illumina®

TruSight™ Oncology 500 and TruSight Oncology 500 High-Throughput

Enabling flexible, scalable comprehensive genomic profiling from FFPE samples.

Highlights

- **Consolidated assay**
  Save time and sample by analyzing multiple tumor variant types in 523 genes, from both DNA and RNA, in a single assay
- **Comprehensive content**
  Access current and emerging biomarkers identified in guidelines and clinical trials
- **Proven, reliable results**
  Generate accurate data using an assay shown to meet demanding performance specifications
- **Value-adding in-house solution**
  Keep samples and obtain data that is most relevant to the local institution and community

Introduction

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

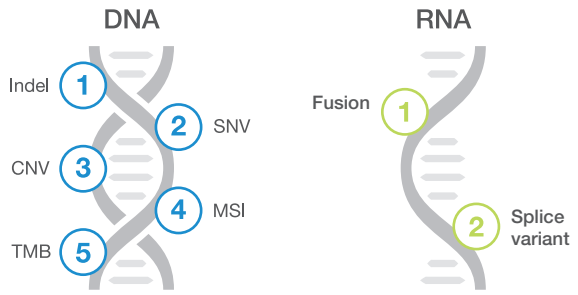

Figure 1: Variant types detected by TruSight Oncology 500 and TruSight Oncology 500 High-Throughput

One workflow analyzes multiple tumor types and biomarkers

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

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

Parameter	TruSight Oncology 500	TruSight Oncology 500 High-Throughput
System	NextSeq 500 System or NextSeq 550Dx (research mode)	NovaSeq 6000 System
Panel size	1.94 Mb DNA, 358 kb RNA	1.94 Mb DNA, 358 kb RNA
DNA input requirement	40 ng	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%

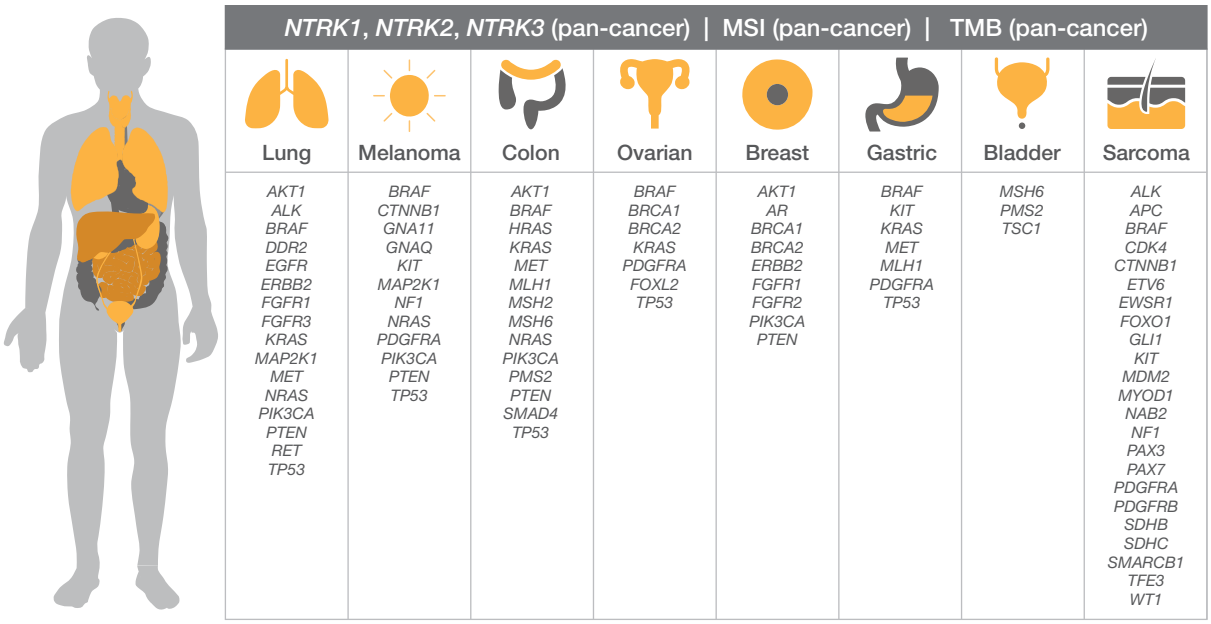

Figure 2: Genomic tumor profiling biomarkers for mulitple cancer types—Content for TruSight Oncology 500 and TruSight Oncology 500 High-Throughput includes key guideline biomarkers for multiple cancer types, plus current and emerging pan-cancer biomarkers such as MSI, *NTRK1*, *NTRK2*, *NTRK3*, and TMB.

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

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

Comprehensive content design

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

Panel content comprises genes listed in current guidelines with significant coverage of key guidelines for multiple tumor types (Figure 3) and genes involved in over 1000 clinical trials.

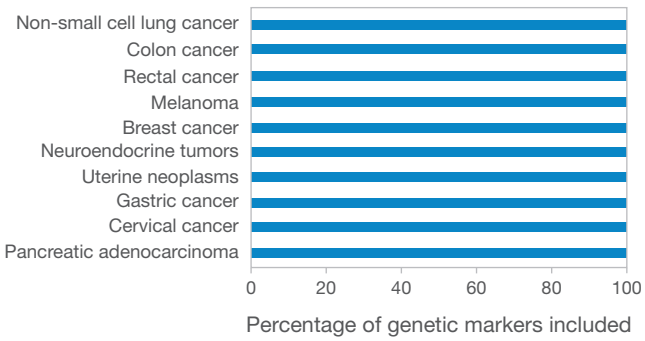

Figure 3: TruSight Oncology 500 content alignment to key guidelines by cancer type—The graph provides examples of content alignment; it is not meant to be all-inclusive.

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*	*1L7R*	*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*	*PRKC1*	*SETBP1*	*TET3*
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*	*ARF2*	*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*	*PDGRFB*	*RAD51B*	*SMC3*	*TSC2*
AURKB	*CDKN1A*	*EPHA3*	*FGF9*	*HIST1H3F*	*KEAP1*	*MST1R*	*PDK1*	*RAD51C*	*SMO*	*TSHR*
AXIN1	*CDKN1B*	*EPHA5*	*FGFR1*	*HIST1H3G*	*KEL*	*MTOR*	*PDPK1*	*RAD51D*	*SNCAIP*	*U2AF1*
AXIN2	*CDKN2A*	*EPHA7*	*FGFR2*	*HIST1H3H*	*KIF5B*	*MUTYH*	*PGR*	*RAD52*	*SOCS1*	*VEGFA*
AXL	*CDKN2B*	*EPHB1*	*FGFR3*	*HIST1H3I*	*KIT*	*MYB*	*PHF6*	*RAD54L*	*SOX10*	*VHL*
B2M	*CDKN2C*	*ERBB2*	*FGFR4*	*HIST1H3J*	*KLF4*	*MYC*	*PHOX2B*	*RAF1*	*SOX17*	*VTCN1*
BAP1	*CEBPA*	*ERBB3*	*FH*	*HIST2H3A*	*KLHL6*	*MYCL1*	*PIK3C2B*	*RANBP2*	*SOX2*	*WISP3*
BARD1	*CENPA*	*ERBB4*	*FLCN*	*HIST2H3C*	*KMT2B*	*MYCN*	*PIK3C2G*	*RARA*	*SOX9*	*WT1*
BBC3	*CHD2*	*ERCC1*	*FLI1*	*HIST2H3D*	*KMT2C*	*MYD88*	*PIK3C3*	*RASA1*	*SPEN*	*XIAP*
BCL10	*CHD4*	*ERCC2*	*FLT1*	*HIST3H3*	*KMT2D*	*MYOD1*	*PIK3CA*	*RB1*	*SPOP*	*XPO1*
BCL2	*CHEK1*	*ERCC3*	*FLT3*	*HLA-A*	*KRAS*	*NAB2*	*PIK3CB*	*RBM10*	*SPTA1*	*XRCC2*
BCL2L1	*CHEK2*	*ERCC4*	*FLT4*	*HLA-B*	*LAMP1*	*NBN*	*PIK3CD*	*RECQL4*	*SRC*	*YAP1*
BCL2L11	*CIC*	*ERCC5*	*FOXA1*	*HLA-C*	*LATS1*	*NCOA3*	*PIK3CG*	*REL*	*SRSF2*	*YES1*
BCL2L2	*CREBBP*	*ERG*	*FOXL2*	*HNF1A*	*LATS2*	*NCOR1*	*PIK3R1*	*RET*	*STAG1*	*ZBTB2*
BCL6	*CRKL*	*ERRFI1*	*FOXO1*	*HNRNPK*	*LMO1*	*NEGR1*	*PIK3R2*	*RFWD2*	*STAG2*	*ZBTB7A*
BCOR	*CRLF2*	*ESR1*	*FOXP1*	*HOXB13*	*LRP1B*	*NF1*	*PIK3R3*	*RHEB*	*STAT3*	*ZFHX3*
BCORL1	*CSF1R*	*ETS1*	*FRS2*	*HRAS*	*LYN*	*NF2*	*PIM1*	*RHOA*	*STAT4*	*ZNF217*
BCR	*CSF3R*	*ETV1*	*FUBP1*	*HSD3B1*	*LZTR1*	*NFE2L2*	*PLCG2*	*RICTOR*	*STAT5A*	*ZNF703*
BIRC3	*CSNK1A1*	*ETV4*	*FYN*	*HSP90AA1*	*MAGI2*	*NFKBIA*	*PLK2*	*RIT1*	*STAT5B*	*ZRSR2*
BLM	*CTCF*	*ETV5*	*GABRA6*	*ICOSLG*	*MALT1*	*NKX2-1*	*PMAIP1*	*RNF43*	*STK11*	
BMPR1A	*CTLA4*	*ETV6*	*GATA1*	*ID3*	*MAP2K1*	*NKX3-1*	*PMS1*	*ROS1*	*STK40*	
BRAF	*CTNNA1*	*EWSR1*	*GATA2*	*IDH1*	*MAP2K2*	*NOTCH1*	*PMS2*	*RPS6KA4*	*SUFU*	
BRCA1	*CTNNB1*	*EZH2*	*GATA3*	*IDH2*	*MAP2K4*	*NOTCH2*	*PNRC1*	*RPS6KB1*	*SUZ12*	
BRCA2	*CUL3*	*FAM123B*	*GATA4*	*IFNGR1*	*MAP3K1*	*NOTCH3*	*POLD1*	*RPS6KB2*	*SYK*	

Content shaded in grey is analyzed for CNV detction.

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

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

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

Integrated workflow

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

Start with DNA or RNA

The TruSight Oncology 500 assays can use RNA or DNA extracted from the same sample as input material. If using DNA, sample preparation starts with shearing the genomic DNA (gDNA). If starting from RNA, the first step is to reverse transcribe the sample into cDNA. Sheared gDNA and cDNA are converted simultaneously into sequence-ready libraries.

Add tags for analytical specificity

During library preparation, unique molecular identifiers (UMIs)[10] are added to the gDNA or cDNA fragments. These UMIs enable detection of variants at low variant allele frequency (VAF) while simultaneously suppressing errors, providing high analytical specificity.

Enrich libraries to focus efforts

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

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

Sequence 8-192 samples

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

Table 5: Scalable solution

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

a. NextSeq 550Dx System in Research Mode

Analyze data

Variant calling for TruSight Oncology 500 and TruSight Oncology 500 High-Throughput uses five sophisticated, proprietary algorithms that remove errors, artifacts, and germline variants. The result is highly

* NextSeq 550Dx System in research mode

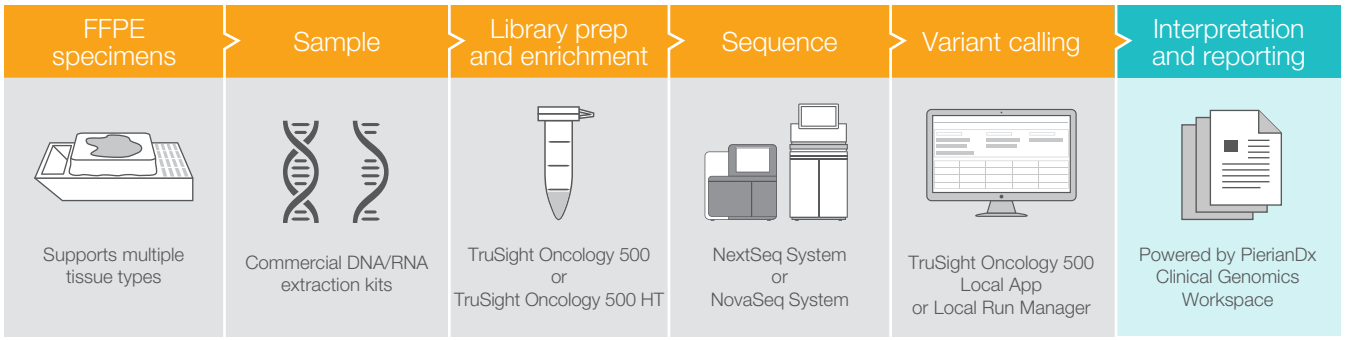

Figure 4: TruSight Oncology 500 workflow—Both TruSight Oncology 500 assays integrate into current lab workflows, going from nucleic acids to a variant calls in 4-5 days. Local Run Manager (LRM) is available only with TruSight Oncology 500.

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

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

Proven, reliable results

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

Accurate assessment of TMB and MSI

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

MSI status has been traditionally analyzed with PCR (MSI-PCR) and immunohistochemistry. While other methods deliver a qualitative result describing samples as either MSI-stable or MSI-high, NGS-based assessment with the TruSight Oncology 500 assays interrogates 130 homopolymer MSI marker sites to calculate an accurate quantitative score for MSI status (Figure 5).[9]

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

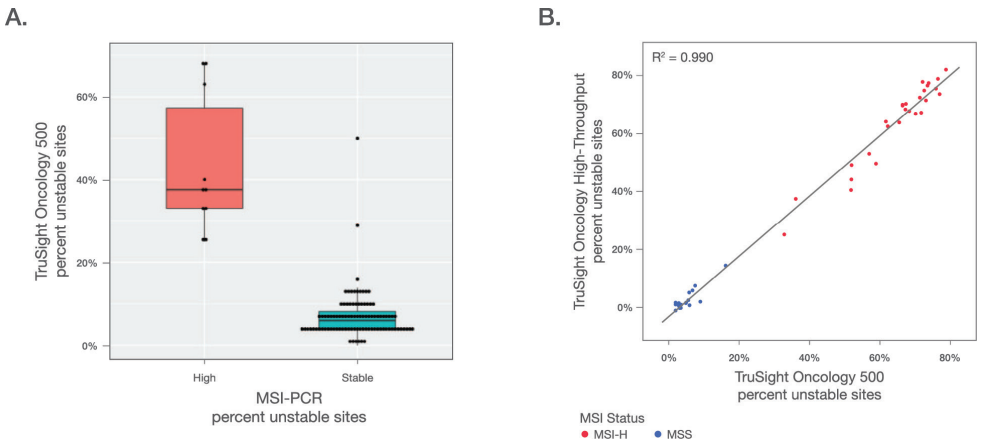

Figure 5: Accurate assessment of MSI status—(A) FFPE tissue samples analyzed using TruSIght Oncology 500 produce a quantitative score (y-axis) compared to a qualitative score using MSI-PCR (x-axis). (B) High concordance of MSI analysis between TruSight Oncology 500 and TruSight Oncology 500 High-Throughput.

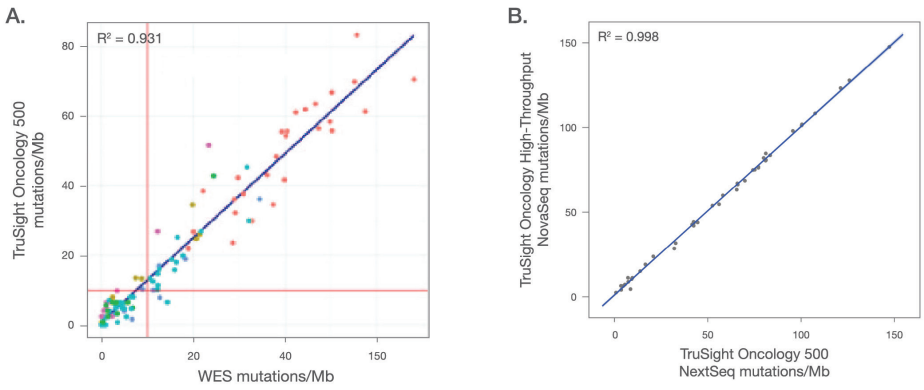

Figure 6: Accurate assessment of TMB status—(A) Analysis of 108 FFPE tissue samples shows high concordance between TMB measurements using WES and TruSight Oncology 500.Red line indicates the threshold value (10 mutations/Mb). (B) High concordance of TMB analysis between TruSight Oncology 500 and TruSight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

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

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

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

Sensitive detection of CNVs

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

Table 7: Sensitive CNV detection

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

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

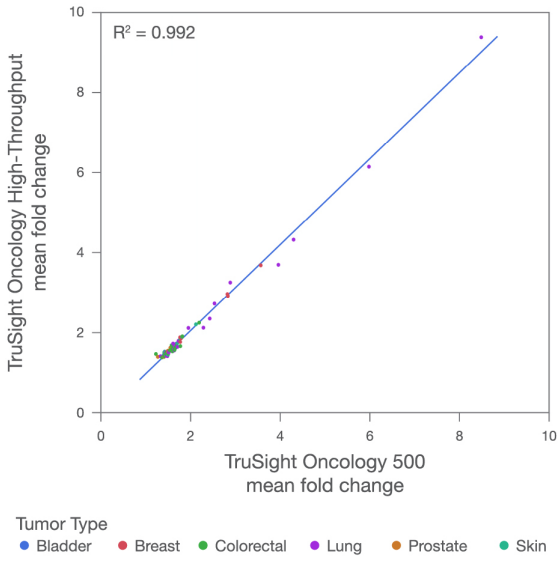

Figure 7: High concordance of CNV detection between TruSight Oncology 500 and TruSight Oncology 500 High-Throughput

Highly sensitive variant detection from FFPE samples

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

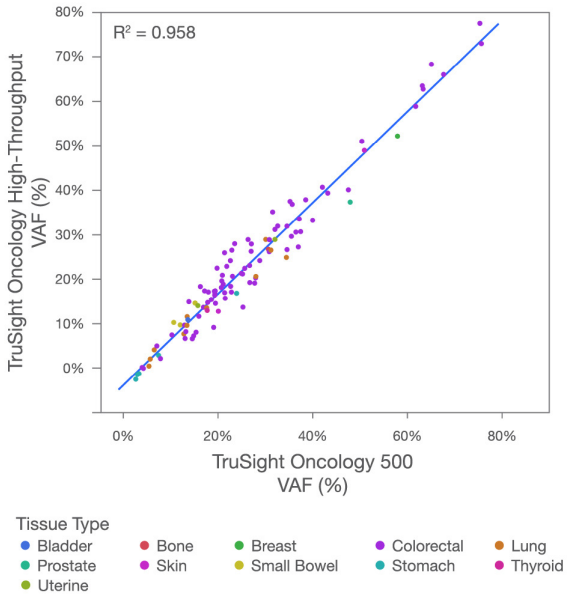

Figure 8: Highly sensitive variant detection—High VAF concordance between TruSight Oncology 500 and TruSight Oncology 500 High-Throughput.

Table 8: Highly sensitive DNA small variant detection

Gene	Mutation	Detected	VAF		Variant type
			TruSight Oncology 500	TruSight Oncology 500 High-Throughput	
AKT1	E17K	√	20%	18%	SNV
BRAF	V600E	√	19%	19%	SNV
CDKN2	R58*	√	12%	14%	SNV
CTNBB1	G34E	√	16%	18%	SNV
EGFR	L858R	√	18%	17%	SNV
EGFR	T790M	√	13%	12%	SNV
FBXW7	R465C	√	8%	7%	SNV
FGFR2	S252W	√	32%	32%	SNV
GNAS	R876*	√	5%	5%	SNV
H3F3B	K37M	√	31%	30%	SNV
IDH2	R140Q	√	23%	22%	SNV
KRAS	G12D	√	6%	6%	SNV
NRAS	Q61K	√	15%	18%	SNV
PIK3CA	E542K	√	14%	15%	SNV
PTCH1	A563V	√	4%	4%	SNV
SMARCA4	R973W	√	3%	3%	SNV
TP53	R248Q	√	29%	27%	SNV
RET	A845V	√	7%	8%	MNV
APC	T1556Nfs*3	√	21%	20%	Insertion
ARID1A	D1850Tfs*33	√	4%	5%	Deletion
EP300	H2324fs*29	√	24%	20%	Deletion
KMT2A	K3828Rfs*31	√	3%	3%	Deletion
PTEN	K267Rfs*9	√	21%	21%	Deletion
RNF43	G659Vfs*41	√	18%	18%	Deletion

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

Robust detection of RNA fusions

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

To achieve comparable results with RNA analysis, 40 ng RNA is recommended for use with TruSight Oncology 500 while a range of 40-80 ng RNA is recommended for use with TruSight Oncology 500 High-Throughput. In cases where FFPE RNA yields from FFPE tissues are low, 40 ng RNA input can still be used to detect variants expressed at mid-to-high levels with TruSight Oncology 500 High-Throughput. However, when sufficient RNA is available, 80 ng input helps maximize sensitivity for fusions present at very low concentrations (Table 9).

Table 9: Robust detection of fusions and splice variants

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

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

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.

Ordering information

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

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

Learn more

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

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