VARIANT*Plex* BRCA v3

Description

The VARIANT*Plex* BRCA v3 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* standard protocol for Illumina® (RA-DOC-057), VARIANT*Plex* HS/HGC protocol for Illumina® (RA-DOC-056), or VARIANT*Plex*–LAC protocol for Illumina® (RA-DOC-470).

VARIANT*Plex* BRCA v3 contains **377** GSPs with coverage of all coding exons of the BRCA1 and BRCA2 genes for the detection of SNVs, Indels, and large intragenic structural variants.

Description	Part number	Storage
VARIANT*Plex* BRCA v3 GSP1 - 8 reactions	SA24462081	–20°C ± 10°C
VARIANT*Plex* BRCA v3 GSP2 - 8 reactions	SA24462082	

Required reagent volumes

Protocol reference	Protocol step	Reagent	Volume per reaction (µL) per standard protocol (RA-DOC-057)
A	Cleanup after Adapter Ligation	10mM Tris-HCl pH 8.0	20
B	First PCR	VARIANT*Plex* BRCA v3 GSP1	2
C	First PCR	Purified DNA	18
D	Cleanup after First PCR	10mM Tris-HCl pH 8.0	20
E	Cleanup after First PCR	Purified DNA	18
F	Second PCR	VARIANT*Plex* BRCA v3 GSP2	2

Protocol reference	Protocol step	Reagent	Volume per reaction (µL) per HS/HGC protocol (RA-DOC-056)
A	Ligation Step 2 Elution	5mM NaOH	36
B	First PCR	VARIANT*Plex* BRCA v3 GSP1	4
C	First PCR	10mM Tris-HCl pH 8.0	38
D	First PCR	Purified PCR1 eluate	36
E	Second PCR	VARIANT*Plex* BRCA v3 GSP2	4

Protocol reference	Protocol step	Reagent	Volume per reaction (µL) per -LAC protocol (RA-DOC-470)
A	Ligation Step 2 Elution	5mM NaOH	36
B	First PCR	VARIANT*Plex* BRCA v3 GSP1	4
C	First PCR	10mM Tris-HCl pH 8.0	34
D	First PCR	Purified PCR1 eluate	32
E	Second PCR	VARIANT*Plex* BRCA v3 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

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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* BRCA v3 libraries should be sequenced to a minimum of 200,000 reads for germline applications and 1M reads for standard tumor profiling applications. 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 (v7.3, or greater). The VARIANT*Plex* BRCA v3 panel is compatible with the ***SNV/Indel, Structural Variations and CNV 2.0*** pipelines, found under the ***DNA*** Input Type. Selection of the DNA Target Coverage pipeline is optional and requires a region of interest BED file. See the Archer Analysis User Guide for more details on setting up your analysis.

Processing of VARIANT*Plex* BRCA v3 libraries requires a one-time upload of a Target Region file (a text file, in GTF format, which directs the software on how to analyze data from the panel). For SNV/Indel detection it is recommended analysis is performed using a Targeted Mutations File. Files can be obtained by contacting archer-tech@idtdna.com

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.

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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-068/REV01	September 2024	Initial release.

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