Pan-cancer: *BRAF, NTRK1, NTRK2, NTRK3, RET*, MSI, TMB													
		Genes with biomarkers of clinical significance*											Genes with biomarkers of potential clinical significance†
	Breast	*BRCA1*	*BRCA2*	*ERBB2*	*ESR1*	*PALB2*	*PIK3CA*						180
	Colorectal	*ERBB2*	*KRAS*	*NRAS*									166
	Bone	*EGFR*	*ERG*	*ETV1*	*ETV4*	*EWSR1*	*FEV*	*FLI1*	*FUS*	*H3F3A*	*HEY1*	*IDH1*	140
		MDM2	*NCOA2*	*SMARCB1*									
	Lung	*ALK*	*EGFR*	*ERBB2*	*KRAS*	*MET*	*NUTM1*	*ROS1*					223
	Melanoma	*KIT*	*NRAS*	*ROS1*									172
	Ovarian	*BRCA1*	*BRCA2*	*FOXL2*									149
	CNS‡	*APC*	*ATRX*	*CDKN2A*	*CDKN2B*	*EGFR*	*H3F3A*	*HIST1H3B*	*HIST1H3C*	*IDH1*	*IDH2*	*MYCN*	140
		PTCH1	*RELA*	*TERT*	*TP53*								
	Prostate	*AR*	*ATM*	*BARD1*	*BRCA1*	*BRCA2*	*BRIP1*	*CDK12*	*CHEK1*	*CHEK2*	*FANCL*	*FGFR2*	151
		FGFR3	*PALB2*	*PTEN*	*RAD51B*	*RAD51C*	*RAD51D*	*RAD54L*					
	Thyroid	*HRAS*	*KRAS*	*NRAS*	*TERT*								165
	Uterine & cervical	*BRCA2*	*EPC1*	*ERBB2*	*ESR1*	*FOXO1*	*GREB1*	*JAZF1*	*NCOA2*	*NCOA3*	*NUTM2A*	*NUTM2B*	138
		PAX3	*PAX7*	*PHF1*	*POLE*	*SMARCA4*	*SUZ12*	*TP53*	*YWHAE*				
	Other solid tumors	*ALK*	*APC*	*ARID1A*	*ASPSCR1*	*ATF1*	*ATIC*	*BAP1*	*BCOR*	*BRCA1*	*BRCA2*	*CAMTA1*	152
		CARS	*CCNB3*	*CDK4*	*CDKN2A*	*CIC*	*CITED2*	*CLTC*	*COL1A1*	*COL6A3*	*CREB1*	*CREB3L1*	
		CREB3L2	*CSF1*	*CTNNB1*	*DDIT3*	*DDX3X*	*DNAJB1*	*DUX4*	*EED*	*EGFR*	*ERBB2*	*ERG*	
		ETV1	*ETV4*	*ETV6*	*EWSR1*	*FEV*	*FGFR2*	*FGFR3*	*FLI1*	*FOXL2*	*FOXO1*	*FOXO4*	
		FUS	*GLI1*	*HEY1*	*HGF*	*HMGA2*	*IDH1*	*KRAS*	*LEUTX*	*MAML3*	*MDM2*	*MYB*	
		MYOD1	*NAB2*	*NCOA2*	*NF1*	*NFATC2*	*NFIB*	*NR4A3*	*NRAS*	*NUTM1*	*NUTM2A*	*NUTM2B*	
		PALB2	*PATZ1*	*PAX3*	*PAX7*	*PDGFB*	*PDGFRA*	*PRKACA*	*PRKD1*	*RANBP2*	*ROS1*	*SDHA*	
		SDHB	*SDHC*	*SDHD*	*SMARCB1*	*SS18*	*SSX1*	*SSX2*	*SSX4*	*STAT6*	*SUZ12*	*TAF15*	
		TCF12	*TERT*	*TFE3*	*TFEB*	*TFG*	*TP53*	*TPM3*	*TPM4*	*TRAF7*	*TSPAN31*	*VGLL2*	
		WT1	*WWTR1*	*YAP1*	*YWHAE*	*ZC3H7B*							

Figure 3: Subset of genomic tumor profiling biomarkers for multiple cancer types—TruSight Oncology 500 and TruSight Oncology 500 High-Throughput include key guideline biomarkers, emerging biomarkers, and pan-cancer biomarkers such as *BRAF*, *NTRK1*, *NTRK2*, *NTRK3*, *RET*, MSI, and TMB. Content analysis provided by Velsera based on software Knowledge Base v8.5 (February 2023).

* Genes with biomarkers of clinical significance linked to current drug labels or guidelines.

† Genes with biomarkers of potential clinical significance based on presence in clinical trials.

‡ CNS, central nervous system.

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*[a]	*CRLF2*	*ERCC1*	*FGF7*	*HIST1H1C*	*INSR*	*MAPK3*	*NOTCH1*	*PIK3R2*	*RARA*	*SMC3*	*TOP1*
AKT3	*BRCA2*[a]	*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*[b]	
BCL2L11	*CDKN2C*	*EML4*	*FGF9*	*GPR124*	*HSD3B1*	*MAGI2*	*NBN*	*PHF6*	*RAD21*	*SHQ1*	*TET1*	
BCL2L2	*CEBPA*	*EP300*	*FGF10*	*GPS2*	*HSP90AA1*	*MALT1*	*NCOA3*	*PHOX2B*	*RAD50*	*SLIT2*	*TET2*	
BCL6	*CENPA*	*EPCAM*	*FGF14*	*GREM1*	*ICOSLG*	*MAP2K1*	*NCOR1*	*PIK3C2B*	*RAD51*	*SLX4*	*TFE3*	
BCOR	*CHD2*	*EPHA3*	*FGF19*	*GRIN2A*	*ID3*	*MAP2K2*	*NEGR1*	*PIK3C2G*	*RAD51B*	*SMAD2*	*TFRC*	
BCORL1	*CHD4*	*EPHA5*	*FGF2*	*GRM3*	*IDH1*	*MAP2K4*	*NF1*	*PIK3C3*	*RAD51C*	*SMAD3*	*TGFBR1*	

a. Large rearrangements (exon-level CNVs) detected for *BRCA1* and *BRCA2*.

b. *TERT* promoter region only covered for variant calling.

CNV calling is available for all genes except: *DNAJB1, FANCF, FOXL2, HIST1H3A, HIST1H3C, HIST1H3D, HIST1H3E, HIST1H3F, HIST1H3G, HIST1H3H, HIST1H3I, HIST1H3J, HIST2H3A, HIST2H3C, HIST2H3D, HLA-A, HLA-B, HLA-C, KMT2B, KMT2C, KMT2D, TERC, TERT*

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.

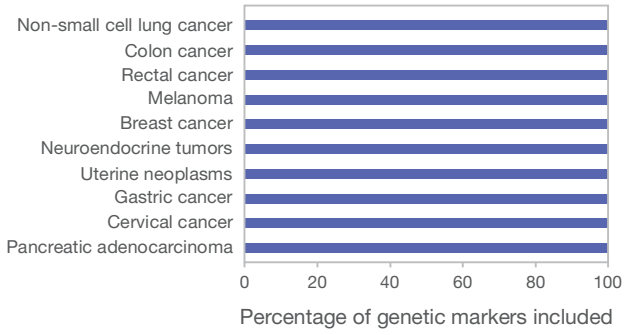

Figure 4: TruSight Oncology 500 content alignment to key guidelines by cancer type—The graph provides examples of content alignment; it is not meant to be all-inclusive.

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 to purify selected targets from DNA- and RNA-based libraries. Biotinylated probes hybridize to regions of interest, which are pulled down using streptavidin-coated magnetic beads, and then eluted to enrich the library pool. Hybridization-based enrichment is a useful strategy for analyzing specific genetic variants in a given sample and reliably sequencing exomes or large numbers of genes (eg, > 50 genes). It delivers dependable results across a wide range of input types and quantities. Hybrid–capture chemistry offers several advantages over amplicon sequencing, including yielding data with fewer artifacts and dropouts. Additionally, hybrid-capture chemistry is fusion agnostic, enabling detection and characterization of known and novel fusions. Unlike amplicon-based approaches, which require confirmatory tests as false-positives can arise, the hybrid-capture method is highly sensitive and can accurately characterize gene fusions with both known and novel partners.