

## FUSION*Plex*-HT Lymphoma

### Description

The FUSION*Plex*-HT Lymphoma panel is a balanced pool of gene-specific primer (GSP) oligonucleotides that is optimized for use with FUSION*Plex*-HT reagents and molecular barcode (MBC) adapters to produce targeted NGS libraries. This product insert should be used in conjunction with FUSION*Plex*-HT protocol for Illumina® (RA-DOC-049).

FUSION*Plex*-HT Lymphoma contains **716** GSPs targeting **125** genes commonly mutated in hematological malignancies.

Description	Part number	Storage
FUSION <i>Plex</i> -HT Lymphoma GSP1, 24 reactions or FUSION <i>Plex</i> -HT Lymphoma GSP1, 96 reactions	SA0077241 or SA0077961	-20°C ± 10°C
FUSION <i>Plex</i> -HT Lymphoma GSP2, 24 reactions or FUSION <i>Plex</i> -HT Lymphoma GSP2, 96 reactions	SA0077242 or SA0077962	
10X VCP Primer Mix, 24 reactions or 10X VCP Primer Mix, 96 reactions	SA0840 or SA0841	

### 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

## Recommended PCR cycling (cont.)

	Step	Temperature (°C)	Time	Cycles
Second PCR reaction	1	95	3 min	1
	2	95	30 sec	
	3	60	10 sec	20 <sup>†</sup>
	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

FUSIONPlex-HT Lymphoma libraries should be sequenced to a minimum of **2M** 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, or greater). The FUSIONPlex-HT Lymphoma panel requires selection of the **Fusion** pipeline and (optional) **SNV/Indel** pipeline, found under the **RNA** Input Type. See the Archer Analysis User Guide for more details on setting up your analysis.

Processing of FUSIONPlex-HT Lymphoma libraries requires a one-time upload of the Custom Panel GTF. Files can be obtained by contacting [archer-tech@idtdna.com](mailto:archer-tech@idtdna.com).

## Assay targets

Gene	Accession	Exon	Variant Type	Description*
AICDA	NM_020661	N/A	Expression	N/A
AKT3	NM_005465	N/A	Mutation	K172
ALK	NM_004304	N/A	Mutation	T1151-C1156, F1174, L1196-S1206, G1269

<b>Gene</b>	<b>Accession</b>	<b>Exon</b>	<b>Variant Type</b>	<b>Description*</b>
<i>ALK</i>	NM_004304	2, 4, 6, 10, 16, 17, 18, 19, 20, 21, 22, 23	Fusion	5'
<i>ASB13</i>	NM_024701	N/A	Expression	N/A
<i>BATF3</i>	NM_018664	N/A	Expression	N/A
<i>BAX</i>	NM_138761	N/A	Mutation	G179
<i>BCL2</i>	NM_000633	N/A	Mutation	F104
<i>BCL2</i>	NM_000633	N/A	Expression	N/A
<i>BCL2</i>	NM_000633	3	Fusion	3'
<i>BCL2</i>	NM_000633	2	Fusion	5'
<i>BCL2A1</i>	NM_004049	N/A	Expression	N/A
<i>BCL3</i>	NM_005178	N/A	Expression	N/A
<i>BCL6</i>	NM_001706	N/A	Expression	N/A
<i>BCL6</i>	NM_001706	2, 3	Fusion	5'
<i>BCR</i>	NM_004327	1, 2, 3, 8, 12, 13, 14, 15, 16	Fusion	3'
<i>BIRC3</i>	NM_001165	N/A	Mutation	Q547
<i>BIRC3</i>	NM_001165	4, 5, 6, 7	Fusion	3'
<i>BLNK</i>	NM_013314	N/A	Expression	N/A
<i>BMF</i>	NM_033503	N/A	Expression	N/A
<i>BMP7</i>	NM_001719	N/A	Expression	N/A
<i>BRAF</i>	NM_004333	N/A	Mutation	V600

Gene	Accession	Exon	Variant Type	Description*
<i>BTK</i>	NM_000061	N/A	Mutation	C481
<i>CARD11</i>	NM_032415	N/A	Mutation	D230
<i>CBFB</i>	NM_022845	4,5	Fusion	3'
<i>CCDC50</i>	NM_174908	N/A	Expression	N/A
<i>CCND1</i>	NM_053056	N/A	Expression	N/A
<i>CCND1</i>	NM_053056	5	Fusion	3'
<i>CCND1</i>	NM_053056	N/A	Mutation	E36, V42-C47
<i>CCND2</i>	NM_001759	N/A	Expression	N/A
<i>CCND3</i>	NM_001760	N/A	Expression	N/A
<i>CCND3</i>	NM_001760	2	Fusion	5'
<i>CD274</i>	NM_014143	N/A	Expression	N/A
<i>CD44</i>	NM_000610	N/A	Expression	N/A
<i>CD79B</i>	NM_000626	N/A	Mutation	Y196
<i>CDC25A</i>	NM_001789	N/A	Expression	N/A
<i>CDK6</i>	NM_001259	1, 2, 3, 4	Fusion	3'
<i>CDKN2A</i>	NM_000077	N/A	Expression	N/A
<i>CDKN2B</i>	NM_004936	N/A	Expression	N/A
<i>CEBPD</i>	NM_005195	N/A	Expression	N/A
<i>CEBPE</i>	NM_001805	N/A	Expression	N/A

Gene	Accession	Exon	Variant Type	Description*
<i>CEBPG</i>	NM_001806	N/A	Expression	N/A
<i>CHIC2</i>	NM_012110	1, 2, 3	Fusion	3'
<i>CIITA</i>	NM_000246	1, 2	Fusion	3'
<i>CREB3L2</i>	NM_194071	N/A	Expression	N/A
<i>CREBBP</i>	NM_004380	N/A	Mutation	P1053, C1240, R1446, S1680-L1681
<i>CREBBP</i>	NM_004380	2, 3, 4, 5, 6	Fusion	5'
<i>CTLA4</i>	NM_005214	N/A	Expression	N/A
<i>CYB5R2</i>	NM_016229	N/A	Expression	N/A
<i>DEK</i>	NM_003472	2, 3	Fusion	3'
<i>DENND3</i>	NM_014957	N/A	Expression	N/A
<i>DLEU1</i>	NR_002605	N/A	Expression	N/A
<i>DNMT3A</i>	NM_175629	N/A	Mutation	R688-K693, R882
<i>DNMT3B</i>	NM_006892	N/A	Expression	N/A
<i>DNTT</i>	NM_004088	N/A	Expression	N/A
<i>DUSP22</i>	NM_020185	1, 2	Fusion	3'
<i>E2F2</i>	NM_004091	N/A	Expression	N/A
<i>EIF4A1</i>	NM_001416	2, 3	Fusion	5'
<i>ENTPD1</i>	NM_001776	N/A	Expression	N/A
<i>ETV6</i>	NM_001987	N/A	Mutation	Y104-R105

Gene	Accession	Exon	Variant Type	Description*
<i>ETV6</i>	NM_001987	1, 2, 3, 4, 5, 6	Fusion	3'
<i>ETV6</i>	NM_001987	2, 3, 4, 5, 6	Fusion	5'
<i>EXOC2</i>	NM_018303	N/A	Expression	N/A
<i>EZH2</i>	NM_004456	N/A	Mutation	Y646, R690
<i>FAM216A</i>	NM_013300	N/A	Expression	N/A
<i>FBXW7</i>	NM_033632	N/A	Mutation	R224-T226, R338-I347, T385, R465, R479, R505
<i>FGFR1</i>	NM_023110	12, 17	Fusion	3'
<i>FGFR1</i>	NM_023110	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 17	Fusion	5'
<i>FOXP1</i>	NM_032682	N/A	Expression	N/A
<i>FUT8</i>	NM_004480	N/A	Expression	N/A
<i>IDH1</i>	NM_005896	N/A	Mutation	R132
<i>IDH2</i>	NM_002168	N/A	Mutation	R140, R172
<i>IL16</i>	NM_004513	N/A	Expression	N/A
<i>IRF4</i>	NM_002460	N/A	Expression	N/A
<i>IRF8</i>	NM_002163	N/A	Expression	N/A
<i>ITPKB</i>	NM_002221	N/A	Expression	N/A
<i>JAK1</i>	NM_002227	N/A	Mutation	V658, S703, R724
<i>JAK2</i>	NM_004972	N/A	Mutation	F537-F547, V617-C618, L681-R683, L855, V863, A880, V911, M929- R938, I960, R980-E985, D994

Gene	Accession	Exon	Variant Type	Description*
<i>JAK2</i>	NM_004972	6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20	Fusion	5'
<i>JAK2</i>	NM_004972	9, 10, 11, 12	Fusion	3'
<i>JAK3</i>	NM_000215	N/A	Mutation	M511, A572-A573, R657, S789
<i>KIAA0101</i>	NM_014736	N/A	Expression	N/A
<i>KMT2A</i>	NM_005933	2, 3	Fusion	5'
<i>KMT2A</i>	NM_005933	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	Fusion	3'
<i>KRAS</i>	NM_004985	N/A	Mutation	G12-G13, Q61, A146
<i>LIMD1</i>	NM_014240	N/A	Expression	N/A
<i>LMO2</i>	NM_005574	N/A	Expression	N/A
<i>LRMP</i>	NM_006152	N/A	Expression	N/A
<i>LZTS1</i>	NM_021020	N/A	Expression	N/A
<i>MAL</i>	NM_002371	N/A	Expression	N/A
<i>MALT1</i>	NM_006785	9	Fusion	3'
<i>MAML3</i>	NM_018717	N/A	Expression	N/A
<i>MKL1</i>	NM_020831	4, 5, 6	Fusion	5'
<i>MLF1</i>	NM_022443	2, 3, 4	Fusion	5'
<i>MLLT10</i>	NM_004641	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18	Fusion	5'
<i>MLLT10</i>	NM_004641	7, 8, 9, 10	Fusion	3'

Gene	Accession	Exon	Variant Type	Description*
<i>MME</i>	NM_000902	N/A	Expression	N/A
<i>MUC1</i>	NM_002456	N/A	Expression	N/A
<i>MYBL1</i>	NM_001080416	N/A	Expression	N/A
<i>MYC</i>	NM_002467	N/A	Expression	N/A
<i>MYC</i>	NM_002467	1, 2	Fusion	5'
<i>MYD88</i>	NM_002468	N/A	Mutation	V217-S219, M232, S243, L265
<i>NEK6</i>	NM_014397	N/A	Expression	N/A
<i>NFKB1</i>	NM_003998	N/A	Expression	N/A
<i>NFKB2</i>	NM_002502	14, 15, 16, 17, 18, 19, 20, 21	Fusion	3'
<i>NME1</i>	NM_000269	N/A	Expression	N/A
<i>NOTCH1</i>	NM_017617	N/A	Mutation	L1574, V1578, L1585, F1592-L1593, R1598-L1600, L1678-I1680, P2514-E2515, P2525
<i>NOTCH1</i>	NM_017617	24, 25, 26, 27, 28, 29	Fusion	5'
<i>NOTCH1</i>	NM_017617	24	Fusion	3'
<i>NOTCH1</i>	NM_017617	34	Exon Skipping	N/A
<i>NOTCH2</i>	NM_024408	N/A	Mutation	R2400
<i>NRAS</i>	NM_002524	N/A	Mutation	G12-G13, G60-Q61
<i>P2RY8</i>	NM_178129	1	Fusion	3'
<i>PAICS</i>	NM_006452	N/A	Expression	N/A
<i>PDCD1</i>	NM_005018	N/A	Expression	N/A



<b>Gene</b>	<b>Accession</b>	<b>Exon</b>	<b>Variant Type</b>	<b>Description*</b>
<i>PDCD1LG2</i>	NM_025239	1, 2, 3	Fusion	5'
<i>PDCD1LG2</i>	NM_025239	5, 6	Fusion	3'
<i>PDCD1LG2</i>	NM_025239	N/A	Expression	N/A
<i>PDGFRA</i>	NM_006206	N/A	Mutation	T674
<i>PDGFRA</i>	NM_006206	9, 10, 11, 12, 13, 14	Fusion	5'
<i>PIM1</i>	NM_002648	N/A	Expression	N/A
<i>PIM2</i>	NM_006875	N/A	Expression	N/A
<i>PLCG1</i>	NM_002660	N/A	Mutation	S345
<i>PLCG2</i>	NM_002661	N/A	Mutation	R665, L845
<i>PPAT</i>	NM_002703	N/A	Expression	N/A
<i>PRDM16</i>	NM_022114	1, 2, 3, 4	Fusion	5'
<i>PRKAR2B</i>	NM_002736	N/A	Expression	N/A
<i>PTPN1</i>	NM_002827	N/A	Expression	N/A
<i>PYCR1</i>	NM_006907	N/A	Expression	N/A
<i>RAB29</i>	NM_003929	N/A	Expression	N/A
<i>RAG1</i>	NM_000448	N/A	Expression	N/A
<i>RAG2</i>	NM_000536	N/A	Expression	N/A
<i>RANBP1</i>	NM_002882	N/A	Expression	N/A
<i>RHOA</i>	NM_001664	N/A	Mutation	G17

Gene	Accession	Exon	Variant Type	Description*
<i>S1PR2</i>	NM_004230	N/A	Expression	N/A
<i>SERPINA9</i>	NM_175739	N/A	Expression	N/A
<i>SF3B1</i>	NM_012433	N/A	Mutation	E622-N626, H662-K666, K700, G742
<i>SH3BP5</i>	NM_004844	N/A	Expression	N/A
<i>STAT3</i>	NM_003150	N/A	Mutation	Y640-N647, D661
<i>STAT5B</i>	NM_012448	N/A	Mutation	N642
<i>STAT6</i>	NM_003153	N/A	Mutation	D419
<i>STIL</i>	NM_003035	1, 2	Fusion	3'
<i>STRBP</i>	NM_018387	N/A	Expression	N/A
<i>TCF3</i>	NM_003200	11, 12, 13, 14, 15, 16, 17, 18	Fusion	3'
<i>TNFRSF13B</i>	NM_012452	N/A	Expression	N/A
<i>TNFSF4</i>	NM_003326	N/A	Expression	N/A
<i>TP63</i>	NM_003722	3, 4, 5	Fusion	5'
<i>WT1</i>	NM_000378	N/A	Mutation	Q301-T303, G352-Q354, V359-T365, Q445-F448
<i>WT1</i>	NM_000378	N/A	Expression	N/A
<i>XPO1</i>	NM_003400	N/A	Mutation	E571

\*The mutations listed under the Description column are targeted by the assay design. Version 6.2 and earlier of Archer Analysis may not support RNA SNV/Indel variant calling at exon junctions depending on the sequence context (SNVs ≤5bp, indels ≤30bp). *De Novo* RNA SNV/Indel and Internal Tandem Duplication mutation detection are not supported on the Ion Torrent Sequencing System.

Note: Fusions involving BCR and TCR loci, including IGH, IGL and IGK, are targeted for expression and may not be explicitly called as a fusion because these often do not result in chimeric transcripts. For the “Expression” assay type, unique molecules originating from probes across these genes can be counted and normalized to target control genes to enable relative expression level detection. Results are visualized in Archer Analysis.

## 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-451/REV01	October 2023	Initial release.
RA-DOC-451/REV02	November 2023	Updated First and Second PCR cycling conditions to include separate anneal and extended steps.  Updated branding.