TruSight™ Oncology Comprehensive (EU)

A CE-marked, IVD, kitted solution for comprehensive genomic profiling (CGP)

Detect actionable biomarkers across > 28 solid tumor types using minimal patient biopsy

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

Deliver 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. That said, CGP can be a complex undertaking when implemented as a laboratory-developed test (LDT). TruSight Oncology Comprehensive (EU) facilitates this onerous task. As a validated, CE-marked, IVD, kitted solution, TruSight Oncology Comprehensive (EU) 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 (EU)

TruSight Oncology Comprehensive (EU) is the first commercially available, *in vitro* diagnostic (IVD), kitted CGP test containing both DNA and RNA content. 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 CE-marked IVD NextSeq™ 550Dx System. 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.

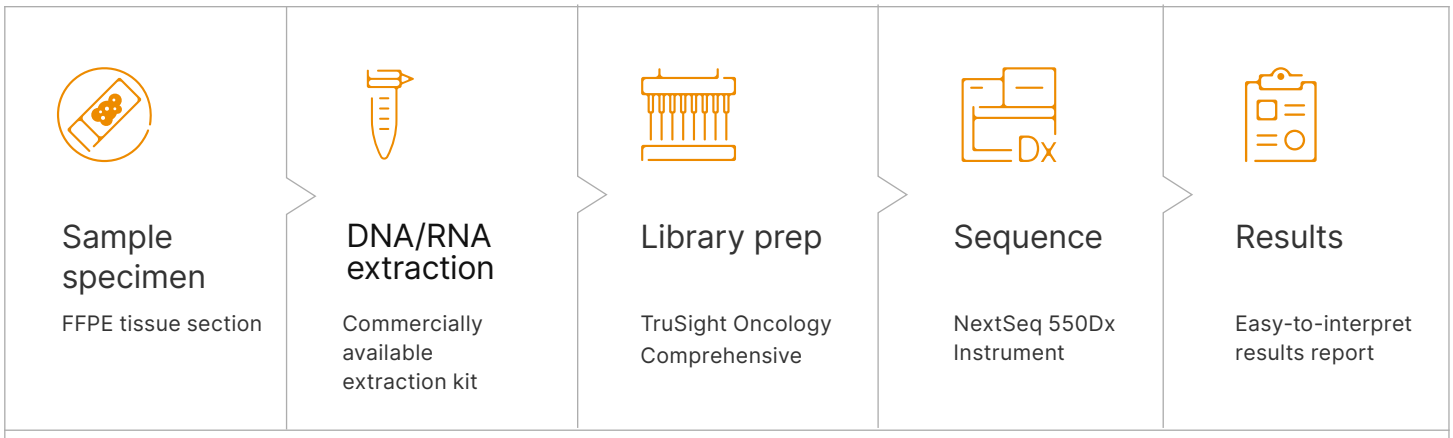


Figure 1: TruSight Oncology Comprehensive (EU) workflow
Batch up to seven patient samples and two control samples per run using TruSight Oncology Comprehensive (EU). The library preparation and enrichment steps take 2 days. The fully automated workflow on the NextSeq 550Dx Instrument sequences samples; 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 (EU) 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	• 517 genes for small variants • 23 genes for fusions • 2 genes for splice variants (*MET*, *EGFR*) • 2 genes for amplifications (*ERBB2*, *MET*) • TMB and MSI
Variant types detected	• DNA variants: SNVs, MNVs, insertions, deletions, gene amplifications • RNA variants: fusions, splice variants • Complex genomic signatures: TMB and MSI
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, ≥ 30% tumor content required to detect MSI-high
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	Gene amplifications, 0% Small DNA variants, 0.0001% MSI, 0% 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 (EU) overcomes these content limitations and simultaneously analyzes 517 genes with known cancer associations across > 28 solid tumor types in a single assay (Tables 3–5). The test calls multiple DNA and RNA variant types, including single nucleotide variants (SNVs), multiple nucleotide variants (MNVs), insertion/deletions (indels), gene amplifications, fusions, and splice variants (Figure 2). In addition, the test detects emerging immunotherapy biomarkers (ie, TMB[7] and microsatellite instability (MSI)[8-10]). Content provides significant coverage of key guidelines for multiple tumor types and genes linked to clinical trials (Table 2, Table 6). The inclusive nature of TruSight Oncology Comprehensive (EU) maximizes the chances of finding a positive biomarker.

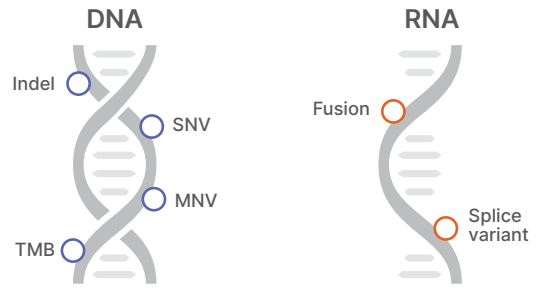


Figure 2: Variant types and genomic signatures detected by TruSight Oncology Comprehensive (EU)

Companion diagnostic indications

Illumina has established multiple partnerships with several pharma companies to develop a growing pipeline of companion diagnostic (CDx) indications. This information will help identify patients who are likely to respond to specific therapies. TruSight Oncology Comprehensive (EU) 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) in accordance with the approved therapeutic labeling.[11-13]

Additional CDx indications, currently under development, will be included once they receive the appropriate regulatory approvals (Table 7).

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

Tumor type		Select genes with biomarkers of clinical significance[a]
	Pan-cancer	*BRAF, FGFR1, FGFR2, FGFR3,* MSI*, NTRK1, NTRK2, NTRK3, RET*, TMB
	Breast cancer	*AKT1, BRCA1, BRCA2, ERBB2, ESR1, PALB2, PIK3CA, PTEN*
	Colorectal cancer	*BRAF, ERBB2, KRAS*, MSI, *NRAS*, *POLE*
	Melanoma	*BRAF, KIT, NRAS*
	Non-small cell lung cancer	*ALK, BRAF, EGFR, ERBB2, KRAS, MET, NRG1, RET, ROS1*
	Ovarian cancer	*BRCA1*, *BRCA2*
	Pancreatic cancer	*BRCA1*, *BRCA2, KRAS, PALB2, NRG1*
	Prostate cancer	*ATM*, *BRCA1*, *BRCA2*, *PALB2, PTEN*

a. Genes with biomarkers of clinical significance linked to major oncology guidelines.

MSI, microsatellite instability; TMB, tumor mutational burden.

Table 3: DNA content included in TruSight Oncology Comprehensive (EU)

ABL1	*BRCA2*	*CTNNB1*	*EWSR1*	*GATA1*	*IDH2*	*MAP3K13*	*NOTCH3*	*PNRC1*	*RPS6KA4*	*STK40*
ABL2	*BRD4*	*CUL3*	*EZH2*	*GATA2*	*IFNGR1*	*MAP3K14*	*NOTCH4*	*POLD1*	*RPS6KB1*	*SUFU*
ACVR1	*BRIP1*	*CUX1*	*FAM123B*	*GATA3*	*IGF1*	*MAP3K4*	*NPM1*	*POLE*	*RPS6KB2*	*SUZ12*
ACVR1B	*BTG1*	*CXCR4*	*FAM175A*	*GATA4*	*IGF1R*	*MAPK1*	*NRAS*	*PPARG*	*RPTOR*	*SYK*
AKT1	*BTK*	*CYLD*	*FAM46C*	*GATA6*	*IGF2*	*MAPK3*	*NRG1*	*PPM1D*	*RUNX1*	*TAF1*
AKT2	*C11orf30*	*DAXX*	*FANCA*	*GEN1*	*IKBKE*	*MAX*	*NSD1*	*PPP2R1A*	*RUNX1T1*	*TBX3*
AKT3	*CALR*	*DCUN1D1*	*FANCC*	*GID4*	*IKZF1*	*MCL1*	*NTRK1*	*PPP2R2A*	*RYBP*	*TCEB1*
ALK	*CARD11*	*DDR2*	*FANCD2*	*GLI1*	*IL10*	*MDC1*	*NTRK2*	*PPP6C*	*SDHA*	*TCF3*
ALOX12B	*CASP8*	*DDX41*	*FANCE*	*GNA11*	*IL7R*	*MDM2*	*NTRK3*	*PRDM1*	*SDHAF2*	*TCF7L2*
ANKRD11	*CBFB*	*DHX15*	*FANCF*	*GNA13*	*INHA*	*MDM4*	*NUP93*	*PREX2*	*SDHB*	*TERC*
ANKRD26	*CBL*	*DICER1*	*FANCG*	*GNAQ*	*INHBA*	*MED12*	*NUTM1*	*PRKAR1A*	*SDHC*	*TERT*
APC	*CCND1*	*DIS3*	*FANCI*	*GNAS*	*INPP4A*	*MEF2B*	*PAK1*	*PRKCI*	*SDHD*	*TET1*
AR	*CCND2*	*DNAJB1*	*FANCL*	*GPR124*	*INPP4B*	*MEN1*	*PAK3*	*PRKDC*	*SETBP1*	*TET2*
ARAF	*CCND3*	*DNMT1*	*FAS*	*GPS2*	*INSR*	*MET*	*PAK7*	*PRSS8*	*SETD2*	*TFE3*
ARFRP1	*CCNE1*	*DNMT3A*	*FAT1*	*GREM1*	*IRF2*	*MGA*	*PALB2*	*PTCH1*	*SF3B1*	*TFRC*
ARID1A	*CD274*	*DNMT3B*	*FBXW7*	*GRIN2A*	*IRF4*	*MITF*	*PARK2*	*PTEN*	*SH2B3*	*TGFBR1*
ARID1B	*CD276*	*DOT1L*	*FGF1*	*GRM3*	*IRS1*	*MLH1*	*PARP1*	*PTPN11*	*SH2D1A*	*TGFBR2*
ARID2	*CD74*	*E2F3*	*FGF10*	*GSK3B*	*IRS2*	*MLL/KMT2A*	*PAX3*	*PTPRD*	*SHQ1*	*TMEM127*
ARID5B	*CD79A*	*EED*	*FGF14*	*H3F3A*	*JAK1*	*MLLT3*	*PAX5*	*PTPRS*	*SLIT2*	*TMPRSS2*
ASXL1	*CD79B*	*EGFL7*	*FGF19*	*H3F3B*	*JAK2*	*MPL*	*PAX7*	*PTPRT*	*SLX4*	*TNFAIP3*
ASXL2	*CDC73*	*EGFR*	*FGF2*	*H3F3C*	*JAK3*	*MRE11A*	*PAX8*	*QKI*	*SMAD2*	*TNFRSF14*
ATM	*CDH1*	*EIF1AX*	*FGF23*	*HGF*	*JUN*	*MSH2*	*PBRM1*	*RAB35*	*SMAD3*	*TOP1*
ATR	*CDK12*	*EIF4A2*	*FGF3*	*HIST1H1C*	*KAT6A*	*MSH3*	*PDCD1*	*RAC1*	*SMAD4*	*TOP2A*
ATRX	*CDK4*	*EIF4E*	*FGF4*	*HIST1H2BD*	*KDM5A*	*MSH6*	*PDCD1LG2*	*RAD21*	*SMARCA4*	*TP53*
AURKA	*CDK6*	*EML4*	*FGF5*	*HIST1H3A*	*KDM5C*	*MST1*	*PDGFRA*	*RAD50*	*SMARCB1*	*TP63*
AURKB	*CDK8*	*EP300*	*FGF6*	*HIST1H3B*	*KDM6A*	*MST1R*	*PDGFRB*	*RAD51*	*SMARCD1*	*TRAF2*
AXIN1	*CDKN1A*	*EPCAM*	*FGF7*	*HIST1H3C*	*KDR*	*MTOR*	*PDK1*	*RAD51B*	*SMC1A*	*TRAF7*
AXIN2	*CDKN1B*	*EPHA3*	*FGF8*	*HIST1H3D*	*KEAP1*	*MUTYH*	*PDPK1*	*RAD51C*	*SMC3*	*TSC1*
AXL	*CDKN2A*	*EPHA5*	*FGF9*	*HIST1H3E*	*KEL*	*MYB*	*PGR*	*RAD51D*	*SMO*	*TSC2*
B2M	*CDKN2B*	*EPHA7*	*FGFR1*	*HIST1H3F*	*KIF5B*	*MYC*	*PHF6*	*RAD52*	*SNCAIP*	*TSHR*
BAP1	*CDKN2C*	*EPHB1*	*FGFR2*	*HIST1H3G*	*KIT*	*MYCL1*	*PHOX2B*	*RAD54L*	*SOCS1*	*U2AF1*
BARD1	*CEBPA*	*ERBB2*	*FGFR3*	*HIST1H3H*	*KLF4*	*MYCN*	*PIK3C2B*	*RAF1*	*SOX10*	*VEGFA*
BBC3	*CENPA*	*ERBB3*	*FGFR4*	*HIST1H3I*	*KLHL6*	*MYD88*	*PIK3C2G*	*RANBP2*	*SOX17*	*VHL*
BCL10	*CHD2*	*ERBB4*	*FH*	*HIST1H3J*	*KRAS*	*MYOD1*	*PIK3C3*	*RARA*	*SOX2*	*VTCN1*
BCL2	*CHD4*	*ERCC1*	*FLCN*	*HIST2H3A*	*LAMP1*	*NAB2*	*PIK3CA*	*RASA1*	*SOX9*	*WISP3*
BCL2L1	*CHEK1*	*ERCC2*	*FLI1*	*HIST2H3C*	*LATS1*	*NBN*	*PIK3CB*	*RB1*	*SPEN*	*WT1*
BCL2L11	*CHEK2*	*ERCC3*	*FLT1*	*HIST2H3D*	*LATS2*	*NCOA3*	*PIK3CD*	*RBM10*	*SPOP*	*XIAP*
BCL2L2	*CIC*	*ERCC4*	*FLT3*	*HIST3H3*	*LMO1*	*NCOR1*	*PIK3CG*	*RECQL4*	*SPTA1*	*XPO1*
BCL6	*CREBBP*	*ERCC5*	*FLT4*	*HNF1A*	*LRP1B*	*NEGR1*	*PIK3R1*	*REL*	*SRC*	*XRCC2*
BCOR	*CRKL*	*ERG*	*FOXA1*	*HNRNPK*	*LYN*	*NF1*	*PIK3R2*	*RET*	*SRSF2*	*YAP1*
BCORL1	*CRLF2*	*ERRFI1*	*FOXL2*	*HOXB13*	*LZTR1*	*NF2*	*PIK3R3*	*RFWD2*	*STAG1*	*YES1*
BCR	*CSF1R*	*ESR1*	*FOXO1*	*HRAS*	*MAGI2*	*NFE2L2*	*PIM1*	*RHEB*	*STAG2*	*ZBTB2*
BIRC3	*CSF3R*	*ETS1*	*FOXP1*	*HSD3B1*	*MALT1*	*NFKBIA*	*PLCG2*	*RHOA*	*STAT3*	*ZBTB7A*
BLM	*CSNK1A1*	*ETV1*	*FRS2*	*HSP90AA1*	*MAP2K1*	*NKX2-1*	*PLK2*	*RICTOR*	*STAT4*	*ZFHX3*
BMPR1A	*CTCF*	*ETV4*	*FUBP1*	*ICOSLG*	*MAP2K2*	*NKX3-1*	*PMAIP1*	*RIT1*	*STAT5A*	*ZNF217*
BRAF	*CTLA4*	*ETV5*	*FYN*	*ID3*	*MAP2K4*	*NOTCH1*	*PMS1*	*RNF43*	*STAT5B*	*ZNF703*
BRCA1	*CTNNA1*	*ETV6*	*GABRA6*	*IDH1*	*MAP3K1*	*NOTCH2*	*PMS2*	*ROS1*	*STK11*	*ZRSR2*

Content shaded in grey is analyzed for gene amplifications.

Table 4: RNA content included in TruSight Oncology Comprehensive (EU)

ALK	*BRAF*	*ERG*	*ETV4*	*FGFR3*	*NTRK1*	*PAX3*	*ROS1*
AXL	*EGFR*	*ESR1*	*FGFR1*	*KIF5B*	*NTRK2*	*RAF1*	*TMPRSS2*
BCL2	*EML4*	*ETV1*	*FGFR2*	*NRG1*	*NTRK3*	*RET*	

Table 5: Splice variants included in TruSight Oncology Comprehensive (EU)

EGFR	*MET*

Table 6: TruSight Oncology Comprehensive (EU) content coverage

49 Clinical practice guidelines
117 Drug labels
~680 European clinical trials
Analysis provided by Velsera based on the TruSight Oncology Comprehensive (EU) software Knowledge Base. Current as of February 2023.

Table 7: CDx indications

CDx indication	**Partner**
Solid tumors positive for *NTRK1*, *NTRK2*, or *NTRK3* gene fusions for treatment with VITRAKVI (larotrectinib)	Bayer[11-13]
Under development	
RET	Lilly[11]
EGFR	Teligene[14]
HRD	Myriad Genetics, Merck[15,16]
TP53	Kartos Therapeutics[17]
MSI	Bristol Myers Squibb[15]
CDx developments apply to the TruSight Oncology Comprehensive (EU) portfolio. Availability of each CDx will vary by geography and is based upon variable timelines for therapy and test approvals by region.	

More information, less sample, less time

TruSight Oncology Comprehensive (EU) provides more information from less sample, in less total time compared to current iterative testing methods. For example, a potential journey for a patient diagnosed with non-small cell lung carcinoma (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.[18-23] In contrast, a CGP test using TruSight Oncology Comprehensive (EU) 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).

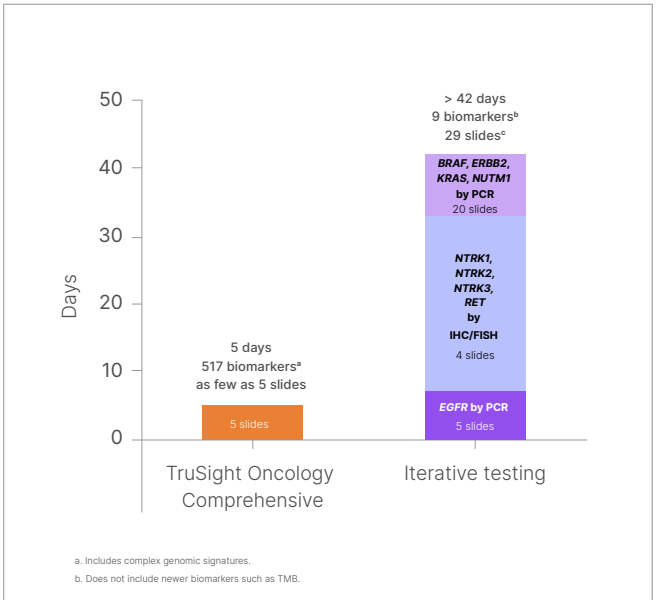


Figure 3: Advantages of TruSight Oncology Comprehensive (EU) compared to iterative testing
Example showing potential journeys for NSCLC patient. CGP with TruSight Oncology Comprehensive (EU) provides substantially more coverage with less time and less sample compared to single-gene iterative testing.[18-23]

One easy-to-interpret results report

TruSight Oncology Comprehensive (EU) results, supported by an expertly curated Knowledge Base, are presented in a single, streamlined results report. There's no need to search multiple reports from tests performed over a period of time in an attempt to identify significant variants. The TruSight Oncology Comprehensive (EU) 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
- Genomic Findings 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, EMA-approved drug labels, ASCO Clinical Practice Guidelines, or ESMO Clinical Practice Guidelines for the patient's tumor type, as specified by the Knowledge Base[24]
- Genomic Findings with Potential Clinical Significance—detected variants that have potential clinical significance (therapeutic, prognostic or diagnostic) based on information in FDA-approved drug labels, EMA-approved drug labels, ASCO Clinical Practice Guidelines, or ESMO 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[24*]

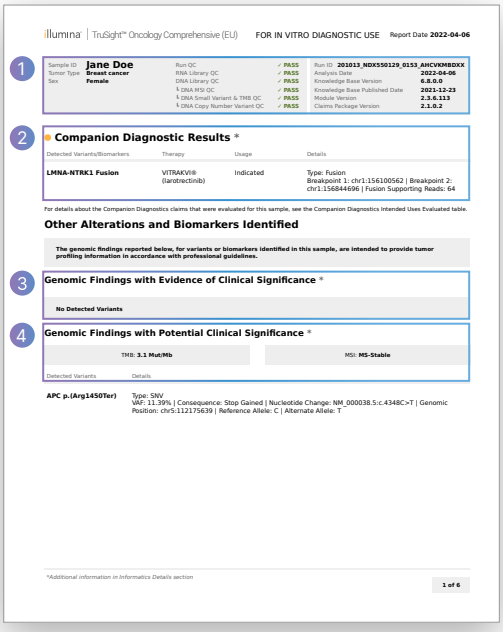
* ASCO, American Society of Clinical Oncology; EMA, European Medicines Agency; ESMO, European Society for Medical Oncology; FDA, Food and Drug Administration.

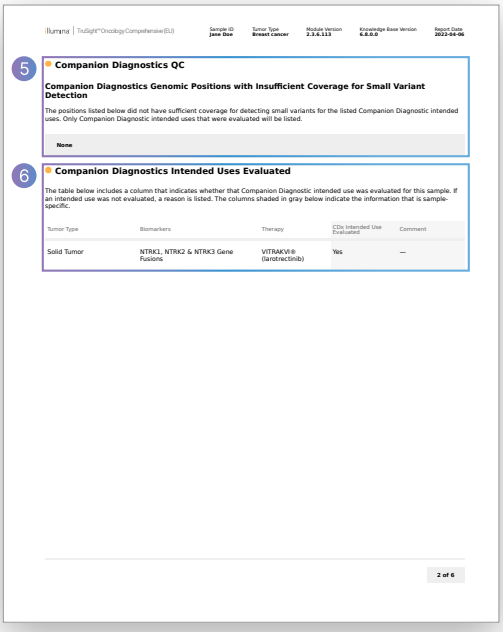
Validated solution

TruSight Oncology Comprehensive (EU) is a validated (Table 8), sample-to-answer CGP test that includes kitted reagents, a sequencing system, and analysis software. The test was developed using a rigorous design control process and validated across > 350 unique FFPE samples and > 55 different tumor types. Results were compared to orthogonal methods to ensure accurate, reproducible, and consistent data.

Table 8: Validation studies using TruSight Oncology Comprehensive (EU)

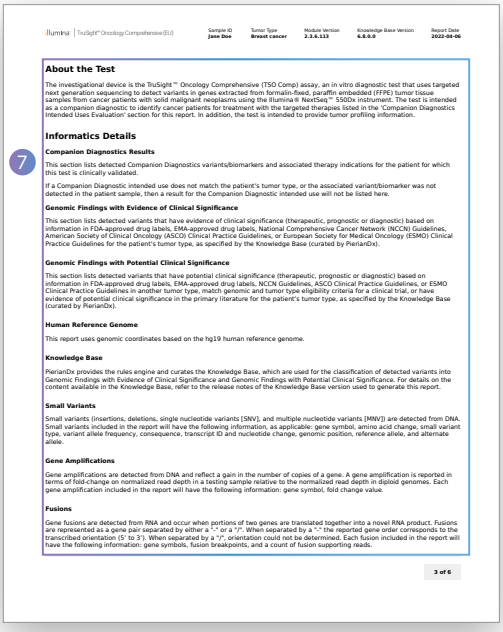
Accuracy and clinical bridging studies for *NTRK1/2/3* and *RET* fusion detection	Library stability
Analytical accuracy	Limit of blank
Assay workflow guardbanding	Limit of detection
Cross contamination	Nucleic acid extraction kit evaluation
External controls evaluation	Real-time stability
Nucleic acid input titration guardbanding	Reproducibility
Interfering substances	Slide-mounted FFPE tissue stability
Kit in-use stability	Within-laboratory precision
Kit transport stability	

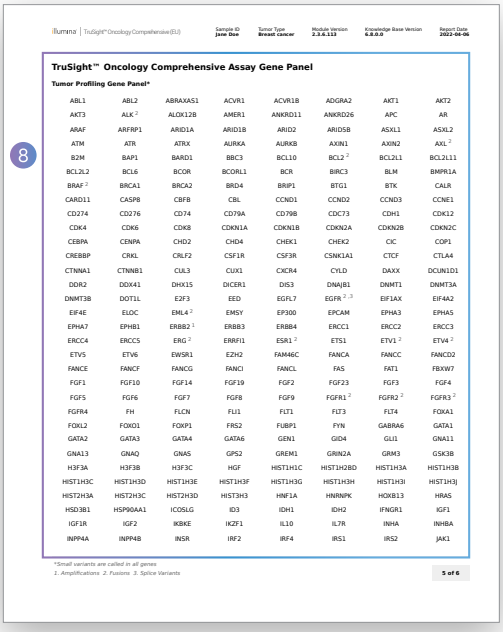




1. Patient sample information
2. Companion diagnostic results
   - Detected Companion Diagnostics variants/biomarkers and associated therapy indications
3. Genomic findings with evidence of clinical significance
   - Variant name and genomic details
4. Genomic findings with potential clinical significance
   - Includes TMB, MSI

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
   - Genomics findings 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 (EU)

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.

Using TruSight Oncology Comprehensive (EU)

TruSight Oncology Comprehensive (EU) 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).

Prepare libraries

TruSight Oncology Comprehensive (EU) 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)[25] 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 efforts

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 (EU) libraries are sequenced on the NextSeq 550Dx System (Figure 5). The NextSeq 550Dx System is a CE-marked 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 sequencing of small panels 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 roughly 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.[26]

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 (EU) workflow from sequencing through results report generation.

Patient batching throughput

Using TruSight Oncology Comprehensive (EU) 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 (EU) is performed automatically on the NextSeq 550Dx System using the Local Run Manager TruSight Oncology Comprehensive (EU) 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, gene amplifications, TMB, and MSI 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 and MSI calculations, tumor profiling of variants into two levels of clinical significance, and report generation. The interpreted variant results, as well as the TMB and MSI 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 (EU) Software is supported by a purpose-built over time, clinically derived rules engine and Knowledge Base to maximize report actionability. The rules engine and supported Knowledge Base, both provided by Velsera,[27] comprise extensive coverage of peer-reviewed publications, actionable variant information, and the most recent guidelines, drug labels, and clinical trials (Table 9, Figure 6). The TruSight Oncology Comprehensive (EU) Software uses this rich content to determine classifications of the detected genetic variants.

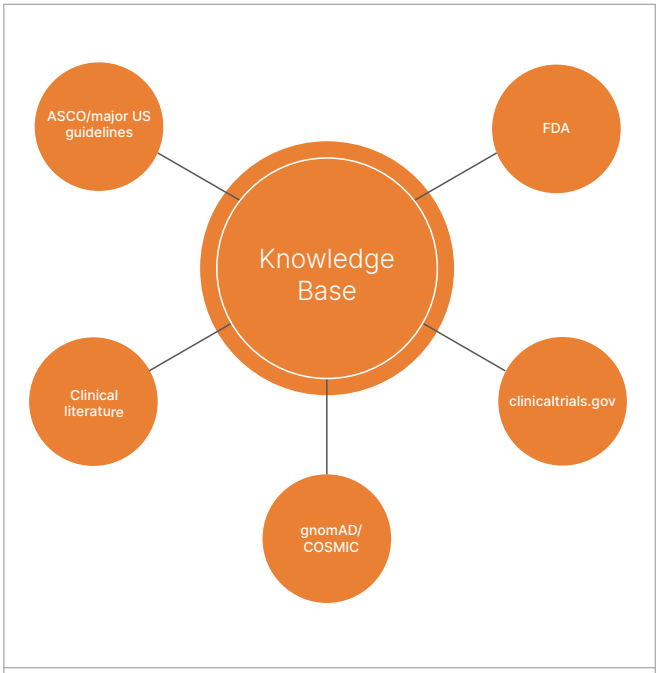


Figure 6: Knowledge Base creation
The TruSight Oncology Comprehensive (EU) Tumor Profiling software is built on a foundation of extensively reviewed rules. Source rules, derived from clinical practice guidelines, drug labels, and primary literature, identify and classify actionable variants. Data from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

Table 9: 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]

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 clinical practice guidelines (eg, ASCO, ESMO), approved drug labels (FDA, EMA), clinical trial registries (clincialtrials.gov, EUCTR), 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 make sure 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 and the 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 regularly made available24 to account for new biomarkers; changes to guidelines, drug labels, and clinical trials; and newly published clinical research studies. 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 (EU) 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).13 Qualitative studies across multiple operators, instruments, reagent lots, and days showed high concordance with minimal variance.[13] For detailed information on the studies performed, refer to the Illumina TruSight Oncology Comprehensive (EU) Package Insert.[13]

‡ ASCO, American Society of Clinical Oncology; COSMIC, Catalogue of Somatic Mutations In Cancer; EMA, European Medicines Agency; ESMO, European Society for Medical Oncology; EUCTR, European Clinical Trials Registry; FDA, Food and Drug Administration; gnomAD, Genome Aggregation Database.

Bring CGP 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

Facilitated implementation

Implementing a CGP test can require significant time and effort. With the introduction of TruSight Oncology Comprehensive (EU), Illumina has addressed some of the biggest challenges, streamlining the process. Starting with a highly validated, CE-marked, IVD, kitted solution:

- Reduces 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
- Provides an *In Vitro* Diagnostic Directive (IVDD)-compliant test that is on the path to meeting *In Vitro* Diagnostic Regulation (IVDR) requirements, helping labs prepare to meet the 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 training, 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

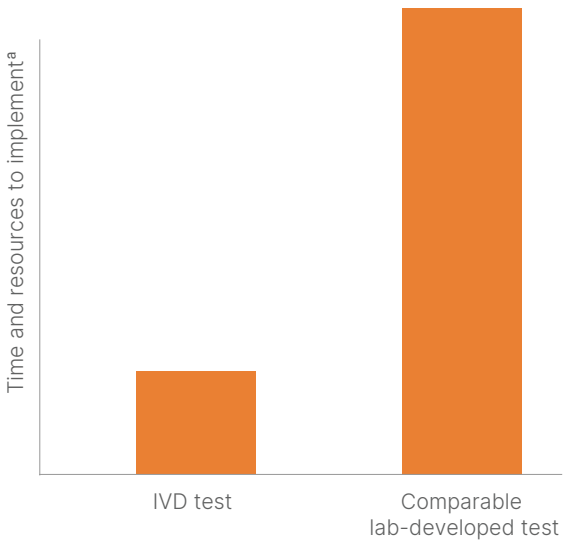


Figure 7: Simpler, less burdensome test implementation

TruSight Oncology Comprehensive (EU) is a CE-marked IVD test that requires only ISO 15189 performance verification, 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.

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. Reimbursement differs based on the country, clinical setting, and services provided. Currently, national or regional funding is available in some European countries (Figure 8). 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.

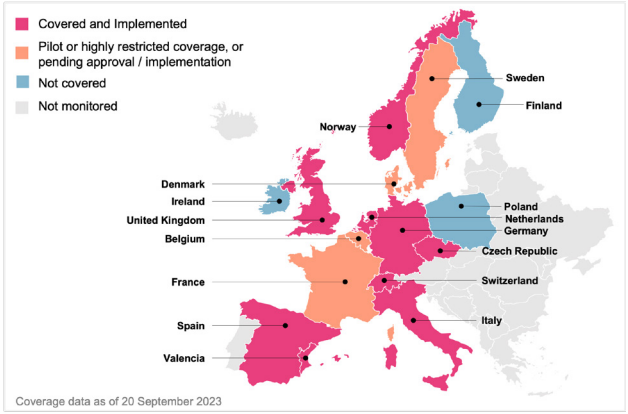


Figure 8: CGP test coverage options across Europe
Data current as of September 20, 2023.

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, use TruSight Oncology Comprehensive (EU) to analyze multiple variant types in 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 (EU)

Comprehensive genomic profiling (CGP)

NextSeq 550Dx System

Ordering information

Product	Catalog no.
TruSight Oncology Comprehensive (EU) Kit	20063092
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

Appendix

Limit of blank study

Low false positives for TruSight Oncology Comprehensive (EU)

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

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.

Limit of detection (LoD) studies

LoD—splice variants

Splice variant	LoD
MET	18.7

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—RNA fusions and splice variants

Fusion	LoD
NCOA4-RET	10
TMPRSS2-ERG	13.2
KIF5B-RET	14.5
ACPP-ETV1	17.2
FGFR3-TACC3	17.5
EML4-ALK	20.2
FGFR1-GSR	23.7
EGFR-GALNT13	24
ESR1-CCDC170	24.3
FGFR2-SRPK2	24.7
HNRNPUL1-AXL	26.3
CD74-ROS1;GOPC	28.2
SPIDR-NRG1	28.2
RAF1-VGLL4	28.5
DHX8;ETV4-STAT3	30.5
MKRN1-BRAF	31.2
BCL2-IGHJ5	44.2
PAX3-FOXO1	54.7

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—small DNA variants and gene amplifications

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	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
Gene amplifications (fold-change)	By gene (*ERBB2, MET*)	2	2.034–2.195

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%).

Reproducibility for tumor profiling studies

Reproducibility for tumor profiling—gene amplifications

Targeted gene	Mean fold-change[a]	PPC	95% CI[b]
MET	5.14	100.0%	92.6%, 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 fold-change calculated from observed assay results.
b. 95% two-sided CI calculated via the Wilson Score Method.
PPC, percent positive call; CI, confidence interval.

Reproducibility for tumor profiling—MSI

Panel member	Mean MSI score[a]	PPC	95% CI[b]
TPSBD4	60.5	100.0% (36/36)	90.4%, 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. Mean MSI score calculated from observed assay results.
a. Mean MSI score calculated from observed assay results.
b. 95% two-sided CI calculated via the Wilson Score Method.
PPC, percent positive call; CI, confidence interval.

Reproducibility for tumor profiling—small DNA variants

Gene	Variant type	Targeted variant (amino acid)	Mean VAF[a]	PPC	95% CI[b]
APC	Deletion	L1488fsTer19	0.181	100.0% (28/28)	87.9%, 100.0%
APC	Deletion	S1465WfsTer3	0.166	100.0% (40/40)	91.2%, 100.0%
APC	Insertion	T1556NfsTer3	0.227	100.0% (32/32)	89.3%, 100.0%
APC	Insertion	S1465fs*9	0.100	100.0% (48/48)	92.6%, 100.0%
ARID1A	Insertion	Q372fs*28	0.084	100.0% (4/4)	51.0%, 100.0%
BRAF	SNV	V600E	0.045	91.3% (42/46)	79.7%, 96.6%
EGFR	Deletion	E746_A750del	0.112	100.0% (46/46)	92.3%, 100.0%
EGFR	SNV	L858R	0.045	100.0% (38/38)	90.8%, 100.0%
EP300	Deletion	H2324fs*29	0.245	100.0% (44/44)	92.0%, 100.0%
ERBB2	Insertion	Y772_A775dup	0.075	100.0% (36/36)	90.4%, 100.0%
IDH1	SNV	R132H	0.155	100.0% (36/36)	90.4%, 100.0%
KRAS	MNV	G12I	0.111	100.0% (38/38)	90.8%, 100.0%
NOTCH1	Insertion	R1598fs*12	0.146	100.0% (48/48)	92.6%, 100.0%
PTEN	Deletion	T319fs*1	0.157	100.0% (44/44)	92.0%, 100.0%
TP53	Insertion	P152_P153dup	0.157	100.0% (2/2)	34.2%, 100.0%
TP53	Insertion	R333HfsTer5	0.154	100.0% (48/48)	92.6%, 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	Variant type	Mean supporting reads[a]	PPC	95% CI[b]
ACPP-ETV1	Fusion	44.7	100.0% (46/46)	92.3%, 100.0%
BCL2-IGHJ5	Fusion	124.9	100.0% (46/46)	92.3%, 100.0%
CD74-ROS1;GOPC	Fusion	56.6	100.0% (48/48)	92.6%, 100.0%
DHX8;ETV4-STAT3	Fusion	48.9	100.0% (46/46)	92.3%, 100.0%
EGFR-GALNT13	Fusion	49.8	100.0% (46/46)	92.3%, 100.0%
EML4-ALK	Fusion	49.3	100.0% (48/48)	92.6%, 100.0%
ESR1-CCDC170	Fusion	45.1	100.0% (46/46)	92.3%, 100.0%
FGFR1-GSR	Fusion	61.1	100.0% (46/46)	92.3%, 100.0%
FGFR2-SRPK2	Fusion	53.4	100.0% (48/48)	92.6%, 100.0%
FGFR3-TACC3	Fusion	53.5	100.0% (48/48)	92.6%, 100.0%
HNRNPUL1-AXL	Fusion	58.0	100.0% (48/48)	92.6%, 100.0%
KIF5B-RET	Fusion	11.6	91.7% (44/48)	80.4%, 96.7%
MKRN1-BRAF	Fusion	33.4	100.0% (48/48)	92.6%, 100.0%
PAX3-FOXO1	Fusion	70.1	100.0% (48/48)	92.6%, 100.0%
RAF1-VGLL4	Fusion	15.9	100.0% (46/46)	92.3%, 100.0%
SPIDR-NRG1	Fusion	51.5	100.0% (48/48)	92.6%, 100.0%
TMPRSS2-ERG	Fusion	43.5	97.9% (47/48)	89.1%, 99.6%
EGFRvIII	Splice variant	64.0	100.0% (46/46)	92.3%, 100.0%
MET exon 14 skipping	Splice variant	61.2	100.0% (48/48)	92.6%, 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. 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%).

a. Mean supporting reads calculated from observed assay results.

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

PPC, percent positive call; CI, confidence interval.

Analytical accuracy studies

Analytical accuracy—DNA variants and MSI

Variant type	Orthogonal method	PPA	NPA
Small DNA variants (somatic)	WES	85% (382/451) (95% CI: 81%-87%)	99.999% (70,000,481/70,000,907) (95% CI: 99.999%-99.999%)
Small DNA variants (germline)	WES	99.8% (33,163/33,224)(95% CI: 99.8%-99.9%)	99.999% (70,000,481/70,000,907) (95% CI: 99.999%-99.999%)
Gene amplifications	WES	92% (337/365) (95% CI: 89%, 95%)	98.3% (24,000/24,415) (95% CI: 98.1%, 98.5%)
MSI	MSI-PCR	93% (40/43) (95% CI: 81%, 98%)	99% (150/152) (95% CI: 95%, > 99%)

The ability of TruSight Oncology Comprehensive (EU) to detect alterations in hundreds of FFPE samples was compared to the results achieved with the indicated reference method. At least 48% of the somatic variants detected by TruSight Oncology Comprehensive (EU) were not detected by WES due to allele frequencies being below the WES threshold. WES data also showed evidence for the presence of additional variants detected by TruSight Oncology Comprehensive (EU), but with low support from WES calls. This suggests that these variants were missed in the tumor by WES because of normal contamination.

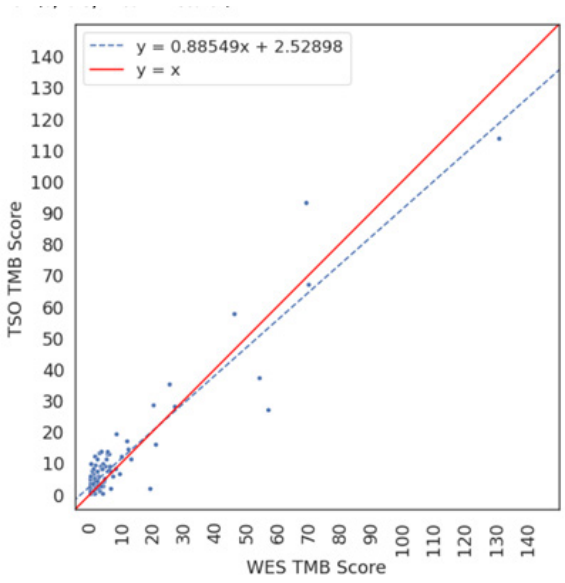
MSI, microsatellite instability; NPA, negative percent agreement; PPA, positive percent agreement; WES, whole-exome sequencing.

Analytical accuracy—RNA variants

Variant type	Orthogonal method	PPA	NPA
Fusions	• RNA whole-exome sequencing (RNGS1) • Targeted NGS fusion panel (RNGS2) • Droplet digital PCR (ddPCR)	82% (63/77) (95% CI: 72%, 89%)	99.9% (13821/13839) (95% CI: 99.8%, 99.9%)
Splice variants	qPCR	57% (4/7) (95% CI: 25%, 84%)	100% (230/230) (95% CI: 98%, 100%)

The ability of TruSight Oncology Comprehensive (EU) to detect alterations in hundreds of FFPE samples was compared to the results achieved with the indicated reference method. TruSight Oncology Comprehensive (EU) detected 41 fusions missed by orthogonal approaches. LoD for RNGS1 was 4-8× that of TruSight Oncology Comprehensive (EU), prompting use of additional methods with greater sensitivity, but less breadth of fusions. Confirmed additional 41 fusions detected by TruSight Oncology Comprehensive using ddPCR. PPA and NPA scores for fusions represent a composite of the three orthogonal methods. Three samples were called positive for MET Exon 14 deletions by qPCR but not by TruSight Oncology Comprehensive (EU) had an average Ct > 37, which is below the TruSight OncologyComprehensive (EU) LoD level.

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



Tumor profiling accuracy–TMB

The ability of TruSight Oncology Comprehensive (EU) 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.

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Intended use statement

TruSight™ Oncology Comprehensive (EU) is an *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, multinucleotide variants, insertions, deletions and gene amplifications from DNA, and gene fusions and splice variants from RNA. The test also reports a Tumor Mutational Burden (TMB) score and Microsatellite Instability (MSI) status.

The test is intended as a companion diagnostic to identify cancer patients for treatment with the targeted therapy 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 healthcare professionals in accordance with professional guidelines and is not conclusive or prescriptive for labeled use of any specific therapeutic product.

Table 1: Companion diagnostics indication

Tumor type	Biomarkers	Targeted therapy
Solid tumors	*NTRK1, NTRK2, NTRK3* gene fusions	VITRAKVI (larotrectinib)

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