VARIANT*Plex* -HT Comprehensive Tissue & Blood

Description

The VARIANT*Plex* Comprehensive Tissue & Blood panel is a balanced pool of gene-specific primer (GSP) oligonucleotides that is optimized for use with VARIANT*Plex*™ reagents and molecular barcode (MBC) adapters to produce targeted NGS libraries. This product insert should be used in conjunction with VARIANT*Plex* -HT protocol for Illumina® (RA-DOC-058).

VARIANT*Plex* Comprehensive Tissue & Blood contains **26,535** GSPs targeting **460** genes commonly mutated in solid tumor and blood cancer types as well as microsatellite instability (**MSI**), tumor mutational burden (**TMB**), and homologous recombination deficiency (**HRD**) status.

Description	Part number	Storage
VARIANT*Plex* Comprehensive Tissue & Blood GSP1 - 8 reactions	SA24315081	–20°C ± 10°C
VARIANT*Plex* Comprehensive Tissue & Blood GSP2 - 8 reactions	SA24315082	

Required reagent volumes

Protocol reference	Protocol step	Reagent	Volume per reaction (µL)
A	Ligation Step 2 Elution	5mM NaOH	20
B	First PCR	VARIANT*Plex* Comprehensive Tissue & Blood GSP1	8
C	First PCR	10mM Tris-HCl pH 8.0	18
D	First PCR	Purified PCR1 eluate	16
E	Second PCR	VARIANT*Plex* Comprehensive Tissue & Blood GSP2	8

Recommended PCR cycling

	Step	Temperature (°C)	Time	Cycles
First PCR reaction	**1**	95	3 min	1
	2	95	30 sec	15
	3	58	10 sec	
	4	60	10 min (100% ramp rate)	
	5	72	3 min	1
	6	4	Hold	1
Second PCR reaction	**1**	95	3 min	1
	2	95	30 sec	20†
	3	58	10 sec	
	4	65	10 min (100% ramp rate)	
	5	72	3 min	1
	6	4	Hold	1

†The number of PCR2 cycles may be decreased if you regularly experience library yields greater than 200 nM.

Recommended reads and multiplexing

VARIANT*Plex* Comprehensive Tissue & Blood libraries should be sequenced to a minimum of **61M** reads. Starting read depth recommendations for standard profiling may be adjusted based on user needs.

Archer™ Analysis settings

Sequencing data should be processed using Archer Analysis (v7.4, or greater). The VARIANT*Plex* Comprehensive Tissue & Blood panel is compatible with the ***SNV/Indel, Copy Number Variation, CNV 2.0, ASCN, Structural Variation, MSI, TMB, and HRD*** pipelines, found under the ***DNA*** Input Type**.** The recommended TMB settings in Archer Analysis are *High Threshold for TMB* = 17.5 and *Low Threshold* for TMB = 6 for VARIANT*Plex*

Comprehensive Tissue & Blood. As with most Analysis settings, these may be adjusted based on user needs. Selection of the DNA Target Coverage pipeline is optional (see the Archer Analysis User Guide for more details on setting up your analysis).

Archer Analysis processing of VARIANT*Plex* Comprehensive Tissue & Blood libraries requires a one-time upload of the Panel GTF. When performing DNA Target Coverage analysis, users must also select a Region of Interest BED file. Users may optionally add a Targeted Mutations VCF file for targeted SNV/Indel detection. Files can be obtained by contacting archer-tech@idtdna.com

Assay targets

Integrative genomic biomarkers

MSI, TMB, HRD

Genes

The following genes in the panel have full coding sequence coverage for SNV/Indel analysis. Coding sequence was determined using NCBI RefSeq transcripts†. These genes are also enabled for Copy Number Variation (CNV) analysis.

ABL1	*CBLB*	*DMD*	*FGFR3*	*IRF6*	*MLLT3*	*PIK3CG*	*RHOA*	*SYK*
ABL2	*CBLC*	*DNMT3A*	*FGFR4*	*IRS2*	*MPL*	*PIK3R1**	*RICTOR*	*TAF1*
ACVR1	*CCND1*	*DOT1L*	*FH*	*JAK1*	*MRE11A*	*PIK3R2*	*RIT1*	*TBL1XR1*
ACVR2A	*CCND2*	*EEF1A1*	*FLCN*	*JAK2*	*MSH2*	*PIK3R3*	*RNF43*	*TBX3*
AKT1	*CCND3*	*EEF2*	*FLNA*	*JAK3*	*MSH3*	*PIM1*	*ROS1*	*TCEB1*
AKT2	*CCNE1*	*EGFR*	*FLT1*	*JUN*	*MSH6*	*PLCB4*	*RPL5*	*TCF3*
AKT3	*CD28*	*EGR3*	*FLT3*	*KANSL1*	*MST1R*	*PLCG1*	*RPS6KB1*	*TCF7L2*
ALK	*CD70*	*EIF1AX*	*FLT4*	*KDM5A*	*MTOR*	*PLCG2*	*RPS14*	*TERT*
ANKRD26	*CD79A*	*ELF3*	*FOXA1*	*KDM5C*	*MUC6*	*PLXNB2*	*RPTOR*	*TET1*
APC	*CD79B*	*EP300*	*FOXA2*	*KDM6A*	*MUC16*	*PMS1*	*RRAS2*	*TET2*
AR	*CD274 (PD-L1)*	*EPAS1*	*FOXL2*	*KDR*	*MUTYH*	*PMS2*	*RUNX1*	*TFRC*
ARAF	*CDC25C*	*EPCAM*	*FOXO1*	*KEAP1*	*MYC*	*POLD1*	*RUNX1T1*	*TGFBR1*
ARHGAP35	*CDC73*	*EPHA2*	*FOXP1*	*KEL*	*MYCL (MYCL1)*	*POLE*	*RXRA*	*TGFBR2*
ARID1A	*CDH1*	*EPHA3*	*FOXQ1*	*KIF1A*	*MYCN*	*POLQ*	*SAMD9*	*TGIF1*
ARID1B	*CDK4*	*EPHA7*	*FUBP1*	*KIT**	*MYD88*	*POLRMT*	*SAMD9L*	*THRAP3*

ARID2	*CDK6*	*EPHB1*	*GATA1*	*KLF2*	*MYH9*	*PPARG*	*SCAF4*	*TLR4*
ARID5B	*CDK8*	*ERBB2*	*GATA2*	*KLF4*	*NBN*	*PPM1D*	*SDHA*	*TMSB4X*
ASXL1	*CDK12*	*ERBB3*	*GATA3*	*KLF5*	*NCOR1*	*PPP2R1A*	*SDHB*	*TNFAIP3*
ASXL2	*CDKN1A*	*ERBB4*	*GEN1*	*KMT2A (MLL)*	*NF1*	*PPP2R2A*	*SDHC*	*TNFRSF14*
*ATM**	*CDKN1B*	*ERCC1*	*GLI1*	*KMT2B*	*NF2*	*PPP6C*	*SDHD*	*TP53*
ATR	*CDKN2A*	*ERCC2*	*GNA11*	*KMT2C*	*NFE2L2*	*PRDM1*	*SETBP1*	*TP63*
ATRX	*CDKN2B*	*ERCC4*	*GNA13*	*KMT2D (MLL2)*	*NFKBIA*	*PRKAR1A*	*SETD2*	*TRAF2*
AURKA	*CDKN2C*	*ERG*	*GNAQ*	*KRAS*	*NFKBIE*	*PRKD1*	*SF3B1*	*TRAF3*
AURKB	*CEBPA*	*ERRFI1*	*GNAS*	*KRT222*	*NIPBL*	*PRKDC*	*SH2B3*	*TRAF7*
AXIN1	*CHD1*	*ESR1*	*GPS2*	*LAMP1*	*NKX2-1*	*PSIP1*	*SH2D1A*	*TSC1*
AXIN2	*CHD3*	*ETNK1*	*GRIN2D*	*LATS1*	*NOTCH1**	*PTCH1*	*SLC29A1*	*TSC2*
AXL	*CHD4*	*ETV6*	*GRM3*	*LATS2*	*NOTCH2*	*PTEN*	*SLX4*	*TSHR*
B2M	*CHD8*	*EWSR1*	*H3F3A*	*LEMD2*	*NOTCH3*	*PTMA*	*SMAD2*	*TXNIP*
BAP1	*CHEK1*	*EZH2*	*H3F3B*	*LRP1B*	*NOTCH4*	*PTPDC1*	*SMAD3*	*U2AF1*
BARD1	*CHEK2**	*FAM46C*	*H3F3C*	*LUC7L2*	*NPM1*	*PTPN11*	*SMAD4*	*U2AF2*
BCL2	*CIC*	*FAM46D*	*HGF*	*LZTR1*	*NRAS*	*PTPRC*	*SMARCA4*	*UBA1*
BCL2L1	*COL5A1*	*FAM175A*	*HIST1H1C*	*MACF1*	*NSD1*	*PTPRD*	*SMARCB1*	*UNCX*
BCL2L11	*CREBBP*	*FANCA*	*HIST1H1E*	*MAP2K1 (MEK1)*	*NTRK1*	*PTPRT*	*SMC1A*	*USP9X*
BCL6	*CRKL*	*FANCC*	*HIST1H2BD*	*MAP2K2 (MEK2)*	*NTRK2*	*RAC1*	*SMC3*	*VHL*
BCOR	*CSDE1*	*FANCD2*	*HIST1H3B*	*MAP2K4*	*NTRK3*	*RAD21*	*SMO*	*WT1*
BCORL1	*CSF1R*	*FANCE*	*HIST1H3C*	*MAP3K1*	*NUP93*	*RAD50*	*SOCS1*	*XPO1*
BIRC3	*CSF3R*	*FANCF*	*HNF1A*	*MAP3K4*	*PALB2*	*RAD51*	*SOX2*	*XRCC2*
BLM	*CTCF*	*FANCG*	*HOXB13*	*MAP3K13*	*PARP1*	*RAD51B*	*SOX9*	*XRCC3*
BMPR1A	*CTLA4*	*FANCI*	*HRAS*	*MAPK1*	*PAX5*	*RAD51C*	*SOX17*	*ZBTB20*
*BRAF**	*CTNNA1*	*FANCL*	*HUWE1*	*MAX*	*PAX8*	*RAD51D*	*SPEN*	*ZFHX3*
*BRCA1**	*CTNNB1**	*FAT1*	*ID3*	*MCL1*	*PBRM1*	*RAD52*	*SPOP*	*ZMYM2*
*BRCA2**	*CUL3*	*FBXW7*	*IDH1*	*MDM2*	*PDCD1 (PD-1)*	*RAD54L*	*SPTA1*	*ZMYM3*
BRIP1	*CUX1*	*FGF1*	*IDH2*	*MDM4*	*PDCD1LG2 (PD-L2)*	*RAF1*	*SPTAN1*	*ZNF750*
BTG1	*CXCR4*	*FGF2*	*IGF1R*	*MECOM*	*PDGFRA**	*RARA*	*SRC*	*ZRSR2*
BTG2	*CYSLTR2*	*FGF3*	*IKBKE*	*MED12*	*PDGFRB*	*RASA1*	*SRSF2*	
BTK	*DAXX*	*FGF4*	*IKZF1*	*MEF2B*	*PGR*	*RB1*	*STAG1*	

CACNA1A	*DCK*	*FGF7*	*IL6ST*	*MEN1*	*PHF6*	*RBBP6*	*STAG2*
*CALR**	*DDR2*	*FGF8*	*IL7R*	*MET**	*PIK3C2B*	*RBM10*	*STAT3*
CARD11	*DDX3X*	*FGF9*	*INPP4B*	*MGA*	*PIK3C2G*	*RECQL4*	*STAT5B*
CASP8	*DDX41*	*FGF19*	*INPPL1*	*MGMT*	*PIK3CA*	*REL*	*STAT6*

†Contact archer-tech@idtdna.com for the panel target file to view complete list of targeted regions.
*Indicates that select regions of this gene are enabled for Structural Variant analysis in the GTF.

SNP sites targeted for sample tracking

rs560681	rs430046	rs987640	rs10776839	rs12393891
rs740598	rs8078417	rs6444724	rs6530357	chrX:4429309
rs1498553	rs9951171	rs6811238	rs5971553	chrX:11314433
rs10773760	rs576261	rs13182883	rs5953060	chrY:6738552
rs1058083	rs1109037	rs214955	rs6524626	chrY:19490214
rs4530059	rs1523537	rs321198	rs5940270	
rs1821380	rs221956	rs4606077	rs722847	

SNPs may be used in combination to uniquely tag and track samples over time. Contact archer-tech@idtdna.com for further details.

Limitations of use

For research use only. Not for use in diagnostic procedures. Unless otherwise agreed to in writing, IDT does not intend these products to be used in clinical applications and does not warrant their fitness or suitability for any clinical diagnostic use. Purchaser is solely responsible for all decisions regarding the use of these products and any associated regulatory or legal obligations.

Safety data sheets pertaining to this product are available upon request.

Revision History

Document Number	Date	Description of change
RA-DOC-481/REV01	August 2024	Initial release.