Product Insert

VARIANT*Plex*™ MPN Focus Panel

VARIANT*Plex* MPN Focus

Description

The VARIANT*Plex* MPN 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*Plex* MPN Focus GSP1, 8 reactions	dSA09814081	–20°C ± 10°C
VARIANT*Plex* MPN Focus GSP2, 8 reactions	dSA09814082	
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*Plex* MPN Focus GSP1	4
C	First PCR	10mM Tris-HCl pH 8.0	38
D	First PCR	Purified PCR1 eluate	36
E	Second PCR	VARIANT*Plex* MPN Focus GSP2	4

Recommended PCR cycling

	Step	Temperature (°C)	Time	Cycles
First PCR reaction	**1**	95	3 min	1
	2	95	30 sec	15
	3	60	10 sec	
	4	65	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	20†
	3	60	10 sec	
	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* MPN 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* MPN Focus panel requires selection of the ***SNV/Indel and DNA 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* MPN 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
ASXL1	NM_015338	11,12,13
CALR	NM_004343	8,9
CSF3R	NM_000760	10,14,15,16
CSF3R	NM_156039	17
CSF3R	NM_172313	18
DNMT3A	NM_022552	2,3,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23
DNMT3A	NM_153759	1,2
DNMT3A	NM_175630	4
EZH2	NM_004456	2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20
IDH1	NM_005896	3,4
IDH2	NM_002168	4,6
JAK2	NM_004972	12,13,14,15,16
KIT	NM_000222	2,8,9,10,11,12,13,14,15,17,18
MPL	NM_005373	10,12
SETBP1	NM_015559	4 (p.799-p.950)
TET2	NM_001127208	4,5,6,7,8,9,10,11

Gene	Accession	Exon
TET2	NM_017628	3

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.

Revision History

Document Number	Date	Description of change
RA-DOC-038/REV01	June 2023	Initial release.
RA-DOC-038/REV02	November 2023	Updated First and Second PCR cycling conditions to include separate anneal and extended steps. Updated branding.