illumina®

TruSight™ Oncology 500 and TruSight Oncology 500 High-Throughput

Enabling flexible, scalable comprehensive genomic profiling from FFPE samples.

Highlights

- **Consolidated, comprehensive assay**
  Analyze multiple tumor variant types in 523 genes, across DNA and RNA, from guidelines and clinical trials in a single assay
- **Fast, integrated workflow**
  Go from sample to results in 4-5 days using automation-friendly library prep kits and optimized data analysis solutions
- **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.

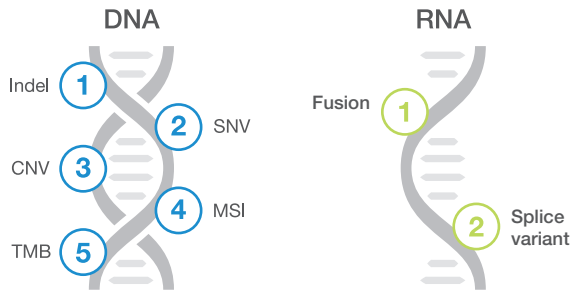


Figure 1: Variant types detected by TruSight Oncology 500 and TruSight Oncology 500 High-Throughput

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 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%

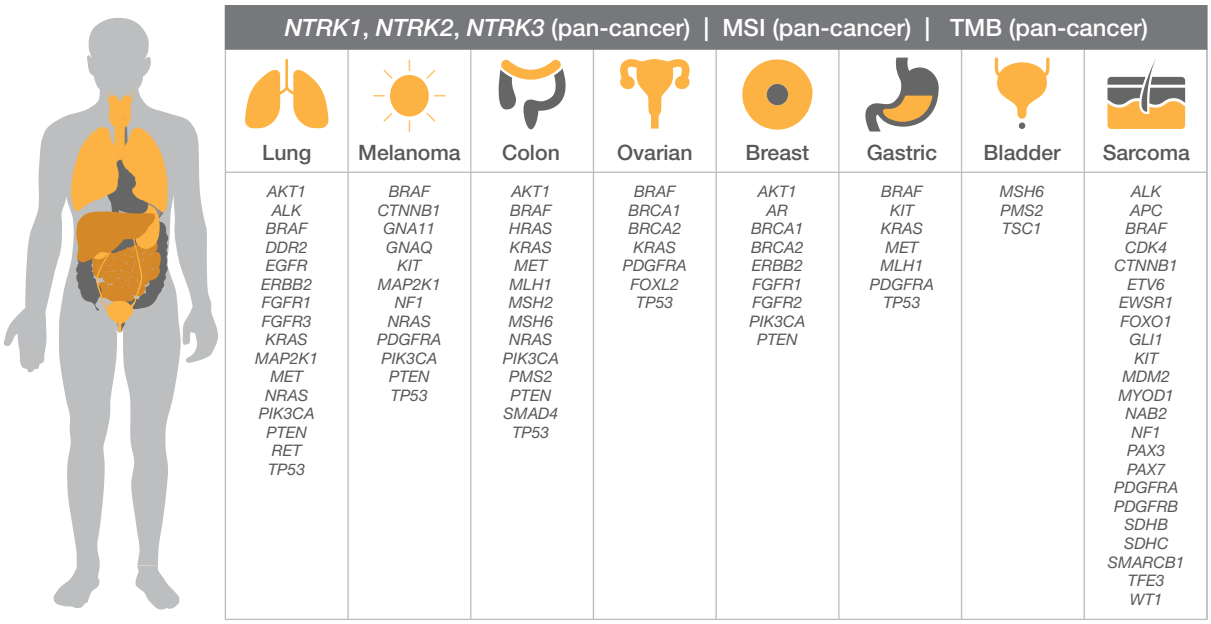


Figure 2: Genomic tumor profiling biomarkers for mulitple cancer types—Content for TruSight Oncology 500 and TruSight Oncology 500 High-Throughput includes key guideline biomarkers for multiple cancer types, plus pan-cancer biomarkers such as MSI, *NTRK1*, *NTRK2*, *NTRK3*, and TMB.

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	√	

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 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 (Table 3, Table 4). 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. 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.[10]

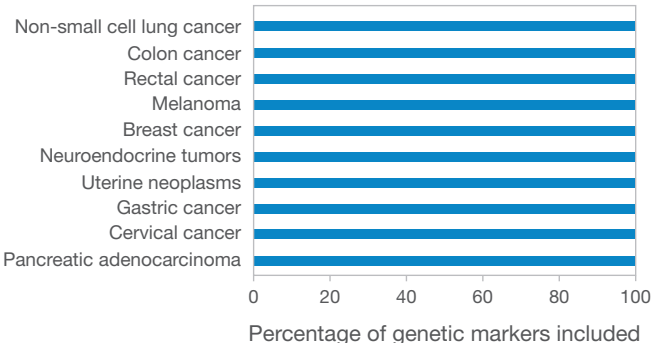


Figure 3: TruSight Oncology 500 content alignment to key guidelines by cancer type—The graph provides examples of content alignment; it is not meant to be all-inclusive.

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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

Implementing CGP in house is simplified with the availability of a comprehensive, streamlined workflow that spans from sample input to final report (Figure 4). Using automated library preparation kits and methods, variant calling tools, and interpretation and reporting software can enable 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 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.

Automate for speed

Automation-friendly TruSight 500 Oncology Kits and TruSight Qualified Methods support library preparation scalability without greatly impacting cost. The automation kits contain increased reagent amounts to absorb the additional reagent volumes required by liquid-handling robots, at a cost similar to that of the original kits. TruSight Qualified Methods, developed by the Illumina R&D team in partnership with leading liquid-handling robot manufacturers, provide protocols and scripts optimized to make sure that the same high-quality results produced by the manual protocols are achieved when using automation. Following an automated protocol can reduce hands-on time by ~ 50%, helping labs save on labor costs and reduce manual errors.

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 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

* NextSeq 550Dx System in research mode

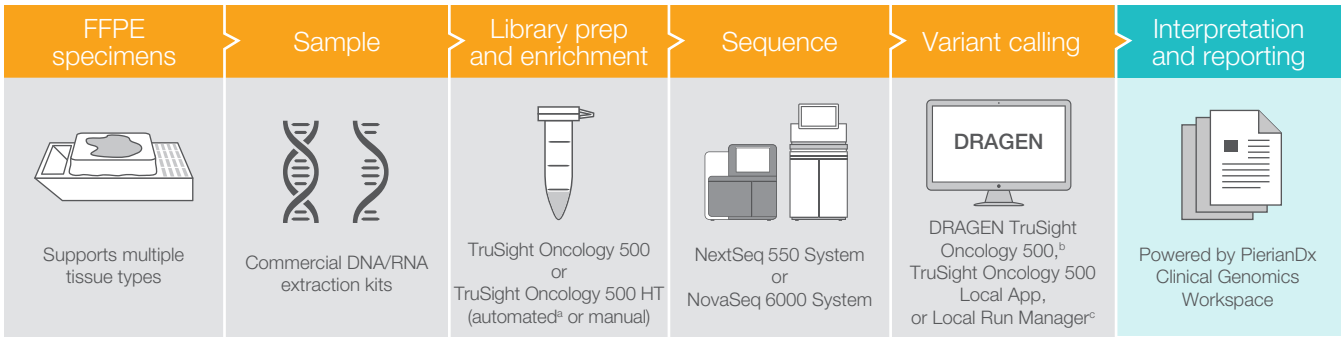


Figure 4: TruSight Oncology 500 workflow—Both TruSight Oncology 500 assays integrate into current lab workflows, going from nucleic acids to a variant calls in four days. Local Run Manager (LRM) is available only with TruSight Oncology 500.

a. TruSight Oncology 500 Kits are available in an automation-friendly format; automating reduces library preparation hands-on time by ~50%. Automation-friendly kits for TruSight Oncology 500 High-Throughput coming Q1 2021.

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.

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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 System in research mode

Analyze data

Variant calling for TruSight Oncology 500 and TruSight Oncology 500 High-Throughput is currently 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	
	Local App[b]	DRAGEN App[c]
8	5.5 hours	2 hours
16	12 hours	3 hours
32	18 hours	5 hours
72	24 hours	10 hours

Analysis times are based on actual runs and will vary from run to run

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

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).[13]

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

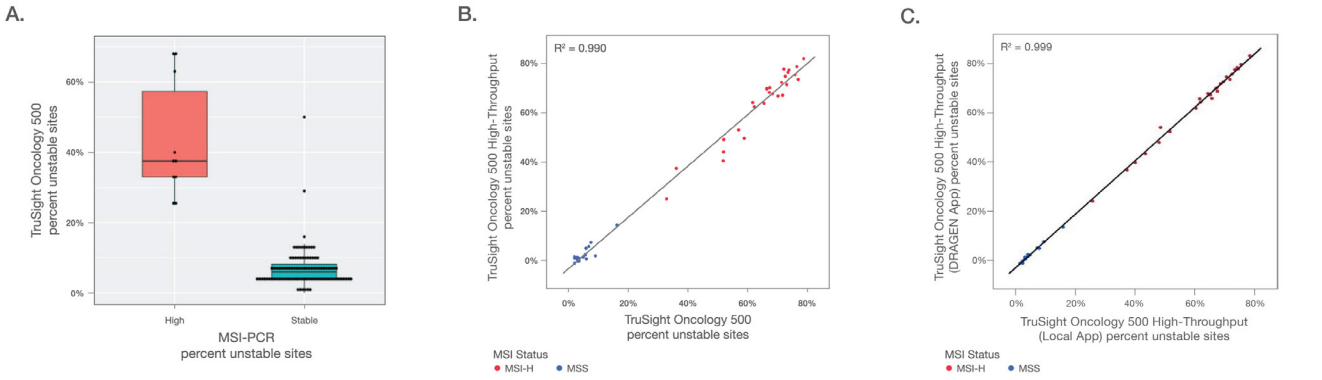


Figure 2: Figure 5: Accurate assessment of MSI status—(A) FFPE tissue samples analyzed using TruSIght Oncology 500 produce a quantitative score (y-axis) compared to a qualitative score using MSI-PCR (x-axis). (B) High concordance of MSI analysis between TruSight Oncology 500 and TruSight Oncology 500 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.

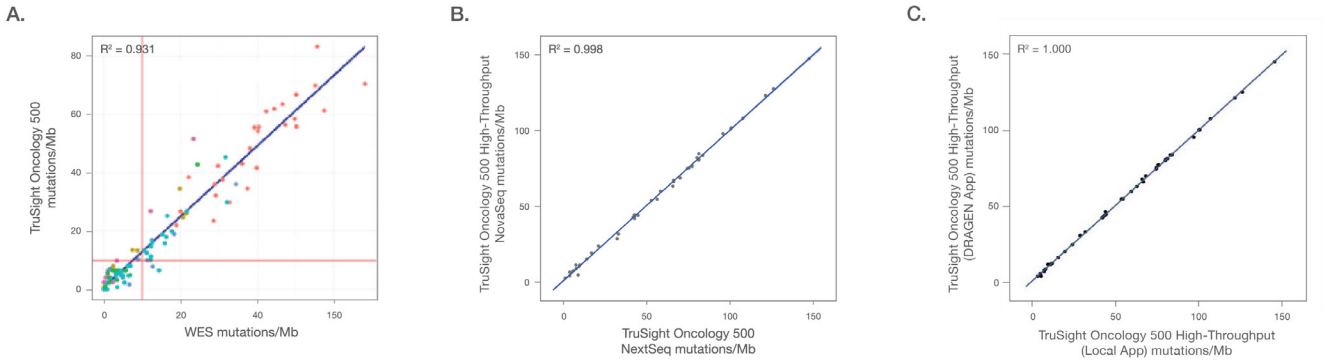


Figure 6: Accurate assessment of TMB status—(A) Analysis of 108 FFPE tissue samples shows high concordance between TMB measurements using WES and TruSight Oncology 500. Red line indicates the threshold value (10 mutations/Mb). (B) High concordance of TMB analysis between TruSight Oncology 500 and TruSight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

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%
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.[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).

Table 8: Sensitive CNV detection[a]

Gene	Detected	Fold change			Tissue
		TruSight Oncology 500	TruSight Oncology 500 High-Throughput		
			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

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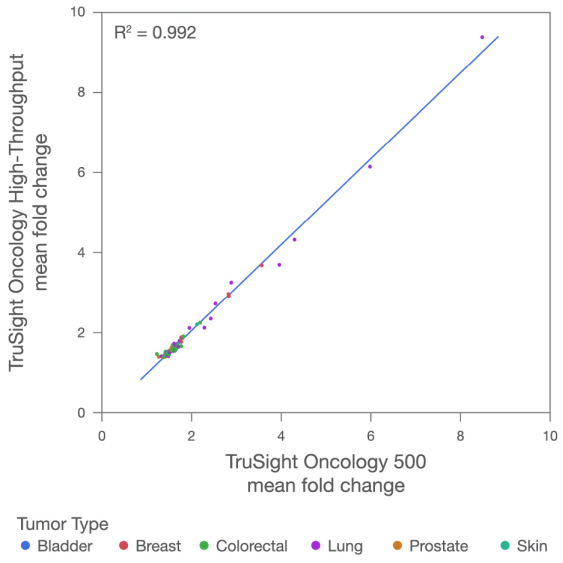


Figure 7: High concordance of CNV detection between TruSight Oncology 500 and TruSight Oncology 500 High-Throughput

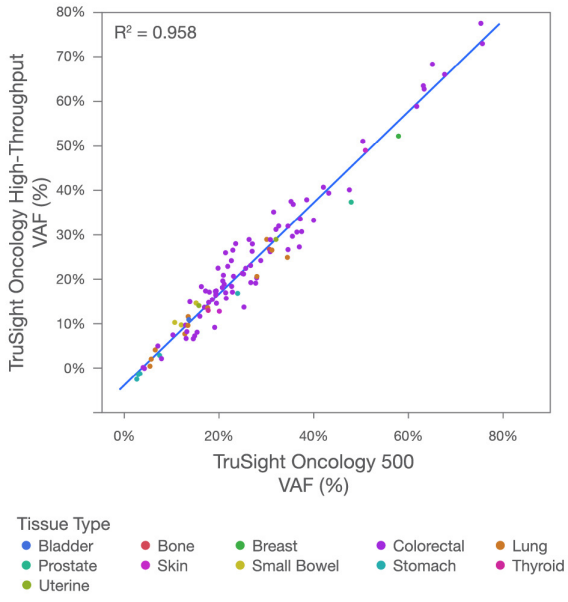


Figure 8: Highly sensitive variant detection—High VAF concordance between TruSight Oncology 500 and TruSight Oncology 500 High-Throughput.

Robust detection of RNA 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 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 10).

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

Gene	Mutation	Detected	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. 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

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Table 10: 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 High-Throughput on the NovaSeq 6000 System. Data analyzed using the TruSight Oncology 500 Local App. 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.

Plan for the future

TruSight Oncology 500 and TruSight Oncology 500 High-Throughout 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.

Learn more

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

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Ordering information: TruSight Oncology 500 workflow

	Sample type	Library prep		PierianDx included
		Product	Catalog no.	
Manual	DNA	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	√
	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	√
Automated	DNA	TruSight Oncology 500 DNA Automation Kit	20045504	
		TruSight Oncology 500 DNA Automation Kit, plus PierianDx	20045506	√
		TruSight Oncology 500 DNA Automation Kit, for Use with NextSeq	20045505	
		TruSight Oncology 500 DNA Automation Kit, for Use with NextSeq, plus PierianDx	20045507	√
	DNA/RNA	TruSight Oncology 500 DNA/RNA Automation Kit	20045508	
		TruSight Oncology 500 DNA/RNA Automation Kit, plus PierianDx	20045509	√
		TruSight Oncology 500 DNA/RNA Automation Kit, for Use with NextSeq	20045990	
		TruSight Oncology 500 DNA/RNA Automation Kit, for Use with NextSeq, plus PierianDx	20045991	√

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

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

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Ordering information: TruSight Oncology 500 High-Throughput workflow

Sample type	Library prep		PierianDx included	Consumables		Variant calling	
	Product	Catalog no.		Product	Catalog no.	Product	Catalog no.
DNA	TruSight Oncology 500 DNA High-Throughput Kit (48 samples)	20040765		Index kits		1-year Licenses	
	TruSight Oncology 500 DNA High-Throughput Kit (144 samples)	20040767		IDT for Illumina UMI DNA/RNA UD Indexes Set A, Ligation (96 indexes, 96 samples)	20034701	DRAGEN TruSight Oncology 500 Analysis Software, On-Premise, Level 1 (120 DNA or 96 DNA/RNA samples)	Coming soon
	TruSight Oncology 500 DNA High-Throughput Kit, with PierianDx (48 samples)	20040769	√	IDT for Illumina UMI DNA/RNA UD Indexes Set B, Ligation (96 indexes, 96 samples)	20034702	DRAGEN TruSight Oncology 500 Analysis Software, On-Premise, Level 2 (360 DNA or 288 DNA/RNA samples)	Coming soon
	TruSight Oncology 500 DNA High-Throughput Kit, with PierianDx (144 samples)	20040771	√	Sequencing reagent kits		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 High-Throughput Kit (24 samples)	20040764		NovaSeq 6000 SP Reagent Kit v1.5 (200 cycles)	20040719	DRAGEN TruSight Oncology 500 Analysis Software, On-Premise, Level 4 (925 DNA or 740 DNA/RNA samples)	Coming soon
	TruSight Oncology 500 DNA/RNA High-Throughput Kit (72 samples)	20040766		NovaSeq 6000 S1 Reagent Kit v1.5 (200 cycles)	20028318	DRAGEN TruSight Oncology 500 Analysis Software, On-Premise, Level 5 (1850 DNA or 1480 DNA/RNA samples)	Coming soon
	TruSight Oncology 500 DNA/RNA High-Throughput Kit, with PierianDx (24 samples)	20040768	√	NovaSeq 6000 S2 Reagent Kit v1.5 (200 cycles)	20028315	DRAGEN TruSight Oncology 500 Analysis Software, On-Premise, Level 6 (5550 DNA or 4440 DNA/RNA samples)	Coming soon
	TruSight Oncology 500 DNA/RNA High-Throughput Kit, with PierianDx (72 samples)	20040770	√	NovaSeq 6000 S4 Reagent Kit v1.5 (200 cycles)	20028313	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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