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Variant calling on the GRCh38 assembly with the data from phase three of the 1000 Genomes Project [version 1; referees: awaiting peer review]

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Abstract
We present biallelic SNVs called from 2,548 samples across 26 populations from the 1000 Genomes Project, called directly on GRCh38. We believe this will be a useful reference resource for those using GRCh38, representing an improvement over the "lift-overs" of the 1000 Genomes Project data that have been available to date and providing a resource necessary for the full adoption of GRCh38 by the community. Here, we describe how the call set was created and provide benchmarking data describing how our call set compares to that produced by the final phase of the 1000 Genomes Project on GRCh37.

Keywords
Genomics, population genetics, variant calling, single nucleotide variation, variant discovery

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Author roles: Lowy-Gallego E: Data Curation, Formal Analysis, Methodology, Software, Validation, Writing – Original Draft Preparation; Fairley S: Methodology, Project Administration, Supervision, Writing – Review & Editing; Zheng-Bradley X: Data Curation, Software; Ruffier M: Supervision; Clarke L: Conceptualization, Funding Acquisition, Project Administration, Supervision; Flicek P: Conceptualization, Funding Acquisition, Supervision;

Competing interests: No competing interests were disclosed.

Grant information: This work was completed thanks to the funding from the Wellcome Trust (grant number 104947) and the European Molecular Biology Laboratory. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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How to cite this article: Lowy-Gallego E, Fairley S, Zheng-Bradley X et al. Variant calling on the GRCh38 assembly with the data from phase three of the 1000 Genomes Project [version 1; referees: awaiting peer review] Wellcome Open Research 2019, 4:50 (https://doi.org/10.12688/wellcomeopenres.15126.1)

First published: 11 Mar 2019, 4:50 (https://doi.org/10.12688/wellcomeopenres.15126.1)
Introduction

The 1000 Genomes Project started in 2008 with the aim of producing a deep catalogue of human genomic variation and, for this, more than 2600 samples from 26 different populations were sequenced. The project completed its final phase (named phase three) in 2015, with the release of more than 85 million variants of various types and phased haplotypes for those variants. This data has been widely used by the scientific community for genotype imputation and many other applications. The strategy adopted by the project consisted of sequencing samples using whole genome sequencing (WGS) and whole exome sequencing (WES), and the alignment of that sequence data to a version of the GRCh37 human reference genome, which included decoy sequences for optimal read mapping.

While the 1000 Genomes Project was based on GRCh37, the latest version of the human reference assembly (GRCh38) was released by the Genome Reference Consortium (GRC) in 2013 and is the current best representation of the human genome available [PMID: 28396521]. This updated assembly has the following fundamental improvements with respect to its predecessors:

- corrects thousands of small sequencing artifacts and misassembled regions in addition to filling or reducing more than 100 gaps
- includes synthetic centromeric sequences that previously were represented in the reference by gaps of three million base pairs
- improves the diversity of the reference by including new alternate sequences, to address the fact that some genomic regions are highly variable

By improving the reference genome, GRCh38 improves the foundation for calling variation by providing both a more accurate and more diverse representation of the genome, thereby enabling better read mapping and reducing opportunities for erroneous variation calls.

To make full use of GRCh38, there has been a need for widely used genomic reference data sets, like the 1000 Genomes data, to be made available on the assembly, so that pipelines and analyses that rely on such additional reference materials can use GRCh38 and benefit from its improvements.

dbSNP have facilitated the use of the 1000 Genomes variation data on GRCh38 by “lifting-over” the calls, using a method relying on an alignment created between GRCh37 and GRCh38. The alignment is then used to determine equivalent locations between the two assemblies, allowing variation data to be “lifted-over”. Files from dbSNP are reformatted into a standard VCF by the European Variation Archive (EVA) and shared as part of our resources through the 1000 Genomes FTP site and also via the Ensembl genome browser [PMID: 30407521].

Lift-over approaches, however, have several limitations. 1) Necessarily, they rely on an equivalent region existing in the new genome, so new sequence in the improved assembly is effectively excluded. 2) Reliable transfer requires a good mapping between the assemblies, covering not just a given variation but the context that was used to make that call—it may be possible to “lift-over” a SNP where the data supporting the original call would not lift-over. Where the context of a call alters, the data becomes less reliable. 3) While lift-overs can give an approximation of the variant sites on the new assembly the results will differ from calling directly on the new assembly, the latter taking advantage of the increased representation of genomic sequence, assembly corrections and making calls from the underlying read data in context. With the above in mind, and given that the 1000 Genomes data is a heavily used resource, we decided to create a new call set from alignments of the original 1000 Genomes read data to GRCh38.

The first step was alignment of the 1000 Genomes sequence data to the GRCh38 as previously described. These alignments were taken as the starting point in creating the variation calls described in this data note.

To generate calls from the 1000 Genomes data, we adopted a multi-caller approach, aiming to produce a similar quality reference call set to that produced by the 1000 Genomes Project while using a simpler methodology, reflecting both practical considerations and the improved understanding of the process developed by the 1000 Genomes Project itself.
Four supporting call sets were created, using different callers and combinations of the exome and WGS sequence data.

For the final call set, biallelic SNVs (single nucleotide variants) only were selected from the four supporting call sets. These represent the major part of the SNVs present in the human genome.

The inclusion of only biallelic SNVs generates a data set useful for many purposes, while enabling more streamlined data processing than is possible when handling indels and multi-allelic variants. We are thus able to share what we believe to be a useful data set while planning to revisit our supporting call sets and, in future, produce updated call sets including a broader spectrum of variation.

**Methods**

**Input data**

The methods used for sample collection, library construction, and sequencing are described in the previous 1000 Genomes Project publications. The read data used for this analysis followed the same criteria as the final phase of the 1000 Genomes Project, namely only sequence data generated by Illumina sequencing and only reads longer than 70 bp (WGS) and 68 bp (WES). This data was aligned to GRCh38 as previously described. The complete list of the whole genome and whole exome sequencing alignment files used as the input for generating the callsets can be found on our FTP site at ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/data_collections/1000_genomes_project/1000genomes.low_coverage.GRCh38DH.alignment.index and at ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/data_collections/1000_genomes_project/1000genomes.exome.GRCh38DH.alignment.index.

**Reference genome**

We used the full GRCh38 reference, including ALT contigs, decoy and EBV sequences (accession GCA_000001405). In addition, more than 500 HLA sequences compiled by Heng Li from the IMGT/HLA database provided by the Immuno Polymorphism Database (IPD) [PMID: 27899604] are included. The reference genome can be accessed at ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/technical/reference/GRCh38_reference_genome/.

**Ethical considerations**

Information concerning ethical approval and the informed consent procedure for the 1000 Genomes project can be found here.

**Quality control of the alignment files**

We adopted a similar quality control process to that used in the final phase of the 1000 Genomes Project. Chk_indel_rg was applied to discard alignment files with an unbalanced ratio of short insertions and deletions (greater than 5). Picard CollectWgsMetrics was used with the whole genome files and those with mean non-duplicated aligned coverage level ≤2x were discarded. In the case of the exome files, we used Picard CollectHsMetrics using the exome target coordinates that can be found at ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/data_collections/1000_genomes_project/working/20190125_coords_exon_target/, and we kept the files having more than 70% of the target regions covered by 20x or greater of sequence reads.

In addition, VerifyBAMID was used to assess sample contamination and sample mix-ups and the following cutoffs were used:

- free_mix > 0.03 and chip_mix > 0.02 for whole genome files
- free_mix > 0.035 and chip_mix > 0.02 for exome files

Only files passing the quality assessment were used in subsequent variant calling.

**Variant discovery**

A total of 2,659 WGS and 2,498 WES BAMs were generated corresponding to 2,698 samples were used for variant identification. Figure 1 details the analysis of the alignment files with three established methods (BCFtools version 1.3.1-220-g9f38991, Freebayes version v1.0.2-58-g054b257 and GATK UnifiedGenotyper version 3.5-0-g36282e4). BCFtools was used to analyse WGS and WES files in two independent runs, GATK UnifiedGenotyper
was used only with WGS files and Freebayes was used to analyse everything together (WGS+WES). The following command lines were used for each of the methods:

- **BCFtools with the WGS files:**
  
  ```bash
  bcftools mpileup -E -a DP -a SP -a AD -P ILLUMINA -pm3 -F0.2 -C50 -d 700000 -f $ref.fa $file.bam | bcftools call -mv -O z --ploidy GRCh38 -S $samples.ped -o $out.vcf.gz
  ```

- **GATK UnifiedGenotyper with the WGS files:**
  
  ```java
  java -Xmx6g -jar GenomeAnalysisTK.jar -T UnifiedGenotyper -R $ref.fa -I $file.bam -o Out.vcf.gz -dcov 250 -stand_emit_conf 10 -glm both --genotyping_mode GENOTYPE GIVEN ALLELES --dbsnp ALL_20141222.dbSNP142_human_GRCh38.snps.vcf.gz -stand_call_conf 10
  ```

- **BCFtools with the WES files:**
  
  ```bash
  bcftools mpileup -E -a DP -a SP -a AD -P ILLUMINA -pm3 -F0.2 -C50 -d 1400000 -f $ref.fa $file.bam | bcftools call -mv -O z --ploidy GRCh38 -S $samples.ped -o $out.vcf.gz
  ```

- **Freebayes with the WGS+WES files:**
  
  ```bash
  freebayes --genotyping-max-iterations 10 --min-alternate-count 3 --max-coverage 2000000 --min-mapping-quality 1 --min-alternate-coverage 50 --min-base-quality 3 -f $ref.fa -b $file.bam | bgzip -c > $out.vcf.gz
  ```
Variant filtering

Our variant discovery pipeline produced four initial call sets as described above. To create the final call set, we discarded the variants falling in the centromeres, as these are regions of low complexity that hinder variant calling. Variants on the chromosome Y or in regions of the chromosome X not corresponding to the pseudoautosomal regions (PAR) were also discarded due to the ploidy settings used in this work. Additionally, the initial call sets contained spurious variants filtered using different methods and parameters depending on the call set:

**GATK UnifiedGenotyper call set.** We used the VariantScoreRecalibration (VQSR) method following the GATK best practices and GATK training call sets. The combination of commands and parameters we used were different depending on the the variant type being analysed. For SNPs we used GATK VariantRecalibrator and ApplyRecalibration as follows:

```java
java -jar GenomeAnalysisTK.jar \
-T VariantRecalibrator \\
-R $ref.fa \ 
-input $file.vcf.gz \ -resource:hapmap,known=false,training=true,truth=true ,prior=15.0 hapmap_3.3.hg38.vcf.gz \ -resource:omni,known=false,training=true,truth=true ,prior=12.0 1000G_omni2.5.hg38.vcf.gz \ -resource:1000G,known=false,training=true,truth=false,prior=10.0 1000G_phase1.snps.high_confidence. hg38.vcf.gz \ -resource:dbsnp,known=true,training=false,truth=false,prior=2.0 dbsnp_146.hg38.vcf.gz \ -an DP \ -an QD \ -an FS \ -an SOR \ -an MQ \ -an MQRankSum \ -an ReadPosRankSum \ -an InbreedingCoeff \ -mode SNP \ -tranche 100.0 -tranche 99.0 -tranche 99.0 -tranche 98.0 -tranche 97.0 -tranche 96.0 -tranche 95.0 -tranche 92.0 -tranche 90.0 -tranche 85.0 -tranche 80.0 -tranche 75.0 -tranche 70.0 -tranche 65.0 -tranche 60.0 -tranche 55.0 -tranche 50.0 \ -recalFile recalibrate_SNP.recal \ -tranchesFile recalibrate_SNP.tranches \ -rscriptFile recalibrate_SNP_plots.R
```

And:

```java
java -jar GenomeAnalysisTK.jar \
-T ApplyRecalibration \ 
-R $ref.fa \ 
-input $file.vcf.gz \ 
-mode SNP \ 
--ts_filter_level 99.9 \ 
-recalFile recalibrate_SNP.recal \ 
-tranchesFile recalibrate_SNP.tranches | bgzip -c > recalibrated_snps_raw_indels.vcf.gz
```

And for INDELs we used:

```java
java -jar GenomeAnalysisTK.jar \
-T VariantRecalibrator \\
-R $ref.fa \ 
-input recalibrated_snps_raw_indels.vcf.gz \ -resource:mills,known=false,training=true,truth=true,prior=12.0 Mills_and_1000G_gold_standard.indels.hg38.vcf.gz \ -resource:dbsnp,known=true,training=false,truth=false,prior=2.0 dbsnp_146.hg38.vcf.gz \ -an QD \ -an DP \ -an FS
```
And:
java -jar GenomeAnalysisTK.jar 
-T ApplyRecalibration 
-R $ref.fa 
-input recalibrated_snps_raw_indels.vcf 
-mode INDEL 
--ts_filter_level 80.0 
-recalFile recalibrate_INDEL.recal 
-tranchesFile recalibrate_INDEL.tranches 
-rscriptFile recalibrate_INDEL_plots.R 
--maxGaussians 4

BCFTools call sets. We compared the distribution of the values for different variant annotations in the set of true positive and false positive sites and established the set of variant annotations and cutoff values used in the filtering. We considered true positives the sites identified in our call set for genome NA12878 that were also present in the gold-standard call set generated for the same sample by Genome in a Bottle (GIAB). GIAB’s calls for NA12878 are the result of an effort to integrate data generated by 13 different sequencing technologies and analysis methods. Sites that were present in our call sets and absent in GIAB were considered false positive sites. Table 1 and Table 2 show the variant annotations and cutoff values used for the SNPs and INDELs with the low coverage data and Table 3 and Table 4 show the annotations and cutoff values used for the exome data with the SNPs and INDELs respectively. These cutoff values were applied using the following command:

- SNPs from the low coverage data:
bcftools filter -s GIABFILTER 
-e 'INFO/DP>24304 | MQ<34 | MQ0F>0.049737 | HOB>0.1643732 | SGB>2347.043 | SGB<-64440.286 | QUAL<20' "$file.snps.vcf.gz" 
-o $out.snps.filtered.vcf.gz -O z

- INDELs from the low coverage data:
bcftools filter -s GIABFILTER 
-e 'INFO/DP>23758 | MQ<41 | MQ0F>0.009913696 | HOB>0.20265508 | SGB>2143.8876 | SGB<-29513.557 | IDV>51 | IMF<0.387097 | QUAL<20' "$file.indels.vcf.gz" 
-o $out.indels.filtered.vcf.gz -O z

- SNPs from the exome data:
bcftools filter -s GIABFILTER 
-e 'INFO/DP>656519 | MQ<41 | MQ0F>0.0146629 | HOB>0.1536016 | SGB>57489.21 | SGB<-226326.93 | QUAL<20' "$file.snps.vcf.gz" 
-o $out.snps.filtered.vcf.gz -O z

- INDELs from the exome data:
bcftools filter -s GIABFILTER 
-e 'MQ<45 | MQ0F>0.002034686 | HOB>0.269603 | SGB>53165.5 | SGB<-85919.729 | IMF<0.3323922 | QUAL<20' "$file.indels.vcf.gz" 
-o $out.indels.filtered.vcf.gz -O z
## Table 1. Variant annotations and cutoff values used for SNPs identified using the low coverage data.

<table>
<thead>
<tr>
<th>Annotation</th>
<th>Description</th>
<th>Cutoff value</th>
</tr>
</thead>
<tbody>
<tr>
<td>INFO/DP</td>
<td>Raw read depth</td>
<td>&gt;24,304</td>
</tr>
<tr>
<td>INFO/MQ</td>
<td>Average mapping quality</td>
<td>&lt;34</td>
</tr>
<tr>
<td>INFO/MQ0F</td>
<td>Fraction of MQ0 reads (smaller is better)</td>
<td>&gt;0.049737</td>
</tr>
<tr>
<td>INFO/HOB</td>
<td>Bias in the number of HOMs number (smaller is better)</td>
<td>&gt;0.1643732</td>
</tr>
<tr>
<td>INFO/SGB</td>
<td>Segregation based metric</td>
<td>&gt;2347.043</td>
</tr>
<tr>
<td>INFO/SGB</td>
<td>Segregation based metric</td>
<td>&lt;-64440.286</td>
</tr>
<tr>
<td>QUAL</td>
<td>Variant quality</td>
<td>&lt;20</td>
</tr>
</tbody>
</table>

## Table 2. Variant annotations and cutoff values used for INDELs identified using the low coverage data.

<table>
<thead>
<tr>
<th>Annotation</th>
<th>Description</th>
<th>Cutoff value</th>
</tr>
</thead>
<tbody>
<tr>
<td>INFO/DP</td>
<td>Raw read depth</td>
<td>&gt;23,758</td>
</tr>
<tr>
<td>INFO/MQ</td>
<td>Average mapping quality</td>
<td>&lt;41</td>
</tr>
<tr>
<td>INFO/MQ0F</td>
<td>Fraction of MQ0 reads (smaller is better)</td>
<td>&gt;0.009913696</td>
</tr>
<tr>
<td>INFO/HOB</td>
<td>Bias in the number of HOMs number (smaller is better)</td>
<td>&gt;0.20265508</td>
</tr>
<tr>
<td>INFO/SGB</td>
<td>Segregation based metric</td>
<td>&gt;2143.8876</td>
</tr>
<tr>
<td>INFO/SGB</td>
<td>Segregation based metric</td>
<td>&lt;-29513.557</td>
</tr>
<tr>
<td>INFO/IDV</td>
<td>Maximum number of reads supporting an indel</td>
<td>&gt;51</td>
</tr>
<tr>
<td>INFO/IMF</td>
<td>Maximum fraction of reads supporting an indel</td>
<td>&lt;0.387097</td>
</tr>
<tr>
<td>QUAL</td>
<td>Variant quality</td>
<td>&lt;20</td>
</tr>
</tbody>
</table>

## Table 3. Variant annotations and cutoff values used for SNPs identified using the exome data.

<table>
<thead>
<tr>
<th>Annotation</th>
<th>Description</th>
<th>Cutoff value</th>
</tr>
</thead>
<tbody>
<tr>
<td>INFO/DP</td>
<td>Raw read depth</td>
<td>&gt;656,519</td>
</tr>
<tr>
<td>INFO/MQ</td>
<td>Average mapping quality</td>
<td>&lt;38</td>
</tr>
<tr>
<td>INFO/MQ0F</td>
<td>Fraction of MQ0 reads (smaller is better)</td>
<td>&gt;0.0146629</td>
</tr>
<tr>
<td>INFO/HOB</td>
<td>Bias in the number of HOMs number (smaller is better)</td>
<td>&gt;0.20265508</td>
</tr>
<tr>
<td>INFO/SGB</td>
<td>Segregation based metric</td>
<td>&gt;57489.21</td>
</tr>
<tr>
<td>INFO/SGB</td>
<td>Segregation based metric</td>
<td>&lt;-226326.93</td>
</tr>
<tr>
<td>QUAL</td>
<td>Variant quality</td>
<td>&lt;20</td>
</tr>
</tbody>
</table>

## Table 4. Variant annotations and cutoff values used for INDELs identified using the exome data.

<table>
<thead>
<tr>
<th>Annotation</th>
<th>Description</th>
<th>Cutoff value</th>
</tr>
</thead>
<tbody>
<tr>
<td>INFO/MQ</td>
<td>Average mapping quality</td>
<td>&lt;45</td>
</tr>
<tr>
<td>INFO/MQ0F</td>
<td>Fraction of MQ0 reads (smaller is better)</td>
<td>&gt;0.009913696</td>
</tr>
<tr>
<td>INFO/HOB</td>
<td>Bias in the number of HOMs number (smaller is better)</td>
<td>&gt;0.269629</td>
</tr>
<tr>
<td>INFO/SGB</td>
<td>Segregation based metric</td>
<td>&gt;53165.5</td>
</tr>
<tr>
<td>INFO/SGB</td>
<td>Segregation based metric</td>
<td>&lt;-85919.729</td>
</tr>
<tr>
<td>INFO/IMF</td>
<td>Maximum fraction of reads supporting an indel</td>
<td>&lt;0.3323922</td>
</tr>
<tr>
<td>QUAL</td>
<td>Variant quality</td>
<td>&lt;20</td>
</tr>
</tbody>
</table>
**Freebayes call set.** We used a simple hard filter that discarded variants having a QUAL value less than or equal to 1. This filter was applied using the following command:

```bash
bcftools filter -sQUALFILTER -e'QUAL<1' $file.vcf.gz \
-o $file.filtered.vcf.gz -O z
```

**Generation of the consensus call set**

First, each call set was normalized using a combination of `vt normalize` (version 0.5) and `vcflib vcfallelicprimitives` (version v1.0.0-rc1). This procedure was necessary because sometimes the different variant callers describe the same variant in a different way, which makes comparison difficult and affects the integration of the different initial call sets. Additionally, GATK VariantsToAllelicPrimitives was used to decompose the multi-nucleotide polymorphisms (MNPs) that were present in the Freebayes call set.

Finally, and in order to take advantage of the strengths of each method used for the variant identification, we generated a consensus call set by the union of the biallelic sites from each call set and by the calculation of the genotype likelihoods for each site using GATK UnifiedGenotyper in `genotype_given_alleles` (GGA) mode using the following command line:

```bash
java -jar GenomeAnalysisTK.jar \
  -T UnifiedGenotyper \
  -R $ref.fa \
  -I input.$chr:$start-$end.bam \
  -glm SNP \
  --intervals $chr:$start-$end \
  --intervals integrated.biallelic.sites.vcf.gz \
  --output_mode EMIT_ALL_SITES \
  --alleles integrated.biallelic.sites.vcf.gz \
  --interval_set_rule INTERSECTION \
  --genotyping_mode GENOTYPE_GIVEN_ALLELES \
  --max_deletion_fraction 1.5
```

Where `$chr:$start-$end` is the genomic chunk that is being analysed and `integrated.biallelic.sites.vcf.gz` is the VCF containing the union of the biallelic sites for which the genotype likelihoods will be calculated.

We then filtered the spurious variants resulting of the union of the sites using Variant Quality Score Recalibration (VQSR) and the same parameters and training call sets that were described above used for filtering the supporting call set generated using GATK UnifiedGenotyper. GATK ApplyRecalibrator was used with the same `--ts_filter_level` value of 99.9 used for SNPs.

**Phasing and imputation of the consensus call set.** The VCF file containing the genotype likelihoods obtained following the procedure described in previous section was divided into single chromosome VCF files that were further divided into genomic chunks containing 2,100 sites of which 600 were shared between consecutive chunks. These chunks were processed by in parallel by `Beagle` by using the following command:

```bash
java -jar beagle.08Jun17.d8b.jar \
  chrom=$chr:$start-$end \
  gl=$chr.biallelic.GL.vcf.gz \
  out=$chr.$start.$end.beagle \
  niterations=15
```

Where `$chr.biallelic.GL.vcf.gz` is the VCF file containing the genotype likelihoods.

After processing all the chunks with Beagle we obtained an initial set of genotypes and haplotypes used in the next step consisting of phasing the genotype likelihoods onto a highly accurate haplotype scaffold obtained using `SHAPEIT2` (version v2.r837) with microarray genotype data available on the same samples. This scaffold was obtained by leveraging family information and running SHAPEIT2 in two different independent runs on either the Illumina Omni 2.5 or Affymetrix 6.0 microarray data that was generated as part of the 1000 Genomes Project. SHAPEIT2 was run using the following settings (``--window 0.5, --states 200, --burn 10, --prune 10, --main 50, --duohmm) and SNPs with a missing data rate above 10% and a Mendel error rate above 5% were removed before phasing.
In order to phase the genotype likelihoods obtained from Beagle onto the haplotype scaffold we used SHAPEIT2. Genotypes called by Beagle with a posterior probability greater than 0.995 were fixed as known genotypes and the haplotypes estimated by Beagle were used to initialize the SHAPEIT2 phasing. This phasing was run in chunks of 12,250 sites with 3,500 sites overlapping between consecutive chunks. SHAPEIT2 was run using the following command:

```
shapeit -call \
--input-gen input.shapeit.$chr.gen.gz input.shapeit.$chr.gen.sample \
--input-init input.shapeit.$chr.hap.gz input.shapeit.$chr.hap.sample \
--input-scaffold chip.omni.snps.$chr.haps chip.omni.snps.$chr.sample chip.affy.snps.$chr.haps chip.affy.snps.$chr.sample \
--input-map $chr.gmap.gz \
--window 0.1 \ 
--states 400 \ 
--states-random 200 \ 
--burn 0 \ 
--run 12 \ 
--prune 4 \ 
--main 20 \ 
--input-from $chunk_start \ 
--input-to $chunk_end \ 
--output-max out.$chr.$chunk_start.$chunk_end.haps.gz out.$chr.$chunk_start.$chunk_end.haps.sample
```

Where --input-gen specifies specifies the genotype/GL input data from Beagle, --input-init specifies the haplotypes from Beagle, --input-map specifies the genetic map used in the estimation, --input-scaffold gives the SNP-array derived haplotype scaffold obtained from SHAPEIT2. The genetic map used was downloaded from [https://data.broadinstitute.org/alkesgroup/Eagle/downloads/tables/genetic_map_hg38_withX.txt.gz](https://data.broadinstitute.org/alkesgroup/Eagle/downloads/tables/genetic_map_hg38_withX.txt.gz). Each of the phased chunks resulting from running SHAPEIT2 were joined together using the program `ligateHAPLOTYPES`

The strategy described here was used in the final phase of the 1000 Genomes Project and has been shown to produce low error rates for genotype calls13.

The pipelines used in this work were implemented using the eHive workflow system14 and modules developed in Perl and Python, which have been packaged for ease of deployment. All the analyses were run in parallel on a high-throughput compute cluster to ensure completion in a reasonable timeframe. Code is publicly available via GitHub (see software availability section)14-16.

**Data set validation**

To assess our call set and compare it to the released data from the final phase of the 1000 Genomes Project, we utilised resources from GIAB. Our strategy compares our GRCh38 calls for NA12878 with those on the same assembly for that sample from GIAB. In addition, we compared the 1000 Genomes variant calls for NA12878 to the set of calls from GIAB for NA12878 on GRCh37. NA12878 was selected for this due to the availability of high quality call sets. In the sequence data used in generating our call set, NA12878 has lower coverage (4.6x) than the average coverage (6.2x) for the WGS alignment files and has higher coverage (144.1x) than the average coverage (84.9x) for the WES alignment files. We assume that the conclusions derived from NA12878 can be extrapolated to the rest of the samples and are likely to be conservative regarding the accuracy of our calls on GRCh38. Our comparison approach has benefits of both enabling us to benchmark the performance on a given sample with an independently produced gold-standard call set and allowing us to apply the equivalent benchmark to data from the 1000 Genomes Project, which gives a direct indication of how our call set compares to that produced by the 1000 Genomes Project.

In order to validate our data set we used the variants for NA12878 from the multi-sample phased VCF and compared them with the GIAB sites on GRCh38 downloaded from [ftp://ftp-trace.ncbi.nlm.nih.gov/giab/ftp/release/NA12878_HG001/latest](ftp://ftp-trace.ncbi.nlm.nih.gov/giab/ftp/release/NA12878_HG001/latest) (version 3.3.2). For comparative purposes we also compared the GRCh37 variants from the final phase of the 1000 Genomes Project (downloaded here) with the GRCh37 GIAB variants obtained here (version 3.3.2). Our comparison is restricted to regions of the genomes for which GIAB considers calls to be high confidence and was performed using the Nextflow17 workflow accessible from the link in the software availability section.
The result of our comparison is shown in Table 5. The average percentage of sites among all the chromosomes identified in our work that were also present in GIAB represents 96.4% of the total GIAB sites. This percentage is comparable to 97.9% resulting from the comparison with the final phase of the 1000 Genomes Project.

Table 5. Site comparison for NA12878 between our call set and Genome in a Bottle (GIAB)-mapped to GRCh38 and between the 1000 Genomes Project phase 3 (P3) call set and GIAB mapped to GRCh37. Results are shown for each chromosome. ‘Shared (TP)’ are the true positive variants identified in the compared call sets. ‘giab_only (FN)’ are the false negative variants identified by GIAB only. ‘Thiswork_only (FP)’ are the false positive variants identified in our call set only.

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Shared (TP)</th>
<th>%shared (TP)</th>
<th>giab only (FN)</th>
<th>%giab only (FN)</th>
<th>Thiswork only (FP)</th>
<th>%Thiswork only (FP)</th>
<th>Total (GIAB)</th>
<th>Total thiswork only</th>
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<tr>
<td>Chr1 (b38)</td>
<td>238,323</td>
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Additionally, the percentage of sites identified in our call set but not in GIAB is 0.5%, which is comparable to the 0.4% obtained in the comparison with 1000 Genomes P3. Taken together, these results demonstrate both the high sensitivity and high specificity of our callset.

**Data availability**

The variants resulting from this work are available in the European Variation Archive. Accession number PRJEB30460.

This call set is also available from the International Genome Sample Resource (IGSR) [PMID: 27638885] at: http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/data_collections/1000_genomes_project/release/20181203_biallelic_SNV/.
Software availability

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<th>Documentation</th>
<th>Licence</th>
<th>DOI</th>
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Grant information
This work was completed thanks to the funding from the Wellcome Trust (grant number 104947) and the European Molecular Biology Laboratory.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements
We would like to thank Petr Danecek (Matthew Hurles Group, Wellcome Sanger Institute), Erik Garrison (Durbin Group, Wellcome Sanger Institute) and Tommy Carstensen (Global Health & Population Science, Department of Medicine, University of Cambridge) for participating in discussions on the methodology used in this work. Shane McCarthy (Department of Genetics, University of Cambridge) for detailed advice and discussion of the project plan. We would also like to thank Zamin Iqbal (Iqbal group, EMBL-EBI) for discussions on the project methodology and outputs. In addition, our thanks go to the Systems Infrastructure team of EMBL-EBI Shane McCarthy (Department of Genetics, University of Cambridge) for participating in discussions on the methodology used in this work. Finally, we would like to thank Tommy Carstensen for providing the liftover of the array data used for the phasing of the variants identified in this work.

Members of the 1000 Genomes Project Consortium are listed in the Supplementary Note, contained within the Supplementary Text and Figures of Poznik et al. 19.

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References