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Supplementary information

Haplotype-aware variant calling with PEPPER-Margin-DeepVariant enables high accuracy in nanopore long-reads

In the format provided by the authors and unedited

Haplotype-aware variant calling with PEPPER-Margin-DeepVariant enables high accuracy in nanopore long-reads
Supplementary Results

Supplementary Notes

Data availability

Analysis data

We have made all of our analysis outputs i.e. variant calling VCF, phased VCF, and genome assemblies publicly available:
https://console.cloud.google.com/storage/browser/pepper-deepvariant-public/analysis_data/

Reference Sequences

We have used `GRCh38` and `GRCh37` human genome reference sequence that are publicly available:

`GRCh38:` ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/001/405/GCA_000001405.15_GRCh38/seqs_for_alignment_pipelines.ucsc_ids/GCA_000001405.15_GRCh38_no_alt_analysis_set.fna.gz

`GRCh37:` ftp://ftp-trace.ncbi.nih.gov/1000genomes/ftp/technical/reference/phase2_reference_assembly_sequence/hs37d5.fa.gz

Oxford Nanopore Sequencing data

We used publicly available data of ten samples to evaluate different methods. Following are the links to the fastq files that we used:

`HG001:` https://s3-us-west-2.amazonaws.com/human-pangenomics/index.html?prefix=NHGRI_UCSC_panel/HG001/nanopore/Guppy_4.2.2/

- `HG001_Circulomics_Guppy_4.2.2.fastq.gz`
- `HG001_NBT2018_Guppy_4.2.2.fastq.gz`

`HG002:` https://s3-us-west-2.amazonaws.com/human-pangenomics/index.html?prefix=NHGRI_UCSC_panel/HG002/nanopore/Guppy_4.2.2/

- `GM24385_1_Guppy_4.2.2_prom.fastq.gz`
- `GM24385_2_Guppy_4.2.2_prom.fastq.gz`
- `GM24385_3_Guppy_4.2.2_prom.fastq.gz`

`HG003:` https://s3-us-west-2.amazonaws.com/human-pangenomics/index.html?prefix=NHGRI_UCSC_panel/HG003/nanopore/Guppy_4.2.2/

- `GM24149_1_Guppy_4.2.2_prom.fastq.gz`
- `GM24149_2_Guppy_4.2.2_prom.fastq.gz`
- `GM24149_3_Guppy_4.2.2_prom.fastq.gz`

`HG004:` https://s3-us-west-2.amazonaws.com/human-pangenomics/index.html?prefix=NHGRI_UCSC_panel/HG004/nanopore/Guppy_4.2.2/

- GM24143_1_Guppy_4.2.2_prom.fastq.gz
- GM24143_2_Guppy_4.2.2_prom.fastq.gz
- GM24143_3_Guppy_4.2.2_prom.fastq.gz

HG005: https://s3-us-west-2.amazonaws.com/human-pangenomics/index.html?prefix=NHGRI_UCSC_panel/HG005/nanopore/Guppy_4.2.2/

- 01_09_20_R941_GM24631_1_Guppy_4.2.2_prom.fastq.gz
- 01_09_20_R941_GM24631_2_Guppy_4.2.2_prom.fastq.gz
- 01_09_20_R941_GM24631_3_Guppy_4.2.2_prom.fastq.gz

HG006: https://s3-us-west-2.amazonaws.com/human-pangenomics/index.html?prefix=NHGRI_UCSC_panel/HG006/nanopore/Guppy_4.2.2/

- 01_09_20_R941_GM24694_1_Guppy_4.2.2_prom.fastq.gz
- 01_09_20_R941_GM24694_2_Guppy_4.2.2_prom.fastq.gz
- 01_09_20_R941_GM24694_3_Guppy_4.2.2_prom.fastq.gz

HG007: https://s3-us-west-2.amazonaws.com/human-pangenomics/index.html?prefix=NHGRI_UCSC_panel/HG007/nanopore/Guppy_4.2.2/

- 01_09_20_R941_GM24695_1_Guppy_4.2.2_prom.fastq.gz
- 01_09_20_R941_GM24695_2_Guppy_4.2.2_prom.fastq.gz
- 01_09_20_R941_GM24695_3_Guppy_4.2.2_prom.fastq.gz

HG00733: https://s3-us-west-2.amazonaws.com/human-pangenomics/index.html?prefix=NHGRI_UCSC_panel/HG00733/nanopore/Guppy_4.2.2/

- HG00733_1_Guppy_4.2.2_prom.fastq.gz
- HG00733_2_Guppy_4.2.2_prom.fastq.gz
- HG00733_3_Guppy_4.2.2_prom.fastq.gz

HG02723: https://s3-us-west-2.amazonaws.com/human-pangenomics/index.html?prefix=NHGRI_UCSC_panel/HG02723/nanopore/Guppy_4.2.2/

- HG02723_1_Guppy_4.2.2_prom.fastq.gz
- HG02723_2_Guppy_4.2.2_prom.fastq.gz
- HG02723_2_Guppy_4.2.2_prom.fastq.gz

CHM13: https://s3.amazonaws.com/nanopore-human-wgs/chm13/nanopore/rel6/rel6.fastq.gz

PacBio HiFi sequencing data

We used publicly available PacBio HiFi sequencing data of eight samples to evaluate different methods. Following are the links to the data locations:

HG001: ftp://ftp-trace.ncbi.nlm.nih.gov/giab/ftp/data/NA12878/

HG002: https://storage.googleapis.com/pepper-deepvariant-public/sequencing_data/PacBio_HiFi/HG002/HG002_35x_PacBio_14kb-15kb.fastq.gz

HG003: https://storage.googleapis.com/pepper-deepvariant-public/sequencing_data/PacBio_HiFi/HG003/HG003_35x_PacBio_14kb-15kb.fastq.gz

HG004: https://storage.googleapis.com/pepper-deepvariant-public/sequencing_data/PacBio_HiFi/HG004/HG004_35x_PacBio_14kb-15kb.fastq.gz

HG005: https://s3-us-west-2.amazonaws.com/human-pangenomics/index.html?prefix=submissions/8ced218e-9699-458e-9708-eef6969a8065--EXTRAMURAL_SAMPLES/HG005/PacBio_HiFi/

- PBmixSequel788_1_A01_PBXX_30hours_19kbV2PD_70pM_HumanHG005_CCS/m64109_200304_195708.fastq.gz

- PBmixSequel789_1_A01_PBXW_30hours_15kbV2PD_70pM_HumanHG005_CCS/m64109_200309_192110.fastq.gz
- PBmixSequel789_2_B01_PBXX_30hours_19kbV2PD_70pM_HumanHG005_CCS/m64109_200311_013444.fastq.gz
- PBmixSequel840_1_A01_PCCD_30hours_15kbV2PD_70pM_HumanHG005_CCS/m64017_200723_190224.fastq.gz
- PBmixSequel842_1_A01_PCCD_30hours_15kbV2PD_70pM_HumanHG005_CCS/m64017_200730_190124.fastq.gz
- PBmixSequel842_2_B01_PCCD_30hours_15kbV2PD_70pM_HumanHG005_CCS/m64017_200801_011415.fastq.gz
- PBmixSequel842_3_C01_PCCD_30hours_15kbV2PD_70pM_HumanHG005_CCS/m64017_200802_073944.fastq.gz

HG00733: https://s3-us-west-2.amazonaws.com/human-pangenomics/index.html?prefix=submissions/8ced218e-9699-458e-9708-eef6969a8065--EXTRAMURAL_SAMPLES/HG00733/hifi/

- HG00733_20190925_EEE_m54329U_190607_185248.Q20.fastq.gz
- HG00733_20190925_EEE_m54329U_190615_010947.Q20.fastq.gz
- HG00733_20190925_EEE_m54329U_190617_231905.Q20.fastq.gz
- HG00733_20190925_EEE_m54329U_190619_052546.Q20.fastq.gz
- HG00733_20190925_EEE_m54329U_190629_180018.Q20.fastq.gz
- HG00733_20190925_EEE_m54329U_190701_222759.Q20.fastq.gz
- HG00733_20190925_EEE_m54329U_190827_173812.Q20.fastq.gz

HG02723: https://s3-us-west-2.amazonaws.com/human-pangenomics/index.html?prefix=submissions/8fa7bde9-be6f-4160-97a9-b639a8962c66--WUSTL_OTHER_HiFi/HG02723/PacBio_HiFi/

- m64043_191221_024136.ccs.bam
- m64043_191223_180311.ccs.bam
- m64043_191225_001554.ccs.bam
- m64043_191226_064057.ccs.bam

CHM13: https://github.com/nanopore-wgs-consortium/CHM13/

Illumina sequencing data

We used publicly available Illumina short read data of three samples to perform variant calling assessment.
HG002:

- https://storage.googleapis.com/pepper-deepvariant-public/sequencing_data/Illumina_novaseq/HG002/HG002.novaseq.pcr-free.35x.R1.fastq.gz
- https://storage.googleapis.com/pepper-deepvariant-public/sequencing_data/Illumina_novaseq/HG002/HG002.novaseq.pcr-free.35x.R2.fastq.gz

HG003:

- https://storage.googleapis.com/pepper-deepvariant-public/sequencing_data/Illumina_novaseq/HG003/HG003.novaseq.pcr-free.35x.R1.fastq.gz
- https://storage.googleapis.com/pepper-deepvariant-public/sequencing_data/Illumina_novaseq/HG003/HG003.novaseq.pcr-free.35x.R2.fastq.gz

HG004:

- https://storage.googleapis.com/pepper-deepvariant-public/sequencing_data/Illumina_novaseq/HG004/HG004.novaseq.pcr-free.35x.R1.fastq.gz
- https://storage.googleapis.com/pepper-deepvariant-public/sequencing_data/Illumina_novaseq/HG004/HG004.novaseq.pcr-free.35x.R2.fastq.gz

We used publicly available Illumina short read data for assembly quality assessment.
`HG005-HG006-HG007:`

- https://s3-us-west-2.amazonaws.com/human-pangenomics/index.html?prefix=submissions/00c8916e-a869-473a-9a03-e39867b35499--HG005_CHILD/HG005/Illumina/
- https://s3-us-west-2.amazonaws.com/human-pangenomics/index.html?prefix=submissions/8ced218e-9699-458e-9708-eef6969a8065--EXTRAMURAL_SAMPLES/HG005/parents/HG006/Illumina/
- https://s3-us-west-2.amazonaws.com/human-pangenomics/index.html?prefix=submissions/8ced218e-9699-458e-9708-eef6969a8065--EXTRAMURAL_SAMPLES/HG005/parents/HG007/Illumina/

`HG00731-HG00732-HG00733:`

- https://s3-us-west-2.amazonaws.com/human-pangenomics/index.html?prefix=submissions/325b4b1c-9f20-49be-b03a-596da89c466e--1000G_CHILDREN/HG00733/1000G_data/HG00733.final.cram
- https://s3-us-west-2.amazonaws.com/human-pangenomics/index.html?prefix=submissions/54D542ED-6650-4A12-8B40-0F92CC320486--1000G_parents/HG00733/parents/HG00732/HG00732.final.cram
- https://s3-us-west-2.amazonaws.com/human-pangenomics/index.html?prefix=submissions/54D542ED-6650-4A12-8B40-0F92CC320486--1000G_parents/HG00733/parents/HG00731/HG00731.final.cram

`HG02721-HG02722-HG02723:`

- https://s3-us-west-2.amazonaws.com/human-pangenomics/index.html?prefix=submissions/325b4b1c-9f20-49be-b03a-596da89c466e--1000G_CHILDREN/HG02723/1000G_data/HG02723.final.cram
- https://s3-us-west-2.amazonaws.com/human-pangenomics/index.html?prefix=submissions/54D542ED-6650-4A12-8B40-0F92CC320486--1000G_parents/HG02723/parents/HG02722/HG02722.final.cram
- https://s3-us-west-2.amazonaws.com/human-pangenomics/index.html?prefix=submissions/54D542ED-6650-4A12-8B40-0F92CC320486--1000G_parents/HG02723/parents/HG02721/HG02721.final.cram

`CHM13:` https://github.com/nanopore-wgs-consortium/CHM13

PacBio CLR data
`HG002:`

- https://www.ncbi.nlm.nih.gov/sra/SRR9972588
- https://www.ncbi.nlm.nih.gov/sra/SRR9972589
- https://www.ncbi.nlm.nih.gov/sra/SRR9972590
- https://www.ncbi.nlm.nih.gov/sra/SRR9972591
- https://www.ncbi.nlm.nih.gov/sra/SRR9972592
- https://www.ncbi.nlm.nih.gov/sra/SRR9972593
- https://www.ncbi.nlm.nih.gov/sra/SRR9972594
- https://www.ncbi.nlm.nih.gov/sra/SRR9972595
- https://www.ncbi.nlm.nih.gov/sra/SRR9972596

`HG003:`

- https://github.com/genome-in-a-bottle/giab_data_indexes/blob/master/AshkenazimTrio/sequence.index.AJtrio_PacBio_MtSinai_NIST_subreads_fasta_10082018.HG003

`HG004:`

- https://github.com/genome-in-a-bottle/giab_data_indexes/blob/master/AshkenazimTrio/sequence.index.AJtrio_PacBio_MtSinai_NIST_subreads_fasta_10052018.HG004

Genome-In-A-Bottle (GIAB) benchmarking data

We used Genome-In-A-Bottle (GIAB) truth set for seven genomes HG001-HG007. For each genome, we have a VCF file describing the truth variants and a bed file describing regions to use for benchmarking. Both these files are input to `hap.py` when benchmarking a query VCF. In this truth set, `v3.3.2` was generated for `GRCh37` reference and `v4.2.1` was generated for `GRCh38` human reference genome. While evaluating our pipeline, we used associated reference while generating the benchmarking results.

The GIAB benchmarking data is publicly available:
`HG001`
ftp://ftp-trace.ncbi.nlm.nih.gov/giab/ftp/release/NA12878_HG001/NISTv3.3.2/GRCh37/
`HG002`
ftp://ftp-trace.ncbi.nlm.nih.gov/giab/ftp/release/AshkenazimTrio/HG002_NA24385_son/NISTv4.2.1/GRCh38/
`HG003`
ftp://ftp-trace.ncbi.nlm.nih.gov/giab/ftp/release/AshkenazimTrio/HG003_NA24149_father/NISTv4.2.1/GRCh38/
`HG004`
ftp://ftp-trace.ncbi.nlm.nih.gov/giab/ftp/release/AshkenazimTrio/HG004_NA24143_mother/NISTv4.2.1/GRCh38/
`HG005`
ftp://ftp-trace.ncbi.nlm.nih.gov/giab/ftp/release/ChineseTrio/HG005_NA24631_son/NISTv3.3.2/GRCh37/
`HG006`
ftp://ftp-trace.ncbi.nlm.nih.gov/giab/ftp/release/ChineseTrio/HG006_NA24694_father/NISTv3.3.2/GRCh37/
`HG007`
ftp://ftp-trace.ncbi.nlm.nih.gov/giab/ftp/release/ChineseTrio/HG007_NA24695_mother/NISTv3.3.2/GRCh37/
`HG005 v4.2 draft`
https://storage.googleapis.com/pepper-deepvariant-public/analysis_data/6_updates/HG005_evaluation/HG005_GRCh38_1_22_draft_2_v4.2.1_benchmark.bed
https://storage.googleapis.com/pepper-deepvariant-public/analysis_data/6_updates/HG005_evaluation/HG005_GRCh38_1_22_draft_2_v4.2.1_benchmark.vcf.gz

`Stratification regions of GRCh38`
https://ftp-trace.ncbi.nlm.nih.gov/giab/ftp/release/genome-stratifications/v2.0/

Gencode annotation files

We used gencode version `v35` annotation of `GRCh38` and `GRCh37` to determine variant calling accuracy in gene regions. The gencode annotation file is publicly available:

`Gencode v35`
ftp://ftp.ebi.ac.uk/pub/databases/gencode/Gencode_human/release_35/gencode.v35.annotation.gtf.gz

Execution Parameters

Minimap2, PBMM2

`Minimap2` version `2.17-r941` from `https://github.com/lh3/minimap2`. We used `samtools` version `1.10` for sorting and filtering.

We used the following command to map nanopore reads with minimap2:

```
minimap2 -ax map-ont -t 32 \
  Reference.fasta \
  reads.fastq.gz | samtools view -hb -F 0x904 > unsorted.bam
samtools sort -@32 -o sorted.bam unsorted.bam
samtools index -@32 sorted.bam
```

For PacBio HiFi data we used `pbmm2` version `1.4.0` form `https://github.com/PacificBiosciences/pbmm2`. We used the following command to map PacBio HiFi reads with pbmm2:

```
docker run --ipc=host \
  -v /data:/data \
  quay.io/biocontainers/pbmm2:1.4.0--h56fc30b_0 pbmm2 align \
  --sort -j <THREADS> -J <THREADS> \
  --preset CCS \
  /data/reference.fa /data/reads.fastq /data/reads_2_ref.bam
```

Subsampling with samtools

We used `samtools` version `1.10` to subsample the alignment files at different coverages. We used the following command:

```
    samtools view -s 0.FRAC -@<THREDS> -b INPUT.bam > OUTPUT_DOWNSAMPLED.sam
```

PEPPER-Margin-DeepVariant variant calling

We used the following command to run the PEPPER-Margin-DeepVariant variant calling pipeline:

```
docker run --ipc=host \
  -v /data:/data \
  -u (id -u $USER):(id -g $USER) \
  kishwars/pepper_deepvariant:r0.4 \
  run_pepper_margin_deepvariant call_variant \
  -b <BAM> \
  -f <FASTA>\
  -o <OUTPUT_DIR> \
  -t <THREADS> \
  --ont # --ccs if using PacBio HiFi reads
```

When running the PEPPER-Margin-DeepVariant assembly polishing pipeline, we ran it with these arguments:

```
time docker run --ipc=host \
  -v /data:/data \
  kishwars/pepper_deepvariant:r0.4 \
  run_pepper_margin_deepvariant polish_assembly \
  -b <READS_2_ASSEMBLY_BAM> \
  -f <ASSEMBLY> \
  -o <OTUPUT_DIR> \
  -t <THREADS> \
  -s <SAMPLE_NAME>
  --ont # --ccs if using PacBio HiFi reads
```

Medaka

We used Medaka `v1.2.0` available from `https://github.com/nanoporetech/medaka`. We used the following command to variant call using Medaka:

```
medaka_variant -i INPUT.bam -f reference.fna -t THREADS -o OUTPUT_DIR/
```

Clair

We used Clair `v2.1.1` available from `https://github.com/HKU-BAL/Clair`. We used the following command to run `Clair`:

```
conda activate clair-env

python clair.py callVarBamParallel \
  --chkpnt_fn clair_model/ont/model \
  --ref_fn reference.fna \
  --bam_fn INPUT.bam \
  --threshold 0.2 \
  --sampleName SAMPLE \
  --output_prefix OUTPUT_DIR/OUTPUT > command_clair.sh
```

```
cat command_clair.sh | parallel -j16

for i in OUTPUT.*.vcf; do if ! [ -z "$(tail -c 1 "$i")" ]; then echo "$i"; fi ; done | \
grep -f - command.sh | sh

vcfcat OUTPUT.*.vcf | bcftools sort -m 2G | bgzip > OUTPUT.wgs.vcf.gz
bcftools filter -s LowQual -e '%QUAL<748' -Oz OUTPUT.wgs.vcf.gz > OUTPUT.wgs.FILT.vcf.gz
```

We evaluated the filtered file. The filter cutoff value is adopted from the github suggestion.

Longshot

We used longshot `v0.4.2` available from `https://github.com/pjedge/longshot`. We used the following command to run longshot:

```
longshot --strand_bias_pvalue_cutoff 0.01 \
  --bam INPUT.bam --ref reference.fna --out OUTPUT.VCF
```

DeepVariant (Illumina and PacBio HiFi)

We used `DeepVariant v1.1.0` available in `https://github.com/google/deepvariant` to produce variant calls using Illumina and PacBio HiFi data. Following are the commands we used. For PacBio HiFi variant calling, we used:

- **DeepVariant for Illumina**:

```
docker run \
  -v /data:/data \
  google/deepvariant:1.1.0 \
  /opt/deepvariant/bin/run_deepvariant \
  --model_type=WGS \
  --ref=/data/reference.fna \
  --reads=/data/INPUT.bam \
  --output_vcf=/data/OUTPUT.vcf.gz \
  --num_shards=THREADS
```

- **DeepVariant for PacBio HiFi**:
  - Initial calling on unphased bam:

```
docker run \
  -v /data:/data \
  google/deepvariant:1.1.0 \
  /opt/deepvariant/bin/run_deepvariant \
  --model_type=PACBIO \
  --ref=/data/reference.fna \
  --reads=/data/INPUT.PHASED.bam \
  --output_vcf=/data/OUTPUT.vcf.gz \
  --num_shards=THREADS
```

  - Haplotype-aware calling on phased bam:

```
docker run \
  -v /data:/data \
  google/deepvariant:1.1.0 \
  /opt/deepvariant/bin/run_deepvariant \
  --model_type=PACBIO \
  --ref=/data/reference.fna \
  --reads=/data/INPUT.PHASED.bam \
  --output_vcf=/data/OUTPUT.vcf.gz \
  --num_shards=THREADS \
  --use_hp_information
```

Hap.py

We used `hap.py` version `v0.3.12` to assess the variant calls against GIAB truth set. The `hap.py` program is available via `jmcdani20/hap.py:v0.3.12` docker image.

```
docker run -it -v /data/:/data/ \
  jmcdani20/hap.py:v0.3.12 /opt/hap.py/bin/hap.py \
  /data/GIAB_benchmark.vcf.gz \
  /data/INPUT.vcf.gz \
  -f /data/GIAB_benchmark.bed \
  -r /data/reference.fna \
  -o /data/output/prefix \
  --stratification /data/genome_stratification/v2.0-GRCh38-stratifications.tsv \
  --pass-only \
  --engine=vcfeval \
  --threads=<THREADS>
```

The parameters we used for `hap.py`:

- `GIAB_benchmark.vcf.gz`: GIAB truth VCF file.
- `INPUT.vcf.gz`: The input VCF to compare against GIAB.
- `-f`: GIAB regions defined for variant assessment.
- `-r`: Reference FASTA file (GRCh38 or GRCh37).
- `--stratification`: Stratification file list for generating stratified results.
- `--pass-only`: Only use the PASS variants from input for assessment.
- `--engine`: Comparison engine.
- `--threads`: Number of threads to use.

Mendelian Analysis

To generate the low-confidence region for this analysis, we used `bedtools subtract` to invert the GIAB v4.2.1 high confidence regions. We further removed all centromeric regions as defined by the `http://hgdownload.cse.ucsc.edu/goldenPath/hg38/database/centromeres.txt.gz`.

To run the analysis we used RTG 3.12 with the following commands:

```
rtg vcffilter \
  -i MERGED.vcf.gz \
  -o MERGED.CONF.vcf \
  --include-bed CONF.bed
rtg mendelian
  -i MERGED.CONF.vcf.gz \
  -o MERGED.CONF.MENDELIAN.vcf.gz
  -t GCA_000001405.15_GRCh38_no_alt_analysis_set.sdf/
```

Margin

We used `Margin v2.0` available from `https://github.com/UCSC-nanopore-cgl/margin`.

To haplotag a bam, we used the following command:

```
margin phase \
  INPUT.bam \
  REFERENCE.fasta \
  INPUT.vcf \
  params/misc/allParams.XXX_haplotag.json \
  --threads THREAD_COUNT \
  --skipPhasedVCF
```

To phase a vcf, we used the following command:

```
margin phase \
  INPUT.bam \
```

```
  REFERENCE.fasta \
  INPUT.vcf \
  params/misc/allParams.phase_vcf.json \
  --threads THREAD_COUNT \
  --skipHaplotypeBAM
```

WhatsHap

We used `WhatsHap v1.0` available from `https://github.com/whatshap/whatshap`. To phased a VCF file, we used the following command:

```
whatshap phase \
  -o PHASED.OUTPUT.vcf \
  --ignore-read-groups \
  --reference REFERENCE \
  INPUT.vcf \
  INPUT.bam
```

To produce haplotags for reads, we used the following command after compressing and indexing the phased VCF:

```
whatshap haplotag \
  --reference REFERENCE \
  --regions CONTIG \
  --ignore-read-groups \
  --output OUTPUT.phased.bam \
  PHASED.OUTPUT.vcf.gz \
  INPUT.bam
```

Local phasing correctness

We used the `calcLocalPhasingCorrectness` executable in `Margin v2.0` to calculate local phasing correctness. We used the following command to generate LPC statistics:

```
calcLocalPhasingCorrectness -m 1 -M 100000000 -d -s TRUTH.vcf.gz QUERY.vcf.gz > OUTPUT.tsv
```

We plotted the results of LPC with `plot_haplotagging_lpc.py` script found in the `https://github.com/tpesout/genomics_scripts` repository. We used the following commands to generate the plots:

```
plot_haplotagging_lpc.py -f OUTPUT_1.png -b \
  -i DATA-1_TOOL-1.tsv -i DATA-1_TOOL-2.tsv -i DATA-2_TOOL-1.tsv ..
plot_haplotagging_lpc.py -f OUTPUT_2.png -b -l \
  -i DATA-1_TOOL-1.tsv -i DATA-1_TOOL-2.tsv -i DATA-2_TOOL-1.tsv ..
plot_haplotagging_lpc.py -f OUTPUT_3.png -b -l 100000 -L 10  \
  -i DATA-1_TOOL-1.tsv -i DATA-1_TOOL-2.tsv -i DATA-2_TOOL-1.tsv ..
```

Trio-Binning

We used `canu` to trio-bin the reads to generate the admixture sample used in Fig 3.

The command used for Nanopore data was:

```
canu -haplotype -p trio_Binned -d trio_Binned_dir genomeSize=3.3g \
-haplotypeFather Father.fastq -haplotypeMother Mother.fastq \
-nanopore-raw nanopore.fastq
```

The command used for PacBio HiFi data was:

```
canu -haplotype -p trio_Binned -d trio_Binned_dir genomeSize=3.3g \
-haplotypeFather Father.fastq -haplotypeMother Mother.fastq -pacbio-hifi HiFi.fastq
```

Natural switch error

We calculated the natural switch was determined using `haplotagging_stats.py` available in `https://github.com/tpesout/genomics_scripts`. We used the following command to calculate the values:

```
haplotagging_stats.py \
  --input HAPLOTAGGED.bam \
```

```
  --truth_hap1 HAP1.read_ids.txt \
  --truth_hap2 HAP2.read_ids.txt \
  --threads THREAD_COUNT
```

Natural switch visualization

We used `compare_read_phasing_hapBam.py` available in `https://github.com/tpesout/genomics_scripts`. We used the following command to visualize the natural switch:

```
compare_read_phasing_hapBam.py  \
  -1 HAP1.read_idx.txt  \
  -2 HAP2.read_idx.txt  \
  -i INPUT.chr1.bam  \
  -p INPUT.phaseset.bed  \
  -F  \
  -d 100  \
  -N
```

Shasta

We used `Shasta` version `0.7.0` (commit `06a639d36d26a4203c0b934d6e63c719750c5398`) available from `https://github.com/chanzuckerberg/shasta` to generate nanopore-based *de novo* assemblies. We used the following command to run Shasta:

```
    shasta --input /reads/SAMPLE_READS*.fasta  \
    --conf shasta/conf/Nanopore-Sep2020.conf \
    --memoryMode filesystem --memoryBacking 2M --assemblyDirectory SAMPLE_OUTPUT_DIR
```

Flye

We used `Flye` version `2.8.2` available from `https://github.com/fenderglass/Flye` to generate nanopore-based *de novo* assemblies. We invoked Flye the following way:

```
flye --nano-raw \
  file1.fastq.gz \
  file2.fastq.gz \
  file3.fastq.gz
  --out-dir FLYE_OUTPUT/
```

Trio-Hifiasm

We used `hifiasm` version `0.14` available from `https://github.com/chhylp123/hifiasm`. We also used `YAK` in this pipeline which is available in `https://github.com/lh3/yak`. We generated the assembly in two-steps:

Step 1: Generate maternal and paternal k-mer counts from paired-end short-reads from the parent samples:

```
yak count \
  -b37 -t16 -o pat.yak <(cat pat_1.fq.gz pat_2.fq.gz) < (cat pat_1.fq.gz pat_2.fq.gz)

yak count \
  -b37 -t16 -o mat.yak <(cat mat_1.fq.gz mat_2.fq.gz) < (cat mat_1.fq.gz mat_2.fq.gz)
```

Step 2: Generate the assembly using sample-specific PacBio HiFi reads (`SAMPLE-HiFi_reads.fa.gz`):

```
hifiasm -o SAMPLE.asm -t<THREADS> -1 pat.yak -2 mat.yak SAMPLE-HiFi_reads.fa.gz
```

A WDL workflow of this pipeline is available in
`https://github.com/human-pangenomics/hpp_production_workflows/`.

SV Analysis

To align assemblies to the reference we used the command:

```
minimap2 hg19_ref.fasta  test_assmebly.fasta -r2k -ax asm5 --cs -t 10 \
     | samtools sort > hg19_mapping.bam
```

To call diploid variants we used the command:

```
svim-asm diploid . hg19_mapping_hp1.bam hg19_mapping_hp2.bam \
    hg19_ref.fasta --min_sv_size 50 --query_names
```

To call haploid variants we used the command:

```
svim-asm haploid . hg19_mapping.bam hg19_ref.fasta --min_sv_size 50 \
    --query_names
```

Given two sets of SV calls, to quantify the accuracy we used the command:

```
SVbenchmark --ref hg19_ref.fasta --test test_sv.vcf --truth truth_sv.vcf \
    --prefix unique_prefix --includebed GIAB_HG002_SVs_Tier1_v0.6_chr.bed
```

Homopolymer run-length analysis

We used `runLengthMatrix` module of `margin` to perform the homopolymer run-length analysis. We used the following command to generate the run-length statistics:

```
./runLengthMatrix \
  <READS_2_ASSEMBLY.BAM> \
  <ASSEMBLY.FASTA> \
  <margin/params/base_params.json> \
  -o <OUTPUT_PATH> \
  -t <THREADS> \
  -l <MAX_RUN_LENGTH>
```

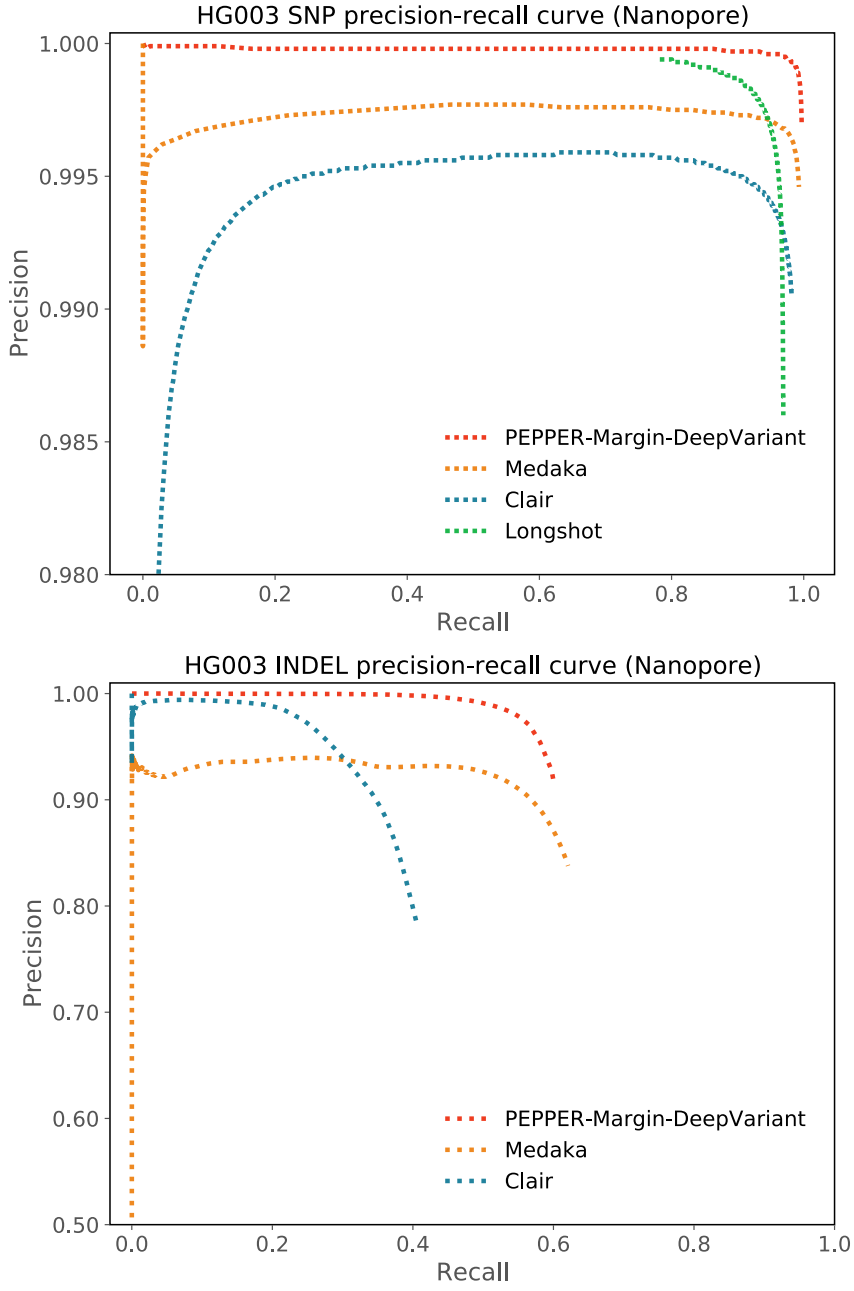
Dipcall

We used `dipcall` available in https://github.com/lh3/dipcall to generate small variant call from the assemblies.

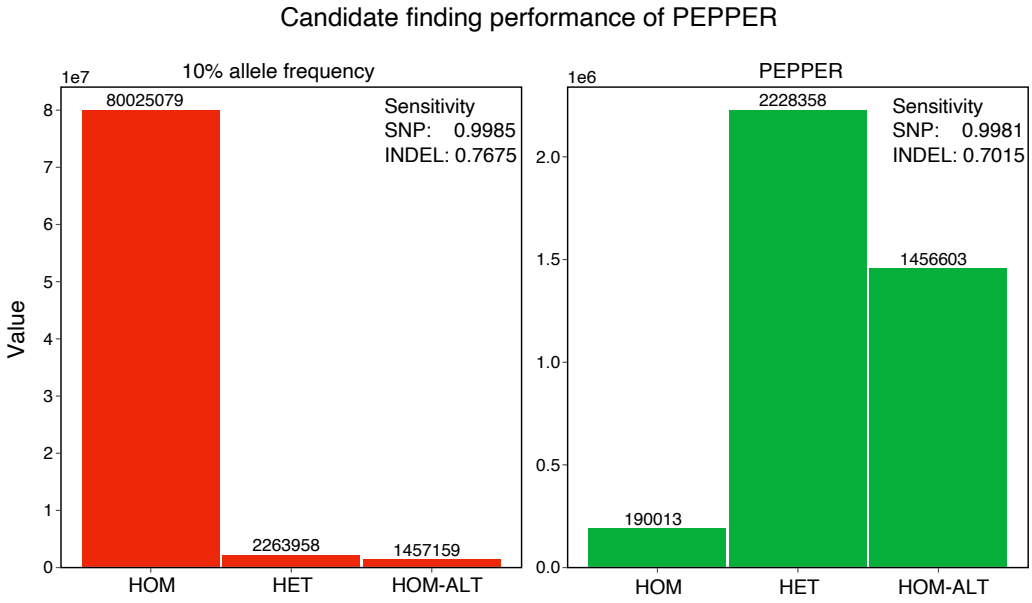
```
./run-dipcall \
OUTPUT_DIR/OUTPUT_PREFIX \
REFERENCE.fa \
Assembly.hp1.fa \
Assembly.hp2.fa \
-t THREADS > ASSEMBLY_2_REFERENCE.mak

make -j2 -f ASSEMBLY_2_REFERENCE.mak
```

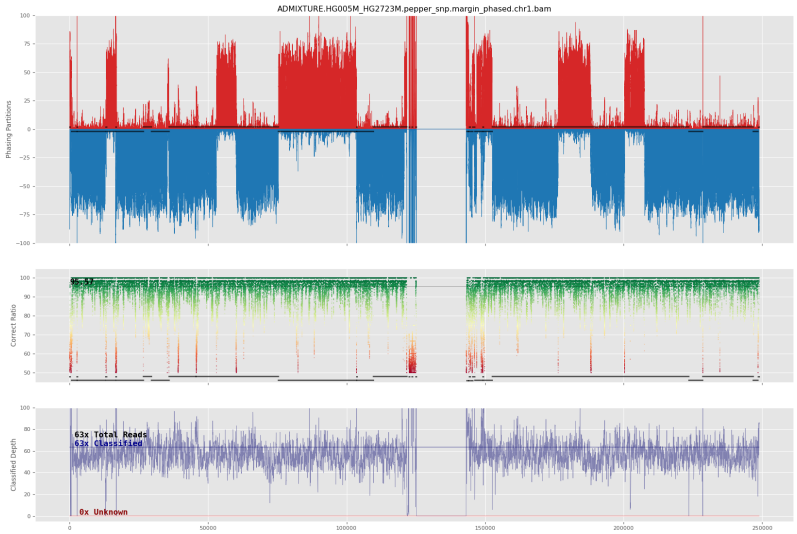
Supplementary Figures



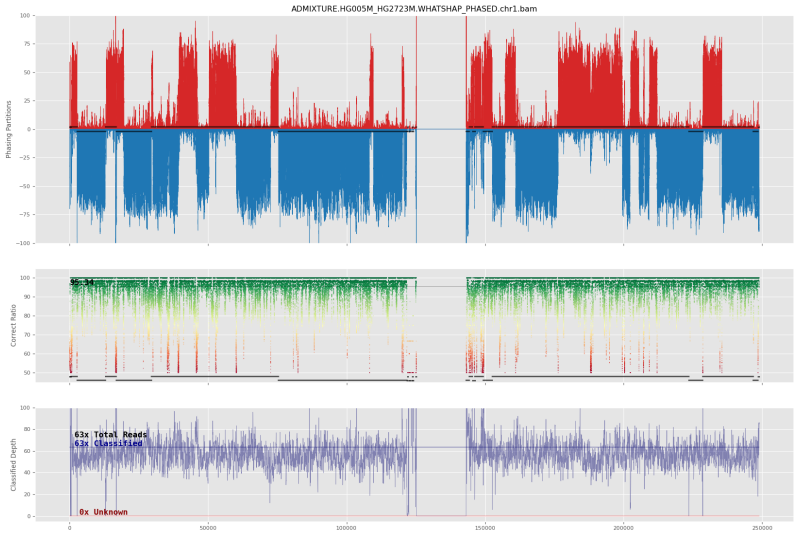
Supplementary Figure 1: Precision-Recall plot of HG003 for nanopore-based variant callers.



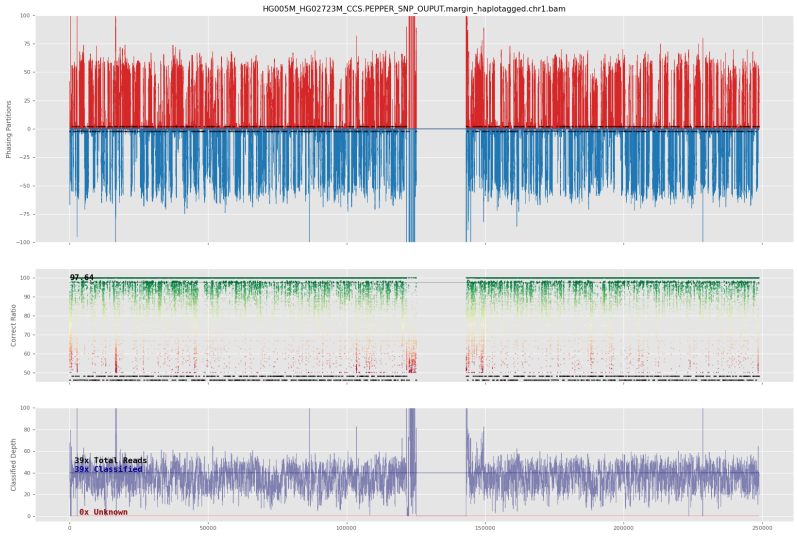
Supplementary Figure 2: HG003 ONT 90x candidate finding performance comparison between 10% heuristic based approach and PEPPER.



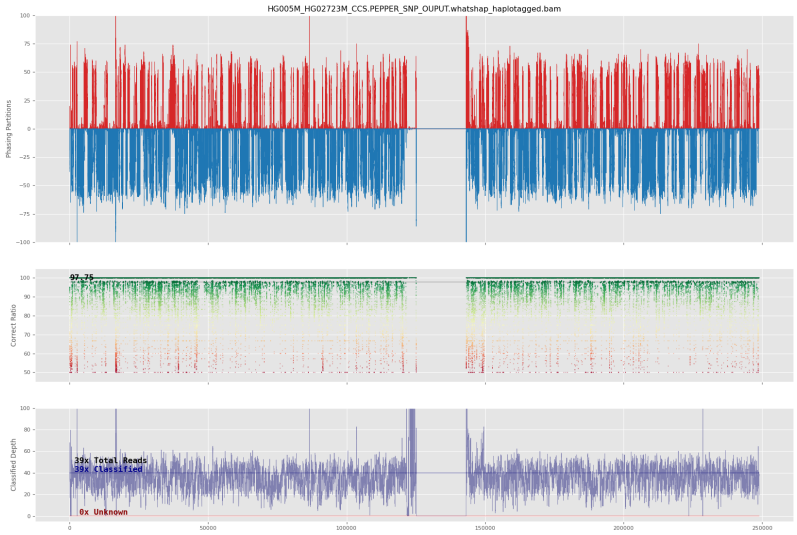
Supplementary Figure 3: Full Natural Switch plot for chr1 of an admixture of HG005 and HG02723's maternal haplotypes from nanopore data phased by Margin



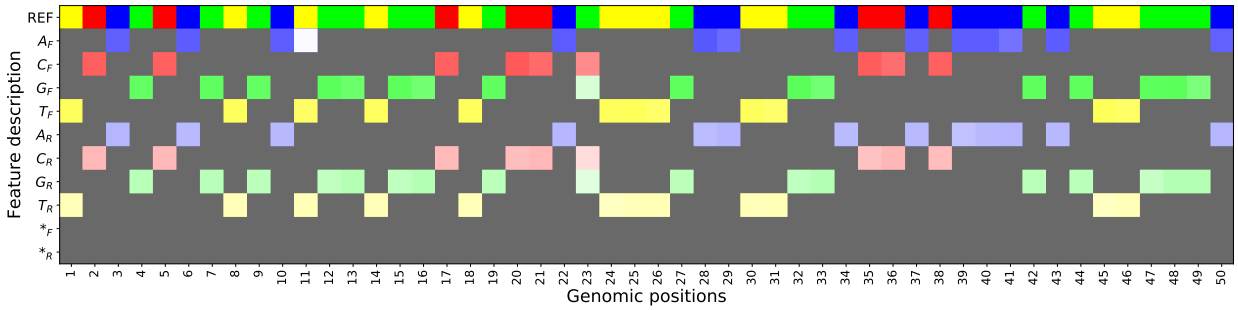
Supplementary Figure 4: Full Natural Switch plot for chr1 of an admixture of HG005 and HG02723's maternal haplotypes from nanopore data phased by WhatsHap



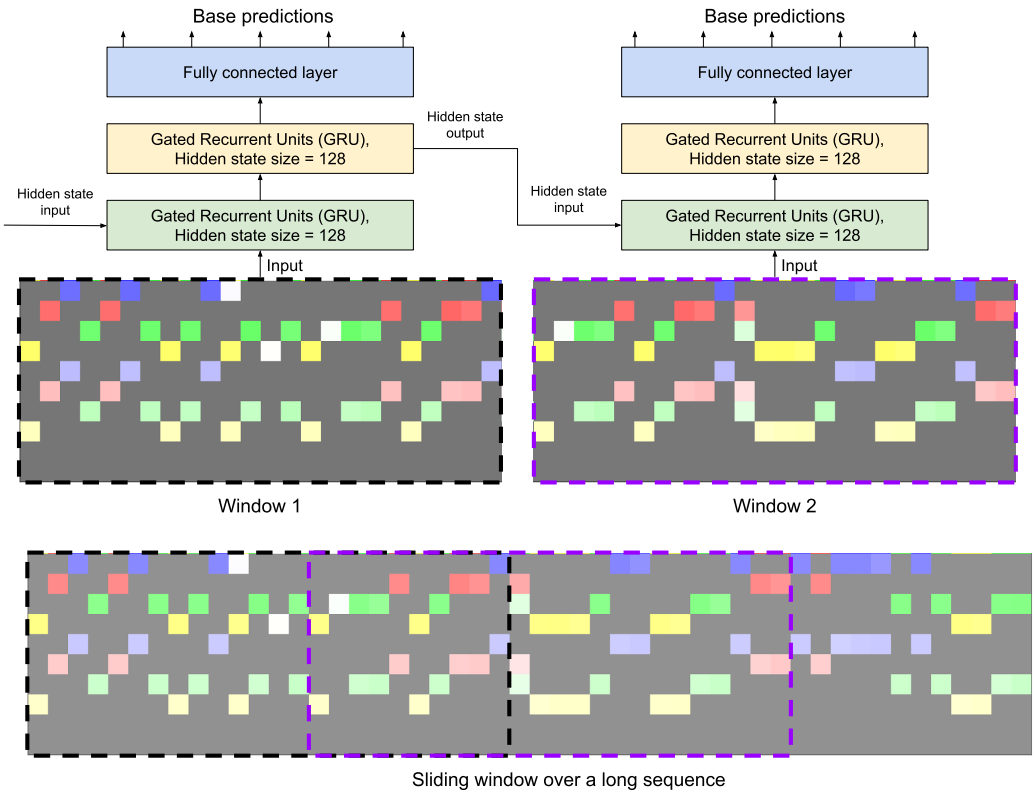
Supplementary Figure 5: Full Natural Switch plot for chr1 of an admixture of HG005 and HG02723's maternal haplotypes from PacBio HiFi data phased by Margin



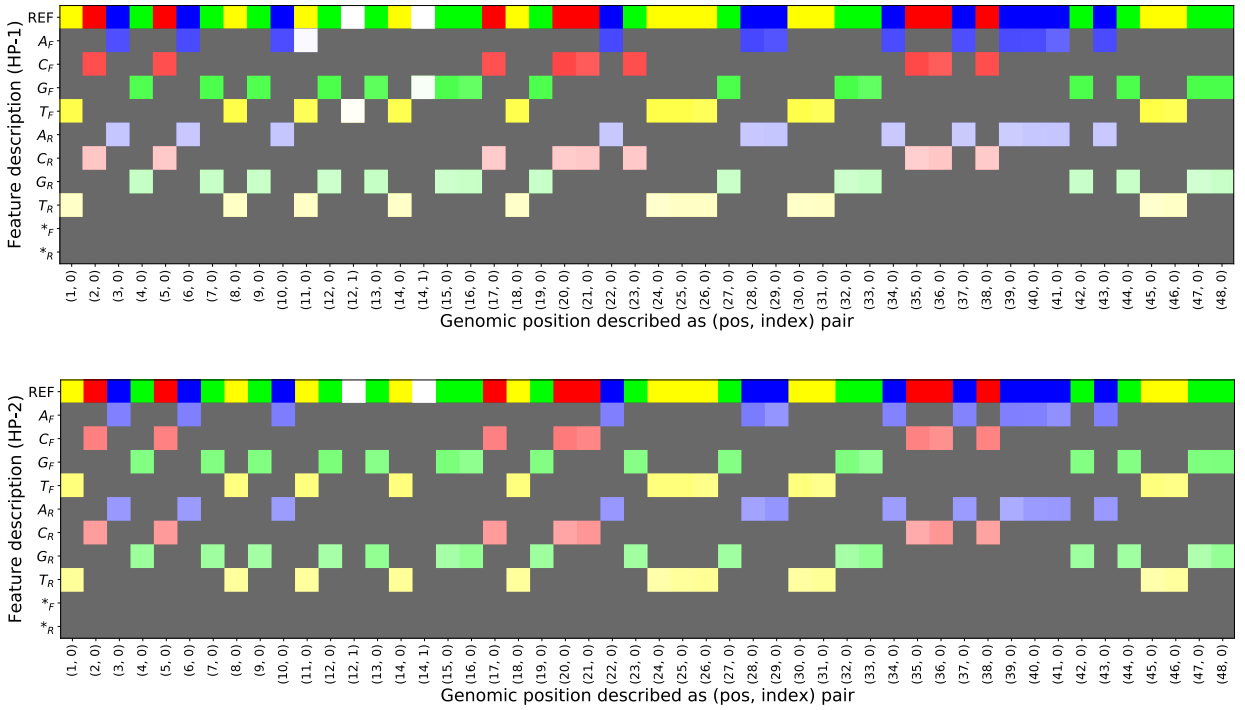
Supplementary Figure 6: Full Natural Switch plot for chr1 of an admixture of HG005 and HG02723's maternal haplotypes from PacBio HiFi data phased by WhatsHap



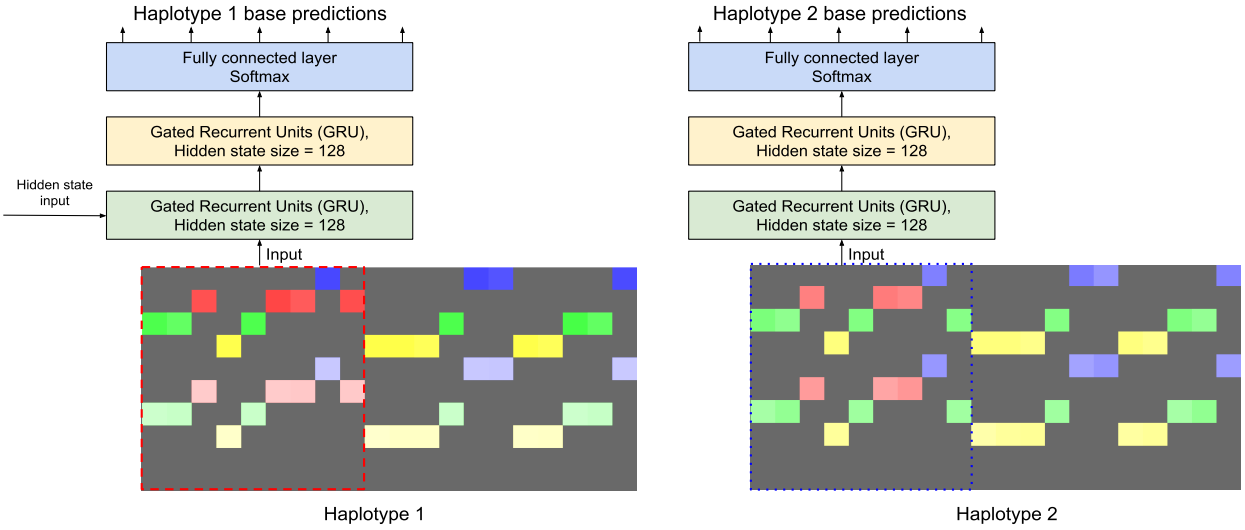
Supplementary Figure 7: PEPPER-SNP image generation scheme.



Supplementary Figure 8: PEPPER-SNP inference scheme.



Supplementary Figure 9: PEPPER-HP haplotype specific image generation scheme. Each row describes an encoded feature and each column describes a reference position. The top summary is derived from reads with haplotag 1 (HP-1) and the bottom is derived from reads with haplotag 2 (HP-2).



Supplementary Figure 10: PEPPER-HP haplotype-specific inference scheme.

Supplementary Results

Variant calling results

Sample Name	**Type**	**Method**	**True positives**	**False negatives**	**False positives**	**Recall**	**Precision**	**F1-score**
HG003	SNP	P-M-DV	3317032	10463	9958	0.9969	0.9970	0.9969
		Medaka	3293174	22716	26549	0.9931	0.9920	0.9926
		Clair	3266489	61006	31220	0.9817	0.9905	0.9861
		Longshot	3224643	102852	45780	0.9691	0.9860	0.9775
	INDEL	P-M-DV	303643	200858	29400	0.6019	0.9136	0.7257
		Medaka	313033	189639	69434	0.6227	0.8226	0.7089
		Clair	205364	299137	58367	0.4071	0.7812	0.5352
HG004	SNP	P-M-DV	3338882	7728	7474	0.9977	0.9978	0.9977
		Medaka	3286457	20743	23309	0.9937	0.9930	0.9933
		Clair	3285625	60985	32021	0.9818	0.9903	0.9860
		Longshot	3243183	103427	45387	0.9691	0.9862	0.9776
	INDEL	P-M-DV	300258	210261	32429	0.5881	0.9046	0.7128
		Medaka	306050	198390	88346	0.6067	0.7807	0.6828
		Clair	203825	306694	61454	0.3993	0.7708	0.5260

Supplementary Table 1: Oxford nanopore variant calling performance comparison between Medaka, Clair, Longshot and PEPPER-Margin-DeepVariant (P-M-DV) on HG003 and HG004 with 90× coverage.

Sample	Ref. GRC	GIAB version	Variant Type	Method	True pos.	False neg.	False pos.	Recall	Precision	F1-score
HG005	h37	v3.3.2	SNP	P-M-DV	3036676	5947	11807	**0.9980**	**0.9961**	**0.9971**
				Medaka	3026174	16361	21414	0.9946	0.9930	0.9938
				Clair	2982163	60460	68238	0.9801	0.9776	0.9789
				Longshot	2980529	62094	57698	0.9796	0.9810	0.9803
			INDEL	P-M-DV	258720	131438	30008	**0.6631**	**0.8981**	**0.7629**
				Medaka	264300	125852	66019	0.6774	0.8043	0.7354
				Clair	175540	214617	54654	0.4499	0.7650	0.5666
	h38	v4.2.1 (draft)	SNP	P-M-DV	3269767	7563	9624	**0.9977**	**0.9971**	**0.9974**
				Medaka	3256035	21207	26871	0.9935	0.9918	0.9927
				Clair	3210942	66388	73550	0.9797	0.9776	0.9787
				Longshot	3189831	87499	64687	0.9733	0.9801	0.9767
			INDEL	P-M-DV	278726	138235	30957	**0.6685**	**0.9019**	**0.7678**
				Medaka	284407	132546	68363	0.6821	0.8100	0.7406
				Clair	187276	229685	56443	0.4491	0.7706	0.5675

Supplementary Table 2: Oxford nanopore variant calling performance comparison between Medaka, Clair, Longshot and PEPPER-Margin-DeepVariant (P-M-DV) on HG005 sample between two reference (GRCh37 and GRCh38) and GIAB truth set (v3.3.2 and v4.2.1).

Confidence	Total Region Size	Paternal Concordance	Maternal Concordance	Concordance	Checked Records	Indeterminate Consistency	Mendelian Violations
GIAB High	2.504 Gb	$\frac{2899287}{2902160}$ (99.9%)	$\frac{2906561}{2909420}$ (99.9%)	$\frac{2257125}{2262783}$ (99.75%)	$\frac{3588024}{4791528}$ (74.88%)	$\frac{1323587}{3588024}$ (36.89%)	$\frac{7312}{3588024}$ (0.20%)
GIAB Low	0.315 Gb	$\frac{544760}{554736}$ (98.20%)	$\frac{536611}{548668}$ (97.80%)	$\frac{394059}{412533}$ (95.52%)	$\frac{775185}{1052572}$ (73.65%)	$\frac{356121}{775185}$ (45.94%)	$\frac{25005}{775185}$ (3.23%)

Supplementary Table 3: Mendelian consistency for HG005, HG006, HG007 trio in and out of GIAB high confidence regions v4.2.1 using `rtg mendelian` on GRCh38. "Checked records" denotes output from the tool with description "Records were variant in at least 1 family member and checked for Mendelian constraints", "Indeterminate Consistency" denotes output from the tool with description "Records had indeterminate consistency status due to incomplete calls", and "Mendelian Violoations" denotes output from the tool with description "Records contained a violation of Mendelian constraints". GIAB Low was generated by excluding GIAB's high confidence BED from GRCh38 as well as centromeric regions.

HG003 coverage	Method	True positives	False negatives	False positives	Recall	Precision	F1-score (INDEL)
10x	P-M-DV	169072	335430	248485	0.3351	0.4074	0.3677
	Medaka	147181	351183	2222020	0.2953	0.0631	0.1039
	Clair	78015	426486	31587	0.1546	0.7133	0.2542
20x	P-M-DV	239619	264882	96644	0.4750	0.7164	0.5712
	Medaka	229787	268549	182403	0.4611	0.5628	0.5069
	Clair	142647	361854	40955	0.2827	0.7785	0.4148
30x	P-M-DV	265318	239183	64214	0.5259	0.8083	0.6372
	Medaka	264877	233132	119190	0.5319	0.6949	0.6025
	Clair	167998	336503	44982	0.3330	0.7905	0.4686
40x	P-M-DV	278902	225599	51381	0.5528	0.8472	0.6691
	Medaka	284431	217108	103944	0.5671	0.7373	0.6411
	Clair	180964	323537	48517	0.3587	0.7905	0.4935
50x	P-M-DV	288480	216021	43169	0.5718	0.8723	0.6908
	Medaka	297390	206752	91135	0.5899	0.7702	0.6681
	Clair	189538	314963	51278	0.3757	0.7891	0.5090
60x	P-M-DV	294414	210087	38056	0.5836	0.8878	0.7042
	Medaka	301161	198584	82479	0.6026	0.7896	0.6835
	Clair	195079	309422	53275	0.3867	0.7877	0.5187
70x	P-M-DV	298553	205948	34079	0.5918	0.8997	0.7139
	Medaka	306842	194792	76519	0.6117	0.8048	0.6951
	Clair	199070	305431	55055	0.3946	0.7856	0.5253
80x	P-M-DV	301312	203189	31269	0.5972	0.9079	0.7205
	Medaka	309376	191507	72591	0.6177	0.8142	0.7024
	Clair	202551	301950	56606	0.4015	0.7840	0.5310
90x	P-M-DV	303643	200858	29400	0.6019	0.9136	0.7257
	Medaka	313033	189639	69434	0.6227	0.8226	0.7089
	Clair	205364	299137	58367	0.4071	0.7812	0.5352

Supplementary Table 4: Comparison on INDEL performance between Medaka, Clair and PEPPER-Margin-DeepVariant (P-M-DV) variant callers at different coverages of HG003 sample.

HG003 coverage	Method	True positives	False negatives	False positives	Recall	Precision	F1-score (SNP)
10x	P-M-DV	3015493	312002	2510007	0.9062	0.5458	0.6813
	Medaka	2998322	288605	8528164	0.9122	0.2602	0.4049
	Clair	2067633	1259862	585625	0.6214	0.7793	0.6914
20x	P-M-DV	3286124	41371	475385	0.9876	0.8736	0.9271
	Medaka	3228058	59716	369037	0.9818	0.8974	0.9377
	Clair	3026716	300779	229952	0.9096	0.9294	0.9194
30x	P-M-DV	3308068	19427	60871	0.9942	0.9819	0.9880
	Medaka	3248842	34884	59816	0.9894	0.9819	0.9856
	Clair	3194577	132918	121085	0.9601	0.9635	0.9618
40x	P-M-DV	3312504	14991	20633	0.9955	0.9938	0.9947
	Medaka	3279473	29155	39141	0.9912	0.9882	0.9897
	Clair	3237789	89706	80265	0.9730	0.9758	0.9744
50x	P-M-DV	3314808	12687	13806	0.9962	0.9959	0.9960
	Medaka	3298374	26404	33504	0.9921	0.9899	0.9910
	Clair	3254017	73478	58229	0.9779	0.9824	0.9802
60x	P-M-DV	3315655	11840	12062	0.9964	0.9964	0.9964
	Medaka	3271294	24807	30926	0.9925	0.9906	0.9916
	Clair	3260364	67131	46813	0.9798	0.9858	0.9828
70x	P-M-DV	3316257	11238	11217	0.9966	0.9966	0.9966
	Medaka	3283443	24010	28991	0.9927	0.9913	0.9920
	Clair	3263513	63982	39636	0.9808	0.9880	0.9844
80x	P-M-DV	3316750	10745	10219	0.9968	0.9969	0.9969
	Medaka	3280595	23263	27321	0.9930	0.9917	0.9924
	Clair	3265361	62134	34616	0.9813	0.9895	0.9854
90x	P-M-DV	3317032	10463	9958	0.9969	0.9970	0.9969
	Medaka	3293174	22716	26549	0.9931	0.9920	0.9926
	Clair	3266489	61006	31220	0.9817	0.9905	0.9861

Supplementary Table 5: Comparison on SNP performance between Medaka, Clair and PEPPER-Margin-DeepVariant (P-M-DV) variant callers at different coverages of HG003 sample.

File	Read N50	Gb	Coverage
HG001	21443	309.88	93.91
HG002	50317	160.39	48.6
HG003	44550	277.38	84.05
HG004	47996	284.32	86.16
HG005	49297	182.53	55.31
HG006	50019	163.87	49.66
HG007	50423	132.75	40.23

Supplementary Table 6: Sample-wise nanopore read coverage for seven Genome-In-A-Bottle (GIAB) samples.

Sample Name	Reference	Version	Regions covered by benchmark (bp)
HG001	GRCh37	v3.3.2	2437907771
HG002	GRCh38	v4.2.1	2542242843
HG003	GRCh38	v4.2.1	2528531102
HG004	GRCh38	v4.2.1	2524487531
HG005	GRCh37	v3.3.2	2376855757
HG006	GRCh37	v3.3.2	2393652163
HG007	GRCh37	v3.3.2	2394471248

Supplementary Table 7: Details of Genome-In-A-Bottle truth set used for each genome.

Sample	**Type**	**Total truth**	**True positives**	**False negatives**	**False positives**	**Recall**	**Precision**	**F1-score**
HG001	SNP	3209309	3203740	5569	11466	0.9983	0.9964	0.9973
	INDEL	481841	292565	189276	37718	0.6072	0.8883	0.7213
HG003	SNP	3327495	3317032	10463	9958	0.9969	0.9970	0.9969
	INDEL	504501	303643	200858	29400	0.6019	0.9136	0.7257
HG004	SNP	3346610	3338882	7728	7474	0.9977	0.9978	0.9977
	INDEL	510519	300258	210261	32429	0.5881	0.9046	0.7128
HG005	SNP	3042623	3036676	5947	11807	0.9980	0.9961	0.9971
	INDEL	390158	258720	131438	30008	0.6631	0.8981	0.7629
HG006	SNP	3053660	3047013	6647	13802	0.9978	0.9955	0.9967
	INDEL	394727	242909	151818	30518	0.6154	0.8901	0.7277
HG007	SNP	3069407	3060423	8984	18351	0.9971	0.9940	0.9956
	INDEL	397103	236816	160287	34295	0.5964	0.8753	0.7094

Supplementary Table 8: PEPPER-Margin-DeepVariant performance on six GIAB samples with Oxford nanopore data.

Sample	Variant Type	Method	True positives	False negatives	False positives	Recall	Precision	F1-score
HG003	SNP	P-M-DV	3317032	10463	9958	**0.9969**	**0.9970**	**0.9969**
		Medaka	3293174	22716	26549	0.9931	0.9920	0.9926
		PEPPER-HP	3311863	15632	27711	0.9953	0.9917	0.9935
	INDEL	P-M-DV	303643	200858	29400	**0.6019**	**0.9136**	**0.7257**
		Medaka	313033	189639	69434	0.6227	0.8226	0.7089
		PEPPER-HP	310722	193779	151334	0.6159	0.6784	0.6456
HG004	SNP	P-M-DV	3338882	7728	7474	**0.9977**	**0.9978**	**0.9977**
		Medaka	3286457	20743	23309	0.9937	0.9930	0.9933
		PEPPER-HP	3333967	12643	25961	0.9962	0.9923	0.9942
	INDEL	P-M-DV	300258	210261	32429	**0.5881**	**0.9046**	**0.7128**
		Medaka	306050	198390	88346	0.6067	0.7807	0.6828
		PEPPER-HP	307935	202584	190089	0.6032	0.6247	0.6137

Supplementary Table 9: Oxford nanopore variant calling performance comparison between PEPPER-HP (tuned for balanced precision and recall), Medaka and PEPPER-Margin-DeepVariant on HG003 and HG004 with 90× coverage.

Sample	Haplotype-aware pipeline	Runtime hh:mm:ss	Cost USD($)	INDEL F1-Score	SNP F1-Score
HG003	DeepVariant-Whatshap-DeepVariant	15:11:52	$36.24	0.9942	0.9990
	DeepVariant-Margin-DeepVariant	08:03:43	$36.71	0.9945	0.9991
	PEPPER-Margin-DeepVariant	05:55:28	$26.99	0.9944	0.9990
HG004	DeepVariant-Whatshap-DeepVariant	15:47:29	$37.35	0.9940	0.9992
	DeepVariant-Margin-DeepVariant	08:26:36	$38.45	0.9942	0.9992
	PEPPER-Margin-DeepVariant	05:58:57	$27.26	0.9941	0.9992

Supplementary Table 10: PacBio HiFi variant calling performance and runtime comparison between three haplotype-aware pipelines on 35× coverage HG003 and HG004 samples. For PEPPER, Margin and DeepVariant we used $4.56/h `n1-standard-96` and for WhatsHap we used $0.09/h `n1-standard-2` instance types on google cloud platform. The F1-scores are derived by comparing the variant calls against GIAB v4.2.1 benchmark variants for HG003 and HG004.

Sample	Method	CPUs	Memory	GPUs	Instance cost/h	Total runtime	Total cost
HG001 50x ONT	Longshot	16vCPUs	104 GB	-	$0.95	51:25:31	$48.84
	Clair	96vCPUs	360 GB	-	$4.56	02:30:05	$11.40
	Medaka	16vCPUs	104 GB	1x NVIDIA Tesla P100	$2.41	40:21:11	$97.24
		16vCPUs	104 GB	-	$0.95	95:14:01	$90.47
	PEPPER Margin DeepVariant	96vCPUs	360 GB	-	$4.56	12:59:19	$59.28
		96vCPUs	360 GB	4x NVIDIA Tesla P100	$10.4	6:41:56	$70
HG001 75x ONT	Longshot	32vCPUs	208 GB	-	$1.89	73:56:43	$139.73
	Clair	96vCPUs	360 GB	-	$4.56	03:05:46	$14.13
	Medaka	32vCPUs	206GB	2x NVIDIA Tesla P100	$4.81	46:58:11	$225.87
		32vCPUs	206GB	-	$1.50	116:41:04	$175.025
	PEPPER Margin DeepVariant	96vCPUs	360 GB	-	$4.56	14:44:40	$68.4
		96vCPUs	360 GB	4x NVIDIA Tesla P100	$10.4	9:05:01	$94.4

Supplementary Table 11: Run-time and cost analysis of Oxford nanopore-based variant calling pipelines on 50x and 75x HG001 data. We used various `n1-series` instance types available on Google Cloud Platform (GCP). The we calculated the cost using the GCP cost calculator (`https://cloud.google.com/products/calculator`). Logs of all the runs are publicly available (See supplementary Notes).

Sample name	Pipeline	Type	True positives	False negatives	False positives	Recall	Precision	F1-score
HG003 35x	DeepVariant	INDEL	499277	5224	4901	0.9896	0.9907	0.9902
		SNP	3323609	3886	2883	0.9988	0.9991	0.9990
	DV-WH-DV	INDEL	501509	2992	2935	0.9941	0.9944	0.9942
		SNP	3323655	3840	2734	0.9988	0.9992	0.9990
	DV-M-DV	INDEL	501567	2934	2746	**0.9942**	**0.9948**	**0.9945**
		SNP	3323586	3909	1841	**0.9988**	**0.9994**	**0.9991**
	P-M-DV	INDEL	501539	2962	2816	0.9941	0.9946	0.9944
		SNP	3323607	3888	2501	0.9988	0.9992	0.9990
HG004 35x	DeepVariant	INDEL	504939	5580	5217	0.9891	0.9902	0.9896
		SNP	3343142	3468	2274	0.9990	0.9993	0.9991
	DV-WH-DV	INDEL	507288	3231	2966	0.9937	0.9944	0.9940
		SNP	3343074	3536	1771	0.9989	0.9995	0.9992
	DV-M-DV	INDEL	507351	3168	2846	**0.9938**	**0.9946**	**0.9942**
		SNP	3342966	3644	1491	**0.9989**	**0.9996**	**0.9992**
	P-M-DV	INDEL	507313	3206	2903	0.9937	0.9945	0.9941
		SNP	3342928	3682	1721	0.9989	0.9995	0.9992

Supplementary Table 12: PacBio HiFi variant calling perfomance comparison between PEPPER-Margin-DeepVariant (P-M-DV), DeepVariant-WhatsHap-DeepVariant (DV-WH-DV), DeepVariant-Margin-DV (DV-M-DV), DeepVariant only.

Sample	Pipeline	SNP calling runtime	Phasing runtime	Variant calling runtime	Total runtime	Cost
HG003 35x PacBio HiFi	DeepVariant Whatshap DeepVariant	03:48:45 (n1-std-96)	07:32:35 (n1-std-2)	03:50:32 (n1-std-96)	15:11:52	$36.24
	DeepVariant Margin DeepVariant	03:48:45 (n1-std-96)	00:24:51 (n1-std-96)	03:50:07 (n1-std-96)	08:03:43	$36.71
	PEPPER Margin DeepVariant	1:28:49 (n1-std-96)	00:23:25 (n1-std-96)	4:03:14 (n1-std-96)	05:55:28	$26.99
HG004 35x PacBio HiFi	DeepVariant Whatshap DeepVariant	04:03:58 (n1-std-96)	07:44:56 (n1-std-2)	03:58:35 (n1-std-96)	15:47:29	$37.35
	DeepVariant Margin DeepVariant	04:03:58 (n1-std-96)	00:26:09 (n1-std-96)	03:56:29 (n1-std-96)	08:26:36	$38.45
	PEPPER Margin DeepVariant	1:25:03 (n1-std-96)	0:23:41 (n1-std-96)	4:10:13 (n1-std-96)	05:58:57	$27.26

Supplementary Table 13: PacBio HiFi variant calling run-time comparison between three haplotype-aware pipelines on 35× coverage HG003 and HG004 samples. We used $4.56/h `n1-standard-96 (n1-std-96)` and $0.09/h `n1-standard-2 (n1-std-2)` instance types on google cloud platform for this analysis.

Sample	Coverage	Method	True positives	False negatives	False positives	Recall	Precision	F1-score
HG003	26.5x	P-M-DV	3278007	20943	50763	0.9937	0.9847	0.9892
		Longshot	3220875	53788	107929	0.9836	0.9676	0.9755
HG004	10.9x	P-M-DV	2862862	208230	484946	0.9322	0.8551	0.8920

Supplementary Table 14: SNP Variant accuracy statistics for HG003 and HG004 against GIAB v4.2.1 on GRCh38 using PacBio CLR data

Sample	Variant type	Sequencing technology	True positives	False negatives	False positives	Recall	Precision	F1-score
HG003	SNP	Nanopore	3317032	10463	9958	0.9969	0.9970	0.9969
		Illumina	3307988	19508	4808	0.9941	0.9985	0.9963
		PacBio HiFi	3323607	3888	2501	0.9988	0.9992	0.9990
	INDEL	Nanopore	303643	200858	29400	0.6019	0.9136	0.7257
		Illumina	501546	2955	1276	0.9941	0.9976	0.9959
		PacBio HiFi	501539	2962	2816	0.9941	0.9946	0.9944
HG004	SNP	Nanopore	3338882	7728	7474	0.9977	0.9978	0.9977
		Illumina	3326040	20570	4476	0.9939	0.9987	0.9962
		PacBio HiFi	3342928	3682	1721	0.9989	0.9995	0.9992
	INDEL	Nanopore	300258	210261	32429	0.5881	0.9046	0.7128
		Illumina	507418	3101	1284	0.9939	0.9976	0.9958
		PacBio HiFi	507313	3206	2903	0.9937	0.9945	0.9941

Supplementary Table 15: Variant calling performance comparison in all benchmark regions between Oxford Nanopore Technology (ONT), Illumina NovaSeq (Illumina) and PacBio HiFi sequencing technology. Illumina variant calls are generated with DeepVariant v1.1 and ONT and PacBio HiFi variant calls are generated with PEPPER-Margin-DeepVariant.

Region	Sample	Platform	Total truth	True positives	False negatives	False positives	Recall	Precision	F1-score
MHC (SNP)	HG003	ONT	19543	19437	106	56	0.9946	0.9971	0.9958
		Illumina	19544	19336	208	31	0.9894	0.9984	0.9939
		PacBio	19543	19391	152	39	0.9922	0.9980	0.9951
	HG004	ONT	19271	19181	90	42	0.9953	0.9978	0.9966
		Illumina	19271	18998	273	31	0.9858	0.9984	0.9921
		PacBio	19271	19112	159	12	0.9917	0.9994	0.9955
Seg. Dup. (SNP)	HG003	ONT	121960	119838	2122	2288	0.9826	0.9813	0.9819
		Illumina	121960	112293	9667	2905	0.9207	0.9748	0.9470
		PacBio	121960	119003	2957	818	0.9758	0.9932	0.9844
	HG004	ONT	122191	120107	2084	1693	0.9829	0.9861	0.9845
		Illumina	122191	112296	9895	2710	0.9190	0.9764	0.9469
		PacBio	122191	119713	2478	672	0.9797	0.9944	0.9870
Low map. (SNP)	HG003	ONT	192520	190380	2140	2152	0.9889	0.9888	0.9889
		Illumina	192520	174763	17757	3627	0.9078	0.9797	0.9423
		PacBio	192520	189453	3067	888	0.9841	0.9953	0.9897
	HG004	ONT	192653	190671	1982	1634	0.9897	0.9915	0.9906
		Illumina	192653	174196	18457	3510	0.9042	0.9803	0.9407
		PacBio	192653	190118	2535	653	0.9868	0.9966	0.9917
250bp+ non-unique (SNP)	HG003	ONT	13608	12594	1014	592	0.9255	0.9552	0.9401
		Illumina	13608	7420	6188	1377	0.5453	0.8436	0.6624
		PacBio	13608	11613	1995	380	0.8534	0.9684	0.9072
	HG004	ONT	13492	12615	877	413	0.9350	0.9683	0.9514
		Illumina	13492	7235	6257	1323	0.5362	0.8455	0.6563
		PacBio	13492	11847	1645	284	0.8781	0.9766	0.9247

Supplementary Table 16: SNP performance in difficult-to-map regions with Illumina, PacBio HiFi and Oxford nanopore data.

Region	Sample	Platform	Total truth	True positives	False negatives	False positives	Recall	Precision	F1-score
H.poly. (7bp-11bp)	HG003	ONT	70736	68620	2116	2464	0.9701	0.9654	0.9677
		Illumina	70737	70632	105	60	0.9985	0.9992	0.9988
		PacBio	70736	70645	91	93	0.9987	0.9987	0.9987
	HG004	ONT	71141	68912	2229	2665	0.9687	0.9628	0.9657
		Illumina	71141	71045	96	39	0.9987	0.9995	0.9991
		PacBio	71142	71032	110	127	0.9985	0.9982	0.9983
H.Poly. 11bp+	HG003	ONT	12187	10708	1479	1359	0.8786	0.8879	0.8832
		Illumina	12188	12176	12	16	0.9990	0.9987	0.9989
		PacBio	12187	12005	182	203	0.9851	0.9841	0.9846
	HG004	ONT	12494	10751	1743	1441	0.8605	0.8823	0.8713
		Illumina	12494	12478	16	38	0.9987	0.9971	0.9979
		PacBio	12494	12332	162	182	0.9870	0.9861	0.9866
Di-Mer repeat (11bp-50bp)	HG003	ONT	18817	18322	495	668	0.9737	0.9654	0.9695
		Illumina	18817	18778	39	34	0.9979	0.9982	0.9981
		PacBio	18817	18763	54	120	0.9971	0.9939	0.9955
	HG004	ONT	18925	18417	508	676	0.9732	0.9652	0.9692
		Illumina	18925	18880	45	41	0.9976	0.9979	0.9978
		PacBio	18925	18873	52	109	0.9973	0.9945	0.9959
Tri-Mer repeat (15bp-50bp)	HG003	ONT	4179	4129	50	89	0.9880	0.9791	0.9835
		Illumina	4179	4172	7	3	0.9983	0.9993	0.9988
		PacBio	4179	4153	26	22	0.9938	0.9948	0.9943
	HG004	ONT	4213	4175	38	92	0.9910	0.9785	0.9847
		Illumina	4213	4210	3	2	0.9993	0.9995	0.9994
		PacBio	4213	4196	17	18	0.9960	0.9958	0.9959

Supplementary Table 17: SNP performance in low-complexity regions with Illumina, PacBio HiFi and Oxford nanopore data.

Region	Sample	Type	Total truth	Platform	F1-score	Recall	Precision	True positives	False neg.	False pos.
Not in all difficult regions (76% genome fraction)	HG003	SNP	2717833	Illumina	0.9997	0.9997	0.9997	2717119	713	796
				ONT	0.9988	0.9986	0.9989	2714093	3740	2857
				CCS	0.9999	0.9999	0.9998	2717567	265	416
		INDEL	155063	Illumina	0.9996	0.9995	0.9997	154988	74	54
				ONT	0.9719	0.9535	0.9910	147852	7211	1343
				CCS	0.9997	0.9997	0.9997	155023	42	39
	HG004	SNP	2732800	Illumina	0.9998	0.9997	0.9998	2732050	750	600
				ONT	0.9996	0.9996	0.9997	2731662	1138	903
				CCS	0.9999	0.9998	0.9999	2732330	470	322
		INDEL	156444	Illumina	0.9997	0.9996	0.9998	156385	59	31
				ONT	0.9700	0.9503	0.9905	148676	7768	1429
				CCS	0.9997	0.9996	0.9998	156388	59	38

Supplementary Table 18: Performance in not all difficult regions (easy regions) with Illumina, PacBio HiFi and Oxford nanopore data.

Region	Sample	Type	Total truth	Platform	F1-score	Recall	Precision	True pos.	False neg.	False pos.
Not in all tandem repeats and hom. (86% genome fraction)	HG003	SNP	3161145	Illumina	0.9962	0.9939	0.9985	3141934	19209	4642
				ONT	0.9981	0.9980	0.9982	3154668	6477	5534
				CCS	0.9992	0.9989	0.9996	3157593	3550	1396
		INDEL	184877	Illumina	0.9962	0.9937	0.9987	183717	1159	237
				ONT	0.9704	0.9516	0.9900	175933	8944	1784
				CCS	0.9991	0.9989	0.9994	184667	212	105
	HG004	SNP	3180117	Illumina	0.9961	0.9936	0.9986	3159831	20286	4291
				ONT	0.9990	0.9989	0.9991	3176662	3455	2774
				CCS	0.9993	0.9990	0.9997	3176818	3299	1068
		INDEL	186340	Illumina	0.9961	0.9935	0.9988	185125	1215	226
				ONT	0.9684	0.9479	0.9898	176633	9707	1825
				CCS	0.9992	0.9988	0.9995	186120	224	92
In all tandem repeats and hom. (4% genome fraction)	HG003	SNP	166352	Illumina	0.9986	0.9982	0.9990	166054	299	166
				ONT	0.9748	0.9760	0.9736	162364	3988	4425
				CCS	0.9976	0.9978	0.9974	165994	358	445
		INDEL	320072	Illumina	0.9957	0.9944	0.9969	318271	1800	1039
				ONT	0.5401	0.4002	0.8305	128093	191979	27622
				CCS	0.9918	0.9915	0.9922	317355	2719	2663
	HG004	SNP	166493	Illumina	0.9986	0.9983	0.9989	166209	284	185
				ONT	0.9731	0.9743	0.9720	162220	4273	4700
				CCS	0.9977	0.9979	0.9974	166147	346	440
		INDEL	324622	Illumina	0.9956	0.9942	0.9969	322730	1892	1059
				ONT	0.5193	0.3820	0.8108	123993	200629	30601
				CCS	0.9914	0.9908	0.9920	321655	2971	2774

Supplementary Table 19: Performance comparson of Illumina, PacBio HiFi and Oxford nanopore data in repeat and non-repeat regions.

Data	Tool	Phased Variants	Unphased Variants	Blocks	Median Variants per Block	Average Variants per Block	Median BP per Block	Average BP per Block	Block N50
ONT 25x	Margin	2293009	1008276	2536	347	904	503808	1056597	2067806
	WhatsHap	2452395	849215	2297	395	1068	523694	1177941	2372651
ONT 50x	Margin	2275697	875317	1376	613	1654	853602	1993709	4211518
	WhatsHap	2391670	759715	1172	822	2041	1049089	2355537	4900234
ONT 75x	Margin	2091713	1023259	1167	496	1792	769148	2372510	6126250
	WhatsHap	2393421	722297	812	955	2948	1167915	3430964	8266083
HiFi 35x	Margin	2327420	1035855	14069	15	165	48362	154095	242226
	WhatsHap	2412900	954503	14061	14	172	48362	155745	252972

Supplementary Table 20: Details of Margin and WhatsHap phasing output on HG001 sample with Oxford Nanopore (ONT) and PacBio HiFi data. Results are generated with `whatshap stats` command.

Data	Tool	Data	Tool	Assessed Pairs	Switches	Switch Rate	Hamming	Hamming Rate
ONT 25x	Margin	ONT 25x	Margin	1901418	16639	0.00875	162341	0.0854
	WhatsHap	ONT 25x	WhatsHap	1917571	17696	0.00923	177660	0.0926
ONT 50x	Margin	ONT 50x	Margin	1895721	16252	0.00857	195897	0.1033
	WhatsHap	ONT 50x	WhatsHap	1926257	17513	0.00909	161079	0.0836
ONT 75x	Margin	ONT 75x	Margin	1759253	14356	0.00816	174052	0.0989
	WhatsHap	ONT 75x	WhatsHap	1927665	17462	0.00906	179655	0.0932
HiFi 35x	Margin	HiFi 35x	Margin	1908770	17077	0.00895	24187	0.0127
	WhatsHap	HiFi 35x	WhatsHap	1914368	17801	0.00930	28973	0.0151

Supplementary Table 21: Comparison of Margin and WhatsHap phasesets of HG001 sample with Oxford Nanopore (ONT) and PacBio HiFi data. Comparison is performed with `whatshap compare` command.

Data	Tool	Average Accuracy	Average Reads per 1kb	Average Tagged Reads per 1kb
ONT 55x Chr1	Margin	96.26	56.9	56.9
	WhatsHap	95.71	56.9	56.9
CCS 35x Chr1	Margin	98.00	35.5	35.5
	WhatsHap	97.99	35.5	35.5

Supplementary Table 22: Haplotagging results comparing Margin and WhatsHap on an Admixed sample with an approximately equal amount of reads from the maternal haplotypes of HG005 and HG02723. Accuracy is determined for each kilobase bucket by comparing the number of direct-matched reads R_d (truth H1 to tagged H1 or truth H2 to tagged H2) and cross-matched reads R_c (truth H1 to tagged H2 or truth H2 to tagged H1) and calculating $\max(R_c, R_d)/(R_c + R_d)$, then averaging this value across all buckets in the HG003 high confidence regions.

Sample	Variant Type	Variant caller	True positives	False negatives	False positives	Recall	Precision	F1-score
HG003	SNP	P-M-DV	3317032	10463	9958	0.9969	0.9970	0.9969
		P-WH-DV	3316452	11043	11716	0.9967	0.9965	0.9966
	INDEL	P-M-DV	303643	200858	29400	0.6019	0.9136	0.7257
		P-WH-DV	301732	202769	29507	0.5981	0.9128	0.7227
HG004	SNP	P-M-DV	3338882	7728	7474	0.9977	0.9978	0.9977
		P-WH-DV	3338354	8256	10522	0.9975	0.9969	0.9972
	INDEL	P-M-DV	300258	210261	32429	0.5881	0.9046	0.7128
		P-WH-DV	298389	212130	32383	0.5845	0.9041	0.7100

Supplementary Table 23: Oxford Nanopore variant calling perfomance comparison between PEPPER-Margin-DeepVariant (P-M-DV) and PEPPER-WhatsHap-DeepVariant (P-WH-DV) only.

Data	Tool	Module	Max Threads	Max Memory	Runtime (min)	Instance Type	Instance Cost ($/hr)	Cost ($)
ONT 25x	Margin	Haplotag	64	20	21	n1-highcpu-64	2.267	0.79
		Phase VCF	64	15	15	n1-highcpu-64	2.267	0.56
		Total	–	–	36	n1-highcpu-64	2.267	1.36
	WhatsHap	Phase	2	3	347	n1-standard-2	0.095	0.54
		Haplotag	2	3	247	n1-standard-2	0.095	0.39
		Total	–	–	941	n1-standard-2	0.095	1.48
ONT 50x	Margin	Haplotag	64	28	54	n1-highcpu-64	2.267	2.04
		Phase VCF	64	18	30	n1-highcpu-64	2.267	1.13
		Total	–	–	84	n1-highcpu-64	2.267	3.17
	WhatsHap	Phase	2	3	446	n1-standard-2	0.095	0.7
		Haplotag	2	3	444	n1-standard-2	0.095	0.7
		Total	–	–	1336	n1-standard-2	0.095	2.11
ONT 75x	Margin	Haplotag	64	35	80	n1-highcpu-64	2.267	3.02
		Phase VCF	64	22	43	n1-highcpu-64	2.267	1.62
		Total	–	–	123	n1-highcpu-64	2.267	4.64
	WhatsHap	Phase	2	3	522	n1-standard-2	0.095	0.82
		Haplotag	2	3	644	n1-standard-2	0.095	1.01
		Total	–	–	1688	n1-standard-2	0.095	2.67
HiFi 35x	Margin	Haplotag	64	19	19	n1-highcpu-64	2.267	0.71
		Phase VCF	64	18	14	n1-highcpu-64	2.267	0.52
		Total	–	–	33	n1-highcpu-64	2.267	1.24
	WhatsHap	Phase	2	3	277	n1-standard-2	0.095	0.43
		Haplotag	2	3	210	n1-standard-2	0.095	0.33
		Total	–	–	764	n1-standard-2	0.095	1.2

Supplementary Table 24: Margin/WhatsHap Runtimes. Total runtimes are sum of Haplotag and Phase VCF runtimes for Margin, and sum of 2x Phase and 1x Haplotag for WhatsHap, as `whatshap haplotag` requires a phased VCF.

Type	Data	Gene Type	Subset	Recall	Precision	F1 Score
SNP	Nanopore	all regions	all regions	0.998169	0.996314	0.997241
		all genes	all genes	0.998097	0.996481	0.997289
		protein coding	all cds	0.998641	0.997887	0.998263
			all exons	0.998158	0.997675	0.997916
			all genes	0.99799	0.996751	0.99737
	PacBio HiFi	all regions	all regions	0.999391	0.998062	0.998726
		all genes	all genes	0.999384	0.998197	0.99879
		protein coding	all cds	0.999446	0.998994	0.99922
			all exons	0.999502	0.999117	0.99931
			all genes	0.999382	0.998441	0.998912
INDEL	Nanopore	all regions	all regions	0.60077	0.878512	0.713567
		all genes	all genes	0.595042	0.877943	0.709325
		protein coding	all cds	0.799544	0.926893	0.858522
			all exons	0.632435	0.896594	0.741696
			all genes	0.584914	0.876731	0.701692
	PacBio HiFi	all regions	all regions	0.948736	0.92602	0.937241
		all genes	all genes	0.947847	0.922887	0.935201
		protein coding	all cds	0.984055	0.909278	0.94519
			all exons	0.955149	0.927624	0.941186
			all genes	0.946128	0.918991	0.932362

Supplementary Table 25: Accuracy stats for ONT and CCS calls made on GRCh37 with HG001 data in high confidence regions against GIAB v3.3.2 stratified by all gene and protein coding gene, further stratified by whole gene, exon, CDS as annotated by GENCODE v35lift37. CDS regions are coding sequences, and include start and stop codons for this analysis.

Gene Region	Subset	Subset Size	Subset High Confidence Size	High Confidence Ratio	High Confidence Whole Genome Ratio
Genome	–	2951332653	2579466415	0.874001	0.874001
All Genes	–	1982798080	1591767788	0.802789	0.539339
Protein Coding	Coding Sequence	114906140	31986772	0.278373	0.010838
Protein Coding	Exon	283314507	92457254	0.326341	0.031327
Protein Coding	Gene Regions	1367165648	1201166019	0.878581	0.406991

Supplementary Table 26: Size of GENCODE Gene Regions

Phasing Coverage	Switch Errors Present	SNP, INDEL Errors	Gene Region	Count	25 Quartile Gene Size	Median Gene Size	75 Quartile Gene Size
wholly	no error	no error	gene	1738	1438	3332	8005
			exon	3121	2845	10303	34848
			cds	3481	3163	11478	39123
		error	gene	1764	15676	35729	80335
			exon	381	7533	27218	67507
			cds	21	3161	5190	31102
	error	no error	gene	15	1685	4868	15383
			exon	33	8703	19682	86981
			cds	37	8703	20549	72326
		error	gene	23	18072	63546	107182
			exon	5	7680	47627	63546
			cds	1	7680	7680	7680
partially	no error	no error	gene	6	4760	10482	36920
			exon	29	25339	102612	147661
			cds	37	25339	102612	161893
		error	gene	31	44745	110698	223634
			exon	8	54807	99792	318869
			cds	0	–	–	–
	error	all	all	0	–	–	–
not	–	no error	gene	125	1769	3060	8819
			exon	201	2352	8744	29504
			cds	214	2478	9365	29958
		error	gene	91	19316	33429	69811
			exon	15	10690	23836	35712
			cds	2	44794	53073	61351

Supplementary Table 27: Gencode protein coding genes with coding sequence (CDS, start_codon, and stop_codon) 80% spanned by high confidence stratified by how phased it is by Margin, whether there were switch errors, whether there were SNP or INDEL errors, and gene region for HG001 with 75x Nanopore data on GRCh37. Three gene length quartiles are presented for the groupings.

Phasing Coverage	Switch Errors Present	SNP, INDEL Errors	Gene Region	Count	25 Quartile Gene Size	Median Gene Size	75 Quartile Gene Size
wholly	no error	no error	gene	2086	2184	5907	17294
			exon	2446	2586	8471	27219
			cds	2474	2615	8536	27351
		error	gene	390	23230	51309	102861
			exon	30	7098	15068	65957
			cds	2	60929	108132	155335
	error	no error	gene	18	1690	6798	11879
			exon	23	2321	8703	23001
			cds	24	2567	9247	19731
		error	gene	6	13297	23861	43535
			exon	1	12242	12242	12242
			cds	0	–	–	–
partially	no error	no error	gene	190	13338	34319	67099
			exon	354	28087	68206	146543
			cds	360	28289	68510	145331
		error	gene	170	76695	137702	255101
			exon	6	55573	75267	104898
			cds	0	–	–	–
	error	no error	gene	2	20915	21281	21647
			exon	5	22014	109387	127176
			cds	5	22014	109387	127176
		error	gene	3	118281	127176	138247
			exon	0	–	–	–
			cds	0	–	–	–
not	no error	no error	gene	741	2565	8419	23809
			exon	917	3533	13187	43784
			cds	928	3639	13351	43785
		error	gene	187	30101	75166	156763
			exon	11	10504	29953	68614
			cds	0	–	–	–

Supplementary Table 28: Gencode protein coding genes with coding sequence (CDS, start_codon, and stop_codon) 80% spanned by high confidence stratified by how phased it is by Margin, whether there were switch errors, whether there were SNP or INDEL errors, and gene region for HG001 with 35x PacBio HiFi data on GRCh37. Three gene length quartiles are presented for the groupings.

Sample	Assembler	Polisher	Assembly haplotype	NG50	Estimated QV YAK (k=31)	Switch error rate	Hamming error
HG005	Flye	-	Haploid	37254637	31.08	0.146333	0.319502
	Shasta	-	Haploid	39831103	32	0.16431	0.293283
		P-M-DV (ONT)	HP-1	39820763	35.06	0.058178	0.207221
			HP-2	39820481	35.06	0.059199	0.218271
		P-M-DV (PacBio HiFi)	HP-1	39808277	43.54	0.028165	0.26687
			HP-2	39809097	43.5	0.028253	0.264903
	Trio-hifiasm	-	mat	51324672	51.81	0.007056	0.009601
			pat	50669010	51.72	0.003106	0.004542
HG00733	Flye	-	Haploid	36602095	31.93	0.226708	0.455478
	Shasta	-	Haploid	42512208	32.7	0.263731	0.4387
		P-M-DV (ONT)	HP-1	42497702	35.83	0.09903	0.319373
			HP-2	42498275	35.84	0.098502	0.320807
		P-M-DV (PacBio HiFi)	HP-1	42475072	43.83	0.050551	0.401146
			HP-2	42476106	43.85	0.049385	0.406129
	Trio-hifiasm	-	mat	32479553	53.6	0.0102	0.012044
			pat	35318917	53.35	0.010144	0.010069
HG02723	Flye	-	Haploid	39652856	31.88	0.24692	0.454764
	Shasta	-	Haploid	49185987	32.52	0.28754	0.424615
		P-M-DV (ONT)	HP-1	49165039	35.8	0.104264	0.248018
			HP-2	49164831	35.79	0.103455	0.238674
		P-M-DV (PacBio HiFi)	HP-1	49146102	43.46	0.046367	0.365246
			HP-2	49143792	43.38	0.046215	0.363784
	Trio-hifiasm	-	mat	19737990	56.27	0.005794	0.007677
			pat	22214675	55.94	0.006683	0.009111

Supplementary Table 29: Diploid assembly polishing results of PEPPER-Margin-DeepVariant (P-M-DV) pipeline on HG005, HG00733 and HG02723 samples. We report estimated quality value (QV), switch error rate and hamming error using YAK assembly assessment tool.

Sample	Assembler	Polisher	Estimated QV YAK (k=31)
CHM13 chrX	Flye	-	32.85
	Shasta	-	34.601
		P-M-DV (ONT)	36.91
		P-M-DV (PacBio HiFi)	42.765
	Hifiasm	-	53.039

Supplementary Table 30: Haploid assembly polishing results of PEPPER-Margin-DeepVariant (P-M-DV) pipeline on CHM13-chrX. We report estimated quality value (QV) using YAK assembly assessment tool.

Genome	Test SV set	Truth SV set	Hom. SV recall	Het. SV recall	Precision
HG002	Shasta	GIAB curated	94.4%	46.7%	80.1%
	PEPPER-Margin-DV		94.9%	49.0%	81.1%
	hifiasm		97.8%	97.0%	93.0%
	Shasta	hifiasm	95.2%	48.7%	83.2%
	PEPPER-Margin-DV		96.0%	50.9%	84.7%
HG005	Shasta	hifiasm	93.7%	49.6%	82.3%
	PEPPER-Margin-DV		95.2%	51.7%	84.0%
HG00733	Shasta	hifiasm	95.1%	47.7%	80.1%
	PEPPER-Margin-DV		95.9%	49.7%	81.7%
HG02733	Shasta	hifiasm	94.6%	48.7%	80.7%
	PEPPER-Margin-DV		95.8%	51.3%	82.4%

Supplementary Table 31: Evaluation of the accuracy and completeness of SV reconstruction of Shasta and PEPPER-Margin-DeepVariant assemblies. Recall and precision were computed using the SVbenchmark tool inside the Tier1 high-confidence regions defined in the HG002 curated set of SVs. Since the set of curated SVs was only available for the HG002 genome, for the remaining genomes SVs recovered from the hifiasm assemblies were used as reference.