illumina®

TruSight™ Oncology 500 and TruSight Oncology 500 High-Throughput

Enabling flexible, scalable comprehensive genomic profiling from FFPE samples.

Highlights

- **Consolidated assay**
  Save time and sample by analyzing multiple tumor variant types in 523 genes, from both DNA and RNA, in a single assay

- **Comprehensive content**
  Access current and emerging biomarkers identified in guidelines and clinical trials

- **Proven, reliable results**
  Generate accurate data using an assay shown to meet demanding performance specifications

- **Value-adding in-house solution**
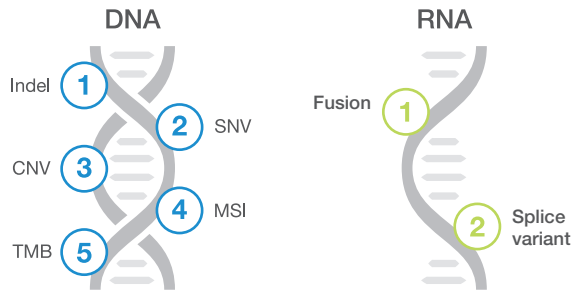 Keep samples and obtain data that is most relevant to the local institution and community

Introduction

Recent large-cohort studies show that comprehensive genomic profiling has the potential to identify relevant genetic alterations in up to 90% of samples.[1-6] Using a single, comprehensive assay to assess a wide range of biomarkers offers the added advantages of using less sample and returning results more quickly compared to multiple, iterative tests. To help researchers working with limited tissue supply and time, Illumina offers TruSight Oncology 500 and TruSight Oncology 500 High-Throughput (Table 1). With proven technology, relevant biomarker content, and multiple established pharma partnerships, these assays are well-positioned to be the foundation for future tumor profiling diagnostic assays.

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

One workflow analyzes multiple tumor types and biomarkers

TruSight Oncology 500 and TruSight Oncology 500 High-Throughput are next-generation sequencing (NGS) assays that analyze 523 cancer-relevant genes from both DNA and RNA in one integrated workflow (Table 2). The assays simultaneously assess multiple variant types (Figure 1) for DNA and RNA, eliminating the need to spend precious tissue sample and time on iterative testing.

Table 1: TruSight Oncology 500 and TruSight Onclology 500 High-Throughput at a glance

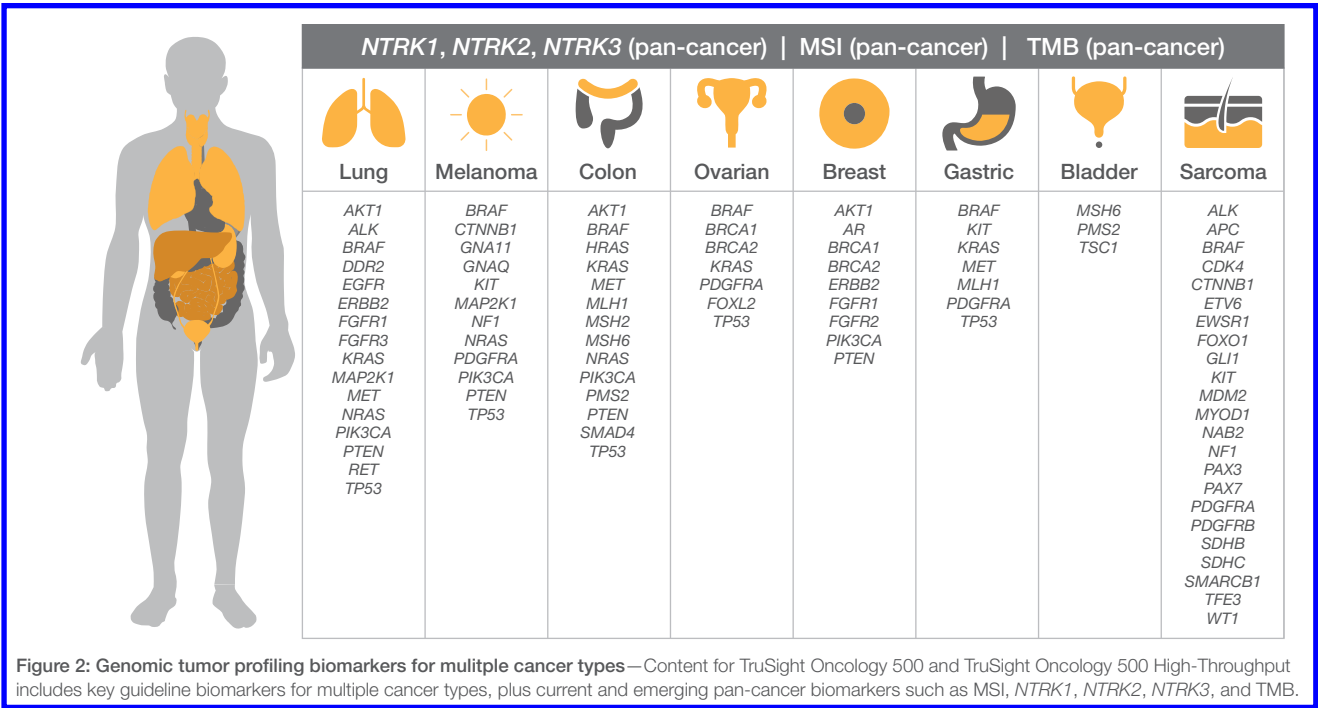| Parameter	TruSight Oncology 500	TruSight Oncology 500 High-Throughput
System	NextSeq 500, NextSeq 550, or NextSeq 550Dx (research mode) Systems	NovaSeq 6000 System
Panel size	1.94 Mb DNA, 358 kb RNA	1.94 Mb DNA, 358 kb RNA
DNA input requirement	40 ng	40 ng
RNA input requirement	40 ng	40-80 ng
FFPE input requirement	Minimum recommendation of 2 mm³ from FFPE tissue samples	Minimum recommendation of 2 mm³ from FFPE tissue samples
Total assay time	4-5 days from nucleic acid to variant report	4-5 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
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 2.2× fold-change for CNVs	5% VAF for small variants 5 copies per ng RNA input for fusions (80 ng input) 2.2× fold-change for CNVs
Analytical sensitivity	> 96% (for all variant types at 5% VAF)	> 96% (for all variant types at 5% VAF)

Tumors and genes tested in kit

NTRK1, NTRK2, NTRK3 (pan-cancer)	MSI (pan-cancer)	TMB (pan-cancer)							
Lung	**Melanoma**	**Colon**	**Ovarian**	**Breast**	**Gastric**	**Bladder**	**Sarcoma**
AKT1 ALK BRAF DDR2 EGFR ERBB2 FGFR1 FGFR3 KRAS MAP2K1 MET NRAS PIK3CA PTEN RET TP53	BRAF CTNNB1 GNA11 GNAQ KIT MAP2K1 NF1 NRAS PDGFRA PIK3CA PTEN TP53	AKT1 BRAF HRAS KRAS MET MLH1 MSH2 MSH6 NRAS PIK3CA PMS2 PTEN SMAD4 TP53	BRAF BRCA1 BRCA2 KRAS PDGFRA FOXL2 TP53	AKT1 AR BRCA1 BRCA2 ERBB2 FGFR1 FGFR2 PIK3CA PTEN	BRAF KIT KRAS MET MLH1 PDGFRA TP53	MSH6 PMS2 TSC1	ALK APC BRAF CDK4 CTNNB1 ETV6 EWSR1 FOXO1 GLI1 KIT MDM2 MYOD1 NAB2 NF1 PAX3 PAX7 PDGFRA PDGFRB SDHB SDHC SMARCB1 TFE3 WT1

Figure 2: Genomic tumor profiling biomarkers for mulitple cancer types—Content for TruSight Oncology 500 and TruSight Oncology 500 High-Throughput includes key guideline biomarkers for multiple cancer types, plus current and emerging pan-cancer biomarkers such as MSI, NTRK1, NTRK2, NTRK3, and TMB.

Table 2: Simultaneous analysis of multiple lung cancer biomarkers using DNA and RNA in the same sample

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

Details of chip

Comprehensive content design

Illumina partnered with recognized authorities in the oncology community to design content for the TruSight Oncology 500 and TruSight Oncology 500 High-Throughput panels. The resulting panels provide comprehensive coverage of biomarkers commonly mutated in numerous cancer types (Figure 2), including 523 genes for single nucleotide variants (SNVs), insertions/deletions (indels), copy number variations (CNVs); and 55 genes for known and novel fusion and splice variants (Tables 3 and 4). In addition, the TruSight Oncology 500 panels include the key immunotherapy biomarker, microsatellite instability (MSI), with known correlations to responses,[7,8] and the emerging biomarker, tumor mutational burden (TMB).

Panel content comprises genes listed in current guidelines with significant coverage of key guidelines for multiple tumor types (Figure 3) and genes involved in over 1000 clinical trials.
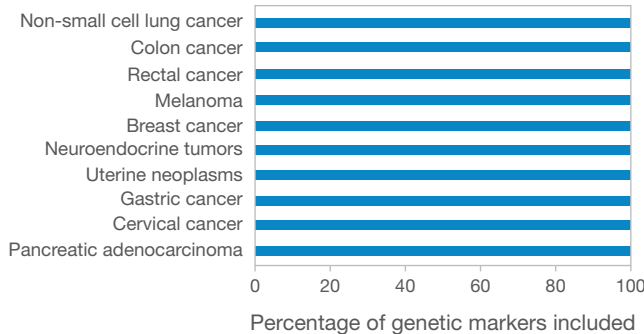
Figure 3: TruSight Oncology 500 content alignment to key guidelines by cancer type—The graph provides examples of content alignment; it is not meant to be all-inclusive.

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Mutant gene alleles tested; genes with the same prefix have multiple causative alleles related to different cancers

Table 3: DNA content included in the TruSight Oncology 500 and TruSight Oncology High Throughput panels

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	

Content shaded in grey is analyzed for CNV detction.

Table 4: RNA content included in the TruSight Oncology 500 and TruSight Oncology High Throughput panels

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*

All genes listed are assessed for known and novel fusions. In addition, the content shaded in grey is analyzed for splice variants.

Integrated workflow

TruSight Oncology 500 and TruSight Oncology 500 High-Throughput are part of a comprehensive, nucleic acid to final annotated report, tumor-only workflow solution available through Illumina and PierianDx (Figure 4). The entire workflow can be completed in as little as four days. If following an automated protocol, hands-on time is expected to be reduced by ~50%.

Start with DNA or RNA

The TruSight Oncology 500 assays can use RNA or DNA extracted 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.

Add tags for analytical specificity

During library preparation, unique molecular identifiers (UMIs)[10] 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 analytical specificity.

Enrich libraries to focus efforts

Library preparation is based on proven hybrid-capture chemistry using biotinylated probes and streptavidin-coated magnetic beads to purify selected targets from DNA- and RNA-based libraries. Regions of interest hybridize to the biotinylated probes, are magnetically pulled down, and then eluted to enrich the library pool. Hybridization-based enrichment is a useful strategy for analyzing specific genetic variants in a given sample and provides the ability to reliably sequence exomes or large numbers of genes (eg, > 50 genes) using robust and straightforward workflows. It delivers dependable results across a wide range of input types and quantities.

Hybrid-capture chemistry offers several advantages over amplicon sequencing, including yielding data with less artifacts and less dropouts. Additionally, hybrid-capture chemistry is fusion agnostic, enabling detection of and characterization of known and novel fusions.

Sequence 8-192 samples

TruSight Oncology 500 and TruSight Oncology 500 High-Throughput follow the same sample and library preparation workflow. The primary difference between the assays is scale. TruSight Oncology 500 runs on the NextSeq™ 500 or NextSeq 550Dx* Systems, which can batch up to eight samples at a time. TruSight Oncology 500 High-Throughput assay provides scalability to extremely high sample throughput. When run on the NovaSeq™ 6000 System, customers can batch from 16 to 192 samples. This flexibility is enabled by the availability of 192 unique indexes for TruSight Oncology 500 High-Throughput, and NovaSeq flow cells that accommodate varying throughput levels (Table 5). Each sample index performs consistently to produce sequencing metrics above quality control (QC) expectations.

Table 5: Scalable solution

Assay	TruSight Oncology 500	TruSight Oncology 500 High-Throughput			
System	NextSeq 550 or NextSeq 550Dx[a]	NovaSeq 6000 System			
Flow cell	High-output	SP	S1	S2	S4
No. samples	8	16	32	72	192

a. NextSeq 550Dx System in Research Mode

Analyze data

Variant calling for TruSight Oncology 500 and TruSight Oncology 500 High-Throughput uses five sophisticated, proprietary algorithms that remove errors, artifacts, and germline variants. The result is highly
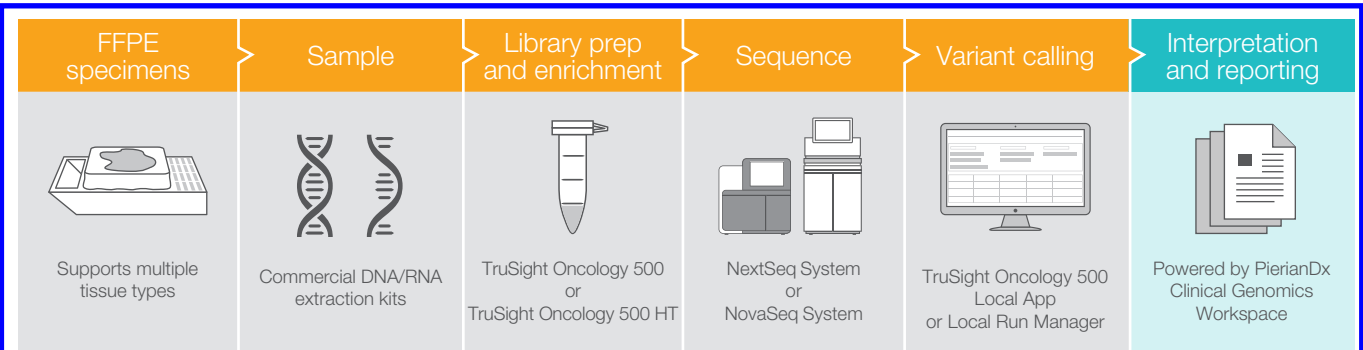
* NextSeq 550Dx System in research mode

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 4: TruSight Oncology 500 workflow—Both TruSight Oncology 500 assays integrate into current lab workflows, going from nucleic acids to a variant calls in 4-5 days. Local Run Manager (LRM) is available only with TruSight Oncology 500.

Work flow for testing samples

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accurate variant calling performance with an analytical specificity of 99.9998%. This level of specificity is particularly beneficial when it is critical to know the exact number of mutations per Mb, as in TMB evaluation with a tumor-only workflow.

For interpretation and reporting, variant report files can be uploaded into the PierianDx Clinical Genomics Workspace (CGW) cloud directly from the sequencing system. CGW performs variant annotation and filtering for smooth interpretation and reporting. From thousands of variants in the genome, the PierianDx CGW filters and prioritizes biologically relevant variants for the final automated, customizable genomic report.

Proven, reliable results

Although TruSight Oncology 500 and TruSight Oncology 500 High-Throughput were designed to run on separate sequencing platforms with different throughput options, the assays have the same genomic content and performance expectations for variant calling. Both assays demonstrate high concordance when detecting MSI, TMB, CNVs, small variants, and fusions.

Accurate assessment of TMB and MSI

TruSight Oncology 500 and TruSight Oncology 500 High-Throughput are well suited to interrogate MSI and TMB, which rely upon analysis of multiple genomic loci.

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 the TruSight Oncology 500 assays interrogates 130 homopolymer MSI marker sites to calculate an accurate quantitative score for MSI status (Figure 5).[9]

Obtaining a precise and reproducible TMB value at low mutation levels can be challenging with smaller panels. TruSight Oncology 500 panels combine comprehensive genomic content with sophisticated informatics algorithms to provide accurate TMB estimation that is highly concordant with whole-exome studies (Figure 6, Table 6).[9] The addition of UMIs during library preparation coupled with proprietary Illumina informatics reduces sequencing error rates by 10-20 fold.[10] Removing FFPE artifacts (such as deamination, oxidation) enables analytical sensitivity as low as 5% VAF from low-quality DNA samples.
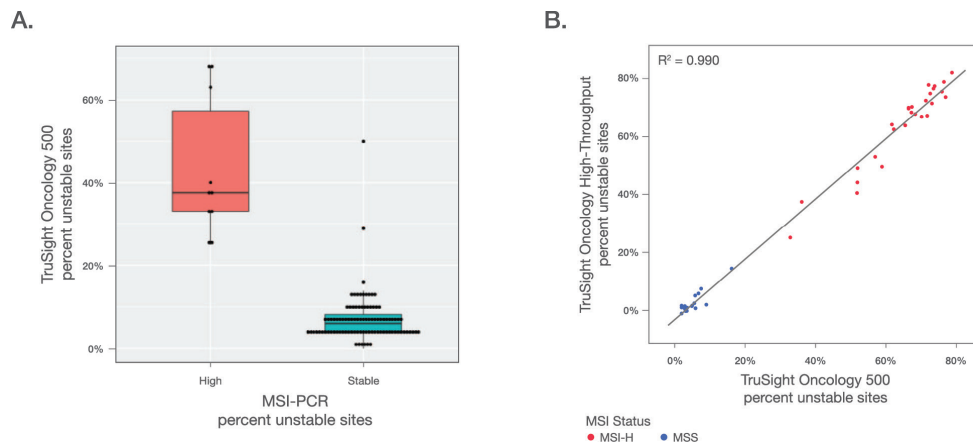

Figure 5: Accurate assessment of MSI status—(A) FFPE tissue samples analyzed using TruSight Oncology 500 produce a quantitative score (y-axis) compared to a qualitative score using MSI-PCR (x-axis). (B) High concordance of MSI analysis between TruSight Oncology 500 and TruSight Oncology 500 High-Throughput.
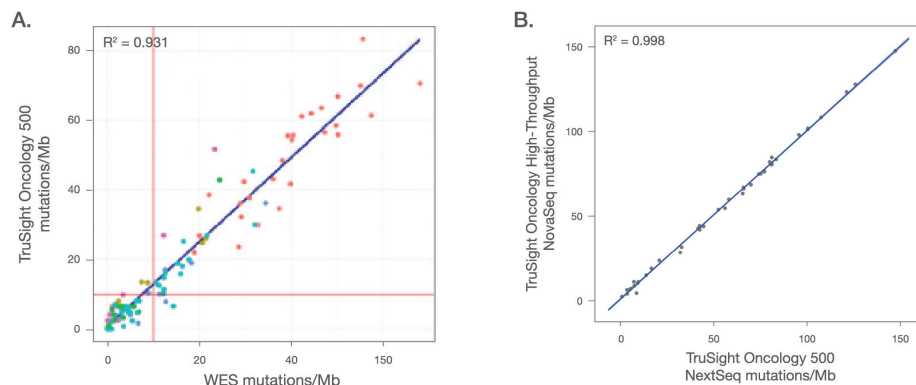

Figure 6: Accurate assessment of TMB status—(A) Analysis of 108 FFPE tissue samples shows high concordance between TMB measurements using WES and TruSight Oncology 500.Red line indicates the threshold value (10 mutations/Mb). (B) High concordance of TMB analysis between TruSight Oncology 500 and TruSight Oncology 500 High-Throughput.

Table 6: High concordance between WES and TruSight Oncology 500 for TMB classification at 10 mutations/Mb

Metric	Value
Percent positive agreement	94.7%
Negative percent agreement	96.1%
Overall percent agreement	95.4%

Based on TMB values from 108 FFPE tissue samples. Percent agreement is shown for TMB-high or TMB-low classifications, with 10 mutations/Mb as the threshold value.

Sensitive detection of CNVs

Copy-number changes in several genes and tumor types can be associated with tumorigenesis.[11] Both TruSight Oncology 500 assays include analysis of 59 CNV-associated genes, and can call amplifications with a limit of detection at 2.2× fold-change (Figure 7, Table 7).

Table 7: Sensitive CNV detection

Gene	Detected	Fold change		Tissue
		TruSight Oncology 500	TruSight Oncology 500 High-Throughput	
ERBB2	√	23.43	23.37	Breast
MDM2	√	8.50	9.34	Lung
EGFR	√	6.00	6.12	Lung
EGFR	√	4.32	4.31	Lung
MET	√	3.98	3.68	Lung
MYC	√	3.59	3.67	Breast
ERBB2	√	2.86	2.91	Breast
BRAF	√	2.31	2.12	Lung
MYC	√	2.22	2.24	Colorectal
CCND1	√	2.15	2.20	Skin
KRAS	√	1.82	1.86	Breast
MDM4	√	1.80	1.77	Breast
CCNE1	√	1.76	1.79	Lung
FGF19	√	1.73	1.74	Skin
AR	√	1.72	1.68	Colorectal
MET	√	1.69	1.62	Colorectal
KRAS	√	1.64	1.73	Lung
MYCN	√	1.63	1.66	Colorectal
CDK6	√	1.62	1.60	Colorectal
CHEK2	√	1.58	1.54	Lung
FGF10	√	1.54	1.51	Lung
BRCA2	√	1.53	1.53	Breast
FGF7	√	1.49	1.50	Colorectal
FGFR1	√	1.39	1.38	Colorectal

Note: The information in this table shows examples of concordance between TruSight Oncology 500 and TruSight Oncology 500 High-Throughput and is not a comprehensive list of the CNVs detected.
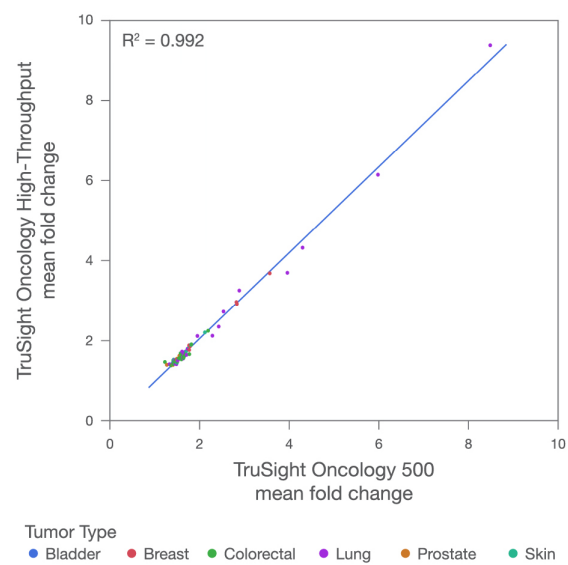

Figure 7: High concordance of CNV detection between TruSight Oncology 500 and TruSight Oncology 500 High-Throughput

Highly sensitive variant detection from FFPE samples

One benefit of target enrichment chemistry is the use of probes designed large enough to impart high binding specificity, but also allow 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, Table 8).
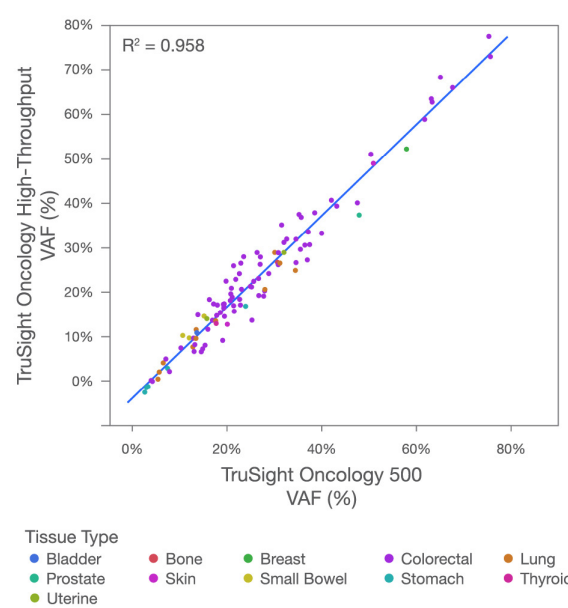

Figure 8: Highly sensitive variant detection—High VAF concordance between TruSight Oncology 500 and TruSight Oncology 500 High-Throughput.

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Table 8: Highly sensitive DNA small variant detection

| Gene | Mutation | Detected | VAF | | Variant type |
			TruSight Oncology 500	TruSight Oncology 500 High-Throughput	
AKT1	E17K	√	20%	18%	SNV
BRAF	V600E	√	19%	19%	SNV
CDKN2A	R58*	√	12%	14%	SNV
CTNNB1	G34E	√	16%	18%	SNV
EGFR	L858R	√	18%	17%	SNV
EGFR	T790M	√	13%	12%	SNV
FBXW7	R465C	√	8%	7%	SNV
FGFR2	S252W	√	32%	32%	SNV
GNAS	R876*	√	5%	5%	SNV
H3F3B	K37M	√	31%	30%	SNV
IDH2	R140Q	√	23%	22%	SNV
KRAS	G12D	√	6%	6%	SNV
NRAS	Q61K	√	15%	18%	SNV
PIK3CA	E542K	√	14%	15%	SNV
PTCH1	A563V	√	4%	4%	SNV
SMARCA4	R973W	√	3%	3%	SNV
TP53	R248Q	√	29%	27%	SNV
RET	A845V	√	7%	8%	MNV
APC	T1556Nfs*3	√	21%	20%	Insertion
ARID1A	D1850Tfs*33	√	4%	5%	Deletion
EP300	H2324fs*29	√	24%	20%	Deletion
KMT2A (MLL)	K3828Rfs*31	√	3%	3%	Deletion
PTEN	K267Rfs*9	√	21%	21%	Deletion
RNF43	G659Vfs*41	√	18%	18%	Deletion

Information in this table shows examples of concordance between TruSight Oncology 500 and TruSight Oncology 500 High-Throughput and is not a comprehensive list of the SNVs and indels detected. SNV = single nucleotide variant, MNV = multiple nucleotide variant

Table 9: Robust detection of fusions and splice variants

| RNA fusion | Detected | RNA input amount | | | Tissue |
		40 ng	60 ng	80 ng	
ALK-EML4	√	15	21	40	Lung
EGFR-RAB3IP	√	5	9	19	Brain
EGFR-METTL1	√	25	84	71	Brain
BRCA1-MPP2	√	25	28	29	Unknown
ALK-BRE	√	75	112	128	Sarcoma
CCDC170-ESR1	√	122	59	168	Kidney
MYC-MRPL13	√	27	35	52	Breast
MYC-STK3	√	11	39	28	Breast
ROS1;GOPC-ENC1	√	32	53	93	Lung
ROS1;GOPC-CD74	√	104	92	141	Lung
ANKUB1;RNF13-ETV5;DGKG	√	29	45	72	Uterus
NTRK3-SEMA6A	√	7	16	25	Skin
RET-NCOA4	√	74	78	154	Thyroid
EWSR1-ATF1	√	19	30	32	Sarcoma
EWSR1-CBY1	√	44	30	97	Sarcoma
BRCA2-NRXN3	√	33	60	84	Bone
FLT3-SMOX	√	50	72	54	Bone
FLT3-VWA8	√	29	51	69	Bone
FLT3-LCP1	√	12	32	47	Bone
Splice variant					
ARv7	√	26	38	46	Breast
EGFR v3	√	567	884	937	Brain
EGFR v3	√	1249	1614	2049	Brain

Fusion and splice variants detected using TruSight Oncology 500 on the NextSeq 500 System. Values represent the number of supporting reads for each sample at the indicated RNA input amount. Cut-off value for RNA fusions = 5; cut-off value for splice variants = 10.

Robust detection of RNA fusions

Cancer can arise from epigenetic changes, expression level changes, and gene fusions that are undetectable by standard sequencing.[12,13] The TruSight Oncology 500 assays use a hybrid-capture approach for targeted RNA-Seq to detect and characterize fusions agnostic from the partner. Unlike amplicon-based approaches, which require confirmatory tests as false-positives can arise, the hybrid-capture method is highly sensitive and can accurately characterize both gene fusions from both known and novel fusion gene partners.

To achieve comparable results with RNA analysis, 40 ng RNA is recommended for use with TruSight Oncology 500 while a range of 40-80 ng RNA is recommended for use with 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 9).

Plan for the future

TruSight Oncology 500 and TruSight Oncology 500 High-Throughput integrate easily into labs currently using NGS, enabling them to offer comprehensive genomic profiling capabilities without exploring an entirely new technology. By consolidating multiple independent, single biomarker assays into one assay, labs can save sample, time, and money, while increasing the chances of identifying a positive biomarker. In addition, bringing tumor assays in house allows labs to keep sample and raw data and become a more active part of molecular tumor boards.

Summary

TruSight Oncology 500 and TruSight Oncology 500 High-Throughput are NGS-based, hybrid-capture assays that enable comprehensive genomic profiling through analysis of all key biomarkers present in guidelines and clinical trials, in a single assay using a small amount of sample. Combining DNA and RNA hybrid-capture with sophisticated informatics reduces errors and yields high-quality data, even from FFPE samples. With TruSight Oncology 500 High-Throughput, labs can increase their batching sizes and process more samples per week. Leverage the power of TruSight Oncology 500 to improve lab efficiency and produce meaningful results.

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Ordering information

Product	Indexes/ samples	Catalog no.
TruSight Oncology 500 DNA only		
TruSight Oncology 500 DNA Kit[a]	16 indexes 48 samples	20028213
TruSight Oncology 500 DNA Kit, plus PierianDx[a]	16 indexes 48 samples	20032624
TruSight Oncology 500 DNA Kit, for Use with NextSeq[b]	16 indexes 48 samples	20028214
TruSight Oncology 500 DNA Kit, for Use with NextSeq, plus PierianDx[b]	16 indexes 48 samples	20032625
TruSight Oncology 500 DNA/RNA		
TruSight Oncology 500 DNA/RNA Bundle[a]	16 indexes 24 samples	20028215
TruSight Oncology 500 DNA/RNA Bundle, plus PierianDx[a]	16 indexes 24 samples	20032626
TruSight Oncology 500 DNA/RNA Bundle, for Use with NextSeq[b]	16 indexes 24 samples	20028216
TruSight Oncology 500 DNA/RNA Bundle, for Use with NextSeq, plus PierianDx[b]	16 indexes 24 samples	20032627
TruSight Oncology 500 High-Throughput DNA only		
TruSight Oncology 500 DNA High-Throughput Kit	48 samples	20040765
TruSight Oncology 500 DNA High-Throughput Kit, with PierianDx	48 samples	20040769
TruSight Oncology 500 DNA High-Throughput Kit	144 samples	20040767
TruSight Oncology 500 DNA High-Throughput Kit, with PierianDx	144 samples	20040771
TruSight Oncology 500 High-Throughput DNA/RNA		
TruSight Oncology 500 DNA/RNA High-Throughput Kit	24 samples	20040764
TruSight Oncology 500 DNA/RNA High-Throughput Kit, with PierianDx	24 samples	20040768
TruSight Oncology 500 DNA/RNA High-Throughput Kit	72 samples	20040766
TruSight Oncology 500 DNA/RNA High-Throughput Kit, with PierianDx	72 samples	20040770
Index kits for use with TruSight Oncology 500 High-Throughput		
IDT for Illumina UMI DNA Index Anchors–Set A	96 indexes	20034701
IDT for Illumina UMI DNA Index Anchors–Set B	96 indexes	20034702
NovaSeq Reagent Kits for use with TruSight Oncology 500 High-Throughput		
NovaSeq SP Reagent Kit (200 cycles)		20040326
NovaSeq S1 Reagent Kit (200 cycles)		20012864
NovaSeq S2 Reagent Kit (200 cycles)		20012861
NovaSeq S4 Reagent Kit (200 cycles)		20027466

a. Includes DNA library prep and enrichment reagents; does not include NextSeq System sequencing reagents
b. Includes DNA library prep and enrichment reagents, and NextSeq System sequencing reagents

Learn more

For more information about TruSight Oncology 500 and TruSight Oncology 500 High-Throughput, visit www.illumina.com/tso500

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