

VARIANT*Plex* Pan Solid Tumor

Description

The VARIANT*Plex* Pan Solid Tumor panel is a balanced pool of gene-specific primer (GSP) oligonucleotides that is optimized for use with VARIANT*Plex* HGC 2.0 reagents and molecular barcode (MBC) adapters to produce targeted NGS libraries. This product insert should be used in conjunction with VARIANT*Plex* HS/HGC protocol for Illumina® (RA-DOC-056).

VARIANT*Plex* Pan Solid Tumor contains **9878** GSPs targeting **185** genes commonly mutated in solid tumors as well as microsatellite instability (**MSI**) and tumor mutational burden (**TMB**) status.

Description	Part number	Storage
VARIANT <i>Plex</i> Pan Solid Tumor GSP1, 8 reactions	SA20196081	-20°C ± 10°C
VARIANT <i>Plex</i> Pan Solid Tumor GSP2, 8 reactions	SA20196082	
PreSeq [™] DNA QC Assay Standard, 32 µL	SA0597	
PreSeq [™] DNA QC Assay 10X Primer Mix, 120 µL	SA0598	

Required reagent volumes

Protocol reference	Protocol step	Reagent	Volume per reaction (µL)
A	Ligation Step 2 Elution	5mM NaOH	16
B	First PCR	VARIANT <i>Plex</i> Pan Solid Tumor GSP1	24
C	First PCR	10mM Tris-HCl pH 8.0	18
D	First PCR	Purified PCR1 eluate	16
E	Second PCR	VARIANT <i>Plex</i> Pan Solid Tumor GSP2	24

Recommended PCR cycling

	Step	Temperature (°C)	Time	Cycles
First PCR reaction	1	95	3 min	1
	2	95	30 sec	
	3	58	10 sec	15
	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	
	3	58	10 sec	20 [†]
	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 Pan Solid Tumor libraries should be sequenced to a minimum of **25M** 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 (v7.2, or greater). The VARIANT[™]Plex Pan Solid Tumor panel requires selection of the **SNV/Indel, Copy Number Variation, Structural Variation, MSI, and TMB** pipelines, found under the **DNA** Input Type. The recommended TMB settings in Archer Analysis are *High Threshold for TMB = 20* and *Low Threshold for TMB = 5* for VARIANT[™]Plex Pan Solid Tumor. As with most Analysis settings, these may be adjusted based on user needs. Selection of the DNA Target Coverage pipeline is also optional and requires a region of interest bed file from supporting. See the Archer Analysis User Guide for more details on setting up your analysis.

Processing of VARIANTPlex Pan Solid Tumor libraries requires a one-time upload of a Target Region file (a text file, in GTF format, which directs the software on how to analyze data from the panel). For SNV/Indel detection it is recommended analysis is performed using a Targeted Mutations File. Files can be obtained by contacting archer-tech@idtdna.com

Assay targets

Integrative genomic biomarkers

MSI, TMB

Genes

The following genes in the panel have full coding sequence coverage for SNV/Indel analysis. Coding sequence was determined using NCBI RefSeq transcripts†.

<i>ABL1</i>	<i>BMPR1A</i>	<i>CTNNB1*</i>	<i>FGF19</i>	<i>HNF1A</i>	<i>MDM4</i>	<i>NOTCH2</i>
<i>ACVR1</i>	<i>BRAF*</i>	<i>DAXX</i>	<i>FGFR1</i>	<i>HRAS</i>	<i>MED12</i>	<i>NOTCH3</i>
<i>AKT1</i>	<i>BRCA1*</i>	<i>DDR2</i>	<i>FGFR2</i>	<i>IDH1</i>	<i>MEN1</i>	<i>NOTCH4</i>
<i>AKT2</i>	<i>BRCA2*</i>	<i>DDX3X</i>	<i>FGFR3</i>	<i>IDH2</i>	<i>MET*</i>	<i>NPM1</i>
<i>AKT3</i>	<i>BRIP1</i>	<i>DICER1</i>	<i>FGFR4</i>	<i>JAK1</i>	<i>MLH1</i>	<i>NRAS</i>
<i>ALK</i>	<i>CCND1</i>	<i>EGFR</i>	<i>FH</i>	<i>JAK2</i>	<i>MPL</i>	<i>NTRK1</i>
<i>APC</i>	<i>CCND2</i>	<i>EIF1AX</i>	<i>FLCN</i>	<i>JAK3</i>	<i>MRE11A</i>	<i>NTRK2</i>
<i>AR</i>	<i>CCND3</i>	<i>EP300</i>	<i>FLT1</i>	<i>KDM6A</i>	<i>MSH2</i>	<i>NTRK3</i>
<i>ARID1A</i>	<i>CCNE1</i>	<i>EPCAM</i>	<i>FLT3</i>	<i>KDR</i>	<i>MSH3</i>	<i>PALB2</i>
<i>ARID1B</i>	<i>CDH1</i>	<i>ERBB2</i>	<i>FLT4</i>	<i>KEAP1</i>	<i>MSH6</i>	<i>PBRM1</i>
<i>ARID2</i>	<i>CDK12</i>	<i>ERBB3</i>	<i>FOXA1</i>	<i>KIT*</i>	<i>MTOR</i>	<i>PDGFRA*</i>
<i>ATM*</i>	<i>CDK4</i>	<i>ERBB4</i>	<i>FOXL2</i>	<i>KLF4</i>	<i>MUC16</i>	<i>PIK3CA</i>
<i>ATR</i>	<i>CDK6</i>	<i>ERCC1</i>	<i>FUBP1</i>	<i>KMT2C</i>	<i>MUTYH</i>	<i>PIK3CB</i>
<i>ATRX</i>	<i>CDKN2A</i>	<i>ERCC2</i>	<i>GNA11</i>	<i>KMT2D (MLL2)</i>	<i>MYC</i>	<i>PIK3R1*</i>
<i>AURKA</i>	<i>CDKN2B</i>	<i>ESR1</i>	<i>GNAQ</i>	<i>KRAS</i>	<i>MYCN</i>	<i>PLCB4</i>
<i>B2M</i>	<i>CHD1</i>	<i>EZH2</i>	<i>GNAS</i>	<i>LZTR1</i>	<i>NBN</i>	<i>PMS2</i>
<i>BAP1</i>	<i>CHEK1</i>	<i>FANCA</i>	<i>H3F3A</i>	<i>MAP2K1 (MEK1)</i>	<i>NF1</i>	<i>POLD1</i>
<i>BARD1</i>	<i>CHEK2*</i>	<i>FANCI</i>	<i>H3F3B</i>	<i>MAP2K2 (MEK2)</i>	<i>NF2</i>	<i>POLE</i>
<i>BCOR</i>	<i>CIC</i>	<i>FANCL</i>	<i>HIST1H3B</i>	<i>MAP3K1</i>	<i>NKX2-1</i>	<i>PPP2R1A</i>

<i>BLM</i>	<i>CSF1R</i>	<i>FBXW7</i>	<i>HIST1H3C</i>	<i>MDM2</i>	<i>NOTCH1*</i>	<i>PPP2R2A</i>
<i>PRKD1</i>	<i>RAD51C</i>	<i>RICTOR</i>	<i>SETD2</i>	<i>SRC</i>	<i>TP53</i>	<i>VHL</i>
<i>PTCH1</i>	<i>RAD51D</i>	<i>RNF43</i>	<i>SF3B1</i>	<i>SRSF2</i>	<i>TP63</i>	<i>XRCC2</i>
<i>PTEN</i>	<i>RAD54L</i>	<i>ROS1</i>	<i>SMAD2</i>	<i>STAG2</i>	<i>TRAF7</i>	<i>XRCC3</i>
<i>PTPN11</i>	<i>RAF1</i>	<i>SDHA</i>	<i>SMAD4</i>	<i>STK11</i>	<i>TSC1</i>	
<i>RAD50</i>	<i>RB1</i>	<i>SDHB</i>	<i>SMARCA4</i>	<i>SUFU</i>	<i>TSC2</i>	
<i>RAD51</i>	<i>RET*</i>	<i>SDHC</i>	<i>SMARCB1</i>	<i>TERT</i>	<i>TSHR</i>	
<i>RAD51B</i>	<i>RHOA</i>	<i>SDHD</i>	<i>SMO</i>	<i>TGFBR2</i>	<i>U2AF1</i>	

†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.

Genes targeted for CNV

<i>AKT1</i>	<i>BRIP1</i>	<i>ERBB2</i>	<i>JAK2</i>	<i>PDGFRA</i>
<i>AKT2</i>	<i>CCND1</i>	<i>ERBB3</i>	<i>KDR</i>	<i>PIK3CA</i>
<i>AKT3</i>	<i>CCND2</i>	<i>ERCC1</i>	<i>KEAP1</i>	<i>PIK3CB</i>
<i>ALK</i>	<i>CCND3</i>	<i>ERCC2</i>	<i>KIT</i>	<i>PTEN</i>
<i>AR</i>	<i>CCNE1</i>	<i>ESR1</i>	<i>KRAS</i>	<i>RAD51B</i>
<i>ATM</i>	<i>CDK12</i>	<i>FGF19</i>	<i>MDM2</i>	<i>RAF1</i>
<i>AURKA</i>	<i>CDK4</i>	<i>FGFR1</i>	<i>MDM4</i>	<i>RET</i>
<i>BARD1</i>	<i>CDK6</i>	<i>FGFR2</i>	<i>MET</i>	<i>RICTOR</i>
<i>BRAF</i>	<i>CHEK1</i>	<i>FGFR3</i>	<i>MYC</i>	<i>STK11</i>
<i>BRCA1</i>	<i>CHEK2</i>	<i>FGFR4</i>	<i>MYCN</i>	<i>TERT</i>
<i>BRCA2</i>	<i>EGFR</i>	<i>FLT3</i>	<i>NRAS</i>	

Please contact archer-tech@idtdna.com to inquire about enabling additional genes for CNV detection.

SNPs and 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

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

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Revision History

Document Number	Date	Description of change
RA-DOC-031/REV01	June 2023	Initial release.
RA-DOC-031/REV02	August 2023	Added TMB pipeline information to “Archer Analysis settings” section.
RA-DOC-031/REV03	November 2023	Updated First and Second PCR cycling conditions to include separate anneal and extended steps. Updated branding.