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TruSight™ Oncology 500

A comprehensive next-generation sequencing assay that targets somatic variants, TMB, and MSI status from the same FFPE tumor only sample.

Highlights

- **Unlock immuno-oncology with TMB and MSI**
  1.94 Mb of content with sophisticated algorithms enable accurate analysis of TMB and MSI
- **Save time and sample with a multiplex assay**
  Comprehensive coverage of pan-cancer content, aligned with key guidelines spanning 523 cancer-relevant genes
- **Achieve confidence in results**
  Enrichment chemistry including UMIs coupled with an informatics pipeline for high accuracy in variant detection
- **Address the needs of the oncology community both today and tomorrow**
  Relevant and emerging biomarkers support a future-proof foundation for new solutions

One workflow for multiple tumor types and multiple biomarkers

Comprehensive genomic profiling used in recent studies with large cohorts has shown that 30-49% of samples may have informative alterations.[1-6] With limited time to return results and limited amounts of tissues, a comprehensive single assay that assesses a wide range of biomarkers increases the chances of obtaining relevant information. To help researchers address this challenge, Illumina offers TruSight Oncology 500, a next-generation sequencing (NGS) assay that analyzes 523 cancer-relevant genes from both DNA and RNA* in one integrated workflow. TruSight Oncology 500 assesses multiple variant types in a single assay (Table 1), including small nucleotide variants (SNVs), indels, splice variants, fusions, and emerging immunotherapy biomarkers that rely on analysis of multiple genomic loci, such as tumor mutational burden (TMB) and microsatellite instability (MSI).

Table 1: Variant types detected by TruSight Oncology 500

Variant type	TruSight Oncology 500	Relevant examples
SNVs and indels	✓	*KRAS* G12D, *EGFR* exon 19 deletions, *BRAF* V600E
Fusions	✓	*ALK*, *ROS1*, *NTRK1*, *NTRK2*, *NTRK3*
Splice variants	✓	*MET* exon 14
MSI	✓	MSI-High
TMB	✓	TMB-High

During library preparation, enrichment chemistry is optimized to capture nucleic acid targets from formalin-fixed, paraffin-embedded (FFPE) tissues. Addition of unique molecular identifiers (UMIs)[7] during DNA library preparation, enables detection of variants at low variant allele frequency (VAF) while simultaneously suppressing errors, thus providing high specificity. Variant-calling software, developed in concert with the assay reagents, is included with the kit and can be run on external hardware provided by the laboratory.

Illumina has established partnerships with several academic centers, pharmaceutical companies, and advocacy groups, to assist with the design, development, and evaluation of new oncology applications. To facilitate such endeavors, TruSight Oncology 500 is easily integrated into current lab workflows (Figure 1) and is amenable to automation. Using proven Illumina technology, with gene content relevant across multiple tumor types and including emerging biomarkers, TruSight Oncology 500 is well-positioned to be the foundation for developing future oncology diagnostic solutions.

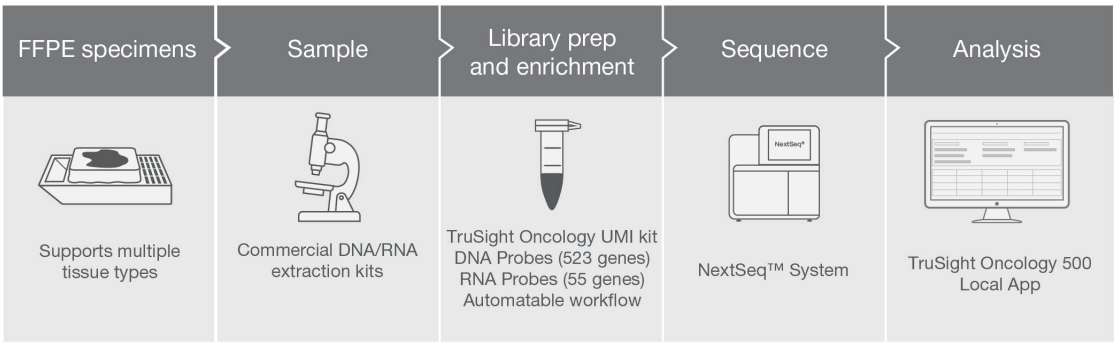

Figure 1: TruSight Oncology 500 workflow— TruSight Oncology 500 integrates into current lab workflows, going from nucleic acids to a variant calls in 3–4 days.

* The products to evaluate DNA and RNA variants consist of the TruSight Oncology 500 DNA panel and the TruSight Tumor 170 RNA panel.

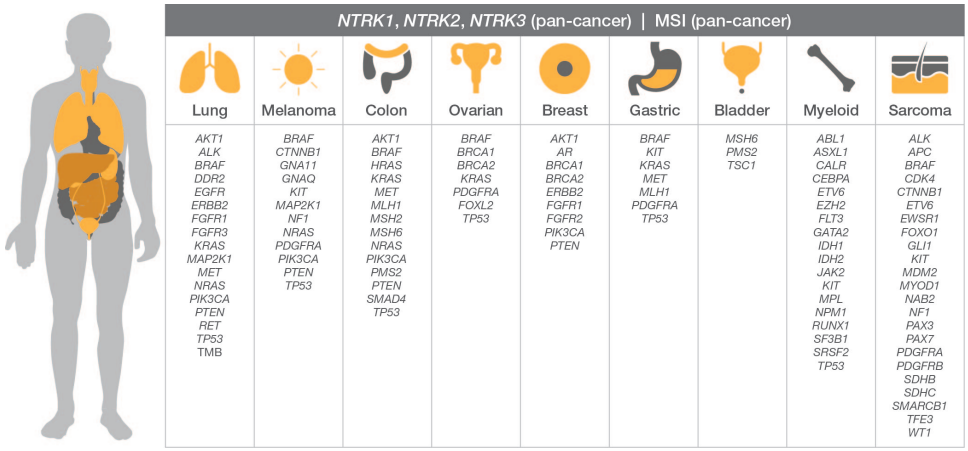

Figure 2: Tumor profiling biomarkers for multiple cancer types — Content for TruSight Oncology 500 includes key biomarkers for multiple cancer types, and emerging pan-cancer biomarkers such as MSI, *NTRK1*, *NTRK2*, and *NTRK3*.

Comprehensive content design

The TruSight Oncology 500 panel includes a comprehensive list of biomarkers commonly mutated in numerous neoplasm types (Figure 2). With simultaneous analysis of both DNA and RNA* various types of biomarkers relevant to a given tumor type (SNVs, indels, fusions, splice variants, TMB, MSI) can be assessed from the same sample in a single assay (Table 2). The panel uses a probe design that enables capture of both known fusions and novel fusions. The TruSight Oncology 500 panel includes 523 genes for SNV and indel detection and 55 genes for fusion and splice variant detection (Table 3).

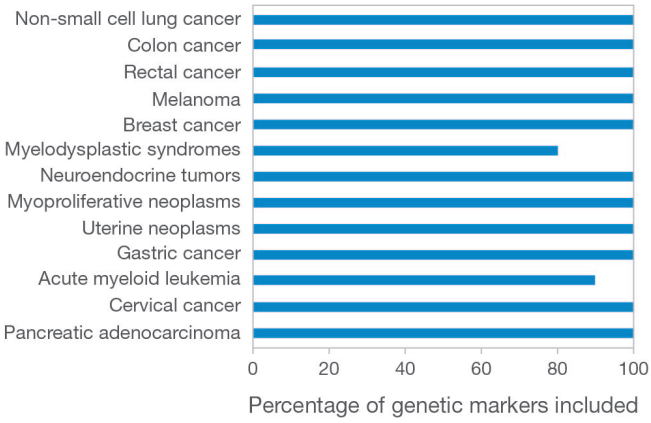

Figure 3: TruSight Oncology 500 content alignment to National Comprehensive Cancer Network (NCCN) guidelines — For each cancer type, the percentage of genetic markers in current NCCN guidelines[3] that are included in the gene panel is indicated.

By harnessing expertise from recognized authorities in the oncology community, content was designed to include both current guidelines and emerging biomarkers, including 100% coverage of NCCN guidelines for 11 tumor types (Figure 3),[8] and coverage of genes involved in over 1600 clinical trials. With comprehensive coverage of variants most likely to play a role in tumorigenesis, TruSight Oncology 500 is well-positioned as a foundational assay from which new companion diagnostics can be developed.

Table 2: Multiple types of relevant biomarkers assessed for a comprehensive lung tumor assay

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

* The products to evaluate DNA and RNA variants consist of the TruSight Oncology 500 DNA panel and the TruSight Tumor 170 RNA panel.

Table 3: Genes included in the TruSight Oncology 500 panel

DNA content										
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	*ClC*	*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*	
RNA content*										
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*

* The products to evaluate DNA and RNA variants consist of the TruSight Oncology 500 DNA panel and the TruSight Tumor 170 RNA panel.

Accurate assessment of TMB and MSI

TMB and MSI are emerging biomarkers that correlate with response to immunotherapies.[9] TruSight Oncology 500 is well suited to interrogate both biomarkers, which rely upon analysis of multiple genomic loci.

Obtaining a precise and reproducible TMB value at low mutation levels can be challenging with smaller panels. Recent studies have shown that panels with larger genomic content (at least 1.5 Mb) perform well with samples containing less than 30 Mb/mutation.[10,11] With 1.94 Mb of genomic content, TruSight Oncology 500 surpasses this requirement, demonstrating accurate TMB estimation that is highly concordant with whole-exome studies (Figure 4, Table 4).[12] The addition of UMIs during library preparation coupled with proprietary Illumina informatics reduces sequencing error rates by 10–20 fold.[7] Removing FFPE artifacts (such as deamination, oxidation) enables analytical sensitivity as low as 5% VAF from low-quality DNA samples.

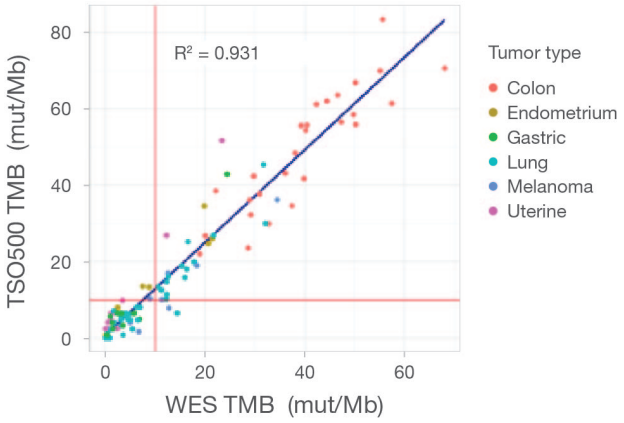

Figure 4: High concordance between TMB measurements from TruSight Oncology 500 and WES— 108 FFPE tissue samples.were analyzed by TruSight Oncology 500 (TSO500) in tumor-only workflow, and WES using tumor-normal pairs. TMB values from both assays are plotted to display high concordance (R^2 = 0.931).

TruSight Oncology 500 analysis uses a sophisticated proprietary algorithm to perform TMB analysis. Measuring both nonsynonymous and synonymous SNVs and indels increases analytical sensitivity by using more variants. After variant calling and error correction, germline variants and variants in low-confidence regions are filtered to deliver accurate results. Filtering germline variants also allows TMB evaluation with a tumor-only workflow (Figure 5), further supporting high-throughput sample processing.

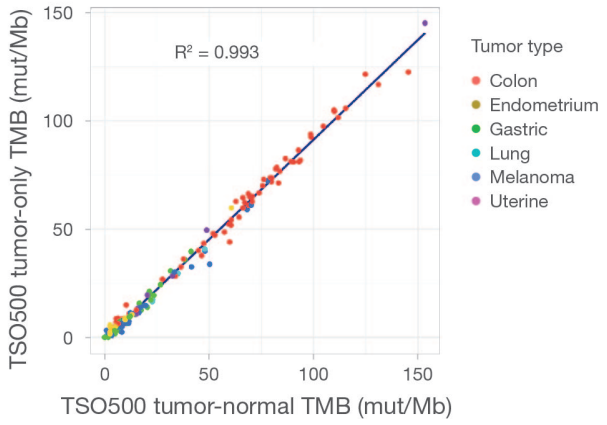

Figure 5: Reproducible TMB evaluation with tumor-only or tumor-normal workflow— TruSight Oncology 500 employs an algorithm that filters germline variants, enabling TMB analysis with a tumor-only workflow. To demonstrate concordance with tumor-normal workflows, 170 FFPE samples from six different tissue types were analyzed, and TMB values from both workflows plotted to display high concordance (R^2 = 0.993).

Table 4: Concordance between WES and TruSight Oncology 500 TMB classification at low value range (10 mut/Mb)

Metric	Value
Positive percent agreement	94.7%
Negative percent agreement	96.1%
Overall percent agreement	95.4%

TMB values were plotted from 108 FFPE tissue samples. Percent agreement between WES and TruSight Oncology 500 is shown for TMB-high or TMB-low classifications, with 10 mut/Mb as the threshhold value (red lines in Figure 2).

MSI status is also a biomarker correlated with response to checkpoint inhibition therapy. However, high TMB does not correlate to high MSI status in many tissues tested, warranting development as an independent biomarker with new MSI profiles in previously uncharacterized cancer types.[13,14] 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 TruSight Oncology 500 interrogates 130 homopolymer MSI marker sites to calculate an accurate quantitative score for MSI status (Figure 6).[12]

As a multiplex assay, TruSight Oncology 500 can deliver answers for both TMB and MSI status from a single assay, eliminating the need to spend precious tissue sample on iterative testing.

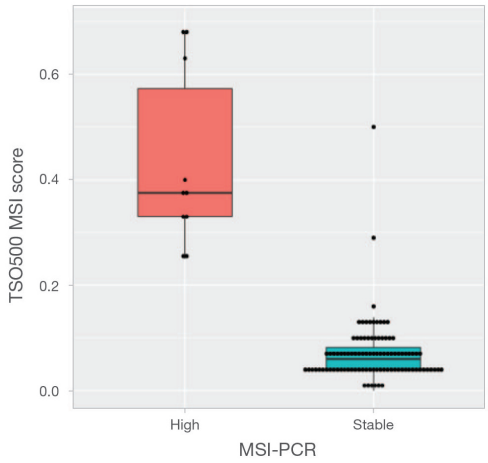

Figure 6: Quantitative assessment of MSI status— The TruSight Oncology 500 tumor-only workflow was used to analyze FFPE samples. A quantitative MSI score (Y-axis) is plotted against a qualitative score (high vs stable) obtained by analyzing the same samples with MSI-PCR (Promega MSI Analysis System).

Enrichment chemistry enables low-level variant detection from FFPE samples

TruSight Oncology 500 library preparation is based on proven target enrichment chemistry using biotinylated probes and streptavidin-coated magnetic beads to purify selected targets from DNA- and RNA-based* libraries. A benefit of target enrichment chemistry is the use of probes designed large enough to impart high binding specificity, but also allowing 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. TruSight Oncology 500 can reproducibly detect variants in FFPE samples as low as 5% VAF (Figure 7).

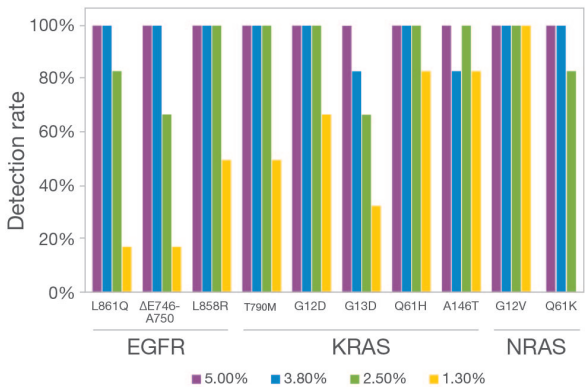

Figure 7: Small variant detection at low variant allele frequency— FFPE cell line samples with known VAF for each variant were diluted to values ranging from 1.30–5.00% VAF. Six replicates of each sample were analyzed by TruSight Oncology 500 using 40 ng DNA input.

Combined workflow for DNA and RNA*

TruSight Oncology 500 library preparation uses an enrichment method that can be simultaneously applied to DNA and RNA* extracted from the same sample. After the initial steps, in which genomic DNA is sheared and RNA* is converted to cDNA, library prep becomes a combined workflow. Sheared DNA and cDNA are converted simultaneously into sequenceable libraries. Then regions of interest are hybridized to biotinylated probes, magnetically pulled down with streptavidin-coated beads, and eluted to enrich the library pool. Finally, libraries are normalized using a simple bead-based protocol before pooling and sequencing.

Table 5: TruSight Oncology 500 product specifications

Parameter	Details
System	NextSeq 550Dx or NextSeq System
Panel size	1.94 Mb DNA 358 kb RNA*
DNA input requirement	40 ng total
RNA* input requirement	40 ng total
FFPE input requirement	Minimum recommendation of 0.65 mm^3 from FFPE tissue samples (5 slides cut at 10 microns thick)
Total assay time	3–4 days from nucleic acid to variant report
Sequence run time	24 hours (NextSeq Systems)
Sequence run	2 × 101 cycles
Kit size	24 or 48 samples
Sample throughput	8 samples per run (NextSeq Systems)
Limit of detection	5% VAF For fusions, 5 copies per ng RNA* input
Analytical sensitivity	>96% (for all variant types at 5% VAF)
Analytical specificity	>99.99%

Summary

TruSight Oncology 500 is an NGS-based, multiplex assay that analyzes hundreds of cancer-related biomarkers in a single assay. Assessing DNA and RNA* in the same workflow supports identification of a wide range of variants implicated in various tumor types. Taking advantage of extensive genomic content, TruSight Oncology 500 also provides assessment of immunotherapy biomarkers (TMB and MSI) without requiring multiple samples for iterative testing. Using target-enrichment chemistry with sophisticated tools to reduce errors, high-quality data is obtainable from FFPE samples. Leverage the power of TruSight Oncology 500 to accelerate your research goals today.

Learn more

For more information about TruSight Oncology 500, visit www.illumina.com/tso500

* The products to evaluate DNA and RNA variants consist of the TruSight Oncology 500 DNA panel and the TruSight Tumor 170 RNA panel.

Table 6: Ordering information

Library prep kit	No. of indexes/samples	Catalog no.
TruSight Oncology 500 DNA Kit (Includes DNA library prep and enrichment reagents. Does not include NextSeq System core reagents)	16 indexes 48 samples	20028213
TruSight Oncology 500 DNA Kit, for Use with NextSeq (Includes DNA library prep and enrichment reagents, and NextSeq System core reagents)	16 indexes 48 samples	20028214
TruSight Oncology 500 DNA/RNA* Bundle[a] (Includes DNA library prep and enrichment reagents. Does not include NextSeq System core reagents)	16 indexes 24 samples	20028215
TruSight Oncology 500 DNA/RNA* Bundle,[a] for Use with NextSeq (Includes DNA library prep and enrichment reagents, and NextSeq System core reagents)	16 indexes 24 samples	20028216

a. The products to evaluate DNA and RNA variants (PN: 20028215/20028216) consist of the TruSight Oncology 500 DNA panel and the TruSight Tumor 170 RNA panel.

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