

VARIANT*Plex*-HT Myeloid

Description

The VARIANT*Plex*-HT Myeloid panel is a balanced pool of gene-specific primer (GSP) oligonucleotides that is optimized for use with VARIANT*Plex*-HT 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*-HT Myeloid contains **1800** GSPs targeting **75** genes commonly mutated in myeloid malignancies.

Description	Part number	Storage
VARIANT <i>Plex</i> -HT Myeloid GSP1, 24 reactions or VARIANT <i>Plex</i> -HT Myeloid GSP1, 96 reactions	SA5031241 or SA5031961	-20°C ± 10°C
VARIANT <i>Plex</i> -HT Myeloid GSP2, 24 reactions or VARIANT <i>Plex</i> -HT Myeloid GSP2, 96 reactions	SA5031242 or SA5031962	

Required reagent volumes

Protocol reference	Protocol step	Reagent	Volume per reaction (µL)
A	Ligation Step 2 Elution	5mM NaOH	20
B	First PCR	VARIANT <i>Plex</i> -HT Myeloid GSP1	8
C	First PCR	10mM Tris-HCl pH 8.0	18
D	First PCR	Purified PCR1 eluate	16
E	Second PCR	VARIANT <i>Plex</i> -HT Myeloid GSP2	8

Recommended PCR cycling

	Step	Temperature (°C)	Time	Cycles
First PCR reaction	1	95	3 min	1
	2	95	30 sec	
	3	60	10 sec	15
	4	62	5 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	60	10 sec	20†
	4	65	5 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*-HT Myeloid libraries should be sequenced to a minimum of **4M** 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 (v7.0, or greater). The VARIANT*Plex*-HT Myeloid panel requires selection of the **SNV/Indel, Copy Number Variation, and Structural Variation** pipelines found under the **DNA** Input Type. See the Archer Analysis User Guide for more details on setting up your analysis.

Processing of VARIANT*Plex*-HT Myeloid 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

Gene	Accession	Exon
<i>ABL1</i>	NM_005157	4,5,6,7,8,9,10
<i>ANKRD26</i>	NM_014915	1 (c.-113-c.-134)
<i>ASXL1</i>	NM_015338.5	1,2,3,4,5,6,7,8,9,10,11,12,13
<i>ASXL1</i>	NM_001164603.1	5
<i>ATRX</i>	NM_000489	8,9,10,11,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32
<i>BCOR</i>	NM_017745	2,3,4,5,6,7,9,10,11,12,13,14,15
<i>BCOR</i>	NM_001123385	8
<i>BCORL1</i>	NM_021946	1,2,3,4,5,6,7,8,9,10,11,12
<i>BRAF</i>	NM_004333	3,10,11,12,13,15
<i>BTK</i>	NM_000061	15
<i>CALR</i>	NM_004343	8,9
<i>CBL</i>	NM_005188	2,3,4,5,7,8,9,16
<i>CBLB</i>	NM_170662	3,9,10
<i>CBLC</i>	NM_012116	9,10
<i>CCND2</i>	NM_001759	5
<i>CDKN2A</i>	NM_058197	1
<i>CDKN2A</i>	NM_058195	1
<i>CDKN2A</i>	NM_000077	2,3
<i>CDKN2A</i>	NM_001195132	3
<i>CEBPA</i>	NM_004364	1
<i>CSF3R</i>	NM_156039	17
<i>CSF3R</i>	NM_172313	10,18
<i>CSF3R</i>	NM_000760	14,15,16
<i>CUX1</i>	NM_001202543	15,16,17,18,19,20,21,22,23,24
<i>CUX1</i>	NM_001913	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23
<i>CUX1</i>	NM_181552	1
<i>CXCR4</i>	NM_003467	1,2
<i>DCK</i>	NM_000788	2,3
<i>DDX41</i>	NM_016222	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17
<i>DHX15</i>	NM_001358	3
<i>DNMT3A</i>	NM_022552	2,3,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23
<i>DNMT3A</i>	NM_153759	1,2
<i>DNMT3A</i>	NM_175630	4

Gene	Accession	Exon
<i>ETNK1</i>	NM_018638	3
<i>ETV6</i>	NM_001987	1,2,3,4,5,6,7,8
<i>EZH2</i>	NM_004456	2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20
<i>FBXW7</i>	NM_018315	1,2,3,4,5,6,7,8,9,10,11
<i>FLT3</i>	NM_004119	8,9,10,11,12,13,14,15,16,17,19,20,21
<i>GATA1</i>	NM_002049	2
<i>GATA2</i>	NM_032638	2,3,4,5,6
<i>GNAS</i>	NM_000516	8,9,10,11
<i>HRAS</i>	NM_005343	2,3,4
<i>IDH1</i>	NM_005896	3,4
<i>IDH2</i>	NM_002168	4,6
<i>IKZF1</i>	NM_001291845	4
<i>IKZF1</i>	NM_001291846	5
<i>IKZF1</i>	NM_006060	2,3,4,5,6,7,8
<i>JAK2</i>	NM_004972	12,13,14,15,16,19,20,21,22,23,24,25
<i>JAK3</i>	NM_000215	3,11,13,15,18,19
<i>KDM6A</i>	NM_021140	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29
<i>KDM6A</i>	NM_001291415	14
<i>KIT</i>	NM_000222	1,2,5,8,9,10,11,12,13,14,15,17,18
<i>KMT2A</i>	NM_005933	1,2,3,4,5,6,7,8,9,10,11,12,13,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36
<i>KMT2A</i>	NM_001197104	14
<i>KRAS</i>	NM_004985	2,3,4
<i>LUC7L2</i>	NM_016019	1,2,3,4,5,6,7,8,9,10
<i>LUC7L2</i>	NM_001244585	2
<i>MAP2K1</i>	NM_002755	2,3
<i>MPL</i>	NM_005373	10,12
<i>MYC</i>	NM_002467	1,2,3
<i>MYD88</i>	NM_002468	4,5
<i>MYD88</i>	NM_001172567	3
<i>NF1</i>	NM_000267	1,2,3,4,5,6,7,8,9,10,11,12,13,14,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56, 57
<i>NF1</i>	NM_001128147	15
<i>NF1</i>	NM_001042492	31
<i>NOTCH1</i>	NM_017617	26,27,28,34,c.*370 to c.*380

Gene	Accession	Exon
<i>NPM1</i>	NM_002520	11
<i>NRAS</i>	NM_002524	2,3,4,5
<i>PDGFRA</i>	NM_006206	12,14,15,18
<i>PHF6</i>	NM_032335	2,3,4,5,6,7,8
<i>PHF6</i>	NM_001015877	10
<i>PHF6</i>	NM_032458	9
<i>PPM1D</i>	NM_003620	6
<i>PTEN</i>	NM_000314	1,2,3,4,5,6,7,8,9
<i>PTPN11</i>	NM_002834	3,4,7,8,12,13
<i>PTPN11</i>	NM_080601	11
<i>RAD21</i>	NM_006265	2,3,4,5,6,7,8,9,10,11,12,13,14
<i>RBBP6</i>	NM_006910	p.1444,p.1451,p.1569,p.1654,p.1673
<i>RUNX1</i>	NM_001754	2,3,5,6,7,8,9
<i>RUNX1</i>	NM_001122607	1,5
<i>SETBP1</i>	NM_015559	4 (p.799-p.950)
<i>SF3B1</i>	NM_012433	13,14,15,16,17,18,19,20,21
<i>SH2B3</i>	NM_005475	2,3,4,5,6,7,8
<i>SLC29A1</i>	NM_001078175	4,13
<i>SMC1A</i>	NM_006306	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25
<i>SMC1A</i>	NM_001281463	2
<i>SMC3</i>	NM_005445	10,13,19,23,25,28
<i>SRSF2</i>	NM_003016	1,2
<i>STAG2</i>	NM_006603	2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33
<i>STAG2</i>	NM_001042749	32
<i>STAT3</i>	NM_003150	20
<i>STAT3</i>	NM_139276	21
<i>TET2</i>	NM_001127208	4,5,6,7,8,9,10,11
<i>TET2</i>	NM_017628	3
<i>TP53</i>	NM_000546	1,2,3,4,5,6,7,8,9,10,11
<i>TP53</i>	NM_001276696	10
<i>TP53</i>	NM_001276695	10
<i>U2AF1</i>	NM_006758	2,6,7
<i>U2AF1</i>	NM_001025204	6
<i>U2AF2</i>	NM_007279	1,2,3,4,5,6,7,8,9,10,11,12

Gene	Accession	Exon
<i>WT1</i>	NM_000378	1,2,3,4,5,6,7,9
<i>WT1</i>	NM_001198552	8
<i>XPO1</i>	NM_003400	15,16,18
<i>ZRSR2</i>	NM_005089	1,2,3,4,5,6,7,8,9,10,11

Genes targeted for CNV

<i>ASXL1</i>	<i>CDKN2A</i>	<i>FLT3</i>	<i>MYC</i>	<i>RUNX1</i>	<i>U2AF2</i>
<i>BCOR</i>	<i>CUX1</i>	<i>IKZF1</i>	<i>NF1</i>	<i>TET2</i>	<i>WT1</i>
<i>CBL</i>	<i>ETV6</i>	<i>KDM6A</i>	<i>RAD21</i>	<i>TP53</i>	<i>ZRSR2</i>
<i>CDC25C</i>	<i>EZH2</i>	<i>LUC7L2</i>	<i>RPS14</i>	<i>U2AF1</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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Revision History

Document Number	Date	Description of change
<i>RA-DOC-063/REV01</i>	October 2023	Initial release.
<i>RA-DOC-063/REV02</i>	November 2023	Updated First and Second PCR cycling conditions to include separate anneal and extended steps. Updated typo in “Genes targeted for CNV” table. Updated branding.
<i>RA-DOC-063/REV03</i>	March 2025	Updated IKZF1 assay targets