type

LT Type	Measure of Agreement	% Agreement (n/N)	95% CI[a]
DNA NGS	PPA	84.2% (32/38)	68.7%–94.0%
	NPA	88.9% (16/18)	65.3%–98.6%
RNA NGS[b]	PPA	91.5% (75/82)	83.2%–96.5%
	NPA	96.9% (218/225)[c]	93.7%–98.7%
FISH	PPA	80.0% (8/10)	44.4%–97.5%
	NPA	Not calculated (1/1)	Not calculated
PCR	PPA	100.0% (8/8)	63.1%–100.0%
	NPA	Not calculated (0/0)	Not calculated

Note: for subgroups with sample count < 5, agreement statistics were not calculated
[a]The two-sided 95% CIs were calculated using the (exact) Clopper-Pearson method.

[b]Includes NGS methods that use RNA only and both DNA and RNA.
[c]Five of the TSO Comprehensive assay positive results were due to an isolated contamination event during the study

Of the 382 samples with valid TSO Comprehensive assay and LT results, 24 had discordant results with the LTs: 15 were positive by the LTs and negative by the TSO Comprehensive assay and 9 were negative by the LTs and positive by the TSO Comprehensive assay. Of the 24 samples with discordant results, 8 were tested with a DNA NGS LT, 14 with an RNA NGS LT, and 2 with FISH.

A validated independent NGS method was concordant with TSO Comprehensive assay results in 14 of the 24 samples with discordant results. For the remaining 10 samples, TSO Comprehensive assay results were discordant with both the LT and the independent NGS method.

Testing of discordant results was performed for samples in both the *NTRK* Clinical Accuracy and Bridging studies due to low NPA values in both studies, possibly due to a suspected contamination event. A total of 142 RNA samples from both studies, comprised of 107 concordant samples (53 concordant negative, 54 concordant positive), 26 discordant samples, and 9 samples with unknown *NTRK* status from any of the methods used (TSO Comprehensive, comparator method and LT), were re-tested with the TSO Comprehensive assay. Of the 142 samples, 133 produced valid results. Of the 5 suspected contaminated samples that tested *NTRK*-fusion positive by TSO Comprehensive assay, but negative by the LTs, in the original Bridging study (Table 75), 3 of them were available for re-testing. All 3 of those samples were *NTRK*-fusion negative when re-tested by the TSO Comprehensive assay. Additionally, 6 out of the remaining 127 samples with valid retest results changed TSO Comprehensive assay status upon retesting. Of those samples, 3 were concordant *NTRK* fusion-positives by original testing, changing to negative upon re-test. None of the concordant *NTRK* fusion-negatives from the original testing changed to positives. In a sensitivity analysis based on the testing of discordant samples, the PPA in the imputed dataset was estimated to be 86.5% (95% CI: 80.2%, 92.8%) and NPA was estimated at 98.8% (95% CI: 97.5%, 100%). The PPA and NPA from the imputed dataset are within the confidence limits of the original estimates (Table 75). Looking at the totality of the bridging concordance data, there were explanations for the 8 of the 9 "discordant" putative false positives; therefore, the NPA may be underestimated in this bridging study. A limitation in the device labeling addresses the low overall NPA value [96.3% (95% CI: 93.1%, 98.3%] between drug trial enrollment assays and TSO Comprehensive for *NTRK* fusion detection. False positive results for gene fusions such as NTRK may be due to non-specific detection.

Sensitivity Analysis for Concordance Against the Missing CDx Results

Sensitivity analysis against the missing CDx results was performed by imputing missing CDx results for the LT fusion positive patients (70), using a logistic regression model. Agreement estimates including the imputed values were PPA= 85.2%, 95% CI [78.6%, 91.7%] and NPA=96.3%, 95% CI [93.9%, 98.7%] (Table 78), and were similar to the agreement results of the primary concordance analysis (PPA = 89.1%, 95% CI [82.7%, 93.8%], NPA = 96.3%, 95% CI [93.1%, 98.3%]) (Table 75).

Table 77. Concordance Between the CDx and LT for Detection of *NTRK* Gene Fusions including Imputed Values for LT Fusion Positive Patients with Missing CDx Results Using the MI Boot Method (FPAS, FN-CES)

Measure of agreement	% Agreement	95% CI *
Positive percent agreement	85.2%	78.6% -91.7%
Negative percent agreement	96.3%	93.9% -98.7%

Abbreviations: CDx = TSO Comprehensive assay Companion Diagnostic; FN-CES = Fusion Negative CDx Evaluable Set; FPAS = Fusion Positive Analysis Set; MI = Multiple Imputation; *NTRK* = Neurotrophic Tyrosine Receptor Kinase.
Note: The MI Boot method is a bootstrap step nested in the multiple imputation (Schomaker and Heumann).
Note 2: Missing CDx results for LT fusion negative patients were not imputed.
*The two-sided 95% CI was calculated based on the MI Boot method.

Effectiveness Results – Efficacy

Primary Efficacy Results in the *NTRK* Gene Fusion Positive Populations
Efficacy of larotrectinib in the TSO Comprehensive assay positive, LT positive population (34 patients, ORR=85%, 95% CI [69%, 95%]) was higher than the efficacy of larotrectinib in the NDA efficacy population (55 patients, ORR=75%, 95% CI [61%, 85%]) with overlapping confidence intervals (Table 78). The lower limit of the two-sided 95% CI exceeds 30% for the TSO Comp positive, LT positive population, which was considered clinically significant for the larotrectinib clinical study integrated analysis.

Of the 34 TSO Comprehensive assay positive patients in the efficacy set, 10 (29%) patients had achieved a complete response/surgical complete response and 19 (56%) patients had achieved a partial response.

Of the 5 TSO Comprehensive assay negative patients in the *NTRK* fusion positive clinical trial population, one showed complete response and one showed partial response with larotrectinib therapy. The remaining 3 TSO Comprehensive assay negative patients were non-responders.

Of the 55 patients in the PAS, 16 had missing CDx results. The ORR of these 16 missing patients (CDx non-evaluable) in the PAS is lower (ORR=63%) than the ORR of the CDx evaluable population (39 patients, ORR=80%) and the total PAS population (55 patients, ORR=75%). The higher proportion of responders in the CDx evaluable population is likely contributing to the higher ORR observed for the TSO Comp positive population relative to the total PAS population.

Table 78. Primary Efficacy Results: Best Overall Response and Overall Response Rate for LT *NTRK* Fusion Positive Patients by LT and CDx Results in the Efficacy Primary Analysis Set (PAS)

		LT Fusion Positive N=55 (100%)	CDx Fusion Positive and LT Fusion Positive N=34 (100%)	CDx Fusion Negative and LT Fusion Positive N=5 (100%)
	Complete response	11 (20.0%)	8 (23.5%)	1 (20.0%)

		LT Fusion Positive N=55 (100%)	CDx Fusion Positive and LT Fusion Positive N=34 (100%)	CDx Fusion Negative and LT Fusion Positive N=5 (100%)
Best overall response, n (%)	Surgical complete response	3 (5.5%)	2 (5.9%)	0
	Partial response	27 (49.1%)	19 (55.9%)	1 (20.0%)
	Stable disease	7 (12.7%)	4 (11.8%)	1 (20.0%)
	Progressive disease	5 (9.1%)	1 (2.9%)	2 (40.0%)
	Not evaluable	2 (3.6%)	0	0
Overall response rate	Number of patients, n	55	34	5
	Number of patients with CR + sCR + PR, n	41	29	2
	ORR% (95% CI*)	74.5% (61.0%, 85.3%)	85.3% (68.9%, 95.0%)	40.0% (5.3%, 85.3%)

Abbreviations: CDx = TSO Comprehensive assay Companion Diagnostic; CI = Confidence Interval; CR = Complete Response; *NTRK* = Neurotrophic Tyrosine Receptor Kinase; ORR = Overall Response Rate; PAS = Primary Analysis Set; PR = Partial Response; sCR = Surgical Complete Response.
16 patients have missing CDx results.
Data cut-off 15 JUL 2019.
*The two-sided 95% confidence interval was calculated using the Clopper-Pearson exact method.

Sensitivity Analysis for Efficacy Against Missing CDx Results

Sensitivity analysis against the 16 missing CDx results was performed to estimate the impact on ORR for the TSO Comprehensive assay positive, LT positive population. Missing CDx results for the LT fusion positive patients in the efficacy set were imputed 100 times using a logistic regression model. Clinical efficacy including the imputed values was ORR=82%, 95% CI [69%, 95%] (Table 79) with the point estimate reduced relative to the primary efficacy analysis (ORR=85%, 95% CI [69%, 95%]) (Table 78). In the sensitivity analysis, the lower limit of the two-sided CI exceeds 30% for the TSO Comprehensive assay positive, LT positive population, which was considered clinically significant for the larotrectinib clinical study integrated analysis.

Table 79. Sensitivity Analysis for Overall Response Rate by CDx Result for LT *NTRK* Fusion Positive Patients including Imputed Values for Missing CDx Results in the Efficacy Primary Analysis Set (PAS) Using the MI Boot Method

	CDx Fusion Positive and LT Fusion Positive	CDx Fusion Negative and LT Fusion Positive
ORR% (95% CI *)	81.8% (68.6% - 95.0%)	55.6% (26.1% - 85.2%)

Abbreviations: CDx = TSO Comprehensive assay Companion Diagnostic; CI = Confidence Interval; MI = Multiple Imputation; *NTRK* = Neurotrophic Tyrosine Receptor Kinase; ORR = Overall Response Rate; PAS = Primary Analysis Set.
The MI Boot method is a bootstrap step nested in the multiple imputation (Schomaker and Heumann).
Data cut-off 15 JUL 2019
*The two-sided 95% CI was calculated based on the MI Boot method.

Sensitivity Analysis for Estimation of ORR in the Total TSO Comprehensive Assay Positive Population

Sensitivity analysis was performed to estimate ORR in the total TSO Comprehensive assay positive population (CDx+) including the TSO Comprehensive assay positive, LT positive

(CDx+, LT+) and the TSO Comprehensive assay positive, LT negative (CDx+, LT-) sub-populations. ORR was estimated by the method proposed by Li[2], including consideration of the frequency of *NTRK* gene fusions in the population, PPA of 89% as determined from the concordance analysis, and NPA varying from 93% to 99%, which is inclusive of the 95% CI determined from the primary concordance analysis. The ORR for the missing CDx+, LT- population (ORR (CDx+, LT-) = c * ORR (CDx+, LT+) was varied from 0 to 85% (c = 0 to 1), where ORR (CDx+, LT+) = 85%, the ORR observed from the primary efficacy analysis.

With an assumed *NTRK* frequency of 0.32%, sensitivity analysis against the missing population showed ORR in the total CDx *NTRK* positive population (Table 80) varying from 4% to 85% and highly dependent on the assumed value of c and ORR (CDx+, LT-).

For the larotrectinib clinical study PAS population, the ORR lower limit of the two-sided CI exceeding 30% was considered clinically significant. From Table 80, this condition is met at any value of NPA within the observed 95% CI [93%, 98%] and c ≥ 0.5 (when ORR (CDx+, LT-) is approximately 0.5 or greater than ORR (CDx+, LT+)).

Table 80. Estimated Efficacy in CDx Positive Population

	Estimated Overall Response Rate and 95% CI[a] in the Total CDx Fusion Positive Population				
NPA (%)	**c[b] = 0**	**c = 0.25**	**c = 0.5**	**c = 0.75**	**c = 1**
99.0%	19% (0%, 38%)	36% (19%, 53%)	52% (39%, 66%)	69% (58%, 80%)	85% (75%, 95%)
98.0%	11% (2%, 19%)	29% (17%, 42%)	48% (37%, 59%)	67% (56%, 77%)	85% (75%, 96%)
97.0%	7% (2%, 12%)	27% (15%, 38%)	46% (35%, 58%)	66% (55%, 77%)	85% (74%, 96%)
96.0%	6% (2%, 9%)	26% (14%, 37%)	45% (34%, 57%)	65% (54%, 77%)	85% (74%, 96%)
95.0%	5% (2%, 7%)	25% (13%, 36%)	45% (34%, 56%)	65% (54%, 76%)	85% (74%, 97%)
94.0%	4% (2%, 6%)	24% (13%, 36%)	45% (33%, 56%)	65% (54%, 76%)	85% (74%, 97%)
93.0%	3% (2%, 5%)	24% (12%, 35%)	44% (33%, 56%)	65% (53%, 76%)	85% (74%, 97%)

Abbreviations: CDx = TSO Comprehensive assay Companion Diagnostic; CI = Confidence Interval; NPA = Negative Percent Agreement; *NTRK* = Neurotrophic Tyrosine Receptor Kinase; ORR = Overall Response Rate; PPA = Positive Percent Agreement.
ORR in the TSO Comprehensive assay positive population (CDx+) estimated by sensitivity analysis was based on:
The ORR = 85% in the CDx positive, LT positive population from the primary efficacy analysis.
The ORR in the CDx positive but LT negative population was assumed to be c-times the ORR in the CDx positive, LT positive population, where c varies in the set (0, 0.25, 0.5, 0.75, 1).
The NPA was varied to be inclusive of the confidence interval calculated from the primary concordance analysis.
The PPA = 89% based on the complete case concordance analysis excluding the invalid CDx results.
NTRK fusion frequency = 0.32% based on literature.
[a]The two-sided 95% confidence interval was calculated using the delta method.
[b]c is the ratio of efficacy in TSO Comprehensive assay positive, LT negative over TSO Comprehensive assay positive, LT positive patient populations.

Extended Efficacy Analysis: ORR Results

Within the ePAS4, the efficacy of larotrectinib in the TSO Comprehensive assay positive, LT positive population (97 patients, ORR=78.4%, 95% CI [68.8%, 86.1%]) was similar to the efficacy of larotrectinib in the total ePAS4 population (164 patients, ORR=72.6%, 95% CI [65.1%, 79.2%]) (Table 81). Of the 97 TSO Comprehensive assay positive patients in ePAS4, 28 (28.9%) patients had achieved a complete response/surgical complete response and 48 (49.5%) patients had achieved a partial response.

Of the 13 TSO Comprehensive assay negative, LT positive population, 1 (7.7%) showed complete response and 2 (15.4%) showed partial response with larotrectinib therapy.

Table 81. *NTRK* Clinical Bridging Study: ORR for LT Positive Patients by LT and CDx Results in ePAS4

		LT Positive N=164	CDx Positive and LT Positive N=97	CDx Negative and LT Positive N=13
Overall response, n (%)	Complete response	31 (18.9%)	22 (22.7%)	1 (7.7%)
	Surgical complete response	8 (4.9%)	6 (6.2%)	0
	Partial response	80 (48.8%)	48 (49.5%)	2 (15.4%)
	Stable disease	25 (15.2%)	13 (13.4%)	4 (30.8%)
	Progressive disease	13 (7.9%)	6 (6.2%)	5 (38.5%)
	Not evaluable	7 (4.3%)	2 (2.1%)	1 (7.7%)
Overall response rate	Number of patients, n	164	97	13
	Number of patients with CR + sCR + PR, n	119	76	3
	ORR% (95% CI*)	72.6% (65.1%, 79.2%)	78.4% (68.8%, 86.1%)	23.1% (5.0%, 53.8%)

Abbreviations: CDx = TSO Comprehensive assay Companion Diagnostic; CR = Complete Response, PR = Partial Response, sCR = Surgical Complete Response.
Note: 54 patients have missing TSO Comprehensive assayassay results.
*The two-sided 95% confidence interval was calculated using the (exact) Clopper-Pearson method.

Pediatric Extrapolation

VITRAKVI (larotrectinib) was approved by the FDA in 2018 for treatment of adult and pediatric patients with solid tumors that have an *NTRK* gene fusion without a known acquired resistance mutation, are metastatic, or where surgical resection is likely to result in severe morbidity and have no satisfactory alternative treatments or that have progressed following treatment.

The safety and effectiveness of larotrectinib in pediatric patients was established based upon data from three multicenter, open-label, single-arm clinical trials in adult or pediatric patients 28 days and older. The efficacy of larotrectinib was evaluated in 12 pediatric patients and the safety of larotrectinib was evaluated in 44 pediatric patients who received larotrectinib. Of these 44 patients, 27% were 1 month to < 2 years (n = 12), 43% were 2 years to < 12 years (n = 19), and 30% were 12 years to < 18 years (n = 13); 43% had metastatic disease and 57% had locally advanced disease; and 91% had received prior treatment for their cancer, including surgery, radiotherapy, or systemic therapy. The most common cancers were infantile fibrosarcoma (32%), soft tissue sarcoma (25%), primary CNS tumors (20%), and thyroid cancer (9%). The median duration of exposure was 5.4 months (range: 9 days to 1.9 years).

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included 1 investigator. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

2. Clinical Bridging Study to Identify RET Fusions in NSCLC patients to be treated with selpercatinib

A. Study Design

To validate the TSO Comprehensive assay as a companion diagnostic (TSO Comprehensive CDx, hereafter "CDx") for the selection of patients for treatment with RETEVMO® (selpercatinib), RET fusion-positive samples from 105 patients enrolled in the LIBRETTO-001 clinical trial (LOXO-RET-17001, NCT03157128), using a data cutoff of Dec 2019, and 129 RET-fusion negative supplemental samples from commercial sources, were used in both clinical accuracy and bridging studies.

The bridging study was conducted to assess the concordance between the TSO Comprehensive assay and LTs (LTs) (used to determine RET gene fusion status for enrollment in the LIBRETTO-001) in the intent-to-treat population, and to assess the clinical

efficacy of the selpercatinib in the RET fusion-positive patients identified by the TSO Comprehensive assay. RET fusion status of the patients was determined by testing tissue or blood using various LTs (including NGS [next-generation sequencing], PCR [polymerase chain reaction], or FISH [fluorescent in situ hybridization]).

Key Inclusion Criteria for Clinical Study Samples

Loxo at Lilly identified eligible LIBRETTO-001 subjects with NSCLC based on the criteria described below.

Study Inclusion Criteria – LIBRETTO-001 Subjects

- Subject was enrolled in LIBRETTO-001.
- Subject had RET fusion positive NSCLC.
- Subject had available residual FFPE NSCLC tissue (in block or slide form) and/or sufficient purified RNA extracted from the FFPE NSCLC tissue that was collected/retained under informed consent.

Eligible LIBRETTO-001 samples were based on the criteria described below. Samples consisted of FFPE tumor tissue and/or extracted RNA. A hematoxylin and eosin (H&E) slide/scanned image of the H&E slide, or an extra slide to be used for H&E staining for samples provided as tissue, were made available to assess eligibility for testing.

- Sample was collected/retained under informed consent and was available.
- Sample could be FFPE NSCLC tissue (in block or slide form) and/or purified RNA extracted from the FFPE NSCLC tissue.

Key Exclusion Criteria for Clinical Study Samples

Study Exclusion Criteria – LIBRETTO-001 Subjects

- Subject had withdrawn from LIBRETTO-001 or did not have appropriate consent.

LIBRETTO-001 Sample Exclusion Criteria

- Sample did not meet FFPE tumor and/or RNA requirements outlined in the TSO Comp assay package insert.
- Sample was withdrawn/rejected in LIBRETTO-001 or did not have appropriate consent.

Follow-up Schedule
The bridging study involved retrospective testing of samples; as such, no additional patient follow-up was conducted in regard to the clinical bridging study.

Primary Objective and Endpoints
The primary objective for the clinical bridging study was to demonstrate safety and effectiveness of the TSO Comprehensive assay for the selection of NSCLC patients with RET fusions for treatment with RETEVMO.

The primary endpoint for the LIBRETTO-001 trial was the objective response rate (ORR), which is the proportion of patients with a best overall response of confirmed complete response (CR) or confirmed partial response (PR) based on IRC assessment using RECIST 1.1 criteria.

The bridging study was used to establish concordance between enrollment LTs and TSO Comprehensive results (Positive, Negative, and Unknown) and to determine the clinical outcomes (specifically with respect to the primary efficacy endpoint of overall response rate (ORR)) based on TSO Comprehensive assay results from LIBRETTO-001 NSCLC specimens. Sensitivity analyses for the clinical concordance and clinical outcomes (ORR) were performed.

B. Accountability of PMA Cohort

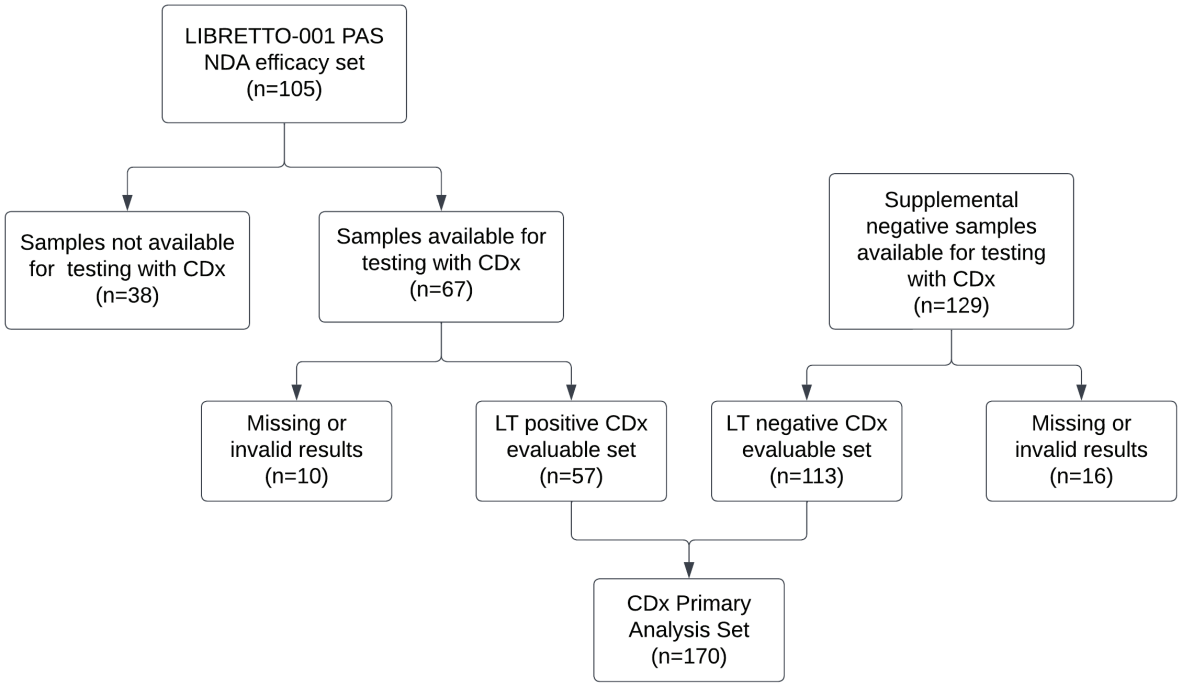
The primary analysis was performed using 57 samples from the LIBRETTO-001 clinical trial from NSCLC patients who had received prior therapy and were LT positive and enrolled in the study (CDx Primary Analysis Set). A smaller secondary analysis set (n=29, 21 of 29 patients with clinical outcome data) of LT positive samples from NSCLC patients who were treatment naïve and enrolled in the clinical trial were also evaluated (CDx Secondary Analysis Set). The combined analysis set including both the previously treated with platinum-based chemotherapy and treatment naïve patient subpopulations were evaluated as well.

One hundred and twenty-nine (129) LT negative samples tested with a representative NGS-based LT assay were supplemented in the bridging study, as there were no screen failed samples collected in the LIBRETTO-001 clinical trial. Of these supplemental samples, 113 samples had valid TSO Comprehensive assay results and were included in the analysis (LT Negative CDx Evaluable Set).

CDx Primary Analysis Set
Of the 105 LIBRETTO-001 samples in the NDA primary analysis set (PAS), 67 were available for testing with 10 having missing or invalid results (LT Positive, Unevaluable Set). Therefore, 57 samples were included in CDx primary analysis set that had valid TSO Comprehensive assay results (LT Positive CDx Evaluable Set). In addition, 113 supplemental LT negative samples were tested and had valid TSO Comprehensive assay

results (LT Negative CDx Evaluable Set). Therefore, the CDx primary analysis set was made up of a total 0f 170 samples as shown in Figure 5.

Figure 5. Sample Accounting for the CDx Primary Analysis Set



PAS: Primary Analysis Set, CDx: TSO Comprehensive Companion Diagnostic

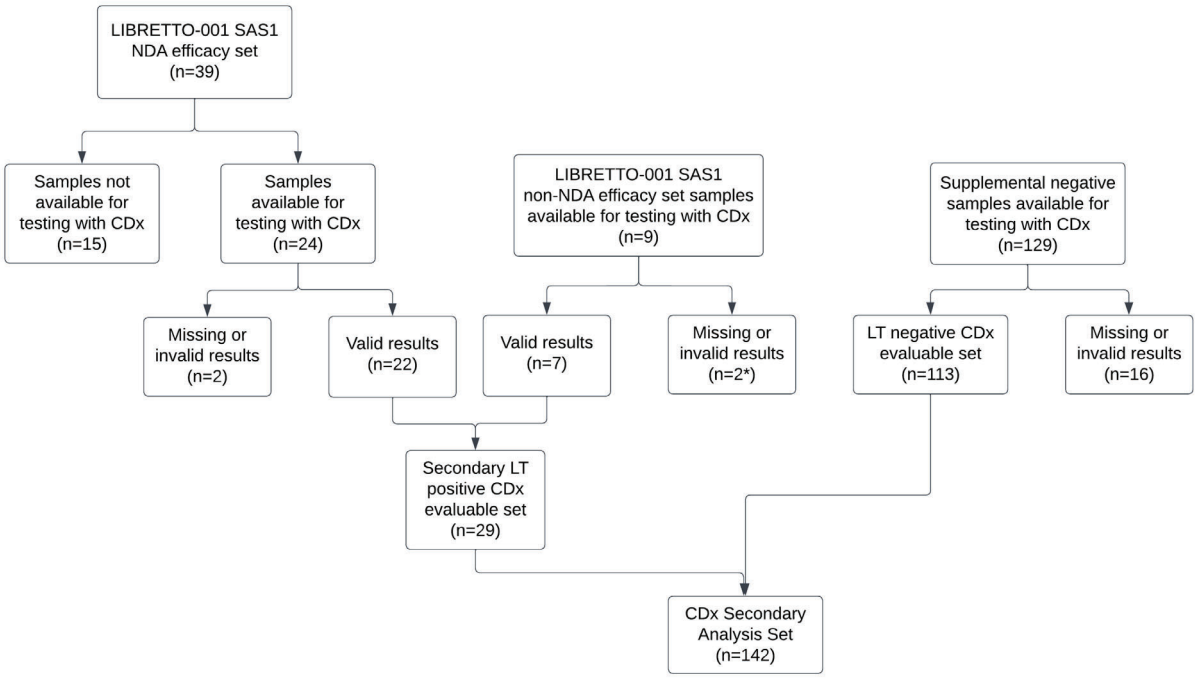
CDx Secondary Analysis Set

Figure 6 shows a diagram of the sample accounting for the CDx Secondary Analysis Set. There were 39 samples from the LIBRETTO-001 SAS1 New Drug Application (NDA) efficacy set. Of these, 24 were available for testing. In addition, 9 samples were available for testing from the LIBRETTO-001 supplemental analysis set 1 (SAS1) non-NDA efficacy set.

29 samples were tested and had valid results (Secondary LT Positive CDx Evaluable Set) and were included in the CDx Secondary Analysis Set.

The 113 supplemental negative samples included in the LT Negative CDx Evaluable Set were included in the CDx Secondary Analysis Set.

Figure 6. Sample Accounting for the CDx Secondary Analysis Set



SAS1: Supplemental Analysis Set 1, CDx: TSO Comprehensive Companion Diagnostic
*Note: one sample had sufficient RNA but was not tested.

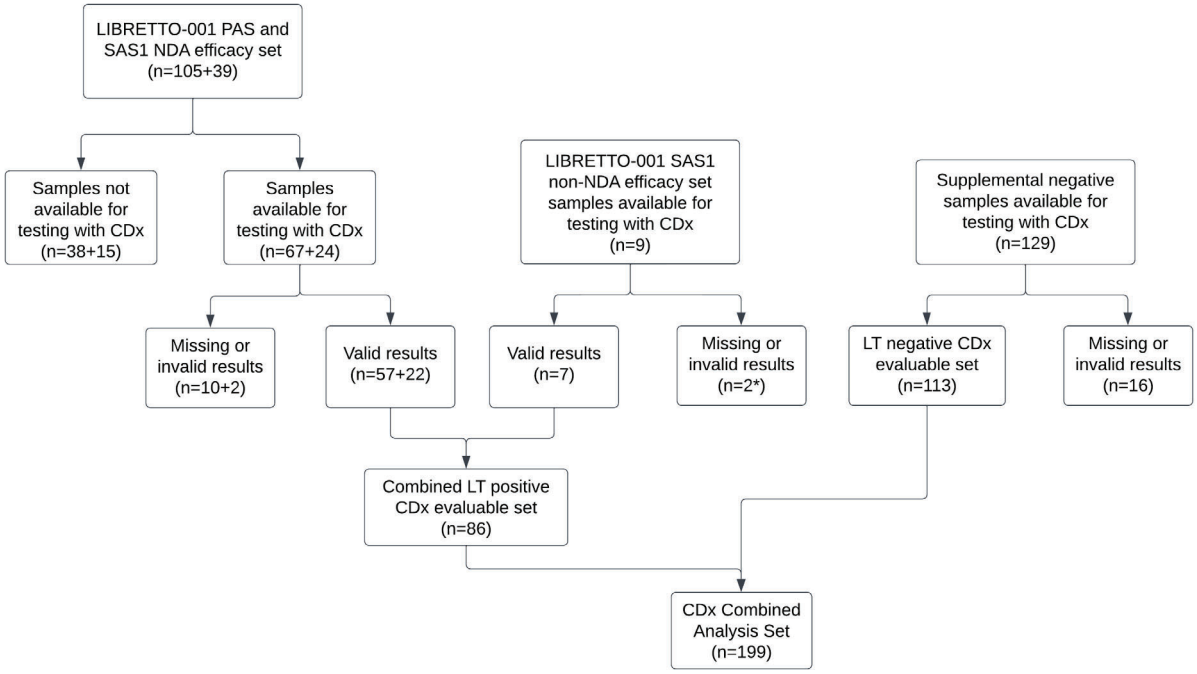
CDx Combined Analysis Set

Figure 7 shows a diagram of the sample accounting for the CDx Combined Analysis Set. There were 144 positive samples from the LIBRETTO-001 PAS and SAS1 NDA efficacy sets. Of these, 91 were available for testing. In addition, 9 samples were available for testing from the LIBRETTO-001 SAS1 non-NDA efficacy set. Fourteen (14) of these samples that were available for testing had missing or invalid TSO Comprehensive assay results. Eighty-six were tested and had valid TSO Comprehensive assay results (LT Positive Evaluable Set

and Secondary LT Positive Evaluable Set) and were included in the CDx Combined Analysis Set.

The 113 supplemental negative samples included in the LT Negative CDx Evaluable Set were included in the CDx Combined Analysis Set.

Figure 7. Sample Accounting for the CDx Combined Analysis Set



PAS: Primary Analysis Set, SAS1: Supplemental Analysis Set 1, CDx: TSO Comprehensive assay Companion Diagnostic
*Note: one sample had sufficient RNA but was not tested.

C. Study Population Demographics and Baseline Parameters

Within LT positive LIBRETTO-001 PAS, the baseline characteristics of the subset of CDx Evaluable set was compared to the CDx Unevaluable set. Table 82 shows the baseline demographics comparison between LT positive CDx evaluable set and LT positive CDx unevaluable set. Table 83 shows the baseline disease characteristics between LT positive CDx evaluable set and LT positive CDx unevaluable set. The demographic and baseline clinical characteristics of patients were well-balanced between LT Positive CDx Evaluable and LT Positive CDx Unevaluable sets, with the exception of age (p-value <0.2), which was about 5 years older in average in the evaluable set than in the unevaluable set.

Table 82. RET Clinical Bridging Study: Baseline Demographics Comparison Between LT Positive CDx Evaluable Set and LT Positive CDx Unevaluable Set

	LT+	LT Positive CDx Evaluable Set	LT Positive CDx Unevaluable Set	P-value
Number of Patients	(N=105)	(N=57)	(N=48)	

	LT+	LT Positive CDx Evaluable Set	LT Positive CDx Unevaluable Set	P-value
Age (years)				0.03
Mean (SD)	58.6 (11.9)	61.0 (11.2)	55.8 (12.3)	
Median [Min, Max]	61.0 [23.0, 81.0]	62.0 [34.0, 80.0]	57.5 [23.0, 81.0]	
Gender, n (%)				0.43
Female	62 (59.0 %)	36 (63.2 %)	26 (54.2 %)	
Male	43 (41.0 %)	21 (36.8 %)	22 (45.8 %)	
Race, n (%)				0.49
White	55 (52.4 %)	28 (49.1 %)	27 (56.2 %)	
Asian	40 (38.1 %)	21 (36.8 %)	19 (39.6 %)	
Other	8 (7.6 %)	6 (10.5 %)	2 (4.2 %)	
Missing	2 (1.9%)	2 (3.5%)	0 (0.0 %)	
Ethnicity, n (%)				0.33
Hispanic Or Latino	4 (3.8 %)	1 (1.8 %)	3 (6.2 %)	
Not Hispanic Or Latino	98 (93.3 %)	54 (94.7 %)	44 (91.7 %)	
Missing	3 (2.9%)	2 (3.5%)	1 (2.1%)	
Country, n (%)				1.00
USA	57 (54.3 %)	31 (54.4 %)	26 (54.2 %)	
Other	48 (45.7 %)	26 (45.6 %)	22 (45.8 %)	
Weight (kg)				0.73
Mean (SD)	67.8 (17.1)	67.9 (16.1)	67.6 (18.5)	
Median [Min, Max]	64.0 [42.2, 148.0]	64.4 [42.2, 110.2]	63.8 [44.4, 148.0]	
Height (cm)				0.23
Mean (SD)	165.8 (9.3)	164.8 (9.3)	166.9 (9.2)	
Median [Min, Max]	164.0 [149.0, 188.0]	163.5 [149.0, 188.0]	167.0 [149.0, 185.0]	
BMI (kg/m2)				0.35
Mean (SD)	24.4 (4.8)	24.7 (4.6)	24.0 (5.0)	
Median [Min, Max]	23.1 [17.3, 43.7]	23.9 [18.3, 41.9]	22.7 [17.3, 43.7]	
Smoking Status, n (%)				0.52
Former Smoker/ Current Smoker	30 (28.6 %)	18 (31.6 %)	12 (25.0 %)	
Never A Smoker	75 (71.4 %)	39 (68.4 %)	36 (75.0 %)	

Abbreviation: CDx = TSO Comprehensive Companion Diagnostic
Statistical comparison is based on non-missing values.

Table 83. RET Clinical Bridging Study: Baseline Disease Characteristics Between LT Positive CDx Evaluable Set and LT Positive CDx Unevaluable Set

	LT+	LT Positive CDx Evaluable Set	LT Positive CDx Unevaluable Set	P-value
Number of Patients	(N=105)	(N=57)	(N=48)	
ECOG, n (%)				0.52
0	31 (29.5 %)	15 (26.3 %)	16 (33.3 %)	
1/2	74 (70.5 %)	42 (73.7 %)	32 (66.7 %)	
Tumor Stage at Diagnosis, n (%)				1.00
Stage IA/IIIA/IIIB	4 (3.8 %)	2 (3.5 %)	2 (4.2 %)	
Stage IV/IVA/IVB/IVC	101 (96.2 %)	55 (96.5 %)	46 (95.8 %)	
Tumor Grade, n (%)				0.92
Well Differentiated	3 (2.9 %)	1 (1.8 %)	2 (4.2 %)	
Moderately Differentiated	6 (5.7 %)	3 (5.3 %)	3 (6.2 %)	
Poorly Differentiated	21 (20.0 %)	11 (19.3 %)	10 (20.8 %)	
Not Applicable	13 (12.4 %)	8 (14.0 %)	5 (10.4 %)	
Unknown	61 (58.1 %)	34 (59.6 %)	27 (56.2 %)	
Missing	1 (1.0%)	0 (0.0 %)	1 (2.1%)	
Tumor Subtype, n (%)				1.00
Adenocarcinoma	90 (85.7 %)	49 (86.0 %)	41 (85.4 %)	
Other*	15 (14.3 %)	8 (14.0 %)	7 (14.6 %)	
Metastasis Status, n (%)				0.21
Yes	103 (98.1 %)	57 (100.0 %)	46 (95.8 %)	
No	2 (1.9 %)	0 (0.0 %)	2 (4.2 %)	

Abbreviation: CDx = TSO Comprehensive assay Companion Diagnostic
*Other contains Large Cell Neuroendocrine Carcinoma, Squamous Cell Carcinoma, and Unknown; Statistical comparison is based on non-missing values.

D. Safety and Effectiveness Results

Safety Results

The safety with respect to treatment with selpercatinib was addressed during the review of the NDA. Testing for the TSO Comprehensive assay bridging study was conducted retrospectively on banked study specimens and the test results were not reported to patients. No patient treatment decisions were made as a result of any of the TSO Comprehensive assay test results; therefore, as expected, there were no Unanticipated Adverse Device Effect (UADE) events related to the study products or procedures to patients or operators. Please refer to fda.gov/drugs for complete safety information on RETEVMO® (selpercatinib).

Effectiveness Results – Concordance

Concordance Results: Primary Analysis Set

For the primary analysis group including the previously treated NSCLC patients and supplemental negative samples, there were 186 samples with valid LT results tested with the TSO Comprehensive assay. Of those, 16 had invalid TSO Comprehensive assay results (invalid rate of 8.6%). Agreement of TSO Comprehensive assay results relative to LT results among samples with valid results are shown in Table 84. Of the 57 RET fusion-positive by the LT, 53 were RET fusion-positive by the TSO Comprehensive assay. The PPA was 93.0% (53/57; 95% CI: 83.0%–98.1%). The remaining 113 of 170 samples were RET fusion-negatives by both the representative LT and the TSO Comprehensive assay. The NPA was 100.0% (113/113; 95% CI: 96.8% - 100.0%).

Table 84. RET Clinical Bridging Study: Concordance Between the TSO Comprehensive Assay and LT for Detection of RET Fusions in Previously Treated NSCLC Patients and Supplemental Negative Samples

		LT Result		
		RET Fusion Positive	**RET Fusion Negative**	**Total**
TSO Comprehensive Assay Result	RET Fusion Positive	53	0	53
	RET Fusion Negative	4	113	117
	Total	57	113	170
Agreement Statistics	PPA% (n/N; 95% CI*)	93.0% (53/57; 83.0% - 98.1%)		
	NPA% (n/N; 95% CI*)	100.0% (113/113; 96.8% -100.0%)		

*CI based on Clopper-Pearson (exact) method.

Concordance Results: Secondary Analysis Set

For the secondary analysis group with the treatment naïve patients and supplemental negative samples, there were 156 samples with valid LT results tested with TSO Comprehensive assay. Of those, 14 had invalid TSO Comprehensive assay results (invalid rate of 9.0%). Agreement of TSO Comprehensive assay results relative to LT results among samples with valid results are shown in Table 85. Of the 29 RET fusion-positive by the LT, 28 were RET fusion-positive by the TSO Comprehensive assay. The PPA was 96.6% (28/29; 95% CI: 82.2%–99.9%). The remaining 113 of 142 samples were RET fusion-negatives by both the representative LT and the TSO Comprehensive assay. The NPA was 100.0% (113/113; 95% CI: 96.8%–100.0%).

Table 85. RET Clinical Bridging Study: Concordance Between the TSO Comprehensive Assay and LT for Detection of RET Fusions in the Treatment-Naïve NSCLC Patients and Supplemental Negative Samples

		LT Result		
		RET Fusion Positive	**RET Fusion Negative**	**Total**
TSO Comprehensive Assay Result	RET Fusion Positive	28	0	28
	RET Fusion Negative	1	113	114
	Total	29	113	142
Agreement Statistics	PPA% (n/N; 95% CI*)	96.6% (28/29; 82.2% - 99.9%)		
	NPA% (n/N; 95% CI*)	100.0% (113/113; 96.8% -100.0%)		

*CI based on Clopper-Pearson (exact) method.

Concordance Results: Combined Analysis Set

For the combined group which includes both previously treated, treatment-naïve patients and supplemental negative samples, there were 215 samples with valid LT results tested with TSO Comprehensive assay. Of those, 16 had invalid TSO Comprehensive assay results (invalid rate of 7.4%). Agreement of the TSO Comprehensive assay relative to the LT among samples with valid results are shown in Table 86. Of the 86 RET fusion-positive by the LT, 81 were RET fusion-positive by the TSO Comprehensive assay. The PPA was 94.2% (81/86; 95% CI: 87.0%–98.1%). The remaining 113 of 199 samples were RET fusion-negatives by both the representative LT and the TSO Comprehensive assay. The NPA was 100.0% (113/113; 95% CI: 96.8%–100.0%).

Table 86. RET Clinical Bridging Study: Concordance Between TSO Comprehensive and LT for Detection of RET Fusions in the Previously Treated, Treatment-Naïve NSCLC Patients and Supplemental Negative Samples

		LT Result		
		RET Fusion Positive	**RET Fusion Negative**	**Total**
TSO Comprehensive Assay Result	RET Fusion Positive	81	0	81
	RET Fusion Negative	5	113	118
	Total	86	113	199
Agreement Statistics	PPA% (n/N; 95% CI*)	94.2% (81/86; 87.0% - 98.1%)		
	NPA% (n/N; 95% CI*)	100.0% (113/113; 96.8% -100.0%)		

*CI based on Clopper-Pearson (exact) method.

Sensitivity Analysis for Concordance

Missing TSO Comprehensive assay results for the LT fusion positive patients in the primary analysis set were imputed 500 times using a logistic regression model including age, gender, tumor subtype, and ECOG status. Table 87 shows the PPA from the sensitivity analysis for concordance based on 500 simulations. No sensitivity analysis was performed on the LT

negative samples because the observed NPA was 100.0%. The median PPA including imputed results were 93.3% (95% CI: 89.5% to 95.2%).

Table 87. RET Clinical Bridging Study: Sensitivity Analysis for PPA

	Median of Estimates	Minimum	Maximum	Empirical 95% CI for the Estimates
PPA	93.3%	87.6%	96.2%	(89.5%, 95.2%)

Effectiveness Results – Efficacy

Efficacy Results in the RET Fusion Positive Populations

Table 88 shows the ORR for selpercatinib in the RET-fusion-positive patient populations as detected by the TSO Comprehensive assay. The percentage of patients with a response in the primary analysis set was 71.7% (38/53; 95% CI: 57.7% to 83.2%). The primary analysis set included patients who had previously received at least one platinum-based chemotherapy. The ORR in the secondary analysis set, which included only treatment naïve patients, was 81.0% (17/21; 95% CI: 58.1%, 94.6%). The ORR in the combined analysis set was 74.3% (55/74; 95% CI: 62.8%, 83.8%), and the combined analysis set included both the previously treated with platinum-based chemotherapy and treatment naïve patient subpopulations.

Table 88. RET Clinical Bridging Study: Clinical Efficacy in the TSO Comprehensive assay Positive Patients

	ORR (n/N) (TSO)	95% CI [a] (TSO)	ORR (95%CI) LT+ NDA Drug Efficacy Population
Primary Analysis Set[b]	71.7% (38/53)	(57.7%, 83.2%)	63.8% (53.9%-73.0%)
Secondary Analysis Set[c]	81.0% (17/21)	(58.1%, 94.6%)	84.6% (69.5%-94.1%)
Combined Analysis Set[d]	74.3% (55/74)	(62.8%, 83.8%)	

[a]CI based on Clopper-Pearson (exact) method.
[b]Patients previously treated with platinum-based chemotherapy.
[c]Treatment-naïve patients.
[d]Patients previously treated with platinum-based chemotherapy plus treatment naïve patients.

Sensitivity Analysis for Efficacy Results

Sensitivity analysis was performed to evaluate the impact of missing TSO Comprehensive assay results for the LT fusion positive patients in LIBRETTO-001 PAS on the ORR estimate. Using the 500 imputed sets of TSO Comprehensive assay results, ORR was calculated for the LT positive, TSO Comprehensive assay positive population (LT+, CDx+) and LT positive, TSO Comprehensive assay negative population (LT+, CDx-). Summary statistics for ORRs from the 500 imputed sets are reported for the 2 populations in Table 89. The median ORR for the LT positive, TSO Comprehensive assay positive population (LT+, CDx+) including the imputed results was 65.6% (95% CI: 63.9% to 67.7%), which was slightly reduced relative to the ORR in the TSO Comprehensive assay positive population in the CDx primary analysis set (ORR = 71.7%; 95% CI: 57.7% to 83.2%), but comparable to the ORR of the LIBRETTO-001 PAS (ORR = 64%; 95% CI: 54% to 73%). The ORR for LT positive, TSO Comprehensive assay negative population (LT+, CDx-) including the imputed results was 40.0% (95% CI: 16.7% to 62.5%).

Table 89. RET Clinical Bridging Study: Sensitivity Analysis on Clinical Efficacy (ORR)

	Median of ORR	Minimum	Maximum	Empirical 95% CI
(LT+ CDx+)	65.6%	63.3%	68.1%	(63.9%, 67.3)
(LT+ CDx-)	40.0%	12.5%	66.7%	(16.7%, 62.5%)

Abbreviation: CDx = TSO Comprehensive Companion Diagnostic

Pediatric Extrapolation

Existing clinical data was not leveraged to support approval of a pediatric patient population.

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included one investigator. This clinical investigator did not have disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XI. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Not applicable.

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

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

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

For the intended use to identify the *NTRK*1, *NTRK*2, and *NTRK*3 fusions in patients with solid tumor for therapy with larotrectinib, the effectiveness of the TSO Comprehensive assay is supported by the results of a clinical bridging study using specimens from the following larotrectinib clinical trials: LOXO-TRK-14001 (NCT02122913), NAVIGATE (NCT02576431, LOXO-TRK-15002), SCOUT (NCT02637687, LOXO-TRK-15003). The data from the analytical validation and clinical studies support the reasonable assurance of safety and effectiveness of the TSO Comprehensive assay when used in accordance with the indications for use. Data show that patients who had a qualifying *NTRK*1, *NTRK*2, or *NTRK*3 fusion received benefit from treatment with larotrectinib, and support this CDx indication for the TSO Comprehensive assay.

For the intended use to identify RET fusions in tumors from NSCLC patients for therapy with selpercatinib, the effectiveness of the TSO Comprehensive assay is supported by the results of a clinical bridging study using specimens from the LIBRETTO-001 clinical study. The data from the analytical validation and clinical studies support the reasonable assurance of safety and effectiveness of the TSO Comprehensive assay when used in accordance with the indications for use. Data shows that NSCLC patients who had qualifying RET fusions received benefit from treatment with selpercatinib and support this CDx indication to the TSO Comprehensive assay.

B. Safety Conclusions

The risks of the device are based on data collected in the analytical studies conducted to support PMA approval as described above. The TSO Comprehensive assay is an in vitro diagnostic test, which involves testing of DNA and/or RNA extracted from FFPE tumor tissue.

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

C. Benefit-Risk Determination

The probable benefit of the TSO Comprehensive assay, for selection of solid tumor patients with NTRK1, NTRK2, and NTRK3 fusions, for treatment with larotrectinib, was demonstrated through a clinical bridging study using specimens from patients screened for enrollment into the LOXO-TRK-14001 (NCT02122913), NAVIGATE (NCT02576431, LOXO-TRK-15002), SCOUT (NCT02637687, LOXO-TRK-15003) clinical trials. This bridging study showed clinically meaningful ORR in patients with metastatic NTRK1, NTRK2, and NTRK3 fusion-positive solid tumors detected by the TSO Comprehensive assay. The clinical efficacy of larotrectinib for the TSO Comprehensive NTRK fusion positive population (34 patients, ORR=85%, 95% CI [69%, 95%]) compared favorably to the clinical efficacy of larotrectinib in the currently approved NDA efficacy population (55 patients, ORR=75%, 95% CI [61%, 85%]), with the 15 JUL 2019 cutoff. In addition, the concordance portion of the bridging study, showed a PPA of 89.1% and an NPA of 96.3%, conditional on the local enrolling tests. A sensitivity analysis was also conducted on this data, indicating a PPA of 86.5% and an NPA of 98.8%. Additional factors, including the analytical accuracy study and analytical performance of the device included in this submission, have been considered and have demonstrated acceptable performance, further supporting the clinical bridging study. Thus, the totality of the analytical and clinical data indicate that the TSO Comprehensive assay has probable benefit in selecting patients with solid tumors which harbor NTRK1, NTRK2, or NTRK3 fusions for treatment with larotrectinib.

The probable benefit of the TSO Comprehensive assay, for the selection of RET fusion positive NSCLC patients, for treatment with selpercatinib was demonstrated through a clinical bridging study using specimens from patients screened for enrollment into the LIBRETTO-001 clinical trial. This bridging study showed clinically meaningful ORR in patients with metastatic RET fusions-positive NSCLC detected by TSO Comprehensive assay. The clinical bridging efficacy data, indicated that the RET fusion positive population by the TSO Comprehensive assay had clinically meaningful ORR in the primary analysis set (pre-treated), with an ORR of 71.7% (95% CI: 57.7-83.2%), as well as in the secondary analysis set (treatment naïve), with an ORR of 81.0% (95% CI: 58.1-94.5%); also the efficacies observed in the NDA primary and secondary analysis sets were maintained. The concordance study, to enrolling tests, showed a PPA of 93.0% and an NPA of 100.0%, excluding unknown results, in the LIBRETTO-001 primary efficacy analysis population. Additional factors, including the analytical accuracy study and analytical performance of the device for RET fusions, included in this submission, have been considered and have demonstrated acceptable performance, further supporting the clinical bridging study. Thus, the totality of the analytical and clinical data indicate that the TSO Comprehensive assay has probable benefit in selecting patients with NSCLC with RET fusions for treatment with selpercatinib.

This test also demonstrated probable benefit, in the tumor profiling of 517 genes using nucleic acids extracted from formalin-fixed, paraffin embedded (FFPE) tumor tissue samples from cancer patients with solid malignant neoplasms, to detect single nucleotide variants, multi-nucleotide variants, insertions, and deletions from DNA, and fusions in 24 genes, 1 splice variant (EGFRvIII) from RNA and the reporting of the Tumor Mutational Burden (TMB) score, based on a series of analytical studies conducted for this validation. This tumor profiling provides a significant added probable benefit, beyond the TSO companion diagnostic claims discussed above, because it allows for the simultaneous interrogation of numerous alterations in genes and variants and other molecular signatures (i.e., TMB), that have either evidence of clinical significance or evidence of potential clinical significance in tissue. The results of this tumor profiling panel are intended to be used by qualified health care professionals in accordance with professional guidelines and are not conclusive or prescriptive for labeled use of any specific therapeutic product. The totality of the scope of the TSO Comprehensive Assay, factors prominently in the overall assessment of the magnitude and probability of the probable benefit of this device.

There is also potential risk associated with the use of this device, mainly due to 1) false positives, false negatives, and failure to provide a result and 2) incorrect interpretation of test results by the user. The risks of TSO Comprehensive assay for selection of solid tumor patients with NTRK 1, NTRK2, or NTRK3 fusions or NSCLC patients with RET fusions are associated with the potential mismanagement of patient's treatment resulting from false results of the test. Patients who are determined to be false positive by the test may be exposed to a drug that is not beneficial and may lead to adverse events or may have delayed access to other treatments that could be more beneficial. The false positivity for NTRK1/2/3 is identified as a key risk of this device, and is addressed by labeling in the form of the following limitation, that serves to partially mitigate this risk:

"The overall negative percent agreement for NTRK fusions between drug trial enrollment assays and TSO Comprehensive was 96.3% (95% CI: 93.1%-98.3%). False positive results for gene fusions such as NTRK may be due to non-specific detection."

A false negative result may prevent a patient from accessing a potentially beneficial therapeutic regimen. The risks of erroneous results are partially mitigated by the analytical performance of the device. The likelihood of false results was assessed by analytical and clinical validation studies, which partially mitigate the probable risk of the TSO Comprehensive assay.

1. Patient Perspective
   This submission either did not include specific information on patient perspectives or the information did not serve as part of the basis of the decision to approve or deny the PMA for this device.

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

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from the analytical and clinical validation studies support the performance of TSO Comprehensive assay as an aid for identification of *NTRK* fusion solid tumor cancer patients who may benefit from VITRAKVI (larotrectinib) treatment, and NSCLC patients with *RET* fusions who may benefit from RETEVMO (selpercatinib) treatment.

XIV. CDRH DECISION

CDRH issued an approval order on August 21, 2024.

The final non-clinical condition of approval cited in the approval letter is described below.

1. Illumina must appropriately modify the test results report to include the agreed upon modifications for reporting variant results. Illumina must conduct and provide necessary software verification documentation, including executed test protocols and reports, risk assessment, any new assay unresolved anomalies identified, and regression test reports, to demonstrate that these modifications do not adversely affect the safety and effectiveness of the device. The final documentation must be submitted within 6 months of the PMA approval date.

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

XV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.

XVI. REFERENCES

1. Gelman, Andrew. Bayesian Data Analysis, Third Edition. United Kingdom, Taylor & Francis, 2014.

2. Li M. Statistical consideration and challenges in bridging study of personalized medicine. J Biopharm Stat. 2015;25(3):397-407. doi: 10.1080/10543406.2014.920340. PMID: 24897254.

APPENDICES

Appendix 1. List of Genes included in TSO Comprehensive Assay

No.	Entrez ID	Gene	Variant Type	No.	Entrez ID	Gene	Variant Type	No.	Entrez ID	Gene	Variant Type
1	25	ABL1	S	176	2261	FGFR3	S, F	351	7849	PAX8	S
2	27	ABL2	S	177	2264	FGFR4	S	352	55193	PBRM1	S
3	84142	ABRAXAS1	S	178	2271	FH	S	353	5133	PDCD1	S
4	90	ACVR1	S	179	201163	FLCN	S	354	80380	PDCD1LG2	S
5	91	ACVR1B	S	180	2313	FLI1	S	355	5156	PDGFRA	S
6	25960	ADGRA2	S	181	2321	FLT1	S, F	356	5159	PDGFRB	S
7	207	AKT1	S	182	2322	FLT3	S	357	5163	PDK1	S
8	208	AKT2	S	183	2324	FLT4	S	358	5170	PDPK1	S
9	10000	AKT3	S	184	3169	FOXA1	S	359	5241	PGR	S
10	238	ALK	S	185	668	FOXL2	S	360	84295	PHF6	S
11	242	ALOX12B	S	186	2308	FOXO1	S	361	8929	PHOX2B	S
12	139285	AMER1	S	187	27086	FOXP1	S	362	5287	PIK3C2B	S
13	29123	ANKRD11	S	188	10818	FRS2	S	363	5288	PIK3C2G	S
14	22852	ANKRD26	S	189	8880	FUBP1	S	364	5289	PIK3C3	S
15	324	APC	S	190	2534	FYN	S	365	5290	PIK3CA	S
16	367	AR	S	191	2559	GABRA6	S	366	5291	PIK3CB	S
17	369	ARAF	S	192	2623	GATA1	S	367	5293	PIK3CD	S
18	10139	ARFRP1	S	193	2624	GATA2	S	368	5294	PIK3CG	S
19	8289	ARID1A	S	194	2625	GATA3	S	369	5295	PIK3R1	S
20	57492	ARID1B	S	195	2626	GATA4	S	370	5296	PIK3R2	S
21	196528	ARID2	S	196	2627	GATA6	S	371	8503	PIK3R3	S
22	84159	ARID5B	S	197	348654	GEN1	S	372	5292	PIM1	S
23	171023	ASXL1	S	198	79018	GID4	S	373	5336	PLCG2	S
24	55252	ASXL2	S	199	2735	GLI1	S	374	10769	PLK2	S
25	472	ATM	S	200	2767	GNA11	S	375	5366	PMAIP1	S
26	545	ATR	S	201	10672	GNA13	S	376	5378	PMS1	S
27	546	ATRX	S	202	2776	GNAQ	S	377	5395	PMS2	S
28	6790	AURKA	S	203	2778	GNAS	S	378	10957	PNRC1	S
29	9212	AURKB	S	204	2874	GPS2	S	379	5424	POLD1	S
30	8312	AXIN1	S	205	26585	GREM1	S	380	5426	POLE	S
31	8313	AXIN2	S	206	2903	GRIN2A	S	381	5468	PPARG	S
32	558	AXL	S, F	207	2913	GRM3	S	382	8493	PPM1D	S
33	567	B2M	S	208	2932	GSK3B	S	383	5518	PPP2R1A	S
34	8314	BAP1	S	209	3020	H3F3A	S	384	5520	PPP2R2A	S
35	580	BARD1	S	210	3021	H3F3B	S	385	5537	PPP6C	S
36	27113	BBC3	S	211	440093	H3F3C	S	386	639	PRDM1	S
37	8915	BCL10	S	212	3082	HGF	S	387	80243	PREX2	S
38	596	BCL2	S, F	213	3006	HIST1H1C	S	388	5573	PRKAR1A	S
39	598	BCL2L1	S	214	3017	HIST1H2BD	S	389	5584	PRKCI	S

No.	Entrez ID	Gene	Variant Type	No.	Entrez ID	Gene	Variant Type	No.	Entrez ID	Gene	Variant Type
40	10018	BCL2L11	S	215	8350	HIST1H3A	S	390	5591	PRKDC	S
41	599	BCL2L2	S	216	8358	HIST1H3B	S	391	5071	PRKN	S
42	604	BCL6	S	217	8352	HIST1H3C	S	392	5652	PRSS8	S
43	54880	BCOR	S	218	8351	HIST1H3D	S	393	5727	PTCH1	S
44	63035	BCORL1	S	219	8353	HIST1H3E	S	394	5728	PTEN	S
45	613	BCR	S	220	8968	HIST1H3F	S	395	5781	PTPN11	S
46	330	BIRC3	S	221	8355	HIST1H3G	S	396	5789	PTPRD	S
47	641	BLM	S	222	8357	HIST1H3H	S	397	5802	PTPRS	S
48	657	BMPR1A	S	223	8354	HIST1H3I	S	398	11122	PTPRT	S
49	673	BRAF	S, F	224	8356	HIST1H3J	S	399	9444	QKI	S
50	672	BRCA1	S	225	333932	HIST2H3A	S	400	11021	RAB35	S
51	675	BRCA2	S	226	126961	HIST2H3C	S	401	5879	RAC1	S
52	23476	BRD4	S	227	653604	HIST2H3D	S	402	5885	RAD21	S
53	83990	BRIP1	S	228	8290	HIST3H3	S	403	10111	RAD50	S
54	694	BTG1	S	229	6927	HNF1A	S	404	5888	RAD51	S
55	695	BTK	S	230	3190	HNRNPK	S	405	5890	RAD51B	S
56	811	CALR	S	231	10481	HOXB13	S	406	5889	RAD51C	S
57	84433	CARD11	S	232	3265	HRAS	S	407	5892	RAD51D	S
58	841	CASP8	S	233	3283	HSD3B1	S	408	5893	RAD52	S
59	865	CBFB	S	234	3320	HSP90AA1	S	409	8438	RAD54L	S
60	867	CBL	S	235	23308	ICOSLG	S	410	5894	RAF1	S, F
61	595	CCND1	S	236	3399	ID3	S	411	5903	RANBP2	S
62	894	CCND2	S	237	3417	IDH1	S	412	5914	RARA	S
63	896	CCND3	S	238	3418	IDH2	S	413	5921	RASA1	S
64	898	CCNE1	S	239	3459	IFNGR1	S	414	5925	RB1	S
65	29126	CD274	S	240	3479	IGF1	S	415	8241	RBM10	S
66	80381	CD276	S	241	3480	IGF1R	S	416	9401	RECQL4	S
67	972	CD74	S	242	3481	IGF2	S	417	5966	REL	S
68	973	CD79A	S	243	9641	IKBKE	S	418	5979	RET	S, F
69	974	CD79B	S	244	10320	IKZF1	S	419	6009	RHEB	S
70	79577	CDC73	S	245	3586	IL10	S	420	387	RHOA	S
71	999	CDH1	S	246	3575	IL7R	S	421	253260	RICTOR	S
72	51755	CDK12	S	247	3623	INHA	S	422	6016	RIT1	S
73	1019	CDK4	S, F	248	3624	INHBA	S	423	54894	RNF43	S
74	1021	CDK6	S	249	3631	INPP4A	S	424	6098	ROS1	S
75	1024	CDK8	S	250	8821	INPP4B	S	425	8986	RPS6KA4	S
76	1026	CDKN1A	S	251	3643	INSR	S	426	6198	RPS6KB1	S
77	1027	CDKN1B	S	252	3660	IRF2	S	427	6199	RPS6KB2	S
78	1029	CDKN2A	S	253	3662	IRF4	S	428	57521	RPTOR	S
79	1030	CDKN2B	S	254	3667	IRS1	S	429	861	RUNX1	S
80	1031	CDKN2C	S	255	8660	IRS2	S	430	862	RUNX1T1	S
81	1050	CEBPA	S	256	3716	JAK1	S	431	23429	RYBP	S

No.	Entrez ID	Gene	Variant Type	No.	Entrez ID	Gene	Variant Type	No.	Entrez ID	Gene	Variant Type
82	1058	CENPA	S	257	3717	JAK2	S	432	6389	SDHA	S
83	1106	CHD2	S	258	3718	JAK3	S	433	54949	SDHAF2	S
84	1108	CHD4	S	259	3725	JUN	S	434	6390	SDHB	S
85	1111	CHEK1	S	260	7994	KAT6A	S	435	6391	SDHC	S
86	11200	CHEK2	S	261	5927	KDM5A	S	436	6392	SDHD	S
87	23152	CIC	S	262	8242	KDM5C	S	437	26040	SETBP1	S
88	64326	COP1	S	263	7403	KDM6A	S	438	29072	SETD2	S
89	1387	CREBBP	S	264	3791	KDR	S	439	23451	SF3B1	S
90	1399	CRKL	S	265	9817	KEAP1	S	440	10019	SH2B3	S
91	64109	CRLF2	S	266	3792	KEL	S	441	4068	SH2D1A	S
92	1436	CSF1R	S	267	3799	KIF5B	S, F	442	55164	SHQ1	S
93	1441	CSF3R	S	268	3815	KIT	S	443	9353	SLIT2	S
94	1452	CSNK1A1	S	269	9314	KLF4	S	444	84464	SLX4	S
95	10664	CTCF	S	270	89857	KLHL6	S	445	4087	SMAD2	S
96	1493	CTLA4	S	271	4297	KMT2A	S	446	4088	SMAD3	S
97	1495	CTNNA1	S	272	3845	KRAS	S	447	4089	SMAD4	S
98	1499	CTNNB1	S	273	3916	LAMP1	S	448	6597	SMARCA4	S
99	8452	CUL3	S	274	9113	LATS1	S	449	6598	SMARCB1	S
100	1523	CUX1	S	275	26524	LATS2	S	450	6602	SMARCD1	S
101	7852	CXCR4	S	276	4004	LMO1	S	451	8243	SMC1A	S
102	1540	CYLD	S	277	53353	LRP1B	S	452	9126	SMC3	S
103	1616	DAXX	S	278	4067	LYN	S	453	6608	SMO	S
104	54165	DCUN1D1	S	279	8216	LZTR1	S	454	9627	SNCAIP	S
105	4921	DDR2	S	280	9863	MAGI2	S	455	8651	SOCS1	S
106	51428	DDX41	S	281	10892	MALT1	S	456	6663	SOX10	S
107	1665	DHX15	S	282	5604	MAP2K1	S	457	64321	SOX17	S
108	23405	DICER1	S	283	5605	MAP2K2	S	458	6657	SOX2	S
109	22894	DIS3	S	284	6416	MAP2K4	S	459	6662	SOX9	S
110	3337	DNAJB1	S	285	4214	MAP3K1	S	460	23013	SPEN	S
111	1786	DNMT1	S	286	9175	MAP3K13	S	461	8405	SPOP	S
112	1788	DNMT3A	S	287	9020	MAP3K14	S	462	6708	SPTA1	S
113	1789	DNMT3B	S	288	4216	MAP3K4	S	463	6714	SRC	S
114	84444	DOT1L	S	289	5594	MAPK1	S	464	6427	SRSF2	S
115	1871	E2F3	S	290	5595	MAPK3	S	465	10274	STAG1	S
116	8726	EED	S	291	4149	MAX	S	466	10735	STAG2	S
117	51162	EGFL7	S	292	4170	MCL1	S	467	6774	STAT3	S
118	1956	EGFR	S, F, Sp	293	9656	MDC1	S	468	6775	STAT4	S
119	1964	EIF1AX	S	294	4193	MDM2	S	469	6776	STAT5A	S
120	1974	EIF4A2	S	295	4194	MDM4	S	470	6777	STAT5B	S
121	1977	EIF4E	S	296	9968	MED12	S	471	6794	STK11	S
122	6921	ELOC	S	297	100271849	MEF2B	S	472	83931	STK40	S
123	27436	EML4	S, F	298	4221	MEN1	S	473	51684	SUFU	S

No.	Entrez ID	Gene	Variant Type	No.	Entrez ID	Gene	Variant Type	No.	Entrez ID	Gene	Variant Type
	124	56946	EMSY	S	299 4233	MET	S	474	23512	SUZ12	S
125	2033	EP300	S	300	23269	MGA	S	475	6850	SYK	S
126	4072	EPCAM	S	301	4286	MITF	S	476	6872	TAF1	S
127	2042	EPHA3	S	302	4292	MLH1	S	477	6926	TBX3	S
128	2044	EPHA5	S	303	4300	MLLT3	S	478	6929	TCF3	S
129	2045	EPHA7	S	304	4352	MPL	S	479	6934	TCF7L2	S
130	2047	EPHB1	S	305	4361	MRE11	S	480	7012	TERC	S
131	2064	ERBB2	S	306	4436	MSH2	S	481	7015	TERT	S
132	2065	ERBB3	S	307	4437	MSH3	S	482	80312	TET1	S
133	2066	ERBB4	S	308	2956	MSH6	S	483	54790	TET2	S
134	2067	ERCC1	S	309	4485	MST1	S	484	7030	TFE3	S
135	2068	ERCC2	S	310	4486	MST1R	S	485	7037	TFRC	S
136	2071	ERCC3	S	311	2475	MTOR	S	486	7046	TGFBR1	S
137	2072	ERCC4	S	312	4595	MUTYH	S	487	7048	TGFBR2	S
138	2073	ERCC5	S	313	4602	MYB	S	488	55654	TMEM127	S
139	2078	ERG	S, F	314	4609	MYC	S	489	7113	TMPRSS2	S, F
140	54206	ERRFI1	S	315	4610	MYCL	S	490	7128	TNFAIP3	S
141	2099	ESR1	S, F	316	4613	MYCN	S	491	8764	TNFRSF14	S
142	2113	ETS1	S	317	4615	MYD88	S	492	7150	TOP1	S
143	2115	ETV1	S, F	318	4654	MYOD1	S	493	7153	TOP2A	S
144	2118	ETV4	S, F	319	4665	NAB2	S	494	7157	TP53	S
145	2119	ETV5	S	320	4683	NBN	S	495	8626	TP63	S
146	2120	ETV6	S	321	8202	NCOA3	S	496	7186	TRAF2	S
147	2130	EWSR1	S, F	322	9611	NCOR1	S	497	84231	TRAF7	S
148	2146	EZH2	S	323	257194	NEGR1	S	498	7248	TSC1	S
149	54855	FAM46C	S	324	4763	NF1	S	499	7249	TSC2	S
150	2175	FANCA	S	325	4771	NF2	S	500	7253	TSHR	S
151	2176	FANCC	S	326	4780	NFE2L2	S	501	7307	U2AF1	S
152	2177	FANCD2	S	327	4792	NFKBIA	S	502	7422	VEGFA	S
153	2178	FANCE	S	328	7080	NKX2-1	S	503	7428	VHL	S
154	2188	FANCF	S	329	4824	NKX3-1	S	504	79679	VTCN1	S
155	2189	FANCG	S	330	4851	NOTCH1	S	505	8838	WISP3	S
156	55215	FANCI	S	331	4853	NOTCH2	S	506	7490	WT1	S
157	55120	FANCL	S	332	4854	NOTCH3	S	507	331	XIAP	S
158	355	FAS	S	333	4855	NOTCH4	S	508	7514	XPO1	S
159	2195	FAT1	S	334	4869	NPM1	S	509	7516	XRCC2	S
160	55294	FBXW7	S	335	4893	NRAS	S	510	10413	YAP1	S
161	2246	FGF1	S	336	3084	NRG1	S, F	511	7525	YES1	S
162	2255	FGF10	S	337	64324	NSD1	S	512	57621	ZBTB2	S
163	2259	FGF14	S	338	4914	*NTRK*1	S, F	513	51341	ZBTB7A	S
164	9965	FGF19	S	339	4915	*NTRK*2	S, F	514	463	ZFHX3	S
165	2247	FGF2	S	340	4916	*NTRK*3	S, F	515	7764	ZNF217	S

No.	Entrez ID	Gene	Variant Type	No.	Entrez ID	Gene	Variant Type	No.	Entrez ID	Gene	Variant Type
166	8074	FGF23	S	341	9688	NUP93	S	516	80139	ZNF703	S
167	2248	FGF3	S	342	256646	NUTM1	S	517	8233	ZRSR2	S
168	2249	FGF4	S	343	5058	PAK1	S				
169	2250	FGF5	S	344	5063	PAK3	S				
170	2251	FGF6	S	345	57144	PAK5	S				
171	2252	FGF7	S	346	79728	PALB2	S				
172	2253	FGF8	S	347	142	PARP1	S				
173	2254	FGF9	S	348	5077	PAX3	S, F				
174	2260	FGFR1	S, F	349	5079	PAX5	S				
175	2263	FGFR2	S, F	350	5081	PAX7	S				

Abbreviations: S = small DNA variants (includes SNVs, MNVs, insertions and deletions), F = fusions, Sp = splice variants

Appendix 2. Concordance of Small DNA Variants by Gene and Variant Type from Accuracy for Tumor Profiling Markers Study

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
ABL1	3904	36	88.9% (74.7%-95.6%)	100.0% (>99.9%-100.0%)	27	96.3% (81.7%-99.3%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	2	50.0% (9.5%-90.5%)	100.0% (>99.9%-100.0%)
ABL2	3758	14	84.6% (57.8%-95.7%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
ABRAXAS1	1266	5	40.0% (11.8%-76.9%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
ACVR1	1566	8	100.0% (67.6%-100.0%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
ACVR1B	1681	13	100.0% (77.2%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
AKT1	1495	12	100.0% (75.8%->99.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
AKT2	1498	3	66.7% (20.8%-93.9%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
AKT3	1702	7	100.0% (64.6%->99.9%)	100.0% (99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
ALK	5333	36	86.1% (71.3%-93.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
AMER1	3412	30	75.0% (56.6%-87.3%)	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
APC	8814	75	98.7% (92.8%-99.8%)	100.0% (>99.9%-100.0%)	5	100.0% (56.6%-100.0%)	100.0% (>99.9%->99.9%)	17	100.0% (81.6%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)
AR	2826	29	65.4% (46.2%-80.6%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	1	--	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
ARAF	1893	9	77.8% (45.3%-93.7%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	1	--	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
ARID1A	6898	50	100.0% (92.9%-100.0%)	100.0% (>99.9%-100.0%)	6	100.0% (61.0%-100.0%)	100.0% (>99.9%-100.0%)	19	100.0% (83.2%-100.0%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
ARID1B	6902	43	87.9% (72.7%-95.2%)	>99.9% (>99.9%->99.9%)	6	66.7% (30.0%-90.3%)	100.0% (>99.9%->99.9%)	5	25.0% (4.6%-69.9%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
ARID2	5614	21	100.0% (83.9%->99.9%)	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	4	100.0% (51.0%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
ARID5B	3615	7	100.0% (64.6%->99.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)
ASXL1	4684	30	96.7% (83.3%-99.4%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	3	100.0% (43.9%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)
ATM	9417	45	90.9% (78.8%-96.4%)	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	3	100.0% (43.9%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
ATR	8120	21	81.0% (60.0%-92.3%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
ATRX	7602	30	90.0% (74.4%-96.5%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
AURKA	1243	3	100.0% (43.9%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
AURKB	1113	3	100.0% (34.2%-100.0%)	>99.9% (99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	1	--	>99.9% (99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
AXIN1	2629	10	100.0% (72.2%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	5	100.0% (56.6%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
AXIN2	2646	11	70.0% (39.7%-89.2%)	>99.9% (>99.9%->99.9%)	3	100.0% (43.9%-100.0%)	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
AXL	2953	14	78.6% (52.4%-92.4%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
B2M	395	7	100.0% (64.6%->99.9%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	7	100.0% (64.6%->99.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)
BAP1	2258	12	83.3% (55.2%-95.3%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
BARD1	2378	14	100.0% (78.5%->99.9%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	5	0.0% (0.0%-79.3%)	>99.9% (>99.9%->99.9%)
BCL10	712	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (99.9%-100.0%)
BCL2	856	3	100.0% (43.9%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
BCL2L1	710	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
BCL2L11	1029	6	83.3% (43.6%-97.0%)	100.0% (>99.9%-100.0%)	1	0.0% (0.0%-79.3%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
BCL6	2153	6	100.0% (61.0%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
BCOR	5340	35	73.5% (56.9%-85.4%)	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%->99.9%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%->99.9%)
BCORL1	5183	21	50.0% (29.9%-70.1%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	9	100.0% (64.6%->99.9%)	>99.9% (>99.9%->99.9%)	1	0.0% (0.0%-79.3%)	100.0% (>99.9%->99.9%)
BCR	5172	17	80.0% (54.8%-93.0%)	>99.9% (>99.9%->99.9%)	3	66.7% (20.8%-93.9%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	2	--	>99.9% (>99.9%->99.9%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
BIRC3	1846	11	90.9% (62.3%-98.4%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
BLM	4334	17	94.1% (73.0%-99.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	1	0.0% (0.0%-79.3%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)
BMPR1A	1639	5	100.0% (43.9%-100.0%)	>99.9% (99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)
BRAF	2419	58	96.6% (88.3%-99.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	4	100.0% (51.0%-100.0%)	100.0% (>99.9%->99.9%)
BRCA1	6041	28	96.3% (81.7%-99.3%)	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	3	100.0% (43.9%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
BRCA2	10358	42	100.0% (91.4%->99.9%)	>99.9% (>99.9%->99.9%)	4	100.0% (51.0%-100.0%)	100.0% (>99.9%->99.9%)	8	85.7% (48.7%-97.4%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
BRD4	4391	15	86.7% (62.1%-96.3%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
BRIP1	3826	24	95.8% (79.8%-99.3%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)
BTG1	524	1	100.0% (20.7%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
BTK	2082	12	75.0% (46.8%-91.1%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
CALR	1290	6	83.3% (43.6%-97.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
CARD11	3557	25	92.0% (75.0%-97.8%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
CASP8	1795	9	100.0% (70.1%->99.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	3	100.0% (43.9%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)
CBFB	619	3	100.0% (43.9%-100.0%)	100.0% (99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)
CBL	2777	18	88.9% (67.2%-96.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
CCND1	2222	8	100.0% (64.6%->99.9%)	>99.9% (>99.9%->99.9%)	1	0.0% (0.0%-79.3%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
CCND2	890	2	100.0% (34.2%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)
CCND3	1050	6	100.0% (61.0%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
CCNE1	1299	4	75.0% (30.1%-95.4%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
CD274	897	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
CD79A	701	1	0.0% (0.0%-79.3%)	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
CD79B	714	1	100.0% (20.7%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
CDC73	1664	3	100.0% (43.9%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
CDH1	2709	15	85.7% (60.1%-96.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)
CDK12	4526	25	84.0% (65.3%-93.6%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)
CDK4	1025	4	100.0% (51.0%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
CDK6	1008	5	80.0% (37.6%-96.4%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
CDK8	1443	4	100.0% (51.0%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
CDKN1A	729	9	100.0% (70.1%->99.9%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
CDKN1B	622	6	100.0% (61.0%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
CDKN2A	1370	18	100.0% (82.4%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)
CDKN2B	506	1	100.0% (20.7%-100.0%)	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
CDKN2C	515	6	100.0% (61.0%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
CEBPA	1138	7	57.1% (25.0%-84.2%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)
CHD2	5634	22	95.5% (78.2%-99.2%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
CHD4	5883	22	77.3% (56.6%-89.9%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
CHEK1	1478	6	100.0% (61.0%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
CHEK2	1778	13	76.9% (49.7%-91.8%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
CIC	4905	18	87.5% (64.0%-96.5%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	7	100.0% (64.6%->99.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)
CREBBP	7449	40	97.5% (87.1%-99.6%)	100.0% (>99.9%->99.9%)	4	75.0% (30.1%-95.4%)	100.0% (>99.9%-100.0%)	3	100.0% (20.7%-100.0%)	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
CRKL	924	4	100.0% (51.0%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
CRLF2	792	6	66.7% (30.0%-90.3%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
CSF1R	3003	15	80.0% (54.8%-93.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
CSF3R	2761	12	91.7% (64.6%-98.5%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
CTCF	2224	11	81.8% (52.3%-94.9%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
CTLA4	688	1	100.0% (20.7%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
CTNNA1	2791	20	94.7% (75.4%-99.1%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	3	100.0% (43.9%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
CTNNB1	2401	27	100.0% (87.5%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
CUL3	2458	5	80.0% (37.6%-96.4%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
CUX1	6810	25	95.0% (76.4%-99.1%)	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
CXCR4	1094	6	83.3% (43.6%-97.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
CYLD	2930	3	100.0% (43.9%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
DAXX	2246	6	100.0% (56.6%-100.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
DDR2	2631	9	100.0% (70.1%->99.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
DICER1	5926	23	100.0% (85.7%->99.9%)	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
DIS3	3031	7	100.0% (64.6%->99.9%)	100.0% (>99.9%-100.0%)	4	100.0% (51.0%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
DNMT3A	3143	14	85.7% (60.1%-96.0%)	100.0% (>99.9%->99.9%)	1	0.0% (0.0%-79.3%)	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
DOT1L	5316	23	95.7% (79.0%-99.2%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
EGFR	4985	34	91.2% (77.0%-97.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	4	66.7% (20.8%-93.9%)	>99.9% (>99.9%->99.9%)	1	--	>99.9% (>99.9%->99.9%)
EIF1AX	463	3	100.0% (43.9%-100.0%)	100.0% (99.8%->99.9%)	0	--	100.0% (99.8%-100.0%)	0	--	100.0% (99.8%-100.0%)	0	--	100.0% (99.8%-100.0%)
ELOC	350	1	100.0% (20.7%-100.0%)	100.0% (99.8%-100.0%)	0	--	100.0% (99.8%-100.0%)	0	--	100.0% (99.8%-100.0%)	0	--	100.0% (99.8%-100.0%)
EMSY	4052	10	90.0% (59.6%-98.2%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
EP300	7368	27	88.9% (71.9%-96.1%)	100.0% (>99.9%-100.0%)	2	100.0% (20.7%-100.0%)	>99.9% (>99.9%->99.9%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
EPCAM	965	5	100.0% (56.6%-100.0%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
EPHA7	3065	12	91.7% (64.6%-98.5%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
EPHB1	3019	30	86.7% (70.3%-94.7%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
ERBB2	4082	40	90.0% (76.9%-96.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
ERBB3	4333	45	84.4% (71.2%-92.3%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
ERBB4	4039	33	93.8% (79.9%-98.3%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
ERCC1	1775	6	100.0% (56.6%-100.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	--	>99.9% (>99.9%->99.9%)
ERCC2	2883	32	87.5% (71.9%-95.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%->99.9%)
ERCC3	2405	8	50.0% (18.8%-81.2%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
ERCC4	2812	19	100.0% (82.4%-100.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
ERCC5	3629	23	100.0% (85.7%->99.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
ERG	1873	8	75.0% (40.9%-92.9%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)
ERRFI1	1401	3	100.0% (43.9%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
ESR1	1823	19	84.2% (62.4%-94.5%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	0.0% (0.0%-79.3%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
ETS1	1790	6	100.0% (61.0%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
ETV1	2442	11	90.9% (62.3%-98.4%)	100.0% (>99.9%-100.0%)	1	--	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
ETV4	1830	7	71.4% (35.9%-91.8%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
ETV5	1747	6	100.0% (61.0%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
ETV6	1687	6	100.0% (61.0%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)
EWSR1	2874	6	100.0% (56.6%-100.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	6	0.0% (0.0%-39.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
EZH2	2330	12	100.0% (75.8%->99.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
FAM46C	1180	3	66.7% (20.8%-93.9%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
FANCA	4565	28	92.9% (77.4%-98.0%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
FANCC	1887	12	100.0% (75.8%->99.9%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
FANCD2	4652	16	92.9% (68.5%-98.7%)	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
FANCE	1651	3	66.7% (20.8%-93.9%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
FANCF	1127	3	100.0% (43.9%-100.0%)	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)
FANCG	1915	6	66.7% (30.0%-90.3%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
FANCI	4134	10	90.0% (59.6%-98.2%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
FANCL	1198	4	100.0% (51.0%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
FAS	1044	6	100.0% (61.0%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
FAT1	13869	37	88.9% (74.7%-95.6%)	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	3	100.0% (34.2%-100.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
FBXW7	2812	22	81.8% (61.5%-92.7%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	3	66.7% (20.8%-93.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
FGF1	477	1	100.0% (20.7%-100.0%)	100.0% (99.8%-100.0%)	0	--	100.0% (99.8%-100.0%)	0	--	100.0% (99.8%-100.0%)	0	--	100.0% (99.8%-100.0%)
FGF10	635	4	100.0% (51.0%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
FGF14	976	3	100.0% (43.9%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
FGF19	222	1	0.0% (0.0%-79.3%)	100.0% (99.8%-100.0%)	0	--	100.0% (99.8%-100.0%)	0	--	100.0% (99.8%-100.0%)	0	--	100.0% (99.8%-100.0%)
FGF2	801	0	--	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%->99.9%)
FGF23	814	7	85.7% (48.7%-97.4%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
FGF3	1087	10	88.9% (56.5%-98.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
FGF4	741	2	100.0% (34.2%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
FGF5	1013	5	100.0% (51.0%-100.0%)	>99.9% (99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
FGF6	877	7	71.4% (35.9%-91.8%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
FGF7	290	0	--	100.0% (99.1%-100.0%)	0	--	100.0% (99.1%-100.0%)	0	--	100.0% (99.1%-100.0%)	0	--	100.0% (99.1%-100.0%)
FGF8	850	3	66.7% (20.8%-93.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
FGF9	639	2	100.0% (34.2%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
FGFR1	2707	22	90.9% (72.2%-97.5%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
FGFR2	3617	18	94.4% (74.2%-99.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
FGFR3	2690	16	100.0% (80.6%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
FGFR4	2583	17	88.2% (65.7%-96.7%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
FH	1596	3	33.3% (6.1%-79.2%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
FLCN	2009	13	69.2% (42.4%-87.3%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
FLT1	4415	20	89.5% (68.6%-97.1%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
FLT3	3078	21	95.2% (77.3%-99.2%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
FLT4	4343	27	88.9% (71.9%-96.1%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
FOXA1	1693	6	100.0% (61.0%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
FOXL2	1131	3	100.0% (43.9%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)
FOXO1	1986	9	88.9% (56.5%-98.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
FOXP1	2583	15	92.9% (68.5%-98.7%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
FRS2	1547	4	100.0% (51.0%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
FUBP1	2077	5	75.0% (30.1%-95.4%)	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
GABRA6	1398	17	100.0% (78.5%->99.9%)	>99.9% (99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
GATA1	1262	4	100.0% (51.0%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
GATA2	1463	9	88.9% (56.5%-98.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
GATA3	1355	13	100.0% (77.2%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
GATA4	1347	3	100.0% (43.9%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
GATA6	1800	8	80.0% (37.6%-96.4%)	>99.9% (99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
GEN1	2778	8	87.5% (52.9%-97.8%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
GLI1	3365	6	83.3% (43.6%-97.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	3	100.0% (34.2%-100.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
GNA11	1108	7	85.7% (48.7%-97.4%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
GNA13	1148	3	100.0% (43.9%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
GNAQ	909	4	100.0% (51.0%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
GNAS	4070	32	100.0% (89.3%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	3	100.0% (34.2%-100.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
GPS2	1024	4	75.0% (30.1%-95.4%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
GREM1	606	1	100.0% (20.7%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
GRIN2A	4443	47	91.3% (79.7%-96.6%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	1	--	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
GRM3	2659	17	93.3% (70.2%-98.8%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)
H3F3A	503	2	100.0% (34.2%-100.0%)	100.0% (99.8%-100.0%)	0	--	100.0% (99.8%-100.0%)	0	--	100.0% (99.8%-100.0%)	0	--	100.0% (99.8%-100.0%)
HGF	2282	14	85.7% (60.1%-96.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
HIST1H3B	413	6	100.0% (61.0%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%-100.0%)
HNF1A	1934	12	81.8% (52.3%-94.9%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	3	100.0% (43.9%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
HNRNPK	130	1	100.0% (20.7%-100.0%)	100.0% (99.5%-100.0%)	0	--	100.0% (99.5%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (99.5%-100.0%)	0	--	100.0% (99.5%-100.0%)
HOXB13	863	7	85.7% (48.7%-97.4%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
HRAS	672	8	100.0% (67.6%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
HSD3B1	1134	6	100.0% (61.0%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
HSP90AA1	2609	8	50.0% (15.0%-85.0%)	>99.9% (99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
IDH1	1277	11	100.0% (74.1%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
IDH2	1403	9	88.9% (56.5%-98.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
IFNGR1	1597	8	87.5% (52.9%-97.8%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)
IKBKE	2235	8	87.5% (52.9%-97.8%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
IKZF1	1588	15	100.0% (79.6%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
IL7R	1412	10	80.0% (49.0%-94.3%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
INHBA	118	1	100.0% (20.7%-100.0%)	100.0% (99.7%->99.9%)	0	--	100.0% (99.7%-100.0%)	0	--	100.0% (99.7%-100.0%)	0	--	100.0% (99.7%-100.0%)
INPP4B	2866	12	100.0% (75.8%->99.9%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
IRF2	1082	6	83.3% (43.6%-97.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
IRF4	1426	7	83.3% (43.6%-97.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
IRS2	4006	16	86.7% (62.1%-96.3%)	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
JAK1	3561	16	87.5% (64.0%-96.5%)	100.0% (>99.9%->99.9%)	2	100.0% (20.7%-100.0%)	>99.9% (>99.9%->99.9%)	5	100.0% (34.2%-100.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
JAK2	3491	11	88.9% (56.5%-98.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
JAK3	3467	12	81.8% (52.3%-94.9%)	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
JUN	1120	7	85.7% (48.7%-97.4%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
KAT6A	6074	19	89.5% (68.6%-97.1%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)
KDM5A	5178	19	83.3% (60.8%-94.2%)	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
KDM5C	4880	22	81.0% (60.0%-92.3%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
KDM6A	4480	13	76.9% (49.7%-91.8%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
KDR	4190	28	96.4% (82.3%-99.4%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
KEAP1	1895	21	81.0% (60.0%-92.3%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
KEL	2275	21	95.2% (77.3%-99.2%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
KIT	3179	20	100.0% (83.9%->99.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
KLF4	1558	3	100.0% (43.9%-100.0%)	100.0% (>99.9%-100.0%)	1	--	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
KLHL6	1894	6	83.3% (43.6%-97.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
KMT2A	12054	40	87.2% (73.3%-94.4%)	>99.9% (>99.9%->99.9%)	3	100.0% (43.9%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
KRAS	999	73	100.0% (95.0%->99.9%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
LATS1	3513	8	85.7% (48.7%-97.4%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
LMO1	513	4	100.0% (43.9%-100.0%)	>99.9% (99.9%->99.9%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
LRP1B	14161	108	95.3% (89.5%-98.0%)	>99.9% (>99.9%->99.9%)	3	100.0% (20.7%-100.0%)	>99.9% (>99.9%->99.9%)	3	100.0% (43.9%-100.0%)	100.0% (>99.9%->99.9%)	3	100.0% (43.9%-100.0%)	100.0% (>99.9%->99.9%)
LYN	1587	3	100.0% (43.9%-100.0%)	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)
LZTR1	2606	4	100.0% (51.0%-100.0%)	100.0% (>99.9%-100.0%)	1	0.0% (0.0%-79.3%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
MAGI2	4454	14	100.0% (78.5%->99.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	--	>99.9% (>99.9%->99.9%)
MALT1	2539	1	0.0% (0.0%-79.3%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
MAP2K1	1170	4	75.0% (30.1%-95.4%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	3	100.0% (43.9%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
MAP2K2	1246	6	100.0% (61.0%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
MAP2K4	1276	3	100.0% (43.9%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
MAP3K1	4611	6	83.3% (43.6%-97.0%)	100.0% (>99.9%-100.0%)	1	0.0% (0.0%-79.3%)	100.0% (>99.9%-100.0%)	7	100.0% (64.6%->99.9%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
MAPK1	1109	3	100.0% (43.9%-100.0%)	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)
MAX	843	1	100.0% (20.7%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)
MCL1	1075	3	100.0% (43.9%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
MDM2	1584	6	66.7% (30.0%-90.3%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)
MDM4	1513	8	100.0% (67.6%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
MED12	6695	33	66.7% (49.6%-80.2%)	100.0% (>99.9%->99.9%)	2	0.0% (0.0%-65.8%)	100.0% (>99.9%->99.9%)	4	33.3% (6.1%-79.2%)	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
MEF2B	1139	3	100.0% (34.2%-100.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	3	100.0% (43.9%-100.0%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
MEN1	1907	8	100.0% (64.6%->99.9%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	3	100.0% (43.9%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
MET	4578	18	88.9% (67.2%-96.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
MITF	1873	8	100.0% (67.6%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
MLH1	2366	12	83.3% (55.2%-95.3%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
MLLT3	1713	7	100.0% (56.6%-100.0%)	>99.9% (99.9%->99.9%)	1	0.0% (0.0%-79.3%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
MPL	1956	21	85.7% (65.4%-95.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
MRE11	2198	5	50.0% (15.0%-85.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
MSH2	2885	16	75.0% (50.5%-89.8%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	3	100.0% (43.9%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
MSH3	3500	16	100.0% (80.6%-100.0%)	100.0% (>99.9%->99.9%)	1	0.0% (0.0%-79.3%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
MSH6	4196	21	95.2% (77.3%-99.2%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	4	100.0% (51.0%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
MTOR	7878	44	93.2% (81.8%-97.7%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
MUTYH	1714	3	100.0% (34.2%-100.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)
MYB	3303	6	75.0% (30.1%-95.4%)	>99.9% (>99.9%->99.9%)	3	50.0% (9.5%-90.5%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
MYC	1513	17	100.0% (81.6%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	3	100.0% (43.9%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
MYCL	1860	8	57.1% (25.0%-84.2%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
MYCN	1611	17	93.8% (71.7%-98.9%)	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
MYD88	974	1	100.0% (20.7%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)
NBN	2329	4	75.0% (30.1%-95.4%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
NCOR1	7725	19	82.4% (59.0%-93.8%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
NF1	8242	36	91.7% (78.2%-97.1%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
NF2	1892	13	69.2% (42.4%-87.3%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
NFE2L2	1907	9	100.0% (67.6%-100.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	3	50.0% (9.5%-90.5%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
NFKBIA	978	2	100.0% (34.2%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
NKX2-1	1214	3	66.7% (20.8%-93.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	2	100.0% (20.7%-100.0%)	>99.9% (99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
NOTCH1	7800	71	98.6% (92.4%-99.8%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
NOTCH2	6861	32	87.5% (71.9%-95.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
NOTCH3	7073	25	91.3% (73.2%-97.6%)	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)
NOTCH4	6205	24	91.3% (73.2%-97.6%)	>99.9% (>99.9%->99.9%)	3	0.0% (0.0%-65.8%)	>99.9% (>99.9%->99.9%)	2	0.0% (0.0%-79.3%)	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
NPM1	954	4	100.0% (51.0%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
NRAS	790	13	100.0% (77.2%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
NRG1	5260	35	85.3% (69.9%-93.6%)	>99.9% (>99.9%->99.9%)	1	--	>99.9% (>99.9%->99.9%)	3	100.0% (43.9%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
NSD1	8179	20	80.0% (58.4%-91.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
*NTRK*1	3105	30	96.7% (83.3%-99.4%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
*NTRK*2	14079	52	85.4% (72.8%-92.8%)	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)	3	100.0% (20.7%-100.0%)	>99.9% (>99.9%->99.9%)
*NTRK*3	4898	42	90.2% (77.5%-96.1%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)
PAK1	1765	4	25.0% (4.6%-69.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
PALB2	3612	14	92.9% (68.5%-98.7%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	3	100.0% (43.9%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
PAX3	2322	6	80.0% (37.6%-96.4%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	2	50.0% (9.5%-90.5%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
PAX5	1259	6	66.7% (30.0%-90.3%)	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)
PAX7	1889	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
PAX8	3797	13	90.9% (62.3%-98.4%)	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
PBRM1	5240	18	94.1% (73.0%-99.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
PDCD1	877	2	100.0% (20.7%-100.0%)	>99.9% (99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)
PDCD1LG2	846	3	100.0% (43.9%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
PDGFRA	3376	25	96.0% (80.5%-99.3%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
PDGFRB	3409	26	88.5% (71.0%-96.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
PDK1	1354	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
PDPK1	1780	0	--	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)
PHF6	1582	2	50.0% (9.5%-90.5%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
PHOX2B	942	2	100.0% (20.7%-100.0%)	>99.9% (99.9%->99.9%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
PIK3C2B	5033	16	87.5% (64.0%-96.5%)	100.0% (>99.9%-100.0%)	1	--	>99.9% (>99.9%->99.9%)	6	100.0% (34.2%-100.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
PIK3CA	3287	54	96.2% (87.2%-99.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
PIK3CB	3378	14	92.9% (68.5%-98.7%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
PIK3CD	3223	9	100.0% (70.1%->99.9%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
PIK3CG	3349	22	100.0% (85.1%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
PIK3R1	2612	21	95.0% (76.4%-99.1%)	>99.9% (>99.9%->99.9%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)	9	87.5% (52.9%-97.8%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
PIK3R2	2227	8	100.0% (67.6%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
PIM1	966	2	100.0% (34.2%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
PLCG2	3974	30	86.7% (70.3%-94.7%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
PMS1	2847	3	100.0% (34.2%-100.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	1	--	>99.9% (>99.9%->99.9%)
PMS2	1126	9	88.9% (56.5%-98.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	3	66.7% (20.8%-93.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)
PNRC1	74	1	0.0% (0.0%-79.3%)	100.0% (99.1%-100.0%)	0	--	100.0% (99.1%-100.0%)	0	--	100.0% (99.1%-100.0%)	0	--	100.0% (99.1%-100.0%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
POLD1	3507	15	86.7% (62.1%-96.3%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
POLE	7054	52	90.2% (79.0%-95.7%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
PPARG	1713	6	66.7% (30.0%-90.3%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
PPM1D	1842	5	80.0% (37.6%-96.4%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
PPP2R1A	1950	14	78.6% (52.4%-92.4%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
PPP2R2A	1456	1	100.0% (20.7%-100.0%)	100.0% (99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
PPP6C	1061	7	83.3% (43.6%-97.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
PRDM1	2518	8	87.5% (52.9%-97.8%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
PREX2	4981	30	96.2% (81.1%-99.3%)	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	2	100.0% (20.7%-100.0%)	>99.9% (>99.9%->99.9%)	9	100.0% (56.6%-100.0%)	>99.9% (>99.9%->99.9%)
PRKAR1A	1231	2	100.0% (34.2%-100.0%)	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
PRKDC	12725	51	82.6% (69.3%-90.9%)	>99.9% (>99.9%->99.9%)	3	33.3% (6.1%-79.2%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
PRKN	1513	9	100.0% (70.1%->99.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
PTCH1	4930	29	89.7% (73.6%-96.4%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	13	100.0% (75.8%->99.9%)	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
PTEN	1449	17	94.1% (73.0%-99.0%)	100.0% (>99.9%-100.0%)	5	100.0% (56.6%-100.0%)	100.0% (>99.9%-100.0%)	12	91.7% (64.6%-98.5%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
PTPN11	1910	6	100.0% (56.6%-100.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
PTPRD	5941	49	91.8% (80.8%-96.8%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
PTPRT	4509	48	83.0% (69.9%-91.1%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	2	--	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
QKI	1160	2	100.0% (34.2%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
RAC1	664	5	100.0% (56.6%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
RAD21	1947	9	87.5% (52.9%-97.8%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
RAD50	4034	13	100.0% (77.2%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	1	0.0% (0.0%-79.3%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
RAD51	1058	1	--	>99.9% (99.9%->99.9%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
RAD51B	1170	8	25.0% (7.1%-59.1%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
RAD51C	1243	4	100.0% (51.0%-100.0%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
RAD51D	1202	7	57.1% (25.0%-84.2%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
RAD54L	2316	6	60.0% (23.1%-88.2%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
RAF1	2011	11	81.8% (52.3%-94.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
RANBP2	9779	23	87.5% (64.0%-96.5%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)
RARA	1809	6	83.3% (43.6%-97.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	2	--	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
RASA1	3334	8	100.0% (67.6%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
RB1	2890	17	94.1% (73.0%-99.0%)	100.0% (>99.9%-100.0%)	1	0.0% (0.0%-79.3%)	100.0% (>99.9%-100.0%)	4	100.0% (51.0%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
RBM10	2915	20	85.0% (64.0%-94.8%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
RECQL4	3709	20	60.0% (38.7%-78.1%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	3	66.7% (20.8%-93.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
RET	3785	41	97.5% (87.1%-99.6%)	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	4	100.0% (51.0%-100.0%)	100.0% (>99.9%-100.0%)
RHEB	587	1	100.0% (20.7%-100.0%)	100.0% (99.8%-100.0%)	0	--	100.0% (99.8%->99.9%)	0	--	100.0% (99.8%->99.9%)	0	--	100.0% (99.8%->99.9%)
RHOA	598	4	100.0% (51.0%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
RICTOR	5352	9	88.9% (56.5%-98.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
RIT1	733	6	100.0% (61.0%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
RNF43	2542	20	75.0% (53.1%-88.8%)	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)	17	100.0% (79.6%-100.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
ROS1	8012	26	83.3% (64.1%-93.3%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
RPS6KB1	1909	3	100.0% (43.9%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
RPTOR	4148	23	85.0% (64.0%-94.8%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
RUNX1	1657	9	100.0% (70.1%->99.9%)	100.0% (>99.9%->99.9%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%->99.9%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
RUNX1T1	1995	20	87.5% (64.0%-96.5%)	>99.9% (99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
SDHA	2119	10	90.0% (59.6%-98.2%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
SDHAF2	517	0	--	100.0% (99.8%->99.9%)	0	--	100.0% (99.8%->99.9%)	0	--	100.0% (99.8%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (99.8%-100.0%)
SDHB	872	1	100.0% (20.7%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
SDHC	641	4	75.0% (30.1%-95.4%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
SDHD	496	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
SETBP1	5225	35	94.3% (81.4%-98.4%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	2	100.0% (20.7%-100.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
SETD2	7778	31	90.0% (74.4%-96.5%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	3	0.0% (0.0%-79.3%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
SF3B1	4088	10	100.0% (72.2%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
SH2B3	1882	11	100.0% (74.1%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	2	100.0% (20.7%-100.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
SLIT2	4737	20	95.0% (76.4%-99.1%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
SLX4	5559	14	76.9% (49.7%-91.8%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
SMAD2	1550	5	100.0% (56.6%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
SMAD3	1392	6	100.0% (56.6%-100.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	1	--	>99.9% (>99.9%->99.9%)
SMAD4	1703	13	100.0% (77.2%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	3	100.0% (43.9%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)
SMARCA4	5328	40	90.0% (76.9%-96.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	5	100.0% (56.6%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
SMARCB1	1194	7	100.0% (61.0%-100.0%)	>99.9% (99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
SMC1A	4773	16	43.8% (23.1%-66.8%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
SMC3	4890	14	64.3% (38.8%-83.7%)	100.0% (>99.9%->99.9%)	1	0.0% (0.0%-79.3%)	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
SMO	2330	16	93.8% (71.7%-98.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)
SOCS1	640	6	83.3% (43.6%-97.0%)	100.0% (99.9%-100.0%)	1	0.0% (0.0%-79.3%)	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
SOX10	1406	4	100.0% (51.0%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)
SOX2	958	1	100.0% (20.7%-100.0%)	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
SOX9	1537	7	100.0% (61.0%-100.0%)	>99.9% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
SPEN	11332	41	84.6% (70.3%-92.8%)	>99.9% (>99.9%->99.9%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%->99.9%)
SPOP	1161	9	88.9% (56.5%-98.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
SRC	1655	11	100.0% (74.1%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
SRSF2	674	3	100.0% (43.9%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)
STAG2	3937	12	75.0% (46.8%-91.1%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
STAT3	2405	14	100.0% (78.5%->99.9%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
STAT4	2338	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
STAT5A	2460	8	75.0% (40.9%-92.9%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)
STAT5B	2436	4	100.0% (51.0%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
STK11	1338	14	92.9% (68.5%-98.7%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	6	100.0% (61.0%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
SUFU	1658	8	71.4% (35.9%-91.8%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)
SUZ12	2275	3	66.7% (20.8%-93.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
SYK	1960	10	88.9% (56.5%-98.0%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
TAF1	5827	20	63.2% (41.0%-80.9%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
TBX3	2331	20	95.0% (76.4%-99.1%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
TCF3	2481	12	90.9% (62.3%-98.4%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
TCF7L2	2162	10	100.0% (72.2%-100.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
TERT	78	2	100.0% (34.2%-100.0%)	100.0% (99.5%->99.9%)	0	--	100.0% (99.5%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (99.5%-100.0%)	0	--	100.0% (99.5%-100.0%)
TET2	6419	34	90.9% (76.4%-96.9%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
TFE3	992	3	33.3% (6.1%-79.2%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
TGFBR1	1554	5	100.0% (43.9%-100.0%)	>99.9% (99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
TGFBR2	1809	4	75.0% (30.1%-95.4%)	100.0% (>99.9%->99.9%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
TMEM127	724	1	100.0% (20.7%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%->99.9%)
TMPRSS2	2169	8	87.5% (52.9%-97.8%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%->99.9%)
TNFAIP3	2405	10	87.5% (52.9%-97.8%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
TNFRSF14	888	3	66.7% (20.8%-93.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
TOP1	2382	4	75.0% (30.1%-95.4%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
TOP2A	4735	3	66.7% (20.8%-93.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
TP53	2180	159	95.5% (91.1%-97.8%)	>99.9% (>99.9%->99.9%)	10	77.8% (45.3%-93.7%)	>99.9% (>99.9%->99.9%)	14	92.9% (68.5%-98.7%)	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)

Gene	Total Bases	SNVs			Insertions			Deletions			MNVs		
		# of SNVs	SNV PPA (95%CI)	SNV NPA (95%CI)	# of Ins	Ins PPA (95%CI)	Ins NPA (95%CI)	# of Del	Del PPA (95%CI)	Del NPA (95%CI)	# of MNVs	MNV PPA (95%CI)	MNV NPA (95%CI)
TP63	2268	15	92.9% (68.5%-98.7%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
TRAF7	2089	8	87.5% (52.9%-97.8%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	1	0.0% (0.0%-79.3%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
TSC1	3572	16	81.2% (57.0%-93.4%)	100.0% (>99.9%-100.0%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%->99.9%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
TSC2	5587	44	97.7% (88.2%-99.6%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%->99.9%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%-100.0%)	1	0.0% (0.0%-79.3%)	100.0% (>99.9%->99.9%)
TSHR	2476	9	88.9% (56.5%-98.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
U2AF1	829	6	83.3% (43.6%-97.0%)	100.0% (>99.9%-100.0%)	1	100.0% (20.7%-100.0%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
VEGFA	1335	7	85.7% (48.7%-97.4%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	3	100.0% (43.9%-100.0%)	100.0% (>99.9%->99.9%)	2	100.0% (34.2%-100.0%)	100.0% (>99.9%->99.9%)
VHL	654	4	100.0% (51.0%-100.0%)	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
WT1	1598	10	80.0% (49.0%-94.3%)	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)
XPO1	3310	4	66.7% (20.8%-93.9%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
XRCC2	854	3	66.7% (20.8%-93.9%)	100.0% (99.9%->99.9%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)	0	--	100.0% (99.9%-100.0%)
ZFHX3	11092	40	80.6% (65.0%-90.2%)	>99.9% (>99.9%->99.9%)	10	25.0% (7.1%-59.1%)	>99.9% (>99.9%->99.9%)	11	100.0% (70.1%->99.9%)	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%-100.0%)
ZNF217	3163	12	100.0% (75.8%->99.9%)	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)	0	--	100.0% (>99.9%-100.0%)
ZRSR2	1491	3	100.0% (34.2%-100.0%)	>99.9% (>99.9%->99.9%)	2	0.0% (0.0%-65.8%)	100.0% (>99.9%-100.0%)	1	--	>99.9% (>99.9%->99.9%)	0	--	100.0% (>99.9%->99.9%)

Appendix 3. Panel-wide precision by specimen and variant from Reproducibility for Tumor Profiling Markers Study

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSAD1	SNV	2	EGFR: EGFR-AS1	chr7_55259515_T_G	38	4.5	4.3	2.5	7.8	0.013 (28.8)	100.0 (38/38)
TPSAD1	SNV	3	TP53	chr17_7577498_C_G	38	2.6	2.7	0.9	4.1	0.010 (36.9)	55.3 (21/38)
TPSAD2	SNV	3	AKT1	chr14_105246551_C_T	46	2	1.7	0.5	4.7	0.011 (54.9)	67.4 (31/46)
TPSAD2	SNV	3	APC	chr5_112174685_G_T	42	3.7	3.7	0.6	6.6	0.016 (42.1)	59.5 (25/42)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSAD2	SNV	2	BRAF	chr7_140453136_A_T	46	4.5	4.7	0	7.1	0.017 (36.9)	91.3 (42/46)
TPSAD2	DELETION	3	FBXW7	chr4_153332539_AT_A	46	4.4	4.4	1.6	6.8	0.013 (30.5)	82.6 (38/46)
TPSAD2	DELETION	3	MSH2	chr2_47639587_GA_G	16	5.6	4.7	3.2	11.1	0.028 (50.5)	81.3 (13/16)
TPSAD3	SNV	2	IDH1	chr2_209113112_C_T	36	15.5	15.9	6.9	24.3	0.039 (25.2)	100.0 (36/36)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSAD3	SNV	2	TERT	chr5_1295228_G_A	20	11.5	8	3.8	25	0.081 (70.5)	70.0 (14/20)
TPSAD4	MNV	2	BRAF	chr7_140453136_AC_TT	46	13	13.2	8.6	16.7	0.019 (14.8)	100.0 (46/46)
TPSAD5	SNV	3	APC	chr5_112116592_C_T	38	11.7	11.8	5.2	15.6	0.027 (23.3)	100.0 (38/38)
TPSAD5	SNV	3	APC	chr5_112175426_G_T	38	6.8	6.6	4.4	10.8	0.018 (25.7)	100.0 (38/38)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSAD5	MNV	2	KRAS	chr12_25398284_CC_AT	38	11.1	11.1	7.9	14.9	0.020 (18.5)	100.0 (38/38)
TPSAD5	DELETION	3	TCF7L2	chr10_114711231_CGCCATGTTAGCG_C	38	11.7	11.2	8.8	15.4	0.018 (15.1)	100.0 (38/38)
TPSAD6	INSERTION	3	ARID1A:LOC101928728	chr1_27024001_C_CG	4	8.4	8.2	5.8	11.3	0.027	100.0 (4/4)
TPSAD6	SNV	3	CDKN2A:CDKN2A-AS1	chr9_21971186_G_A	4	18.3	18.4	15.7	20.6	0.025	100.0 (4/4)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSAD6	SNV	2	CSDE1:NRAS	chr1_115256530_G_T	4	15.9	16.2	14.6	16.5	0.008	100.0 (4/4)
TPSAD7	DELETION	3	APC	chr5_112175751_CTTTA_C	32	17	16.7	12.4	21	0.024 (14.4)	100.0 (32/32)
TPSAD7	INSERTION	3	APC	chr5_112175951_G_GA	32	22.7	23	15.5	30.2	0.036 (16.1)	100.0 (32/32)
TPSAD7	SNV	2	KRAS	chr12_25398284_C_T	32	28.9	28.8	24.6	33.6	0.022 (7.7)	100.0 (32/32)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSAD8	DELETION	3	APC	chr5_112175479_TGA_T	48	10.5	10.8	8	13.4	0.013 (12.6)	100.0 (48/48)
TPSAD8	INSERTION	3	APC	chr5_112175675_A_AAG	48	10	10.1	7.6	12.3	0.011 (11.3)	100.0 (48/48)
TPSAD8	DELETION	3	ARID1A:LOC101928728	chr1_27023903_TG_T	48	16.6	16.9	7.5	23.3	0.040 (24.0)	100.0 (48/48)
TPSAD8	MNV	3	BRAF	chr7_140453136_AC_TT	48	11.1	11.1	7.6	15.9	0.019 (17.0)	100.0 (48/48)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSAD8	DELETION	3	CDKN2A:CDKN2A-AS1	chr9_21971123_TGA_T	48	21	21	14.6	28.6	0.037 (17.7)	100.0 (48/48)
TPSAD8	MNV	3	CSDE1:NRAS	chr1_115256529_TG_CT	48	9.5	9.4	6.4	13.5	0.017 (18.2)	100.0 (48/48)
TPSAD8	DELETION	3	EGFR	chr7_55242464_AGGAATTAAGAGAAGC_A	48	17.8	18	13.6	21	0.015 (8.4)	100.0 (48/48)
TPSAD8	DELETION	3	EGFR	chr7_55242467_AATTAAGAGAAGCAACATC_T	48	8.3	8.1	5.3	12	0.017 (20.0)	100.0 (48/48)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSAD8	DELETION	3	EGFR	chr7_55242469_TTAAGAGAAG_C	48	14.3	14.2	6.5	21	0.037 (26.1)	100.0 (48/48)
TPSAD8	INSERTION	3	EGFR:EGFR-AS1	chr7_55248998_A_ATGGCCAGCG	48	16.3	16.5	13.5	18.3	0.013 (7.7)	100.0 (48/48)
TPSAD8	INSERTION	3	EGFR:EGFR-AS1	chr7_55249010_G_GACAACCCCC	48	16.3	16.4	13.9	18.8	0.012 (7.5)	100.0 (48/48)
TPSAD8	SNV	3	FBXO11:MSH6	chr2_48033753_G_T	48	13.2	12.9	8.3	21.1	0.028 (20.9)	100.0 (48/48)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSAD8	SNV	3	IDH1	chr2_209113112_C_T	48	12.1	11.8	7.9	16.4	0.020 (16.7)	100.0 (48/48)
TPSAD8	INSERTION	3	KIT	chr4_55592178_C_CTGCCTA	48	9.9	9.6	6.2	14.5	0.020 (19.8)	100.0 (48/48)
TPSAD8	DELETION	3	KIT	chr4_55593600_CAGTGGAAGGTTGTTG_C	48	6.3	6.4	3.3	9	0.016 (25.7)	100.0 (48/48)
TPSAD8	SNV	3	KRAS	chr12_25378647_T_G	48	16.8	16.2	11.2	24.1	0.030 (17.8)	100.0 (48/48)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSAD8	MNV	2	KRAS	chr12_25398284_CC_AA	48	11.2	11.4	6.9	15.8	0.019 (17.1)	100.0 (48/48)
TPSAD8	DELETION	3	MET	chr7_116411879_CTCTTTCTTTCTCTCTGTTTTAAGA_C	48	12.4	12.8	8.4	16.5	0.019 (15.2)	100.0 (48/48)
TPSAD8	SNV	3	MSH2	chr2_47703538_C_T	48	10.8	10.4	8.3	14.5	0.017 (15.7)	100.0 (48/48)
TPSAD8	INSERTION	3	PTEN	chr10_89717695_C_CTT	48	11.8	11.6	8.5	15.6	0.021 (17.4)	100.0 (48/48)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSAD8	DELETION	3	PTEN	chr10_89717718_CTG_C	48	10.9	10.8	7.6	15.9	0.022 (20.5)	100.0 (48/48)
TPSAD8	DELETION	3	PTEN	chr10_89720798_GTACT_G	48	18	17.9	12.9	25.8	0.027 (14.8)	100.0 (48/48)
TPSAD8	DELETION	3	TP53	chr17_7577047_GTGGTGAGGCTCCCCTTTCT_G	48	28.8	28.5	24.7	33.7	0.022 (7.7)	100.0 (48/48)
TPSAD8	DELETION	3	VHL	chr3_10183754_ATCT_A	48	18.7	18.4	15	23.9	0.028 (15.2)	100.0 (48/48)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSAD8	DELETION	3	VHL	chr3_10183824_AC_A	48	21.9	22.3	16.2	25.7	0.026 (11.7)	100.0 (48/48)
TPSAD9	DELETION	3	APC	chr5_112175479_TGA_T	48	6.5	6.4	4.6	8.4	0.011 (16.7)	100.0 (48/48)
TPSAD9	INSERTION	3	APC	chr5_112175675_A_AAG	48	5.7	5.7	3.8	8	0.011 (18.6)	100.0 (48/48)
TPSAD9	DELETION	3	ARID1A:LOC101928728	chr1_27023903_TG_T	37	9.6	9.8	4.4	16.2	0.031 (32.4)	91.9 (34/37)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSAD9	MNV	3	BRAF	chr7_140453136_AC_TT	48	6.6	6.8	2.6	10.7	0.019 (29.5)	100.0 (48/48)
TPSAD9	DELETION	3	CDKN2A:CDKN2A-AS1	chr9_21971123_TGA_T	48	10.2	9.5	6.6	15.1	0.028 (27.7)	100.0 (48/48)
TPSAD9	MNV	3	CSDE1:NRAS	chr1_115256529_TG_CT	48	5.7	5.5	2.6	8.9	0.018 (31.7)	100.0 (48/48)
TPSAD9	DELETION	3	EGFR	chr7_55242464_AGGAATTAAGAGAAGC_A	48	10.9	11	5.9	15.3	0.018 (16.4)	100.0 (48/48)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSAD9	DELETION	3	EGFR	chr7_55242467_AATTAAGAGAAGCAACATC_T	48	5	5	1.7	7.8	0.015 (30.6)	100.0 (48/48)
TPSAD9	DELETION	3	EGFR	chr7_55242469_TTAAGAGAAG_C	48	8.9	8.2	4.1	12.9	0.025 (28.5)	100.0 (48/48)
TPSAD9	INSERTION	3	EGFR:EGFR-AS1	chr7_55248998_A_ATGGCCAGCG	48	9.5	9.5	6.6	11.9	0.014 (14.9)	100.0 (48/48)
TPSAD9	INSERTION	3	EGFR:EGFR-AS1	chr7_55249010_G_GACAACCCCC	48	9.2	9.3	7.4	11.7	0.010 (10.8)	100.0 (48/48)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSAD9	SNV	3	FBXO11:MSH6	chr2_48033753_G_T	46	7	7	4.1	11.3	0.018 (25.2)	95.7 (44/46)
TPSAD9	SNV	3	IDH1	chr2_209113112_C_T	48	7.3	7.2	4.3	10.5	0.016 (21.9)	100.0 (48/48)
TPSAD9	INSERTION	3	KIT	chr4_55592178_C_CTGCCTA	48	6.1	5.8	3	10.1	0.017 (27.5)	97.9 (47/48)
TPSAD9	DELETION	3	KIT	chr4_55593600_CAGTGGAAGGTTGTTG_C	48	4.2	4.2	0.7	8.3	0.015 (36.8)	70.8 (34/48)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSAD9	SNV	3	KRAS	chr12_25378647_T_G	48	10.1	9.9	3.8	18.2	0.033 (32.2)	97.9 (47/48)
TPSAD9	MNV	2	KRAS	chr12_25398284_CC_AA	48	6.4	6.3	3	10.4	0.018 (28.0)	100.0 (48/48)
TPSAD9	DELETION	3	MET	chr7_116411879_CTCTTTCTTTCTCTCTGTTTTAAGA_C	48	6.9	6.5	3.6	10.9	0.021 (31.0)	100.0 (48/48)
TPSAD9	SNV	3	MSH2	chr2_47703538_C_T	48	6.4	6.5	1	9.1	0.016 (24.7)	95.8 (46/48)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSAD9	INSERTION	3	PTEN	chr10_89717695_C_CTT	48	6.7	6.6	3.6	9.9	0.018 (26.5)	100.0 (48/48)
TPSAD9	DELETION	3	PTEN	chr10_89717718_CTG_C	48	6.6	6.6	3.4	9.8	0.014 (20.9)	100.0 (48/48)
TPSAD9	DELETION	3	PTEN	chr10_89720798_GTACT_G	47	12.1	12.2	6.5	18.5	0.026 (21.5)	100.0 (47/47)
TPSAD9	DELETION	3	TP53	chr17_7577047_GTGGTGAGGCTCCCCTTTCT_G	48	16	15.9	12.2	19.1	0.020 (12.5)	100.0 (48/48)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSAD9	DELETION	3	VHL	chr3_10183754_ATCT_A	48	10.5	10.2	6.7	15.4	0.021 (20.4)	100.0 (48/48)
TPSAD9	DELETION	3	VHL	chr3_10183824_AC_A	48	12.8	13.2	6.7	17.9	0.024 (19.0)	100.0 (48/48)
TPSAD10	INSERTION	3	APC	chr5_112175951_G_GA	44	10.4	10.1	4.2	15.6	0.022 (21.5)	100.0 (44/44)
TPSAD11	INSERTION	3	ERBB2:MIEN1:MIR4728	chr17_37880981_A_AGCATACGTGATG	48	32.4	32.6	28	35	0.017 (5.1)	100.0 (48/48)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSAD11	INSERTION	3	TP53	chr17_7574029_C_CGGAT	48	15.4	15.4	11.3	18.9	0.021 (13.4)	100.0 (48/48)
TPSAD12	INSERTION	3	ERBB2:MIEN1:MIR4728	chr17_37880981_A_AGCATACGTGATG	36	7.5	7.6	4	10.3	0.019 (25.5)	100.0 (36/36)
TPSBD1	SNV	3	TERT	chr5_1295228_G_A	2	55.7	55.7	54.2	57.1	0.021	100.0 (2/2)
TPSBD2	DELETION	2	EGFR:EGFR-AS1	chr7_55242465_GGAATTAAGAGAAGCA_G	46	11.2	10.9	7.6	14.7	0.018 (16.3)	100.0 (46/46)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSBD2	SNV	3	TP53	chr17_7578461_C_A	46	12.7	12.2	9.6	17	0.017 (13.8)	100.0 (46/46)
TPSBD2	DELETION	3	TP53	chr17_7579584_AGGGGGAC_A	46	7.9	7.6	4.5	11.6	0.019 (24.1)	100.0 (46/46)
TPSBD3	SNV	3	APC	chr5_112173992_C_T	28	9.4	9.3	5.3	13.2	0.019 (20.3)	100.0 (28/28)
TPSBD3	DELETION	3	APC	chr5_112175751_CT_C	28	18.1	19.2	11.5	23.3	0.036 (19.7)	100.0 (28/28)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSBD3	DELETION	3	BRCA1	chr17_41243512_CT_C	28	44.4	45	38.1	50.9	0.044 (9.8)	100.0 (28/28)
TPSBD3	SNV	2	KRAS	chr12_25398284_C_A	28	3.4	3.5	0.8	4.9	0.014 (41.9)	85.7 (24/28)
TPSBD3	SNV	3	PIK3CA	chr3_178951964_G_C	28	6.9	6.8	2.8	14.3	0.029 (41.8)	89.3 (25/28)
TPSBD3	DELETION	3	TP53	chr17_7578470_CGGGCGGGGTGTG_C	28	29.6	29.4	26.6	33.1	0.019 (6.4)	100.0 (28/28)
TPSBD4	INSERTION	3	ARID1A	chr1_27088681_T_TC	44	18.2	18.5	12.6	24.7	0.029 (16.0)	100.0 (44/44)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSBD4	DELETION	3	ARID1A	chr1_27106073_AG_A	44	20.2	20.1	16	25.7	0.020 (9.7)	100.0 (44/44)
TPSBD4	INSERTION	3	BARD1	chr2_215645974_C_CT	44	17.3	17.1	8.1	27.3	0.037 (21.4)	100.0 (44/44)
TPSBD4	DELETION	3	BRCA1	chr17_41245586_CT_C	44	19.4	18.8	14.3	28.2	0.028 (14.6)	100.0 (44/44)
TPSBD4	SNV	3	FBXW7	chr4_153249385_G_A	44	8.1	7.8	4.1	13.8	0.024 (30.0)	100.0 (44/44)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSBD4	SNV	3	KRAS	chr12_25398281_C_T	44	2.7	2.6	0.7	6	0.013 (47.1)	65.9 (29/44)
TPSBD4	DELETION	3	PTCH1	chr9_98211548_TG_T	44	19.2	19.4	15.1	23.2	0.018 (9.4)	100.0 (44/44)
TPSBD4	DELETION	3	RNF43:TSPOAP1-AS1	chr17_56435160_AC_A	44	2.1	2.1	1.3	3	0.005 (23.1)	100.0 (44/44)
TPSBD5	DELETION	3	ATRX	chrX_76937519_AAG_A	44	13.3	12.9	7.9	21.4	0.028 (21.2)	100.0 (44/44)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSBD5	DELETION	3	TP53	chr17_7574002_CG_C	44	15.8	15.5	11.2	22.4	0.029 (18.4)	100.0 (44/44)
TPSBD6	SNV	3	AKT1	chr14_105246551_C_T	40	18.3	18.3	15	21.7	0.020 (10.7)	100.0 (40/40)
TPSBD6	SNV	3	APC	chr5_112173917_C_T	40	18.3	18.7	14.3	22.6	0.024 (13.3)	100.0 (40/40)
TPSBD6	DELETION	3	APC	chr5_112175675_AAG_A	40	16.6	16.5	9.9	21.6	0.026 (15.7)	100.0 (40/40)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSBD6	DELETION	3	ARID1A:LOC101928728	chr1_27022998_CG_C	34	17.1	17.2	10	24.8	0.043 (25.1)	100.0 (34/34)
TPSBD6	DELETION	3	BARD1	chr2_215645974_CT_C	40	17.2	17.3	10.7	23.5	0.034 (19.8)	100.0 (40/40)
TPSBD6	SNV	3	FBXW7	chr4_153247367_G_A	40	17.3	18	10.7	21.8	0.026 (14.9)	100.0 (40/40)
TPSBD6	DELETION	3	MSH2	chr2_47702162_AG_A	40	16.8	16.6	9	26.6	0.043 (25.6)	100.0 (40/40)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSBD6	SNV	3	MSH2	chr2_47703538_C_T	40	49.7	49.3	40.7	61	0.047 (9.4)	100.0 (40/40)
TPSBD6	DELETION	3	PALB2	chr16_23647027_GT_G	40	17.9	17.8	8.2	23.4	0.035 (19.7)	100.0 (40/40)
TPSBD6	DELETION	3	PTCH1	chr9_98211548_TG_T	40	17.8	17.4	14.9	21.5	0.021 (11.6)	100.0 (40/40)
TPSBD6	DELETION	3	RNF43:TSPOAP1-AS1	chr17_56435160_AC_A	40	35.4	35.1	32	39.6	0.025 (7.0)	100.0 (40/40)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSBD6	DELETION	3	SMARCA4	chr19_11141540_AT_A	40	14.5	14.6	11.9	17.3	0.014 (9.8)	100.0 (40/40)
TPSBD6	DELETION	3	SMARCA4	chr19_11144017_CG_C	40	19.6	19.1	15.7	24.3	0.022 (11.1)	100.0 (40/40)
TPSBD6	SNV	3	VHL	chr3_10191488_C_T	40	16.7	16.9	9.7	21	0.025 (15.1)	100.0 (40/40)
TPSBD7	SNV	3	APC	chr5_112162891_C_T	46	15.3	15.4	6.8	24.3	0.039 (25.3)	100.0 (46/46)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSBD7	DELETION	3	APC	chr5_112175751_CTTTA_C	46	15.5	15.4	11.2	22.4	0.030 (19.4)	100.0 (46/46)
TPSBD7	SNV	2	KRAS	chr12_25398281_C_T	46	31.6	30.7	23.6	40.5	0.045 (14.1)	100.0 (46/46)
TPSBD7	SNV	3	TP53	chr17_7578190_T_C	46	19.6	20.7	14.2	28.6	0.033 (16.9)	100.0 (46/46)
TPSBD8	SNV	3	CTNNB1	chr3_41266104_G_A	44	16.1	15.7	12.3	22	0.023 (14.1)	100.0 (44/44)

Panel Member	Variant Class	Level[1]	Gene	Variant	N	Mean VAF (%)	Median VAF (%)	Min VAF (%)	Max VAF (%)	Total SD (CV%)[2]	Percent Positive Call (%)
TPSBD8	SNV	3	FGFR2	chr10_123279677_G_C	44	33.4	33.1	29.4	38.3	0.021 (6.2)	100.0 (44/44)
TPSBD8	DELETION	3	PTEN	chr10_89653846_CAA_C	44	32.4	32.6	22.4	38.2	0.042 (12.8)	100.0 (44/44)
TPSBD8	DELETION	3	PTEN	chr10_89720798_GTACT_G	44	15.7	15.8	7.9	20.3	0.024 (15.3)	100.0 (44/44)
All Members	All Variant Class	All Levels	All Genes	All Observed Variants	4858						97.6 (4743/4858)

%CV: Percent coefficient of variation.
SD: Standard deviation.
VAF, variant allele frequency.

[1] US Clinical Level
[2] Total variation and coefficient of variation (%CV) computed from variance component analysis. The number of observations of panel members TPSAD6 and TPSBD1 were too small for a variance components model to be fitted. For these 2 panel members, the overall standard deviation is reported.
[3] 95% two-sided confidence interval calculated via the Wilson Score Method.