DATA SHEET

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
- 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

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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 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). 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.

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 for DNA and 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.

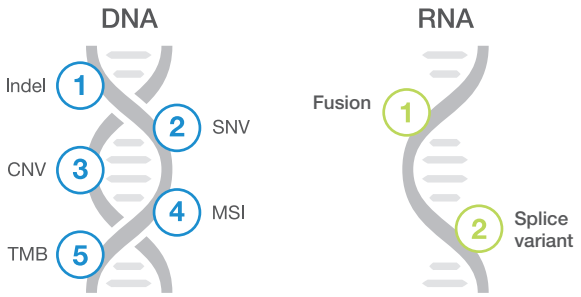

Figure 1: Variant types detected by TruSight Oncology 500 and TruSight Oncology 500 High-Throughput

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

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 mm^3 from FFPE tissue samples	Minimum recommendation of 2 mm^3 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)
Analytical specificity	99.9998%	99.9998%

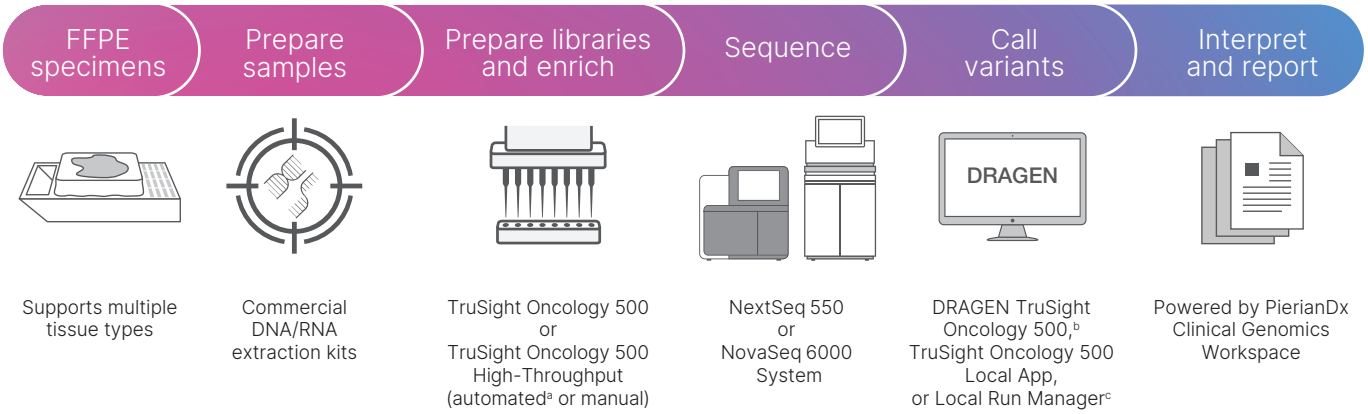

Figure 2: TruSight Oncology 500 workflow—TruSight Oncology 500 and TruSight Oncology 500 High-Throughput integrate into current lab workflows, going from nucleic acids to a variant calls in four days. Local Run Manager is available only with TruSight Oncology 500.

a. TruSight Oncology 500 and TruSight Oncology 500 High-Throughput kits are available in automation-compatible versions.
b. On-premise and cloud-based DRAGEN TruSight Oncology 500 Analysis Software coming soon.
c. Local Run Manager is available on the NextSeq 550 System only.

***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 3: Genomic tumor profiling biomarkers for multiple cancer types—TruSight Oncology 500 and TruSight Oncology 500 High-Throughput include key guideline biomarkers for multiple cancer types, plus pan-cancer biomarkers such as MSI, *NTRK1-3*, and TMB.

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*	*CRLF2*	*ERCC1*	*FGF7*	*HIST1H1C*	*INSR*	*MAPK3*	*NOTCH1*	*PIK3R2*	*RARA*	*SMC3*	*TOP1*
AKT3	*BRCA2*	*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*	*ERRFI1*	*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*	*HNF1A*	*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*	

Content shaded in gray is analyzed for CNV detection

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

ABL1	*EGFR*	*FGFR2*	*MLL*	*PAX3*
AKT3	*EML4*	*FGFR3*	*MLLT3*	*PAX7*
ALK	*ERBB2*	*FGFR4*	*MSH2*	*PDGFRA*
AR	*ERG*	*FLI1*	*MYC*	*PDGFRB*
AXL	*ESR1*	*FLT1*	*NOTCH1*	*PIK3CA*
BCL2	*ETS1*	*FLT3*	*NOTCH2*	*PPARG*
BRAF	*ETV1*	*JAK2*	*NOTCH3*	*RAF1*
BRCA1	*ETV4*	*KDR*	*NRG1*	*RET*
BRCA2	*ETV5*	*KIF5B*	*NTRK1*	*ROS1*
CDK4	*EWSR1*	*KIT*	*NTRK2*	*RPS6KB1*
CSF1R	*FGFR1*	*MET*	*NTRK3*	*TMPRSS2*

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

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,[7-9] and the tumor mutational burden (TMB) biomarker (Table 4).[10]

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**
AKT1	✓	
ALK	✓	✓
BRAF	✓	✓
DDR2	✓	
EGFR	✓	✓
ERBB2	✓	✓
FGFR1	✓	✓
FGFR3	✓	✓
KRAS	✓	
MAP2K1	✓	
MET	✓	✓
NRAS	✓	
NTRK1	✓	✓
NTRK2	✓	✓
NTRK3	✓	✓
PIK3Ca	✓	✓
PTEN	✓	
RET	✓	✓
TP53	✓	

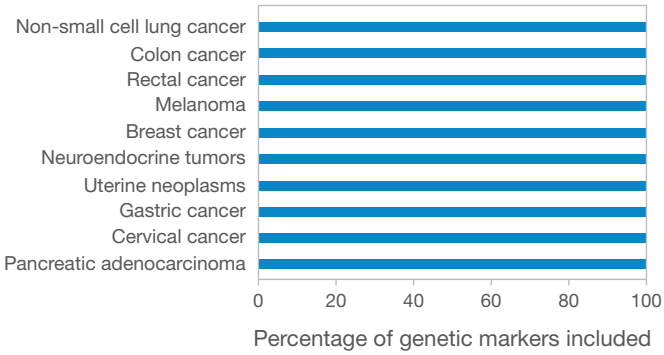

Figure 4: TruSight Oncology 500 content alignment to key guidelines by cancer type—The graph provides examples of content alignment; it is not meant to be all-inclusive.

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.

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 Hamiton 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)[11] 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 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™ 500 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.

Table 5: Scalable solution

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

a. NextSeq 550Dx instrument in research mode

* NextSeq 550Dx instrument in research mode

Analyze data

Variant calling for TruSight Oncology 500 and TruSight Oncology 500 High-Throughput is currently perfomed using a local app on a local server. A DRAGEN™ version of the app, run either on premise using a local DRAGEN Server or as a cloud-based solution, will be coming soon. Both apps 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 99.9998%. 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. DNA variant data analyzed with the TruSight Oncology 500 Local App and TruSight Oncology 500 DRAGEN App show concordant results (Figure 5C, Figure 6C); however, the DRAGEN App completes analysis 2-4× faster than the Local App (Table 6), reducing the time to final results.

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

Table 6: Faster analysis using the TruSight Oncology 500 DRAGEN App[a]

No. tissue biopsy samples	Average time for analysis to complete[b]	
	Local App[c]	DRAGEN App[d]
8	5.5 hours	2 hours
16	12 hours	3 hours
32	18 hours	5 hours
72	24 hours	10 hours

a. On-premise and cloud-based DRAGEN TruSight Oncology 500 Analysis Software coming soon
b. Analysis times are based on actual runs and will vary from run to run
c. Local server specifications: Amazon EC2, c5.9xlarge instance (36 vCPU, 72 GiB memory); analysis time will vary with server specifications
d. 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).[13]

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

Table 7: High concordance between 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

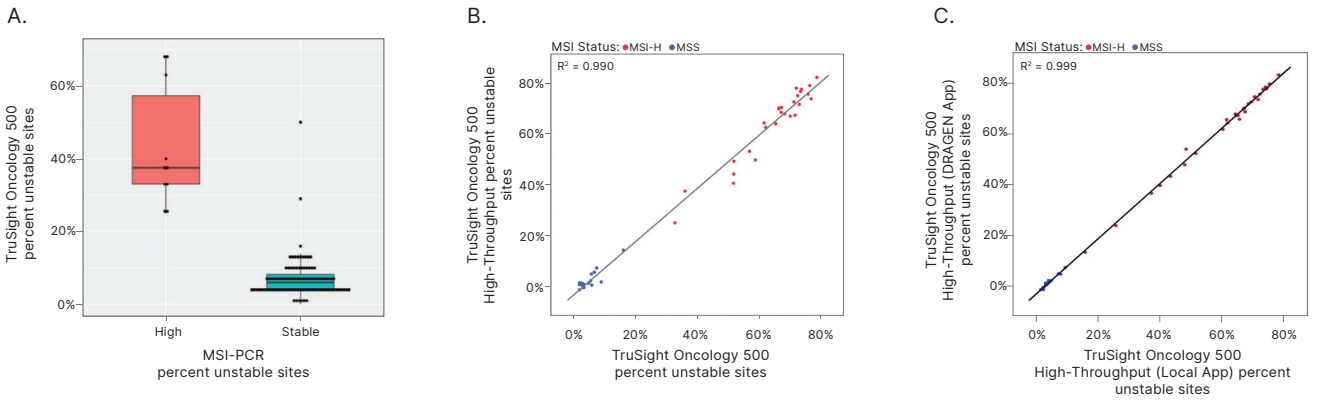

Figure 5: Accurate assessment of MSI status—(A) FFPE tissue samples analyzed using TruSight Oncology 500 produce a quantitative score (Y-axis) compared to a qualitative score using MSI-PCR (X-axis). (B) High concordance of MSI analysis between TruSight Oncology 500 and TruSight Oncology 500 High-Throughput. (C) High concordance between TruSight Oncology 500 High-Throughput data analyzed using the TruSight Oncology 500 DRAGEN App and the TruSight Oncology 500 Local App. Note: On-premise and cloud-based DRAGEN TruSight Oncology 500 Analysis Software coming soon.

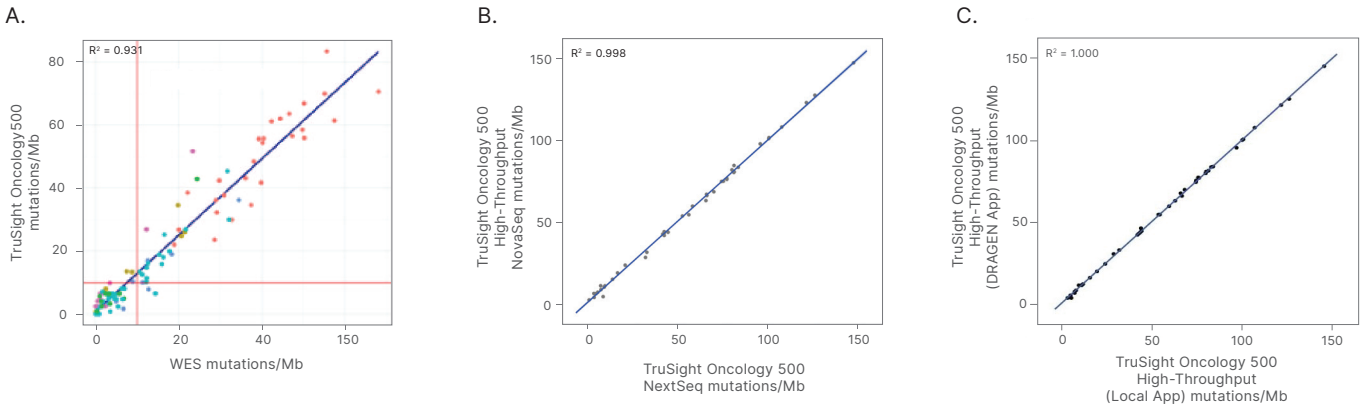

Figure 6: Accurate assessment of TMB status—(A) Analysis of 108 FFPE tissue samples shows high concordance between TMB measurements using WES and TruSight Oncology 500. Red line indicates the threshold value (10 mutations/Mb). (B) High concordance of TMB analysis between TruSight Oncology 500 and TruSight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

Sensitive detection of CNVs

Copy-number changes in several genes and tumor types have been associated with tumorigenesis.[14] 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).

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 9).

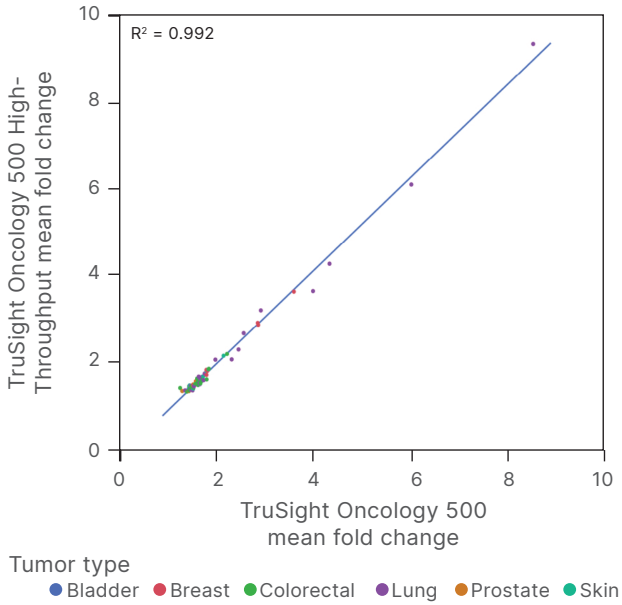

Figure 7: High concordance of CNV detection between TruSight Oncology 500 and TruSight Oncology 500 High-Throughput

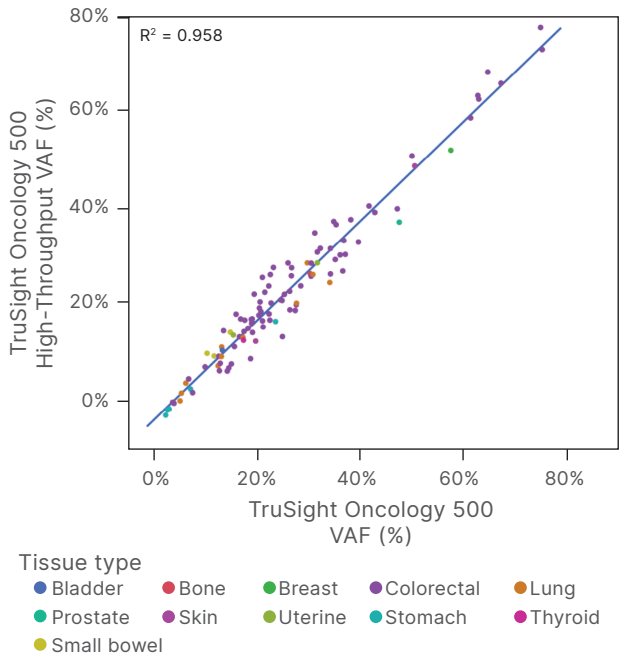

Figure 8: High concordance of VAF between TruSight Oncology 500 and TruSight Oncology 500 High-Throughput

Table 8: Sensitive CNV detection[a]

Gene	Fold change			Tissue
	TruSight Oncology 500	TruSight Oncology 500 HT		
		Local App	DRAGEN App[b]	
ERBB2	23.43	23.37	23.90	Breast
MDM2	8.50	9.34	10.58	Lung
EGFR	6.00	6.12	6.53	Lung
EGFR	4.32	4.31	4.31	Lung
MET	3.98	3.68	3.90	Lung
MYC	3.59	3.67	3.71	Breast
ERBB2	2.86	2.91	2.96	Breast
BRAF	2.31	2.12	2.07	Lung
MYC	2.22	2.24	2.25	Colorectal
CCND1	2.15	2.20	2.15	Skin
KRAS	1.82	1.86	1.87	Breast
MDM4	1.80	1.77	1.85	Breast
CCNE1	1.76	1.79	1.71	Lung
FGF19	1.73	1.74	1.70	Skin
AR	1.72	1.68	1.66	Colorectal
MET	1.69	1.62	1.66	Colorectal
KRAS	1.64	1.73	1.79	Lung
MYCN	1.63	1.66	1.60	Colorectal
CDK6	1.62	1.60	1.62	Colorectal
CHEK2	1.58	1.54	1.49	Lung
FGF10	1.54	1.51	1.58	Lung
BRCA2	1.53	1.53	1.51	Breast
FGF7	1.49	1.50	1.53	Colorectal
FGFR1	1.39	1.38	1.39	Colorectal

a. 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

b. On-premise and cloud-based DRAGEN TruSight Oncology 500 Analysis Software coming soon

Table 9: Highly sensitive DNA small variant detection[a]

Gene	Mutation	VAF		
		TruSIght Oncology 500	TruSIght Oncology 500 High-Throughput	
			Local App	DRAGEN App[b]
Variant type: single nucleotide variant (SNV)				
AKT1	E17K	20%	18%	16%
BRAF	V600E	19%	19%	19%
CDKN2A	R58	12%	14%	14%
CTNNB1	G34E	16%	18%	18%
EGFR	L858R	18%	17%	17%
EGFR	T790M	13%	12%	12%
FBXW7	R465C	8%	7%	7%
FGFR2	S252W	32%	32%	31%
GNAS	R844C	5%	5%	5%
H3F3B	K37M	31%	30%	29%
IDH2	R140Q	23%	22%	22%
KRAS	G12D	6%	6%	6%
NRAS	Q61K	15%	18%	18%
PIK3CA	E542K	14%	15%	15%
PTCH1	A563V	4%	4%	4%
SMARCA4	R973W	3%	3%	3%
TP53	R248Q	29%	27%	27%
Variant type: multiple nucleotide variant (MNV)				
RET	A845V	7%	8%	8%
Variant type: insertion				
APC	T1556Nfs*3	21%	20%	20%
Variant type: deletion				
ARID1A	D1850Tfs*33	4%	5%	5%
EP300	H2324fs*29	24%	20%	20%
KMT2A (MLL)	K3828Rfs*31	3%	3%	3%
PTEN	K267Rfs*9	21%	21%	19%
RNF43	G659Vfs*41	18%	18%	18%

a. 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
b. On-premise and cloud-based DRAGEN TruSight Oncology 500 Analysis Software coming soon

Robust detection of fusions

Cancer can arise from epigenetic changes, expression level changes, and gene fusions that are undetectable by standard sequencing.[15,16] 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 10).

Plan for the future

TruSight Oncology 500 and TruSight Oncology 500 High-Throughout 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 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 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. Leverage the power of TruSight Oncology 500 to improve lab efficiency and produce meaningful results.

Table 10: Robust detection of fusions and splice variants

RNA fusion	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

a. 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
b. On-premise and cloud-based DRAGEN TruSight Oncology 500 Analysis Software coming soon

Learn more

TruSight Oncology 500 and TruSight Oncology 500 High-Throughput: www.illumina.com/tso500

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Ordering information TruSight Oncology 500

Sample type		Library prep		PierianDx included	Variant calling	
		Product	Catalog no.		Product	Catalog no.
Manual	DNA	TruSight Oncology 500 DNA Kit[a] (16 indexes, 48 samples)	20028213		1-year Licenses	
		TruSight Oncology 500 DNA Kit, plus PierianDx[a] (16 indexes, 48 samples)	20032624	✓	DRAGEN TruSight Oncology 500 Analysis Software, On-Premise, Level 1 (120 DNA or 96 DNA/RNA samples)	Coming soon
		TruSight Oncology 500 DNA Kit, for Use with NextSeq[b] (16 indexes, 48 samples)	20028214		DRAGEN TruSight Oncology 500 Analysis Software, On-Premise, Level 2 (360 DNA or 288 DNA/RNA samples)	Coming soon
		TruSight Oncology 500 DNA Kit, for Use with NextSeq, plus PierianDx[b] (16 indexes, 48 samples)	20032625	✓	DRAGEN TruSight Oncology 500 Analysis Software, On-Premise, Level 3 (600 DNA or 480 DNA/RNA samples)	Coming soon
	DNA/RNA	TruSight Oncology 500 DNA/RNA Bundle[a] (16 indexes, 24 samples)	20028215		DRAGEN TruSight Oncology 500 Analysis Software, On-Premise, Level 4 (925 DNA or 740 DNA/RNA samples)	Coming soon
		TruSight Oncology 500 DNA/RNA Bundle, plus PierianDx[a] (16 indexes, 24 samples)	20032626	✓	DRAGEN TruSight Oncology 500 Analysis Software, On-Premise, Level 5 (1850 DNA or 1480 DNA/RNA samples)	Coming soon
		TruSight Oncology 500 DNA/RNA Bundle, for Use with NextSeq[b] (16 indexes, 24 samples)	20028216		DRAGEN TruSight Oncology 500 Analysis Software, On-Premise, Level 6 (5550 DNA or 4440 DNA/RNA samples)	Coming soon
		TruSight Oncology 500 DNA/RNA Bundle, for Use with NextSeq, plus PierianDx[b] (16 indexes, 24 samples)	20032627	✓	DRAGEN TruSight Oncology 500 Analysis Software, On-Premise, Level 7 (9250 DNA or 7400 DNA/RNA samples)	Coming soon
Automated	DNA	TruSight Oncology 500 DNA Automation[a] Kit (16 indexes, 64 samples)	20045504		DRAGEN TruSight Oncology 500 Analysis Software, On-Premise, Level 8 (18,500 DNA or 14,800 DNA/RNA samples)	Coming soon
		TruSight Oncology 500 DNA Automation Kit, plus PierianDx[a] (16 indexes, 64 samples)	20045506	✓	Hardware (for on-premise analysis)	
		TruSight Oncology 500 DNA Automation Kit, for Use with NextSeq[b] (16 indexes, 64 samples)	20045505		DRAGEN S3 Server	20040619
		TruSight Oncology 500 DNA Automation Kit, for Use with NextSeq, plus PierianDx[b] (16 indexes, 64 samples)	20045507	✓		
	DNA/RNA	TruSight Oncology 500 DNA/RNA[a] Automation Kit (16 indexes, 32 samples)	20045508			
		TruSight Oncology 500 DNA/RNA Automation Kit, plus PierianDx[a] (16 indexes, 32 samples)	20045509	✓		
		TruSight Oncology 500 DNA/RNA Automation Kit, for Use with NextSeq[b] (16 indexes, 32 samples)	20045990			
		TruSight Oncology 500 DNA/RNA Automation Kit, for Use with NextSeq, plus PierianDx[b] (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		PierianDx included	Automation	
		Product	Catalog no.		Product	Catalog no.
Manual	DNA	TruSight Oncology 500 DNA High-Throughput Kit[a] (48 samples)	20040765		Beckman Coulter i-Series	Contact Illumina sales
		TruSight Oncology 500 DNA High-Throughput Kit, with PierianDx[a] (48 samples)	20040769	✓	Hamilton Microlab STAR	Contact Illumina sales
		TruSight Oncology 500 DNA High-Throughput Kit[a] (144 samples)	20040767			
		TruSight Oncology 500 DNA High-Throughput, with PierianDx[a] (144 samples)	20040771	✓		
	DNA/RNA	TruSight Oncology 500 DNA/RNA High-Throughput Kit[a] (24 samples)	20040764			
		TruSight Oncology 500 DNA/RNA High-Throughput Kit, with PierianDx[a] (24 samples)	20040768	✓		
		TruSight Oncology 500 DNA/RNA High-Throughput Kit[a] (72 samples)	20040766			
		TruSight Oncology 500 DNA/RNA High-Throughput Kit, with PierianDx[a] (72 samples)	20040770	✓		
Automated	DNA	TruSight Oncology 500 DNA High-Throughput Automation Kit[a] (64 samples)	20049283			
		TruSight Oncology 500 DNA High-Throughput Automation Kit[a] (64 samples) plus PierianDx	20049277	✓		
		TruSight Oncology 500 DNA High-Throughput Automation Kit[a] (144 samples)	Coming soon			
		TruSight Oncology 500 DNA High-Throughput Automation Kit[a] (144 samples) plus PierianDx	Coming soon	✓		
	DNA/RNA	TruSight Oncology 500 DNA/RNA High-Throughput Automation Kit[a] (32 samples)	20049282			
		TruSight Oncology 500 DNA/RNA High-Throughput Automation Kit[a] (32 samples) plus PierianDx	20049276	✓		
		TruSight Oncology 500 DNA/RNA High-Throughput Automation Kit[a] (72 samples)	Coming soon			
		TruSight Oncology 500 DNA/RNA High-Throughput Automation Kit[a] (72 samples) plus PierianDx	Coming soon	✓		

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		
Product		Catalog no.
Index kits		
Manual	IDT for Illumina UMI DNA/RNA UD Indexes Set A, Ligation (96 indexes, 96 samples)	20034701
Manual	IDT for Illumina UMI DNA/RNA UD Indexes Set B, Ligation (96 indexes, 96 samples)	20034702
Automated	IDT for Illumina UMI DNA/RNA UD Indexes for Automation Set A, Ligation (96 indexes, 96 samples)	Coming soon
Automated	IDT for Illumina UMI DNA/RNA UD Indexes for Automation Set B, Ligation (96 indexes, 96 samples)	20063213
Sequencing reagent kits		
NovaSeq 6000 SP Reagent Kit v1.5 (200 cycles)		20040719
NovaSeq 6000 S1 Reagent Kit v1.5 (200 cycles)		20028318
NovaSeq 6000 S2 Reagent Kit v1.5 (200 cycles)		20028315
NovaSeq 6000 S4 Reagent Kit v1.5 (200 cycles)		20028313

Variant calling	
Product	Catalog no.
1-year Licenses	
DRAGEN TruSight Oncology 500 Analysis Software, On-Premise, Level 1 (120 DNA or 96 DNA/RNA samples)	Coming soon
DRAGEN TruSight Oncology 500 Analysis Software, On-Premise, Level 2 (360 DNA or 288 DNA/RNA samples)	Coming soon
DRAGEN TruSight Oncology 500 Analysis Software, On-Premise, Level 3 (600 DNA or 480 DNA/RNA samples)	Coming soon
DRAGEN TruSight Oncology 500 Analysis Software, On-Premise, Level 4 (925 DNA or 740 DNA/RNA samples)	Coming soon
DRAGEN TruSight Oncology 500 Analysis Software, On-Premise, Level 5 (1850 DNA or 1480 DNA/RNA samples)	Coming soon
DRAGEN TruSight Oncology 500 Analysis Software, On-Premise, Level 6 (5550 DNA or 4440 DNA/RNA samples)	Coming soon
DRAGEN TruSight Oncology 500 Analysis Software, On-Premise, Level 7 (9250 DNA or 7400 DNA/RNA samples)	Coming soon
DRAGEN TruSight Oncology 500 Analysis Software, On-Premise, Level 8 (18,500 DNA or 14,800 DNA/RNA samples)	Coming soon
Hardware (for on-premise analysis)	
DRAGEN S3 Server	20040619

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