

VARIANT*Plex*-HT BRCA+PALB2 v2

Description

The VARIANT*Plex*-HT BRCA+PALB2 v2 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*-HT standard protocol for Illumina® (RA-DOC-059) or VARIANT*Plex*-HT protocol for Illumina® (RA-DOC-058).

VARIANT*Plex*-HT BRCA+PALB2 v2 contains **432** GSPs with coverage of all coding exons of the BRCA1, BRCA2, and PALB2 genes for the detection of SNVs, Indels, and large intragenic structural variants.

Description	Part number	Storage
VARIANT <i>Plex</i> -HT BRCA+PALB2 v2 GSP1 - 24 reactions	SA24461241	-20°C ± 10°C
VARIANT <i>Plex</i> -HT BRCA+PALB2 v2 GSP2 - 24 reactions	SA24461242	
VARIANT <i>Plex</i> -HT BRCA+PALB2 v2 GSP1 - 96 reactions	SA24461961	
VARIANT <i>Plex</i> -HT BRCA+PALB2 v2 GSP2 - 96 reactions	SA24461962	

Required reagent volumes

Protocol reference	Protocol step	Reagent	Volume per reaction (µL) per VARIANT <i>Plex</i> -HT standard protocol (RA-DOC-059)
A	Cleanup after Adapter Ligation	10mM Tris-HCl pH 8.0	24
B	First PCR	VARIANT <i>Plex</i> -HT BRCA+PALB2 v2 GSP1	4
C	First PCR	Purified DNA	22
D	Cleanup after First PCR	10mM Tris-HCl pH 8.0	20
E	Cleanup after First PCR	Purified DNA	18
F	Second PCR	VARIANT <i>Plex</i> -HT BRCA+PALB2 v2 GSP2	4

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Protocol reference	Protocol step	Reagent	Volume per reaction (μL) per VARIANT <i>Plex</i> -HT protocol (RA-DOC-058)
A	Ligation Step 2 Elution	5mM NaOH	24
B	First PCR	VARIANT <i>Plex</i> -HT BRCA+PALB2 V2 GSP1	4
C	First PCR	10mM Tris-HCl pH 8.0	22
D	First PCR	Purified PCR1 eluate	20
E	Second PCR	VARIANT <i>Plex</i> -HT BRCA+PALB2 V2 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	15
	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	
	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^{ex}-HT BRCA+PALB2 v2 libraries should be sequenced to a minimum of 250,000 reads for germline applications and 1.5M reads for standard tumor profiling. 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 VARIANTPl^{ex}-HT BRCA+PALB2 v2 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 also 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 VARIANTPl^{ex}-HT BRCA+PALB2 v2 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.

Limitations of use

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Safety data sheets pertaining to this product are available upon request.

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Revision History

Document Number	Date	Description of change
RA-DOC-071/REV01	Septemeber 2024	Initial release.