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TruSight™ Oncology 500 and TruSight Oncology 500 High-Throughput*

Enabling comprehensive genomic profiling from FFPE samples, with flexibility and scalability.

Highlights

- **Save time and sample with a multiplex assay**
  Comprehensive, pan-cancer content spanning 523 cancer-relevant genes aligned with key guidelines and clinical trials

- **Unlock immuno-oncology with TMB and MSI**
  1.94 Mb of content with sophisticated algorithms enable accurate analysis of TMB and MSI

- **Achieve confidence in results**
  Enrichment chemistry including UMIs coupled with an informatics pipeline for high accuracy in variant detection

- **Address the needs of the oncology community both today and tomorrow**
  Relevant and emerging biomarkers support a future-proof foundation for new solutions

One workflow for multiple tumor types and multiple biomarkers

Comprehensive genomic profiling used in recent studies with large cohorts has shown that up to 90% of samples may have informative alterations.[1-6] With limited time to return results and limited amounts of tissues, a single, comprehensive assay that assesses a wide range of biomarkers increases the chances of obtaining relevant information. To help researchers address this challenge, Illumina offers TruSight Oncology 500 and TruSight Oncology 500 High-Throughput, next-generation sequencing (NGS) assays that analyze 523 cancer-relevant genes from both DNA and RNA[‡] in one integrated workflow. The assays assess multiple variant types (Table 1), including small nucleotide variants (SNVs), insertions/deletions (indels), copy-number variations (CNVs), splice variants, fusions, and emerging biomarkers that rely on analysis of multiple genomic loci, such as tumor mutational burden (TMB) and microsatellite instability (MSI).

Hybrid-capture chemistry is optimized to capture nucleic acid targets from formalin-fixed, paraffin-embedded (FFPE) tissues. During DNA library preparation, addition of unique molecular identifiers (UMIs)[7] enables detection of variants at low variant allele frequency (VAF) while simultaneously suppressing errors, thus providing high specificity. Variant-calling software is developed in concert with the assay reagents.

Table 1: Variant types detected by TruSight Oncology 500 and TruSight Oncology 500 High-Throughput

Variant type	Relevant examples
SNVs and indels	*KRAS* G12D, *EGFR* exon 19 deletions, *BRAF* V600E
Fusions	*ALK*, *ROS1*, *NTRK1*, *NTRK2*, *NTRK3*
Splice variants	*MET* exon 14
CNVs	*HER2*
MSI	MSI-High
TMB	TMB-High

Illumina has established partnerships with several academic centers, pharmaceutical companies, and advocacy groups, to assist with the design, development, and evaluation of new oncology applications. To facilitate such endeavors, both TruSight Oncology 500 assays are easily integrated into current lab workflows (Figure 1). Using proven Illumina technology, with gene content relevant across multiple tumor types and including emerging biomarkers, TruSight Oncology 500 is well-positioned to be the foundation for developing future oncology diagnostic solutions.

Flexibility and scalability options

By offering both TruSight Oncology 500 and TruSight Oncology 500 High-Throughput, Illumina provides scalability. Customers can batch a large number of samples if needed. While running TruSight Oncology 500 on the NextSeq System, up to eight samples can be batched. With TruSight Oncology 500 High-Throughput on the NovaSeq System, customers can batch from 16 to 192 samples. This flexibility is enabled by availability of 192 different indices for the TruSight Oncology 500 High-Throughput solution, and NovaSeq flowcells that are designed to accommodate varying throughput levels (Figure 2).
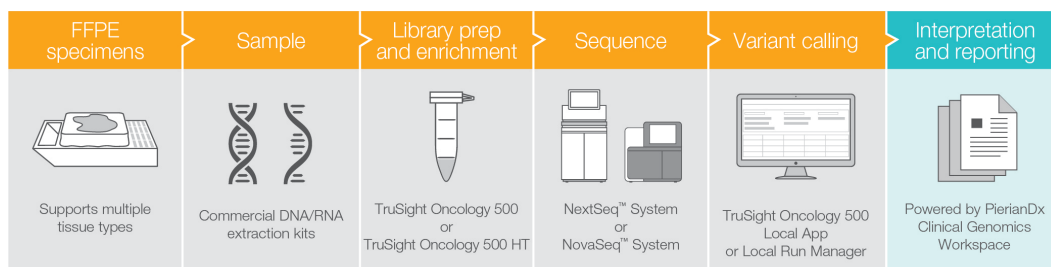

FFPE specimens	Sample	Library prep and enrichment	Sequence	Variant calling	Interpretation and reporting
Supports multiple tissue types	Commercial DNA/RNA extraction kits	TruSight Oncology 500 or TruSight Oncology 500 HT	NextSeq™ System or NovaSeq™ System	TruSight Oncology 500 Local App or Local Run Manager	Powered by PierianDx Clinical Genomics Workspace

Figure 1: TruSight Oncology 500 workflow—Both TruSight Oncology 500 assays integrate into current lab workflows, going from nucleic acids to a variant calls in 3–4 days. Local Run Manager (LRM) is available only with TruSight Oncology 500.

* TruSight Oncology 500 High-Throughput product is under development, coming in 2020.

‡ The products used to evaluate DNA and RNA variants consist of the TruSight Oncology 500 DNA panel and the TruSight Tumor 170 RNA panel.

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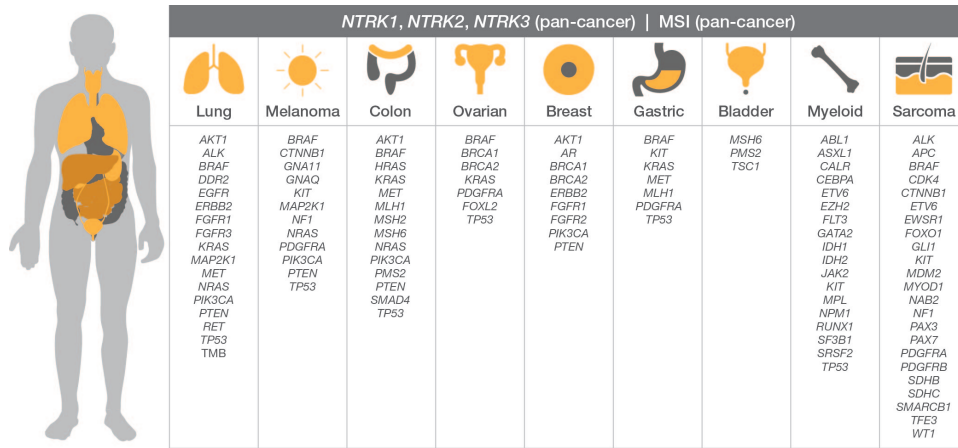| NTRK1, NTRK2, NTRK3 (pan-cancer)	MSI (pan-cancer)							
Lung	Melanoma	Colon	Ovarian	Breast	Gastric	Bladder	Myeloid	Sarcoma
AKT1	BRAF	AKT1	BRAF	AKT1	BRAF	MSH6	ABL1	ALK
ALK	CTNNB1	BRAF	BRCA1	AR	KIT	PMS2	ASXL1	APC
BRAF	GNA11	HRAS	BRCA2	BRCA1	KRAS	TSC1	CALR	BRAF
DDR2	GNAQ	KRAS	KRAS	BRCA2	MET		CEBPA	CDK4
EGFR	KIT	MET	PDGFRA	ERBB2	MLH1		ETV6	CTNNB1
ERBB2	MAP2K1	MLH1	FOXL2	FGFR1	PDGFRA		EZH2	ETV6
FGFR1	NF1	MSH2	TP53	FGFR2	TP53		FLT3	EWSR1
FGFR3	NRAS	MSH6		PIK3CA			GATA2	FOXO1
KRAS	PDGFRA	NRAS		PTEN			IDH1	GLI1
MAP2K1	PIK3CA	PIK3CA					IDH2	KIT
MET	PTEN	PMS2					JAK2	MDM2
NRAS	TP53	PTEN					KIT	MYOD1
PIK3CA		SMAD4					MPL	NAB2
PTEN		TP53					NPM1	NF1
RET							RUNX1	PAX3
TP53							SF3B1	PAX7
TMB							SRSF2	PDGFRA
							TP53	PDGFRB
								SDHB
								SDHC
								SMARCB1
								TFE3
								WT1

Figure 3: Genomic tumor profiling biomarkers for multiple cancer types—Content for TruSight Oncology 500 and TruSight Oncology 500 High-Throughput includes key biomarkers for multiple cancer types, and emerging pan-cancer biomarkers such as MSI, NTRK1, NTRK2, and NTRK3.

NovaSeq 6000 flowcells

NovaSeq	SP	S1	S2	S4
Tissue samples	16 samples	32 samples	72 samples	192 samples

Figure 2: TruSight Oncology 500 High-Throughput scalability—The number of samples that can be batched per run can be adjusted on the NovaSeq System according to which flowcell is used.

Table 2: Multiple types of relevant biomarkers assessed for a comprehensive lung tumor assay

	DNA content	RNA‡ content
Biomarker		
TMB	✓	
MSI	✓	
Biomarker genes	**Small variants**	**Fusions**
AKT1	✓	
ALK	✓	✓
BRAF	✓	✓
DDR2	✓	
EGFR	✓	✓
ERBB2	✓	✓
FGFR1	✓	✓
FGFR3	✓	✓
KRAS	✓	
MAP2K1	✓	
MET	✓	✓
NRAS	✓	
NTRK1	✓	✓
NTRK2	✓	✓
NTRK3	✓	✓
PIK3CA	✓	✓
PTEN	✓	
RET	✓	✓
TP53	✓	

‡ The products used to evaluate DNA and RNA variants consist of the TruSight Oncology 500 DNA panel and the TruSight Tumor 170 RNA panel.

Comprehensive content design

The TruSight Oncology 500 and TruSight Oncology 500 High-Throughput panels include a comprehensive list of biomarkers commonly mutated in numerous neoplasm types (Figure 3). With simultaneous analysis of both DNA and RNA‡ various types of biomarkers relevant to a given tumor type (SNVs, indels, CNVs, fusions, splice variants, TMB, MSI) can be assessed from the same sample in a single assay (Table 2). The panels include 523 genes for SNV, indel, and CNV detection; and 55 genes for fusion and splice variant detection (Table 3), using a probe design that enables capture of both known fusions and novel fusions.

By harnessing expertise from recognized authorities in the oncology community, content was designed to include both current guidelines and emerging biomarkers, including significant coverage of key guidelines for 13 tumor types (Figure 4), and coverage of genes involved in over 1200 clinical trials.
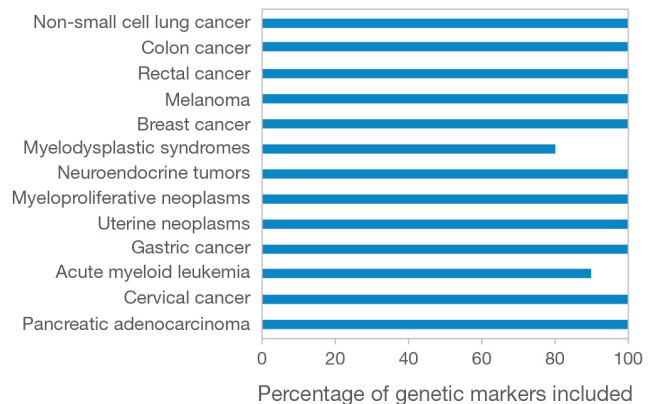
Figure 4: Content alignment to key guidelines—For each cancer type, the percentage of genetic markers in key guidelines that are included in the TruSight Oncology 500 gene panel is indicated.

Table 3: Genes included in the TruSight Oncology 500 panel

DNA content										
ABL1	BRD4	CUX1	FAM175A	GATA6	IGF1	MAP3K13	NOTCH4	POLE	RPTOR	TAF1
ABL2	BRIP1	CXCR4	FAM46C	GEN1	IGF1R	MAP3K14	NPM1	PPARG	RUNX1	TBX3
ACVR1	BTG1	CYLD	FANCA	GID4	IGF2	MAP3K4	NRAS	PPM1D	RUNX1T1	TCEB1
ACVR1B	BTK	DAXX	FANCC	GLI1	IKBKE	MAPK1	NRG1	PPP2R1A	RYBP	TCF3
AKT1	C11orf30	DCUN1D1	FANCD2	GNA11	IKZF1	MAPK3	NSD1	PPP2R2A	SDHA	TCF7L2
AKT2	CALR	DDR2	FANCE	GNA13	IL10	MAX	NTRK1	PPP6C	SDHAF2	TERC
AKT3	CARD11	DDX41	FANCF	GNAQ	IL7R	MCL1	NTRK2	PRDM1	SDHB	TERT
ALK	CASP8	DHX15	FANCG	GNAS	INHA	MDC1	NTRK3	PREX2	SDHC	TET1
ALOX12B	CBFB	DICER1	FANCI	GPR124	INHBA	MDM2	NUP93	PRKAR1A	SDHD	TET2
ANKRD11	CBL	DIS3	FANCL	GPS2	INPP4A	MDM4	NUTM1	PRKCI	SETBP1	TFE3
ANKRD26	CCND1	DNAJB1	FAS	GREM1	INPP4B	MED12	PAK1	PRKDC	SETD2	TFRC
APC	CCND2	DNMT1	FAT1	GRIN2A	INSR	MEF2B	PAK3	PRSS8	SF3B1	TGFBR1
AR	CCND3	DNMT3A	FBXW7	GRM3	IRF2	MEN1	PAK7	PTCH1	SH2B3	TGFBR2
ARAF	CCNE1	DNMT3B	FGF1	GSK3B	IRF4	MET	PALB2	PTEN	SH2D1A	TMEM127
ARFRP1	CD274	DOT1L	FGF10	H3F3A	IRS1	MGA	PARK2	PTPN11	SHQ1	TMPRSS2
ARID1A	CD276	E2F3	FGF14	H3F3B	IRS2	MITF	PARP1	PTPRD	SLIT2	TNFAIP3
ARID1B	CD74	EED	FGF19	H3F3C	JAK1	MLH1	PAX3	PTPRS	SLX4	TNFRSF14
ARID2	CD79A	EGFL7	FGF2	HGF	JAK2	MLL	PAX5	PTPRT	SMAD2	TOP1
ARID5B	CD79B	EGFR	FGF23	HIST1H1C	JAK3	MLLT3	PAX7	QKI	SMAD3	TOP2A
ASXL1	CDC73	EIF1AX	FGF3	HIST1H2BD	JUN	MPL	PAX8	RAB35	SMAD4	TP53
ASXL2	CDH1	EIF4A2	FGF4	HIST1H3A	KAT6A	MRE11A	PBRM1	RAC1	SMARCA4	TP63
ATM	CDK12	EIF4E	FGF5	HIST1H3B	KDM5A	MSH2	PDCD1	RAD21	SMARCB1	TRAF2
ATR	CDK4	EML4	FGF6	HIST1H3C	KDM5C	MSH3	PDCD1LG2	RAD50	SMARCD1	TRAF7
ATRX	CDK6	EP300	FGF7	HIST1H3D	KDM6A	MSH6	PDGFRA	RAD51	SMC1A	TSC1
AURKA	CDK8	EPCAM	FGF8	HIST1H3E	KDR	MST1	PDGFRB	RAD51B	SMC3	TSC2
AURKB	CDKN1A	EPHA3	FGF9	HIST1H3F	KEAP1	MST1R	PDK1	RAD51C	SMO	TSHR
AXIN1	CDKN1B	EPHA5	FGFR1	HIST1H3G	KEL	MTOR	PDPK1	RAD51D	SNCAIP	U2AF1
AXIN2	CDKN2A	EPHA7	FGFR2	HIST1H3H	KIF5B	MUTYH	PGR	RAD52	SOCS1	VEGFA
AXL	CDKN2B	EPHB1	FGFR3	HIST1H3I	KIT	MYB	PHF6	RAD54L	SOX10	VHL
B2M	CDKN2C	ERBB2	FGFR4	HIST1H3J	KLF4	MYC	PHOX2B	RAF1	SOX17	VTCN1
BAP1	CEBPA	ERBB3	FH	HIST2H3A	KLHL6	MYCL1	PIK3C2B	RANBP2	SOX2	WISP3
BARD1	CENPA	ERBB4	FLCN	HIST2H3C	KMT2B	MYCN	PIK3C2G	RARA	SOX9	WT1
BBC3	CHD2	ERCC1	FLI1	HIST2H3D	KMT2C	MYD88	PIK3C3	RASA1	SPEN	XIAP
BCL10	CHD4	ERCC2	FLT1	HIST3H3	KMT2D	MYOD1	PIK3CA	RB1	SPOP	XPO1
BCL2	CHEK1	ERCC3	FLT3	HLA-A	KRAS	NAB2	PIK3CB	RBM10	SPTA1	XRCC2
BCL2L1	CHEK2	ERCC4	FLT4	HLA-B	LAMP1	NBN	PIK3CD	RECQL4	SRC	YAP1
BCL2L11	CIC	ERCC5	FOXA1	HLA-C	LATS1	NCOA3	PIK3CG	REL	SRSF2	YES1
BCL2L2	CREBBP	ERG	FOXL2	HNF1A	LATS2	NCOR1	PIK3R1	RET	STAG1	ZBTB2
BCL6	CRKL	ERRFI1	FOXO1	HNRNPK	LMO1	NEGR1	PIK3R2	RFWD2	STAG2	ZBTB7A
BCOR	CRLF2	ESR1	FOXP1	HOXB13	LRP1B	NF1	PIK3R3	RHEB	STAT3	ZFHX3
BCORL1	CSF1R	ETS1	FRS2	HRAS	LYN	NF2	PIM1	RHOA	STAT4	ZNF217
BCR	CSF3R	ETV1	FUBP1	HSD3B1	LZTR1	NFE2L2	PLCG2	RICTOR	STAT5A	ZNF703
BIRC3	CSNK1A1	ETV4	FYN	HSP90AA1	MAGI2	NFKBIA	PLK2	RIT1	STAT5B	ZRSR2
BLM	CTCF	ETV5	GABRA6	ICOSLG	MALT1	NKX2-1	PMAIP1	RNF43	STK11	
BMPR1A	CTLA4	ETV6	GATA1	ID3	MAP2K1	NKX3-1	PMS1	ROS1	STK40	
BRAF	CTNNA1	EWSR1	GATA2	IDH1	MAP2K2	NOTCH1	PMS2	RPS6KA4	SUFU	
BRCA1	CTNNB1	EZH2	GATA3	IDH2	MAP2K4	NOTCH2	PNRC1	RPS6KB1	SUZ12	
BRCA2	CUL3	FAM123B	GATA4	IFNGR1	MAP3K1	NOTCH3	POLD1	RPS6KB2	SYK	
RNA‡ content										
ABL1	BCL2	CSF1R	ESR1	EWSR1	FLI1	KIF5B	MSH2	NRG1	PAX7	RAF1
AKT3	BRAF	EGFR	ETS1	FGFR1	FLT1	KIT	MYC	NTRK1	PDGFRA	RET
ALK	BRCA1	EML4	ETV1	FGFR2	FLT3	MET	NOTCH1	NTRK2	PDGFRB	ROS1
AR	BRCA2	ERBB2	ETV4	FGFR3	JAK2	MLL	NOTCH2	NTRK3	PIK3CA	RPS6KB1
AXL	CDK4	ERG	ETV5	FGFR4	KDR	MLLT3	NOTCH3	PAX3	PPARG	TMPRSS2

Content shaded in grey is analyzed for CNV detection.

‡ The products to evaluate DNA and RNA variants consist of the TruSight Oncology 500 DNA panel and the TruSight Tumor 170 RNA panel.

Accurate assessment of TMB and MSI

TMB and MSI are emerging biomarkers that correlate with response to immunotherapies.[8,9] TruSight Oncology 500 and TruSight Oncology 500 High-Throughput are well suited to interrogate both biomarkers, which rely upon analysis of multiple genomic loci.

Obtaining a precise and reproducible TMB value at low mutation levels can be challenging with smaller panels. Recent studies have shown that panels with larger genomic content (at least 1.5 Mb) perform well with samples containing less than 30 mutations/Mb.[10,11] With 1.94 Mb of genomic content, TruSight Oncology 500 surpasses this requirement, demonstrating accurate TMB estimation that is highly concordant with whole-exome studies (Figure 5, Table 4).[12] The addition of UMIs during library preparation coupled with proprietary Illumina informatics reduces sequencing error rates by 10–20 fold.[7] Removing FFPE artifacts (such as deamination, oxidation) enables analytical sensitivity as low as 5% VAF from low-quality DNA samples.
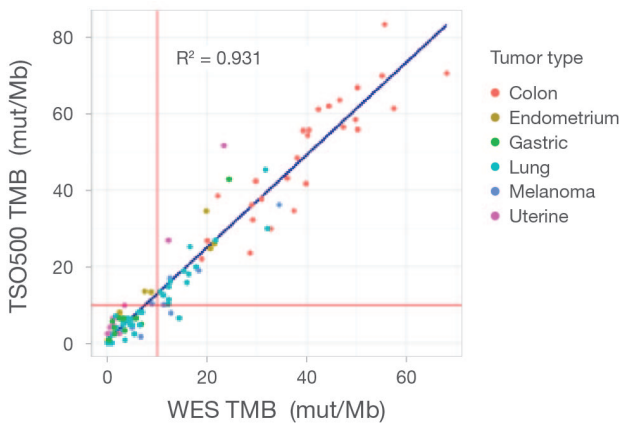
Figure 5: High concordance between TMB measurements from TruSight Oncology 500 and WES—108 FFPE tissue samples.were analyzed by TruSight Oncology 500 (TSO500) in tumor-only workflow, and WES using tumor-normal pairs. TMB values from both assays are plotted to display high concordance (R^2 = 0.931).

Analysis for TruSight Oncology 500 and TruSight Oncology 500 High-Throughput uses a sophisticated proprietary algorithm to perform TMB analysis. Measuring both nonsynonymous and synonymous SNVs and indels increases analytical sensitivity by using more variants. After variant calling and error correction, germline variants and variants in low-confidence regions are filtered to deliver accurate results. Filtering germline variants also allows TMB evaluation with a tumor-only workflow (Figure 6), further supporting high-throughput sample processing.

Table 4: Concordance between WES and TruSight Oncology 500 TMB classification at low value range (10 mut/Mb)

Metric	Value
Positive percent agreement	94.7%
Negative percent agreement	96.1%
Overall percent agreement	95.4%

TMB values plotted from 108 FFPE tissue samples. Percent agreement is shown for TMB-high or TMB-low classifications, with 10 mut/Mb as the threshhold value (red lines in Figure 5).
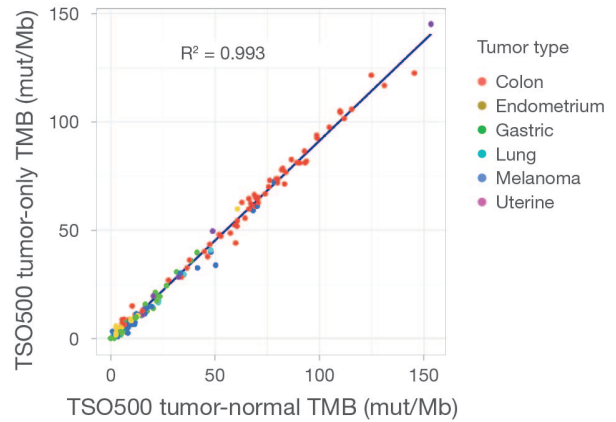
Figure 6: Reproducible TMB evaluation with tumor-only or tumor-normal workflow—TruSight Oncology 500 employs an algorithm that filters germline variants, enabling TMB analysis with a tumor-only workflow. To demonstrate concordance with tumor-normal workflows, 170 FFPE samples from six different tissue types were analyzed, and TMB values from both workflows plotted to display high concordance (R^2 = 0.993).

MSI status is also a biomarker correlated with response to checkpoint inhibition therapy. However, high TMB does not correlate to high MSI status in many tissues tested, warranting development as an independent biomarker with new MSI profiles in previously uncharacterized cancer types.[13,14]

MSI status has been traditionally analyzed with PCR (MSI-PCR) and immunohistochemistry. While other methods deliver a qualitative result describing samples as either MSI-stable or MSI-high, NGS-based assessment with both TruSight Oncology 500 assays interrogates 130 homopolymer MSI marker sites to calculate an accurate quantitative score for MSI status (Figure 7).[12] As a multiplex assay, both TruSight Oncology 500 assays can deliver answers for both TMB and MSI status from a single assay, eliminating the need to spend precious tissue sample on iterative testing.
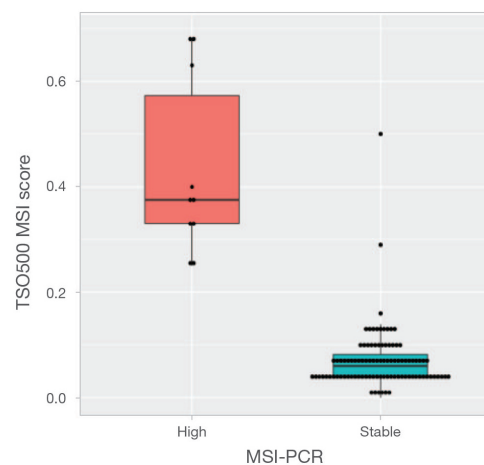
Figure 7: Quantitative assessment of MSI status—The TruSight Oncology 500 tumor-only workflow was used to analyze FFPE samples. A quantitative MSI score (Y-axis) is plotted against a qualitative score (high vs stable) obtained by analyzing the same samples with MSI-PCR (Promega MSI Analysis System).

Enrichment chemistry enables low-level variant detection from FFPE samples

Library preparation for both TruSight Oncology 500 assays 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. A benefit of target enrichment chemistry is the use of probes designed large enough to impart high binding specificity, but also allowing hybridization to targets containing small mutations. This mechanism reduces sample dropouts in the presence of both natural allelic variations and sequence artifacts introduced from FFPE tissue samples. The assay can reproducibly detect variants in FFPE samples as low as 5% VAF (Figure 8).
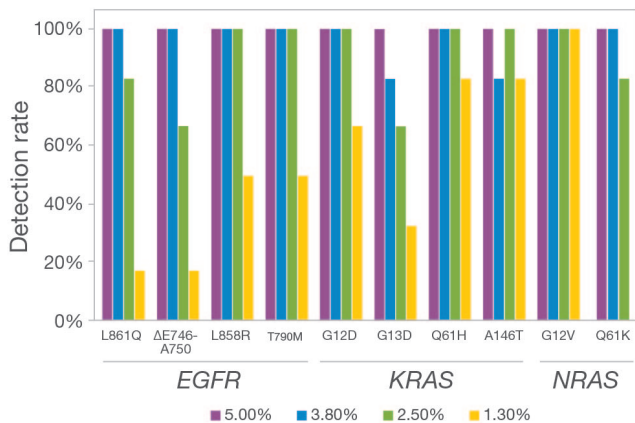

Figure 8: Small variant detection at low variant allele frequency—FFPE cell line samples with known VAF for each variant were diluted to values ranging from 1.30–5.00% VAF. Six replicates of each sample were analyzed by TruSight Oncology 500 using 40 ng DNA input.

Combined workflow for DNA and RNA

Library preparation uses a hybrid-capture method that can be simultaneously applied to DNA and RNA extracted from the same sample. After the initial steps, genomic DNA shearing and cDNA synthesis, the library prep becomes a combined workflow. Sheared DNA and cDNA are converted simultaneously into sequenceable libraries. Regions of interest are hybridized to biotinylated probes, magnetically pulled down with streptavidin-coated beads, and eluted to enrich the library pool. Libraries are then normalized using a simple bead-based protocol before pooling and sequencing.

Detection of CNVs

Copy-number changes associated with tumorigenesis in several genes and several tumor types[15] can be an important component of comprehensive tumor assays. Both TruSight Oncology 500 assays include analysis of 59 CNV-associated genes, and can call amplifications with a limit of detection at 1.5× fold-change (Table 5).

Table 5: CNV detection with TruSight Oncology 500

Gene	Sample	Calls/replicates	Mean fold change
MDM2	4066	2/2	7.967
EGFR	4066	2/2	5.989
MYCN	HD753	10/10	4.678
MDM2	4066	2/2	4.377
MYC	HD753	10/10	4.358
MYCN	HD753	10/10	3.972
MYC	HD753	10/10	3.632
EGFR	4066	2/2	3.462
MYCN	HD753	10/10	2.78
MYC	HD753	10/10	2.535
MYC	4066	2/2	2.526
MDM2	4066	2/2	2.503
EGFR	4066	2/2	2.123
MET	HD753	10/10	1.916
MYC	HD300/HD301	10/10	1.914
MDM2	HD300/HD301	10/10	1.719
MYC	4066	2/2	1.698
MET	HD753	10/10	1.696
PIK3CA	5982	2/2	1.695
FGF23	4138	5/5	1.674
FGF6	4138	5/5	1.671
MDM2	4066	2/2	1.648
MYC	HD300/HD301	10/10	1.617
FGF23	350	5/5	1.518
EGFR	4066	2/2	1.517
MDM2	HD300/HD301	10/10	1.502

Reference DNA samples with known CNV events were examined with the TruSight Oncology 500 assay. Number of replicates (2-10) and recall are indicated in the third column.

Flexibility and reproducibility between sequencing platforms

Although TruSight Oncology 500 and TruSight Oncology 500 High-Throughput were designed to run on separate sequencing platforms with different throughput options, both assays have the same genomic content and performance expectations for variant calling. High concordance has been demonstrated between the two assays for small variant calling (Table 6), MSI (Figure 9), TMB (Figure 9), and RNA fusions (Table 7). Recommended DNA input is the same for each assay (40 ng). However, to achieve comparable results with RNA analysis, 40 ng RNA is recommended for TruSight Oncology 500 while a range of 40-80 ng RNA is recommended for TruSight Oncology 500 High-Throughput. In cases where FFPE RNA yields from FFPE tissues are low, 40 ng RNA input can still be used to detect variants expressed at mid-to-high levels with TruSight Oncology 500 High-Throughput. However, when sufficient RNA is available, 80 ng input helps maximize sensitivity for fusions present at very low concentrations (Table 7).

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Table 6: Concordance between TruSight Oncology 500 and TruSight Oncology 500 High-Throughput for small variant calling.

Gene	Variant	Expected VAF	TSO500	TSO500 HT	Gene	Variant	Expected VAF	TSO500	TSO500 HT
ARID1A	P1562fs	33.50%	3/3	3/3	IDH1	S261L	10.00%	3/3	3/3
PDGFRA	G426D	33.50%	3/3	3/3	KIT	D816V	10.00%	3/3	3/3
FBXW7	G667fs	33.50%	3/3	3/3	PI3KCA	E545K	9.00%	3/3	3/3
BRCA2	A1689fs	33.00%	3/3	3/3	MLH1	L323M	8.50%	3/3	3/3
ALK	P1543S	33.00%	3/3	3/3	FGFR1	P150L	8.50%	3/3	3/3
APC	R2714C	33.00%	3/3	3/3	ABL2	P986fs	8.00%	3/3	3/3
NOTCH1	P668S	31.50%	3/3	3/3	NF2	P275fs	8.00%	3/3	3/3
EGFR	G719S	24.50%	3/3	3/3	EP300	K291fs	8.00%	3/3	3/3
PI3KCA	H1047R	17.50%	3/3	3/3	NF1	L626fs	7.50%	3/3	3/3
KRAS	G13D	15.00%	3/3	3/3	MET	V237fs	6.50%	3/3	3/3
NRAS	Q61K	12.50%	3/3	3/3	KRAS	G12D	6.00%	3/3	3/3
FLT3	V197A	11.50%	3/3	3/3	EGFR	L858R	3.00%	3/3	3/3
BRAF	V600E	10.50%	3/3	3/3	EGFR	ΔE746 - A750	2.00%	3/3	3/3

FFPE samples were run with each assay at 40 ng DNA input for TruSight Oncology 500 (TSO500), or TruSight Oncology 500 High-Throughput (TSO500 HT). TSO500 HT data was produced with developmental workflow.

Table 7: Concordance between TruSight Oncology 500 assays for fusion and splice variant detection.

	TSO500 supporting reads		TSO500 HT supporting reads			
RNA input	40 ng		40 ng		80 ng	
Fusion/splice variant	rep 1	rep 2	rep 1	rep 2	rep 1	rep 2
TRMT61B-ALK	ND	ND	ND	ND	ND	ND
TMPRSS2-ERG (1 007)	93	90	68	82	99	138
TMPRSS2-ERG (2 008)	10	12	ND	6	9	10
SLC34A2-ROS1	37	31	23	22	54	37
RPS6KB1-VMP1	49	76	64	56	71	41
FGFR3-TACC3	83	68	45	64	90	85
FGFR2-COL14A1	218	223	141	170	296	182
EML4-ALK	7	10	ND	ND	9	11
CCDC6-RET	24	13	9	10	20	22
BCR-ABL1	12	16	8	10	18	24
ALK-PTPN3	11	18	7	8	8	14
MET Exon14 Skip	47	25	20	20	39	33

Fragmented RNA reference samples with known fusions were analyzed at 40 ng RNA input for TruSight Oncology 500 (TSO500), or at both 40 ng and 80 ng RNA input for TruSight Oncology 500 High-Throughput (TSO500-HT). ND = not detected. TSO500-HT data was produced with developmental workflow.
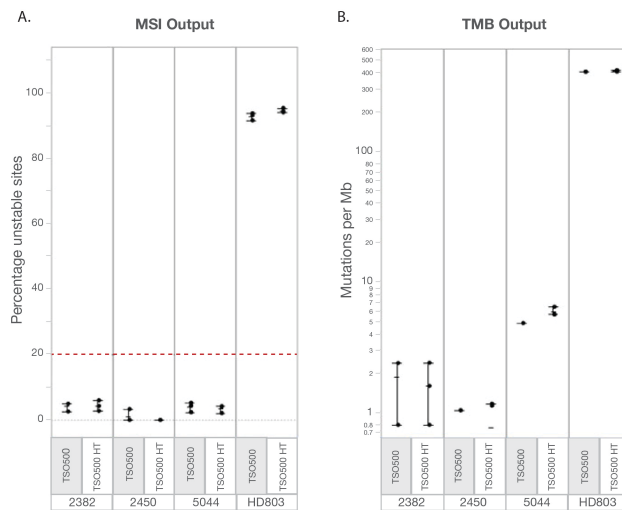

Figure 9: Concordance between TruSight Oncology 500 (TSO500) and TruSight Oncology 500 High-Throughput (TSO500 HT) for MSI (A) and TMB (B) measurement —
Each assay was run with 40 ng DNA input from FFPE tissues (colon: 2382, pancreas: 2450, ovary: 5044) and a formalin-compromised cell line (HD803). Dashed line indicates value cutoff between MSI-stable and MSI-unstable status. TruSight Oncology 500 High-Throughput data was produced with developmental workflow.

High sample throughput with the NovaSeq 6000 System

The TruSight Oncology 500 High-Throughput assay was developed to run on the NovaSeq System to provide scalability to extremely high sample throughput. With 192 indices, multiplexing is optimal when running samples on the S4 flowcell (Figure 2). Each sample index performs consistently to produce sequencing metrics above quality control (QC) expectations (Figure 10).

Summary

TruSight Oncology 500 and TruSight Oncology 500 High-Throughput are NGS-based, multiplex assays that analyze hundreds of cancer-related biomarkers in a single assay. Assessing DNA and RNA‡ in the same workflow supports identification of a wide range of variants implicated in various tumor types. Taking advantage of extensive genomic content, TruSight Oncology 500 also provides assessment of immunotherapy biomarkers (TMB and MSI) without requiring multiple samples for iterative testing. Using target-enrichment chemistry with sophisticated tools to reduce errors, high-quality data is obtainable from FFPE samples. By expanding the TruSight Oncology 500 product franchise with the addition of the TruSight Oncology 500 High-Throughput Kit, we are enabling labs to increase their batching sizes, and process more samples per week. Leverage the power of TruSight Oncology 500 to accelerate your research goals today.

Learn more

For more information about TruSight Oncology 500, visit
www.illumina.com/tso500

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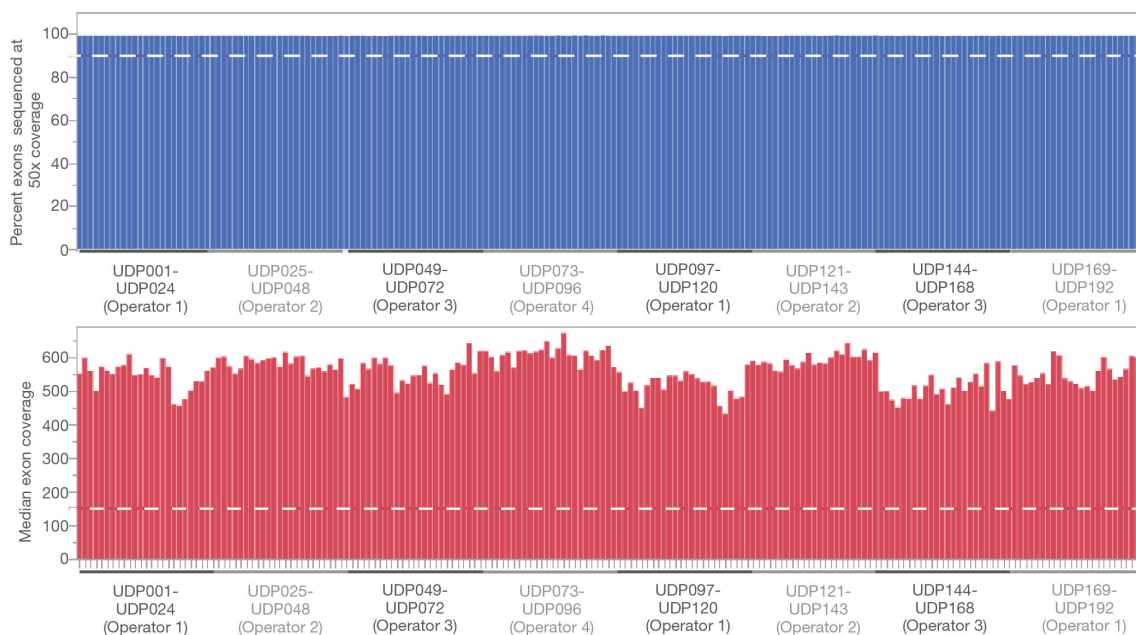
Figure 10: Reproducible performance with 192 indices in the TruSight Oncology 500 High-Throughput assay—Sets of 24 indices were aliquotted and used for independent library preparations among four independent operators. Dashed line indicates lower-level specification value for quality control.

‡ The products used to evaluate DNA and RNA variants consist of the TruSight Oncology 500 DNA panel and the TruSight Tumor 170 RNA panel.

Table 8: Product Specifications

Parameter	TruSight Oncology 500	TruSight Oncology 500 High-Throughput*
System	NextSeq System or NextSeq 550Dx (research mode)	NovaSeq 6000 System
Panel size	1.94 Mb DNA, 358 kb RNA	1.94 Mb DNA, 358 kb RNA
DNA input requirement	40 ng total	40 ng total
RNA[‡] input requirement	40 ng total	40-80 ng total
FFPE input requirement	Minimum recommendation of 0.65 mm^3 from FFPE tissue samples (5 slides cut at 10 microns thick)	Minimum recommendation of 0.65 mm^3 from FFPE tissue samples (5 slides cut at 10 microns thick)
Total assay time	3–4 days from nucleic acid to variant report	3–4 days from nucleic acid to variant report
Sequence run time	24 hours	19 hours (SP and S1), 25 hours (S2), or 36 hours (S4)
Sequence run	2 × 101 cycles	2 × 101 cycles
Kit size	24 or 48 samples	TBD
Sample throughput	8 samples per run	16-192 samples per run
Limit of detection	5% VAF for small variants 5 copies per ng RNA input for fusions 1.5× fold-change for CNVs	5% VAF for small variants 5 copies per ng RNA input for fusions (80 ng input) 1.5× fold-change for CNVs
Analytical sensitivity	>96% (for all variant types at 5% VAF)	TBD
Analytical specificity	>99.99%	>99.99%

* The TruSight Oncology 500 High-Throughput is still under development.

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Ordering information

Library prep kit	Indexes/samples	Catalog no.
TruSight Oncology 500 DNA Kit (Includes DNA library prep and enrichment reagents. Does not include NextSeq System core reagents)	16 indexes 48 samples	20028213
TruSight Oncology 500 DNA/RNA[‡] Bundle (Includes DNA library prep and enrichment reagents. Does not include NextSeq System core reagents)	16 indexes 24 samples	20028215
Library prep kit plus access to the PierianDx Clinical Genomics Workspace	**Indexes/samples**	**Catalog no.**
TruSight Oncology 500 DNA Kit, plus PierianDx (Includes DNA library prep and enrichment reagents. Does not include NextSeq System core reagents)	16 indexes 48 samples	20032624
TruSight Oncology 500 DNA/RNA[‡] Bundle, plus PierianDx (Includes DNA library prep and enrichment reagents. Does not include NextSeq System core reagents)	16 indexes 24 samples	20032626
Library prep kit plus NextSeq System reagents	**Indexes/samples**	**Catalog no.**
TruSight Oncology 500 DNA Kit, for Use with NextSeq (Includes DNA library prep and enrichment reagents, and NextSeq System core reagents)	16 indexes 48 samples	20028214
TruSight Oncology 500 DNA/RNA[‡] Bundle, for Use with NextSeq (Includes DNA library prep and enrichment reagents, and NextSeq System core reagents)	16 indexes 24 samples	20028216
Library prep kit, plus NextSeq System reagents, plus access to the PierianDx Clinical Genomics Workspace	**Indexes/samples**	**Catalog no.**
TruSight Oncology 500 DNA Kit, for Use with NextSeq, plus PierianDx (Includes DNA library prep, enrichment reagents, and NextSeq System core reagents)	16 indexes 48 samples	20032625
TruSight Oncology 500 DNA/RNA[‡] Bundle, for Use with NextSeq, plus PierianDx (Includes DNA library prep, enrichment reagents, and NextSeq System core reagents)	16 indexes 24 samples	20032627

Catalog numbers for TruSight Oncology 500 High-Throughput will be available in 2020.

‡ The products used to evaluate DNA and RNA variants (PN: 20028215, 20032626, 20028216, 20032627) consist of the TruSight Oncology 500 DNA panel and the TruSight Tumor 170 RNA panel.

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