illumina®

DATA SHEET

TruSight™ Oncology Comprehensive

A US FDA–approved, kitted solution for comprehensive genomic profiling (CGP)

Detect actionable biomarkers across solid tumors using minimal patient biopsy tissue

Assess current and emerging biomarkers from clinical practice guidelines, drug labels, and clinical trials simultaneously

Produce an easy-to-interpret results report in 4–5 days

Become a precision medicine provider by offering CGP testing in your institution

Revolutionizing cancer diagnostics

Comprehensive genomic profiling (CGP) is changing the face of cancer diagnostics. As the number of actionable biomarkers, approved therapies, and investigational trials increases, single-biomarker tests and targeted hotspot panels are unable to keep pace, increasing the chances of missing critical information. Furthermore, these methods do not detect certain current or emerging immunotherapy response signatures such as tumor mutational burden (TMB). One option for meeting the challenges of an ever-increasing list of potential therapies and biomarkers is next-generation sequencing (NGS)-based CGP. In a single test, CGP provides a comprehensive view of a tumor's genetics, capturing information on hundreds of biomarkers, and reports clinically actionable results that can lead to molecularly matched therapeutic regimens and better patient outcomes.[1-6]

Offering a CGP test in house provides numerous benefits, including the ability to maintain control over the patient's biopsy and data, further empowering you as a precision medicine provider and increasing your participation in patient care. CGP can be a complex undertaking when implemented as a laboratory-developed test (LDT). As a validated, US FDA–approved, IVD, kitted solution, TruSight Oncology Comprehensive provides a streamlined CGP workflow starting with DNA or RNA and ending with clinically actionable results. All reagents and variant calling pipelines are extensively validated by Illumina, minimizing the time and effort of verifying a new solution and simplifying the implementation process.

About TruSight Oncology Comprehensive

TruSight Oncology Comprehensive is the first distributable CGP *in vitro* diagnostic (IVD) with pan-cancer companion diagnostic (CDx) claims. The test interrogates both DNA and RNA from formalin-fixed, paraffin-embedded (FFPE) tissue. The NGS-based solution simultaneously analyzes 517 cancer-associated genes with known clinical relevance in one integrated workflow (Figure 1, Tables 1–5). The test includes kitted reagents for library preparation and sequencing and automated software pipelines that identify variants, interpret results, and produce results reports. Sequencing is performed on the FDA-registered NextSeq™ 550Dx Instrument. Using this solution, labs can provide CGP testing that yields timely, reliable information regarding relevant biomarkers as noted in primary literature, guidelines, drug labels, and clinical trials in less time and using less biopsy sample than current iterative methods.

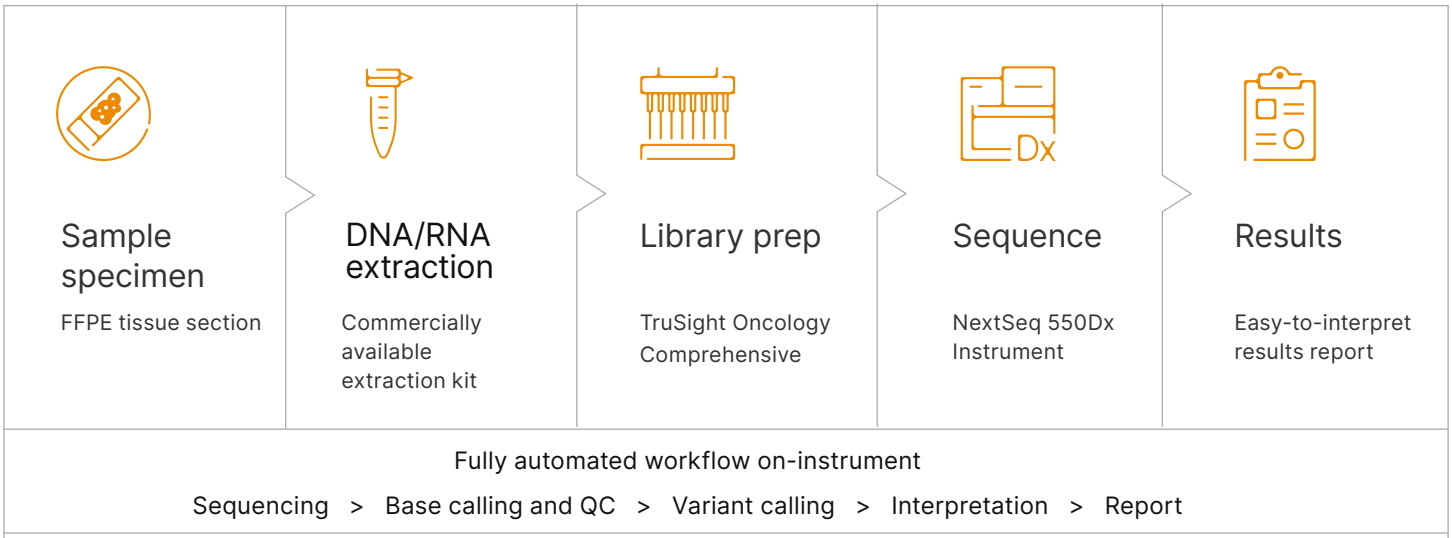


Figure 1: TruSight Oncology Comprehensive workflow
Batch up to seven patient samples and two control samples per run using TruSight Oncology Comprehensive. The library preparation and enrichment steps take 2 days. The fully automated workflow on the NextSeq 550Dx Instrument sequences samples; performs base calling and QC, variant calling, and interpretation, and generates a results report. The entire workflow is complete in 4–5 days.

Table 1: TruSight Oncology Comprehensive at a glance

Feature	Description[a]
Sequencing system	NextSeq 550Dx System
Patient sample throughput	Up to 7 patient and 2 control (1 positive and 1 NTC) samples per sequencing run
Panel content	• Small DNA variants in 517 genes • Fusions in 24 genes • Splice variants in 1 gene • TMB
Variant types detected	• DNA variants: SNVs, MNVs, insertions, deletions • RNA variants: fusions, splice variants • Complex genomic signatures: TMB
Panel size	1.94 Mb DNA, 358 kb RNA
DNA input requirement	40 ng genomic DNA
RNA input requirement	40 ng total RNA
FFPE input requirement	Recommended tissue volume ≥ 1 mm^3 tissue Minimum 20% tumor content (by area) required to detect somatic driver mutations, < 25% necrotic tissue (by area), otherwise macrodissection suggested
No. of biopsy slides	Minimum 5 recommended (10 µm sections, 20 mm^2 tissue area each)
Total assay time	4–5 days from nucleic acid to results report
Limit of detection	See Appendix
False positives by DNA variant type	Small DNA variants, 0.0001% TMB, N/A
False positives by RNA variant type	RNA fusions, 0% RNA splice variants, 0%

a. NTC, no template control; N/A, not applicable.

Comprehensive biomarker profiling

Single-gene tests and targeted hotspot panels are limited in the number of targets they analyze and the type of variants they can detect. CGP with TruSight Oncology Comprehensive overcomes these content limitations and simultaneously analyzes 517 genes with known cancer associations across solid tumors in a single assay (Tables 3–5). The test calls multiple DNA and RNA variant types, including single nucleotide variants (SNVs), multinucleotide variants (MNVs), insertion, deletions, fusions, and splice variants (Figure 2). In addition, the test detects emerging immunotherapy biomarkers (ie, TMB[7-9]) (Table 6). Note that not all clinically actionable content is currently available on the results report and additional tumor profiling claims are under development (Table 7). Content provides significant coverage of key guidelines for multiple tumor types and genes linked to clinical trials (Table 2, Table 8). The inclusive nature of TruSight Oncology Comprehensive maximizes the chances of finding a positive biomarker.

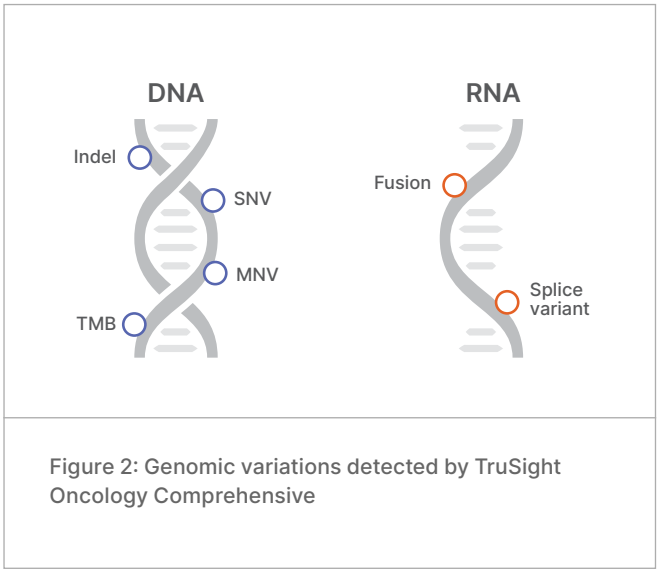


Figure 2: Genomic variations detected by TruSight Oncology Comprehensive

Companion diagnostic indications

Illumina has established multiple partnerships with several pharma companies to develop a growing pipeline of CDx indications. This information will help identify patients who are likely to respond to specific therapies. TruSight Oncology Comprehensive is currently indicated as a CDx test to identify cancer patients with solid tumors who are positive for *NTRK1*, *NTRK2*, or *NTRK3* gene fusions for treatment with VITRAKVI® (larotrectinib) and cancer patients with non-small cell lung cancer (NSCLC) who are positive for *RET* gene fusions for treatment with RETEVMO® (selpercatinib) in accordance with the approved therapeutic labeling.[10-12] Additional CDx indications, currently under development, will be included once they receive the appropriate regulatory approvals (Table 9).

Table 2: Subset of genomic tumor profiling biomarkers for multiple cancer types

Tumor type		Genes with biomarkers of significance[a]
	Pan-cancer	*BRAF*, *NTRK1*, *NTRK2*, *NTRK3*, *RET*, TMB
	Breast cancer	*AKT1*, *BRCA1*, *BRCA2*, *ESR1*, *PIK3CA*, *PTEN*
	Colorectal cancer	*KRAS*, *NRAS*, *POLD1*, *POLE*
	Non-small cell lung cancer	*EGFR*, *KRAS*, *RET*
	Melanoma	*KIT*, *NRAS*
	Ovarian cancer	*BRCA1*, *BRCA2*
	Prostate cancer	*ATM*, *ATR*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CDK12*, *CHEK1*, *CHEK2*, *FANCL*, *MRE11A*, *NBN*, *PALB2*, *RAD51B*, *RAD51C*, *RAD51D*, *RAD54L*
	Uterine cancer	*POLE*

a. Genes listed contain biomarkers of significance linked to major guidelines.

Table 3: Small DNA variants included in TruSight Oncology Comprehensive (US)

ABL1	*BMPR1A*	*CTCF*	*ETS1*	*FUBP1*	*ID3*	*MAP2K4*	*NOTCH1*	*PMS2*	*RPS6KA4*	*STK11*
ABL2	*BRAF*	*CTLA4*	*ETV1*	*FYN*	*IDH1*	*MAP3K1*	*NOTCH2*	*PNRC1*	*ROS1*	*STK40*
ABRAXAS1	*BRCA1*	*CTNNA1*	*ETV4*	*GABRA6*	*IDH2*	*MAP3K13*	*NOTCH3*	*POLD1*	*RPS6KB1*	*SUFU*
ACVR1	*BRCA2*	*CTNNB1*	*ETV5*	*GATA1*	*IFNGR1*	*MAP3K14*	*NOTCH4*	*POLE*	*RPS6KB2*	*SUZ12*
ACVR1B	*BRD4*	*CUL3*	*ETV6*	*GATA2*	*IGF1*	*MAP3K4*	*NPM1*	*PPARG*	*RPTOR*	*SYK*
ADGRA2	*BRIP1*	*CUX1*	*EWSR1*	*GATA3*	*IGF1R*	*MAPK1*	*NRAS*	*PPM1D*	*RUNX1*	*TAF1*
AKT1	*BTG1*	*CXCR4*	*EZH2*	*GATA4*	*IGF2*	*MAPK3*	*NRG1*	*PPP2R1A*	*RUNX1T1*	*TBX3*
AKT2	*BTK*	*CYLD*	*FAM46C*	*GATA6*	*IKBKE*	*MAX*	*NSD1*	*PPP2R2A*	*RYBP*	*TCF3*
AKT3	*CALR*	*DAXX*	*FANCA*	*GEN1*	*IKZF1*	*MCL1*	*NTRK1*	*PPP6C*	*SDHA*	*TCF7L2*
ALK	*CARD11*	*DCUN1D1*	*FANCC*	*GID4*	*IL10*	*MDC1*	*NTRK2*	*PRDM1*	*SDHAF2*	*TERC*
ALOX12B	*CASP8*	*DDR2*	*FANCD2*	*GLI1*	*IL7R*	*MDM2*	*NTRK3*	*PREX2*	*SDHB*	*TERT*
AMER1	*CBFB*	*DDX41*	*FANCE*	*GNA11*	*INHA*	*MDM4*	*NUP93*	*PRKAR1A*	*SDHC*	*TET1*
ANKRD11	*CBL*	*DHX15*	*FANCF*	*GNA13*	*INHBA*	*MED12*	*NUTM1*	*PRKCI*	*SDHD*	*TET2*
ANKRD26	*CCND1*	*DICER1*	*FANCG*	*GNAQ*	*INPP4A*	*MEF2B*	*PAK1*	*PRKDC*	*SETBP1*	*TFE3*
APC	*CCND2*	*DIS3*	*FANCI*	*GNAS*	*INPP4B*	*MEN1*	*PAK3*	*PRKN*	*SETD2*	*TFRC*
AR	*CCND3*	*DNAJB1*	*FANCL*	*GPS2*	*INSR*	*MET*	*PAK5*	*PRSS8*	*SF3B1*	*TGFBR1*
ARAF	*CCNE1*	*DNMT1*	*FAS*	*GREM1*	*IRF2*	*MGA*	*PALB2*	*PTCH1*	*SH2B3*	*TGFBR2*
ARFRP1	*CD274*	*DNMT3A*	*FAT1*	*GRIN2A*	*IRF4*	*MITF*	*PARP1*	*PTEN*	*SH2D1A*	*TMEM127*
ARID1A	*CD276*	*DNMT3B*	*FBXW7*	*GRM3*	*IRS1*	*MLH1*	*PAX3*	*PTPN11*	*SHQ1*	*TMPRSS2*
ARID1B	*CD74*	*DOT1L*	*FGF1*	*GSK3B*	*IRS2*	*MLL/KMT2A*	*PAX5*	*PTPRD*	*SLIT2*	*TNFAIP3*
ARID2	*CD79A*	*E2F3*	*FGF10*	*H3F3A*	*JAK1*	*MLLT3*	*PAX7*	*PTPRS*	*SLX4*	*TNFRSF14*
ARID5B	*CD79B*	*EED*	*FGF14*	*H3F3B*	*JAK2*	*MPL*	*PAX8*	*PTPRT*	*SMAD2*	*TOP1*
ASXL1	*CDC73*	*EGFL7*	*FGF19*	*H3F3C*	*JAK3*	*MRE11A*	*PBRM1*	*QKI*	*SMAD3*	*TOP2A*
ASXL2	*CDH1*	*EGFR*	*FGF2*	*HGF*	*JUN*	*MSH2*	*PDCD1*	*RAB35*	*SMAD4*	*TP53*
ATM	*CDK12*	*EIF1AX*	*FGF23*	*HIST1H1C*	*KAT6A*	*MSH3*	*PDCD1LG2*	*RAC1*	*SMARCA4*	*TP63*
ATR	*CDK4*	*EIF4A2*	*FGF3*	*HIST1H2BD*	*KDM5A*	*MSH6*	*PDGFRA*	*RAD21*	*SMARCB1*	*TRAF2*
ATRX	*CDK6*	*EIF4E*	*FGF4*	*HIST1H3A*	*KDM5C*	*MST1*	*PDGFRB*	*RAD50*	*SMARCD1*	*TRAF7*
AURKA	*CDK8*	*ELOC*	*FGF5*	*HIST1H3B*	*KDM6A*	*MST1R*	*PDK1*	*RAD51*	*SMC1A*	*TSC1*
AURKB	*CDKN1A*	*EML4*	*FGF6*	*HIST1H3C*	*KDR*	*MTOR*	*PDPK1*	*RAD51B*	*SMC3*	*TSC2*
AXIN1	*CDKN1B*	*EMSY*	*FGF7*	*HIST1H3D*	*KEAP1*	*MUTYH*	*PGR*	*RAD51C*	*SMO*	*TSHR*
AXIN2	*CDKN2A*	*EP300*	*FGF8*	*HIST1H3E*	*KEL*	*MYB*	*PHF6*	*RAD51D*	*SNCAIP*	*U2AF1*
AXL	*CDKN2B*	*EPCAM*	*FGF9*	*HIST1H3F*	*KIF5B*	*MYC*	*PHOX2B*	*RAD52*	*SOCS1*	*VEGFA*
B2M	*CDKN2C*	*EPHA3*	*FGFR1*	*HIST1H3G*	*KIT*	*MYCL1*	*PIK3C2B*	*RAD54L*	*SOX10*	*VHL*
BAP1	*CEBPA*	*EPHA5*	*FGFR2*	*HIST1H3H*	*KLF4*	*MYCN*	*PIK3C2G*	*RAF1*	*SOX17*	*VTCN1*
BARD1	*CENPA*	*EPHA7*	*FGFR3*	*HIST1H3I*	*KLHL6*	*MYD88*	*PIK3C3*	*RANBP2*	*SOX2*	*WISP3*
BBC3	*CHD2*	*EPHB1*	*FGFR4*	*HIST1H3J*	*KRAS*	*MYOD1*	*PIK3CA*	*RARA*	*SOX9*	*WT1*
BCL10	*CHD4*	*ERBB2*	*FH*	*HIST2H3A*	*LAMP1*	*NAB2*	*PIK3CB*	*RASA1*	*SPEN*	*XIAP*
BCL2	*CHEK1*	*ERBB3*	*FLCN*	*HIST2H3C*	*LATS1*	*NBN*	*PIK3CD*	*RB1*	*SPOP*	*XPO1*
BCL2L1	*CHEK2*	*ERBB4*	*FLI1*	*HIST2H3D*	*LATS2*	*NCOA3*	*PIK3CG*	*RBM10*	*SPTA1*	*XRCC2*
BCL2L11	*CIC*	*ERCC1*	*FLT1*	*HIST3H3*	*LMO1*	*NCOR1*	*PIK3R1*	*RECQL4*	*SRC*	*YAP1*
BCL2L2	*COP1*	*ERCC2*	*FLT3*	*HNF1A*	*LRP1B*	*NEGR1*	*PIK3R2*	*REL*	*SRSF2*	*YES1*
BCL6	*CREBBP*	*ERCC3*	*FLT4*	*HNRNPK*	*LYN*	*NF1*	*PIK3R3*	*RET*	*STAG1*	*ZBTB2*
BCOR	*CRKL*	*ERCC4*	*FOXA1*	*HOXB13*	*LZTR1*	*NF2*	*PIM1*	*RHEB*	*STAG2*	*ZBTB7A*
BCORL1	*CRLF2*	*ERCC5*	*FOXL2*	*HRAS*	*MAGI2*	*NFE2L2*	*PLCG2*	*RHOA*	*STAT3*	*ZFHX3*
BCR	*CSF1R*	*ERG*	*FOXO1*	*HSD3B1*	*MALT1*	*NFKBIA*	*PLK2*	*RICTOR*	*STAT4*	*ZNF217*
BIRC3	*CSF3R*	*ERRFI1*	*FOXP1*	*HSP90AA1*	*MAP2K1*	*NKX2-1*	*PMAIP1*	*RIT1*	*STAT5A*	*ZNF703*
BLM	*CSNK1A1*	*ESR1*	*FRS2*	*ICOSLG*	*MAP2K2*	*NKX3-1*	*PMS1*	*RNF43*	*STAT5B*	*ZRSR2*

Table 4: Fusions from RNA included in TruSight Oncology Comprehensive[a]

AXL	*CDK4*	*ERG*	*ETV4*	*FGFR2*	*KIF5B*	*NTRK2*	*RAF1*
BCL2	*EGFR*	*ESR1*	*EWSR1*	*FGFR3*	*NRG1*	*NTRK3*	*RET*
BRAF	*EML4*[b]	*ETV1*	*FGFR1*	*FLI1*	*NTRK1*	*PAX3*	*TMPRSS2*

a. Genes listed are assessed for known and novel fusions.
b. *EML4-ALK* fusions are not included.

Table 5: Splice variants from RNA included in TruSight Oncology Comprehensive

EGFR

Table 6: Genomic signatures included in TruSight Oncology Comprehensive

TMB

Table 7: Select gene and variant content not currently included in the results report

MSI
ERBB2 and *MET* gene amplifications from DNA
ALK and *ROS1* fusions from RNA
MET splice variants from RNA

The biomarkers and variants listed above are currently under development in the TruSight Oncology Comprehensive tumor profiling content pipeline. The pipeline is subject to regulatory approval and is not guaranteed.

Table 8: TruSight Oncology Comprehensive content coverage

53 Clinical practice guidelines
67 Drug labels
~820 US clinical trials

Analysis provided by Velsera based on the TruSight Oncology Comprehensive software Knowledge Base v8.20. Current as of September 2024.

Table 9: CDx indications

CDx indication	**Pharmaceutical partner**
Solid tumors positive for *NTRK1*, *NTRK2*, or *NTRK3* gene fusions for treatment with VITRAKVI (larotrectinib)	Bayer[10-12]
Non-small cell lung cancer (NSCLC) patients positive for *RET* gene fusions for treatment with RETEVMO (selpercatinib)	Lilly[10]
Under development	
EGFR	Teligene[13]
RET[a]	Lilly[10]
TP53	Kartos Therapeutics[15]
MSI	Bristol Myers Squibb[14]

CDx developments apply to the TruSight Oncology Comprehensive portfolio. Availability of each CDx will vary by geography and is based on variable timelines for therapy and test approvals by region.

a. TruSight Oncology Comprehensive is currently indicated as a CDx for *RET* fusions in non-small cell lung cancer (NSCLC); additional tumor types (thyroid and medullary thyroid cancer) for this CDx are in development.

More information, less sample, less time

TruSight Oncology Comprehensive provides more information from less sample and in less total time compared to current iterative testing methods. For example, a potential journey for a patient diagnosed with NSCLC following conventional testing methods could involve six different tests, requiring 29 sample slides and upwards of 42 days to obtain results regarding nine biomarkers, followed by analysis and interpretation time to develop a treatment plan.[16-21] In contrast, a CGP test using TruSight Oncology Comprehensive typically requires five slides and up to five days to generate a report with information on 500+ biomarkers and possible therapies and clinical trials (Figure 3).

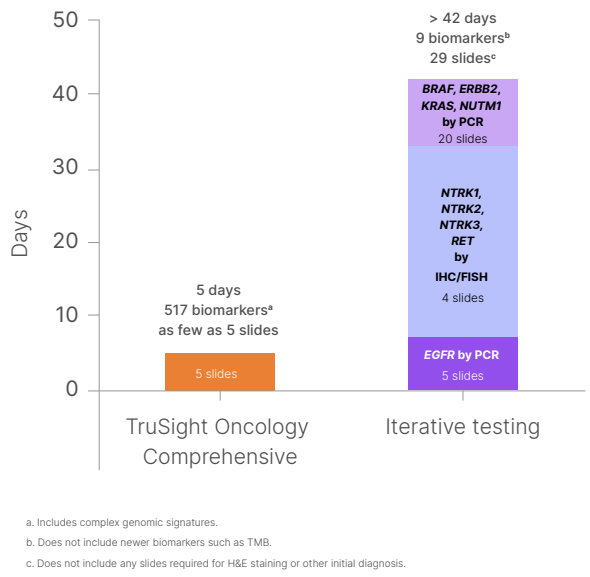


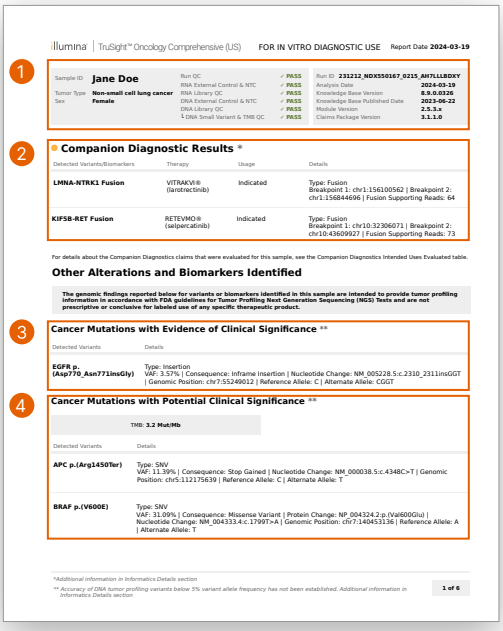
Figure 3: Advantages of TruSight Oncology Comprehensive compared to iterative testing
Example showing potential journeys for NSCLC patient. CGP with TruSight Oncology Comprehensive provides substantially more coverage with less time and less sample compared to single-gene iterative testing.[16-21]

One easy-to-interpret results report

TruSight Oncology Comprehensive results, supported by an expertly curated Knowledge Base, are presented in a single, streamlined results report. The TruSight Oncology Comprehensive results report uses a tiering system to classify variants by clinical relevance level and can help inform therapy decisions according to clinical guidelines (Figure 4). The results report includes:

- Patient sample information—sample ID number, tumor type, sex, QC analysis, run ID, and Knowledge Base details
- Companion Diagnostic Results—detected variants or biomarkers that have a companion diagnostic intended use evaluated for the sample
- Cancer Mutations with Evidence of Clinical Significance—detected variants that have evidence of clinical significance (therapeutic, prognostic, or diagnostic) based on information in FDA-approved drug labels or ASCO and major US clinical practice guidelines for the patient's tumor type, as specified by the Knowledge Base[22*]
- Cancer Mutations with Potential Clinical Significance—detected variants that have potential clinical significance (therapeutic, prognostic or diagnostic) based on information in FDA-approved drug labels or ASCO and major US clinical practice guidelines in another tumor type, match genomic and tumor type eligibility criteria for a clinical trial, or have evidence of potential clinical significance in the primary literature for the patient's tumor type, as specified by the Knowledge Base and supporting rules engine[22*]

* ASCO, American Society of Clinical Oncology; FDA, Food and Drug Administration.

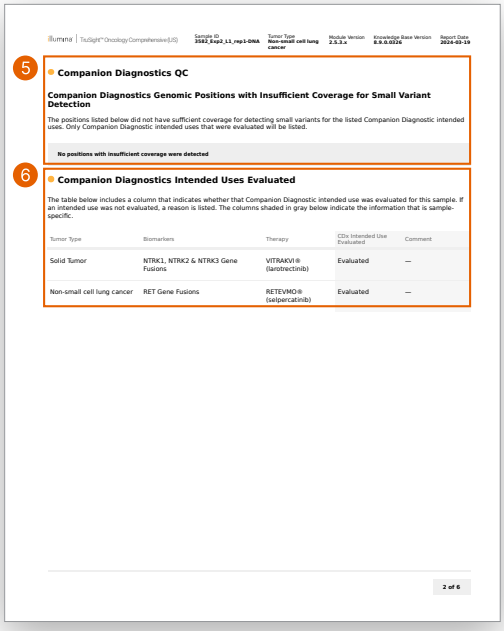


1 Patient sample information

2 Companion diagnostic results
- Detected Companion Diagnostics variants/biomarkers and associated therapy indications

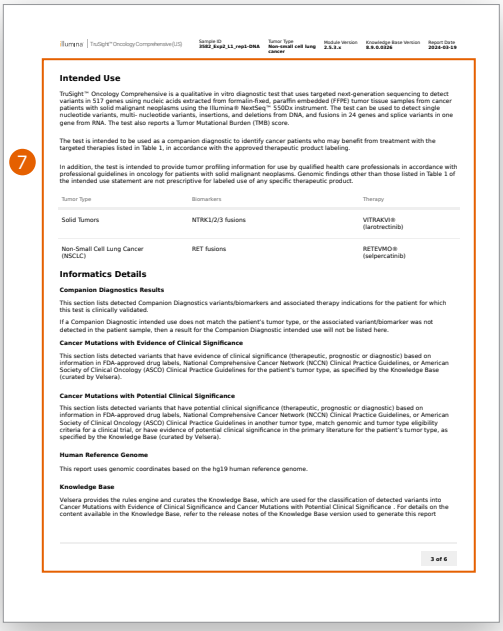
3 Cancer mutations with evidence of clinical significance
- Variant name and genomic details

4 Cancer mutations with potential clinical significance
- Includes TMB

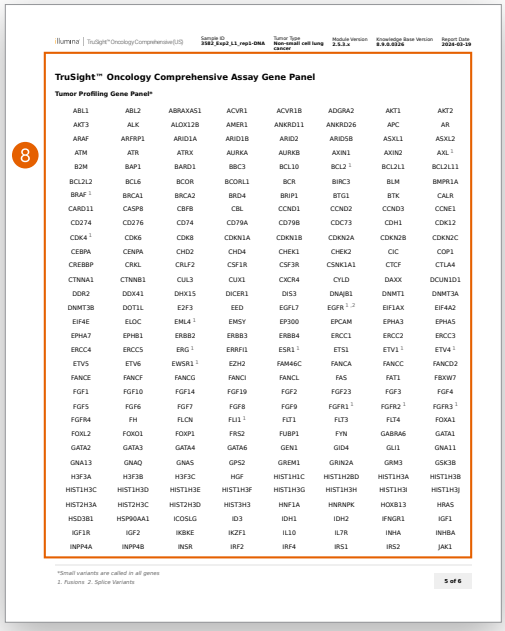


5 Companion diagnostics QC
- Positions with insufficient coverage for small variant detection

6 Companion diagnostics intended uses evaluated
- Includes tumor type, biomarkers, and eligible therapy



7 Test information
- Intended use
- Cancer mutations description
- Review of Knowledge Base
- Variant description
- Limitations of the test



8 Test information, continued
- Genes and variants tested

Figure 4: Results report for TruSight Oncology Comprehensive

Report includes companion diagnostics results and variants reported as clinically significant or potentially clinically significant based on an expertly curated knowledge base that includes clinical guidelines, drug labels, clinical trials, and peer-reviewed literature.

Validated solution

TruSight Oncology Comprehensive is a validated (Table 10), sample-to-answer CGP test that includes kitted reagents, an IVD sequencing system, and analysis software. The test was developed using a rigorous design control process and validated across > 1400 unique FFPE samples and > 15 different tumor types. Results were compared to orthogonal methods to ensure accurate, reproducible, and consistent data.

Using TruSight Oncology Comprehensive

TruSight Oncology Comprehensive provides a streamlined workflow that spans from sample input to final results report. After a two-day library prep protocol, samples are loaded on to a flow cell and into the sequencing system where the remainder of the test is fully automated, including sequencing, variant calling, interpretation, and report generation. The entire test, from nucleic acid extraction to results report can be completed in as few as four days (Figure 1).

Table 10: Validation studies using TruSight Oncology Comprehensive

Accuracy and clinical bridging studies for *NTRK*1/2/3 and *RET* fusion detection	Library stability
Accuracy for tumor profiling	Limit of blank
Assay workflow guardbanding	Limit of detection
Cross contamination	Nucleic acid extraction kit evaluation
External controls evaluation	Nucleic acid stability
Nucleic acid input titration guardbanding	Real-time stability
Interfering substances	Reproducibility
Kit in-use stability	Slide-mounted FFPE tissue stability
Kit transport stability	Within-laboratory precision

Prepare libraries

TruSight Oncology Comprehensive can use DNA and RNA extracted simultaneously from the same sample as input material.

If using DNA, sample preparation starts with shearing the genomic DNA (gDNA). If starting from RNA, the first step is to reverse transcribe the sample into cDNA. Sheared gDNA and cDNA are converted simultaneously into sequence-ready libraries.

During library preparation, unique molecular identifiers (UMIs)[23] are added to the gDNA or cDNA fragments. These UMIs enable detection of variants at low variant allele frequency (VAF) while simultaneously suppressing errors, providing high specificity.

Enrich libraries to focus on clinically relevant content

Library preparation is based on proven hybrid–capture chemistry using biotinylated probes and streptavidin-

coated magnetic beads to purify selected targets from DNA- and RNA-based libraries. Regions of interest hybridize to the biotinylated probes, are magnetically pulled down, and then eluted to enrich the library pool. Hybridization-based enrichment is a useful strategy for analyzing specific genetic variants in a given sample and reliably sequencing exomes or large numbers of genes (eg, > 50 genes).

Hybrid–capture chemistry offers several advantages over amplicon sequencing, including yielding data with fewer artifacts and dropouts and the ability to accommodate larger panel enrichment. Additionally, hybrid–capture chemistry is fusion agnostic, enabling detection and characterization of known and novel fusions.

Sequence with diagnostic power

Prepared TruSight Oncology Comprehensive libraries are sequenced on the NextSeq 550Dx System (Figure 5). The NextSeq 550Dx System is an IVD instrument that enables clinical laboratories to develop and perform NGS-based IVD assays. The NextSeq 550Dx System features:

- A locked configuration with change control enabling laboratories to take advantage of current and future clinical testing options
- High-throughput capabilities to expand operations for larger, deeper studies or increase the number of patient samples run
- Flexible analysis ranging from small panel sequencing to WGS and NGS applications to microarray studies

With prefilled reagent cartridges, starting a run on a NextSeq 550Dx instrument is as easy as thaw, load, and go and takes approximately 30 minutes hands-on time. The intuitive interface allows users to perform various applications with minimal training or instrument set-up time. The NextSeq 550Dx instrument can deliver > 90 Gb of high-quality data with over 75% of bases sequenced with a quality score of Q30 or higher in less than two days.[24]

Figure 5: The NextSeq 550Dx System
Developed under design control and manufactured following good manufacturing practice (GMP) guidelines, the NextSeq 550Dx System (in Dx mode) supports a fully automated TruSight Oncology Comprehensive workflow from sequencing through results report generation.

Patient batching throughput

Using TruSight Oncology Comprehensive with the NextSeq 550Dx System, labs can batch up to seven patient samples† with two controls per sequencing run in 4–5 days.

Variant calling, interpretation, and reporting

All analysis for TruSight Oncology Comprehensive is performed automatically on the NextSeq 550Dx System using the Local Run Manager TruSight Oncology Comprehensive Analysis Module. The on-instrument module facilitates run setup and performs secondary analysis of sequencing results, including demultiplexing, FASTQ file generation, alignment, and variant calling:

- Demultiplexing separates data from pooled libraries based on the unique sequence indexes that were added during the library preparation procedure
- FASTQ intermediate files contain the sequencing reads for each sample and quality scores, excluding reads from any clusters that did not pass filter
- Sequencing reads are aligned against a reference genome to identify a relationship between the sequences and assigned a score based on regions of similarity; aligned reads are written to files in Binary Alignment Map (BAM) format
- Separate algorithms for libraries generated from DNA and RNA samples are used to call small DNA variants, and TMB for DNA samples, and fusions and splice variants for RNA samples with high specificity

The analysis software module generates multiple intermediate files, including sequencing metrics and Variant Call Format (VCF) files. VCF files contain information about variants found at specific positions in a reference genome. Sequencing metrics and individual output files are generated for each sample.

Tertiary analysis, also performed by the Local Run Manager TruSight Oncology Comprehensive Analysis Module, consists of TMB calculations, tumor profiling of variants into two levels of clinical significance, and report generation. Interpreted variant results, as well as TMB biomarker results, are summarized in the TruSight Oncology Comprehensive results report. These results are meant to be incorporated into a clinical report produced by the laboratory and provided to clinicians who make decisions on patient management.

† Number of patient samples varies according to the number of controls run.

Clinically robust Knowledge Base

TruSight Oncology Comprehensive Software is supported by a purpose-built over time, clinically derived rules engine and Knowledge Base to maximize actionability. The rules engine and supported Knowledge Base, both provided by Velsera,[25] comprise extensive coverage of peer-reviewed publications, actionable variant information, and the most recent guidelines, drug labels, and clinical trials (Figure 6, Table 11). The TruSight Oncology Comprehensive Software uses this rich content to determine classifications of the detected genetic variants.

Expertly curated content and rules engine

To deliver accurate interpretations of detected variants, the Knowledge Base relies on a rules engine (both provided by Velsera) that links specific variants or biomarkers to assertions of clinical impact in various tumor types. These assertions are aggregated from various clinical sources, including major clinical practice guidelines (eg, ASCO), approved drug labels (FDA), clinical trial registries (clinicaltrials.gov), primary literature describing clinical studies (PubMed), and biological annotation databases (gnomAD, COSMIC)‡ and can have therapeutic, prognostic, or diagnostic associations.

Supporting evidence for these assertions, known as source rules, are curated by a team of highly trained scientists and undergo extensive review following strict procedures. After this review, source rules are further examined in a Ruleset QC/QA process to ensure the integrity of the rule updates and that all required fields are properly populated. Source rules are then reviewed, ranked, and selected based on their relevance to a genomic finding to develop interpretation rules. Interpretation paragraphs are assembled based on the content associated with the appropriate rules, and the paragraphs include references to the source material as well.

Testing and quality assurance processes are in place to ensure that high-quality content is added and maintained in the Knowledge Base. In addition to the reviews described above, clinical assertions are extracted using independent workflows by trained curators who are not part of the source rule or interpretation rule teams. The

‡ ASCO, American Society of Clinical Oncology; COSMIC, Catalogue of Somatic Mutations In Cancer; FDA, Food and Drug Administration; gnomAD, Genome Aggregation Database.

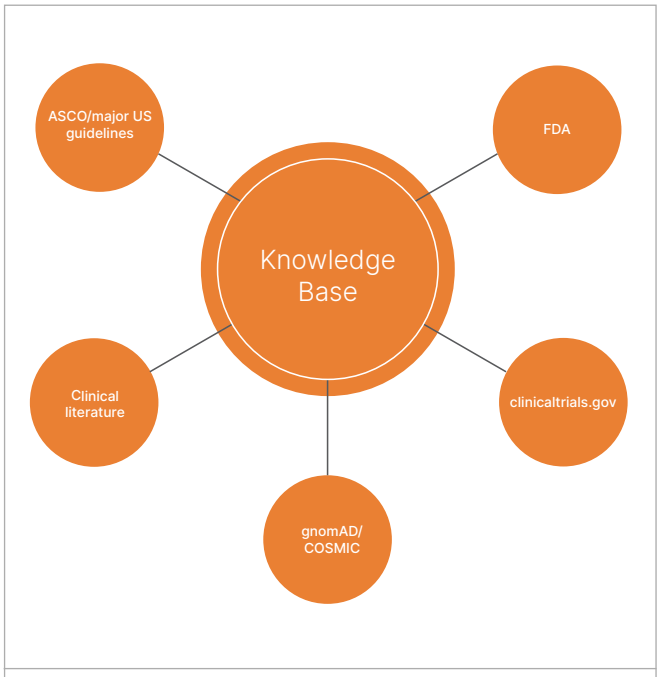


Figure 6: Knowledge Base creation
The TruSight Oncology Comprehensive Tumor Profiling software is built on a foundation of extensively reviewed rules. Source rules, derived from clinical practice guidelines, drug labels, and primary literature, identify and classify actionable variants. Data from clinical trials and biological annotation databases are independent, standalone sources in the Knowledge Base.

Table 11: Knowledge Base facts as of September 2024[a]

Topic	By the numbers
Drug labels	3.9K+ labels reviewed 117K+ pages read
Guidelines	300+ oncology practice guidelines, many updated numerous times annually, reviewed 704K+ pages read
Published literature	220K+ papers reviewed 2M+ pages read
Clinical trials	91K+ trials reviewed
Device compliance	7.4K+ procedures, work instructions, forms, and records reviewed 72K+ pages of device compliance documentation

a. Content is updated by Velsera on a monthly basis to incorporate the latest publications, biomarker discoveries, guidelines, drug labels, and clinical trials.[22]

overall performance of the Tumor Profiling Software and Knowledge Base is assessed for concordance, specificity, and sensitivity. Accuracy of curated content is determined by comparing the classifications derived from the Knowledge Base metadata and the Tumor Profiling Software to classifications previously reported in the Velsera clinical data repository. The Knowledge Base undergoes periodic review by an expert panel of licensed and board-certified medical professionals, molecular pathologists, and medical oncologists.

An updated Knowledge Base is made available regularly to account for new biomarkers; changes to guidelines, drug labels, and clinical trials; and newly published clinical research studies.[22] IVD test providers can readily access the new releases, maximizing their ability to extract actionable information from this CGP test.

Reliable, high performance

The performance characteristics and reliability of TruSight Oncology Comprehensive have been extensively tested to meet rigorous IVD requirements. Evaluations included a limit of blank study, limit of detection (LoD) studies for DNA and RNA variants, reproducibility, and analytical accuracy (Appendix).[12] Qualitative studies across multiple operators, instruments, reagent lots, and days showed high concordance with minimal variance.[12] For detailed information on the studies performed, refer to the Illumina TruSight Oncology Comprehensive (US) Package Insert.[12]

Bring CGP testing into your lab

CGP maximizes the ability to find actionable biomarkers and inform therapy choices that have the potential to improve patient outcomes. CGP in your lab helps you:

- Be a precision medicine provider—Implement a state-of-the-art test and generate clinically actionable results in 4–5 days with reduced quantity not sufficient (QNS) rates and improved test success rates
- Be prepared for the future—Retain access to raw data files and reanalyze as new guidelines, drug labels, and clinical trials are introduced, potentially generating new actionable insights
- Be a trusted partner—Consult with oncologists on therapy decisions and participate in molecular tumor boards

Streamlined implementation

Implementing a CGP test can require significant time and effort. With the introduction of TruSight Oncology Comprehensive, Illumina has addressed some of the biggest challenges, streamlining the process. Starting with a highly validated, FDA-approved, IVD, kitted solution enables users to:

- Verify over validate, reducing the time and expense of test implementation compared to a laboratory-developed test (LDT) (Figure 7)
- Enable faster integration of CGP into standard clinical practice
- Provide an IVD-compliant test, helping prepare labs to meet stricter regulatory guidelines

Comprehensive support

A comprehensive support program is available that will work with labs to expedite implementation and certification to ensure a smooth integration. The program provides:

- Onboarding plan to expedite test verification
- Laboratory trainng, including wet-lab instruction and run assessment from the expert Illumina Field Application Specialist team Verification protocol
- Training certification
- 24/5 technical support
- Ongoing support from the Illumina Medical Affairs team for medical inquiries

In addition, Illumina supplies IVD users with access to ready-to-use marketing and educational assets to share with their local health care providers and help them understand the value of CGP testing.

Access to reimbursement

CGP test coverage is an important consideration when bringing the capability in house. IVD CGP tumor profiling assays with CDx claims across solid, malignant neoplasms are covered for eligible Medicare beneficiaries throughout the US under National Coverage Determination (NCD) 90.2.[26] Commercial coverage for assays with this indication increases by more than a third of US commercially insured lives as compared to assays without CDx claims.[27] Illumina has established a dedicated Market Access team that is actively working with payers to expand CGP test reimbursement across the globe.

Discuss available coverage options with your local Illumina Account Manager.

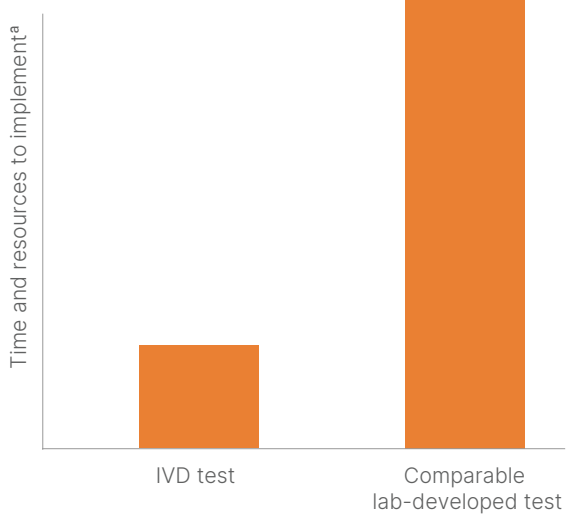


Figure 7: Simpler, less burdensome test implementation

TruSight Oncology Comprehensive is an FDA-approved IVD test that requires performance verification per guidelines set in 42 CFR 493.1253, which is less burdensome than the validation required for a test developed in the laboratory.

a. Illustrative example. Not meant to provide a precise comparison of time and resources

Summary

The use of CGP testing is helping improve patient outcomes, and TruSight Oncology Comprehensive makes implementation in your laboratory straightforward. This verified assay provides a streamlined workflow, validated reagents, and automated clinical software to deliver results in just 4–5 days. Starting from DNA and RNA, it analyzes multiple variant types across 500+ genes in a single assay. Deliver a clear results report, aligned with recognized sources, for integration into the laboratory's clinical report to guide clinicians toward potential matched therapies or clinical trials that may improve patient outcomes.

Learn more →

TruSight Oncology Comprehensive

Comprehensive genomic profiling (CGP)

NextSeq 550Dx System

Appendix

Limit of blank study

False positives were assessed through a limit of blank study using FFPE normal or benign samples from adjacent tissue. False positives were not analyzed for TMB as there is no clinical cut-off value.

Low false positives for TruSight Oncology Comprehensive

Parameter	Value
False positives for small DNA variants	0.0001%
False positives for RNA fusions	0%
False positives for RNA splice variants	0%

Limit of detection (LoD) studies

FFPE samples from 17 tissue types containing variants were diluted to multiple test levels. Six observations were generated per level by two operators, each using a different reagent lot and instrument. LoD is defined as the lowest analyte value (eg, variant allele frequency or supporting reads) that can be detected consistently (95% detection limit or a type II error of 5%).

LoD—splice variants

Splice variant	LoD
EGFR	16.7

LoD—RNA fusions

Fusion	LoD (supporting reads)
EWSR1-FLI1	8.3
TMPRSS2-PMFBP1	9.0
ACPP-ETV1	9.5
CDK4-DPY19L2	10.7
MKRN1-BRAF	11.0
RAF1-VGLL4	11.2
EGFR-GALNT13	12.3
EML4-ALK	12.8
SPIDR-NRG1	12.8
TMPRSS2-ERG	13.2
ESR1-CCDC170	13.5
NCOA4-RET	15.8
DHX8;ETV4-STAT3	16.2
KIF5B-RET	16.6
FGFR3-TACC3	17.5
PAX3-FOXO1	19.0
FGFR1-GSR	23.7
FGFR2-SRPK2	24.7
HNRNPUL1-AXL	26.3
BCL2-IGHJ5	31.3

Data shown represents tumor profiling fusions only.

LoD—small DNA variants

Type (unit of measure for LoD)	Variant class/ Genomic content	No. of variants	Range
Small DNA variants (variant allele frequency)	SNVs	5	0.016–0.064
	MNVs	3	0.022–0.048
	Insertion (1–2 bp) near homopolymer repeats	2	0.086–0.104
	Insertion (1–2 bp) near dinucleotide repeats[a]	2	0.038–0.051
	Insertion (3–5 bp)	2	0.030–0.056
	Insertion (> 5 bp and up to 25 bp)	3	0.034–0.215
	Deletion (1–2 bp) near homopolymer repeats	2	0.094–0.100
	Deletion (1-2 bp) near dinucleotide repeats	2	0.033–0.070
	Deletion (3–5 bp)	2	0.028–0.064
	Deletion (> 5 bp and up to 25 bp)	2	0.047–0.055

a. LoD for this specific variant class was determined using cell lines.

Reproducibility for tumor profiling studies

Reproducibility for tumor profiling—small DNA variants

Gene	Observed variant level	Variant type	Targeted variant (amino acid)	Mean VAF[a]	PPC	95% CI[b]
APC	> 3× LOD	Deletion	L1488fs*18	0.15	100.0% (46/46)	92.3%, 100.0%
APC	~2–3× LOD	Deletion	L1488fsTer19	0.181	100.0% (28/28)	87.9%, 100.0%
APC	~2–3× LOD	Deletion	S1465WfsTer3	0.166	100.0% (40/40)	91.2%, 100.0%
APC	~2–3× LOD	Insertion	T1556NfsTer3	0.227	100.0% (32/32)	89.3%, 100.0%
APC	~2–3× LOD	Insertion	S1465fs*9	0.100	100.0% (48/48)	92.6%, 100.0%
ARID1A	< 2× LOD	Insertion	Q372fs*28	0.084	100.0% (4/4)	51.0%, 100.0%
BRAF	~2–3× LOD	SNV	V600E	0.045	91.3% (42/46)	79.7%, 96.6%
BRAF	> 3× LOD	MNV	V600K	0.13	100.0% (46/46)	92.3%, 100.0%
EGFR	~2–3× LOD	Deletion	E746_A750del	0.112	100.0% (46/46)	92.3%, 100.0%
EGFR	~2–3× LOD	SNV	L858R	0.045	100.0% (38/38)	90.8%, 100.0%
EP300	~2–3× LOD	Deletion	H2324fs*29	0.245	100.0% (44/44)	92.0%, 100.0%
ERBB2	~2–3× LOD	Insertion	Y772_A775dup	0.075	100.0% (36/36)	90.4%, 100.0%
FBXW7	> 3× LOD	Insertion	T15_G16insP	0.13	100.0% (44/44)	92.0%, 100.0%
IDH1	~2–3× LOD	SNV	R132H	0.155	100.0% (36/36)	90.4%, 100.0%
KRAS	~2–3× LOD	MNV	G12I	0.111	100.0% (38/38)	90.8%, 100.0%
NOTCH1	~2–3× LOD	Insertion	R1598fs*12	0.146	100.0% (48/48)	92.6%, 100.0%
PTEN	~2–3× LOD	Deletion	T319fs*1	0.157	100.0% (44/44)	92.0%, 100.0%
TP53	< 2× LOD	Insertion	P152_P153dup	0.157	100.0% (2/2)	34.2%, 100.0%
TP53	~2–3× LOD	Insertion	R333HfsTer5	0.154	100.0% (48/48)	92.6%, 100.0%
TP53	> 3× LOD	Deletion	R342fs*3	0.158	100.0% (44/44)	92.0%, 100.0%

Reproducibility was tested across three sites (one internal, two external), two operators per site, three reagent lots, four testing days, and various sequencing runs per library using 41 FFPE tissue specimens and one cell line.

a. Mean VAF calculated from observed assay results.

b. 95% two-sided CI calculated via the Wilson Score method.

VAF, variant allele frequency; PPC, percent positive call; CI, confidence interval.

Reproducibility for tumor profiling—RNA variants

Targeted variant	Observed variant level[a]	Variant type	Mean supporting reads[b]	PPC	95% CI[c]
ACPP-ETV1	> 3× LOD	Fusion	44.7	100.0% (46/46)	92.3%, 100.0%
BCL2-IGHJ5	> 3× LOD	Fusion	124.9	100.0% (46/46)	92.3%, 100.0%
CDK4-DPY19L2	> 3× LOD	Fusion	55.7	97.8% (45/46)	88.7%, 99.6%
DHX8;ETV4-STAT3	~2–3× LOD	Fusion	48.9	100.0% (46/46)	92.3%, 100.0%
EGFR-GALNT13	> 3× LOD	Fusion	49.8	100.0% (46/46)	92.3%, 100.0%
EML4-ALK	> 3× LOD	Fusion	49.3	100.0% (48/48)	92.6%, 100.0%
ESR1-CCDC170	~2–3× LOD	Fusion	45.1	100.0% (46/46)	92.3%, 100.0%
EWSR1-FLI1	> 3× LOD	Fusion	31.6	100.0% (48/48)	92.6%, 100.0%
FGFR1-GSR	~2–3× LOD	Fusion	61.1	100.0% (46/46)	92.3%, 100.0%
FGFR2-SRPK2	~2–3× LOD	Fusion	53.4	100.0% (48/48)	92.6%, 100.0%
FGFR3-TACC3	~2–3× LOD	Fusion	53.5	100.0% (48/48)	92.6%, 100.0%
HNRNPUL1-AXL	~2–3× LOD	Fusion	58.0	100.0% (48/48)	92.6%, 100.0%
KIF5B-RET	< 2× LOD	Fusion	11.6	91.7% (44/48)	80.4%, 96.7%
MKRN1-BRAF	~2–3× LOD	Fusion	33.4	100.0% (48/48)	92.6%, 100.0%
PAX3-FOXO1	> 3× LOD	Fusion	70.1	100.0% (48/48)	92.6%, 100.0%
RAF1-VGLL4	< 2× LOD	Fusion	15.9	100.0% (46/46)	92.3%, 100.0%
SPIDR-NRG1	> 3× LOD	Fusion	51.5	100.0% (48/48)	92.6%, 100.0%
TMPRSS2-ERG	~2–3× LOD	Fusion	43.5	97.9% (47/48)	89.1%, 99.6%
EGFRvIII	> 3× LOD	Splice variant	64.0	100.0% (46/46)	92.3%, 100.0%

Reproducibility was tested across three sites (one internal, two external), two operators per site, three reagent lots, four testing days, and various sequencing runs per library using 41 FFPE tissue specimens and one cell line. Four ESR1-CCDC170 fusions were found in three RNA panel members with unique breakpoints. Percent negative call (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%). PPC, percent positive call; CI, confidence interval.

a. Variant level calculated from mean observed supporting reads.
b. Mean supporting reads calculated from observed assay results.
c. 95% two-sided CI calculated via the Wilson Score method.

Accuracy for tumor profiling studies

Accuracy for tumor profiling—small DNA variants

Variant class	Clinical significance	PPA (n/N) (95% CI[a])	NPA (n/N) (95% CI[a])
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) (73.0%–80.4%)	>99.9% (212,277/212,311) (> 99.9%– > 99.9%)
	All	80.3% (472/588) (76.9%–83.3%)	> 99.9% (219,211/219,246) (> 99.9%– > 99.9%)
MNVs	Level 2	100.0% (5/5) (56.6%–100.0%)	100.0% (9931/9931) (> 99.9%– > 99.9%)
	Level 3	90.0% (9/10) (59.6%–98.2%)	> 99.9% (212,785/212,786) (> 99.9%– >99.9%)
	All	93.3% (14/15) (70.2%–98.8%)	> 99.9% (219,818/219,819) (> 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% (212,738/212,739) (> 99.9%– > 99.9%)
	All	86.2% (50/58) (75.1%–92.8%)	> 99.9% (219,775/219,776) (> 99.9%– > 99.9%)
Deletions	Level 2	66.7% (2/3) (20.8%–93.9%)	> 99.9% (9932/9933) (99.9%– > 99.9%)
	Level 3	90.3% (139/154) (84.6%–94.0%)	> 99.9% (212,624/212,642) (> 99.9%– >99.9%)
	All	89.8% (141/157) (84.1%–93.6%)	> 99.9% (219,658/219,677) (> 99.9%– > 99.9%)
All variants	Level 2	95.5% (107/112) (90.0%–98.1%)	> 99.9% (9822/9824) (99.9%– > 99.9%)
	Level 3	80.7% (570/706) (77.7%–83.5%)	> 99.9% (212,036/212,090) (> 99.9%– > 99.9%)
	All	82.8% (677/818) (80.0%–85.2%)	> 99.9% (218,960/219,016) (> 99.9%– > 99.9%)

The detection of small DNA variants was compared to another externally validated NGS (evNGS) panel assay (comparator method). The comparison between small DNA variants, consisting of SNVs, MNVs, insertions, and deletions, was based on 414 FFPE samples from 16 different tissue types that were valid for both TruSight Oncology Comprehensive and the comparator method. The evNGS method uses a 5% VAF filter during reporting to prevent artifacts from being output to end users. The evNGS method is therefore not fully validated for variants below 5% VAF; however, calls below 5% VAF are available in the evNGS variant calling output. Therefore, accuracy is assessed across the full VAF range. If only variants ≥ 5% were considered, the PPA for Level 3 SNVs, insertions, and deletions was > 96%. TruSight Oncology Comprehensive detected an *EGFR* clinically significant deletion, but with one nucleotide difference in the alternate sequence relative to the clinically significant deletion found by the comparator method, which caused the PPA for clinically significant deletions to be 66.7% (2/3). Level 2, cancer mutations with evidence of clinical significance; Level 3, cancer mutations with potential clinical significance.

a. 95% two-sided CI calculated via the Wilson Score method.

NPA, negative percent agreement; PPA, positive percent agreement.

Accuracy for tumor profiling—RNA variants

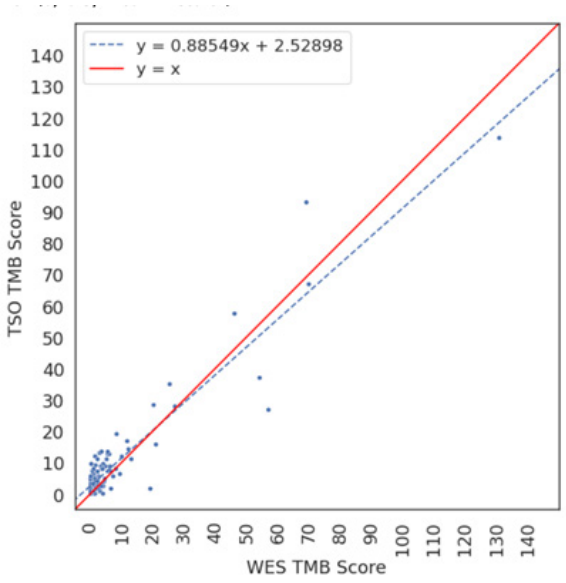
Variant type	Orthogonal method	PPA	NPA
Fusions	• RNA whole-exome sequencing fusions (RNGS1) • Targeted NGS fusion panel (RNGS2) • Droplet digital PCR (ddPCR)	87.29% (95% CI[a]: 67.83%, 96.35%)	99.99% (95% CI[a]: 99.98%, >99.99%)
Splice variant	qPCR	100% (3/3) (95% CI[b]: 44%, 100%)	100% (13/13) (95% CI[b]: 77%–100%)

The ability of TruSight Oncology Comprehensive to detect alterations in hundreds of FFPE samples was compared to results achieved using the indicated reference method. Fusions were compared to a composite method consisting of an RNA whole-exome NGS panel, a targeted NGS fusion panel, and droplet digital PCR (ddPCR). The NGS exome panel overlapped with all the genes for which TruSight Oncology Comprehensive can detect fusions. However, the limit of detection of this method was 4–8× that of TruSight Oncology Comprehensive based on the number of supporting reads observed in the overlapping fusion calls. Hence, a composite method using two additional methods with greater sensitivity but less breadth for fusions was used with the RNA whole-exome panel method. Across the characterized and uncharacterized samples, there were 66 fusions (54 unique fusions) concordant with the composite method covering 43 genes from the TuSight Oncology Comprehensive panel. Accuracy for splice variant detection was calculated by comparing TruSight Oncology Comprehensive results to qPCR assays for EGFRvIII.

a. CI calculated by bootstrap.

b. 95% two-sided CI calculated via the Wilson Score method.

NPA, negative percent agreement; PPA, positive percent agreement; RNGS, RNA next-generation sequencing.



Tumor profiling accuracy–TMB

The ability of TruSight Oncology Comprehensive (TSO) to detect tumor mutational burden (TMB) in > 100 FFPE samples was compared to the results achieved with whole-exome sequencing (WES). Results indicate a Pearson's correlation of 0.94.

Ordering information

Product	Catalog no.
TruSight Oncology Comprehensive Enablement Services	20066472
TruSight Oncology Comprehensive	20032573
TruSight Oncology DNA Control	20065041
TruSight Oncology RNA Control	20065042
NextSeq 550Dx Instrument	20005715
NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles)[a]	20028871

a. Class I sequencing consumables have single lot shipment, kit lot testing, advance change notification, and a Certificate of Analysis available for each lot. Reagents are developed under design control principles, manufactured under current good manufacturing practices (cGMP), and verified to ensure specification compliance.

References

1. Zehir A, Benayed R, Shah RH, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med*. 2017;23(6):703-713. doi:10.1038/nm.4333
2. Soumerai TE, Donoghue MTA, Bandlamudi C, et al. Clinical utility of prospective molecular characterization in advanced endometrial cancer. *Clin Cancer Res*. 2018;24(23):5939-5947. doi:10.1158/1078-0432.CCR-18-0412
3. Gutierrez ME, Choi K, Lanman RB, et al. Genomic profiling of advanced non-small cell lung cancer in community settings: Gaps and opportunities. *Clin Lung Cancer*. 2017;18(6):651-659. doi:10.1016/j.cllc.2017.04.004
4. Singal G, Miller PG, Agarwala V, et al. Association of patient characteristics and tumor genomics with clinical outcomes among patients with non-small cell lung cancer using a clinicogenomic database. *JAMA*. 2019;321(14):1391-1399. doi:10.1001/jama.2019.3241
5. Kato S, Kim KH, Lim HJ, et al. Real-world data from a molecular tumor board demonstrates improved outcomes with a precision N-of-One strategy. *Nat Commun*. 2020;11:4965 (2020). doi.org/10.1038/s41467-020-18613-3
6. Rozenblum AB, Ilouze M, Dudnik E, et al. Clinical impact of hybrid capture-based next-generation sequencing on changes in treatment decisions in lung cancer. *J Thorac Oncol*. 2017;12(2):258-268. doi:10.1016/j.jtho.2016.10.021
7. U.S. Food & Drug Administration. FDA approves pembrolizumab for adults and children with TMB-H solid tumors. fda.gov/drugs/ drug-approvals-and-databases/fda-approves-pembrolizumab-adults-and-children-tmb-h-solid-tumors. Released June 17, 2020. Accessed July 29, 2024.
8. Tray N, Weber JS, Adams S. Predictive biomarkers for checkpoint immunotherapy: Current status and challenges for clinical application. *Cancer Immunol Res*. 2018;6(10):1122-1128. doi:10.1158/2326-6066.CIR-18-0214
9. Samstein RM, Lee CH, Shoushtari AN, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nat Genet*. 2019;51(2):202-206. doi.org/10.1038/s41588-018-0312-8
10. Illumina. Illumina and Loxo Oncology to Partner on Developing Next-Generation Sequencing-Based Pan-Cancer Companion Diagnostics. illumina.com/company/news-center/press-releases/2018/2341745.html. Released April 10, 2018. Accessed July 29, 2024.
11. Biopharmadive. As Lilly deal closes, Bayer secures full rights to Loxo's Vitrakvi. biopharmadive.com/news/as-lilly-deal-closes-bayer-secures-full-rights-to-loxos-vitrakvi/548584/.Published February 15, 2019. Accessed July 29, 2024.
12. Illumina. TruSight Oncology Comprehensive Package Insert. support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/trusight/oncology-comp/200061832_02_1_trusight-oncology-comprehensive-us-ivd-package-insert.pdf. Published 2025. Accessed September 26, 2025.
13. Illumina and Taolue Biopharmaceuticals Collaborate to Help Chinese Innovation Go Global. illumina.com.cn/company/news-center/press-releases/2023/648d0d26-1f0a-4b65-b387-2c272761fbd711111.html. Published June 18, 2019. Accessed July 29, 2024.
14. Illumina Announces New and Expanded Oncology Partnerships with Bristol Myers Squibb, Kura Oncology, Myriad Genetics, and Merck to Advance Comprehensive Genomic Profiling. businesswire.com/news/home/20210111005930/en/Illumina-Announces-New-and-Expanded-Oncology-Partnerships-with-Bristol-Myers-Squibb-Kura-Oncology-Myriad-Genetics-and-Merck-to-Advance-Comprehensive-Genomic-Profiling. Released January 11, 2021. Accessed July 29, 2024.
15. Illumina. Illumina and Kartos Therapeutics Announce New Oncology Partnership to Develop an NGS-Based TP53 Companion Diagnostic. illumina.com/company/news-center/press-releases/2021/12b6e4a6-3f52-407e-8200-8fa72712a980.html. Published 2021. Accessed July 29, 2024.
16. NeoGenomics Laboratories. RET FISH Analysis. neogenomics.com/test-menu/ret-fish. Accessed July 29, 2024.
17. Mayo Clinic Laboratories. EGFR Targeted Mutation Analysis with ALK Reflex, Tumor. testcatalog.org/show/EGFRW. Accessed July 29, 2024.

18. NeoGenomics Laboratories. NTRK 1, 2, 3 FISH Panel. neogenomics.com/test-menu/ntrk-1-2-3-fish-panel. Accessed July 29, 2024.
19. NeoGenomics Laboratories. EGFR Mutation. neogenomics.com/test-menu/egfr-mutation-analysis-pcr. Accessed July 29, 2024.
20. Geisinger Medical Laboratories. Specimen collection and processing instructions for BRAF MUTATION ANALYSIS. geisingermedicallabs.com/catalog/details.cfm. Accessed July 29, 2024.
21. Geisinger Medical Laboratories. Specimen collection and processing instructions for KRAS MUTATION ANALYSIS. geisingermedicallabs.com/catalog/details.cfm. Accessed July 29, 2024.
22. Analysis provided courtesy of Velsera based on the TruSight Oncology Comprehensive Knowledge Base. Current as of March 2024.
23. Illumina. TruSight Oncology UMI Reagents. illumina.com/content/dam/illumina-marketing/documents/products/datasheets/trusight-oncology-umi-reagents-datasheet-1000000050425.pdf. Accessed July 29, 2024.
24. Illumina. NextSeq 550Dx Instrument. illumina.com/systems/ivd-instruments/nextseq-dx.html. Accessed July 29, 2024.
25. Velsera. Genomic Knowledge Base for Clinical Next-Generation Knowledge. pieriandx.com/genomic-knowledge-base. Accessed July 29, 2024.
26. CMS.gov National Coverage Determination (NCD) 90.2. cms.gov/medicare-coverage-database/view/ncd.aspx?NCDId=372. Accessed July 29, 2024.
27. Policy Reporter. Data pulled in 2023.

Intended use statement

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	*NTRK1/2/3* fusions	VITRAKVI® (larotrectinib)
Non-small cell lung cancer (NSCLC)	*RET* fusions	RETEVMO® (selpercatinib)

illumina®

1.800.809.4566 toll-free (US) | +1.858.202.4566 tel
techsupport@illumina.com | www.illumina.com

M-AMR-01466 v4.0