

VARIANT*Plex* AML Focus

Description

The VARIANT*Plex* AML Focus 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).

Description	Part number	Storage
VARIANT <i>Plex</i> AML Focus GSP1, 8 reactions	dSA09192081	-20°C ± 10°C
VARIANT <i>Plex</i> AML Focus GSP2, 8 reactions	dSA09192082	
PreSeq™ DNA QC Assay Standard, 32 µL	SA0597	
PreSeq™ DNA QC Assay Standard, 32 µL	SA0598	

Required reagent volumes

Protocol reference	Protocol step	Reagent	Volume per reaction (µL)
A	Ligation Step 2 Elution	5mM NaOH	36
B	First PCR	VARIANT <i>Plex</i> AML Focus GSP1	4
C	First PCR	10mM Tris-HCl pH 8.0	38
D	First PCR	Purified PCR1 eluate	36
E	Second PCR	VARIANT <i>Plex</i> AML Focus GSP2	4

Recommended PCR cycling

	Step	Temperature (°C)	Time	Cycles
First PCR reaction	1	95	3 min	1
	2	95	30 sec	
	3	60	10 sec	
	4	65	5 min (100% ramp rate)	15
	5	72	3 min	
	6	4	Hold	1
Second PCR reaction	1	95	3 min	1
	2	95	30 sec	
	3	60	10 sec	
	4	65	5 min (100% ramp rate)	20†
	5	72	3 min	
	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* AML Focus libraries should be sequenced to a minimum of **800,000 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* AML Focus panel requires selection of the **SNV/Indel 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 AML Focus 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>ASXL1</i>	NM_015338	11,12,13
<i>CEBPA</i>	NM_004364	1
<i>DNMT3A</i>	NM_022552	23
<i>FLT3</i>	NM_004119	8,9,10,11,12,13,14,15,16,17,19,20,21
<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>NPM1</i>	NM_002520	11
<i>RUNX1</i>	NM_001754	2,3,5,6,7,8,9
<i>RUNX1</i>	NM_001122607	1,5
<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

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

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.

This product or service is licensed under one or more of the following U.S. Patents: 8,835,358; 9,290,808; 9,290,809; 9,315,857; 9,708,659; and 9,816,137 owned by BD and is licensed solely for the use described in the associated product literature. No other rights, implied or otherwise, are granted to purchaser hereunder. Purchaser agrees, by way of example and not limitation, not to use this product to trace back the origin of a nucleic acid to an individual cell as a discrete entity (e.g., single cell analysis).

© 2023 Integrated DNA Technologies, Inc. All rights reserved. FUSION*Plex*, VARIANT*Plex*, LIQUID*Plex*, IMMUNO*Verse*, Archer Analysis, and Archer Assay Marketplace are trademarks of Integrated DNA Technologies, Inc. All other marks are the property of their respective owners. For specific trademark and licensing information, see www.idtdna.com/trademarks.

Revision History

Document Number	Date	Description of change
RA-DOC-037/REV01	June 2023	Initial release.
RA-DOC-037/REV02	November 2023	Updated First and Second PCR cycling conditions to include separate anneal and extended steps. Updated branding.