

VARIANT*Plex* Hereditary Cancer

Description

The VARIANT*Plex* Hereditary Cancer 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* Hereditary Cancer contains **2713** GSPs targeting 55 genes commonly mutated in hereditary cancers.

Description	Part number	Storage
VARIANT <i>Plex</i> Hereditary Cancer GSP1 - 8 reactions	SA24463081	-20°C ± 10°C
VARIANT <i>Plex</i> Hereditary Cancer GSP2 - 8 reactions	SA24463082	

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	18
B	First PCR	VARIANT <i>Plex</i> Hereditary Cancer GSP1	4
C	First PCR	Purified DNA	16
D	Cleanup after First PCR	10mM Tris-HCl pH 8.0	18
E	Cleanup after First PCR	Purified DNA	16
F	Second PCR	VARIANT <i>Plex</i> Hereditary Cancer GSP2	4

Protocol reference	Protocol step	Reagent	Volume per reaction (µL) per HS/HGC protocol (RA-DOC-056)
A	Ligation Step 2 Elution	5mM NaOH	32
B	First PCR	VARIANT <i>Plex</i> Hereditary Cancer 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> Hereditary Cancer GSP2	8

Protocol reference	Protocol step	Reagent	Volume per reaction (µL) per -LAC protocol (RA-DOC-470)
A	Ligation Step 2 Elution	5mM NaOH	32
B	First PCR	VARIANT <i>Plex</i> Hereditary Cancer GSP1	8
C	First PCR	10mM Tris-HCl pH 8.0	30
D	First PCR	Purified PCR1 eluate	28
E	Second PCR	VARIANT <i>Plex</i> Hereditary Cancer 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	15
	4	61	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} Hereditary Cancer libraries should be sequenced to a minimum of 1.5M reads for germline applications and 8M 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} Hereditary Cancer 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} Hereditary Cancer 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

Assay Targets

The following genes in the panel have full coding sequence coverage for SNV/Indel analysis. Coding sequence was determined using NCBI RefSeq transcripts[†]. These genes are also enabled for Copy Number Variation (CNV) analysis.

<i>APC</i> ‡	<i>BRCA2</i> *	<i>DICER1</i>	<i>KIT</i>	<i>MSH3</i>	<i>PMS2</i>	<i>SDHA</i>	<i>STK11</i>
<i>ATM</i>	<i>BRIP1</i>	<i>EGFR</i>	<i>MAX</i>	<i>MSH6</i>	<i>POLD1</i>	<i>SDHAF2</i>	<i>TMEM127</i>
<i>AXIN2</i>	<i>CDH1</i>	<i>EPCAM</i>	<i>MBD4</i>	<i>MUTYH</i>	<i>POLE</i>	<i>SDHB</i>	<i>TP53</i>
<i>BAP1</i>	<i>CDK4</i>	<i>FH</i>	<i>MEN1</i>	<i>NF1</i>	<i>PTEN</i>	<i>SDHC</i>	<i>TSC1</i>
<i>BARD1</i>	<i>CDKN2A</i>	<i>FLCN</i>	<i>MET</i>	<i>NTHL1</i>	<i>RAD51C</i>	<i>SDHD</i>	<i>TSC2</i>
<i>BMPR1A</i>	<i>CHEK2</i>	<i>GREM1</i>	<i>MLH1</i>	<i>PALB2</i>	<i>RAD51D</i>	<i>SMAD4</i>	<i>VHL</i>
<i>BRCA1</i> *	<i>CTNNA1</i>	<i>HOXB13</i>	<i>MSH2</i>	<i>PDGFRA</i>	<i>RET</i>	<i>SMARCA4</i>	

†Contact adx-tech@idtdna.com for the panel target file to view complete list of targeted regions.

‡APC promoter 1A and 1B targeted for CNV

*Indicates that select regions of this gene are enabled for Structural Variant analysis in the GTF.

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-068/REV01	September 2024	Initial release.