

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

Enabling flexible, scalable  
comprehensive genomic  
profiling from FFPE samples

- Analyze multiple variant types and key biomarkers in 500+ genes across DNA and RNA in a single assay
- Go from sample to results in 4-5 days using manual or automated workflows that integrate library prep, sequencing, and data analysis with the DRAGEN™ Bio-IT Platform
- Generate accurate data and reliable results that meet demanding performance specifications
- Keep samples in house and obtain data that is relevant to the local institution and community

illumina®

## Introduction

Large-cohort studies show that comprehensive genomic profiling has the potential to identify relevant genetic alterations in up to 90% of samples.<sup>1-6</sup> A single, comprehensive assay to assess a wide range of biomarkers uses less sample and returns 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).

## Analyze multiple tumor types and biomarkers with a single workflow

TruSight Oncology 500 and TruSight Oncology 500 High-Throughput are next-generation sequencing (NGS) assays that simultaneously analyze both DNA and RNA (Figure 1) in one integrated workflow (Figure 2). Panel content includes multiple variant types and key biomarkers (Figure 3) across 523 cancer-relevant genes from DNA and 55 genes from RNA (Table 2, Table 3, and Table 4), eliminating the need to spend time and precious sample, such as formalin fixed, paraffin embedded (FFPE) tissue blocks, on iterative testing.

Table 1: TruSight Oncology 500 and TruSight Oncology 500 High-Throughput

Parameter	TruSight Oncology 500	TruSight Oncology 500 High-Throughput
System	NextSeq 550 System or NextSeq 550Dx System (research mode)	NovaSeq 6000 System
Sample throughput	8 samples per run	16–192 samples per run
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 mm <sup>3</sup> from FFPE tissue samples	Minimum recommendation of 2 mm <sup>3</sup> 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 hr	19 hr (SP and S1), 25 hr (S2), or 36 hr (S4)
Sequence run	2 × 101 cycles	2 × 101 cycles
Software version	DRAGEN TruSight Oncology v2.1	DRAGEN TruSight Oncology v2.1
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)
Analytical specificity	> 99.9995%	> 99.9995%

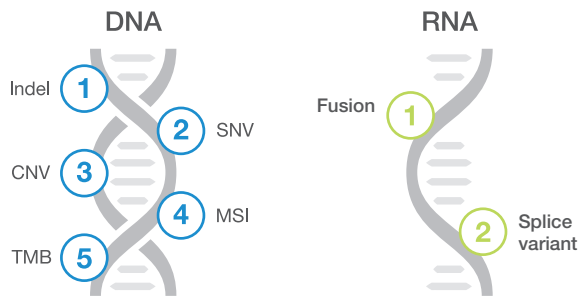


Figure 1: Variant types detected by TruSight Oncology 500 and TruSight Oncology 500 High-Throughput

## Comprehensive content design

Illumina partnered with recognized authorities in the oncology community to design TruSight Oncology 500 and TruSight Oncology 500 High-Throughput content. The resulting panels provide comprehensive coverage of biomarkers commonly mutated in numerous cancer types (Figure 3), 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 (Table 2, Table 3).

Content comprises genes listed in current guidelines with significant coverage of key guidelines for multiple tumor types (Figure 4) and genes involved in over 1000 clinical trials. In addition, the TruSight Oncology 500 panels include the microsatellite instability (MSI) biomarker, with known correlations to responses,<sup>7-9</sup> and the tumor mutational burden (TMB) biomarker (Table 4).<sup>10</sup>

## Integrated workflow

Implementing CGP in house is simplified with the availability of a comprehensive, streamlined workflow that spans from sample input to final report (Figure 2). Using automated library preparation kits and methods, variant calling tools, and interpretation and reporting software enables a smooth workflow that can be completed in as few as four days.

### Start with DNA or RNA

The TruSight Oncology 500 assays can use DNA or RNA 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. Sequencing ready libraries are prepared from sheared gDNA and cDNA simultaneously.

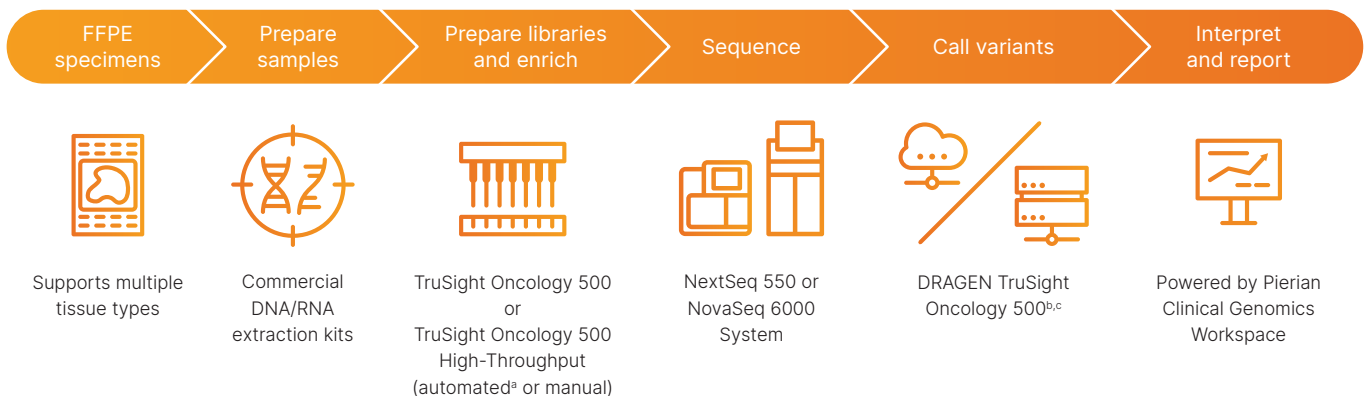


Figure 2: TruSight Oncology 500 workflow—TruSight Oncology 500 and TruSight Oncology 500 High-Throughput integrate into current lab workflows, going from nucleic acids to a variant calls in four days.

- a. TruSight Oncology 500 and TruSight Oncology 500 High-Throughput kits are available in automation-compatible versions.
- b. Local Run Manager is available on the NextSeq 550 System only for use with TruSight Oncology 500.
- c. TruSight Oncology 500 Local App is available on the NextSeq 550 and NovaSeq 6000 Systems.

Table 2: DNA content included in TruSight Oncology 500 and TruSight Oncology 500 High-Throughput

ABL1	BCR	CHEK1	EPHA7	FGF23	GSK3B	IDH2	MAP3K1	NF2	PIK3CA	RAD51D	SMAD4	TGFBR2
ABL2	BIRC3	CHEK2	EPHB1	FGF3	H3F3A	IFNGR1	MAP3K13	NFE2L2	PIK3CB	RAD52	SMARCA4	TMEM127
ACVR1	BLM	CIC	ERBB2	FGF4	H3F3B	INHBA	MAP3K14	NFKBIA	PIK3CD	RAD54L	SMARCB1	TMPRSS2
ACVR1B	BMPR1A	CREBBP	ERBB3	FGF5	H3F3C	INPP4A	MAP3K4	NKX2-1	PIK3CG	RAF1	SMARCD1	TNFAIP3
AKT1	BRAF	CRKL	ERBB4	FGF6	HGF	INPP4B	MAPK1	NKX3-1	PIK3R1	RANBP2	SMC1A	TNFRSF14
AKT2	BRCA1 <sup>a</sup>	CRLF2	ERCC1	FGF7	HIST1H1C	INSR	MAPK3	NOTCH1	PIK3R2	RARA	SMC3	TOP1
AKT3	BRCA2 <sup>a</sup>	CSF1R	ERCC2	FGFR1	HIST1H2BD	IRF2	MAX	NOTCH2	PIK3R3	RASA1	SMO	TOP2A
ALK	BRD4	CSF3R	ERCC3	FGFR2	HIST1H3A	IRF4	MCL1	NOTCH3	PIM1	RB1	SNCAIP	TP53
ALOX12B	BRIP1	CSNK1A1	ERCC4	FGFR3	HIST1H3B	IRS1	MDC1	NOTCH4	PLCG2	RBM10	SOCS1	TP63
ANKRD11	BTG1	CTCF	ERCC5	FGFR4	HIST1H3C	IRS2	MDM2	NPM1	PLK2	RECQL4	SOX10	TRAF2
ANKRD26	BTK	CTLA4	ERG	FH	HIST1H3D	JAK1	MDM4	NRAS	PMAIP1	REL	SOX17	TRAF7
APC	C11orf30	CTNNA1	ERRF1	FLCN	HIST1H3E	JAK2	MED12	NRG1	PMS1	RET	SOX2	TSC1
AR	CALR	CTNNB1	ESR1	FLI1	HIST1H3F	JAK3	MEF2B	NSD1	PMS2	RFWD2	SOX9	TSC2
ARAF	CARD11	CUL3	ETS1	FLT1	HIST1H3G	JUN	MEN1	NTRK1	PNRC1	RHEB	SPEN	TSHR
ARFRP1	CASP8	CUX1	ETV1	FLT3	HIST1H3H	KAT6A	MET	NTRK2	POLD1	RHOA	SPOP	U2AF1
ARID1A	CBFB	CXCR4	ETV4	FLT4	HIST1H3I	KDM5A	MGA	NTRK3	POLE	RICTOR	SPTA1	VEGFA
ARID1B	CBL	CYLD	ETV5	FOXA1	HIST1H3J	KDM5C	MITF	NUP93	PPARG	RIT1	SRC	VHL
ARID2	CCND1	DAXX	ETV6	FOXL2	HIST2H3A	KDM6A	MLH1	NUTM1	PPM1D	RNF43	SRSF2	VTCN1
ARID5B	CCND2	DCUN1D1	EWSR1	FOXO1	HIST2H3C	KDR	MLL	PAK1	PPP2R1A	ROS1	STAG1	WISP3
ASXL1	CCND3	DDR2	EZH2	FOXP1	HIST2H3D	KEAP1	MLLT3	PAK3	PPP2R2A	RPS6KA4	STAG2	WT1
ASXL2	CCNE1	DDX41	FAM123B	FRS2	HIST3H3	KEL	MPL	PAK7	PPP6C	RPS6KB1	STAT3	XIAP
ATM	CD274	DHX15	FAM175A	FUBP1	HLA-A	KIF5B	MRE11A	PALB2	PRDM1	RPS6KB2	STAT4	XPO1
ATR	CD276	DICER1	FAM46C	FYN	HLA-B	KIT	MSH2	PARK2	PREX2	RPTOR	STAT5A	XRCC2
ATRX	CD74	DIS3	FANCA	GABRA6	HLA-C	KLF4	MSH3	PARP1	PRKAR1A	RUNX1	STAT5B	YAP1
AURKA	CD79A	DNAJB1	FANCC	GATA1	HNFI1A	KLHL6	MSH6	PAX3	PRKCI	RUNX1T1	STK11	YES1
AURKB	CD79B	DNMT1	FANCD2	GATA2	HNRNPK	KMT2B	MST1	PAX5	PRKDC	RYBP	STK40	ZBTB2
AXIN1	CDC73	DNMT3A	FANCE	GATA3	HOXB13	KMT2C	MST1R	PAX7	PRSS8	SDHA	SUFU	ZBTB7A
AXIN2	CDH1	DNMT3B	FANCF	GATA4	IGF1	KMT2D	MTOR	PAX8	PTCH1	SDHAF2	SUZ12	ZFHX3
AXL	CDK12	DOT1L	FANCG	GATA6	IGF1R	KRAS	MUTYH	PBRM1	PTEN	SDHB	SYK	ZNF217
B2M	CDK4	E2F3	FANCI	GEN1	IGF2	LAMP1	MYB	PDCD1	PTPN11	SDHC	TAF1	ZNF703
BAP1	CDK6	EED	FANCL	GID4	IKBKE	LATS1	MYC	PDCD1LG2	PTPRD	SDHD	TBX3	ZRSR2
BARD1	CDK8	EGFL7	FAS	GLI1	IKZF1	LATS2	MYCL1	PDGFRA	PTPRS	SETBP1	TCEB1	
BBC3	CDKN1A	EGFR	FAT1	GNA11	IL10	LMO1	MYCN	PDGFRB	PTPRT	SETD2	TCF3	
BCL10	CDKN1B	EIF1AX	FBXW7	GNA13	IL7R	LRP1B	MYD88	PDK1	QKI	SF3B1	TCF7L2	
BCL2	CDKN2A	EIF4A2	FGF1	GNAQ	INHA	LYN	MYOD1	PDPK1	RAB35	SH2B3	TERC	
BCL2L1	CDKN2B	EIF4E	FGF8	GNAS	HRAS	LZTR1	NAB2	PGR	RAC1	SH2D1A	TERT	
BCL2L11	CDKN2C	EML4	FGF9	GPR124	HSD3B1	MAGI2	NBN	PHF6	RAD21	SHQ1	TET1	
BCL2L2	CEBPA	EP300	FGF10	GPS2	HSP90AA1	MALT1	NCOA3	PHOX2B	RAD50	SLIT2	TET2	
BCL6	CENPA	EPCAM	FGF14	GREM1	ICOSLG	MAP2K1	NCOR1	PIK3C2B	RAD51	SLX4	TFE3	
BCOR	CHD2	EPHA3	FGF19	GRIN2A	ID3	MAP2K2	NEGR1	PIK3C2G	RAD51B	SMAD2	TFRC	
BCORL1	CHD4	EPHA5	FGF2	GRM3	IDH1	MAP2K4	NF1	PIK3C3	RAD51C	SMAD3	TGFBR1	

a. Large rearrangements (exon-level CNVs) detected for BRCA1 and BRCA2. Content shaded in gray is analyzed for CNV detection

Table 3: RNA content in the TruSight Oncology 500  
TruSight Oncology 500 High-Throughput panels

<i>ABL1</i>	<i>EGFR</i>	<i>FGFR2</i>	<i>MLL</i>	<i>PAX3</i>
<i>AKT3</i>	<i>EML4</i>	<i>FGFR3</i>	<i>MLL23</i>	<i>PAX7</i>
<i>ALK</i>	<i>ERBB2</i>	<i>FGFR4</i>	<i>MSH2</i>	<i>PDGFRA</i>
<i>AR</i>	<i>ERG</i>	<i>FLI1</i>	<i>MYC</i>	<i>PDGFRB</i>
<i>AXL</i>	<i>ESR1</i>	<i>FLT1</i>	<i>NOTCH1</i>	<i>PIK3CA</i>
<i>BCL2</i>	<i>ETS1</i>	<i>FLT3</i>	<i>NOTCH2</i>	<i>PPARG</i>
<i>BRAF</i>	<i>ETV1</i>	<i>JAK2</i>	<i>NOTCH3</i>	<i>RAF1</i>
<i>BRCA1</i>	<i>ETV4</i>	<i>KDR</i>	<i>NRG1</i>	<i>RET</i>
<i>BRCA2</i>	<i>ETV5</i>	<i>KIF5B</i>	<i>NTRK1</i>	<i>ROS1</i>
<i>CDK4</i>	<i>EWSR1</i>	<i>KIT</i>	<i>NTRK2</i>	<i>RPS6KB1</i>
<i>CSF1R</i>	<i>FGFR1</i>	<i>MET</i>	<i>NTRK3</i>	<i>TMPRSS2</i>

All genes listed are assessed for known and novel fusions; content shaded in gray is analyzed for splice variants

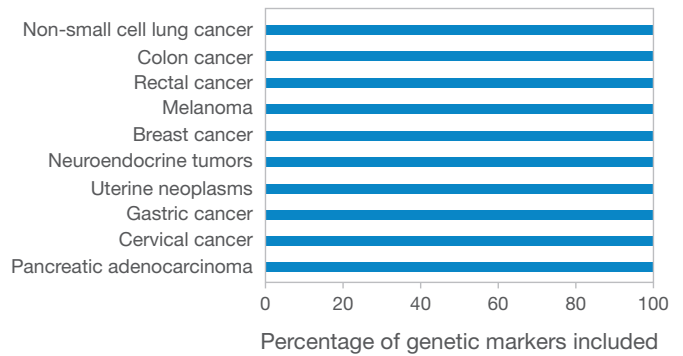


Figure 4: TruSight Oncology 500 content alignment to key guidelines by cancer type—The graph provides examples of content alignment; it is not meant to be all-inclusive.

***NTRK1, NTRK2, NTRK3* (pan-cancer) | *MSI* (pan-cancer) | *TMB* (pan-cancer)**









 Lung	 Melanoma	 Colorectal	 Ovarian	 Breast	 Gastric	 Prostate	 CNS
<i>AKT1</i> <i>ALK</i> <i>ATM</i> <i>BRAF</i> <i>BRCA1</i> <i>BRCA2</i> <i>EGFR</i> <i>ERBB2</i> <i>FGFR1</i> <i>FGFR3</i> <i>KRAS</i> <i>MAP2K1</i> <i>MET</i> <i>NRAS</i> <i>NRG1</i> <i>NUTM1</i> <i>PIK3CA</i> <i>PTEN</i> <i>RET</i> <i>ROS1</i> <i>TP53</i>	<i>ALK</i> <i>BRAF</i> <i>BRCA1</i> <i>BRCA2</i> <i>EGFR</i> <i>ERBB2</i> <i>FGFR1</i> <i>FGFR4</i> <i>HRAS</i> <i>KIT</i> <i>KRAS</i> <i>MAP2K1</i> <i>NRAS</i> <i>NRG1</i> <i>PTEN</i> <i>ROS1</i> <i>TP53</i>	<i>ALK</i> <i>BRAF</i> <i>BRCA1</i> <i>BRCA2</i> <i>EGFR</i> <i>ERBB2</i> <i>FGFR1</i> <i>KRAS</i> <i>MET</i> <i>MLH1</i> <i>MSH2</i> <i>MSH6</i> <i>NRAS</i> <i>NRG1</i> <i>PIK3CA</i> <i>PMS2</i> <i>POLE</i> <i>PTEN</i> <i>RET</i> <i>ROS1</i>	<i>ALK</i> <i>BRAF</i> <i>BRCA1</i> <i>BRCA2</i> <i>EGFR</i> <i>ERBB2</i> <i>FOX2</i> <i>KRAS</i> <i>MET</i> <i>NRG1</i> <i>PDGFRA</i> <i>ROS1</i> <i>TP53</i> <i>HRD</i>	<i>AKT1</i> <i>ALK</i> <i>BRAF</i> <i>BRCA1</i> <i>BRCA2</i> <i>ERBB2</i> <i>ESR1</i> <i>FGFR1</i> <i>FGFR2</i> <i>NRG1</i> <i>NRG1</i> <i>PALB2</i> <i>PIK3CA</i> <i>PTEN</i> <i>ROS1</i> <i>TP53</i> <i>HRD</i>	<i>BRAF</i> <i>EGFR</i> <i>ERBB2</i> <i>FGFR2</i> <i>KIT</i> <i>KRAS</i> <i>MET</i> <i>NRG1</i> <i>PIK3CA</i> <i>PDGFRA</i> <i>TP53</i>	<i>AR</i> <i>ATM</i> <i>BARD1</i> <i>BRAF</i> <i>BRCA1</i> <i>BRCA2</i> <i>BRIP1</i> <i>CDK12</i> <i>CHEK1</i> <i>CHEK2</i> <i>FANCL</i> <i>FGFR2</i> <i>FGFR3</i> <i>NBN</i> <i>NRG1</i> <i>PALB2</i> <i>PPP2R2A</i> <i>PTEN</i> <i>RAD51B</i> <i>RAD51C</i> <i>RAD51D</i> <i>RAD54L</i> <i>RB1</i> <i>ROS1</i> <i>TP53</i>	<i>ATRX</i> <i>BRAF</i> <i>BRIP1</i> <i>EGFR</i> <i>H3F3A</i> <i>HIST1H3B</i> <i>IDH1</i> <i>IDH2</i> <i>MYCN</i> <i>PTCH1</i> <i>RELA</i> <i>TERT</i> <i>TP53</i>

Figure 3: Subset of genomic tumor profiling biomarkers for multiple cancer types—TruSight Oncology 500 and TruSight Oncology 500 High-Throughput include key guideline biomarkers, emerging biomarkers, and pan-cancer biomarkers such as MSI, *NTRK1-3*, and TMB. Content analysis provided by Pierian, based on Knowledge Base v6.10 (February 1, 2022).

Table 4: Simultaneous analysis of multiple lung cancer biomarkers using DNA and RNA in the same sample

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

### Automate for efficiency

TruSight Oncology 500 and TruSight Oncology 500 High-Throughput offer manual and automated options to support scalable library prep. Illumina has partnered with Hamilton and Beckman Coulter Life Sciences, leading liquid-handling manufacturers, to produce fully automated workflows for TruSight Oncology 500 assays that support a range of throughput needs. These automated workflows achieve the same high-quality results produced by manual protocols, while reducing hands-on time by ~50%, enabling labs to save on labor costs and improve efficiency.

### Add tags for analytical specificity

During library preparation, unique molecular identifiers (UMIs)<sup>11</sup> are added to the gDNA or cDNA fragments. These UMIs enable detection of variants at low variant allele frequency (VAF) while simultaneously suppressing errors, providing high analytical specificity.

### Enrich libraries to focus efforts

Library preparation is based on proven hybrid-capture chemistry to purify selected targets from DNA- and RNA-based libraries. Biotinylated probes hybridize to regions of interest, which are pulled down using streptavidin-coated magnetic beads, 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). 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 fewer artifacts and dropouts. Additionally, hybrid-capture chemistry is fusion agnostic, enabling detection and characterization of known and novel fusions. Unlike amplicon-based approaches, which require confirmatory tests as false-positives can arise, the hybrid-capture method is highly sensitive and can accurately characterize gene fusions with both known and novel partners.

### 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™ 550 or NextSeq 550Dx\* Systems, which can batch up to eight samples at a time. TruSight Oncology 500 High-Throughput provides scalability to higher 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.

\* NextSeq 550Dx instrument in research mode


Table 5: Scalable solution

Assay	TruSight Oncology 500	TruSight Oncology 500 High-Throughput			
Instrument	NextSeq 550 or NextSeq 550Dx <sup>a</sup> System	NovaSeq 6000 System			
Flow cell	High-output	SP	S1	S2	S4
No. of samples	8	16	32	72	192

a. NextSeq 550Dx instrument in research mode

## Analyze data

Variant calling for TruSight Oncology 500 and TruSight Oncology 500 High-Throughput is available with the DRAGEN platform either on premise using a local DRAGEN Server or in the cloud using Illumina Connected Analytics (ICA). Both versions take advantage of sophisticated, proprietary algorithms that remove errors, artifacts, and germline variants. The result is highly accurate variant calling performance with an analytical specificity of > 99.9995%. 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. DNA variant data analyzed with the TruSight Oncology 500 Local App and TruSight Oncology 500 DRAGEN pipeline show concordant results (Figure 5C, Figure 6C); however, analysis with the DRAGEN pipeline is completed 2–4× faster than with the local app (Table 6), reducing the time to final results.

 To learn more about ICA, read the [Security, privacy, and compliance with Illumina Connected Analytics technical note](#).

For interpretation and reporting, variant report files can be uploaded into the Pierian Clinical Genomics Workspace cloud. Supported by a comprehensive, continuously updated, expertly curated genomics Knowledgebase,<sup>12</sup> Pierian Clinical Genomics Workspace performs variant annotation and filtering for smooth interpretation and reporting. From thousands of variants in the genome, the Pierian Clinical Genomics Workspace filters and prioritizes biologically relevant variants for the final automated, customizable genomic report.

Table 6: Faster analysis using DRAGEN TruSight Oncology 500 Analysis Software

No. tissue biopsy samples	Average time for analysis to complete <sup>a</sup>	
	Local App <sup>b</sup>	DRAGEN pipeline <sup>c</sup>
8	5.5 hr	2 hr
16	12 hr	3 hr
32	18 hr	5 hr
72	24 hr	10 hr

a. Analysis times are based on actual runs and will vary from run to run  
 b. Local server specifications: Amazon EC2, c5.9xlarge instance (36 vCPU, 72 GiB memory); analysis time will vary with server specifications  
 c. DRAGEN App run on the DRAGEN Server v3

## 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. Traditionally, MSI status has been 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).<sup>13</sup>

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 7).<sup>13</sup> The addition of UMIs during library preparation coupled with proprietary Illumina informatics reduces sequencing error rates by 10–20 fold.<sup>11</sup> Removing FFPE artifacts (such as deamination, oxidation) enables analytical sensitivity as low as 5% VAF from low-quality DNA samples.



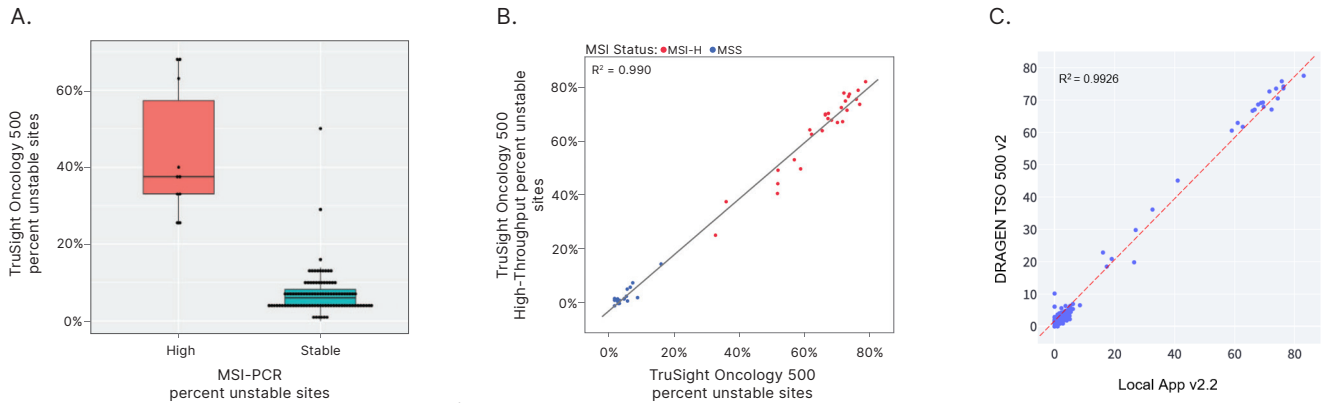


Figure 5: Accurate assessment of MSI status—(A) FFPE tissue samples analyzed using TruSight Oncology 500 produce a quantitative score (Y-axis) compared to a qualitative score using MSI-PCR (X-axis). (B) High concordance of MSI analysis between TruSight Oncology 500 and TruSight Oncology 500 High-Throughput. (C) High concordance between TruSight Oncology 500 data analyzed using DRAGEN TruSight Oncology 500 v2 software and the TruSight Oncology 500 Local App v2.2.

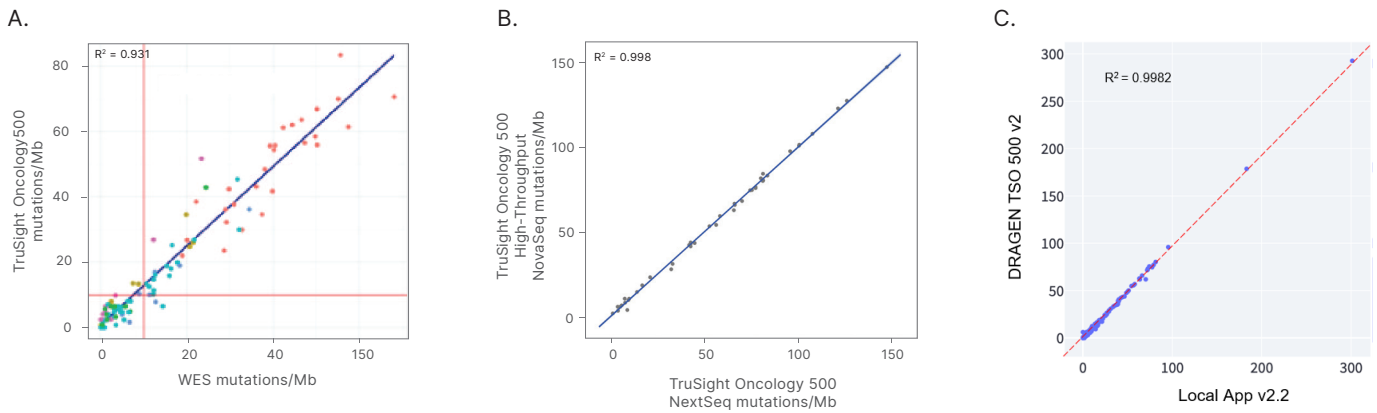


Figure 6: Accurate assessment of TMB status—(A) Analysis of 108 FFPE tissue samples shows high concordance between TMB measurements using WES and TruSight Oncology 500. Red line indicates the threshold value (10 mutations/Mb). (B) High concordance of TMB analysis between TruSight Oncology 500 and TruSight Oncology 500 High-Throughput. (C) High concordance between TruSight Oncology 500 data analyzed using DRAGEN TruSight Oncology 500 v2 software and the TruSight Oncology 500 Local App v2.2.

Table 7: High concordance between whole-exome sequencing (WES) and TruSight Oncology 500 for TMB classification at 10 mutations/Mb

Metric	Value
Percent positive agreement	94.7%
Percent negative 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 have been associated with tumorigenesis.<sup>14</sup> 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 8).



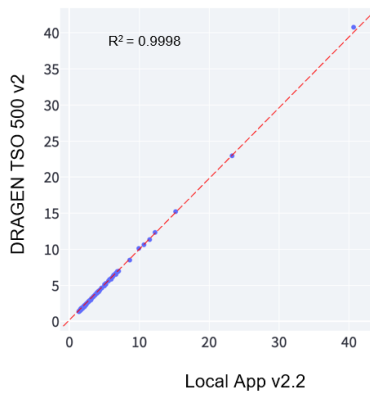


Figure 7: High concordance of CNV detection between TruSight Oncology 500 data analyzed using DRAGEN TruSight Oncology v2 software and TruSight Oncology 500 Local App v2.2

Table 8: Sensitive CNV detection

Gene	Mean fold change	
	DRAGEN TruSight Oncology 500 v2	TruSight Oncology 500 Local App v2.2
AR	2.03	2.17
BRAF	2.09	2.09
BRCA1	1.42	1.42
BRCA2	1.92	1.93
CCND1	4.15	4.14
CCNE1	1.62	1.63
CDK4	3.23	3.24
CDK6	1.85	1.84
CHEK2	1.65	1.68
EGFR	3.55	3.53
ERBB2	8.63	8.66
FGF10	1.60	1.59
FGF19	3.28	3.30
FGFR1	3.57	3.57
KRAS	2.19	2.19
MDM2	2.46	2.47
MDM4	1.65	1.64
MET	1.70	1.69
MYC	1.97	1.98
MYCN	1.45	1.46

The information in this table shows examples of high concordance of data analyzed with DRAGEN TruSight Oncology 500 v2 software and the TruSight Oncology 500 Local App v2.2 and is not a comprehensive list of the CNVs detected.

## Detection of BRCA large rearrangements

A BRCA large rearrangement (LR) step in the DRAGEN TruSight Oncology 500 v2 analysis workflow enables exon-level CNV detection for BRCA 1 and 2 genes. For three or more exons, sensitivity is 43% while under three exons is 50% on the NextSeq 550 System (Table 9).

Table 9: Sensitive detection of BRCA LR

BRCA 1/2 LR detected	Estimated LR VAF
BRCA1 loss exon 8	0.26
BRCA2 loss exon 21-24	0.44
BRCA1 loss exon 14-24	0.51
BRCA1 loss exon 21-24	0.85
BRCA1 loss exon 1-3	0.48
BRCA1 loss exon 1-23	0.70
BRCA2 gain exon 25-27	0.37
BRCA1 loss exon 1-3	0.86
BRCA1 gain exon 5-16	0.83
BRCA1 gain exon 17-18	0.51
BRCA1 gain exon 1-16	0.61
BRCA1 gain exon 13	0.69
BRCA2 gain exon 25	0.40
BRCA2 gain exon 11-27	0.54
BRCA2 gain exon 12-13	0.35
BRCA1 loss exon 22	0.92

a. TruSight Oncology 500 HRD run on NextSeq 550 System. These data were generated with DRAGEN TruSight Oncology 500 v2 software.

## 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 10).

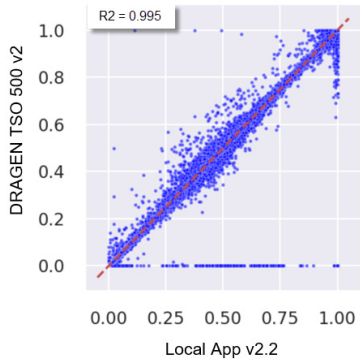


Figure 8: High concordance of VAF between TruSight Oncology 500 data analyzed using DRAGEN TruSight Oncology v2 software and TruSight Oncology 500 Local App v2.2

### Robust detection of fusions

Cancer can arise from epigenetic changes, expression level changes, and gene fusions that are undetectable by standard sequencing.<sup>15,16</sup> The TruSight Oncology 500 assays detect and characterize fusions agnostic from the partner. 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 available, 80 ng RNA input helps maximize sensitivity for fusions present at low concentrations (Table 11).

### Plan for the future

TruSight Oncology 500 and TruSight Oncology 500 High-Throughput integrate easily into labs currently using NGS, enabling them to offer CGP 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.

Table 10: Highly sensitive DNA small variant detection

Gene	Mutation	DRAGEN TruSight Oncology 500 v2 (Mean VAF)
Variant type: single nucleotide variant (SNV)		
<i>AKT1</i>	E17K	5%
<i>APC</i>	R1450*	8%
<i>BRAF</i>	V600E	13%
<i>CTNNB1</i>	T41A	8%
<i>EGFR</i>	L858R	7%
<i>EGFR</i>	T790M	7%
<i>FGFR3</i>	S249C	6%
<i>FOXL2</i>	C134W	7%
<i>GNAS</i>	R201C	7%
<i>IDH1</i>	R132C	7%
<i>KIT</i>	D816V	8%
<i>KRAS</i>	G12D	7%
<i>NOTCH1</i>	P668S	5%
<i>NRAS</i>	Q61R	7%
<i>PIK3CA</i>	E545G	5%
<i>RET</i>	M918T	8%
<i>TP53</i>	R248Q	7%
Variant type: complex variant		
<i>EGFR</i>	L747_P753>Q	3%
Variant type: insertion		
<i>APC</i>	T1556Nfs*3	7%
<i>ERBB2</i>	A775-G776insYVMA	7%
Variant type: deletion		
<i>FBXW7</i>	FBXW7:G667fs	5%
<i>PTEN</i>	PTEN:K267fs*9	7%
<i>TP53</i>	TP53:C242fs*5	6%

The information in this table shows examples of concordance between data analyzed with DRAGEN TruSight Oncology 500 v2 software and the TruSight Oncology 500 Local App v2.2 and is not a comprehensive list of the SNVs and indels detected.

Table 11: Robust detection of fusions and splice variants

RNA fusion	RNA input amount			Tissue
	40 ng	60 ng	80 ng	
<i>ALK-EML4</i>	15	21	40	Lung
<i>EGFR-RAB3IP</i>	5	9	19	Brain
<i>EGFR-METTL1</i>	25	84	71	Brain
<i>BRCA1-MPP2</i>	25	28	29	Unknown
<i>ALK-BRE</i>	75	112	128	Sarcoma
<i>CCDC170-ESR1</i>	122	59	168	Kidney
<i>MYC-MRPL13</i>	27	35	52	Breast
<i>MYC-STK3</i>	11	39	28	Breast
<i>ROS1;GOPC-ENC1</i>	32	53	93	Lung
<i>ROS1;GOPC-CD74</i>	104	92	141	Lung
<i>ANKUB1; RNF13-ETV5;DGKG</i>	29	45	72	Uterus
<i>NTRK3-SEMA6A</i>	7	16	25	Skin
<i>RET-NCOA4</i>	74	78	154	Thyroid
<i>EWSR1-ATF1</i>	19	30	32	Sarcoma
<i>EWSR1-CBY1</i>	44	30	97	Sarcoma
<i>BRCA2-NRXN3</i>	33	60	84	Bone
<i>FLT3-SMOX</i>	50	72	54	Bone
<i>FLT3-VWA8</i>	29	51	69	Bone
<i>FLT3-LCP1</i>	12	32	47	Bone
<b>Splice variant</b>				
<i>ARV7</i>	26	38	46	Breast
<i>EGFR v3</i>	567	884	937	Brain
<i>EGFR v3</i>	1249	1614	2049	Brain

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 SNVs and indels detected. These data were generated with a Local App pipeline (not DRAGEN software).

## Summary

TruSight Oncology 500 and TruSight Oncology 500 High-Throughput are NGS-based, hybrid-capture assays that enable CGP through analysis of 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. Leveraging the power of the DRAGEN platform enables TruSight Oncology 500 to improve lab efficiency and produce meaningful results.

## Learn more

TruSight Oncology 500 and TruSight Oncology 500 High-Throughput, [illumina.com/tso500](https://illumina.com/tso500)

DRAGEN Bio-IT Platform, [illumina.com/products/by-type/informatics-products/dragen-bio-it-platform.html](https://illumina.com/products/by-type/informatics-products/dragen-bio-it-platform.html)

Illumina Connected Analytics, [illumina.com/products/by-type/informatics-products/connected-analytics.html](https://illumina.com/products/by-type/informatics-products/connected-analytics.html)

Ordering information TruSight Oncology 500

	Sample type	Library prep		Pierian included	On-premise variant reporting	
		Product	Catalog no.		Product	Catalog no.
Manual	DNA	TruSight Oncology 500 DNA Kit <sup>a</sup> (16 indexes, 48 samples)	20028213		Illumina DRAGEN Server v3	20040619
		TruSight Oncology 500 DNA Kit, plus Pierian <sup>a</sup> (16 indexes, 48 samples)	20032624	✓	Illumina DRAGEN Server v4	20051343
		TruSight Oncology 500 DNA Kit, for Use with NextSeq <sup>b</sup> (16 indexes, 48 samples)	20028214		Illumina DRAGEN Server Advance Exchange Plan	20032797
		TruSight Oncology 500 DNA Kit, for Use with NextSeq, plus Pierian <sup>b</sup> (16 indexes, 48 samples)	20032625	✓	Cloud-based variant reporting	
	DNA/RNA	TruSight Oncology 500 DNA/RNA Bundle <sup>a</sup> (16 indexes, 24 samples)	20028215		ICA Basic Annual Subscription	20044874
		TruSight Oncology 500 DNA/RNA Bundle, plus Pierian <sup>a</sup> (16 indexes, 24 samples)	20032626	✓	ICA Professional Annual Subscription	20044876
		TruSight Oncology 500 DNA/RNA Bundle, for Use with NextSeq <sup>b</sup> (16 indexes, 24 samples)	20028216		ICA Enterprise Annual Subscription	20038994
		TruSight Oncology 500 DNA/RNA Bundle, for Use with NextSeq, plus Pierian <sup>b</sup> (16 indexes, 24 samples)	20032627	✓	ICA Enterprise Compliance Add-on (applies to Basic only)	20066830
Automated	DNA	TruSight Oncology 500 DNA Automation <sup>a</sup> Kit (16 indexes, 64 samples)	20045504		ICA Training and Onboarding	20049422
		TruSight Oncology 500 DNA Automation Kit, plus Pierian <sup>a</sup> (16 indexes, 64 samples)	20045506	✓	ICA Data Storage: Illumina Analytics, 1 credit	20042038
		TruSight Oncology 500 DNA Automation Kit, for Use with NextSeq <sup>b</sup> (16 indexes, 64 samples)	20045505		ICA Data Storage: Illumina Analytics Starter Pack, 1000 credits	20042039
		TruSight Oncology 500 DNA Automation Kit, for Use with NextSeq, plus Pierian <sup>b</sup> (16 indexes, 64 samples)	20045507	✓	ICA Data Storage: Illumina Analytics, 5000 credits	20042040
	DNA/RNA	TruSight Oncology 500 DNA/RNA <sup>a</sup> Automation Kit (16 indexes, 32 samples)	20045508		ICA Data Storage: Illumina Analytics, 50,000 credits	20042041
		TruSight Oncology 500 DNA/RNA Automation Kit, plus Pierian <sup>a</sup> (16 indexes, 32 samples)	20045509	✓	ICA Data Storage: Illumina Analytics, 100,000 credits	20042042
		TruSight Oncology 500 DNA/RNA Automation Kit, for Use with NextSeq <sup>b</sup> (16 indexes, 32 samples)	20045990			
		TruSight Oncology 500 DNA/RNA Automation Kit, for Use with NextSeq, plus Pierian <sup>b</sup> (16 indexes, 32 samples)	20045991	✓		

a. Includes library prep and enrichment reagents; does not include NextSeq 550 System sequencing reagents  
 b. Includes library prep and enrichment reagents and NextSeq 550 System sequencing reagent

Ordering information: TruSight Oncology 500 and TruSight Oncology 500 High-Throughput

Sample type	Library prep		Pierian included	Automation	
	Product	Catalog no.		Product	Catalog no.
Manual	DNA	TruSight Oncology 500 DNA High-Throughput Kit <sup>a</sup> (48 samples)	20040765		Beckman Coulter i-Series Contact Illumina sales
		TruSight Oncology 500 DNA High-Throughput Kit, with Pierian <sup>a</sup> (48 samples)	20040769	✓	Hamilton Microlab STAR Contact Illumina sales
		TruSight Oncology 500 DNA High-Throughput Kit <sup>a</sup> (144 samples)	20040767		
		TruSight Oncology 500 DNA High-Throughput, with Pierian <sup>a</sup> (144 samples)	20040771	✓	
	DNA/RNA	TruSight Oncology 500 DNA/RNA High-Throughput Kit <sup>a</sup> (24 samples)	20040764		
		TruSight Oncology 500 DNA/RNA High-Throughput Kit, with Pierian <sup>a</sup> (24 samples)	20040768	✓	
		TruSight Oncology 500 DNA/RNA High-Throughput Kit <sup>a</sup> (72 samples)	20040766		
		TruSight Oncology 500 DNA/RNA High-Throughput Kit, with Pierian <sup>a</sup> (72 samples)	20040770	✓	
Automated	DNA	TruSight Oncology 500 DNA High-Throughput Automation Kit <sup>a</sup> (64 samples)	20049283		
		TruSight Oncology 500 DNA High-Throughput Automation Kit <sup>a</sup> (64 samples) plus Pierian	20049277	✓	
		TruSight Oncology 500 DNA High-Throughput Automation Kit <sup>a</sup> (144 samples)	20049285		
		TruSight Oncology 500 DNA High-Throughput Automation Kit <sup>a</sup> (144 samples) plus Pierian	20049279	✓	
	DNA/RNA	TruSight Oncology 500 DNA/RNA High-Throughput Automation Kit <sup>a</sup> (32 samples)	20049282		
		TruSight Oncology 500 DNA/RNA High-Throughput Automation Kit <sup>a</sup> (32 samples) plus Pierian	20049276	✓	
		TruSight Oncology 500 DNA/RNA High-Throughput Automation Kit <sup>a</sup> (72 samples)	20049284		
		TruSight Oncology 500 DNA/RNA High-Throughput Automation Kit <sup>a</sup> (72 samples) plus Pierian	20049278	✓	

a. Includes library prep and enrichment reagents; does not include IDT for Illumina Indexes or NovaSeq 6000 System sequencing reagents

Ordering information: TruSight Oncology 500 High-Throughput

Consumables			On-premise variant reporting	
	Product	Catalog no.	Product	Catalog no.
Index kits			Illumina DRAGEN Server v3	20040619
Manual	IDT for Illumina UMI DNA/RNA UD Indexes Set A, Ligation (96 indexes, 96 samples)	20034701	Illumina DRAGEN Server v4	20051343
	IDT for Illumina UMI DNA/RNA UD Indexes Set B, Ligation (96 indexes, 96 samples)	20034702	Illumina DRAGEN Server Advance Exchange Plan	20032797
Automated	IDT for Illumina UMI DNA/RNA UD Indexes for Automation Set A, Ligation (96 indexes, 96 samples)	20066404	Cloud-based variant reporting	
	IDT for Illumina UMI DNA/RNA UD Indexes for Automation Set B, Ligation (96 indexes, 96 samples)	20063213	ICA Basic Annual Subscription	20044874
Sequencing reagent kits			ICA Professional Annual Subscription	20044876
	NovaSeq 6000 SP Reagent Kit v1.5 (200 cycles)	20040719	ICA Enterprise Annual Subscription	20038994
	NovaSeq 6000 S1 Reagent Kit v1.5 (200 cycles)	20028318	ICA Enterprise Compliance Add-on (applies to Basic only)	20066830
	NovaSeq 6000 S2 Reagent Kit v1.5 (200 cycles)	20028315	ICA Training and Onboarding	20049422
	NovaSeq 6000 S4 Reagent Kit v1.5 (200 cycles)	20028313	ICA Data Storage: Illumina Analytics, 1 credit	20042038
			ICA Data Storage: Illumina Analytics Starter Pack, 1000 credits	20042039
			ICA Data Storage: Illumina Analytics, 5000 credits	20042040
			ICA Data Storage: Illumina Analytics, 50,000 credits	20042041
			ICA Data Storage: Illumina Analytics, 100,000 credits	20042042

## References

1. Stransky N, Cerami E, Schalm S, Kim JL, Lengauer C. [The landscape of kinase fusions in cancer](#). *Nat Commun*. 2014;5:4846. doi:10.1038/ncomms5846.
2. Boland GM, Piha-Paul SA, Subbiah V, et al. [Clinical next generation sequencing to identify actionable aberrations in a phase I program](#). *Oncotarget*. 2015;6(24):20099-20110. doi:10.18632/oncotarget.4040
3. Massard C, Michiels S, Ferte C, et al. [High-Throughput Genomics and Clinical Outcome in Hard-to-Treat Advanced Cancers: Results of the MOSCATO 01 Trial](#). *Cancer Discov*. 2017;7(6):586-595. doi:10.1158/2159-8290.CD-16-1396.
4. Harris MH, DuBois SG, Glade Bender JL, et al. [Multicenter Feasibility Study of Tumor Molecular Profiling to Inform Therapeutic Decisions in Advanced Pediatric Solid Tumors: The Individualized Cancer Therapy \(iCat\) Study](#). *JAMA Oncol*. 2016;2(5):608-615. doi:10.1001/jamaoncol.2015.5689
5. Parsons DW, Roy A, Yang Y, et al. [Diagnostic Yield of Clinical Tumor and Germline Whole-Exome Sequencing for Children With Solid Tumors](#). *JAMA Oncol*. 2016;2(5):616-624. doi:10.1001/jamaoncol.2015.5699
6. 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
7. 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
8. 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:10.1038/s41588-018-0312-8
9. U.S. Food & Drug Administration. FDA Approves First-Line Immunotherapy for Patients with MSI-H/dMMR Metastatic Colorectal Cancer. [fda.gov/news-events/press-announcements/fda-approves-first-line-immunotherapy-patients-msi-hdmmr-metastatic-colorectal-cancer](#). Published 2020. Accessed March 30, 2022.
10. U.S. Food & Drug Administration. FDA approves pembrolizumab for adults and children with TMB-H solid tumors. [fda.gov/drugs/drug-approvals-and-databases/fda-approves-pembrolizumab-adults-and-children-tmb-h-solid-tumors](#). Published 2020. Accessed March 30, 2022.
11. Illumina. TruSight Oncology UMI Reagents technical note. [illumina.com/content/dam/illumina-marketing/documents/products/datasheets/trusight-oncology-umi-reagents-datasheet-1000000050425.pdf](#). Published 2018. Accessed March 30, 2022.
12. Pierian. Genomic Knowledgebase. [pieriandx.com/genomic-knowledgebase](#). Accessed March 30, 2022.
13. Illumina. Analysis of TMB and MSI Status with TruSight Oncology 500. [illumina.com/content/dam/illumina-marketing/documents/products/appnotes/trusight-oncology-500-tmb-analysis-1170-2018-009.pdf](#). Published 2018. Accessed March 30, 2022.
14. Beroukhi R, Mermel CH, Porter D, et al. [The landscape of somatic copy-number alteration across human cancers](#). *Nature*. 2010;463(7283):899-905. doi:10.1038/nature08822
15. Green MR, Vicente-Dueñas C, Romero-Camarero I, et al. [Transient expression of Bcl6 is sufficient for oncogenic function and induction of mature B-cell lymphoma](#). *Nat Commun*. 2014;5:3904. doi:10.1038/ncomms4904
16. Piskol R, Ramaswami G, Li JB. [Reliable identification of genomic variants from RNA-seq data](#). *Am J Hum Genet*. 2013;93(4):641-651. doi:10.1016/j.ajhg.2013.08.008

# illumina®

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

© 2022 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-GL-00173 v4.0