

FUSION*Plex*-HT ALL

Description

The FUSION*Plex*-HT ALL 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 ALL contains **506** GSPs targeting **81** genes commonly mutated in Acute Lymphoblastic Leukemia.

Description	Part number	Storage
FUSION <i>Plex</i> -HT ALL GSP1, 24 reactions or FUSION <i>Plex</i> -HT ALL GSP1, 96 reactions	SA0074241 or SA0074961	-20°C ± 10°C
FUSION <i>Plex</i> -HT ALL GSP2, 24 reactions or FUSION <i>Plex</i> -HT ALL GSP2, 96 reactions	SA0074242 or SA0074962	
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	
	3	60	10 sec	15
	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†
	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

FUSION $Plex$ -HT ALL libraries should be sequenced to a minimum of **1.5M** 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, or greater). The FUSION $Plex$ -HT ALL 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 FUSION $Plex$ -HT ALL libraries requires a one-time upload of the Custom Panel GTF. Files can be obtained by contacting archer-tech@idtdna.com.

Assay targets

Gene	Accession	Exon	Variant Type	Description*
<i>ABL1</i>	NM_005157	N/A	Mutation	Y253-E255, V299, T315-F317, M351-F359
<i>ABL1</i>	NM_005157	1, 2, 3, 4, 5	Fusion	5'
<i>ABL1</i>	NM_005157	N/A	Expression imbalance	N/A
<i>ABL2</i>	NM_005158	2, 3, 4, 5, 6, 7, 8	Fusion	5'

Gene	Accession	Exon	Variant Type	Description*
<i>AICDA</i>	NM_020661	N/A	Expression	N/A
<i>BCL11B</i>	NM_138576	3, 4	Fusion	5'
<i>BCL11B</i>	NM_138576	2, 3	Fusion	3'
<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>BCL6</i>	NM_001706	2, 3	Fusion	5'
<i>BCL6</i>	NM_001706	N/A	Expression	N/A
<i>BCR</i>	NM_004327	1, 2, 3, 8, 12, 13, 14, 15, 16	Fusion	3'
<i>BLNK</i>	NM_013314	N/A	Expression	N/A
<i>BRAF</i>	NM_004333	N/A	Mutation	V600
<i>CD274</i>	NM_014143	N/A	Expression	N/A
<i>CHD1</i>	NM_001270	1,2	Fusion	5'
<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>CRLF2</i>	NM_022148	N/A	Mutation	F232
<i>CRLF2</i>	NM_022148	1	Fusion	5'
<i>CRLF2</i>	NM_022148	N/A	Expression	N/A

Gene	Accession	Exon	Variant Type	Description*
<i>CSF1R</i>	NM_005211	9, 10, 11, 12, 13, 14	Fusion	5'
<i>CTLA4</i>	NM_005214	N/A	Expression	N/A
<i>DNM2</i>	NM_004945	N/A	Mutation	V649, L789
<i>DNTT</i>	NM_004088	N/A	Expression	N/A
<i>EBF1</i>	NM_024007	10, 11, 12, 13, 14, 15	Fusion	3'
<i>EPOR</i>	NM_000121	7, 8	Fusion	3'
<i>ETV6</i>	NM_001987	N/A	Mutation	Y104-R105
<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>EZH2</i>	NM_004456	N/A	Mutation	Y646, R690
<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>FLT3</i>	NM_004119	N/A	Mutation	F590-N609, D835-S838
<i>FLT3</i>	NM_004119	N/A	Expression	N/A
<i>HOXA10</i>	NR_037939	N/A	Expression	N/A
<i>HOXA10</i>	NM_018951	N/A	Expression	N/A
<i>HOXA9</i>	NM_152739	N/A	Expression	N/A
<i>IDH1</i>	NM_005896	N/A	Mutation	R132

Gene	Accession	Exon	Variant Type	Description*
<i>IDH2</i>	NM_002168	N/A	Mutation	R140, R172
<i>IKZF1</i>	NM_006060	1, 2, 3	Exon skipping	3'
<i>IKZF1</i>	NM_006060	7, 8	Exon skipping	5'
<i>IKZF2</i>	NM_016260	3, 4	Fusion	3'
<i>IKZF3</i>	NM_012481	2, 3, 4, 5, 6, 7	Fusion	3'
<i>IKZF3</i>	NM_012481	N/A	Mutation	L162
<i>IL7R</i>	NM_002185	N/A	Mutation	S185, P240-S246
<i>IRF4</i>	NM_002460	N/A	Expression	N/A
<i>IRF8</i>	NM_002163	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
<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>KDM6A</i>	NM_021140	N/A	Mutation	V1113
<i>KLF2</i>	NM_016270	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'

Gene	Accession	Exon	Variant Type	Description*
<i>KRAS</i>	NM_004985	N/A	Mutation	G12-G13, Q61, A146
<i>LMO1</i>	NM_002315	N/A	Expression	N/A
<i>LYL1</i>	NM_005583	N/A	Expression	N/A
<i>MLLT4</i>	NM_001040000	2	Fusion	5'
<i>MPL</i>	NM_005373	N/A	Mutation	S505, W515
<i>MYC</i>	NM_002467	N/A	Expression	N/A
<i>MYC</i>	NM_002467	1, 2	Fusion	5'
<i>NF1</i>	NM_000267	14	Fusion	3'
<i>NF1</i>	NM_000267	36	Fusion	5'
<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>NRAS</i>	NM_002524	N/A	Mutation	G12-G13, G60-Q61
<i>NT5C2</i>	NM_012229	N/A	Mutation	R238, R367
<i>NTRK3</i>	NM_002530	N/A	Expression imbalance	N/A
<i>NTRK3</i>	NM_002530	13, 14, 15	Fusion	5'
<i>NTRK3</i>	NM_001007156	15	Fusion	5'
<i>NUP214</i>	NM_005085	17, 18, 19	Fusion	5'

Gene	Accession	Exon	Variant Type	Description*
<i>NUP98</i>	NM_016320	8, 9, 10, 11, 12, 13, 14, 15, 16, 17	Fusion	3'
<i>NUP98</i>	NM_016320	12, 13	Fusion	5'
<i>P2RY8</i>	NM_178129	1	Fusion	3'
<i>PAG1</i>	NM_018440	2	Fusion	5'
<i>PAX5</i>	NM_016734	N/A	Mutation	P80
<i>PAX5</i>	NM_016734	1, 4, 5, 6, 7, 8	Fusion	3'
<i>PAX5</i>	NM_016734	6, 7, 8	Fusion	5'
<i>PBX1</i>	NM_002585	1, 2, 3, 4, 5, 6, 7, 8, 9	Fusion	5'
<i>PDCD1</i>	NM_005018	N/A	Expression	N/A
<i>PDCD1LG2</i>	NM_025239	N/A	Expression	N/A
<i>PDCD1LG2</i>	NM_025239	1, 2, 3	Fusion	5'
<i>PDCD1LG2</i>	NM_025239	5, 6	Fusion	3'
<i>PDGFRA</i>	NM_006206	N/A	Mutation	T674
<i>PDGFRA</i>	NM_006206	9, 10, 11, 12, 13, 14	Fusion	5'
<i>PDGFRB</i>	NM_002609	8, 9, 10, 11, 12, 13, 14	Fusion	5'
<i>PICALM</i>	NM_007166	16, 17, 18, 19	Fusion	3'
<i>PTK2B</i>	NM_173176	2, 3, 4, 5, 6, 7, 8	Fusion	5'
<i>PTPN11</i>	NM_002834	N/A	Mutation	G60-D61, E69-T73, E76, S502-G503
<i>RAG1</i>	NM_000448	N/A	Expression	N/A

Gene	Accession	Exon	Variant Type	Description*
<i>RAG2</i>	NM_000536	N/A	Expression	N/A
<i>RUNX1</i>	NM_001754	2, 3, 4, 5, 6, 7, 8	Fusion	3'
<i>RUNX1</i>	NM_001754	5, 6, 7, 8, 9	Fusion	5'
<i>RUNX1</i>	NM_001754	N/A	Expression imbalance	N/A
<i>SEMA6A</i>	NM_020796	1, 2	Fusion	3'
<i>SETD2</i>	NM_014159	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	Fusion	3'
<i>SH2B3</i>	NM_005475	N/A	Mutation	E208, D231-D234
<i>SOX11</i>	NM_003108	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>STIL</i>	NM_003035	1, 2	Fusion	3'
<i>TAL1</i>	NM_003189	N/A	Expression	N/A
<i>TAL1</i>	NM_003189	2, 4	Fusion	5'
<i>TAL1</i>	NM_001290404	2, 3	Fusion	5'
<i>TCF3</i>	NM_003200	11, 12, 13, 14, 15, 16, 17, 18	Fusion	3'
<i>TLX1</i>	NM_005521	N/A	Expression	N/A
<i>TLX3</i>	NM_021025	N/A	Expression	N/A
<i>TYK2</i>	NM_003331	16, 18	Fusion	5'
<i>TYK2</i>	NM_003331	N/A	Mutation	W327

Gene	Accession	Exon	Variant Type	Description*
<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>ZCCHC7</i>	NM_032226	1, 2	Fusion	3'
<i>ZCCHC7</i>	NM_032226	2, 3, 4	Fusion	5'

*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 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-447/REV01	October 2023	Initial release.
RA-DOC-447/REV02	November 2023	Updated First and Second PCR cycling conditions to include separate anneal and extended steps. Updated branding.