

## VARIANT*Plex* Myeloid

### Description

The VARIANT*Plex* Myeloid 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* HS/HGC protocol for Illumina® (RA-DOC-056) or VARIANT*Plex*-LAC protocol for Illumina® (RA-DOC-470).

VARIANT*Plex* Myeloid contains GSPs targeting **75** genes frequently mutated in myeloid malignancies.

Description	Part number	Storage
VARIANT <i>Plex</i> Myeloid GSP1, 8 reactions	SA5031081	-20°C ± 10°C
VARIANT <i>Plex</i> Myeloid GSP2, 8 reactions	SA5031082	
PreSeq <sup>™</sup> DNA QC Assay Standard, 32 µL	SA0597	
PreSeq <sup>™</sup> DNA QC Assay 10X Primer Mix, 120 µL	SA0598	

### Required reagent volumes

Protocol reference	Protocol Step	Reagent	HS/HGC Protocol RA-DOC-056 Volume per reaction (µL)	LAC Protocol RA-DOC-470 Volume per reaction (µL)
A	Ligation step 2 elution	5 mM NaOH	32	32
B	First PCR	VARIANT <i>Plex</i> Myeloid GSP1	8	8
C	First PCR	10 mM Tris-HCl pH 8.0	34	30
D	First PCR	Purified First PCR eluate	32	28
E	Second PCR	VARIANT <i>Plex</i> Myeloid GSP2	8	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	16
	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

VARIANTPl<sup>ex</sup> Myeloid libraries should be sequenced to a minimum of **4M reads**. Starting read depth recommendations for standard profiling may be adjusted to increase panel sensitivity.

## Archer™ Analysis settings

Sequencing data should be processed using Archer Analysis (v7.0, or greater). The VARIANTPl<sup>ex</sup> 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). Selection of the DNA Target Coverage pipeline is optional.

Processing of VARIANTPlex 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](mailto: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

<b>Gene</b>	<b>Accession</b>	<b>Exon</b>
<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
<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

<b>Gene</b>	<b>Accession</b>	<b>Exon</b>
<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
<i>NPM1</i>	NM_002520	11
<i>NRAS</i>	NM_002524	2,3,4,5

<b>Gene</b>	<b>Accession</b>	<b>Exon</b>
<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

Gene	Accession	Exon
<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
<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](mailto: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](mailto:archer-tech@idtdna.com) for further details.

## Limitations of use

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## Revision History

Document Number	Date	Description of change
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RA-DOC-022 / REV04

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<i>RA-DOC-022/REV01</i>	June 2023	Initial release.
<i>RA-DOC-022/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-022/REV03</i>	October 2024	Added LAC protocol to Description section and LAC compatible reagent volumes.
<i>RA-DOC-022/REV04</i>	March 2025	Updated IKZF1 assay targets