

# TruSight™ Oncology Comprehensive

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

- Detect actionable biomarkers across solid tumors using minimal patient biopsy
- Assess current and emerging biomarkers from clinical practice guidelines, drug labels, and clinical trials simultaneously
- Deliver an easy-to-interpret, clinically relevant report that can help inform therapy decisions 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.<sup>1-6</sup>

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). TruSight Oncology Comprehensive (TSO Comprehensive)

facilitates this onerous task. As a validated, US FDA-approved, IVD, kitted solution, TSO 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 TSO Comprehensive

TSO 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 FFPE tissue. The NGS-based solution simultaneously analyzes 517 cancer-associated genes with known clinical relevance in one integrated workflow (Figure 1, Tables 1-4). The test includes kitted reagents for library preparation and sequencing and automated software pipelines that identify variants, interpret results, and produce clinically actionable 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.

### Fully automated sequencing and data analysis

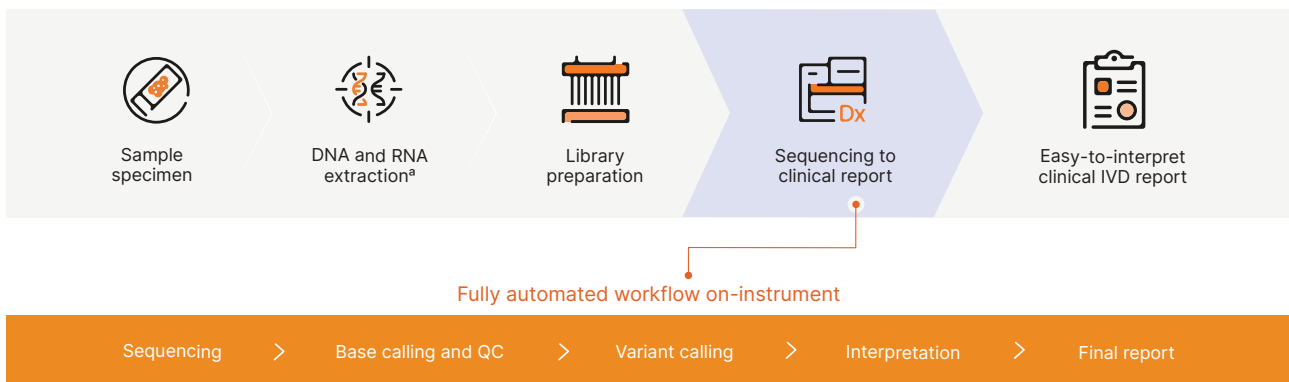


Figure 1: TSO Comprehensive workflow—Batch up to seven patient samples and two control samples per run using TSO Comprehensive. The library preparation and enrichment steps take 2 days. The fully automated workflow on the NextSeq 550Dx Instrument sequences samples; performs base calling and QC, variant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

a. Extraction kits must be purchased separately.

Table 1: TSO Comprehensive at a glance

Feature	Description <sup>a</sup>
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	<ul style="list-style-type: none"> <li>• Small DNA variants in 517 genes</li> <li>• Fusions in 24 genes</li> <li>• Splice variants in 1 gene</li> <li>• TMB</li> </ul>
Variant types detected	<ul style="list-style-type: none"> <li>• DNA variants: SNVs, MNVs, insertions, deletions</li> <li>• RNA variants: fusions, splice variants</li> <li>• Complex genomic signatures: TMB</li> </ul>
DNA input requirement	40 ng genomic DNA
RNA input requirement	40 ng total RNA
FFPE input requirement	Recommended tissue volume $\geq 1 \text{ mm}^3$ 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 $\mu\text{m}$ sections, 20 $\text{mm}^2$ tissue area each)
Total assay time	4-5 days from nucleic acid to clinical 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 TSO Comprehensive overcomes these content limitations and simultaneously analyzes 517 genes with known cancer associations across solid tumors in a single assay (Tables 2-4). The test calls multiple DNA and RNA variant types, including single nucleotide variants (SNVs), multiple nucleotide variants (MNVs), insertion, deletions, fusions, and splice variants (Figure 2). In addition, the test detects emerging immunotherapy biomarkers (ie, TMB<sup>7-9</sup>). Additional tumor profiling claims are under development (Table 5). Content provides significant coverage of key guidelines for multiple tumor types and genes linked to clinical trials (Figure 3, Table 6). The inclusive nature of TSO Comprehensive maximizes the chances of finding a positive biomarker.

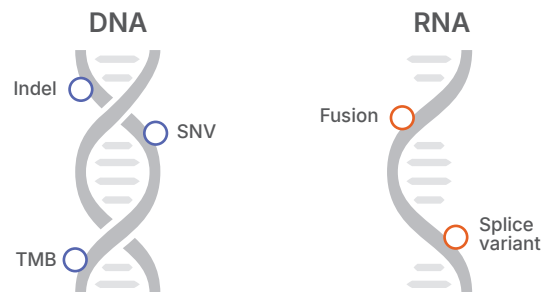


Figure 2: Variant types and genomic signatures detected by TSO Comprehensive.

## 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. TSO 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.<sup>10-12</sup> Additional CDx indications, currently under development, will be included once they receive the appropriate regulatory approvals (Table 7).

Table 2: DNA content included in TSO Comprehensive

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	ERRF1	FOXP1	HSP90AA1	MAP2K1	NKX2-1	PMAIP1	RIT1	STAT5A	ZNF703
BLM	CSNK1A1	ESR1	FRS2	ICOSLG	MAP2K2	NKX3-1	PMS1	RNF43	STAT5B	ZRSR2











Pan-cancer: <i>BRAF, NTRK1, NTRK2, NTRK3, RET, TMB</i>													
Genes with biomarkers of clinical significance <sup>a</sup>											Genes with biomarkers of potential clinical significance <sup>b</sup>		
	<b>Breast</b>	<i>AKT1</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>ERBB2</i>	<i>ESR1</i>	<i>PALB2</i>	<i>PIK3CA</i>	<i>PTEN</i>			104	
	<b>Colorectal</b>	<i>ERBB2</i>	<i>KRAS</i>	<i>NRAS</i>								157	
	<b>Bone</b>	<i>EGFR</i>	<i>ERG</i>	<i>ETV1</i>	<i>ETV4</i>	<i>EWSR1</i>	<i>FEV</i>	<i>FLI1</i>	<i>FUS</i>	<i>IDH1</i>		109	
	<b>Lung</b>	<i>EGFR</i>	<i>ERBB2</i>	<i>KRAS</i>	<i>NUTM1</i>							126	
	<b>Melanoma</b>	<i>KIT</i>	<i>NRAS</i>									116	
	<b>Ovarian</b>	<i>BRCA1</i>	<i>BRCA2</i>	<i>ERBB2</i>								109	
	<b>CNS<sup>c</sup></b>	<i>ATRX</i>	<i>CDKN2A</i>	<i>CDKN2B</i>	<i>EGFR</i>	<i>H3F3A</i>	<i>HIST1H3B</i>	<i>HIST1H3C</i>	<i>IDH1</i>	<i>IDH2</i>	<i>MYCN</i>	<i>NF1</i>	69
	<b>Prostate</b>	<i>ATM</i>	<i>ATR</i>	<i>BARD1</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRIP1</i>	<i>CDK12</i>	<i>CHEK1</i>	<i>CHEK2</i>	<i>FANCA</i>	<i>FANCL</i>	101
	<b>Thyroid</b>	<i>TERT</i>											111
	<b>Uterine and cervical</b>	<i>BRCA2</i>	<i>ERBB2</i>	<i>ESR1</i>	<i>FOXO1</i>	<i>GREB1</i>	<i>NCOA2</i>	<i>NCOA3</i>	<i>PAX3</i>	<i>PAX7</i>	<i>POLE</i>	<i>SMARCA4</i>	130
		<i>TP53</i>											

Figure 3: Genes with key actionable biomarkers for multiple solid tumor types—Genes listed represent a subset of genes present in the TSO Comprehensive panel. Content analysis provided by Velsera based on IVD software Knowledge Base v8.17 (March 2024).

- a. Genes linked to current drug labels or guidelines.
- b. Based on presence in clinical trials.
- c. CNS, central nervous system.

Table 3: RNA content included in TSO Comprehensive<sup>a</sup>

<i>AXL</i>	<i>CDK4</i>	<i>ERG</i>	<i>ETV4</i>	<i>FGFR2</i>	<i>KIF5B</i>	<i>NTRK2</i>	<i>RAF1</i>
<i>BCL2</i>	<i>EGFR</i>	<i>ESR1</i>	<i>EWSR1</i>	<i>FGFR3</i>	<i>NRG1</i>	<i>NTRK3</i>	<i>RET</i>
<i>BRAF</i>	<i>EML4<sup>b</sup></i>	<i>ETV1</i>	<i>FGFR1</i>	<i>FLI1</i>	<i>NTRK1</i>	<i>PAX3</i>	<i>TMPRSS2</i>

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

Table 4: Splice variants included in TSO Comprehensive

<i>EGFR</i>
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Table 5: Tumor profiling content under development

MSI
Gene amplifications from DNA
Additional gene fusions and splice variants from RNA

Table 6: TSO Comprehensive content coverage

53 Clinical practice guidelines
60 Drug labels
~860 US clinical trials
Analysis provided by Velsera based on the TSO Comprehensive software Knowledge Base v8.17. Current as of March 2024.

Table 7: CDx indications

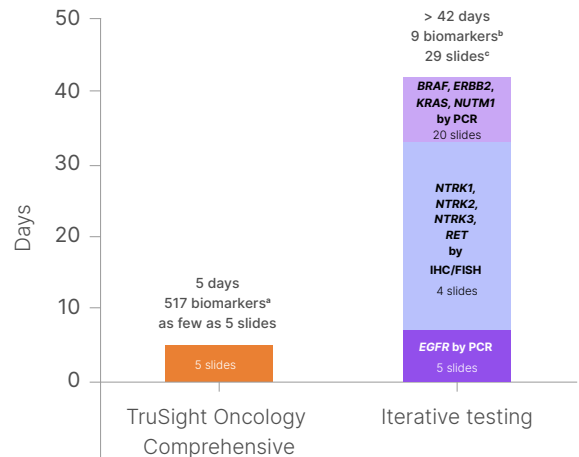
CDx indication	Partner
Solid tumors positive for <i>NTRK1</i> , <i>NTRK2</i> , or <i>NTRK3</i> gene fusions for treatment with VITRAKVI (larotrectinib)	Bayer <sup>10-12</sup>
Non-small cell lung cancer patients positive for <i>RET</i> gene fusions for treatment with RETEVMO (selpercatinib)	Loxo@Lilly <sup>10</sup>
Under development	
<i>EGFR</i>	Teligene <sup>13</sup>
<i>HRD</i>	Myriad Genetics, Merck <sup>14,15</sup>
<i>RET</i> <sup>a</sup>	Loxo@Lilly <sup>10</sup>
<i>TP53</i>	Kartos Therapeutics <sup>16</sup>
MSI	Bristol Myers Squibb <sup>14</sup>

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

a. TSO 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

TSO Comprehensive 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 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.<sup>17-22</sup> In contrast, a CGP test using TSO Comprehensive typically requires five slides and up to five days to generate an actionable report with information on 500+ biomarkers and possible therapies and clinical trials (Figure 4).



a. Includes complex genomic signatures.  
 b. Does not include newer biomarkers such as TMB.  
 c. Does not include any slides required for H&E staining or other initial diagnosis.

Figure 4: Advantages of TSO Comprehensive compared to iterative testing—Example showing potential journeys for NSCLC patient. CGP with TSO Comprehensive provides substantially more coverage with less time and less sample compared to single-gene iterative testing.<sup>17-22</sup>

## One easy-to-interpret, actionable clinical report

TSO Comprehensive results, supported by an expertly curated Knowledge Base, are presented in a single, streamlined, actionable 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 TSO Comprehensive report uses a tiering system to classify variants by clinical relevance level and can help inform therapy decisions according to clinical guidelines (Figure 5). The final 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<sup>23</sup>
- 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<sup>23</sup>

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

## Validated solution

TSO Comprehensive is a validated, sample-to-answer CGP test that includes kitted reagents, an IVD sequencing system (Table 8), 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 TSO Comprehensive

TSO Comprehensive provides a streamlined workflow that spans from sample input to final clinical 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 clinical report can be completed in as few as four days (Figure 1).

Table 8: Verification studies using TSO Comprehensive

Accuracy and clinical bridging studies for <i>NTRK1/2/3</i> and <i>RET</i> 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

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1 **1 Patient sample information**

2 **2 Companion diagnostic results**

3 **3 Cancer mutations with evidence of clinical significance**

4 **4 Cancer mutations with potential clinical significance**

5 **5 Companion diagnostics QC**

6 **6 Companion diagnostics intended uses evaluated**

7 **7 Test information**

8 **8 Test information, continued**

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1 **1 Patient sample information**

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8 **8 Test information, continued**

- 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

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7 **7 Test information**

8 **8 Test information, continued**

**Intended Use**

TruSight™ Oncology Comprehensive is a qualitative in vitro diagnostic test that uses targeted next-generation sequencing to detect variants in 313 genes using nucleic acids extracted from formalin-fixed, paraffin-embedded (FFPE) tumor tissue samples from cancer patients with solid malignant neoplasms using the Illumina® HiSeq™ X5000 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 prescriptive for labeled use of any specific therapeutic product.

Tumor Type	Biomarkers	Therapy
Solid Tumors	NTRK1/3 fusions	VTRAKVIB (larotrectinib)
Non-Small Cell Lung Cancer (NSCLC)	RET fusions	RETENVIB (selpercatinib)

**Informatics Details**

**Companion Diagnostics Results**

This section lists detected Companion Diagnostics variants/biomarkers and associated therapy indications for the patient for which this test is clinically validated.

If a Companion Diagnostic intended use does not match the patient's tumor type, or the associated variant/biomarker was not detected in the patient sample, then a result for the Companion Diagnostic intended use will not be listed here.

**Cancer Mutations with Evidence of Clinical Significance**

This section lists detected variants that have evidence of clinical significance (therapeutic, prognostic, or diagnostic) based on information in FDA-approved drug labels, National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines, or American Society of Clinical Oncology (ASCO) Clinical Practice Guidelines for the patient's tumor type, as specified by the Knowledge Base (curated by Varian).

**Cancer Mutations with Potential Clinical Significance**

This section lists detected variants that have potential clinical significance (therapeutic, prognostic, or diagnostic) based on information in FDA-approved drug labels, National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines, or American Society of Clinical Oncology (ASCO) Clinical Practice Guidelines in another tumor type, multi-genomic, and tumor type eligibility criteria in 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 (curated by Varian).

**Human Reference Genome**

This report uses genomic coordinates based on the hg19 human reference genome.

**Knowledge Base**

Varian provides the rules engine and curates the Knowledge Base, which are used for the classification of detected variants into Cancer Mutations with Evidence of Clinical Significance and Cancer Mutations with Potential Clinical Significance. For details on the content available in the Knowledge Base, refer to the release notes of the Knowledge Base version used to generate this report.

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7 **7 Test information**

8 **8 Test information, continued**

**TruSight™ Oncology Comprehensive Assay Gene Panel**

**Tumor Profiling Gene Panel\***

ABL1	ABL3	ABRAKAS1	ACUR1	ACVR1B	ADORA2	AET1	AET2
AET3	ALK	ALCO12B	AMBR1	ANKRD11	ANKRD26	APC	AR
ARAF	ARFR1	ARID1A	ARID1B	ARID2	ARID5B	ASXL1	ASXL2
ATM	ATR	ATRX	AURKA	AURKB	AXIN1	AXIN2	AXL
B2M	BAP1	BARO1	BBC3	BCL110	BCL2	BCL2L1	BCL2L11
BCL2L2	BCL6	BCOR	BCORL1	BCR	BRCC3	BLM	BMP1L4
BRAF	BRC1	BRC2	BRC4	BRD4	BRP1	BTG1	BTX
CARD11	CASP8	CBF8	CBL	CENPD	CENPD2	CENPD3	CENPE
CD37A	CD37B	CD37C	CD37D	CD37E	CD37F	CD37G	CD37H
CD44	CD46	CD48	CD49A	CD49B	CD49C	CD49D	CD49E
CEBNA	CEBNA	CHD2	CHD4	CHD4L	CHD5	CHD7	CIC
CEP350	CEP350	CEP350	CEP350	CEP350	CEP350	CEP350	CEP350
CTNNA1	CTNNA1	CUL3	CUX1	CACR4	CYLD	DAAX	DCUN1D1
DOR2	DOXA1	DHX15	DICER1	D53	DNAH1	DNMT1	DNMT3A
DNMT3B	DOT1L	EP303	EDD	EGFL7	EGFR	EF1A2	EF1A3
EIF4E	ELOC	EML4	EMSY	EP300	EPCAM	EPHA2	EPHA3
EPHA7	EPHA2	EPHA3	EPHA4	EPHA5	EPHA6	EPHA7	EPHA8
ERCC1	ERCC2	ERCC3	ERCC4	ERCC5	ERCC6	ERCC7	ERCC8
ETV5	ETV6	ETV6L1	ETV7	ETV8	ETV9	ETV10	ETV11
FANCA	FANCB	FANCC	FANCD1	FANCD2	FANCD3	FANCD4	FANCD5
FGF1	FGF10	FGF11	FGF12	FGF13	FGF14	FGF15	FGF16
FGF3	FGF4	FGF5	FGF6	FGF7	FGF8	FGF9	FGFR1
FGFR2	FGFR3	FGFR4	FGFR5	FGFR6	FGFR7	FGFR8	FGFR9
GATA2	GATA3	GATA4	GATA5	GATA6	GATA7	GATA8	GATA9
GATA13	GATA14	GATA15	GATA16	GATA17	GATA18	GATA19	GATA20
HSP90A	HSP90B	HSP90C	HSP90D	HSP90E	HSP90F	HSP90G	HSP90H
HST1H3C	HST1H3D	HST1H3E	HST1H3F	HST1H3G	HST1H3H	HST1H3I	HST1H3J
HST2H3A	HST2H3B	HST2H3C	HST2H3D	HST2H3E	HST2H3F	HST2H3G	HST2H3H
HSD3B1	HSD3B2	HSD3B3	HSD3B4	HSD3B5	HSD3B6	HSD3B7	HSD3B8
IGFBP1	IGFBP2	IGFBP3	IGFBP4	IGFBP5	IGFBP6	IGFBP7	IGFBP8
INPP4A	INPP4B	INSR	IRF2	IRF4	IRS1	IRS2	JAK1

\*Small variants are called in all genes  
† Fusion 2: Splice Variants

- 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 5: Clinical report for TSO 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. The easy-to-read output is intended to increase confidence in treatment decisions.



## Prepare libraries

TSO 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)<sup>24</sup> 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 TSO Comprehensive libraries are sequenced on the NextSeq 550Dx System (Figure 6). 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 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.<sup>25</sup>



Figure 6: 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 TSO Comprehensive workflow from sequencing through final clinical report generation.

### Patient batching throughput

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

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

## Variant calling, interpretation, and reporting

All analysis for TSO 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. Clinicians can use the clinically actionable report to help inform therapy decisions according to clinical practice guidelines, drug labels, and clinical trials.

## Clinically robust Knowledge Base

TSO Comprehensive 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,<sup>26</sup> comprise extensive coverage of peer-reviewed publications, actionable variant information, and the most recent guidelines, drug labels, and clinical trials (Table 9, Figure 7). The TSO Comprehensive Software uses this rich content to determine classifications of the detected genetic variants.

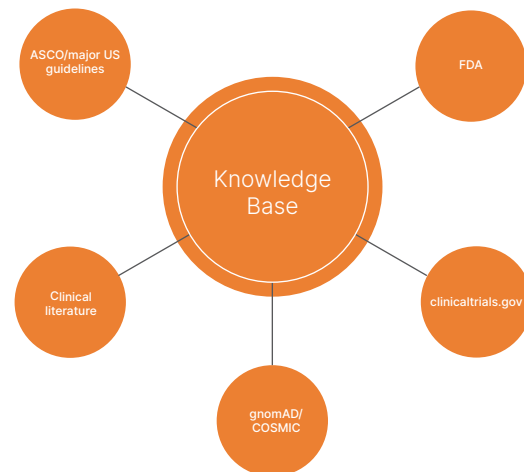


Figure 7: Knowledge Base creation—The TSO Comprehensive Tumor Profiling software is built on a foundation of extensively reviewed rules. Source rules, derived from clinical practice guidelines, drug labels, and primary literature, identify and classify actionable variants. Data from clinical trials and biological annotation databases are independent, standalone sources in the Knowledge Base.

## 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)<sup>†</sup> 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 available<sup>23</sup> 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.

Table 9: Knowledge Base facts as of June 2024<sup>a</sup>

Topic	By the numbers
Drug labels	1700+ labels reviewed 50K+ pages read
Guidelines	300+ oncology practice guidelines, many updated numerous times annually, reviewed 125K+ pages read
Published literature	200K+ papers reviewed 2M+ pages read
Clinical trials	88K+ trials reviewed
Device compliance	7.3K+ procedures, work instructions, forms, and records reviewed 70K+ 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.<sup>23</sup>

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

## Reliable, high performance

The performance characteristics and reliability of TSO 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).<sup>12</sup> Qualitative studies across multiple operators, instruments, reagent lots, and days showed high concordance with minimal variance.<sup>12</sup> For detailed information on the studies performed, refer to the Illumina TruSight Oncology Comprehensive (US) Package Insert.<sup>12</sup>

## 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 TSO Comprehensive, Illumina has addressed some of the biggest challenges, streamlining the process. Starting with a highly validated, FDA-approved, IVD, kitted solution:

- Reduces the time and expense of test implementation compared to a laboratory-developed test (LDT) (Figure 8)
- Expedites CGP moving from a “new” offering to a routine test
- Provides an IVD-compliant test, helping labs prepare 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

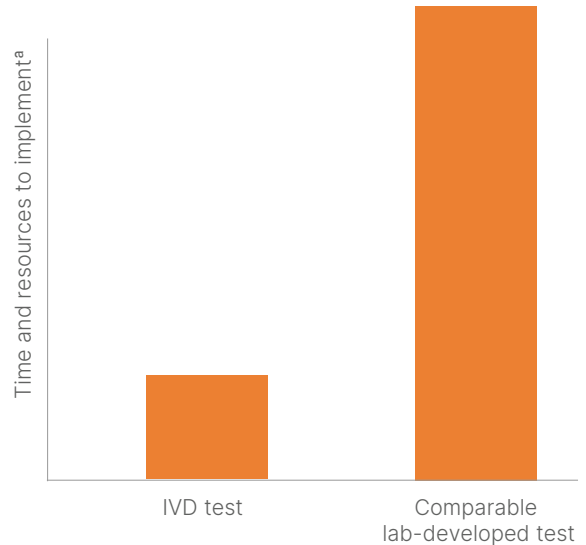


Figure 8: Simpler, less burdensome test implementation—TSO Comprehensive is an FDA-approved IVD test that requires performance verification per guidelines set in 42 CFR 493.1253, which is less burdensome than the validation required for a test developed in the laboratory.

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

- 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

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.<sup>27</sup> 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.<sup>28</sup> 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.

## Summary

The use of CGP testing is leading to improved patient outcomes. Implementing CGP testing in your lab is simplified with TSO Comprehensive. This verified CGP test provides a streamlined workflow, validated reagents, and automated clinical software to take you from sample to clinical report in 4–5 days. Starting from DNA and RNA, use TSO Comprehensive to analyze multiple variant types in 500+ genes in a single test. Produce a clear, clinically relevant final report that accurately identifies actionable mutations that can be used to inform decisions regarding potential matched therapies or clinical trials, according to recognized sources, that might improve patient outcome.

## Learn more

[TruSight Oncology Comprehensive](#)

[Comprehensive genomic profiling \(CGP\)](#)

[NextSeq 550Dx System](#)

## Ordering information

Product	Catalog no.
TruSight Oncology Comprehensive	coming soon
TruSight Oncology DNA Control	coming soon
TruSight Oncology RNA Control	coming soon
NextSeq 550Dx Instrument	20005715
NextSeq 550Dx High-Output Reagent Kit v2.5 (300 cycles) <sup>a</sup>	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.

## 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 TSO 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
<i>EGFR</i>	16.7

LoD—RNA fusions

Fusion	LoD (supporting reads)
<i>EWSR1-FLI1</i>	8.3
<i>TMPRSS2-PMFBP1</i>	9.0
<i>ACPP-ETV1</i>	9.5
<i>CDK4-DPY19L2</i>	10.7
<i>MKRN1-BRAF</i>	11.0
<i>RAF1-VGLL4</i>	11.2
<i>EGFR-GALNT13</i>	12.3
<i>EML4-ALK</i>	12.8
<i>SPIDR-NRG1</i>	12.8
<i>TMPRSS2-ERG</i>	13.2
<i>ESR1-CCDC170</i>	13.5
<i>NCOA4-RET</i>	15.8
<i>DHX8;ETV4-STAT3</i>	16.2
<i>KIF5B-RET</i>	16.6
<i>FGFR3-TACC3</i>	17.5
<i>PAX3-FOXO1</i>	19.0
<i>FGFR1-GSR</i>	23.7
<i>FGFR2-SRPK2</i>	24.7
<i>HNRNPUL1-AXL</i>	26.3
<i>BCL2-IGHJ5</i>	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 <sup>a</sup>	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 <sup>a</sup>	PPC	95% CI <sup>b</sup>
<i>APC</i>	> 3× LOD	Deletion	L1488fs*18	0.15	100.0% (46/46)	92.3%, 100.0%
<i>APC</i>	~2–3× LOD	Deletion	L1488fsTer19	0.181	100.0% (28/28)	87.9%, 100.0%
<i>APC</i>	~2–3× LOD	Deletion	S1465WfsTer3	0.166	100.0% (40/40)	91.2%, 100.0%
<i>APC</i>	~2–3× LOD	Insertion	T1556NfsTer3	0.227	100.0% (32/32)	89.3%, 100.0%
<i>APC</i>	~2–3× LOD	Insertion	S1465fs*9	0.100	100.0% (48/48)	92.6%, 100.0%
<i>ARID1A</i>	< 2× LOD	Insertion	Q372fs*28	0.084	100.0% (4/4)	51.0%, 100.0%
<i>BRAF</i>	~2–3× LOD	SNV	V600E	0.045	91.3% (42/46)	79.7%, 96.6%
<i>BRAF</i>	> 3× LOD	MNV	V600K	0.13	100.0% (46/46)	92.3%, 100.0%
<i>EGFR</i>	~2–3× LOD	Deletion	E746_A750del	0.112	100.0% (46/46)	92.3%, 100.0%
<i>EGFR</i>	~2–3× LOD	SNV	L858R	0.045	100.0% (38/38)	90.8%, 100.0%
<i>EP300</i>	~2–3× LOD	Deletion	H2324fs*29	0.245	100.0% (44/44)	92.0%, 100.0%
<i>ERBB2</i>	~2–3× LOD	Insertion	Y772_A775dup	0.075	100.0% (36/36)	90.4%, 100.0%
<i>FBXW7</i>	> 3× LOD	Insertion	T15_G16insP	0.13	100.0% (44/44)	92.0%, 100.0%
<i>IDH1</i>	~2–3× LOD	SNV	R132H	0.155	100.0% (36/36)	90.4%, 100.0%
<i>KRAS</i>	~2–3× LOD	MNV	G12I	0.111	100.0% (38/38)	90.8%, 100.0%
<i>NOTCH1</i>	~2–3× LOD	Insertion	R1598fs*12	0.146	100.0% (48/48)	92.6%, 100.0%
<i>PTEN</i>	~2–3× LOD	Deletion	T319fs*1	0.157	100.0% (44/44)	92.0%, 100.0%
<i>TP53</i>	< 2× LOD	Insertion	P152_P153dup	0.157	100.0% (2/2)	34.2%, 100.0%
<i>TP53</i>	~2–3× LOD	Insertion	R333HfsTer5	0.154	100.0% (48/48)	92.6%, 100.0%
<i>TP53</i>	> 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. VAF, variant allele frequency; PPC, percent positive call; CI, confidence interval

a. Mean VAF calculated from observed assay results.

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



Reproducibility for tumor profiling—RNA variants

Targeted variant	Observed variant level <sup>a</sup>	Variant type	Mean supporting reads <sup>b</sup>	PPC	95% CI <sup>c</sup>
<i>ACPP-ETV1</i>	> 3× LOD	Fusion	44.7	100.0% (46/46)	92.3%, 100.0%
<i>BCL2-IGHJ5</i>	> 3× LOD	Fusion	124.9	100.0% (46/46)	92.3%, 100.0%
<i>CDK4-DPY19L2</i>	> 3× LOD	Fusion	55.7	97.8% (45/46)	88.7%, 99.6%
<i>DHX8;ETV4-STAT3</i>	~2–3× LOD	Fusion	48.9	100.0% (46/46)	92.3%, 100.0%
<i>EGFR-GALNT13</i>	> 3× LOD	Fusion	49.8	100.0% (46/46)	92.3%, 100.0%
<i>EML4-ALK</i>	> 3× LOD	Fusion	49.3	100.0% (48/48)	92.6%, 100.0%
<i>ESR1-CCDC170</i>	~2–3× LOD	Fusion	45.1	100.0% (46/46)	92.3%, 100.0%
<i>EWSR1-FLI1</i>	> 3× LOD	Fusion	31.6	100.0% (48/48)	92.6%, 100.0%
<i>FGFR1-GSR</i>	~2–3× LOD	Fusion	61.1	100.0% (46/46)	92.3%, 100.0%
<i>FGFR2-SRPK2</i>	~2–3× LOD	Fusion	53.4	100.0% (48/48)	92.6%, 100.0%
<i>FGFR3-TACC3</i>	~2–3× LOD	Fusion	53.5	100.0% (48/48)	92.6%, 100.0%
<i>HNRNPUL1-AXL</i>	~2–3× LOD	Fusion	58.0	100.0% (48/48)	92.6%, 100.0%
<i>KIF5B-RET</i>	< 2× LOD	Fusion	11.6	91.7% (44/48)	80.4%, 96.7%
<i>MKRN1-BRAF</i>	~2–3× LOD	Fusion	33.4	100.0% (48/48)	92.6%, 100.0%
<i>PAX3-FOXO1</i>	> 3× LOD	Fusion	70.1	100.0% (48/48)	92.6%, 100.0%
<i>RAF1-VGLL4</i>	< 2× LOD	Fusion	15.9	100.0% (46/46)	92.3%, 100.0%
<i>SPIDR-NRG1</i>	> 3× LOD	Fusion	51.5	100.0% (48/48)	92.6%, 100.0%
<i>TMPRSS2-ERG</i>	~2–3× LOD	Fusion	43.5	97.9% (47/48)	89.1%, 99.6%
<i>EGFRvIII</i>	> 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 <sup>a</sup> )	NPA (n/N) (95% CI <sup>a</sup> )
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 TSO 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  $\geq$  5% were considered, the PPA for Level 3 SNVs, insertions, and deletions was > 96%. TSO 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; NPA, negative percent agreement; PPA, positive percent agreement.

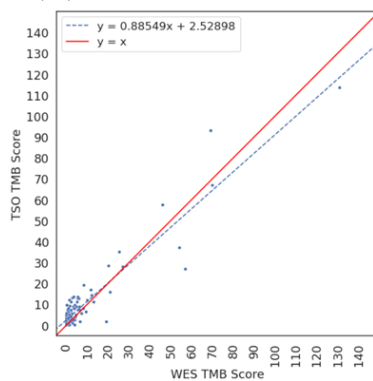
a. 95% two-sided CI calculated via the Wilson Score method.

Accuracy for tumor profiling—RNA variants

Variant type	Orthogonal method	PPA	NPA
Fusions	<ul style="list-style-type: none"> <li>RNA whole-exome sequencing fusions (RNGS1)</li> <li>Targeted NGS fusion panel (RNGS2)</li> <li>Droplet digital PCR (ddPCR)</li> </ul>	87.29% (95% CI <sup>a</sup> : 67.83%, 96.35%)	99.99% (95% CI <sup>a</sup> : 99.98%, >99.99%)
Splice variant	qPCR	100% (3/3) (95% CI <sup>b</sup> : 44%, 100%)	100% (13/13) (95% CI <sup>b</sup> : 77%–100%)

The ability of TSO 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 TSO Comprehensive can detect fusions. However, the limit of detection of this method was 4–8× that of TSO 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 TSO Comprehensive panel. Accuracy for splice variant detection was calculated by comparing TSO Comprehensive results to qPCR assays for *EGFRvIII*. NPA, negative percent agreement; PPA, positive percent agreement; RNGS, RNA next-generation sequencing.

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



Tumor profiling accuracy—TMB—The ability of TSO Comprehensive to detect 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 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, multinucleotide 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 10](#), 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 10](#) of the intended use statement are not conclusive or prescriptive for labeled use of any specific therapeutic product.

Table 10: Companion Diagnostic Indications

Tumor Type	Biomarker(s) Detected	Therapy
Solid Tumors	<i>NTRK1/2/3</i> fusions	VITRAKVI® (larotrectinib)
Non-Small Cell Lung Cancer	<i>RET</i> fusions	RETEVMO® (selpercatinib)



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

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