GWAS summary statistics

Genetics & Genomics Winter School

Module 5

Consensus of sharing GWAS summary statistics (in human genetics research community)

Has Become a standard to share and make publicly available the summary-level data when publishing a GWAS study.

Asking for more

Because of the usefulness of genome-wide association study (GWAS) data for mapping regulatory variation in the human genome, the journal now asks authors to report the co-location of trait-associated variants with gene regulatory elements identified by epigenetic, functional and conservation criteria. We also ask that authors publish or database the genotype frequencies or association *P* values for all SNPs investigated, whether or not they reached genome-wide significance.

—Nat Genet editorial, July 2012

Cell Genomics

CellPress OPEN ACCESS

2021

Perspective

Workshop proceedings: GWAS summary statistics standards and sharing

Jacqueline A.L. MacArthur,[1,2,*] Annalisa Buniello,[1] Laura W. Harris,[1] James Hayhurst,[1] Aoife McMahon,[1] Elliot Sollis,[1] Maria Cerezo,[1] Peggy Hall,[3] Elizabeth Lewis,[1] Patricia L. Whetzel,[1] Orli G. Bahcall,[4] Inês Barroso,[5] Robert J. Carroll,[6] Michael Inouye,[7,8,9] Teri A. Manolio,[3] Stephen S. Rich,[10] Lucia A. Hindorff,[3] Ken Wiley,[3] and Helen Parkinson[1,*]

Table 1. Recommended standard reporting elements for GWAS SumStats

Data element	Column header	Mandatory/Optional
variant id	variant_id	One form of variant ID is mandatory, either rsID or chromosome, base pair location, and genome build[a]
chromosome	chromosome	
base pair location	base_pair_location	
p value	p_value	Mandatory
effect allele	effect_allele	Mandatory
other allele	other_allele	Mandatory
effect allele frequency	effect_allele_frequency	Mandatory
effect (odds ratio or beta)	odds_ratio or beta	Mandatory
standard error	standard_error	Mandatory
upper confidence interval	ci_upper	Optional
lower confidence interval	ci_lower	Optional

PRIMER

Check for updates

Genome-wide association studies

Emil Uffelmann[1], Qin Qin Huang[2], Nchangwi Syntia Munung[3], Jantina de Vries[3], Yukinori Okada[4,5], Alicia R. Martin[6,7,8], Hilary C. Martin[2], Tuuli Lappalainen[9,10,12] and Danielle Posthuma[1,11] ✉

Table 3 | **Databases of GWAS summary statistics**

Database	Content
GWAS Catalog[110]	GWAS summary statistics and GWAS lead SNPs reported in GWAS papers
GeneAtlas[8]	UK Biobank GWAS summary statistics
Pan UKBB	UK Biobank GWAS summary statistics
GWAS Atlas[273]	Collection of publicly available GWAS summary statistics with follow-up in silico analysis
FinnGen results	GWAS summary statistics released from FinnGen, a project that collected biological samples from many sources in Finland
dbGAP	Public depository of National Institutes of Health-funded genomics data including GWAS summary statistics
OpenGWAS database	GWAS summary data sets
Pheweb.jp	GWAS summary statistics of Biobank Japan and cross-population meta-analyses

For a comprehensive list of genetic data resources, see REF.[13]. GWAS, genome-wide association studies; SNP, single-nucleotide polymorphism.

Critical information from GWAS summary data

- SNP ID
- Effect alleles and alternate alleles (A1 and A2)
- Effect allele frequencies
- Marginal SNP effects
- Standard errors
- P value
- (Per-SNP) sample sizes

COJO file (.ma)

```
SNP A1 A2 freq b se p N
rs1001 A G 0.8493 0.0024 0.0055 0.6653 129850
rs1002 C G 0.0306 0.0034 0.0115 0.7659 129799
rs1003 A C 0.5128 0.0045 0.0038 0.2319 129830
```

Yang et al., 2012

What are the minimum data required?

Given the standard GWAS with genotypes being allelic counts (0/1/2), the minimum data required include:

- SNP marginal effect estimates
- Standard errors
- GWAS sample size

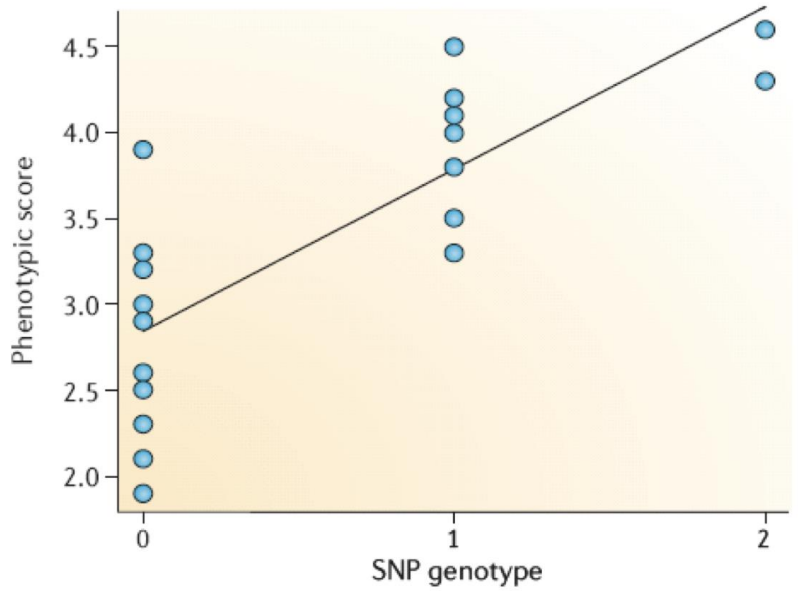
} GWAS sumstats

- LD correlations among SNPs → LD matrix

Other information critical to quality control (QC)

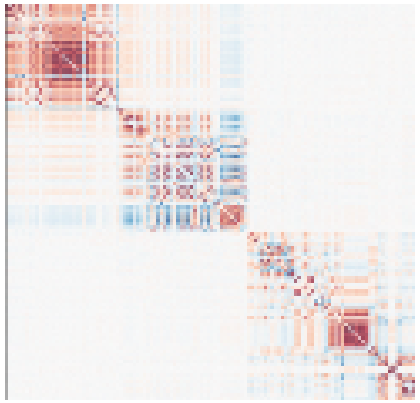
Which allele is the **effect allele** in GWAS?

e.g., A1 allele

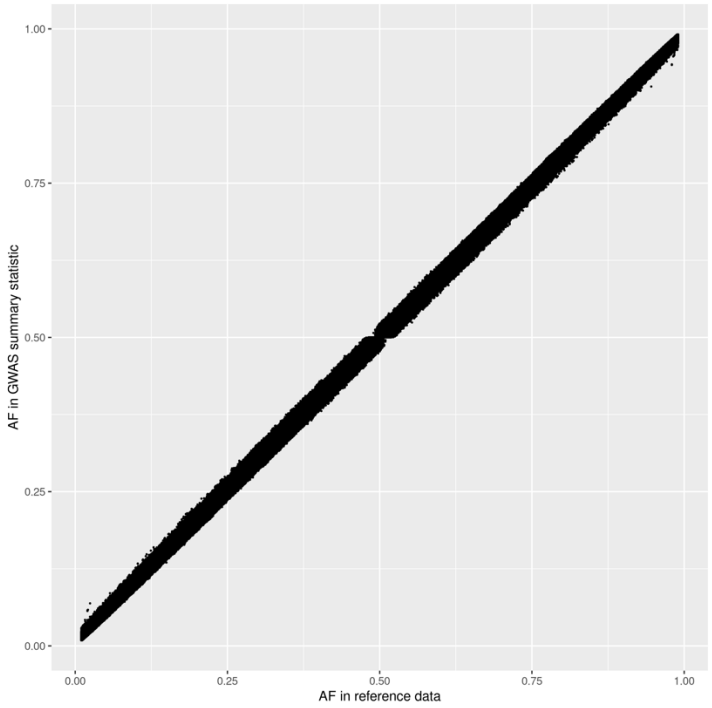


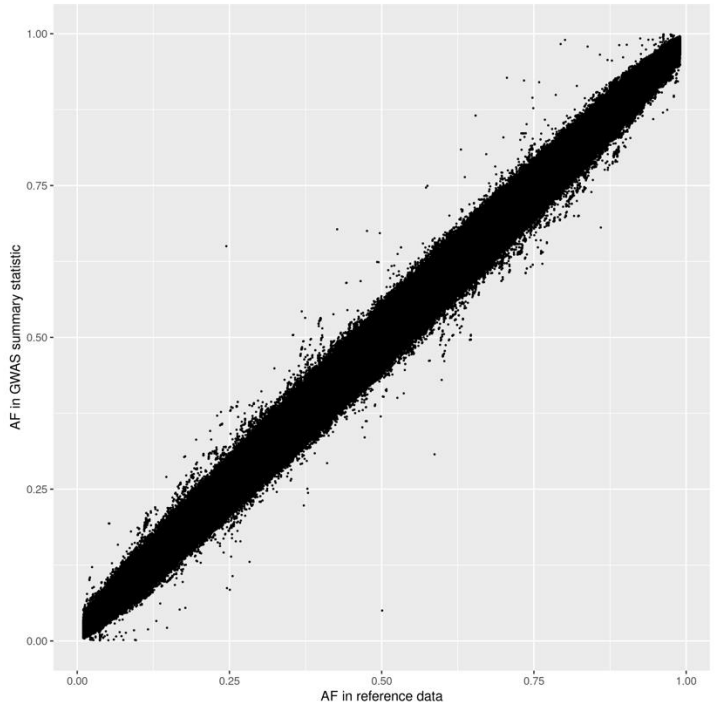
A2A2 A1A2 A1A1

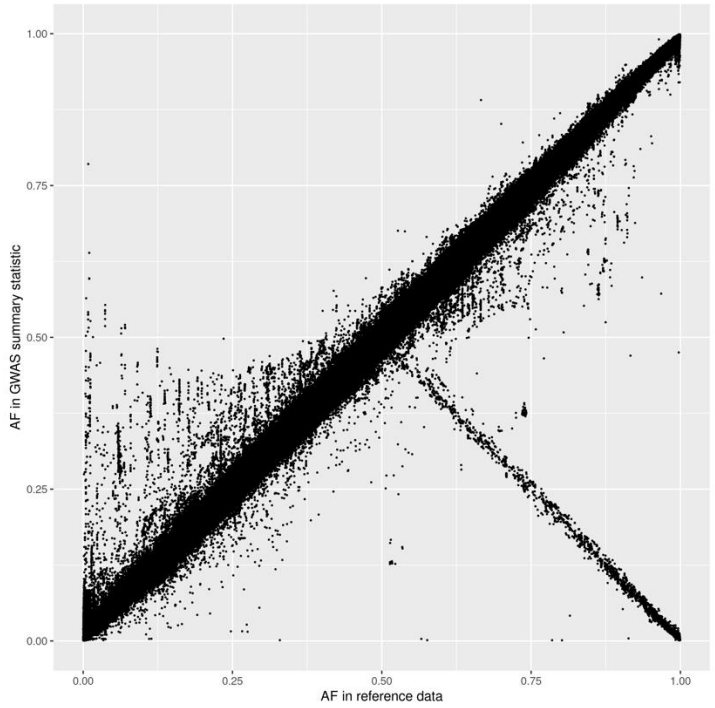
Need to match with the allele used to calculate the LD matrix in the reference sample



Allele Frequency







AF in LD reference →

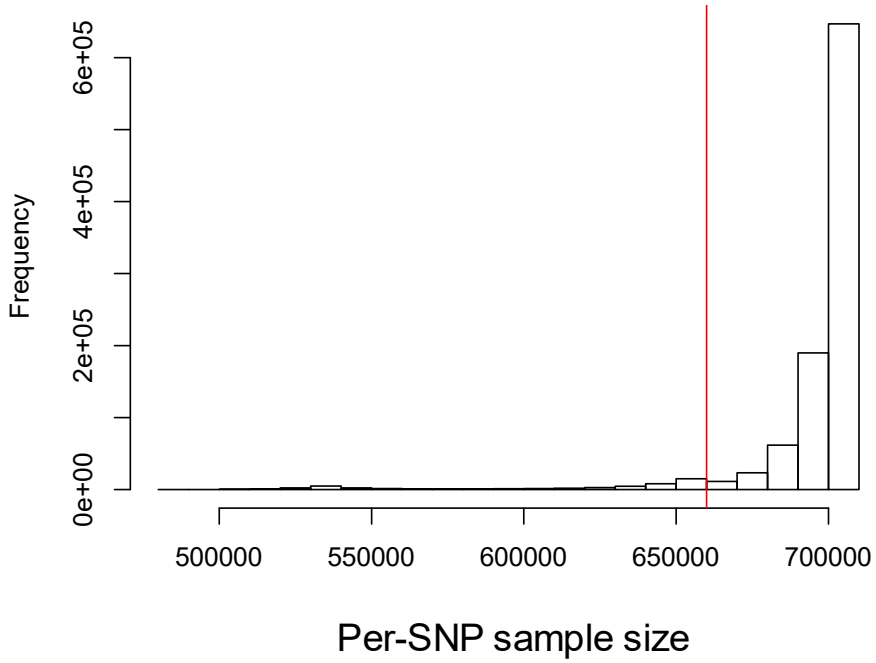
Other information critical to quality control (QC)

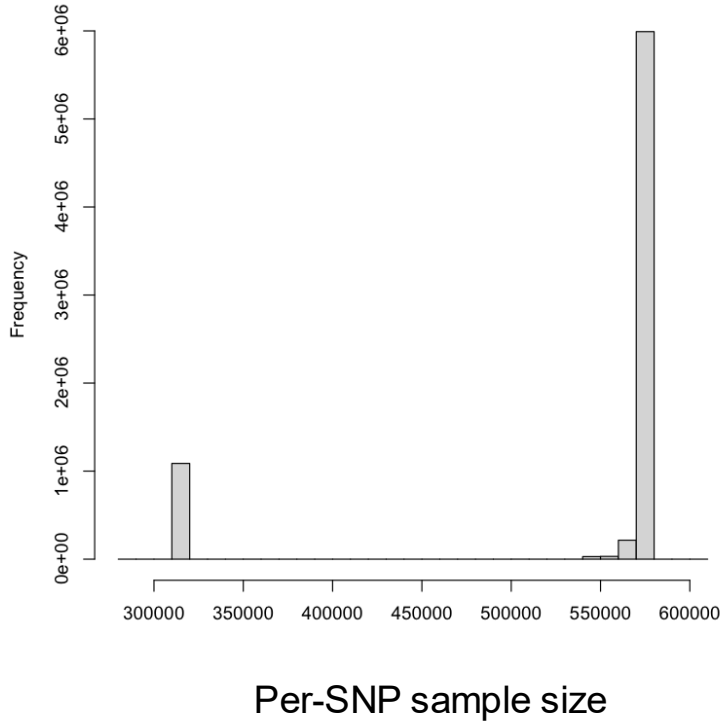
Per-SNP sample size

Heterogeneity in per-SNP sample size (usually due to meta-analysis) may result in a convergence problem in MCMC.

We recommend to visualise the per-SNP sample size distribution and remove the outliers.

Heterogeneity in per-SNP sample size





What should we check prior to the analysis?

THE UNIVERSITY OF QUEENSLAND AUSTRALIA

Raw data file

Item	What could be wrong?	How to fix?
Genome build	Inconsistent coordinates among GWAS summary data and LD reference.	Lift up to the same genome build using *liftover*
SNP ID	rsID not provided.	Use chromosome and position information to find their rsID (from LD reference file).
Alleles	Lower/upper case. Unknown effect allele (A1/A2, REF/ALT).	Check ReadMe file. Check if the predictor is negatively correlated with the phenotype.
Effect allele frequency (p)	Missing data. Provided data are minor allele frequency (MAF). Separate values in cases and controls.	Use data from LD reference. Impute by summary data $2pq = 1/(N * SE + N * b^2)$. Compute $p = \frac{N_{case}\, p_{case} + N_{ctrl}\, p_{ctrl}}{N_{case} + N_{ctrl}}$.
Marginal effect (b)	Provided data are Z-score or odds ratio (OR).	b = Z/SE if SE is provided, or $b = Z/\sqrt{2p(1-p)(N+Z^2)}$ given unit variance. b = log(OR).
Standard error (SE)	Missing data.	SE = b/Z if b is provided, or $SE = 1/\sqrt{2p(1-p)(N+Z^2)}$ given unit variance.
Sample size (N)	Missing data. Separate values in cases and controls.	Check publication/ReadMe file. Some methods require total sample size, while some requires effective sample size.
Incorrect data field format.	Some data field has NA and is non-numeric.	Convert to correct format and filter/impute missing data.

What should we check prior to the analysis? (cont)

Quality control (QC)

Item	What could be wrong?	How to fix?
Missing data	Some SNPs have missing data.	Impute the missing data or remove SNPs.
Mismatched SNPs	SNPs in GWAS are missing in the LD reference, or in reverse.	For applications requiring a perfect match, filter SNPs or impute their marginal effects (e.g., *ImpG*).
Allele discordance	Discordant alleles between data sets, e.g., A/T in GWAS but T/A in LD reference.	Flip the alleles in GWAS and take the opposite sign of the marginal effect size.
Allele frequency differences	Large differences between GWAS and LD reference data.	Remove SNPs with large difference, e.g., > 0.2.
LD differences	LD reference does not match LD in the GWAS sample.	Choose a better LD reference. Remove SNPs with LD heterogeneity (*DENTIST*).
Variable per-SNP sample sizes	Dispersed/skewed/multimodal distribution. Only overall sample size provided in meta-analysis.	Visualise the distribution. Remove long tail/minor mode/ outliers, e.g., > 3*SD. Impute $N = 1/(2pq(SE+b^2))$ if necessary.
Sample size for disease	Total sample size ($N_{case} + N_{ctrl}$) or effective sample size - which one to use?	For *SBayes*, we recommend using the total sample size.

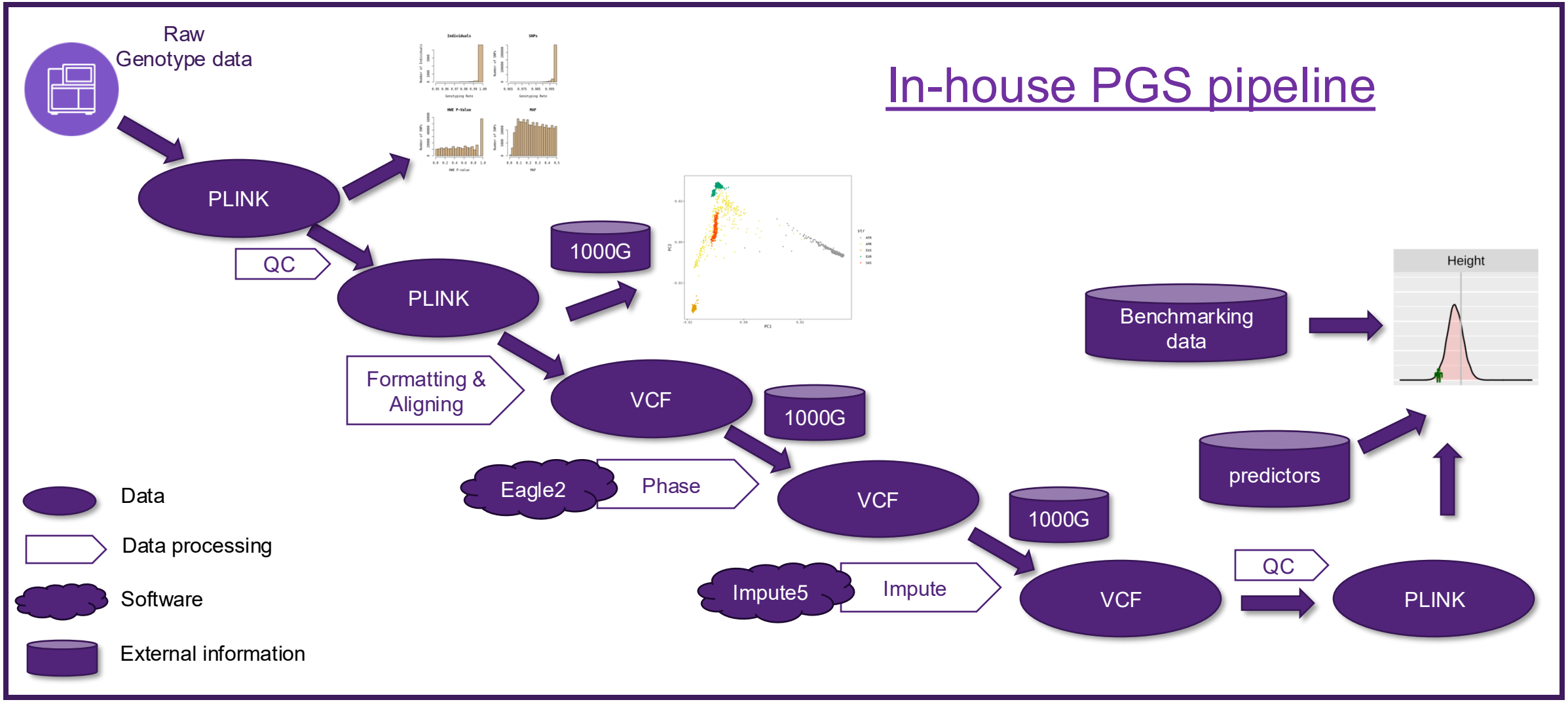
Practical6_SumStat_QC.html

An in-house PGS pipeline

Genetics & Genomics Winter School

Module 5

schematic of technical pipeline



Genotype data from arrays

- Can assay ~1M SNPs per individual with 'SNP chips'
- Data is typically 'counts' of a reference allele

genotype file:

	SNP1	SNP2	SNP3	SNP4
Bob	0	1	0	1
Fred	1	2	0	0
Jose	1	2	2	2
Andy	2	1	1	1

map file:

	chr	position	ref	alt
SNP1	1	52196307	A	T
SNP2	1	52462094	C	T
SNP3	1	52736008	A	G
SNP4	1	53010891	T	C

[borrowed from Keth]

Why a raw data is not ready for PGS profiling?

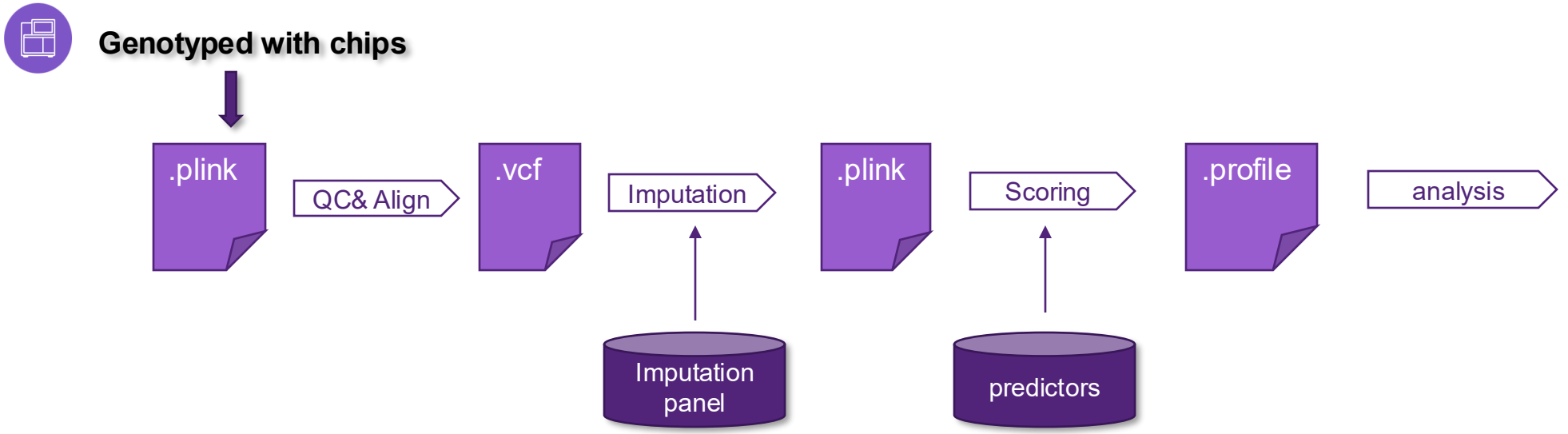
- Quality

- Coverage

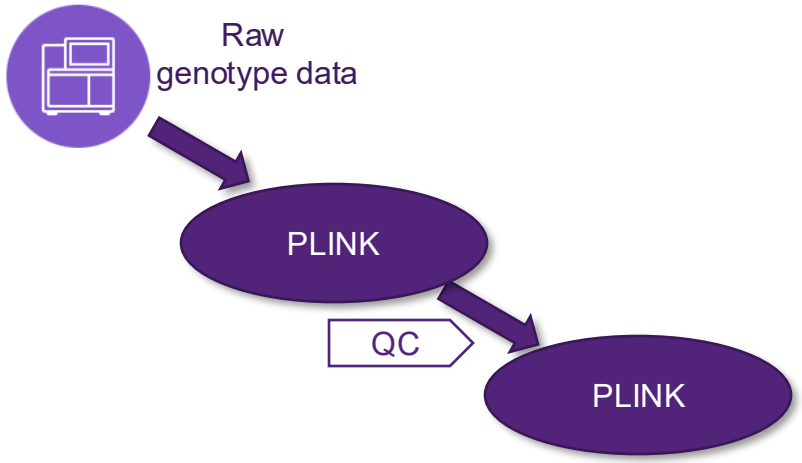
A high density SBayesRC Predictor – 7.3M SNPs

	Number of Nucleotide/Variants
Whole human genome haplotype	3 billion
TopMed	445 million
1000G	80 million
HRC	40 million
HapMap3	1 million
Illumina GSA chip	654 thousand

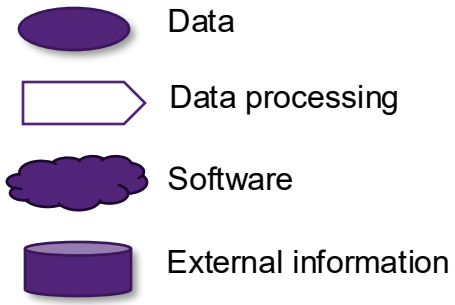
Overview of PGS pipeline



schematic of technical pipeline



In-house PGS pipeline



Revisit Genotype data QC for a GWAS study

- Per Individual QC
  - *1) removal of individuals with excess missing genotypes*
  - *2) removal of individuals with outlying homozygosity values*
  - *3) remove of samples showing a discordant sex*
  - *4) removal of related or duplicate samples, and*
  - *5) removal of ancestry outliers*

- Per SNP QC
  - *1) removal of SNPs with excess missing genotypes*
  - *2) removal of SNPs that deviate from Hardy-Weinberg equilibrium*
  - *3) removal of SNPs with low minor allele frequency*
  - *4) comparing allele frequency to known values*

Extra consideration in practice

- Large number of SNPs with MAF = 0
  - Missing Alleles

- Replicates and relatives can exist

Genotype data QC

- Per Individual QC
  - *1) removal of individuals with excess missing genotypes*
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Extra consideration in practice

- Large number of SNPs with MAF = 0
  - Missing Alleles
- Replicates and relatives can exist
- Different genome build between raw data and imputation panel

Human Genome Assemblies

https://hgdownload.soe.ucsc.edu/downloads.html

Very similar / Same.
GRCh37 names them `chr1`, `chr2`, `chr3`, etc, while hg19 just has `1`, `2`, `3`.
Different Mitochondria contigs.

Human genomes

Jan. 2022 (T2T-CHM13 v2.0/hs1)

- Fileserver (bigBed, maf, fa, etc) annotations
- Standard genome sequence files and select annotations (2bit, GTF, GC-content, etc)
- LiftOver files
- Pairwise alignments

Telomere-to-Telomere

A haploid human genome without gaps

Dec. 2013 (GRCh38/hg38)

- Genome sequence files and select annotations (2bit, GTF, GC-content, etc)
- Sequence data by chromosome
- Annotations
- SNP-masked fasta files
- LiftOver files
- Pairwise alignments
- Multiple alignments
- Patches
- Data archive

hg19ToHg38.over.chain.gz

hg38ToHg19.over.chain.gz

hs1ToHg38.over.chain.gz

hs1ToHg19.over.chain.gz

hg38ToHs1.over.chain.gz

Feb. 2009 (GRCh37/hg19)

- Genome sequence files and select annotations (2bit, GTF, GC-content, etc)
- Sequence data by chromosome
- Annotations
- GC percent data
- Protein database for hg19
- SNP-masked fasta files
- LiftOver files
- Pairwise alignments (primates)
- Pairwise alignments (other mammals)
- Pairwise alignments (other vertebrates)
- Multiple alignments
- Patches
- Data archive

Mar. 2006 (NCBI36/hg18)

- Data and annotations

GRCh = Genome Reference Consortium Human Build

Liftover plink files

Best solution: recommend realigning the manifest files with BCFtools/gtc2vcf (http://github.com/freeseek/gtc2vcf)

Option 1. https://www.strand.org.uk

- update_build.sh <bed-file-stem> <strand-file> <output-file-stem>

Option 2. https://genome.sph.umich.edu/wiki/LiftOver

- python liftMap.py -m data_recoded.map -p data_recoded.ped -o data_recoded_lifted

Option 3. LiftOverPlink

- https://github.com/sritchie73/liftOverPlink/blob/master/README.md

Option 4. use reference file to update dbSNP locations in bim file or GWAS statistics

- Hg38 dbsnp_146.hg38.vcf.gz
- Hg19 dbsnp_138.hg19.vcf.gz

- Available: https://gatk.broadinstitute.org/hc/en-us/articles/360035890811-Resource-bundle

Extra consideration in practice

- Large number of SNPs with MAF = 0
  - Missing Alleles
- Replicates and relatives can exist
- Different genome build between raw data and imputation panel
- SNPs alleles from negative strand

Chromosomes, strands and SNP alleles

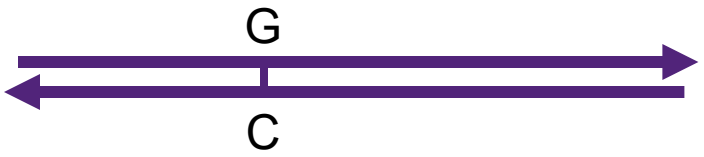
Paternal chromosome

Positive strand

Negative strand

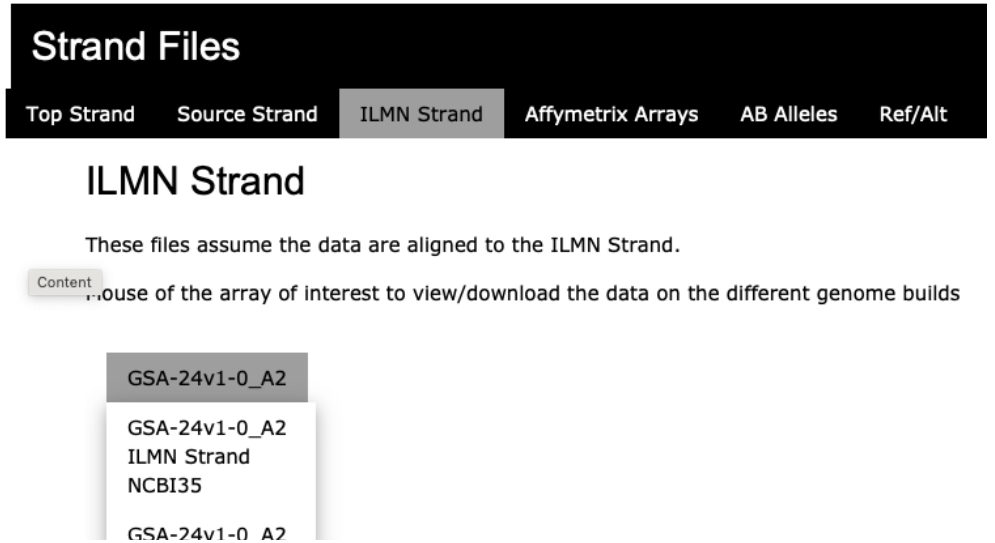
Flip the strand

Maternal chromosome



Strand resource

https://www.strand.org.uk



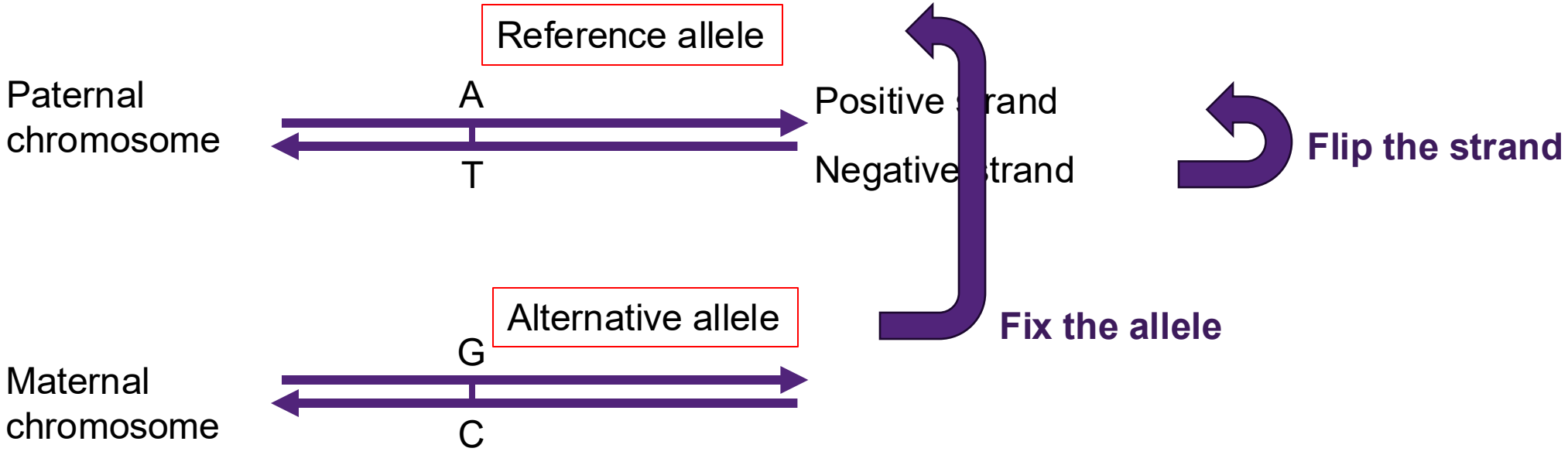
Usage is:
update_build.sh <bed-file-stem> <strand-file> <output-file-stem>
where:

<bed-file-stem>	is the name of your binary ped set minus the .bed, .bim or .fam extension
<strand-file>	is appropriate strand file for you chip and current strand orientation (TOP, SOURCE, ILMN)
<output-file-stem>	is the name of the new output file to create again minus the .bed, .bim or .fam extension

GSA-24v3-0_A2

GSAMD-24v1-0_20011747_A1

Chromosomes, strands and SNP alleles

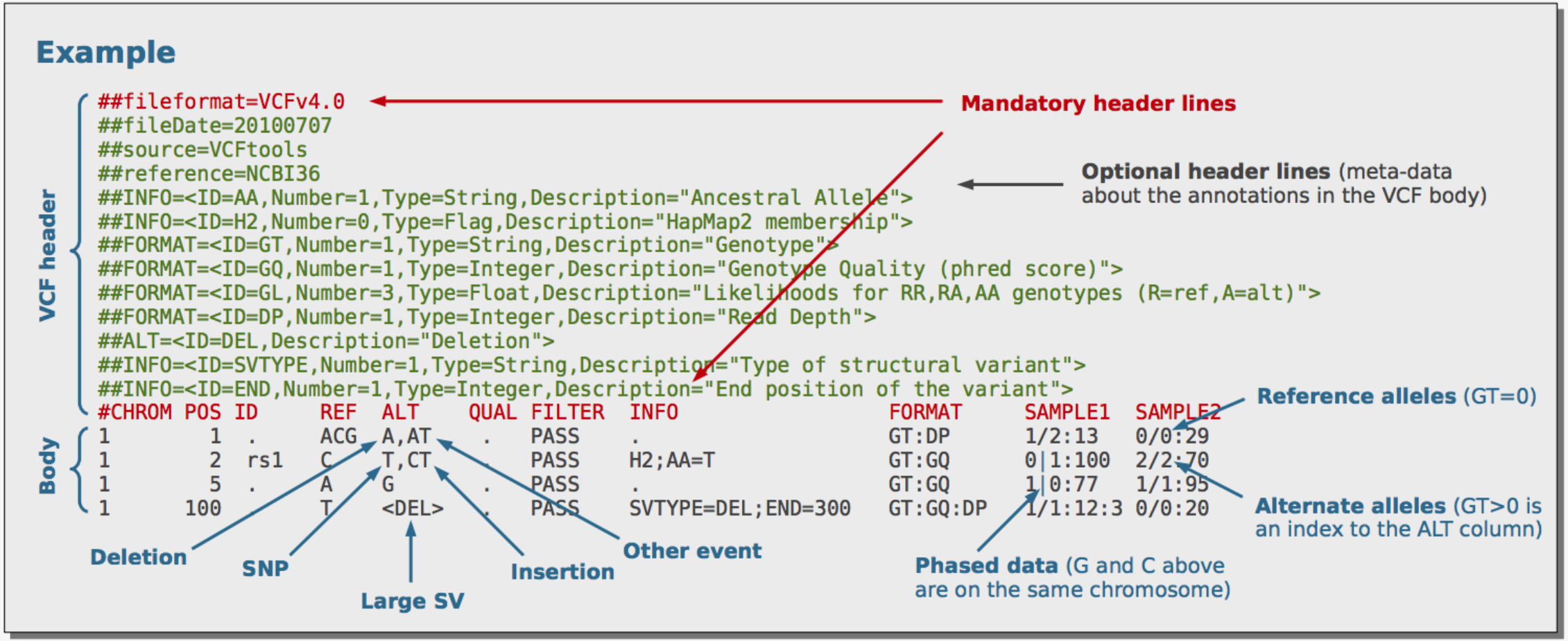


Example script to fix ref allele

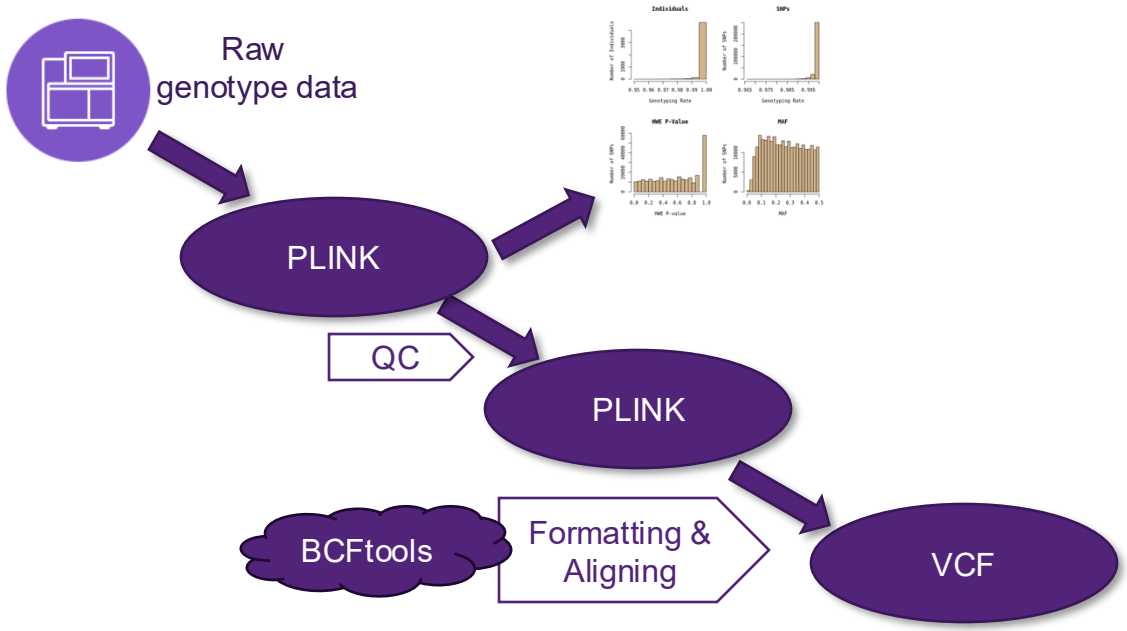
```
chr=22

# Pull out data for relevant chromosome and convert to VCF.
plink --bfile ${data}_chr${chr}  --recode vcf --out  ${data}_chr${chr}

# Sort and compress the VCF file
vcf-sort ${data}_chr${chr}.vcf | bgzip -c > ${data}_chr${chr}.vcf.gz

# Fix the reference allele to match the GRCh37 reference fasta (human_g1k_v37.fasta).
ref2fix=${refpath}/human_g1k_v37.fasta
BCFTOOLS_PLUGINS=/software/bin/
bcftools \
  +fixref \
  ${data}_chr${chr}.vcf.gz \
  -Oz \
  -o fixed_${data}_chr${chr}.vcf.gz  \
  -- -d \
  -f ${ref2fix} \
  -m flip

zcat fixed_${data}_chr${chr}.vcf.gz | bgzip -c > indexed_fixed_${data}_chr${chr}.vcf.gz

# create index file.
tabix indexed_fixed_${data}_chr${chr}.vcf.gz
```

BCFtools
VCFtools

Example VCF files



schematic of technical pipeline



In-house PGS pipeline

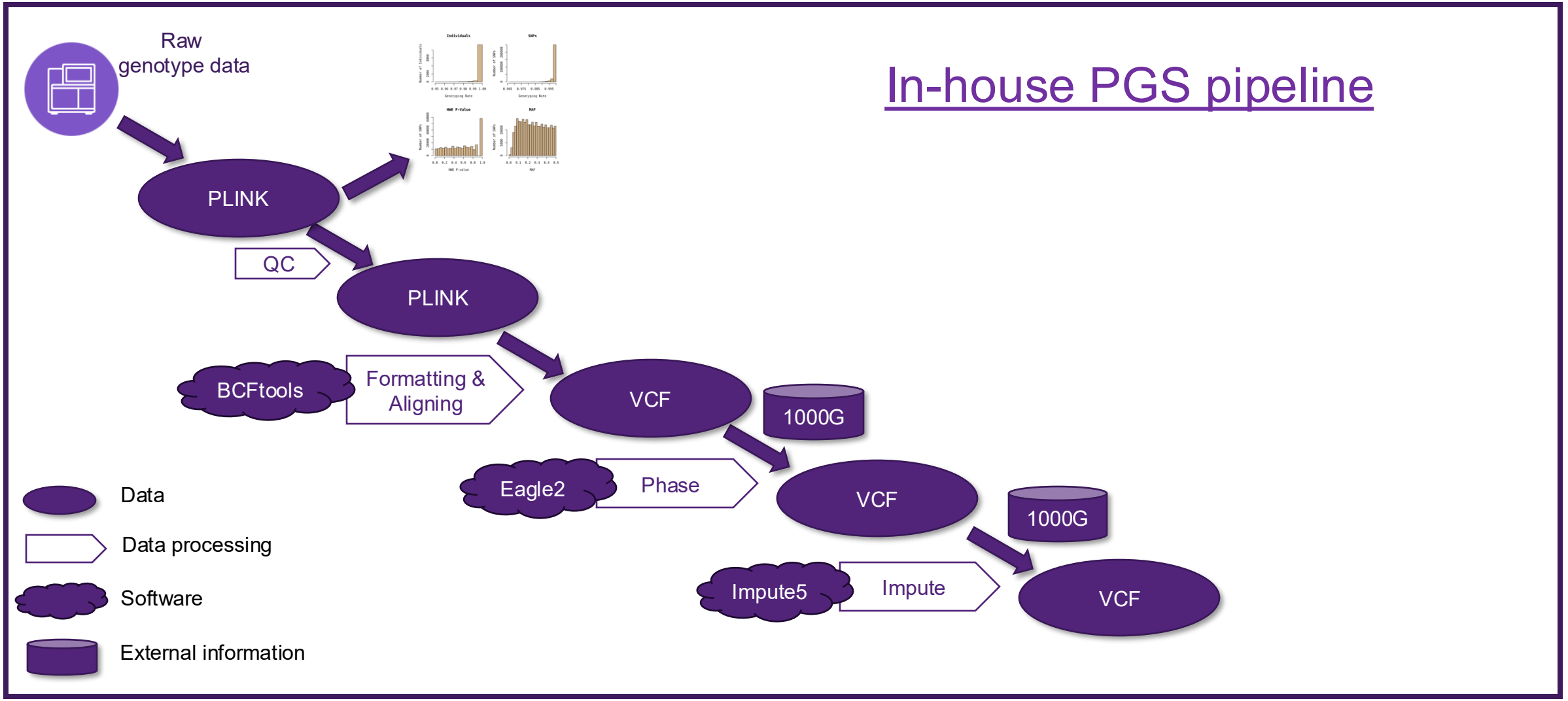
Data

Data processing

Software

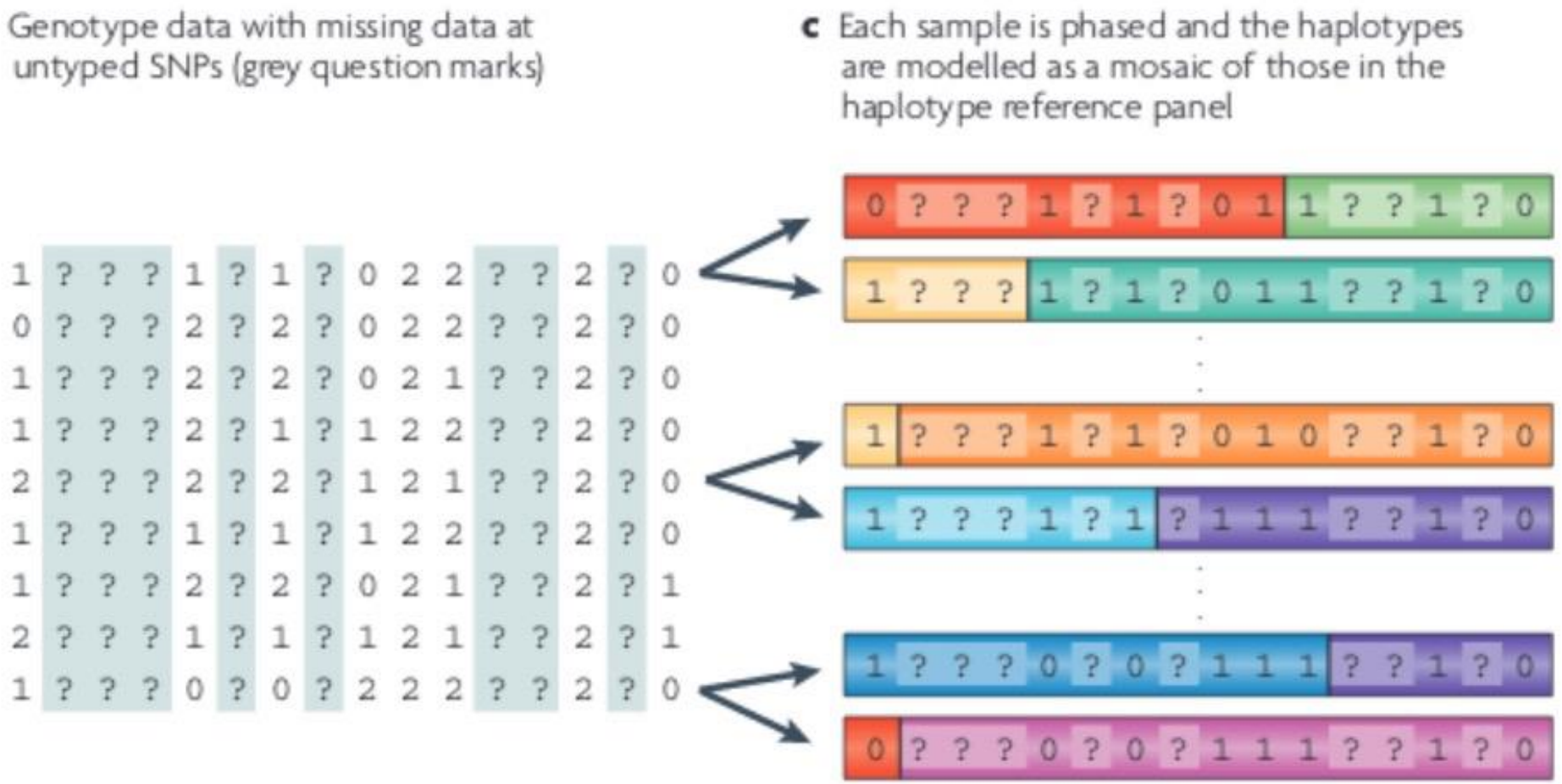
External information

schematic of technical pipeline



In-house PGS pipeline

phasing


a Genotype data with missing data at untyped SNPs (grey question marks)
c Each sample is phased and the haplotypes are modelled as a mosaic of those in the haplotype reference panel

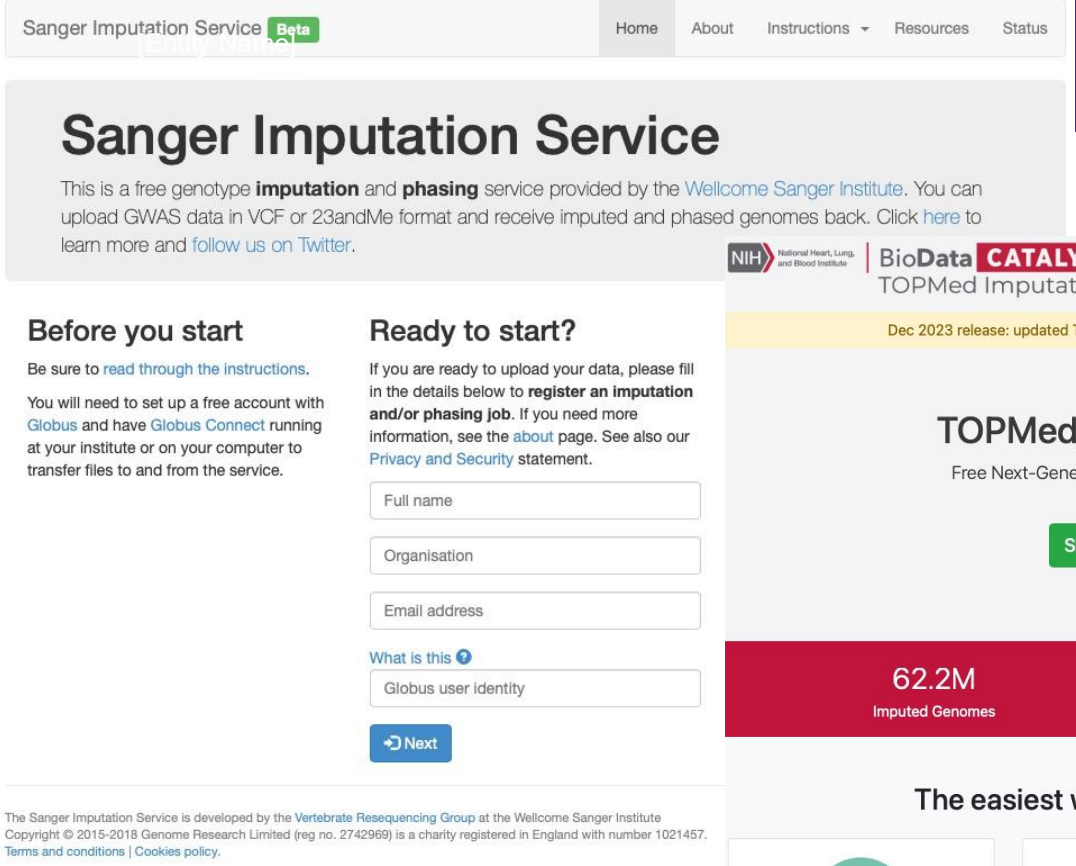
Imputation

Each sample is phased and the haplotypes are modelled as a mosaic of those in the haplotype reference panel

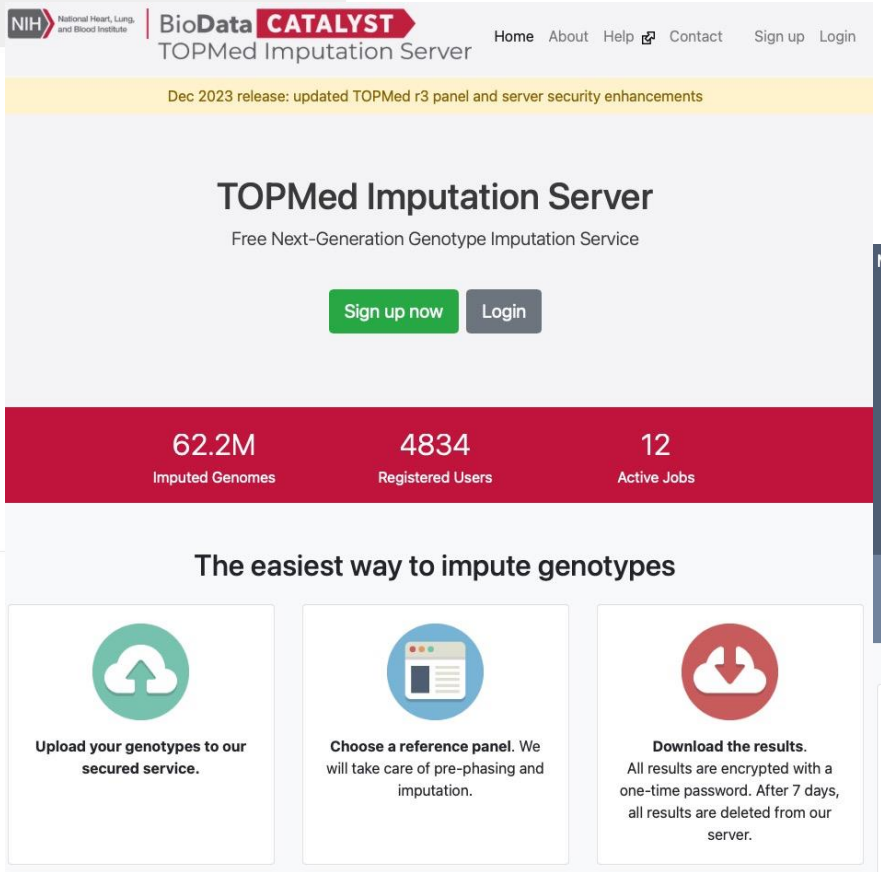
d Reference set of haplotypes, for example, HapMap

Panel options

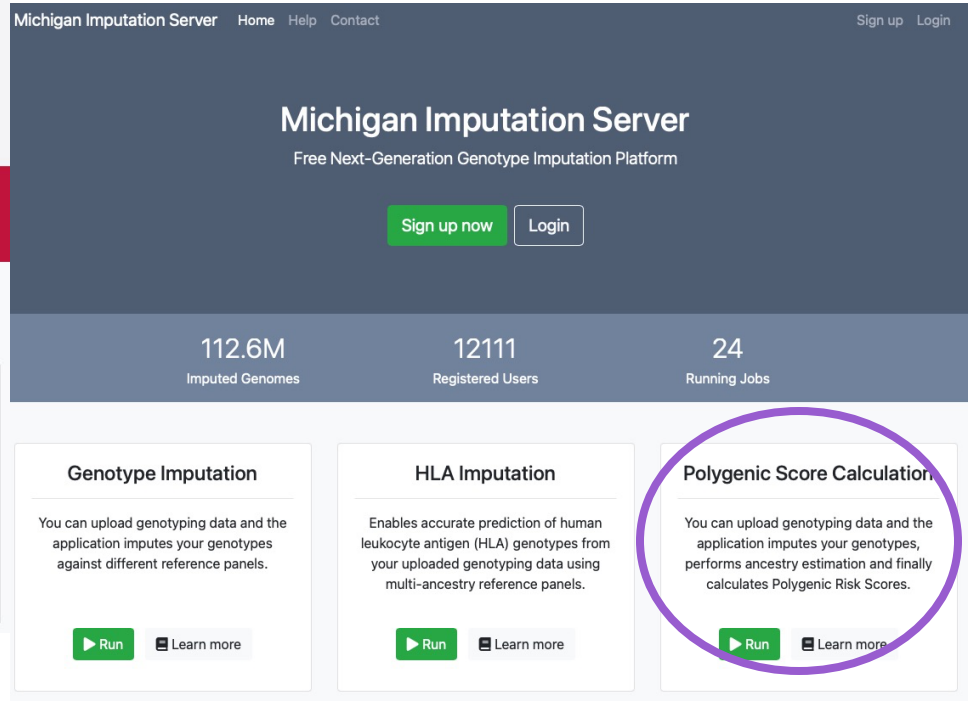
Reference Panel	Number of Individuals	Number of Variants	Population Focus
Haplotype Reference Consortium (HRC)	~32,000	~40 million	European
1000 Genomes Project	2,504	~88 million	Global, diverse
TOPMed	~62,000	>300 million	Diverse, underrepresented
UK10K	~3,800	~30 million	UK, European
GoT2D	~2,657	~20 million	Type 2 Diabetes, Metabolic



Most user friendly



Largest panel



Multiple features

Imputation servers

In house Phasing with Eagle2

```
## Example script
geneticmap=genetic_map_chr${chr}_combined_b37.txt
reference=ALL.chr${chr}.phase3_shapeit2_mvncall_integrated_v5a.20130502.genotypes.vcf.gz

eagle \
 --vcfRef=$reference \
 --vcfTarget=indexed_fixed_${data}_chr${chr}.vcf.gz \
 --geneticMapFile=$geneticmap \
 --vcfOutFormat=z \
 --outPrefix=phased_chr${chr} > phasing.log
```

Alternative: SHAPEIT4

In house Imputation with Impute5

```
## Example script
impute5_1.1.5_static \
 --m $geneticmap \
 --h $reference \
 --g phased_chr${chr}.vcf.gz \
 --r ${chr}:${intstart}-${intend} \
 --ne 20000 \  ## effective sample size, default 10k~20k for human
 --threads 1 \
 --o imputed_chr${chr}_chunk.vcf.gz \
 --l imputed_chr${chr}_chunk.log
```

A chromosome can be imputed as chunks

After imputation

- Format

- Imputed data is output as a zipped VCF file. We usually change the format back to PLINK for following analysis.

Example script

```
## convert the format using plink
plink --vcf  imputed_chr${chr}.vcf.gz   \
            --id-delim '_'  \
            --keep-allele-order \
            --make-bed \
            --out  imputed_chr${chr}
```

After imputation

- Format

- Imputed data is output as a zipped VCF file. We usually change the format back to PLINK for following analysis.

- SNP ID

- Plink does not like duplicate and missing IDs.
  - Fill in dbSNP ID if it's not used, as in files from Michigan and TopMed server
  - Replace missing SNP IDs with "chr_pos"
  - Rename duplicate SNP IDs with "_dup"

After imputation

- Format

- Imputed data is output as a zipped VCF file. We usually change the format back to PLINK for following analysis.

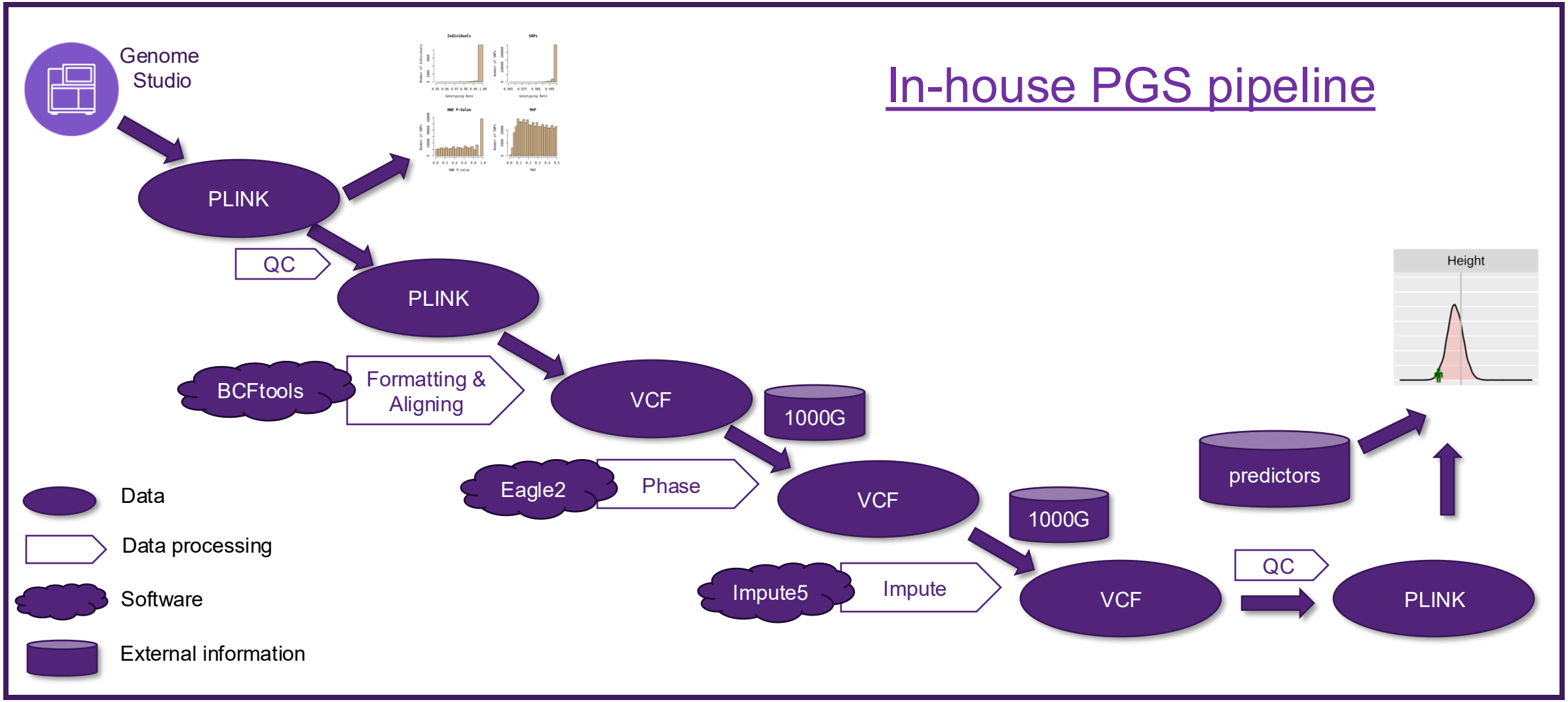
- SNP ID

- Plink does not like duplicate and missing IDs

- Quality

- We suggest to keep all the SNPs regardless of the info score and allele frequency for PGS profiling

schematic of technical pipeline



PGS profiling

```
## Example script
plink \
 --bfile  ${target}_chr${i} \
 --extract overlap_SNPs.txt \
 --score  ${trait}_SBayesRC_predictor.txt 2 5 8 header sum \
 --out ${target}_${trait}_SBayesRC
```

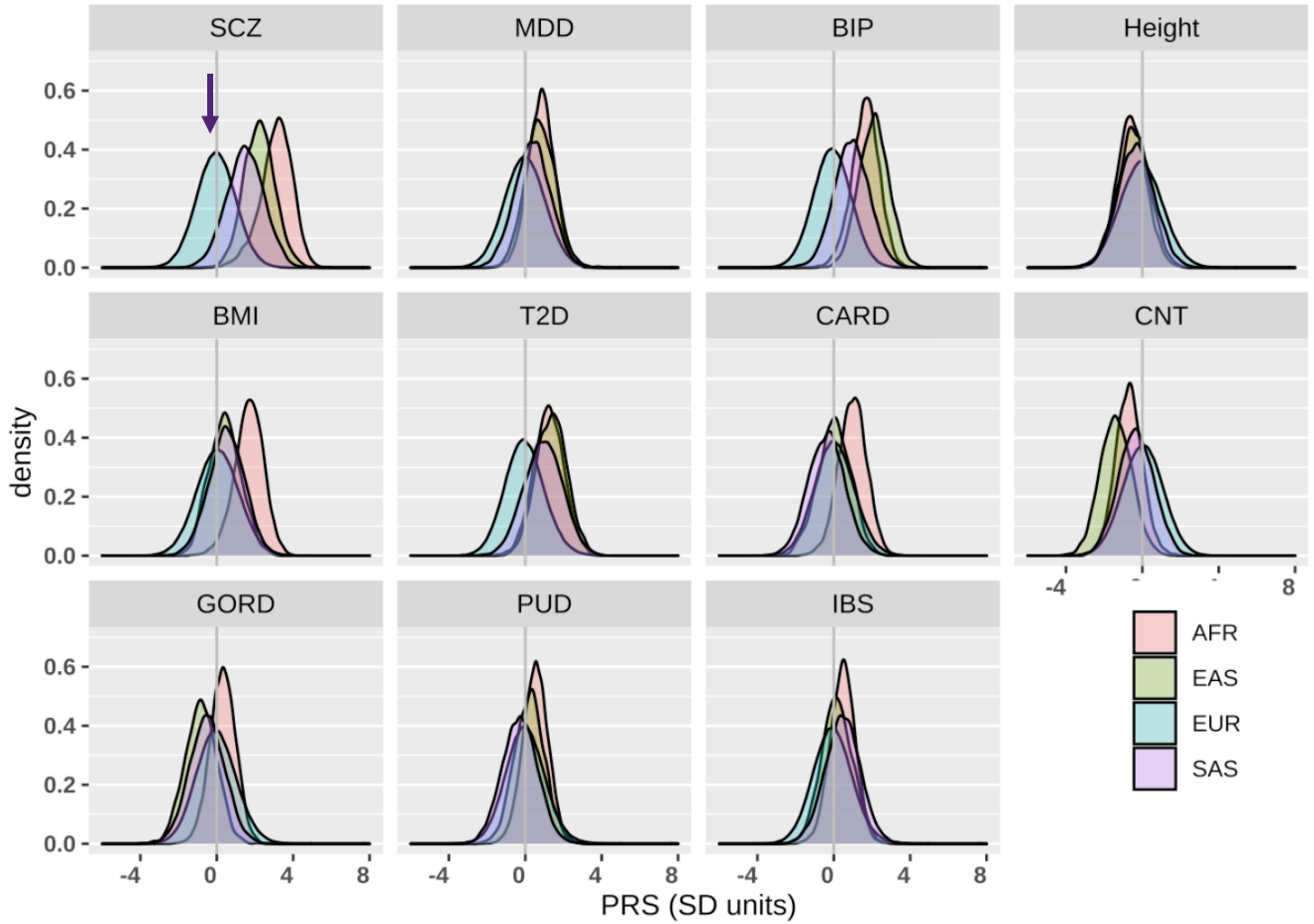
The parameters after your predictor file means

- 2 5 8: Take only the first three columns in the predictor file. The order should be columns of SNP, A1, Effect.
- header: The predictor file has a header row.
- sum: PLINK prefers to divide the score by the number of SNPs in predictor. Using “sum” will prevent the division step.
- It’s suggested to use overlap SNPs if you are going to compare two sets of data.

Interpret PGS

- Case vs. Control?
- Benchmark with population-wise scores

Match ancestry when benchmarking the PGS

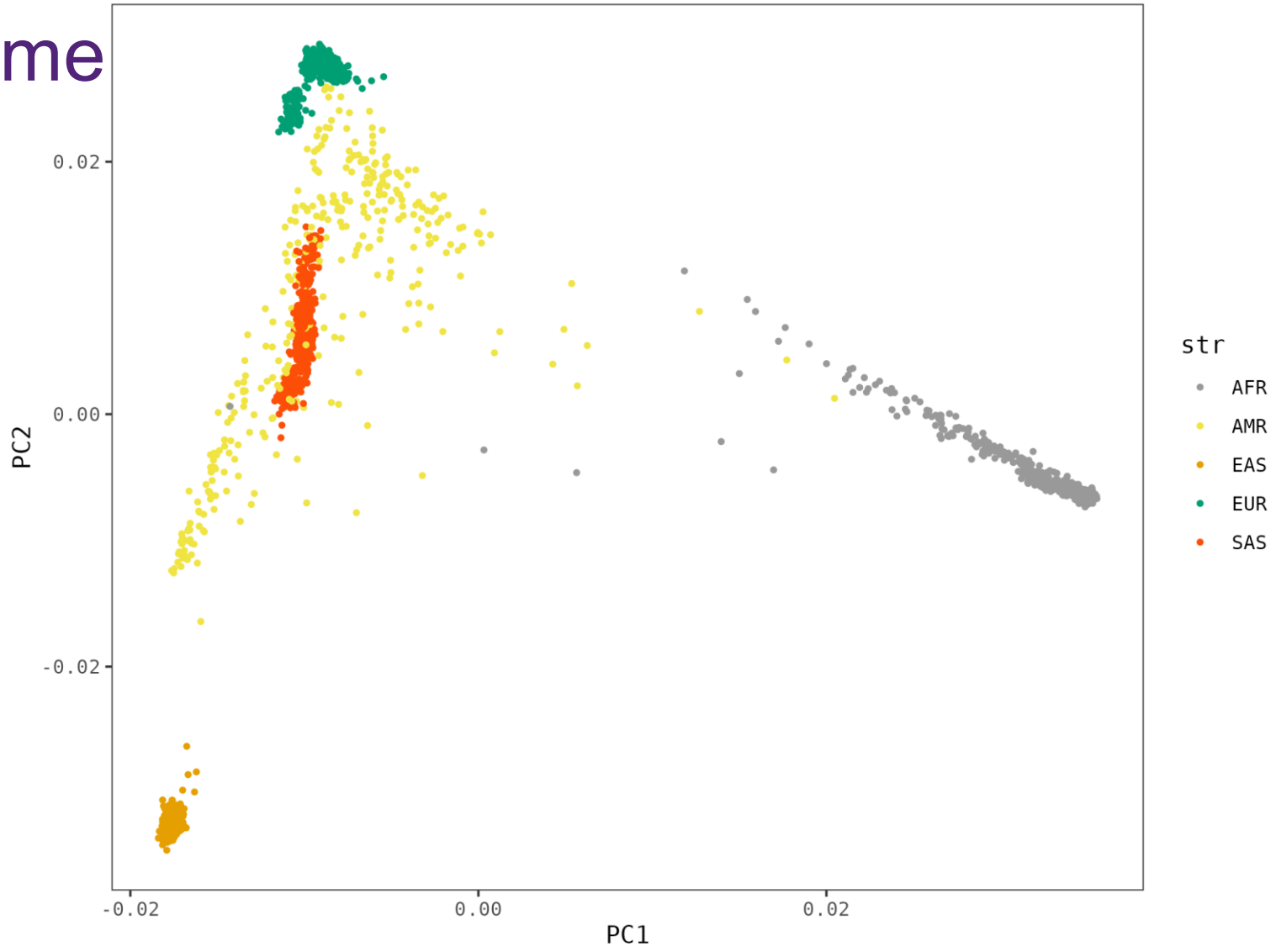


PC calculation using GCTA

- 1000G is the most widely used reference data

```
### generate GRM of reference data
     gcta --bfile ${refpath}/${pcref}.05  \
    --extract common.SNPs.txt \
    --make-grm \
    --out ${pcref}.05.common

### calculate PC of reference data
     gcta --grm     ${pcref}.05.common  --pca 3   --out  ${pcref}.05.common_pca3
```

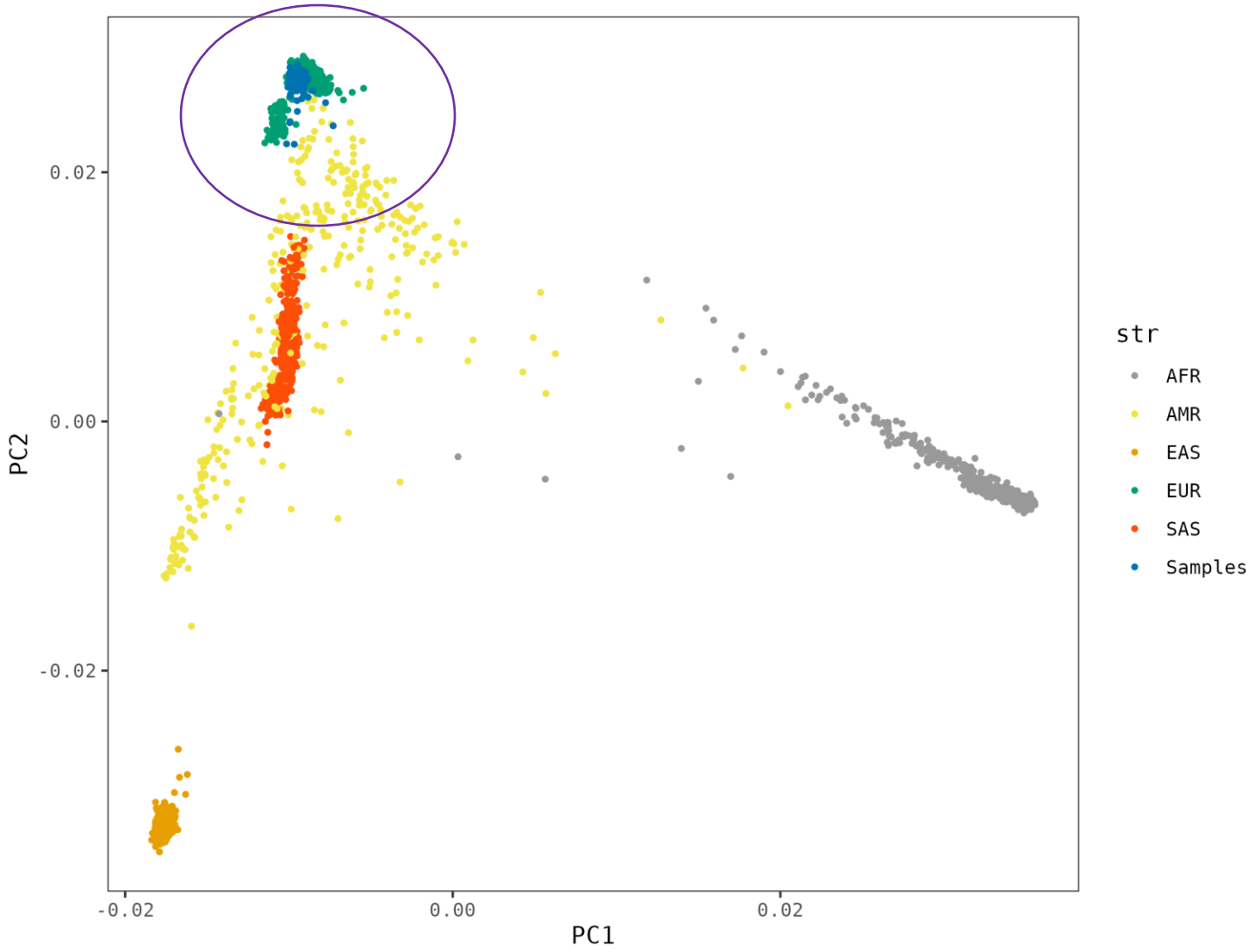
PC plot of 1000Genome



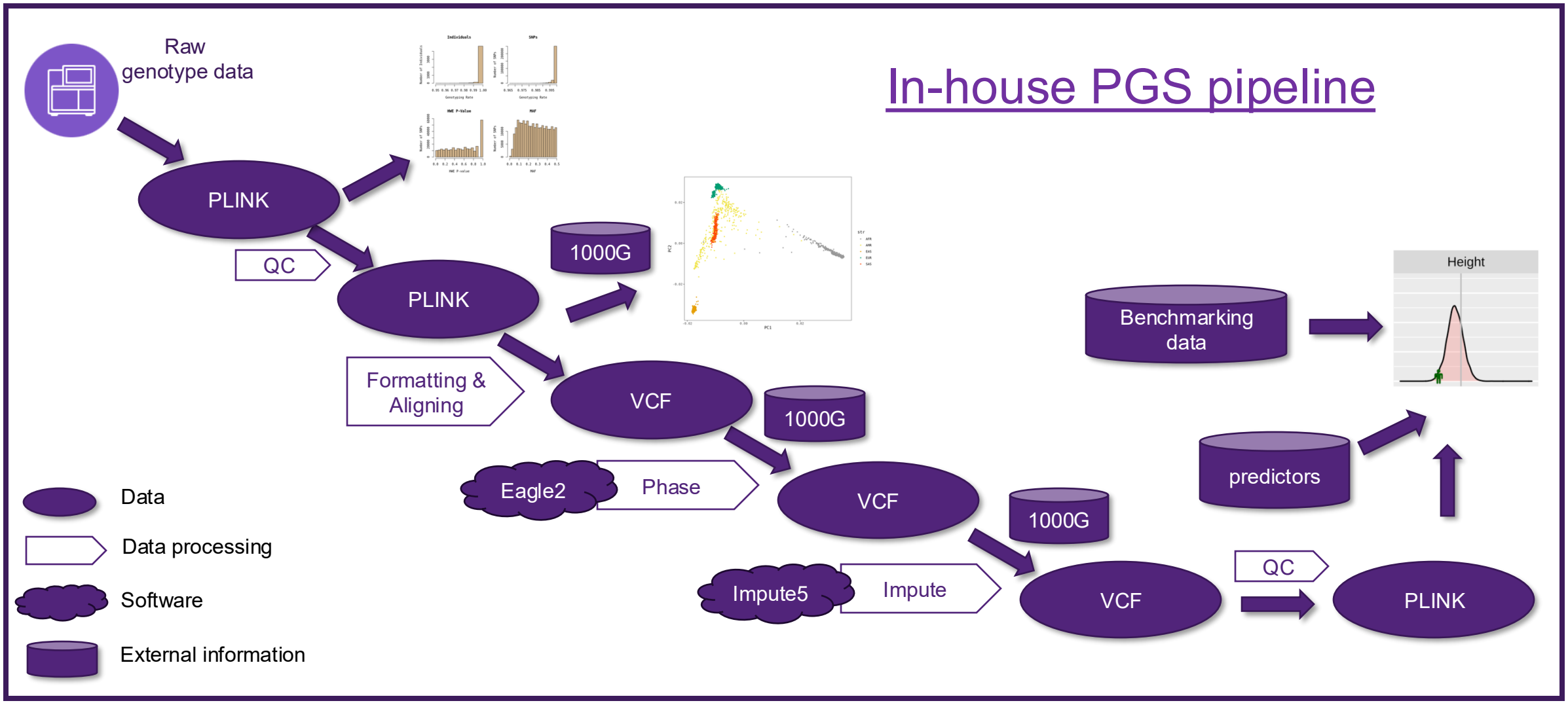
PC projection using GCTA

```
### PC loading
     gcta \
    --bfile  ${refpath}/${pcref}.05    \
    --extract  common.SNPs.txt  \
    --pc-loading   ${pcref}.05.common_pca3   \
    --out  ${pcref}.05.common_pca3_snp_loading

### PC projection
     gcta  \
    --bfile   ${data}  \
    --extract  common.SNPs.txt  \
    --project-loading   ${pcref}.05.common_pca3_snp_loading  3  \
    --out  ${data}_05.common_pca3
```

PC projection



schematic of technical pipeline



Questions and Wrap Up

Survey

https://form.jotform.com/251872762920866

Thank you!!