# FUSION*Plex*-HT Sarcoma v2

## Description

The FUSION*Plex*-HT Sarcoma 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 Sarcoma v2 contains **659** GSPs targeting **63** genes commonly mutated in solid tumors.

| Description | Part number | Storage |
|---|---|---|
| FUSION*Plex*-HT Sarcoma v2 GSP1, 24 reactions<br>or<br>FUSION*Plex*-HT Sarcoma v2 GSP1, 96 reactions | SA19191241<br>or<br>SA19191961 | –20°C ± 10°C |
| FUSION*Plex*-HT Sarcoma v2 GSP2, 24 reactions<br>or<br>FUSION*Plex*-HT Sarcoma v2 GSP2, 96 reactions | SA19191242<br>or<br>SA19191962 | |
| 10X VCP Primer Mix, 24 reactions<br>or<br>10X VCP Primer Mix, 96 reactions | SA0840<br>or<br>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** | 63 | 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 Sarcoma v2 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 FUSION*Plex*-HT Sarcoma 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 Sarcoma 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** |
|---|---|---|---|---|
| *ALK* | NM_004304 | 2, 4, 6, 8, 10, 12, 14, 16, 17, 18, 19, intron19, 20, mid-exon20, 21, 22, 23, 26 | Fusion, ALK ATI$^{\gamma}$, Internal deletion (ALKΔ2-17, ALKΔ2-3) | 5' |
| *ALK* | NM_004304 | 1, 2 | Internal deletion (ALKΔ2-17, ALKΔ2-3) | 3' |
| *ALK* | NM_004304 | 22, 23, 25 | Mutation | p.P1153-p.C1156,p.F1174,p.L1196-p.S1206,p.G1269 |

| Gene | Accession | Exon | Variant Type | Description** |
|---|---|---|---|---|
| *BCOR* | NM_017745 | 8 | Fusion | 5' |
| *BCOR* | NM_001123385 | mid-exon2, 3, 4, mid-exon4, 5, 6, 7, 8, 9, 11, 15 | Fusion, Internal Tandem Duplication | 5' |
| *BCOR* | NM_001123385 | 2, 4, mid-exon4, 6, 7, mid-exon7, 10, 12, 14, 15 | Fusion, Internal Tandem Duplication | 3' |
| *BRAF* | NM_004333 | 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 15, 16 | Fusion, Kinase Domain Duplication, BRAFΔ2-10, BRAFΔ4-10, BRAFΔ2-8, BRAFΔ3-8, BRAFΔ4-8 | 5' |
| *BRAF* | NM_004333 | 1, 2, 3, 7, 8, 10, 13, 14, 18 | Fusion, Kinase Domain Duplication, BRAFΔ2-10, BRAFΔ4-10, BRAFΔ2-8, BRAFΔ3-8, BRAFΔ4-8 | 3' |
| *BRAF* | NM_004333 | 15 | Mutation | p.V600 |
| *CAMTA1* | NM_015215 | 8, 9, mid-exon9, 10 | Fusion | 5' |
| *CAMTA1* | NM_015215 | 3 | Fusion | 3' |
| *CCNB3* | NM_033031 | 2*, 3, 4, 5, 6, mid-exon 6, 7 | Fusion | 5' |
| *CIC* | NM_015125 | 12 | Fusion | 5' |
| *CIC* | NM_015125 | 14, 15, 16, 17, 18, mid-exon19, 19, mid-exon20, 20* | Fusion | 3' |
| *CSF1* | NM_000757 | 2, 3, 4, 5, 6 | Fusion | 5' |
| *CSF1* | NM_000757 | 5, 6, 7, 8*, mid-exon9* | Fusion | 3' |
| *CSF1* | NM_172212 | 9* | Fusion | 3' |
| *CTNNB1* | NM_001904 | 3 | Mutation | p.D32-p.S37 |
| *EGFR* | NM_005228 | 7, 8, 9, 14, 15, 16, 17, 18, 19, 20 | Fusion, Exon 2-7 Skipping (EGFRvIII), Kinase Domain Duplication | 5' |

| Gene | Accession | Exon | Variant Type | Description** |
|---|---|---|---|---|
| *EGFR* | NM_005228 | 1, 24, 25, mid-exon25, 26 | Fusion, Exon 2-7 Skipping (EGFRvIII), Kinase Domain Duplication | 3' |
| *EGFR* | NM_005228 | 18, 19, 20, 21 | Mutation | p.E709-p.G719,p.E746-p.L760,p.V774-p.G796,p.L858-p.L861 |
| *EPC1* | NM_025209 | 9, 10, 11 | Fusion | 3' |
| *ERG* | NM_004449 | 2*, 3*, 4, 5, 6, 7, 8, 9, 10, 11 | Fusion | 5' |
| *ESR1* | NM_000125 | 5, 6, 7, 8 | Fusion | 5' |
| *ESR1* | NM_000125 | 1, 2, 3, 4, 5, 6, 7 | Fusion | 3' |
| *ETV1* | NM_004956 | 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 | Fusion | 5' |
| *ETV4* | NM_001986 | 2, 3, 4, 5, 6, 7, 8, 9, 10 | Fusion | 5' |
| *ETV5* | NM_004454 | 2*, 3, 7, 8, 9 | Fusion | 5' |
| *ETV6* | NM_001987 | 2, 3, 4, 5, 6, 7 | Fusion | 5' |
| *ETV6* | NM_001987 | 1, 2, 3, 4, 5, 6 | Fusion | 3' |
| *ETV6* | NM_001987 | 3 | Mutation | p.Y104-p.R105 |
| *EWSR1* | NM_005243 | 8 | Fusion | 5' |
| *EWSR1* | NM_005243 | 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 | Fusion | 3' |
| *FGFR1* | NM_015850 | 2*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 17 | Fusion, Kinase Domain Duplication | 5' |
| *FGFR1* | NM_015850 | 12, 17 | Fusion, Kinase Domain Duplication | 3' |
| *FGFR1* | NM_023110 | 4, 13, 14 | Mutation | p.T141,p.V561,p.K656 |
| *FGFR2* | NM_000141 | 2*, 3, 5, 6, 7, 8, 9, 10 | Fusion | 5' |

| Gene | Accession | Exon | Variant Type | Description** |
|---|---|---|---|---|
| *FGFR2* | NM_000141 | 16, 17, 18 | Fusion | 3' |
| *FGFR2* | NM_000141 | 7, 9, 12, 13, 14 | Mutation | p.S252-p.P253,p.G305,p.Y375-V395,p.I547-p.N549,p.V564,p.A648-p.K659 |
| *FGFR3* | NM_000142 | 3, 5, 8, 9, 10, 11, 12, 13, 14 | Fusion | 5' |
| *FGFR3* | NM_000142 | 16, 17, intron17, mid-exon18 | Fusion | 3' |
| *FGFR3* | NM_000142 | 7, 9, 13, 14, 16 | Mutation | p.R248-p.S249,p.G370-p.R399,p.V555,p.D641-p.K650,p.G697-p.K715 |
| *FOS* | NM_005252 | mid-exon4 | Fusion | 3' |
| *FOSB* | NM_006732 | 1*, mid-exon1*, 1, 2 | Fusion | 5' |
| *FOXO1* | NM_002015 | 1*, 2, 3* | Fusion | 5' |
| *FOXO1* | NM_002015 | 1*, 2*, 3* | Fusion | 3' |
| *FUS* | NM_004960 | 3, 4, 5, mid-exon6, 6, 7, 8, 9, 10, 11, 13, 14 | Fusion | 3' |
| *GLI1* | NM_005269 | 4, 5, 6, 7 | Fusion | 5' |
| *GLI1* | NM_005269 | 4, 5, mid-exon5, 6, 7 | Fusion | 3' |
| *HMGA2* | NM_003483 | 1, 2, 3, 4, mid-exon5*, 5* | Fusion | 3' |
| *JAZF1* | NM_175061 | 2, 3, 4 | Fusion | 3' |
| *MBTD1* | NM_017643 | 3* | Fusion | 5' |
| *MBTD1* | NM_017643 | 15, 16, 17 | Fusion | 3' |
| *MDM2* | NM_002392 | 5, 9 | Fusion, Expression | 5' |
| *MDM2* | NM_002392 | 2, 4, 6, 8, 10 | Fusion, Expression | 3' |

| Gene | Accession | Exon | Variant Type | Description** |
|---|---|---|---|---|
| *MEAF6* | NM_001270875 | 4, 5 | Fusion | 3' |
| *MET* | NM_000245 | 2, 4, 5, 6, 13, 14, 15, 16, 17, 21 | Fusion, Exon 14 Skipping (METΔex14) | 5' |
| *MET* | NM_000245 | 2, 13 | Fusion, Exon 14 Skipping (METΔex14) | 3' |
| *MGEA5* | NM_012215 | 4, 5, 6, 7, 8, 9, 12, 13, 14, 15 | Fusion, Expression | 5' |
| *MKL2* | NM_014048 | 11, 12, 13 | Fusion | 5' |
| *MYOD1* | NM_002478 | 1 | Mutation | p.L122, full CDS coverage for mutation detection |
| *NCOA1* | NM_147223 | 11, 12, 13, 14, 15 | Fusion | 5' |
| *NCOA2* | NM_006540 | 11, 12, 13, 14, intron14, 15, 16, 22 | Fusion | 5' |
| *NCOA2* | NM_006540 | 14 | Fusion | 3' |
| *NCOA3* | NM_006534 | 2*, 13, 14, 15, 16 | Fusion | 5' |
| *NCOA3* | NM_006534 | 20 | Fusion | 3' |
| *NR4A3* | NM_173200 | 2*, 3*, 4, 5, 7, 9 | Fusion, Expression | 5' |
| *NR4A3* | NM_173200 | 8 | Fusion, Expression | 3' |
| *NTRK1* | NM_001007792 | 1, 2 | Fusion | 5' |
| *NTRK1* | NM_002529 | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 | Fusion | 5' |
| *NTRK1* | NM_002529 | 13, 14, 15, 16, 17 | Mutation | Full Kinase Domain coverage for resistance mutations including p.G595 |
| *NTRK2* | NM_006180 | 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 | Fusion | 5' |
| *NTRK2* | NM_006180 | 11, 14 | Fusion | 3' |

| Gene | Accession | Exon | Variant Type | Description** |
|---|---|---|---|---|
| *NTRK2* | NM_006180 | 16, 17, 18, 19, 20, 21 | Mutation | Full Kinase Domain coverage for resistance mutations |
| *NTRK3* | NM_001007156 | 15 | Fusion | 5' |
| *NTRK3* | NM_002530 | 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 | Fusion | 5' |
| *NTRK3* | NM_002530 | 13, 14, 15, 17 | Fusion | 3' |
| *NTRK3* | NM_002530 | 15, 16, 17, 18, 19 | Mutation | Full Kinase Domain coverage for resistance mutation detection including p.F617,p.G623,p.G696 |
| *NUTM1* | NM_175741 | 2*, 3, mid-exon3, 4, 5, mid-exon6, 6 | Fusion | 5' |
| *PAX3* | NM_181459 | 2, 4, 8 | Fusion, Expression | 5' |
| *PAX3* | NM_181459 | 3, 5, 6, 7, 8 | Fusion, Expression | 3' |
| *PDGFB* | NM_002608 | 2, 3 | Fusion | 5' |
| *PDGFRA* | NM_006206 | 10, 11, 12, mid-exon12, 13, 14, 15 | Fusion,PDGFRAΔ8,9 | 5' |
| *PDGFRA* | NM_006206 | 7 | Fusion,PDGFRAΔ8,9 | 3' |
| *PDGFRA* | NM_006206 | 15, 18 | Mutation | p.T674,p.D842 |
| *PHF1* | NM_024165 | 1*, 2 | Fusion | 5' |
| *PHF1* | NM_024165 | 10, 11, 12 | Fusion | 3' |
| *PLAG1* | NM_002655 | 1, 2, 3, 4 | Fusion | 5' |
| *PRKCA* | NM_002737 | 4, 5, 6, 9, 15 | Fusion | 5' |
| *PRKCB* | NM_002738 | 1, 3, 7, 8, 9 | Fusion | 5' |
| *PRKCD* | NM_006254 | 9, 10, 11, 12, 15 | Fusion | 5' |

| Gene | Accession | Exon | Variant Type | Description** |
|---|---|---|---|---|
| *PRKCD* | NM_006254 | 18 | Fusion | 3' |
| *RAF1* | NM_002880 | 2*, 4, 5, 6, 7, 8, 9, 10, 11, 12 | Fusion | 5' |
| *RAF1* | NM_002880 | 4, 5, 6, 7, 8, 9 | Fusion | 3' |
| *RET* | NM_020630 | 2, 4, 6, 8, 9, 10, 11, mid-exon11, 12, 13, 14 | Fusion | 5' |
| *RET* | NM_020630 | 15, 16 | Mutation | p.A883,p.M918 |
| *ROS1* | NM_002944 | 2, 4, 7, 31, 32, 33, 34, 35, 36, 37 | Fusion | 5' |
| *ROS1* | NM_002944 | 38 | Mutation | p.G2032 |
| *SS18* | NM_001007559 | 2, 3, 4, 5, 6, 10, 11 | Fusion | 5' |
| *SS18* | NM_001007559 | 4, 5, 6, 8, 9, 10 | Fusion | 3' |
| *STAT6* | NM_001178078 | 1*, 2*, 3, 4, 5, 6, 7, 15, 16, 17, 18, 19, 20 | Fusion | 5' |
| *TAF15* | NM_139215 | 6, 7 | Fusion | 5' |
| *TAF15* | NM_139215 | 5, 6, 7, 9 | Fusion | 3' |
| *TCF12* | NM_207036 | 4, 5, 6 | Fusion | 3' |
| *TFE3* | NM_006521 | 2, 3, 4, 5, 6, 7, 8 | Fusion | 5' |
| *TFE3* | NM_006521 | 2, 3, 4, 5, 6 | Fusion | 3' |
| *TFG* | NM_006070 | 6 | Fusion | 5' |
| *TFG* | NM_006070 | 3, 4, 5, 6, 7, mid-exon8 | Fusion | 3' |
| *USP6* | NM_004505 | 1*, mid-exon1*, 2*, 3 | Fusion | 5' |
| *VGLL2* | NM_182645 | 1, 2, 3, intron3, 4 | Fusion | 3' |

| Gene | Accession | Exon | Variant Type | Description** |
|---|---|---|---|---|
| *YAP1* | NM_001130145 | 1, mid-exon1, 2, 3, 4, 8, 9 | Fusion | 5' |
| *YAP1* | NM_001130145 | 1, 2, 3, 4, 5, 6, 7 | Fusion | 3' |
| *YWHAE* | NM_006761 | 5 | Fusion | 3' |

*Indicates exons that are entirely untranslated region (UTR), or for which the UTR is targeted.

**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.

[γ] ALK-ATI currently requires review outside of Archer Analysis.

## Limitations of use

**For research use only. Not for use in diagnostic procedures**. Unless otherwise agreed to in writing, IDT does not intend these products to be used in clinical applications and does not warrant their fitness or suitability for any clinical diagnostic use. Purchaser is solely responsible for all decisions regarding the use of these products and any associated regulatory or legal obligations.

Safety data sheets pertaining to this product are available upon request.

# Revision History

| Document Number | Date | Description of change |
|---|---|---|
| *RA-DOC-455/REV01* | October 2023 | Initial release |
| *RA-DOC-455/REV02* | November 2023 | Updated First and Second PCR cycling conditions to include separate anneal and extended steps.<br>Updated branding. |