

VARIANT*Plex* Core Myeloid

Description

The VARIANT*Plex* Core 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).

VARIANT*Plex* Core Myeloid contains GSPs targeting **37** genes frequently mutated in myeloid malignancies.

Description	Part number	Storage
VARIANT <i>Plex</i> Core Myeloid GSP1, 8 reactions	SA5030081	-20°C ± 10°C
VARIANT <i>Plex</i> Core Myeloid GSP2, 8 reactions	SA5030082	
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	32
B	First PCR	VARIANT <i>Plex</i> Core Myeloid GSP1	8
C	First PCR	10mM Tris-HCl pH 8.0	34
D	First PCR	Purified PCR1 eluate	32
E	Second PCR	VARIANT <i>Plex</i> Core 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	16
	4	63	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* Core Myeloid libraries should be sequenced to a minimum of **3M 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* Core 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 VARIANT*Plex* Core 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	11,12,13
<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>BRAF</i>	NM_004333	11,15
<i>CALR</i>	NM_004343	8,9
<i>CBL</i>	NM_005188	8,9
<i>CEBPA</i>	NM_004364	1
<i>CSF3R</i>	NM_000760	10,14,15,16
<i>CSF3R</i>	NM_156039	17
<i>CSF3R</i>	NM_172313	18
<i>DDX41</i>	NM_016222	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17
<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>FLT3</i>	NM_004119	8,9,10,11,12,13,14,15,16,17,19,20,21

Gene	Accession	Exon
<i>GATA1</i>	NM_002049	2
<i>GATA2</i>	NM_032638	2,3,4,5,6
<i>IDH1</i>	NM_005896	3,4
<i>IDH2</i>	NM_002168	4,6
<i>JAK2</i>	NM_004972	12,13,14,15,16
<i>KIT</i>	NM_000222	2,8,9,10,11,12,13,14,15,17,18
<i>KRAS</i>	NM_004985	2,3,4
<i>MPL</i>	NM_005373	10,12
<i>NPM1</i>	NM_002520	11
<i>NRAS</i>	NM_002524	2,3,4
<i>PHF6</i>	NM_032458	9,10
<i>PHF6</i>	NM_032335	2,3,4,5,6,7,8
<i>PTPN11</i>	NM_002834	3,4,7,8,12,13
<i>PTPN11</i>	NM_080601	11
<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
<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>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

Gene	Accession	Exon
<i>TP53</i>	NM_001276696	10
<i>TP53</i>	NM_001276695	10
<i>U2AF1</i>	NM_006758	2,5,6
<i>WT1</i>	NM_000378	1,2,3,4,5,6,7,9
<i>WT1</i>	NM_001198552	8
<i>ZRSR2</i>	NM_005089	1,2,3,4,5,6,7,8,9,10,11

Genes targeted for CNV

<i>ASXL1</i>	<i>ETV6</i>	<i>RUNX1</i>	<i>U2AF1</i>	<i>BCOR</i>	<i>EZH2</i>
<i>TET2</i>	<i>WT1</i>	<i>CBL</i>	<i>FLT3</i>	<i>TP53</i>	<i>ZRSR2</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
RA-DOC-023/REV01	June 2023	Initial release.
RA-DOC-023/REV02	November 2023	Updated First and Second PCR cycling conditions to include separate anneal and extended steps. Updated branding.