Product Insert

FUSION*Plex*™-HT Myeloid panel

FUSION*Plex*-HT Myeloid

Description

The FUSION*Plex*-HT Myeloid 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 Myeloid contains **507** GSPs targeting **84** genes commonly mutated in myeloid malignancies.

Description	Part number	Storage
FUSION*Plex*-HT Myeloid GSP1, 24 reactions or FUSION*Plex*-HT Myeloid GSP1, 96 reactions	SA0075241 or SA0075961	–20°C ± 10°C
FUSION*Plex*-HT Myeloid GSP2, 24 reactions or FUSION*Plex*-HT Myeloid GSP2, 96 reactions	SA0075242 or SA0075962	
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 Myeloid 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 Myeloid 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 Myeloid 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
AKT3	NM_005465	N/A	Mutation	K172
ASXL1	NM_015338	N/A	Mutation	Y591, H630-E635, I641-G646, R693, E1102

Gene	Accession	Exon	Variant Type	Description*
BCR	NM_004327	1, 2, 3, 8, 12, 13, 14, 15, 16	Fusion	3'
BRAF	NM_004333	N/A	Mutation	V600
CALR	NM_004343	N/A	Mutation	E364-L367, K385
CBFB	NM_022845	4, 5	Fusion	3'
CBL	NM_005188	N/A	Mutation	Q367-Y371, L380-C384, C404-W408, R420
CD274	NM_014143	N/A	Expression	N/A
CEBPA	NM_004364	N/A	Mutation	P23-H24, Q83, K304-L317
CEBPA	NM_004364	N/A	Expression	N/A
CHD1	NM_001270	1, 2	Fusion	5'
CHIC2	NM_012110	1, 2, 3	Fusion	3'
CREBBP	NM_004380	N/A	Mutation	P1053, C1240, R1446, S1680-L1681
CREBBP	NM_004380	2, 3, 4, 5, 6	Fusion	5'
CSF1R	NM_005211	9, 10, 11, 12, 13, 14	Fusion	5'
CSF3R	NM_156039	N/A	Mutation	T618, Q766-Q768, Q776
CTLA4	NM_005214	N/A	Expression	N/A
DCK	NM_000788	N/A	Mutation	A32, E70
DNM2	NM_004945	N/A	Mutation	V649, L789
DNMT3A	NM_175629	N/A	Mutation	R688-K693, R882
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'
EZH2	NM_004456	N/A	Mutation	Y646, R690
FBXW7	NM_033632	N/A	Mutation	R224-T226, R338-I347, T385, R465, R479, R505

Gene	Accession	Exon	Variant Type	Description*
FGFR1	NM_023110	12, 17	Fusion	3'
FGFR1	NM_023110	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 17	Fusion	5'
FGFR2	NM_000141	N/A	Mutation	R6
FGFR3	NM_000142	N/A	Mutation	Y373, K650
FLT3	NM_004119	N/A	Mutation	F590-N609, D835-S838
FLT3	NM_004119	N/A	Expression	N/A
GATA1	NM_002049	N/A	Mutation	M1, S30, Y62-D65
GATA2	NM_032638	N/A	Mutation	A318-L321, R362
GLIS2	NM_032575	2, 3	Fusion	5'
GNAS	NM_000516	N/A	Mutation	D323
ID4	NM_001546	N/A	Expression	N/A
IDH1	NM_005896	N/A	Mutation	R132
IDH2	NM_002168	N/A	Mutation	R140, R172
IKZF1	NM_006060	1, 2, 3	Exon Skipping	3'
IKZF1	NM_006060	7, 8	Exon Skipping	5'
IKZF3	NM_012481	2, 3, 4, 5, 6, 7	Fusion	3'
IKZF3	NM_012481	N/A	Mutation	L162
IRF4	NM_002460	N/A	Expression	N/A
IRF8	NM_002163	N/A	Expression	N/A
JAK1	NM_002227	N/A	Mutation	V658, S703, R724
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'

Gene	Accession	Exon	Variant Type	Description*
JAK3	NM_000215	N/A	Mutation	M511, A572-A573, R657, S789
KAT6A	NM_006766	13, 14, 15, 16	Fusion	3'
KDM6A	NM_021140	N/A	Mutation	V1113
KIT	NM_000222	N/A	Mutation	T417-D419, T670, R796, D816, N822-V825
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'
KRAS	NM_004985	N/A	Mutation	G12-G13, Q61, A146
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'
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'
MPL	NM_005373	N/A	Mutation	S505, W515
MUC1	NM_002456	N/A	Expression	N/A
MYC	NM_002467	N/A	Expression	N/A
MYC	NM_002467	1, 2	Fusion	5'
MYD88	NM_002468	N/A	Mutation	V217-S219, M232, S243, L265
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'

Gene	Accession	Exon	Variant Type	Description*
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
NPM1	NM_002520	N/A	Mutation	W288-W290
NRAS	NM_002524	N/A	Mutation	G12-G13, G60-Q61
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'
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'
PHF6	NM_032458	N/A	Mutation	R116, R274-G275, I314-R319
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'
PTPN11	NM_002834	N/A	Mutation	G60-D61, E69-T73, E76, S502-G503
RARA	NM_000964	N/A	Mutation	E197, R272, T283-M284, L290-M297, R394, Q411

Gene	Accession	Exon	Variant Type	Description*
RARA	NM_000964	N/A	Expression Imbalance	N/A
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'
SETBP1	NM_015559	N/A	Mutation	D868-I871
SETD2	NM_014159	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	Fusion	3'
SF3B1	NM_012433	N/A	Mutation	E622-N626, H662-K666, K700, G742
SLC29A1	NM_001078175	N/A	Mutation	Y11, P419
SRSF2	NM_003016	N/A	Mutation	P95
TCF3	NM_003200	11, 12, 13, 14, 15, 16, 17, 18	Fusion	3'
TFG	NM_006070	2, 3, 4	Fusion	3'
U2AF1	NM_006758	N/A	Mutation	S34, R156-Q157
WT1	NM_000378	N/A	Mutation	Q301-T303, G352-Q354, V359-T365, Q445-F448
WT1	NM_000378	N/A	Expression	N/A
XPO1	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 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-452/REV01	October 2023	Initial release.
RA-DOC-452/REV02	November 2023	Updated First and Second PCR cycling conditions to include separate anneal and extended steps. Updated branding.