

VARIANT*Plex* Expanded Carrier Panel

Description

The VARIANT*Plex* Expanded Carrier 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* standard protocol (RA-DOC-057).

| Description | Part number | Storage |
|--|-------------|--------------|
| VARIANT <i>Plex</i> Expanded Carrier GSP1, 8 reactions | cSA17627081 | -20°C ± 10°C |
| VARIANT <i>Plex</i> Expanded Carrier GSP2, 8 reactions | cSA17627082 | |

Required reagent volumes

| Protocol reference | Protocol Step | Reagent | Volume per reaction (µL) |
|--------------------|------------------|---|--------------------------|
| A | Adapter Ligation | 10mM Tris-HCl pH 8.0 | 18 |
| B | First PCR | VARIANT <i>Plex</i> Expanded Carrier GSP1 | 4 |
| C | First PCR | Purified DNA | 16 |
| D | First PCR | 10mM Tris-HCl pH 8.0 | 18 |
| E | Second PCR | Purified DNA | 16 |
| F | Second PCR | VARIANT <i>Plex</i> Expanded Carrier GSP2 | 4 |

Recommended PCR cycling

| | Step | Temperature (°C) | Time | Cycles |
|---------------------|------|------------------|---------------------------|--------|
| First PCR reaction | 1 | 95 | 3 min | 1 |
| | 2 | 95 | 30 sec | |
| | 3 | 60 | 10 sec | 15 |
| | 4 | 61 | 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* Expanded Carrier 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 (v7.0, or greater). The VARIANT*Plex* Expanded Carrier panel requires selection of the **SNV/Indel, Structural Variation, and Copy Number 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* Expanded Carrier 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>ABCD1</i> | NM_000033 | 1,2,3,4,5,6,7,8,9,10 |
| <i>ABCD4</i> | NM_005050 | 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19 |
| <i>ACAD8</i> | NM_014384 | 1,2,3,4,5,6,7,8,9,10,11 |
| <i>ACADM</i> | NM_000016 | 1,3,4,5,6,7,8,9,10,11,12 |
| <i>ACADM</i> | NM_001127328 | 2 |
| <i>ACADM</i> | NM_001286043 | 5 |
| <i>ACADS</i> | NM_000017 | 1,2,3,4,5,6,7,8,9,10 |
| <i>ACADSB</i> | NM_001609 | 1,2,3,4,5,6,7,8,9,10,11 |
| <i>ACADVL</i> | NM_000018 | 1,2,3,4,5,6,7,8,9,10,11,12,14,15,17,19,20 |
| <i>ACADVL</i> | NM_001270447 | 1,2 |
| <i>ACAT1</i> | NM_000019 | 1,2,3,4,5,6,7,8,9,10,11,12 |
| <i>ACSF3</i> | NM_174917 | 3,4,5,6,7,8,9,10,11 |
| <i>ADA</i> | NM_000022 | 1,2,3,4,5,6,7,8,9,10,11,12 |
| <i>AHCY</i> | NM_000687 | 1,2,3,4,5,6,7,8,9,10 |
| <i>ARG1</i> | NM_000045 | 1,2,4,5,6,7,8 |
| <i>ARG1</i> | NM_001244438 | 3 |
| <i>ASL</i> | NM_000048 | 3,4,5,6,7,8,9,10,11,12,13,14,15,16,17 |
| <i>ASL</i> | NM_001024943 | 1 |
| <i>ASPA</i> | NM_000049 | 1,2,3,4,5,6 |
| <i>ASS1</i> | NM_000050 | 3,4,5,6,7,8,9,10,11,12,13,14,15,16 |
| <i>AUH</i> | NM_001698 | 1,2,3,4,5,6,7,8,9,10 |
| <i>BCKDHA</i> | NM_000709 | 1,2,3,4,5,6,7,8,9 |
| <i>BCKDHB</i> | NM_000056 | 1,2,3,4,5,6,7,8,9 |
| <i>BCKDHB</i> | NM_183050 | 10 |
| <i>BLM</i> | NM_000057 | 2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22 |
| <i>BTD</i> | NM_000060 | Alternate exon 1 (chr3:15643227-15643277),1,2,3,4 |

| Gene | Accession | Exon |
|----------------|------------------|--|
| <i>BTB</i> | NM_001281724 | 3 |
| <i>CBS</i> | NM_000071 | 3,4,5,6,7,8,9,10,11,12,13,14,15,16,17 |
| <i>CD320</i> | NM_016579 | 1,2,3,4,5 |
| <i>CFTR</i> | NM_000492 | 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 |
| <i>CFTR</i> | NM_000492 | Select intronic variants |
| <i>CPT1A</i> | NM_001876 | 2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19 |
| <i>CPT2</i> | NM_000098 | 1,2,3,4,5 |
| <i>CYP21A2</i> | NM_000500 | 1,2,3,4,5,6,7,8,9,10 |
| <i>DBT</i> | NM_001918 | 1,2,3,4,5,6,7,8,9,10,11 |
| <i>DLD</i> | NM_000108 | 1,2,3,4,5,6,7,8,9,10,11,12,13,14 |
| <i>DNAJC19</i> | NM_145261 | 1,3,4,5,6 |
| <i>DUOX2</i> | NM_014080 | 2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34 |
| <i>ETFA</i> | NM_000126 | 1,2,3,4,5,6,7,8,9,10,11,12 |
| <i>ETFB</i> | NM_001014763 | 1 |
| <i>ETFB</i> | NM_001985 | 1,3,4,5,6 |
| <i>ETFDH</i> | NM_004453 | 1,2,3,4,5,6,7,8,9,10,11,12,13 |
| <i>FAH</i> | NM_000137 | 1,2,3,4,5,6,7,8,9,10,11,12,13,14 |
| <i>FANCC</i> | NM_000136 | Select hotspots, see target BED |
| <i>G6PC</i> | NM_000151 | 1,2,3,4,5 |
| <i>G6PD</i> | NM_000402 | 1,2,4,5,6,7,8,9,10,11,12,13 |
| <i>GAA</i> | NM_000152 | 2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20 |
| <i>GALC</i> | NM_000153 | 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17 |
| <i>GALC</i> | NM_001201402 | 1 |
| <i>GALE</i> | NM_000403 | 3,4,5,6,7,9,10,11,12 |
| <i>GALK1</i> | NM_000154 | 1,2,3,4,5,6,7,8 |
| <i>GALT</i> | NM_000155 | 1,2,3,5,6,7,8,9,10,11 |
| <i>GBA</i> | NM_000157 | 1,2,3,4,5,6,7,8,9,10,11 |
| <i>GCDH</i> | NM_000159 | 2,4,5,6,7,8,9,10,11,12 |
| <i>GCH1</i> | NM_000161 | 1,2,3,4,5,6 |

| Gene | Accession | Exon |
|-----------------|------------------|---|
| <i>GJB2</i> | NM_004004 | 2 |
| <i>GJB3</i> | NM_001005752 | 2 |
| <i>GJB6</i> | NM_006783 | 3 |
| <i>GLA</i> | NM_000169 | 1,2,3,4,5,6,7 |
| <i>GNMT</i> | NM_018960 | 1,2,3,4,5,6 |
| <i>HADH</i> | NM_001184705 | 7 |
| <i>HADH</i> | NM_005327 | 1,2,3,4,5,6,7,8 |
| <i>HADHA</i> | NM_000182 | 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20 |
| <i>HADHB</i> | NM_000183 | 2,3,4,5,6,7,8,9,10,11,13,14,15,16 |
| <i>HADHB</i> | NM_001281513 | 4 |
| <i>HBA1</i> | NM_000558 | 1,2,3 |
| <i>HBA2</i> | NM_000517 | 1,2,3 |
| <i>HBB</i> | NM_000518 | 1,2,3 |
| <i>HCFC1</i> | NM_005334 | 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 |
| <i>HEXA</i> | NM_000520 | 1,2,3,4,5,6,7,8,9,10,11,12,13,14 |
| <i>HLCS</i> | NM_000411 | 4,5,6,7,8,9,10,11,12 |
| <i>HMGCL</i> | NM_000191 | 1,2,3,4,5,6,7,8,9 |
| <i>HPD</i> | NM_002150 | 1,3,4,5,6,7,8,9,10,11,12,13,14 |
| <i>HSD17B10</i> | NM_004493 | 1,2,3,4,5,6 |
| <i>IDUA</i> | NM_000203 | 1,2,3,4,5,6,8,9,10,11,12,13,14 |
| <i>IKBKAP</i> | NM_003640 | 2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37 |
| <i>IL2RG</i> | NM_000206 | 1,2,3,4,5,6,7,8 |
| <i>IVD</i> | NM_002225 | 1,2,3,4,5,6,7,8,9,10,11,12 |
| <i>LMBRD1</i> | NM_018368 | 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16 |
| <i>MAT1A</i> | NM_000429 | 1,2,3,4,5,6,7,8,9 |
| <i>MCCC1</i> | NM_020166 | 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19 |
| <i>MCCC2</i> | NM_022132 | 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17 |
| <i>MCEE</i> | NM_032601 | 1,2,3 |
| <i>MCOLN1</i> | NM_020533 | 1,2,3,4,5,6,7,8,9,10,11,12,13,14 |

| Gene | Accession | Exon |
|-----------------|--------------|---|
| <i>MLYCD</i> | NM_012213 | 1,2,3,4,5 |
| <i>MMAA</i> | NM_172250 | 2,3,4,5,6,7 |
| <i>MMAB</i> | NM_052845 | 1,2,3,4,5,6,7,8,9 |
| <i>MMACHC</i> | NM_015506 | 1,2,3,4 |
| <i>MMADHC</i> | NM_015702 | 2,3,4,5,6,7,8 |
| <i>MTHFR</i> | NM_005957 | 2,3,4,5,6,7,8,9,10,11,12 |
| <i>MTR</i> | NM_000254 | 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33 |
| <i>MTRR</i> | NM_002454 | 3,4,5,6,7,8,9,10,11,12,13,14,15 |
| <i>MTRR</i> | NM_024010 | 1,2 |
| <i>MUT</i> | NM_000255 | 2,3,4,5,6,7,8,9,10,11,12,13 |
| <i>NPC1</i> | NM_000271 | 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>NPC2</i> | NM_006432 | 1,2,3,4,5 |
| <i>OPA3</i> | NM_001017989 | 2 |
| <i>OPA3</i> | NM_025136 | 1,2 |
| <i>OTC</i> | NM_000531 | 1,2,3,4,5,6,7,8,9,10 |
| <i>PAH</i> | NM_000277 | 1,2,3,4,5,6,7,8,9,10,11,12,13 |
| <i>PAX8</i> | NM_003466 | 2,3,4,5,6,7,8,9,10,11,12 |
| <i>PCBD1</i> | NM_000281 | 1,2,3,4 |
| <i>PCCA</i> | NM_000282 | 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24 |
| <i>PCCB</i> | NM_000532 | 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15 |
| <i>PCCB</i> | NM_001178014 | 4 |
| <i>PTS</i> | NM_000317 | 1,2,3,4,5,6 |
| <i>QDPR</i> | NM_000320 | 1,2,3,4,5,6,7 |
| <i>SLC22A5</i> | NM_001308122 | 2 |
| <i>SLC22A5</i> | NM_003060 | 1,2,3,4,5,6,7,8,9,10 |
| <i>SLC25A13</i> | NM_001160210 | 10 |
| <i>SLC25A13</i> | NM_014251 | 1,2,3,4,5,6,7,8,9,11,12,13,14,15,16,17,18 |
| <i>SLC25A20</i> | NM_000387 | 1,2,3,4,5,6,7,8,9 |
| <i>SLC26A4</i> | NM_000441 | 2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21 |

| Gene | Accession | Exon |
|---------------|--------------|--|
| <i>SLC5A5</i> | NM_000453 | 1,2,3,4,5,6,7,8,9,11,12,13,14,15 |
| <i>SMPD1</i> | NM_000543 | 1,2,3,4,5,6 |
| <i>TAT</i> | NM_000353 | 2,3,4,5,6,7,8,9,10,11,12 |
| <i>TAZ</i> | NM_000116 | 1,2,3,4,5,6,8,9,10,11 |
| <i>TCN2</i> | NM_000355 | 1,2,3,4,5,6,7,8,9 |
| <i>TG</i> | NM_003235 | 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48 |
| <i>THRA</i> | NM_003250 | 2,3,4,5,6,7,8,10 |
| <i>THRA</i> | NM_199334 | 9 |
| <i>THRB</i> | NM_000461 | 3,4,5,6,7,8,9,10 |
| <i>TPO</i> | NM_000547 | 2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17 |
| <i>TSHB</i> | NM_000549 | 2 |
| <i>TSHB</i> | NM_001277991 | 1 |
| <i>TSHR</i> | NM_000369 | 1,2,3,4,5,6,7,8,9,10 |
| <i>TSHR</i> | NM_001142626 | 9 |

Genes targeted for CNV

| | | | | | |
|---------------|----------------|--------------|-----------------|-----------------|-------------|
| <i>ABCD1</i> | <i>CBS</i> | <i>GALT</i> | <i>HSD17B10</i> | <i>NPC1</i> | <i>TG</i> |
| <i>ABCD4</i> | <i>CD320</i> | <i>GBA</i> | <i>IDUA</i> | <i>NPC2</i> | <i>THRA</i> |
| <i>ACAD8</i> | <i>CFTR</i> | <i>GCDH</i> | <i>IKBKAP</i> | <i>OPA3</i> | <i>THRB</i> |
| <i>ACADM</i> | <i>CPT1A</i> | <i>GCH1</i> | <i>IL2RG</i> | <i>OTC</i> | <i>TPO</i> |
| <i>ACADS</i> | <i>CPT2</i> | <i>GJB2</i> | <i>IVD</i> | <i>PAH</i> | <i>TSHB</i> |
| <i>ACADSB</i> | <i>CYP21A2</i> | <i>GJB3</i> | <i>LMBRD1</i> | <i>PAX8</i> | <i>TSHR</i> |
| <i>ACADVL</i> | <i>DBT</i> | <i>GJB6</i> | <i>MAT1A</i> | <i>PCBD1</i> | <i>TAZ</i> |
| <i>ACAT1</i> | <i>DLD</i> | <i>GLA</i> | <i>MCCC1</i> | <i>PCCA</i> | <i>TCN2</i> |
| <i>ACSF3</i> | <i>DNAJC19</i> | <i>GNMT</i> | <i>MCCC2</i> | <i>PCCB</i> | |
| <i>ADA</i> | <i>DUOX2</i> | <i>HADH</i> | <i>MCEE</i> | <i>PTS</i> | |
| <i>AHCY</i> | <i>ETFA</i> | <i>HADHA</i> | <i>MCOLN1</i> | <i>QDPR</i> | |
| <i>ARG1</i> | <i>ETFB</i> | <i>HADHB</i> | <i>MLYCD</i> | <i>SLC22A5</i> | |
| <i>ASL</i> | <i>ETFDH</i> | <i>HBA1</i> | <i>MMAA</i> | <i>SLC25A13</i> | |
| <i>ASPA</i> | <i>FAH</i> | <i>HBA2</i> | <i>MMAB</i> | <i>SLC25A20</i> | |
| <i>ASS1</i> | <i>G6PC</i> | <i>HBB</i> | <i>MMACHC</i> | <i>SLC26A4</i> | |
| <i>AUH</i> | <i>G6PD</i> | <i>HCFC1</i> | <i>MMADHC</i> | <i>SLC5A5</i> | |
| <i>BCKDHA</i> | <i>GAA</i> | <i>HEXA</i> | <i>MTHFR</i> | <i>SMN1</i> | |
| <i>BCKDHB</i> | <i>GALC</i> | <i>HLCS</i> | <i>MTR</i> | <i>SMN2</i> | |
| <i>BLM</i> | <i>GALE</i> | <i>HMGCL</i> | <i>MTRR</i> | <i>SMPD1</i> | |
| <i>BTD</i> | <i>GALK1</i> | <i>HPD</i> | <i>MUT</i> | <i>TAT</i> | |

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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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-040/REV01 | June 2023 | Initial release |
| RA-DOC-040/REV02 | November 2023 | Updated First and Second PCR cycling conditions to include separate anneal and extended steps. Updated reagent in step A under “Required reagent volumes” section. Updated branding. |
| RA-DOC-030/REV03 | February 2025 | Updated part numbers. |