

TruSight™ Oncology Comprehensive

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

- Detect actionable biomarkers across solid tumors using minimal patient biopsy tissue
- Assess current and emerging biomarkers from clinical practice guidelines, drug labels, and clinical trials simultaneously
- Produce an easy-to-interpret results report in 4–5 days
- Become a precision medicine provider by offering CGP testing in your institution



Revolutionizing cancer diagnostics

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

Offering a CGP test in house provides numerous benefits, including the ability to maintain control over the patient’s biopsy and data, further empowering you as a precision medicine provider and increasing your participation in patient care. CGP can be a complex undertaking when implemented as a laboratory-developed test (LDT). As a validated, US FDA-approved, IVD, kitted solution,

TruSight Oncology Comprehensive (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 results reports. Sequencing is performed on the FDA-registered NextSeq™ 550Dx Instrument. Using this solution, labs can provide CGP testing that yields timely, reliable information regarding relevant biomarkers as noted in primary literature, guidelines, drug labels, and clinical trials in less time and using less biopsy sample than current iterative methods.

Fully automated sequencing and data analysis

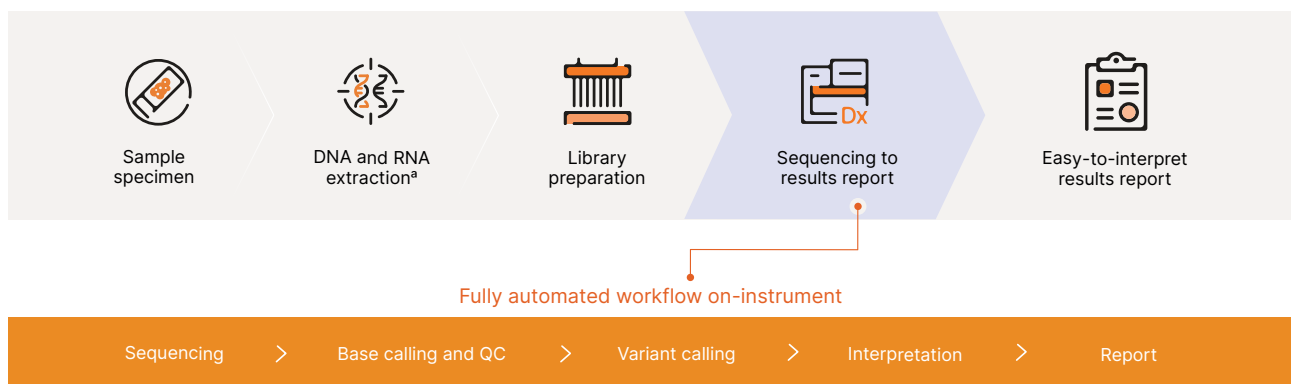


Figure 1: TSO Comprehensive workflow—Batch up to seven patient samples and two control samples per run using TSO Comprehensive. The library preparation and enrichment steps take 2 days. The fully automated workflow on the NextSeq 550Dx Instrument sequences samples; performs base calling and QC, variant calling, and interpretation, and generates a results 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 ^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	<ul style="list-style-type: none"> • Small DNA variants in 517 genes • Fusions in 24 genes • Splice variants in 1 gene • TMB
Variant types detected	<ul style="list-style-type: none"> • DNA variants: SNVs, MNVs, insertions, deletions • RNA variants: fusions, splice variants • Complex genomic signatures: TMB
Panel size	1.94 Mb DNA, 358 kb RNA
DNA input requirement	40 ng genomic DNA
RNA input requirement	40 ng total RNA
FFPE input requirement	Recommended tissue volume $\geq 1 \text{ mm}^3$ 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 μm sections, 20 mm^2 tissue area each)
Total assay time	4-5 days from nucleic acid to results report
Limit of detection	See Appendix
False positives by DNA variant type	Small DNA variants, 0.0001% TMB, N/A
False positives by RNA variant type	RNA fusions, 0% RNA splice variants, 0%

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

Comprehensive biomarker profiling

Single-gene tests and targeted hotspot panels are limited in the number of targets they analyze and the type of variants they can detect. CGP with 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), multi-nucleotide variants (MNVs), insertion, deletions, fusions, and splice variants (Figure 2). In addition, the test detects emerging immunotherapy biomarkers (ie, TMB⁷⁻⁹) (Table 5). Note that not all clinically actionable content is currently available on the results report and additional tumor profiling claims are under development (Table 6). Content provides significant coverage of key guidelines for multiple tumor types and genes linked to clinical trials (Figure 3, Table 7). The inclusive nature of TSO Comprehensive maximizes the chances of finding a positive biomarker.

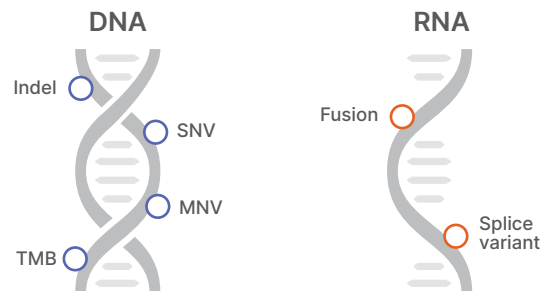


Figure 2: Genomic variations detected by TSO Comprehensive.

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.¹⁰⁻¹² Additional CDx indications, currently under development, will be included once they receive the appropriate regulatory approvals (Table 8).





Pan-cancer: BRAF, ERBB2, ^a NTRK1, NTRK2, NTRK3, RET, TMB													
Genes with biomarkers of clinical significance ^b												Genes with biomarkers of potential clinical significance ^c	
	Breast	AKT1	BRCA1	BRCA2	ESR1	PALB2	PIK3CA	PTEN					94
	Colorectal	KRAS	NRAS	POLD1	POLE								96
	Bone	ERG	ETV1	ETV4	EWSR1	FEV	FLI1	FUS	HEY1	IDH1	NCOA2	PATZ1	97
	Lung	ALK ^a	EGFR	KRAS	MET ^b	MTOR	ROS1 ^c						120
	Melanoma	KIT	NRAS	ROS1 ^c									105
	Ovarian	BRCA1	BRCA2										177
	CNS^d	APC	ATRX	CDKN2A	CDKN2B	CTNNB1	EGFR	H3F3A	HIST1H3B	HIST1H3C	IDH1	IDH2	69
		MYCN	NF1	PTCH1	SMO	TERT	TP53						
	Prostate	ATM	ATR	BARD1	BRCA1	BRCA2	BRIP1	CDK12	CHEK1	CHEK2	FANCA	FANCL	89
		FGFR3	MLH1	MRE11	NBN	PALB2	PTEN	RAD51B	RAD51C	RAD51D	RAD54L	TACC3	
	Thyroid	TERT											96
	Uterine and cervical	BCOR	BRCA2	ESR1	FOXO1	GREB1	NCOA1	NCOA2	NCOA3	NUTM2A	NUTM2B	PAX3	124
		PAX7	POLE	SMARCA4	TP53	YWHAE	ZC3H7B						

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 v8.20 (September 2024).

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

Table 2: Small DNA variants 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	INHAA	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

Table 3: Fusions from RNA included in TSO Comprehensive^a

<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^b</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 from RNA included in TSO Comprehensive

<i>EGFR</i>

Table 5: Genomic signatures included in TSO Comprehensive

TMB

Table 6: Select gene and variant content not currently included in the results report

MSI
<i>ERBB2</i> and <i>MET</i> gene amplifications from DNA
<i>ALK</i> and <i>ROS1</i> fusions from RNA
<i>MET</i> splice variants from RNA

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

Table 7: TSO Comprehensive content coverage

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

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

Table 8: CDx indications

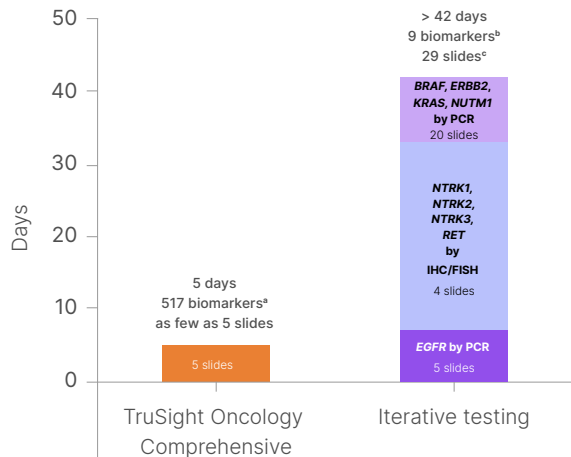
CDx indication	Pharmaceutical partner
Solid tumors positive for <i>NTRK1</i> , <i>NTRK2</i> , or <i>NTRK3</i> gene fusions for treatment with VITRAKVI (larotrectinib)	Bayer ¹⁰⁻¹²
Non-small cell lung cancer (NSCLC) patients positive for <i>RET</i> gene fusions for treatment with RETEVMO (selpercatinib)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

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.¹⁶⁻²¹ In contrast, a CGP test using TSO Comprehensive typically requires five slides and up to five days to generate a report with information on 500+ biomarkers and possible therapies and clinical trials (Figure 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.¹⁶⁻²¹

One easy-to-interpret results report

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

- Patient sample information—sample ID number, tumor type, sex, QC analysis, run ID, and Knowledge Base details
- Companion Diagnostic Results—detected variants or biomarkers that have a companion diagnostic intended use evaluated for the sample
- Cancer Mutations with Evidence of Clinical Significance—detected variants that have evidence of clinical significance (therapeutic, prognostic, or diagnostic) based on information in FDA-approved drug labels or ASCO and major US clinical practice guidelines for the patient’s tumor type, as specified by the Knowledge Base²²
- 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 engine²²

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

illumina | TruSight[™] Oncology Comprehensive (US) FOR IN VITRO DIAGNOSTIC USE Report Date 2024-09-19

1 **1 Patient sample information**

2 **2 Companion diagnostic results**

Detected Variant/Biomarkers	Therapy	Usage	Details
LMNA-NTRK1 Fusion	VITRAKVI® (larotrectinib)	Indicated	Type: Fusion Breakpoint 1: chr1:156100562 Breakpoint 2: chr1:156846691 Fusion Supporting Reads: 64
XIP58-RET Fusion	RETENVO® (selpercatinib)	Indicated	Type: Fusion Breakpoint 1: chr10:32306071 Breakpoint 2: chr10:43629927 Fusion Supporting Reads: 73

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**

illumina | TruSight[™] Oncology Comprehensive (US) Sample ID 2024_0919_01_001-001 Tumor Type Non-small cell lung Knowledge Base Version 8.9.0.026 Report Date 2024-09-19

5 **5 Companion diagnostics QC**

6 **6 Companion diagnostics intended uses evaluated**

Tumor Type	Biomarkers	Therapy	CDU Intended Use Evaluated	Comments
Solid Tumor	NTRK1, NTRK2 & NTRK3 Gene Fusions	VITRAKVI® (larotrectinib)	Evaluated	—
Non-small cell lung cancer	RET Gene Fusions	RETENVO® (selpercatinib)	Evaluated	—

- 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

illumina | TruSight[™] Oncology Comprehensive (US) Sample ID 2024_0919_01_001-001 Tumor Type Non-small cell lung Knowledge Base Version 8.9.0.026 Report Date 2024-09-19

7 **7 Test information**

8 **8 Test information, continued**

illumina | TruSight[™] Oncology Comprehensive (US) Sample ID 2024_0919_01_001-001 Tumor Type Non-small cell lung Knowledge Base Version 8.9.0.026 Report Date 2024-09-19

8 **8 Test information, continued**

TruSight[™] Oncology Comprehensive Assay Gene Panel

Tumor Profiling Gene Panel*

ABL1	ABL2	ABRAKIS1	ACUR1	ACVR1B	ADORA2	AET1	AET2
AET3	ALK	ALCO128	AMBR1	ANKRD11	ANKRD26	APC	AR
ARAF	ARFP1	ARID1A	ARID1B	ARID2	ARID5B	ASXL1	ASXL2
ATM	ATR	ATRX	AURKA	AURKB	AXIN1	AXIN2	AXL
B2M	BAP1	BARO1	BBC3	BCL10	BCL2	BCL2L1	BCL2L11
BCL2L2	BCL6	BCOR	BCORL1	BCR	BRCC3	BLM	BMP1A
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	CHK1	CHK2	CIC	COP1
CREBBP	CRKL	CRU2	CSF1R	CSF3R	CSNK1A1	CTCF	CTSLA
CTNNA1	CTNNA1	CUL3	CUX1	CXCR4	CYLD	DAAX	DCUN1D1
DOR2	DOXA1	DHX15	DICER1	DISE3	DNAB1	DNMT1	DNMT3A
DNMT3B	DOT1L	EP3	EED	EGFL7	EGFR	EJF1A3	EF442
E2F4	ELOC	EML4	EMSY	EP300	EPCAM	EPHA3	EPHA5
EPHA7	EPH81	EPH82	EPH83	EPH84	EPH85	EPH86	EPH87
EPHA8	EPH91	EPH92	EPH93	EPH94	EPH95	EPH96	EPH97
EPHA9	EPH10	EPH11	EPH12	EPH13	EPH14	EPH15	EPH16
EPHA17	EPHA2	EPHA3	EPHA4	EPHA5	EPHA6	EPHA7	EPHA8
EPHA9	EPHA10	EPHA11	EPHA12	EPHA13	EPHA14	EPHA15	EPHA16
EPHA17	EPHA18	EPHA19	EPHA20	EPHA21	EPHA22	EPHA23	EPHA24
EPHA25	EPHA26	EPHA27	EPHA28	EPHA29	EPHA30	EPHA31	EPHA32
EPHA33	EPHA34	EPHA35	EPHA36	EPHA37	EPHA38	EPHA39	EPHA40
EPHA41	EPHA42	EPHA43	EPHA44	EPHA45	EPHA46	EPHA47	EPHA48
EPHA49	EPHA50	EPHA51	EPHA52	EPHA53	EPHA54	EPHA55	EPHA56
EPHA57	EPHA58	EPHA59	EPHA60	EPHA61	EPHA62	EPHA63	EPHA64
EPHA65	EPHA66	EPHA67	EPHA68	EPHA69	EPHA70	EPHA71	EPHA72
EPHA73	EPHA74	EPHA75	EPHA76	EPHA77	EPHA78	EPHA79	EPHA80
EPHA81	EPHA82	EPHA83	EPHA84	EPHA85	EPHA86	EPHA87	EPHA88
EPHA89	EPHA90	EPHA91	EPHA92	EPHA93	EPHA94	EPHA95	EPHA96
EPHA97	EPHA98	EPHA99	EPHA100	EPHA101	EPHA102	EPHA103	EPHA104
EPHA105	EPHA106	EPHA107	EPHA108	EPHA109	EPHA110	EPHA111	EPHA112
EPHA113	EPHA114	EPHA115	EPHA116	EPHA117	EPHA118	EPHA119	EPHA120
EPHA121	EPHA122	EPHA123	EPHA124	EPHA125	EPHA126	EPHA127	EPHA128
EPHA129	EPHA130	EPHA131	EPHA132	EPHA133	EPHA134	EPHA135	EPHA136
EPHA137	EPHA138	EPHA139	EPHA140	EPHA141	EPHA142	EPHA143	EPHA144
EPHA145	EPHA146	EPHA147	EPHA148	EPHA149	EPHA150	EPHA151	EPHA152
EPHA153	EPHA154	EPHA155	EPHA156	EPHA157	EPHA158	EPHA159	EPHA160
EPHA161	EPHA162	EPHA163	EPHA164	EPHA165	EPHA166	EPHA167	EPHA168
EPHA169	EPHA170	EPHA171	EPHA172	EPHA173	EPHA174	EPHA175	EPHA176
EPHA177	EPHA178	EPHA179	EPHA180	EPHA181	EPHA182	EPHA183	EPHA184
EPHA185	EPHA186	EPHA187	EPHA188	EPHA189	EPHA190	EPHA191	EPHA192
EPHA193	EPHA194	EPHA195	EPHA196	EPHA197	EPHA198	EPHA199	EPHA200

- 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: Results 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.

Validated solution

TSO Comprehensive is a validated (Table 9), sample-to-answer CGP test that includes kitted reagents, an IVD sequencing system, and analysis software. The test was developed using a rigorous design control process and validated across > 1400 unique FFPE samples and > 15 different tumor types. Results were compared to orthogonal methods to ensure accurate, reproducible, and consistent data.

Table 9: Validation 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

Using TSO Comprehensive

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

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

Enrich libraries to focus on clinically relevant content

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

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

Sequence with diagnostic power

Prepared 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.²⁴



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 results 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.

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

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

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.

Clinically robust Knowledge Base

TSO Comprehensive Software is supported by a purpose-built over time, clinically derived rules engine and Knowledge Base to maximize actionability. The rules engine and supported Knowledge Base, both provided by Velsera,²⁵ comprise extensive coverage of peer-reviewed publications, actionable variant information, and the most recent guidelines, drug labels, and clinical trials (Figure 7, Table 10). The TSO Comprehensive Software uses this rich content to determine classifications of the detected genetic variants.

Expertly curated content and rules engine

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

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

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

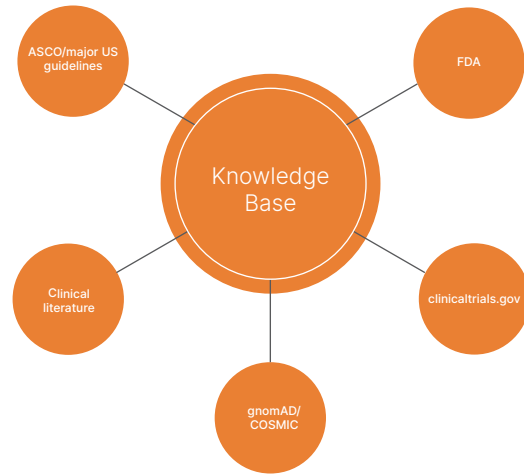


Figure 7: Knowledge Base creation—The TSO Comprehensive Tumor Profiling software is built on a foundation of extensively reviewed rules. Source rules, derived from clinical practice guidelines, drug labels, and primary literature, identify and classify actionable variants. Data from clinical trials and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

Table 10: 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

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 available²² 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 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).¹² Qualitative studies across multiple operators,

instruments, reagent lots, and days showed high concordance with minimal variance.¹² For detailed information on the studies performed, refer to the Illumina TruSight Oncology Comprehensive (US) Package Insert.¹²

Bring CGP testing into your lab

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

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

Streamlined implementation

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

- Verify over validate, reducing the time and expense of test implementation compared to a laboratory-developed test (LDT) (Figure 8)
- Expedite CGP moving from a “new” offering to a routine test
- Provide an IVD-compliant test, helping prepare labs to meet stricter regulatory guidelines

Comprehensive support

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

- Onboarding plan to expedite test verification
- Laboratory 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.

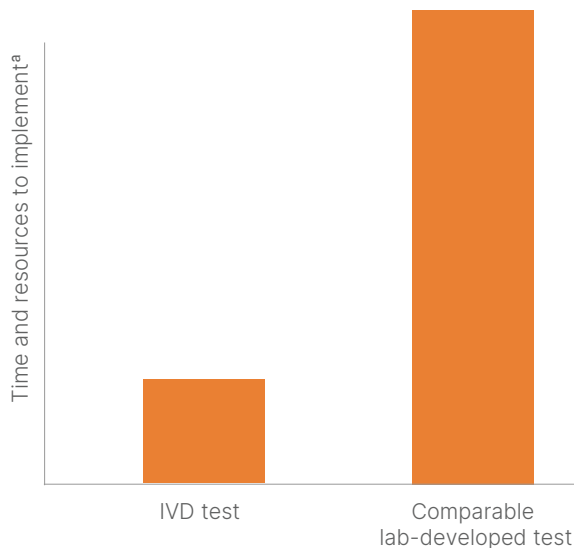


Figure 8: Simpler, less burdensome test implementation—TSO Comprehensive is an FDA-approved IVD test that requires performance verification per guidelines set in 42 CFR 493.1253, which is less burdensome than the validation required for a test developed in the laboratory.

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

Access to reimbursement

CGP test coverage is an important consideration when bringing the capability in house. IVD CGP tumor profiling assays with CDx claims across solid, malignant neoplasms are covered for eligible Medicare beneficiaries throughout the US under National Coverage Determination (NCD) 90.2.²⁶ Commercial coverage for assays with this indication increases by more than a third of US commercially insured lives as compared to assays without CDx claims.²⁷ Illumina has established a dedicated Market Access team that is actively working with payers to expand CGP test reimbursement across the globe.

Discuss available coverage options with your local Illumina Account Manager.

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 results 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 results report that can be incorporated into a report produced by the laboratory and provided to clinicians 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](#)

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 ^a	2	0.038–0.051
	Insertion (3-5 bp)	2	0.030–0.056
	Insertion (> 5 bp and up to 25 bp)	3	0.034–0.215
	Deletion (1-2 bp) near homopolymer repeats	2	0.094–0.100
	Deletion (1-2 bp) near dinucleotide repeats	2	0.033–0.070
	Deletion (3-5 bp)	2	0.028–0.064
	Deletion (> 5 bp and up to 25 bp)	2	0.047–0.055

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

Reproducibility for tumor profiling studies

Reproducibility for tumor profiling—small DNA variants

Gene	Observed variant level	Variant type	Targeted variant (amino acid)	Mean VAF ^a	PPC	95% CI ^b
<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 ^a	Variant type	Mean supporting reads ^b	PPC	95% CI ^c
<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 ^a)	NPA (n/N) (95% CI ^a)
SNVs	Level 2	96.1% (99/103) (90.4%–98.5%)	> 99.9% (9832/9833) (99.9%– > 99.9%)
	Level 3	76.9% (373/485) (73.0%–80.4%)	>99.9% (212,277/212,311) (> 99.9%– > 99.9%)
	All	80.3% (472/588) (76.9%–83.3%)	> 99.9% (219,211/219,246) (> 99.9%– > 99.9%)
MNVs	Level 2	100.0% (5/5) (56.6%–100.0%)	100.0% (9931/9931) (> 99.9%– > 99.9%)
	Level 3	90.0% (9/10) (59.6%–98.2%)	> 99.9% (212,785/212,786) (> 99.9%– >99.9%)
	All	93.3% (14/15) (70.2%–98.8%)	> 99.9% (219,818/219,819) (> 99.9%– > 99.9%)
Insertions	Level 2	100.0% (1/1) (20.7%–100.0%)	100.0% (9935/9935) (> 99.9%–100.0%)
	Level 3	86.0% (49/57) (74.7%–92.7%)	> 99.9% (212,738/212,739) (> 99.9%– > 99.9%)
	All	86.2% (50/58) (75.1%–92.8%)	> 99.9% (219,775/219,776) (> 99.9%– > 99.9%)
Deletions	Level 2	66.7% (2/3) (20.8%–93.9%)	> 99.9% (9932/9933) (99.9%– > 99.9%)
	Level 3	90.3% (139/154) (84.6%–94.0%)	> 99.9% (212,624/212,642) (> 99.9%– >99.9%)
	All	89.8% (141/157) (84.1%–93.6%)	> 99.9% (219,658/219,677) (> 99.9%– > 99.9%)
All variants	Level 2	95.5% (107/112) (90.0%–98.1%)	> 99.9% (9822/9824) (99.9%– > 99.9%)
	Level 3	80.7% (570/706) (77.7%–83.5%)	> 99.9% (212,036/212,090) (> 99.9%– > 99.9%)
	All	82.8% (677/818) (80.0%–85.2%)	> 99.9% (218,960/219,016) (> 99.9%– > 99.9%)

The detection of small DNA variants was compared to another externally validated NGS (evNGS) panel assay (comparator method). The comparison between small DNA variants, consisting of SNVs, MNVs, insertions, and deletions, was based on 414 FFPE samples from 16 different tissue types that were valid for both 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.

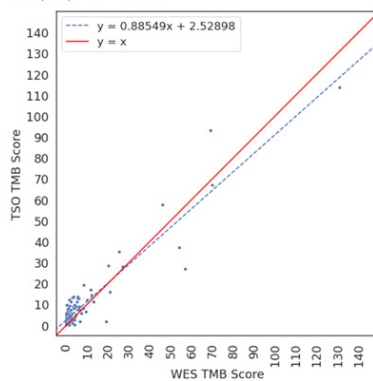
Accuracy for tumor profiling—RNA variants

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

The ability of 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.

Ordering information

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

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

References

- Zehir A, Benayed R, Shah RH, et al. **Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients.** *Nat Med.* 2017;23(6):703-713. doi:10.1038/nm.4333
- Soumerai TE, Donoghue MTA, Bandlamudi C, et al. **Clinical Utility of Prospective Molecular Characterization in Advanced Endometrial Cancer.** *Clin Cancer Res.* 2018;24(23):5939-5947. doi:10.1158/1078-0432.CCR-18-0412
- Gutierrez ME, Choi K, Lanman RB, et al. **Genomic Profiling of Advanced Non-Small Cell Lung Cancer in Community Settings: Gaps and Opportunities.** *Clin Lung Cancer.* 2017;18(6):651-659. doi:10.1016/j.clcc.2017.04.004
- Singal G, Miller PG, Agarwala V, et al. **Association of Patient Characteristics and Tumor Genomics With Clinical Outcomes Among Patients With Non-Small Cell Lung Cancer Using a Clinicogenomic Database.** *JAMA.* 2019;321(14):1391-1399. doi:10.1001/jama.2019.3241
- Kato S, Kim KH, Lim HJ, et al. **Real-world data from a molecular tumor board demonstrates improved outcomes with a precision N-of-One strategy.** *Nat Commun.* 2020;11:4965 (2020). doi.org/10.1038/s41467-020-18613-3
- Rozenblum AB, Ilouze M, Dudnik E, et al. **Clinical Impact of Hybrid Capture-Based Next-Generation Sequencing on Changes in Treatment Decisions in Lung Cancer.** *J Thorac Oncol.* 2017;12(2):258-268. doi:10.1016/j.jtho.2016.10.021
- U.S. Food & Drug Administration. FDA approves pembrolizumab for adults and children with TMB-H solid tumors. FDA website. [fda.gov/drugs/ drug-approvals-and-databases/fda-approves-pembrolizumab-adults-and-children-tmb-h-solid-tumors](https://www.fda.gov/drugs/drug-approvals-and-databases/fda-approves-pembrolizumab-adults-and-children-tmb-h-solid-tumors). Released June 17, 2020. Accessed July 29, 2024.
- Tray N, Weber JS, Adams S. **Predictive Biomarkers for Checkpoint Immunotherapy: Current Status and Challenges for Clinical Application.** *Cancer Immunol Res.* 2018;6(10):1122-1128. doi:10.1158/2326-6066.CIR-18-0214
- Samstein RM, Lee CH, Shoushtari AN, et al. **Tumor mutational load predicts survival after immunotherapy across multiple cancer types.** *Nat Genet.* 2019;51(2):202-206. doi.org/10.1038/s41588-018-0312-8
- Illumina and Loxo Oncology to Partner on Developing Next-Generation Sequencing-Based Pan-Cancer Companion Diagnostics. [businesswire.com/news/home/20180410005649/en/](https://www.businesswire.com/news/home/20180410005649/en/). Released April 10, 2018. Accessed July 29, 2024.
- As Lilly deal closes, Bayer secures full rights to Loxo's Vitrakvi. [biopharmadive.com/news/as-lilly-deal-closes-bayer-secures-full-rights-to-loxos-vitrakvi/548584/](https://www.biopharmadive.com/news/as-lilly-deal-closes-bayer-secures-full-rights-to-loxos-vitrakvi/548584/). Released February 15, 2019. Accessed July 29, 2024.
- Illumina. TruSight Oncology Comprehensive Package Insert. https://support.illumina.com/sequencing/sequencing_kits/tru-sight-oncology-comprehensive-us.html. Accessed November 1, 2024.
- Illumina and Taolue Biopharmaceuticals Collaborate to Help Chinese Innovation Go Global. <https://www.illumina.com/company/news-center/press-releases/2023/648d0d26-1f0a-4b65-b387-2c272761fbd711111.html>. Released June 18, 2019. Accessed July 29, 2024.
- Illumina Announces New and Expanded Oncology Partnerships with Bristol Myers Squibb, Kura Oncology, Myriad Genetics, and Merck to Advance Comprehensive Genomic Profiling. [businesswire.com/news/home/20210111005930/en/Illumina-Announces-New-and-Expanded-Oncology-Partnerships-with-Bristol-Myers-Squibb-Kura-Oncology-Myriad-Genetics-and-Merck-to-Advance-Comprehensive-Genomic-Profilng](https://www.businesswire.com/news/home/20210111005930/en/Illumina-Announces-New-and-Expanded-Oncology-Partnerships-with-Bristol-Myers-Squibb-Kura-Oncology-Myriad-Genetics-and-Merck-to-Advance-Comprehensive-Genomic-Profilng). Released January 11, 2021. Accessed July 29, 2024.
- Illumina. Illumina and Kartos Therapeutics Announce New Oncology Partnership to Develop an NGS-Based TP53 Companion Diagnostic. [illumina.com/company/news-center/press-releases/2021/12b6e4a6-3f52-407e-8200-8fa72712a980.html](https://www.illumina.com/company/news-center/press-releases/2021/12b6e4a6-3f52-407e-8200-8fa72712a980.html). Released 2021. Accessed July 29, 2024.
- NeoGenomics Laboratories. RET FISH Analysis. NeoGenomic Laboratories website. <https://neogenomics.com/test-menu/ret-fish>. Accessed July 29, 2024.
- Mayo Clinic Laboratories. EGFRW - EGFR Targeted Mutation Analysis with ALK Reflex, Tumor. <https://www.mayocliniclabs.com/test-catalog/Overview/614666#Performance>. Accessed July 29, 2024.

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

Intended use statement

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

The test is intended to be used as a companion diagnostic to identify cancer patients who may benefit from treatment with the targeted therapies listed in [Table 11](#), 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 11](#) of the intended use statement are not conclusive or prescriptive for labeled use of any specific therapeutic product.

Table 11: Companion Diagnostic Indications

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



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

© 2024 Illumina, Inc. All rights reserved. All trademarks are the property of Illumina, Inc. or their respective owners. For specific trademark information, see www.illumina.com/company/legal.html.
 M-AMR-01466 v3.0