**Supplemental Figures** 



**Supplemental Figure 1:** Limit of detection (LOD) was established at 5% variant allele fraction (VAF) using Strelka2 callsets from six in-silico mixtures of GIAB reference 300x samples (subset to chromosome 20), HG002 (son) and HG003 (father), as a control. X-axis values indicate the different samples, ranging from 0% AF (HG003 unmixed) to 50% AF (HG002 unmixed). Callsets for each mixture were benchmarked in the precisionFDA app.



**Supplemental Figure 2:** Variant counts from the Strelka2 tumor-normal run with the GIAB AJ trio, benchmarking, and filtering steps to generate a list of potential mosaic variants (366,728 vcfeval false positives) for database creation.



**Supplemental Figure 3:** Mosaic benchmark decision tree for filtering potential mosaic variants to produce a list for manual curation. A series of heuristics were applied to the potential mosaic database (\* starting at bottom left) using combined orthogonal CI thresholds and other attributes for candidate set determination. The tree resulted in three groups of variants: green boxes indicate variants to curate, yellow represents variants excluded from benchmark VCF and BED, and red variants excluded from benchmark VCF but not BED. Values in parentheses are variant counts for each step of the decision tree.



**Supplemental Figure 4:** VAF distributions for all <u>manually curated variants</u> (135) in high-coverage Illumina (300x) and orthogonal (BGI - 100x, Element - 136x, PacBio HiFi - 106x) datasets generated from NIST HG002 reference material.



**Supplemental Figure 5:** VAF distributions for the 85 HG002 <u>mosaic benchmark variants</u> in high-coverage Illumina (300x) and orthogonal (BGI - 100x, Element - 136x, PacBio HiFi - 106x) datasets generated from NIST HG002 reference material.

| uman (GRCh38/hg38)                       | Chr1 Chr1:98,259,099-                       | 98,259,138 Co 音 4 > 🏟 🗋 🤉             | к 📁 I            | chi                                                                   | r1:98259119                                                 | =                                      |            |
|------------------------------------------|---------------------------------------------|---------------------------------------|------------------|-----------------------------------------------------------------------|-------------------------------------------------------------|----------------------------------------|------------|
|                                          | 1584 1.144 1.144 1.284 1.1364 1.2844 1.1844 | p12.1 p11.2 p11.1 p22.3 p22.1 p21.2   | plll plll ql     | 2 921.1 921.3 923.2 9                                                 | 24.1 625.1 625.3 631.2                                      | q32.1 q32.2 q41 q42.12                 | q422 q43 o |
|                                          | •                                           | 06.303.113 im                         | 41 bp            | 20 km                                                                 | 86,215,132 (                                                |                                        |            |
| 1Ch38hmark.vef.gz                        |                                             |                                       | No Visione Frond |                                                                       |                                                             |                                        |            |
| Chillv1.0 wf.gr                          |                                             |                                       | No vanance sana  | Mosaic variant retai                                                  | ned in HG002 draft benr                                     | hmark                                  | -2         |
| Ch38.300x.bam.Cover                      |                                             |                                       | 2                | ● ● ● HG002.GRCh3 )                                                   | 0 0 0 H0003.0RCH38 10 0                                     | H0004.GRCh38                           | -ŏ         |
| Ch38.300x.Algoment                       |                                             | • •                                   | <b>*</b> 9       | chr1:98,259,119<br>Total count: 317                                   | tr1:98,259,119<br>otal count: 382                           | 259,119<br>unt: 351                    |            |
|                                          | p.eq                                        |                                       | ä                | A : 283 (89%, 142+, 141+)<br>C : 0<br>G : 34 (11%, 21+, 13-)<br>G     | : 382 (100%, 180+, 202-) A : 351<br>: 0 C : 0<br>G : 0      | (100%, 163+, 188- )                    |            |
| Di38.300x, Mannent                       |                                             |                                       |                  | T:0<br>N:0                                                            | 0<br>0<br>0                                                 |                                        |            |
| Di 38.330x.bam Cever                     | p. 44                                       |                                       |                  | 0 0 0 H0002_GRCh38_B0                                                 |                                                             | ment                                   |            |
| Dr.38.300x_Alignment                     |                                             | • •                                   | *                | Total count: 121<br>A : 104 (86%, 53+, 51+)                           | Total count: 137                                            |                                        |            |
| CN38_BGI_100X_Cov                        | p. 193                                      |                                       |                  | C:0<br>G:16(13%, 10+, 6-)<br>T:1(1%, 0+, 1-)                          | C:0<br>G:22(16%, 9+, 13-)                                   |                                        |            |
| Ch38_8GI_103X_A6j                        |                                             | • •                                   | Ğ                | N:0                                                                   | N:0                                                         | U                                      |            |
| Ch3am Coverage                           | p. M                                        |                                       |                  | © © © HG002_GRCh38_Per<br>chr1:98,259,119                             | :bio 0 0 0 HG002_ONT-UL                                     |                                        |            |
| Ch3merged.ham                            |                                             |                                       |                  | Total count: 134<br>A : 115 (86%, 52+, 63- )                          | Total count: 35<br>A : 30 (86%, 15+, 15- )                  |                                        |            |
| Ch38_Pachio_Sequel<br>d.bam Coverage     | p. 58                                       |                                       |                  | G : 19 (14%, 10+, 9-)<br>T : 0                                        | G:5(14%, 2+, 3-)<br>T:0                                     |                                        |            |
| Ch36_pactrio_sequel,<br>d.bam            |                                             |                                       | Ğ                | N:0<br>DEL:1                                                          | N:0                                                         |                                        |            |
| T-U., am Coverage                        | p-19                                        |                                       |                  | INS: 1                                                                |                                                             |                                        |            |
| t_UL_d Algoments                         | T A T G G T T G T                           |                                       |                  |                                                                       | T A T T A                                                   |                                        | A C        |
| -45                                      |                                             |                                       | LINC01776        |                                                                       |                                                             |                                        | ·          |
| faet_sda.bed.gz                          |                                             |                                       |                  |                                                                       |                                                             |                                        |            |
| n red ge<br>Honopolynen_gifby            |                                             |                                       |                  |                                                                       |                                                             |                                        |            |
| n_seop5.hed.gz<br>sdod 🔤 🗰 et            | 1.95,250,106                                |                                       |                  |                                                                       |                                                             |                                        | (3,181M of |
| an (GRCh38/hg38                          | ) 😑 chr6 🖸 chr6:126,382,23                  | 24-126,382,263 Go 🚔 ◄ ► 🕸 🗖           | x 📁 I            | chr                                                                   | 6:126382244                                                 | E 00                                   |            |
|                                          | p252 p243 p28 p223 p222 p2133 p2            | 12 p213 p123 p123 p113 q12            | en en en en      | 5 q16.1 q16.3 q21                                                     | e22.1 e22.31 e22.33                                         | e733 e742 e753                         | 4253 426   |
|                                          | -                                           |                                       | 41 bp            |                                                                       |                                                             |                                        |            |
| Made burnered or                         | 595,982,199 00                              | 138,58                                | 12,548 tp-       | 1                                                                     | 1,382,250 to                                                | 10.3                                   | 8,399 to   |
| Chill I mak volgi                        |                                             |                                       | No Variants, our | Variant excluded                                                      | from HG002 mosaic be                                        | nchmark                                |            |
| Kh38.390x.bam.Cover                      |                                             |                                       |                  | 0 0 0 H0002.0RCh3                                                     |                                                             | © 0 H0004.0RCh                         |            |
| Cold Mile Alexand                        | ,                                           |                                       | · · · ·          | Total count: 299<br>A : 28 (9%, 13+, 15-)                             | Total count: 307<br>A : 166 (54%, 104+, 62- ) A :           | al count: 297<br>153 (52%, 70+, 83- )  |            |
|                                          |                                             |                                       |                  | C:0<br>G:1(0%,0+,1-)<br>T:270(90%,1484,127.)                          | C:0<br>C:0<br>T:141 (46%, 74+, 67-)<br>T:                   | 0<br>0<br>144 (48%, 82+, 62- )         |            |
| ICh38.393x ken Cover                     | ,                                           | <b></b>                               |                  | N : 0                                                                 | N:0 N:<br>DEL:2 DE                                          | 0                                      |            |
| 10x38.300x.bam Cover                     | P. M                                        |                                       |                  |                                                                       |                                                             | t 0                                    |            |
| RCh38.390x_Alignment                     | ,                                           | •                                     | × •              | chr6:126,382,244                                                      | chr6:126,382,244<br>Total count: 145                        |                                        |            |
| MCh38_BGL10EK_C#                         |                                             |                                       |                  | A : 4 (3%, 1+, 3-)<br>C : 1 (1%, 1+, 0-)                              | A : 9 (6%, 4+, 5-)<br>C : 1 (1%, 0+, 1-)                    |                                        |            |
| 10000_00_000_000_AQ                      | 6.43.                                       |                                       | <u> </u>         | T : 111 (96%, 62+, 49- )<br>N : 0                                     | T : 135 (93%, 70+, 65- )<br>N : 0                           |                                        |            |
| IRCh3am Coverage                         |                                             |                                       |                  | DEL: 5<br>INS: 0                                                      | 0 0 0 H0002_0N                                              |                                        |            |
| RCh36_Packio_Seque                       | p. tuj                                      |                                       |                  | 0 0 0 H0002_0RCh3                                                     | Criefs 126, 582, 244                                        |                                        |            |
| ped.bam.Coverage<br>IRCh39_packis_seque( | ,                                           | •                                     | · · · · ·        | Total count: 132<br>A : 8 (6%, 5+, 3+)                                | C:0<br>G:0<br>T:35(100%, 14+, 21-)                          |                                        |            |
| NT-U. am Coverage                        | P. N                                        |                                       |                  | C:0<br>G:0<br>T:124(94%, 58+, 66-)                                    | N:0                                                         |                                        |            |
| NT_UL_ d Algements                       | ,                                           | • • • • • • • • • • • • • • • • • • • | <b>*</b> ,       | N:0                                                                   | INS: 0                                                      |                                        |            |
| →<br>                                    | * * * * * * * * * * *                       | • • • • • • •                         | • • • • •        |                                                                       |                                                             |                                        |            |
| ndaeq_odu.bed.ga                         |                                             |                                       | CENPW            |                                                                       |                                                             |                                        |            |
| els bed ge                               |                                             | mak SINE-ALGx                         |                  |                                                                       | makLINEL1PREC2                                              |                                        |            |
| aded                                     | W6 126.382,239                              |                                       |                  |                                                                       |                                                             |                                        | 1.66       |
| un (CRCh38/bol                           | B G chr10 G chr10.106.867                   | 499-106 967 539 Co 🚔 🖌 🔊 🕅            |                  | ch                                                                    | -10-106967510                                               |                                        |            |
|                                          |                                             |                                       |                  | ch                                                                    |                                                             |                                        |            |
|                                          |                                             |                                       | #1bs             | the set of the set of                                                 | and deal deal                                               |                                        | 410.15 428 |
|                                          | 106,667,500 tap                             | 106,867,510 to                        |                  | 96,967,529 to                                                         | 1                                                           | 947,539 bp                             |            |
| RD138. Avreik vol.gz                     |                                             |                                       | No Variants ou   | Variant excluded                                                      | from HG002 mosaic be                                        | enchmark                               |            |
|                                          | A-30.                                       |                                       | No Variants You  | end                                                                   | - To o o Honda GRob., To                                    | 0 0 H0004.GRCh38                       |            |
| Mch38.300x.ban Cover                     |                                             |                                       |                  | chr10:106,867,519<br>Total count: 276                                 | chr10:106,867,519 chr1<br>Total count: 277 Tota             | 0:106,867,519<br>I count: 261          |            |
| RCh38.300x_Alignment                     |                                             |                                       | î                | A : 32 (12%, 12+, 20-)<br>C : 0<br>G : 243 (88%, 113+, 130-)          | A : 132 (48%, 62+, 70-)<br>C : 0<br>G : 145 (52%, 72+, 73-) | (0%, 0+, 1-)<br>(60 (100%, 128+, 132-) |            |
| Ch38.300x.ham Cover                      |                                             |                                       |                  | T : 1 (0%, 1+, 0- )<br>N : 0                                          | T:0<br>N:0                                                  |                                        |            |
| ICh38.300x.ham Cover                     | (- M)                                       |                                       |                  |                                                                       | DEI                                                         | 1                                      |            |
| CD-38-300x_Algement                      |                                             |                                       | · · · ·          | 0 0 0 HG002_GRCh38                                                    | Laci 0 0 0 Hooo2_cach3a                                     | Element                                |            |
| NCH38_BGI_100K_Ce                        |                                             |                                       |                  | chr10:106,867,519<br>Total count: 105<br>A - 21 (200) - 24 MA         | chr10:106,867,519<br>Total count: 98                        |                                        |            |
| RDI38_BGL_100K_AN                        | •                                           |                                       | ×.               | A : 21 (20%, 7+, 14-)<br>C : 2 (2%, 1+, 1-)<br>G : 82 (78%, 37+, 45-) | A : 15 (15%, 7+, 8- )<br>C : 0<br>G : 83 (85%, 35+, 48- )   |                                        |            |
| PIDh3am Covenage                         | (p-9)                                       |                                       |                  | T : 0<br>N : 0                                                        | T : 0<br>N : 0                                              |                                        |            |
| ACh3merged.bam                           |                                             |                                       | â                | DEL: 0<br>INS: 1                                                      |                                                             |                                        |            |
| RCh38_Pachio_Seque<br>ped.bam Coverage   | p. sq                                       |                                       |                  | © © ⊖ H0002_0RCh3                                                     | 18_Pacbio 0 0 0 H0002_0N                                    | T-UL                                   |            |
| PCh38_pachis_seque <br>ped.bam           | •                                           |                                       |                  | Total count: 94<br>A : 15 (16%, 9+, 6- )                              | Total count: 43<br>A : 7 (16%, 4+, 3- )                     |                                        |            |
| NT-Uam Coverage                          |                                             |                                       |                  | C:0<br>G:79(84%, 37+, 42-)<br>T:0                                     | C:0<br>G:36(84%, 21+, 15-)<br>T:0                           |                                        |            |
| NT_ULd Algements                         |                                             | ctcctg a                              | c c t c c        | N-0                                                                   | 0 0 0 0 0                                                   | g e e t t                              |            |
| mes                                      |                                             |                                       | SOROS1           |                                                                       |                                                             |                                        |            |
| ebeq_ofs.bed.gr                          |                                             |                                       |                  |                                                                       |                                                             |                                        |            |
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|                                          |                                             |                                       |                  |                                                                       |                                                             |                                        |            |

**Supplemental Figure 6:** IGV profiles of variants (A) included in and (B,C) excluded from the HG002 mosaic benchmark after manual curation. Profile A depicts an HG002-specific candidate mosaic SNV (chr1:98259119 - not detected in parents), while B and C show a putative and candidate SNV (chr6:126382244 and chr10:106867519). The first three data tracks are the HG002 (son), HG003 (mother), and HG004 (father) 300X coverage Illumina data. The following tracks are for HG002 BGIseq dataset, Element, PacBio Sequel, and ONT-UL datasets.