FUSION*Plex*-HT Heme v2

Description

The FUSION*Plex*-HT Heme v2 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 Heme v2 contains **607** GSPs targeting **87** genes commonly mutated in hematological malignancies.

Description	Part number	Storage
FUSION*Plex*-HT Heme v2 GSP1, 24 reactions or FUSION*Plex*-HT Heme v2 GSP1, 96 reactions	SA0073241 or SA0073961	–20°C ± 10°C
FUSION*Plex*-HT Heme v2 GSP2, 24 reactions or FUSION*Plex*-HT Heme v2 GSP2, 96 reactions	SA0073242 or SA0073962	
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	20†
	3	60	10 sec	
	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 Heme v2 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 Heme v2 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 Heme v2 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*
ABL1	NM_005157	N/A	Mutation	Y253-E255, V299, T315-F317, M351-F359
ABL1	NM_005157	1, 2, 3, 4, 5	Fusion	5'
ABL1	NM_005157	N/A	Expression Imbalance	N/A
ABL2	NM_005158	2, 3, 4, 5, 6, 7, 8	Fusion	5'
ALK	NM_004304	N/A	Mutation	T1151-C1156, F1174, L1196-S1206, G1269

Gene	Accession	Exon	Variant Type	Description*
ALK	NM_004304	2, 4, 6, 10,1 6, 17, 18, 19, 20, 21, 22, 23	Fusion	5'
BCL11B	NM_138576	3, 4	Fusion	5'
BCL11B	NM_138576	2, 3	Fusion	3'
BCL2	NM_000633	N/A	Mutation	F104
BCL2	NM_000633	N/A	Expression	N/A
BCL2	NM_000633	3	Fusion	3'
BCL2	NM_000633	2	Fusion	5'
BCL3	NM_005178	N/A	Expression	N/A
BCL6	NM_001706	2,3	Fusion	5'
BCL6	NM_001706	N/A	Expression	N/A
BCR	NM_004327	1, 2, 3, 8, 12, 13, 14, 15, 16	Fusion	3'
BIRC3	NM_001165	N/A	Mutation	Q547
BIRC3	NM_001165	4, 5, 6, 7	Fusion	3'
CBFB	NM_022845	4, 5	Fusion	3'
CCND1	NM_053056	N/A	Expression	N/A
CCND1	NM_053056	5	Fusion	3'
CCND1	NM_053056	N/A	Mutation	E36, V42-C47
CCND2	NM_001759	N/A	Expression	N/A
CCND3	NM_001760	2	Fusion	5'

Gene	Accession	Exon	Variant Type	Description*
CCND3	NM_001760	N/A	Expression	N/A
CD274	NM_014143	N/A	Expression	N/A
CDK6	NM_001259	1, 2, 3, 4	Fusion	3'
CDKN2A	NM_000077	N/A	Expression	N/A
CEBPA	NM_004364	N/A	Mutation	P23-H24, Q83, K304-L317
CEBPA	NM_004364	N/A	Expression	N/A
CEBPD	NM_005195	N/A	Expression	N/A
CEBPE	NM_001805	N/A	Expression	N/A
CEBPG	NM_001806	N/A	Expression	N/A
CHD1	NM_001270	1, 2	Fusion	5'
CHIC2	NM_012110	1, 2, 3	Fusion	3'
CIITA	NM_000246	1, 2	Fusion	3'
CREBBP	NM_004380	N/A	Mutation	P1053, C1240, R1446, S1680-L1681
CREBBP	NM_004380	2, 3, 4, 5, 6	Fusion	5'
CRLF2	NM_022148	N/A	Mutation	F232
CRLF2	NM_022148	1	Fusion	5'
CRLF2	NM_022148	N/A	Expression	N/A
CSF1R	NM_005211	9, 10, 11, 12, 13, 14	Fusion	5'
CTLA4	NM_005214	N/A	Expression	N/A

Gene	Accession	Exon	Variant Type	Description*
DEK	NM_003472	2, 3	Fusion	3'
DUSP22	NM_020185	1, 2	Fusion	3'
EBF1	NM_024007	10, 11, 12, 13, 14, 15	Fusion	3'
EIF4A1	NM_001416	2, 3	Fusion	5'
EPOR	NM_000121	7, 8	Fusion	3'
ERG	NM_004449	7, 8, 9, 10, 11	Fusion	5'
ETV6	NM_001987	N/A	Mutation	Y104-R105
ETV6	NM_001987	1, 2, 3, 4, 5, 6	Fusion	3'
ETV6	NM_001987	2, 3, 4, 5, 6	Fusion	5'
FGFR1	NM_023110	12, 17	Fusion	3'
FGFR1	NM_023110	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 17	Fusion	5'
FOXP1	NM_032682	N/A	Expression	N/A
GLIS2	NM_032575	2, 3	Fusion	5'
ID4	NM_001546	N/A	Expression	N/A
IKZF1	NM_006060	1, 2, 3	Exon Skipping	3'
IKZF1	NM_006060	7, 8	Exon Skipping	5'
IKZF2	NM_016260	3, 4	Fusion	3'
IKZF3	NM_012481	2, 3, 4, 5, 6, 7	Fusion	3'
IKZF3	NM_012481	N/A	Mutation	L162

Gene	Accession	Exon	Variant Type	Description*
IRF4	NM_002460	N/A	Expression	N/A
IRF8	NM_002163	N/A	Expression	N/A
JAK2	NM_004972	N/A	Mutation	F537-F547, V617-C618, L681-R683, L855, V863, A880, V911, M929-R938, I960, R980-E985, D994
JAK2	NM_004972	6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20	Fusion	5'
JAK2	NM_004972	9, 10, 11, 12	Fusion	3'
KAT6A	NM_006766	13, 14, 15, 16	Fusion	3'
KLF2	NM_016270	2, 3	Fusion	5'
KMT2A	NM_005933	2, 3	Fusion	5'
KMT2A	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'
MALT1	NM_006785	9	Fusion	3'
MECOM	NM_004991	N/A	Expression	N/A
MECOM	NM_004991	1, 2, 3, 4	Fusion	5'
MKL1	NM_020831	4, 5, 6	Fusion	5'
MLF1	NM_022443	2, 3, 4	Fusion	5'
MLLT10	NM_004641	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18	Fusion	5'
MLLT10	NM_004641	7, 8, 9, 10	Fusion	3'
MLLT4	NM_001040000	2	Fusion	5'

Gene	Accession	Exon	Variant Type	Description*
MUC1	NM_002456	N/A	Expression	N/A
MYC	NM_002467	N/A	Expression	N/A
MYC	NM_002467	1, 2	Fusion	5'
MYH11	NM_002474	N/A	Expression Imbalance	N/A
MYH11	NM_002474	7, 8, 9, 10, 11, 14, 15, 16	Fusion	5'
NF1	NM_000267	14	Fusion	3'
NF1	NM_000267	36	Fusion	5'
NFKB2	NM_002502	14, 15, 16, 17, 18, 19, 20, 21	Fusion	3'
NOTCH1	NM_017617	N/A	Mutation	L1574, V1578, L1585, F1592-L1593, R1598-L1600, L1678-I1680, P2514-E2515, P2525
NOTCH1	NM_017617	24, 25, 26, 27, 28, 29	Fusion	5'
NOTCH1	NM_017617	24	Fusion	3'
NOTCH1	NM_017617	34	Exon Skipping	N/A
NTRK3	NM_002530	N/A	Expression Imbalance	N/A
NTRK3	NM_002530	13, 14, 15	Fusion	5'
NTRK3	NM_001007156	15	Fusion	5'
NUP214	NM_005085	17, 18, 19	Fusion	5'
NUP98	NM_016320	8, 9, 10, 11, 12, 13, 14, 15, 16, 17	Fusion	3'
NUP98	NM_016320	12, 13	Fusion	5'
P2RY8	NM_178129	1	Fusion	3'

Gene	Accession	Exon	Variant Type	Description*
PAG1	NM_018440	2	Fusion	5'
PAX5	NM_016734	N/A	Mutation	P80
PAX5	NM_016734	1, 4, 5, 6, 7, 8	Fusion	3'
PAX5	NM_016734	6, 7, 8	Fusion	5'
PDCD1	NM_005018	N/A	Expression	N/A
PDCD1LG2	NM_025239	N/A	Expression	N/A
PDCD1LG2	NM_025239	1, 2, 3	Fusion	5'
PDCD1LG2	NM_025239	5, 6	Fusion	3'
PDGFRA	NM_006206	N/A	Mutation	T674
PDGFRA	NM_006206	9, 10, 11, 12, 13, 14	Fusion	5'
PDGFRB	NM_002609	8, 9, 10, 11, 12, 13, 14	Fusion	5'
PICALM	NM_007166	16, 17, 18, 19	Fusion	3'
PML	NM_002675	N/A	Mutation	C212-S220
PML	NM_002675	2	Fusion	5'
PML	NM_002675	2, 3, 4, 5, 6, 7	Fusion	3'
PRDM16	NM_022114	1, 2, 3, 4	Fusion	5'
PTK2B	NM_173176	2, 3, 4, 5, 6, 7, 8	Fusion	5'
RARA	NM_000964	N/A	Mutation	E197, R272, T283-M284, L290-M297, R394, Q411
RARA	NM_000964	N/A	Expression Imbalance	N/A

Gene	Accession	Exon	Variant Type	Description*
RARA	NM_000964	2, 3, 4, 5	Fusion	5'
RBM15	NM_022768	1	Fusion	3'
ROS1	NM_002944	N/A	Expression Imbalance	N/A
ROS1	NM_002944	31, 32, 33, 34, 35, 36	Fusion	5'
RUNX1	NM_001754	5, 6, 7, 8, 9	Fusion	5'
RUNX1	NM_001754	2, 3, 4, 5, 6, 7, 8	Fusion	3'
RUNX1	NM_001754	N/A	Expression Imbalance	N/A
RUNX1T1	NM_001198679	N/A	Expression Imbalance	N/A
RUNX1T1	NM_001198679	2,3	Fusion	5'
SEMA6A	NM_020796	1,2	Fusion	3'
SETD2	NM_014159	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	Fusion	3'
STIL	NM_003035	1, 2	Fusion	3'
TAL1	NM_003189	N/A	Expression	N/A
TAL1	NM_003189	2, 4	Fusion	5'
TAL1	NM_001290404	2, 3	Fusion	5'
TCF3	NM_003200	11, 12, 13, 14, 15, 16, 17, 18	Fusion	3'
TFG	NM_006070	2, 3, 4	Fusion	3'
TP63	NM_003722	3, 4, 5	Fusion	5'
TYK2	NM_003331	16, 18	Fusion	5'

Gene	Accession	Exon	Variant Type	Description*
TYK2	NM_003331	N/A	Mutation	W327
ZCCHC7	NM_032226	1, 2	Fusion	3'
ZCCHC7	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-449/REV01	October 2023	Initial release.
RA-DOC-449/REV02	November 2023	Updated First and Second PCR cycling conditions to include separate anneal and extended steps. Updated branding.