

VARIANT*Plex* Myeloid

Description

The VARIANT*Plex* Myeloid panel is a balanced pool of gene-specific primer (GSP) oligonucleotides that is optimized for use with VARIANT*Plex* reagents and molecular barcode (MBC) adapters to produce targeted NGS libraries. This product insert should be used in conjunction with VARIANT*Plex* HS/HGC protocol for Illumina® (RA-DOC-056).

VARIANT*Plex* Myeloid contains GSPs targeting **75** genes frequently mutated in myeloid malignancies.

| Description | Part number | Storage |
|---|-------------|--------------|
| VARIANT <i>Plex</i> Myeloid GSP1, 8 reactions | SA5031081 | -20°C ± 10°C |
| VARIANT <i>Plex</i> Myeloid GSP2, 8 reactions | SA5031082 | |
| PreSeq™ DNA QC Assay Standard, 32 µL | SA0597 | |
| PreSeq™ DNA QC Assay 10X Primer Mix, 120 µL | SA0598 | |

Required reagent volumes

| Protocol reference | Protocol Step | Reagent | Volume per reaction (µL) |
|--------------------|-------------------------|----------------------------------|--------------------------|
| A | Ligation step 2 elution | 5 mM NaOH | 32 |
| B | First PCR | VARIANT <i>Plex</i> Myeloid GSP1 | 8 |
| C | First PCR | 10 mM Tris-HCl pH 8.0 | 34 |
| D | First PCR | Purified First PCR eluate | 32 |
| E | Second PCR | VARIANT <i>Plex</i> Myeloid GSP2 | 8 |

Recommended PCR cycling

| | Step | Temperature (°C) | Time | Cycles |
|---------------------|------|------------------|------------------------|--------|
| First PCR reaction | 1 | 95 | 3 min | 1 |
| | 2 | 95 | 30 sec | |
| | 3 | 60 | 10 sec | 16 |
| | 4 | 62 | 5 min (100% ramp rate) | |
| | 5 | 72 | 3 min | 1 |
| | 6 | 4 | Hold | 1 |
| 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

VARIANT $Plex$ Myeloid libraries should be sequenced to a minimum of **4M reads**. Starting read depth recommendations for standard profiling may be adjusted to increase panel sensitivity.

Archer™ Analysis settings

Sequencing data should be processed using Archer Analysis (v7.0, or greater). The VARIANT $Plex$ Myeloid panel requires selection of the **SNV/Indel, Copy Number Variation, and Structural Variation** pipelines found under the **DNA** Input Type (see the Archer Analysis User Guide for more details on setting up your analysis). Selection of the DNA Target Coverage pipeline is optional.

Processing of VARIANT*Plex* Myeloid libraries requires a one-time upload of the Panel GTF. When performing DNA Target Coverage analysis, users must also select a Region of Interest BED file. Users may optionally add a Targeted Mutations VCF file for targeted SNV/Indel detection. Files can be obtained by contacting archer-tech@idtdna.com

Assay targets

| Gene | Accession | Exon |
|----------------|----------------|---|
| <i>ABL1</i> | NM_005157 | 4,5,6,7,8,9,10 |
| <i>ANKRD26</i> | NM_014915 | 1 (c.-113-c.-134) |
| <i>ASXL1</i> | NM_015338.5 | 1,2,3,4,5,6,7,8,9,10,11,12,13 |
| <i>ASXL1</i> | NM_001164603.1 | 5 |
| <i>ATRX</i> | NM_000489 | 8,9,10,11,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32 |
| <i>BCOR</i> | NM_017745 | 2,3,4,5,6,7,9,10,11,12,13,14,15 |
| <i>BCOR</i> | NM_001123385 | 8 |
| <i>BCORL1</i> | NM_021946 | 1,2,3,4,5,6,7,8,9,10,11,12 |
| <i>BRAF</i> | NM_004333 | 3,10,11,12,13,15 |
| <i>BTK</i> | NM_000061 | 15 |
| <i>CALR</i> | NM_004343 | 8,9 |
| <i>CBL</i> | NM_005188 | 2,3,4,5,7,8,9,16 |
| <i>CBLB</i> | NM_170662 | 3,9,10 |
| <i>CBLC</i> | NM_012116 | 9,10 |
| <i>CCND2</i> | NM_001759 | 5 |
| <i>CDKN2A</i> | NM_058197 | 1 |
| <i>CDKN2A</i> | NM_058195 | 1 |
| <i>CDKN2A</i> | NM_000077 | 2,3 |
| <i>CDKN2A</i> | NM_001195132 | 3 |
| <i>CEBPA</i> | NM_004364 | 1 |

| Gene | Accession | Exon |
|---------------|------------------|---|
| <i>CSF3R</i> | NM_156039 | 17 |
| <i>CSF3R</i> | NM_172313 | 10,18 |
| <i>CSF3R</i> | NM_000760 | 14,15,16 |
| <i>CUX1</i> | NM_001202543 | 15,16,17,18,19,20,21,22,23,24 |
| <i>CUX1</i> | NM_001913 | 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23 |
| <i>CUX1</i> | NM_181552 | 1 |
| <i>CXCR4</i> | NM_003467 | 1,2 |
| <i>DCK</i> | NM_000788 | 2,3 |
| <i>DDX41</i> | NM_016222 | 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17 |
| <i>DHX15</i> | NM_001358 | 3 |
| <i>DNMT3A</i> | NM_022552 | 2,3,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23 |
| <i>DNMT3A</i> | NM_153759 | 1,2 |
| <i>DNMT3A</i> | NM_175630 | 4 |
| <i>ETNK1</i> | NM_018638 | 3 |
| <i>ETV6</i> | NM_001987 | 1,2,3,4,5,6,7,8 |
| <i>EZH2</i> | NM_004456 | 2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20 |
| <i>FBXW7</i> | NM_018315 | 1,2,3,4,5,6,7,8,9,10,11 |
| <i>FLT3</i> | NM_004119 | 8,9,10,11,12,13,14,15,16,17,19,20,21 |
| <i>GATA1</i> | NM_002049 | 2 |
| <i>GATA2</i> | NM_032638 | 2,3,4,5,6 |
| <i>GNAS</i> | NM_000516 | 8,9,10,11 |
| <i>HRAS</i> | NM_005343 | 2,3,4 |
| <i>IDH1</i> | NM_005896 | 3,4 |
| <i>IDH2</i> | NM_002168 | 4,6 |
| <i>IKZF1</i> | NM_001220769 | 5 |

| Gene | Accession | Exon |
|---------------|--------------|--|
| <i>IKZF1</i> | NM_001220767 | 2,3,4,5,7 |
| <i>IKZF1</i> | NM_001220771 | 4 |
| <i>IKZF1</i> | NM_001291845 | 4 |
| <i>IKZF1</i> | NM_001291847 | 5 |
| <i>JAK2</i> | NM_004972 | 12,13,14,15,16,19,20,21,22,23,24,25 |
| <i>JAK3</i> | NM_000215 | 3,11,13,15,18,19 |
| <i>KDM6A</i> | NM_021140 | 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29 |
| <i>KDM6A</i> | NM_001291415 | 14 |
| <i>KIT</i> | NM_000222 | 1,2,5,8,9,10,11,12,13,14,15,17,18 |
| <i>KMT2A</i> | NM_005933 | 1,2,3,4,5,6,7,8,9,10,11,12,13,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36 |
| <i>KMT2A</i> | NM_001197104 | 14 |
| <i>KRAS</i> | NM_004985 | 2,3,4 |
| <i>LUC7L2</i> | NM_016019 | 1,2,3,4,5,6,7,8,9,10 |
| <i>LUC7L2</i> | NM_001244585 | 2 |
| <i>MAP2K1</i> | NM_002755 | 2,3 |
| <i>MPL</i> | NM_005373 | 10,12 |
| <i>MYC</i> | NM_002467 | 1,2,3 |
| <i>MYD88</i> | NM_002468 | 4,5 |
| <i>MYD88</i> | NM_001172567 | 3 |
| <i>NF1</i> | NM_000267 | 1,2,3,4,5,6,7,8,9,10,11,12,13,14,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57 |
| <i>NF1</i> | NM_001128147 | 15 |
| <i>NF1</i> | NM_001042492 | 31 |
| <i>NOTCH1</i> | NM_017617 | 26,27,28,34,c.*370 to c.*380 |

| Gene | Accession | Exon |
|----------------|------------------|---|
| <i>NPM1</i> | NM_002520 | 11 |
| <i>NRAS</i> | NM_002524 | 2,3,4,5 |
| <i>PDGFRA</i> | NM_006206 | 12,14,15,18 |
| <i>PHF6</i> | NM_032335 | 2,3,4,5,6,7,8 |
| <i>PHF6</i> | NM_001015877 | 10 |
| <i>PHF6</i> | NM_032458 | 9 |
| <i>PPM1D</i> | NM_003620 | 6 |
| <i>PTEN</i> | NM_000314 | 1,2,3,4,5,6,7,8,9 |
| <i>PTPN11</i> | NM_002834 | 3,4,7,8,12,13 |
| <i>PTPN11</i> | NM_080601 | 11 |
| <i>RAD21</i> | NM_006265 | 2,3,4,5,6,7,8,9,10,11,12,13,14 |
| <i>RBBP6</i> | NM_006910 | p.1444,p.1451,p.1569,p.1654,p.1673 |
| <i>RUNX1</i> | NM_001754 | 2,3,5,6,7,8,9 |
| <i>RUNX1</i> | NM_001122607 | 1,5 |
| <i>SETBP1</i> | NM_015559 | 4 (p.799-p.950) |
| <i>SF3B1</i> | NM_012433 | 13,14,15,16,17,18,19,20,21 |
| <i>SH2B3</i> | NM_005475 | 2,3,4,5,6,7,8 |
| <i>SLC29A1</i> | NM_001078175 | 4,13 |
| <i>SMC1A</i> | NM_006306 | 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25 |
| <i>SMC1A</i> | NM_001281463 | 2 |
| <i>SMC3</i> | NM_005445 | 10,13,19,23,25,28 |
| <i>SRSF2</i> | NM_003016 | 1,2 |
| <i>STAG2</i> | NM_006603 | 2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33 |
| <i>STAG2</i> | NM_001042749 | 32 |

| Gene | Accession | Exon |
|-------|--------------|----------------------------|
| STAT3 | NM_003150 | 20 |
| STAT3 | NM_139276 | 21 |
| TET2 | NM_001127208 | 4,5,6,7,8,9,10,11 |
| TET2 | NM_017628 | 3 |
| TP53 | NM_000546 | 1,2,3,4,5,6,7,8,9,10,11 |
| TP53 | NM_001276696 | 10 |
| TP53 | NM_001276695 | 10 |
| U2AF1 | NM_006758 | 2,6,7 |
| U2AF1 | NM_001025204 | 6 |
| U2AF2 | NM_007279 | 1,2,3,4,5,6,7,8,9,10,11,12 |
| WT1 | NM_000378 | 1,2,3,4,5,6,7,9 |
| WT1 | NM_001198552 | 8 |
| XPO1 | NM_003400 | 15,16,18 |
| ZRSR2 | NM_005089 | 1,2,3,4,5,6,7,8,9,10,11 |

Genes targeted for CNV

| | | | | | |
|--------|--------|--------|-------|-------|-------|
| ASXL1 | CDKN2A | FLT3 | MYC | RUNX1 | U2AF2 |
| BCOR | CUX1 | IKZF1 | NF1 | TET2 | WT1 |
| CBL | ETV6 | KDM6A | RAD21 | TP53 | ZRSR2 |
| CDC25C | EZH2 | LUC7L2 | RPS14 | U2AF1 | |

Please contact archer-tech@idtdna.com to inquire about enabling additional genes for CNV detection.

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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Revision History

| Document Number | Date | Description of change |
|------------------|---------------|--|
| RA-DOC-022/REV01 | June 2023 | Initial release. |
| RA-DOC-022/REV02 | November 2023 | Updated First and Second PCR cycling conditions to include separate anneal and extended steps. Updated typo in “Genes targeted for CNV” table. Updated branding. |