

Product Insert FUSION Plex™ Lymphoma Panel

FUSION Plex Lymphoma

Description

The FUSION*Plex* Lymphoma panel is a balanced pool of gene-specific primer (GSP) oligonucleotides that is optimized for use with FUSION*Plex* reagents and molecular barcode (MBC) adapters to produce targeted NGS libraries. This product insert should be used in conjunction with FUSION*Plex* protocol for Illumina® (RA-DOC-047) or FUSION*Plex* protocol for Ion Torrent™ (RA-DOC-048).

FUSION*Plex* Lymphoma contains **716** GSPs targeting **125** genes commonly mutated in lymphoid malignancies.

| Description | Part number | Storage |
|--|-------------|--------------|
| FUSIONPlex Lymphoma GSP1, 8 reactions | SA0077-8-1 | |
| FUSION Plex Lymphoma GSP2, 8 reactions | SA0077-8-2 | –20°C ± 10°C |
| 10X VCP Primer Mix | SA0126 | |

Recommended PCR cycling

| | Step | Temperature (°C) | Time | Cycles |
|-----------|------|---------------------|------------------------|--------|
| | 1 | 95 | 3 min | 1 |
| | 2 | 95 | 30 sec | |
| First PCR | 3 | 60 | 10 sec | 15 |
| reaction | 4 | 65 | 5 min (100% ramp rate) | |
| | 5 | 72 | 3 min | 1 |
| | 6 | 4 | Hold | 1 |

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Recommended PCR cycling (cont.)

| | Step | Temperature (°C) | Time | Cycles |
|---------------------|------|---------------------|------------------------|-----------------|
| | 1 | 95 | 3 min | 1 |
| | 2 | 95 | 30 sec | |
| Second PCR reaction | 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* 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 (v7.0, or greater). The FUSION*Plex* Lymphoma panel requires selection of the *Fusion* and (optional) *SNV/Indel* pipeline(s), found under the *RNA* Input Type (see the Archer Analysis User Guide for more details on setting up your analysis).

Processing of FUSION Plex Lymphoma libraries requires a one-time upload of the Panel GTF. Files can be obtained by contacting 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 |
| ALK | NM_004304 | 2, 4, 6, 10, 16, 17, 18, 19, 20, 21, 22, 23 | Fusion | 5' |

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| Accession | Exon | Variant Type | Description* |
|-----------|--|---|---|
| NM_024701 | N/A | Expression | N/A |
| NM_018664 | N/A | Expression | N/A |
| NM_138761 | N/A | Mutation | G179 |
| NM_000633 | N/A | Mutation | F104 |
| NM_000633 | N/A | Expression | N/A |
| NM_000633 | 3 | Fusion | 3' |
| NM_000633 | 2 | Fusion | 5' |
| NM_004049 | N/A | Expression | N/A |
| NM_005178 | N/A | Expression | N/A |
| NM_001706 | N/A | Expression | N/A |
| NM_001706 | 2, 3 | Fusion | 5' |
| NM_004327 | 1, 2, 3, 8, 12, 13, 14, 15, 16 | Fusion | 3' |
| NM_001165 | N/A | Mutation | Q547 |
| NM_001165 | 4, 5, 6, 7 | Fusion | 3' |
| NM_013314 | N/A | Expression | N/A |
| NM_033503 | N/A | Expression | N/A |
| NM_001719 | N/A | Expression | N/A |
| NM_004333 | N/A | Mutation | V600 |
| NM_000061 | N/A | Mutation | C481 |
| | NM_024701 NM_018664 NM_138761 NM_000633 NM_000633 NM_000633 NM_004049 NM_005178 NM_001706 NM_001706 NM_001706 NM_001165 NM_013314 NM_033503 NM_001719 NM_004333 | NM_024701 N/A NM_018664 N/A NM_138761 N/A NM_000633 N/A NM_000633 N/A NM_000633 2 NM_000633 2 NM_0005178 N/A NM_001706 N/A NM_001706 2, 3 NM_001706 2, 3 NM_00165 N/A NM_0165 N/A NM_013314 N/A NM_033503 N/A NM_001719 N/A NM_004333 N/A | NM_024701 N/A Expression NM_018664 N/A Expression NM_138761 N/A Mutation NM_000633 N/A Mutation NM_000633 N/A Expression NM_000633 2 Fusion NM_000633 2 Fusion NM_000633 2 Fusion NM_004049 N/A Expression NM_005178 N/A Expression NM_001706 N/A Expression NM_001706 2, 3 Fusion NM_001706 2, 3 Fusion NM_001327 1, 2, 3, 8, 12, 13, 14, Fusion NM_001165 N/A Mutation NM_0013314 N/A Expression NM_033503 N/A Expression NM_001719 N/A Expression NM_004333 N/A Mutation |

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| Gene | Accession | Exon | Variant Type | Description* |
|--------|-----------|------------|--------------|--------------|
| CARD11 | NM_032415 | N/A | Mutation | D230 |
| CBFB | NM_022845 | 4,5 | Fusion | 3' |
| CCDC50 | NM_174908 | N/A | Expression | N/A |
| 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 | N/A | Expression | N/A |
| CCND3 | NM_001760 | 2 | Fusion | 5' |
| CD274 | NM_014143 | N/A | Expression | N/A |
| CD44 | NM_000610 | N/A | Expression | N/A |
| CD79B | NM_000626 | N/A | Mutation | Y196 |
| CDC25A | NM_001789 | N/A | Expression | N/A |
| CDK6 | NM_001259 | 1, 2, 3, 4 | Fusion | 3' |
| CDKN2A | NM_000077 | N/A | Expression | N/A |
| CDKN2B | NM_004936 | 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 |

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

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|---------|-----------|--|--------------|--|
| ETV6 | NM_001987 | 2, 3, 4, 5, 6 | Fusion | 5' |
| EXOC2 | NM_018303 | N/A | Expression | N/A |
| EZH2 | NM_004456 | N/A | Mutation | Y646, R690 |
| FAM216A | NM_013300 | N/A | Expression | N/A |
| FBXW7 | NM_033632 | N/A | Mutation | R224-T226, R338-I347, T385, R465, R479, R505 |
| 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 |
| FUT8 | NM_004480 | N/A | Expression | N/A |
| IDH1 | NM_005896 | N/A | Mutation | R132 |
| IDH2 | NM_002168 | N/A | Mutation | R140, R172 |
| IL16 | NM_004513 | N/A | Expression | N/A |
| IRF4 | NM_002460 | N/A | Expression | N/A |
| IRF8 | NM_002163 | N/A | Expression | N/A |
| ITPKB | NM_002221 | 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' |

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|----------|-----------|--|--------------|--------------------------------|
| JAK2 | NM_004972 | 9, 10, 11, 12 | Fusion | 3' |
| JAK3 | NM_000215 | N/A | Mutation | M511, A572-A573, R657, S789 |
| KIAA0101 | NM_014736 | N/A | Expression | N/A |
| KMT2A | NM_005933 | 2, 3 | Fusion | 5' |
| КМТ2А | 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 |
| LIMD1 | NM_014240 | N/A | Expression | N/A |
| LMO2 | NM_005574 | N/A | Expression | N/A |
| LRMP | NM_006152 | N/A | Expression | N/A |
| LZTS1 | NM_021020 | N/A | Expression | N/A |
| MAL | NM_002371 | N/A | Expression | N/A |
| MALT1 | NM_006785 | 9 | Fusion | 3' |
| MAML3 | NM_018717 | N/A | Expression | N/A |
| 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' |
| MME | NM_000902 | N/A | Expression | N/A |

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|----------|--------------|-----------------------------------|---------------|--|
| MUC1 | NM_002456 | N/A | Expression | N/A |
| MYBL1 | NM_001080416 | 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 |
| NEK6 | NM_014397 | N/A | Expression | N/A |
| NFKB1 | NM_003998 | N/A | Expression | N/A |
| NFKB2 | NM_002502 | 14, 15, 16, 17, 18, 19, 20, 21 | Fusion | 3' |
| NME1 | NM_000269 | N/A | Expression | N/A |
| 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 |
| NOTCH2 | NM_024408 | N/A | Mutation | R2400 |
| NRAS | NM_002524 | N/A | Mutation | G12-G13, G60-Q61 |
| P2RY8 | NM_178129 | 1 | Fusion | 3' |
| PAICS | NM_006452 | N/A | Expression | N/A |
| PDCD1 | NM_005018 | N/A | Expression | N/A |
| PDCD1LG2 | NM_025239 | 1, 2, 3 | Fusion | 5' |
| | | | | |

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| Gene | Accession | Exon | Variant Type | Description* |
|----------|-----------|-----------------------|--------------|--------------|
| PDCD1LG2 | NM_025239 | 5, 6 | Fusion | 3' |
| PDCD1LG2 | NM_025239 | N/A | Expression | N/A |
| PDGFRA | NM_006206 | N/A | Mutation | T674 |
| PDGFRA | NM_006206 | 9, 10, 11, 12, 13, 14 | Fusion | 5' |
| PIM1 | NM_002648 | N/A | Expression | N/A |
| PIM2 | NM_006875 | N/A | Expression | N/A |
| PLCG1 | NM_002660 | N/A | Mutation | S345 |
| PLCG2 | NM_002661 | N/A | Mutation | R665, L845 |
| PPAT | NM_002703 | N/A | Expression | N/A |
| PRDM16 | NM_022114 | 1, 2, 3, 4 | Fusion | 5' |
| PRKAR2B | NM_002736 | N/A | Expression | N/A |
| PTPN1 | NM_002827 | N/A | Expression | N/A |
| PYCR1 | NM_006907 | N/A | Expression | N/A |
| RAB29 | NM_003929 | N/A | Expression | N/A |
| RAG1 | NM_000448 | N/A | Expression | N/A |
| RAG2 | NM_000536 | N/A | Expression | N/A |
| RANBP1 | NM_002882 | N/A | Expression | N/A |
| RHOA | NM_001664 | N/A | Mutation | G17 |
| S1PR2 | NM_004230 | N/A | Expression | N/A |

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| Cono | Accession | Evan | Variant Type | Description* |
|-----------|-----------|-----------------------------------|--------------|---|
| Gene | Accession | Exon | Variant Type | Description* |
| SERPINA9 | NM_175739 | N/A | Expression | N/A |
| SF3B1 | NM_012433 | N/A | Mutation | E622-N626, H662-K666, K700, G742 |
| SH3BP5 | NM_004844 | N/A | Expression | N/A |
| STAT3 | NM_003150 | N/A | Mutation | Y640-N647, D661 |
| STAT5B | NM_012448 | N/A | Mutation | N642 |
| STAT6 | NM_003153 | N/A | Mutation | D419 |
| STIL | NM_003035 | 1, 2 | Fusion | 3' |
| STRBP | NM_018387 | N/A | Expression | N/A |
| TCF3 | NM_003200 | 11, 12, 13, 14, 15, 16, 17, 18 | Fusion | 3' |
| TNFRSF13B | NM_012452 | N/A | Expression | N/A |
| TNFSF4 | NM_003326 | N/A | Expression | N/A |
| TP63 | NM_003722 | 3, 4, 5 | Fusion | 5' |
| 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.

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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-011/REV01 | June 2023 | Initial release. |
| RA-DOC-011/REV02 | November 2023 | Updated First and Second PCR cycling conditions to include separate anneal and extended steps. Updated branding. |

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