

## VARIANT*Plex* Lymphoma

### Description

The VARIANT*Plex* Lymphoma 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) or VARIANT*Plex*-LAC protocol for Illumina® (RA-DOC-470).

VARIANT*Plex* Lymphoma contains **1787** GSPs targeting **51** genes commonly mutated in T- and B- cell lymphomas.

Description	Part number	Storage
VARIANT <i>Plex</i> Lymphoma GSP1, 8 reactions	SA24343081	-20°C ± 10°C
VARIANT <i>Plex</i> Lymphoma GSP2, 8 reactions	SA24343082	

### Required reagent volumes

Protocol reference	Protocol step	Reagent	HS/HGC Protocol RA-DOC-056 Volume per reaction (µL)	LAC Protocol RA-DOC-470 Volume per reaction (µL)
A	Ligation Step 2 Elution	5mM NaOH	32	32
B	First PCR	VARIANT <i>Plex</i> Lymphoma GSP1	8	8
C	First PCR	10mM Tris-HCl pH 8.0	34	30
D	First PCR	Purified PCR1 eluate	32	28
E	Second PCR	VARIANT <i>Plex</i> Lymphoma GSP2	8	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	63	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

VARIANT*Plex* Lymphoma libraries should be sequenced to a minimum of **5M 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 (v7.3 or greater). The VARIANT*Plex* Lymphoma panel requires selection of the **SNV/Indel, Structural Variation, and Copy Number Variation** pipelines, found under the **DNA** Input Type (see the software user manual for further details on setting up analyses). Selection of the DNA Target Coverage pipeline is optional.

Processing of VARIANT<sup>™</sup>Plex Lymphoma libraries requires a one-time upload of the Custom 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](mailto:archer-tech@idtdna.com)

## Assay targets

Gene	Accession	Exon
<i>ARID1A</i>	NM_006015	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20
<i>ATM</i>	NM_000051	1,2,3,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63
<i>ATM</i>	NM_001351835	4
<i>B2M</i>	NM_004048	1,2,3
<i>BCL2</i>	NM_000633	3
<i>BCL2</i>	NM_000657	2
<i>BIRC3</i>	NM_001165	2,3,4,5,6,7,8,9
<i>BRAF</i>	NM_004333	15
<i>BTK</i>	NM_000061	15
<i>CARD11</i>	NM_032415	2,3,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25
<i>CCND1</i>	NM_053056	1,2,3,4,5
<i>CD28</i>	NM_006139	4
<i>CD28</i>	NM_006139	1,2,3,4
<i>CD79A</i>	NM_001783	1,2,3,4,5
<i>CD79B</i>	NM_000626	1,2,3,4,5,6
<i>CDKN2A</i>	NM_000077	2,3
<i>CDKN2A</i>	NM_001195132	3
<i>CDKN2A</i>	NM_058195	1
<i>CDKN2A</i>	NM_058197	1

Gene	Accession	Exon
<i>CDKN2B</i>	NM_004936	2
<i>CDKN2B</i>	NM_078487	1
<i>CREBBP</i>	NM_004380	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31
<i>CXCR4</i>	NM_001008540	1
<i>CXCR4</i>	NM_001348056	2
<i>CXCR4</i>	NM_003467	1
<i>DNMT3A</i>	NM_001320893	1
<i>DNMT3A</i>	NM_022552	2,3,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23
<i>DNMT3A</i>	NM_153759	1,2
<i>DNMT3A</i>	NM_175630	4
<i>EP300</i>	NM_001429	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31
<i>EZH2</i>	NM_004456	2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20
<i>FOXO1</i>	NM_002015	1,2
<i>ID3</i>	NM_002167	1,2
<i>IDH2</i>	NM_002168	4
<i>JAK1</i>	NM_002227	14,17,20,25
<i>JAK3</i>	NM_000215	3,10,11,12,13,14,15,16,17,18,19
<i>KLF2</i>	NM_016270	1,2,3
<i>KMT2D</i>	NM_003482	2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55
<i>MEF2B</i>	NM_001145785	8
<i>MEF2B</i>	NM_005919	4,5,6,7,8,9,10
<i>MYC</i>	NM_002467	1,2,3
<i>MYD88</i>	NM_001172567	3

<b>Gene</b>	<b>Accession</b>	<b>Exon</b>
<i>MYD88</i>	NM_002468	4,5
<i>NFKBIE</i>	NM_004556	1,2,3,4,5,6
<i>NOTCH1</i>	NM_017617	34, c.*370 to c.*380
<i>NOTCH2</i>	NM_024408	34
<i>NRAS</i>	NM_002524	2,3,4,5
<i>PIM1</i>	NM_001243186	1
<i>PIM1</i>	NM_002648	2,3,4,5,6
<i>PLCG1</i>	NM_002660	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32
<i>PLCG2</i>	NM_002661	2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33
<i>PTEN</i>	NM_000314	1,2,3,4,5,6,7,8,9
<i>PTEN</i>	NM_001304717	1,2
<i>PTPRD</i>	NM_001171025	18
<i>PTPRD</i>	NM_002839	12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46
<i>PTPRD</i>	NM_130392	18
<i>PTPRD</i>	NM_130393	12
<i>RHOA</i>	NM_001664	2,3
<i>SF3B1</i>	NM_012433	13,14,15,16,17,18,19,20,21
<i>SOCS1</i>	NM_003745	2
<i>STAT3</i>	NM_003150	19,20
<i>STAT3</i>	NM_139276	21
<i>STAT5B</i>	NM_012448	14,15,16,17
<i>STAT6</i>	NM_003153	8,9,10,11,12,13,14
<i>TCF3</i>	NM_001136139	18

Gene	Accession	Exon
<i>TCF3</i>	NM_003200	2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19
<i>TET2</i>	NM_001127208	4,5,6,7,8,9,10,11
<i>TET2</i>	NM_017628	3
<i>TNFAIP3</i>	NM_006290	2,3,4,5,6,7,8,9
<i>TNFRSF14</i>	NM_003820	1,2,3,4,5,6,7,8
<i>TP53</i>	NM_000546	1,2,3,4,5,6,7,8,9,10
<i>TP53</i>	NM_001126113	10
<i>TP53</i>	NM_001126114	10
<i>TRAF2</i>	NM_021138	2,3,4,5,6,7,8,9,10,11
<i>XPO1</i>	NM_003400	15,16,18

## Genes targeted for CNV

<i>ARID1A</i>	<i>CD28</i>	<i>DNMT3A</i>	<i>KLF2</i>	<i>PIM1</i>	<i>SOCS1</i>	<i>TRAF2</i>
<i>ATM</i>	<i>CD79A</i>	<i>EP300</i>	<i>KMT2D</i>	<i>PLCG1</i>	<i>STAT6</i>	
<i>B2M</i>	<i>CD79B</i>	<i>EZH2</i>	<i>MEF2B</i>	<i>PLCG2</i>	<i>TCF3</i>	
<i>BCL2</i>	<i>CDKN2A</i>	<i>FOXO1</i>	<i>MYC</i>	<i>PTEN</i>	<i>TET2</i>	
<i>BIRC3</i>	<i>CDKN2B</i>	<i>ID3</i>	<i>MYD88</i>	<i>PTPRD</i>	<i>TNFAIP3</i>	
<i>CARD11</i>	<i>CREBBP</i>	<i>JAK1</i>	<i>NFKBIE</i>	<i>RHOA</i>	<i>TNFRSF14</i>	
<i>CCND1</i>	<i>CXCR4</i>	<i>JAK3</i>	<i>NRAS</i>	<i>SF3B1</i>	<i>TP53</i>	

Please contact [archer-tech@idtdna.com](mailto:archer-tech@idtdna.com) to inquire about enabling additional genes for CNV detection.

## 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](mailto:archer-tech@idtdna.com) for further details.

### Limitations of use

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### Revision History

Document Number	Date	Description of change
RA-DOC-066/REV01	July 2024	Initial release.
RA-DOC-066/REV02	October 2024	Updated panel specification information to include PTEN and NRAS gene targets