Human Genetics of Complex Traits Workshop

Institute of Translational Genomics
HELMHOLTZ MUNICH

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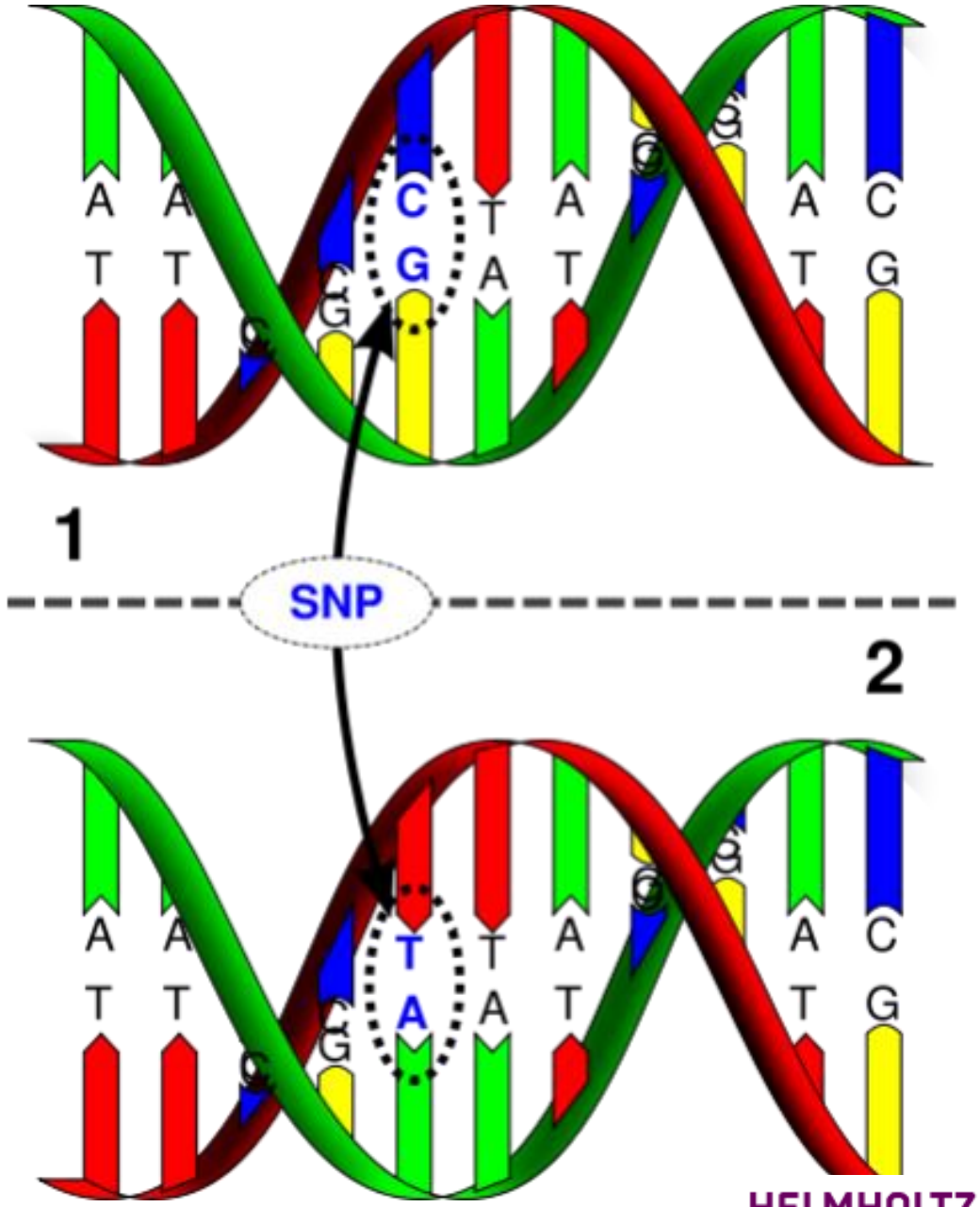
Before we start …

- A **Single Nucleotide Polymorphism** (SNP) is a single base pair at which more than one nucleotide is observed.
- The **Minor Allele Frequency (MAF)** is the relative frequency in a relevant population of the minor (2nd most common) allele.
- For biallelic SNPs, if the MAF of T allele is ***q*** then the frequency of the C allele is ***p=1-q***.

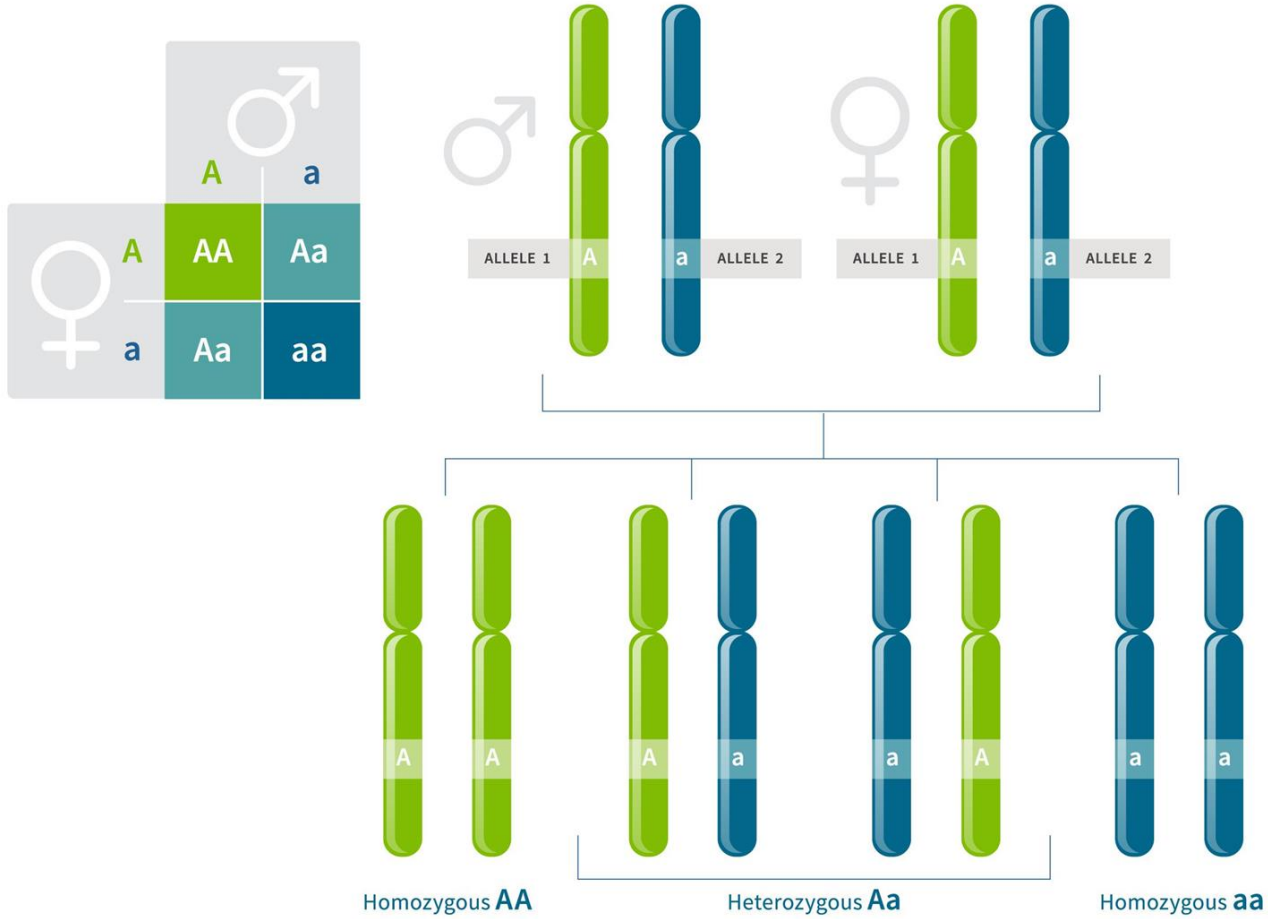


Before we start

At a given position in the DNA (or genetic locus), the pair of alleles from the two chromosomes makes up the **genotype** at that position.

SNP genotypes are **usually encoded as 0, 1 or 2**, based on the number of copies of non-reference alleles.

- genotype TT is coded as 0 (homozygous non-reference)
- genotype CT is coded as 1 (heterozygous)
- genotype TT is coded as 2 (homozygous reference)



https://www.ancestry.com/lp/genotype

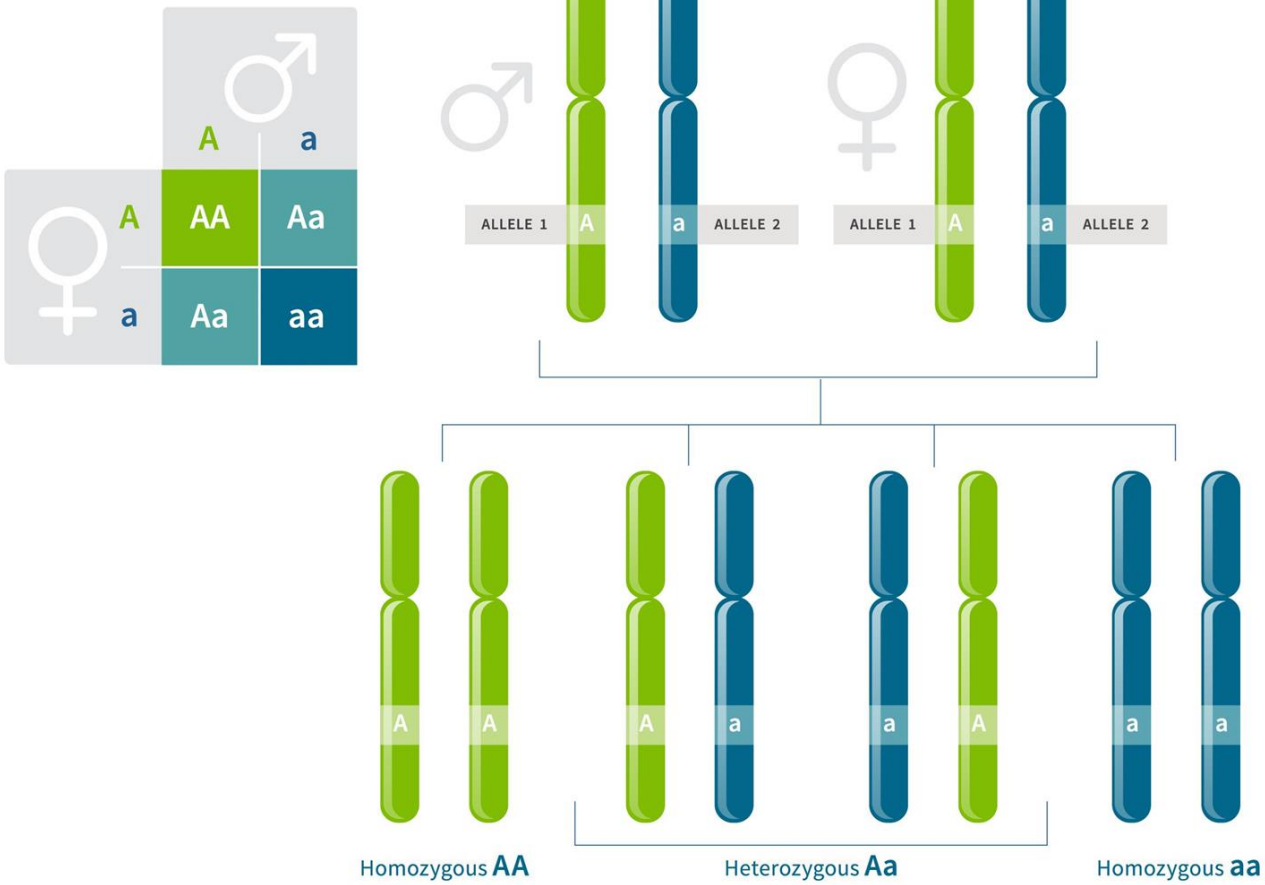
Before we start

SNP genotypes are usually **encoded as 0, 1 or 2**, based on the number of copies of non-reference alleles.

1. genotype TT is coded as 0 (homozygous non-reference)
2. genotype CT is coded as 1 (heterozygous)
3. genotype CC is coded as 2 (homozygous reference)

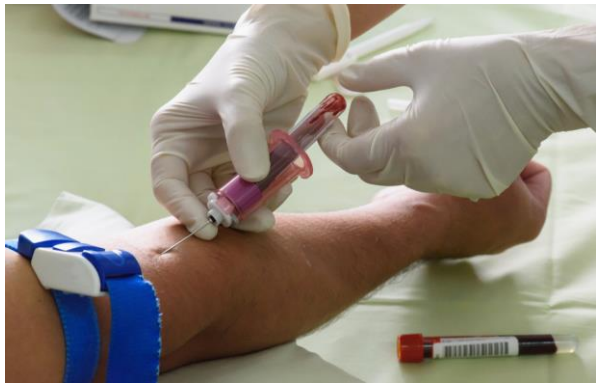
Genotypes frequency:

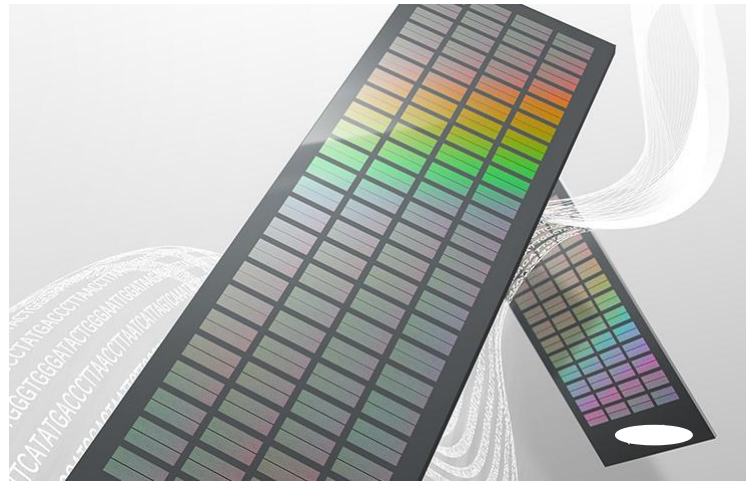
- For 1. = q^2
- For 2.= 2pq
- For 3.= p^2

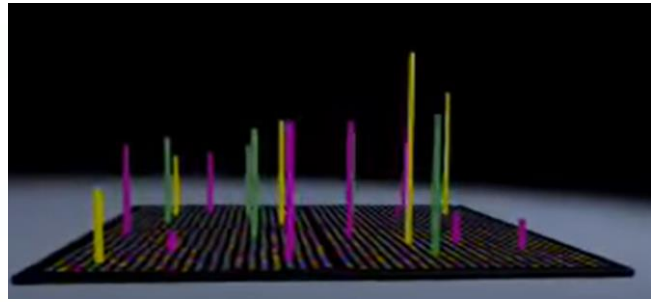


https://www.ancestry.com/lp/genotype

Did you say intensities?







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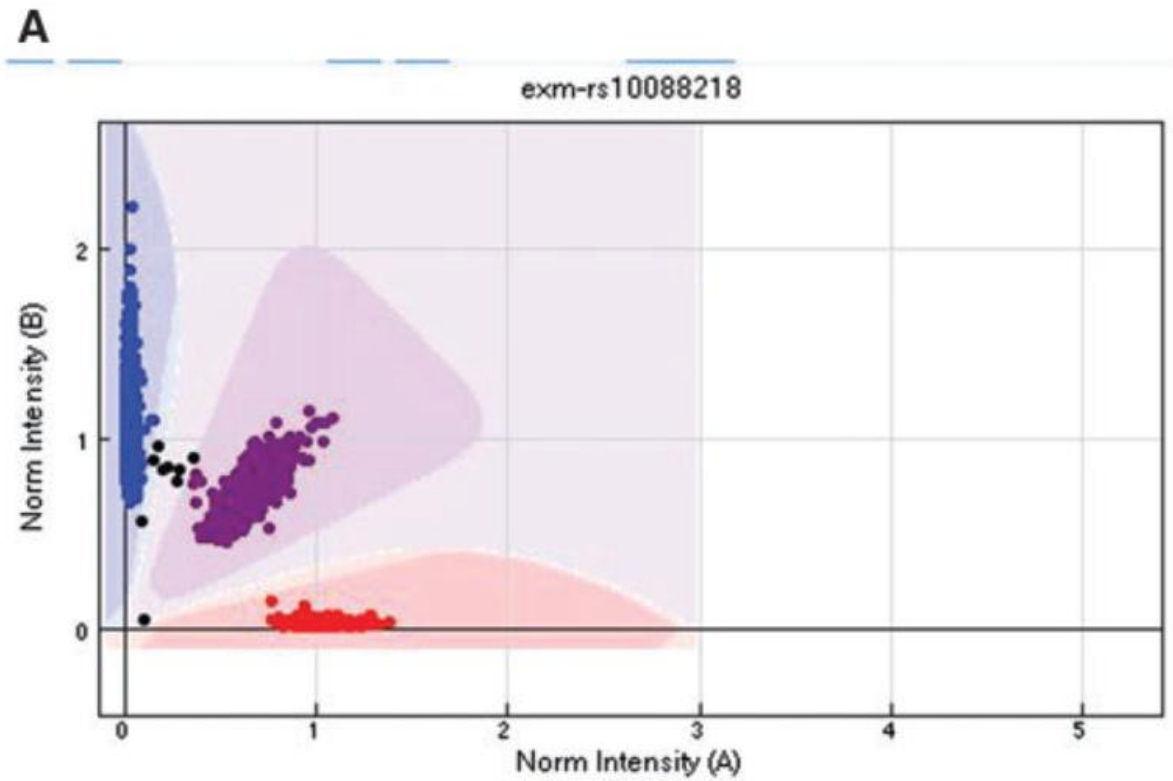
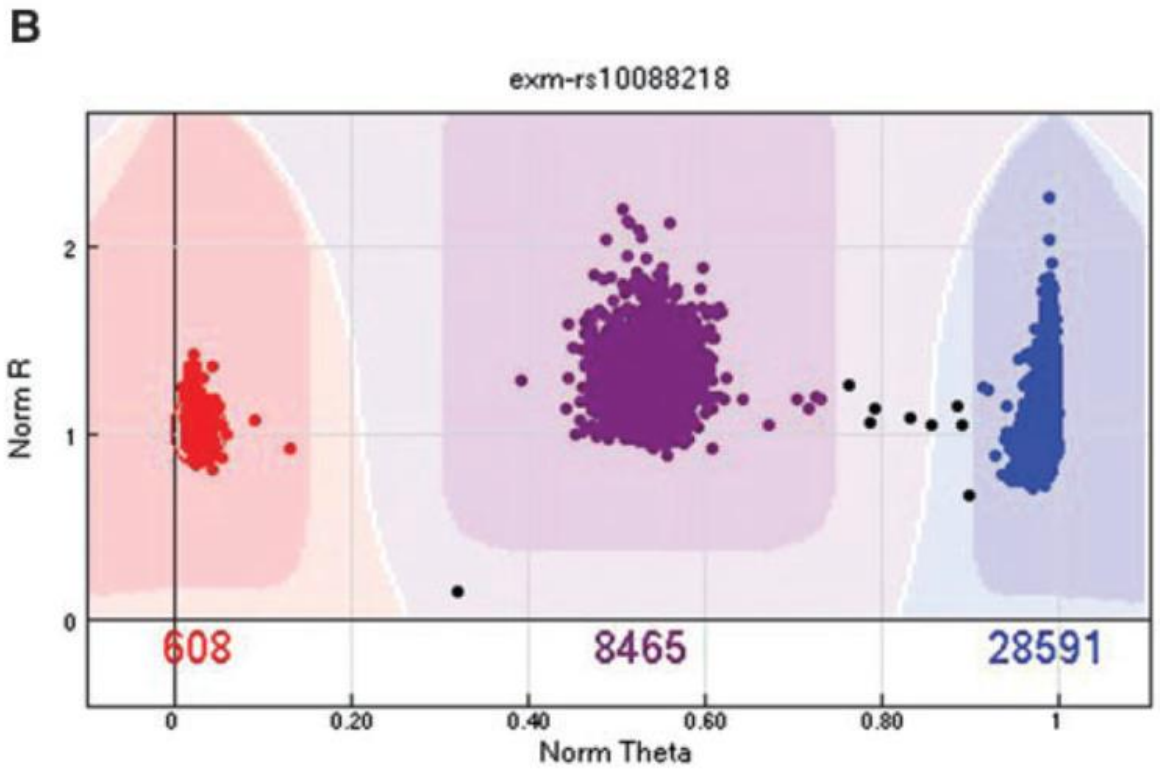
Intensities: the good …



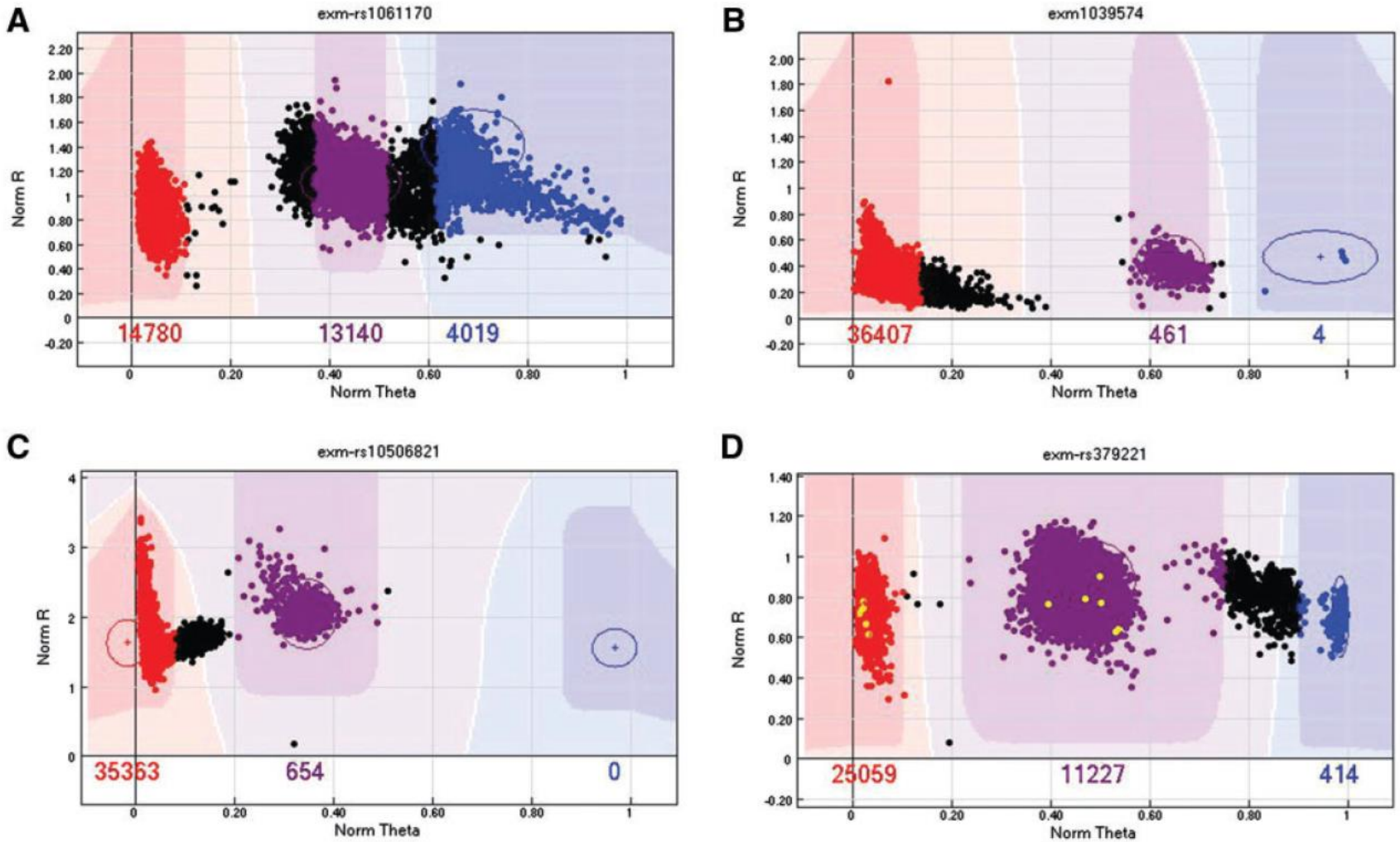
Cartesian coordinates



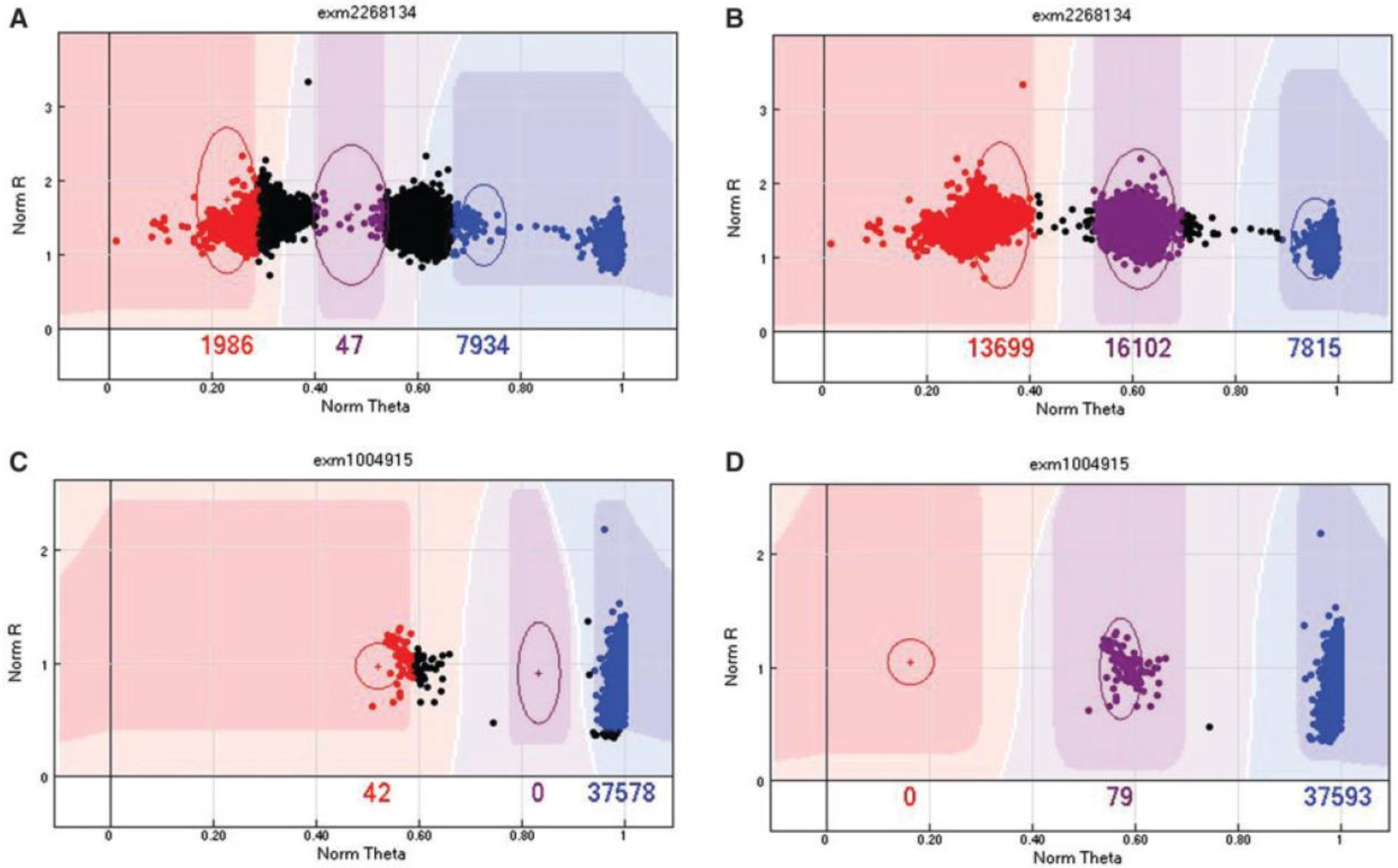
Polar coordinates

Zhao et al., 2018

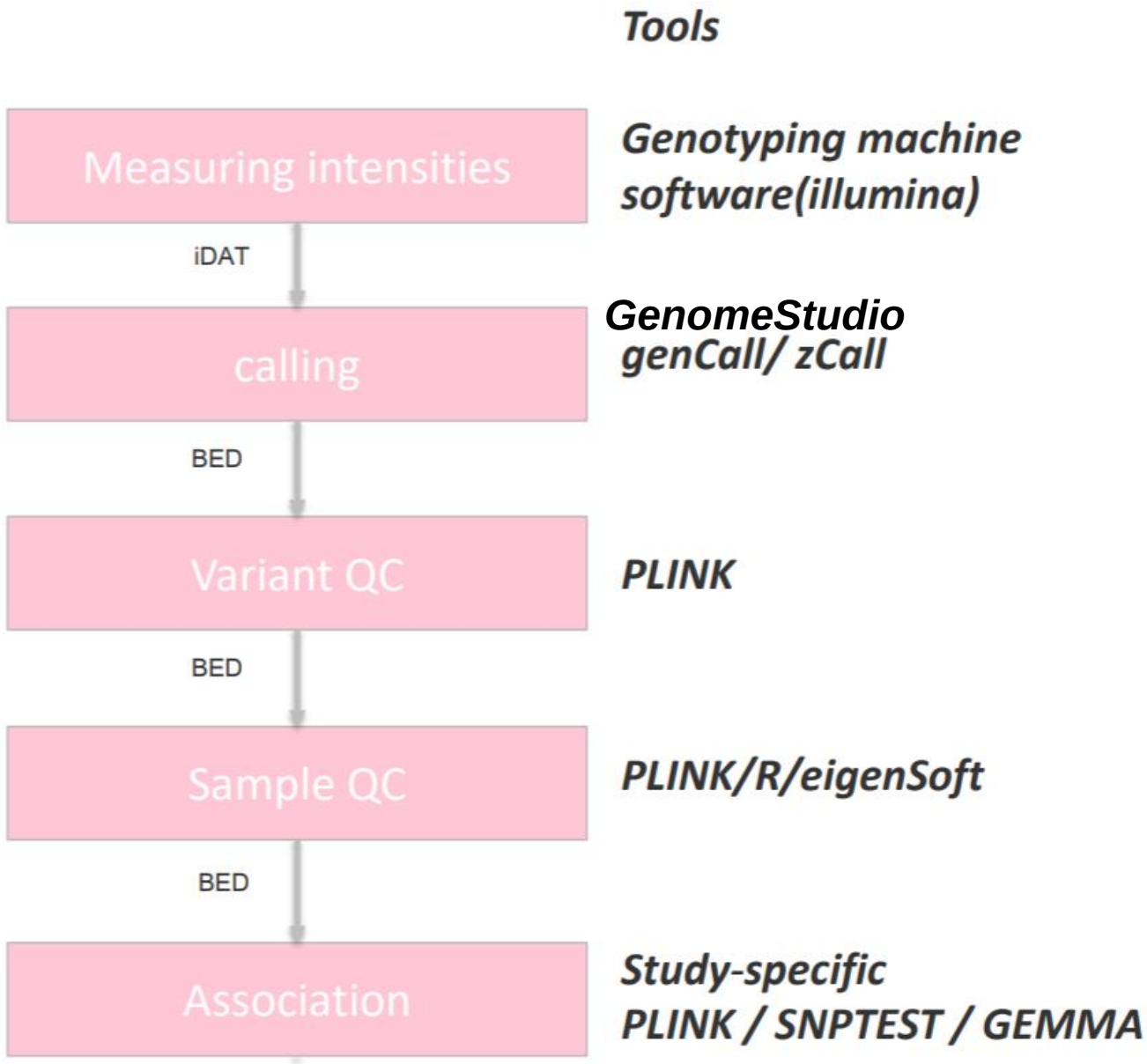
Intensities: the bad …



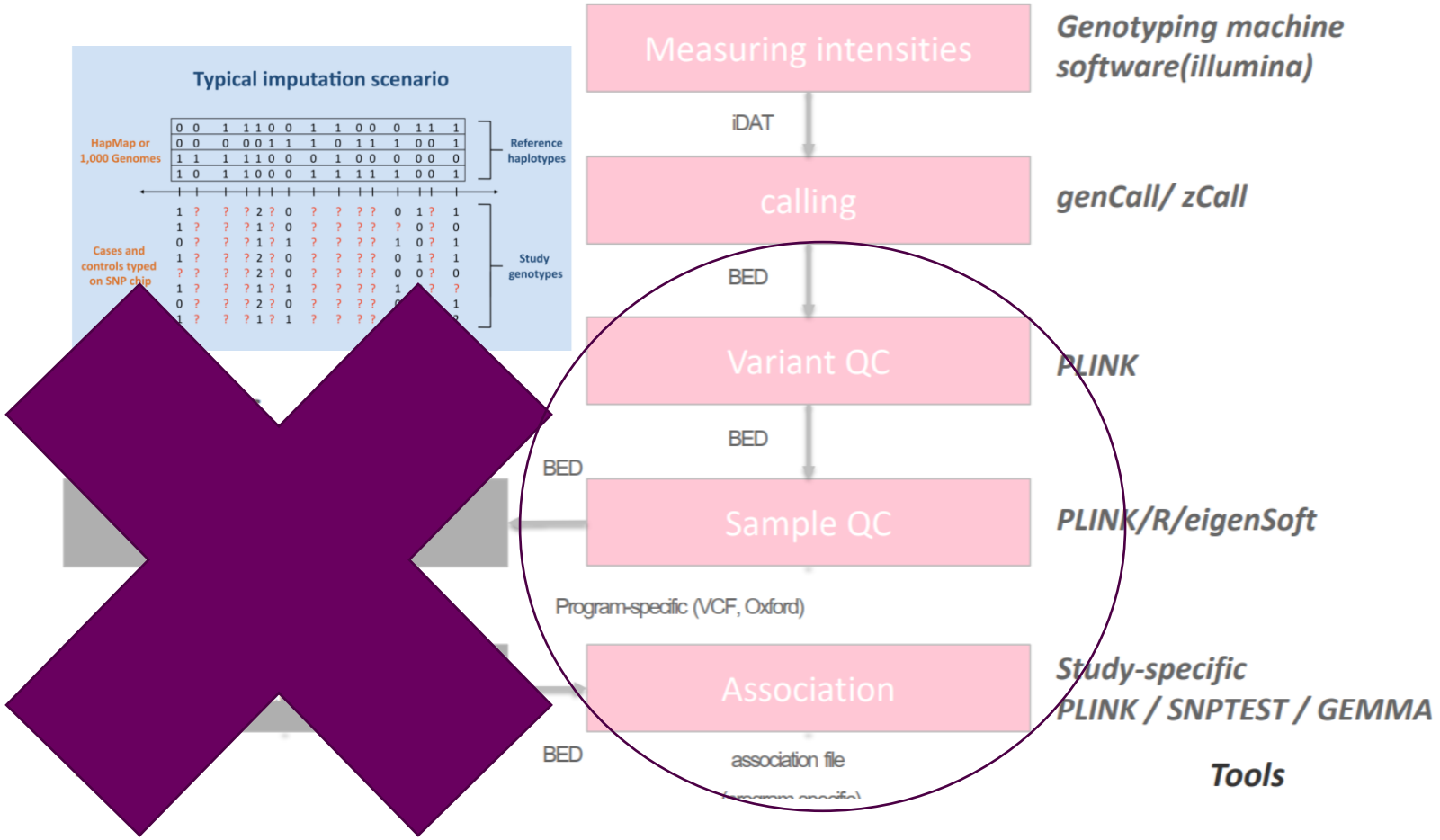
Intensities: the ugly …



The GWAS analysis pipeline



The (imputed) GWAS analysis pipeline

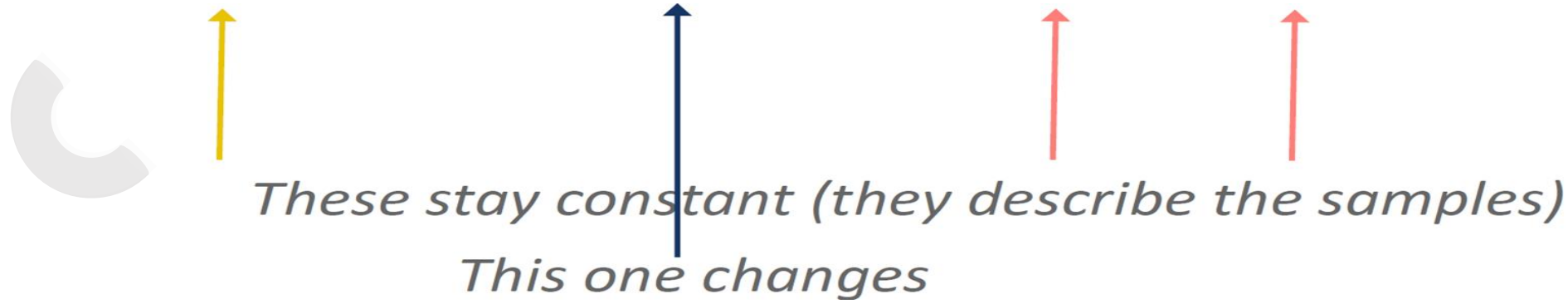


Genotyping data storage

Which data types do we need?

phenotype β genotype + covariates structure ε

$$\begin{bmatrix} pheno_0 \\ \vdots \\ pheno_n \end{bmatrix} \quad \begin{bmatrix} A/T \\ \vdots \\ T/T \end{bmatrix} \quad \begin{bmatrix} male \\ \vdots \\ female \end{bmatrix} \quad \begin{bmatrix} 22\ years \\ \vdots \\ 65\ years \end{bmatrix} \quad \begin{bmatrix} r_{00} & \cdots & r_{0n} \\ \vdots & r_{ij} & \vdots \\ r_{n0} & \cdots & r_{nn} \end{bmatrix}$$



Genotyping data storage: PLINK

Can either be text-format files or binary files.

*.ped

FID	IID	PID	MID	Sex	P	rs1	rs2	rs3
1	1	0	0	2	1	CT	AG	AA
2	2	0	0	1	0	CC	AA	AC
3	3	0	0	1	1	CC	AA	AC

*.map

Chr	SNP	GD	BPP
1	rs1	0	870000
1	rs2	0	880000
1	rs3	0	890000

*.fam

FID	IID	PID	MID	Sex	P
1	1	0	0	2	1
2	2	0	0	1	0
3	3	0	0	1	1

*.bed

Contains binary version of the SNP info of the *.ped file. (not in a format readable for humans)

*.bim

Chr	SNP	GD	BPP	Allele 1	Allele 2
1	rs1	0	870000	C	T
1	rs2	0	880000	A	G
1	rs3	0	890000	A	C

```
10101111 10101111 10100010 10111011 10101000 10000000
00101011 00100000 10101000 10001011 00000011 11111111
11111111 11111111 11111111 11111110 11111111 11111111
11111111 11111110 11111110 11111110 11101111 11111111
```

Legend			
FID	Family ID	rs{x}	Alleles per subject per SNP
IID	Individual ID	Chr	Chromosome
PID	Paternal ID	SNP	SNP name
MID	Maternal ID	GD	Genetic distance (morgans)
Sex	Sex of subject	BPP	Base-pair position (bp units)
P	Phenotype	C{x}	Covariates (e.g., Multidimensional Scaling (MDS) components)

Marees et al., 2017

Genotyping data storage: PLINK

*.ped

FID	IID	PID	MID	Sex	P	rs1	rs2	rs3
1	1	0	0	2	1	CT	AG	AA
2	2	0	0	1	0	CC	AA	AC
3	3	0	0	1	1	CC	AA	AC

*.map

Chr	SNP	GD	BPP
1	rs1	0	870000
1	rs2	0	880000
1	rs3	0	890000

ped(igree) file has **6+2*n***, providing:

1. Family ID
2. Individual ID
3. Paternal ID (0 if father not in dataset)
4. Maternal ID (0 if mother not in dataset)
5. Sex (1=Male, 2=Female, 0 or -9=missing)
6. Phenotype (here 2 or 1, corresponding to case and control)
7. 2 alleles for each SNP (0 = missing)

- **map**(ing) file has **4 columns**, providing:
  1. Chromosome
  2. SNP Name
  3. Genetic distance (in morgans)
  4. Base-pair position (bp unit)

Genotyping data storage: PLINK

*.fam

FID	IID	PID	MID	Sex	P
1	1	0	0	2	1
2	2	0	0	1	0
3	3	0	0	1	1

*.bed

Contains binary version of the SNP info of the *.ped file. (not in a format readable for humans)

*.bim

Chr	SNP	GD	BPP	Allele 1	Allele 2
1	rs1	0	870000	C	T
1	rs2	0	880000	A	G
1	rs3	0	890000	A	C

fam(ily) file consists of the first six columns of ped file

- The **bed** (binary pedigree) file is a matrix of 0s, 1s, 2s or NAs stored in binary format.
  - PLINK uses the following two-bit coding of genotypes:
    - 00 = A1/A1 (Homozygous non-reference)
    - 01 = A1/A2 (Heterozygous)
    - 11 = A2/A2 (Homozygous reference)
    - 10 = 0/0 (Missing)
- **bim** (binary mapping) file is the .map file plus two columns, providing the A1 and A2 alleles

Genotyping data storage: PLINK

What is left?

Matrix file
(program-specific)

$$\begin{bmatrix} r_{00} & \cdots & r_{0n} \\ \vdots & r_{ij} & \vdots \\ r_{n0} & \cdots & r_{nn} \end{bmatrix}$$

Covariate file

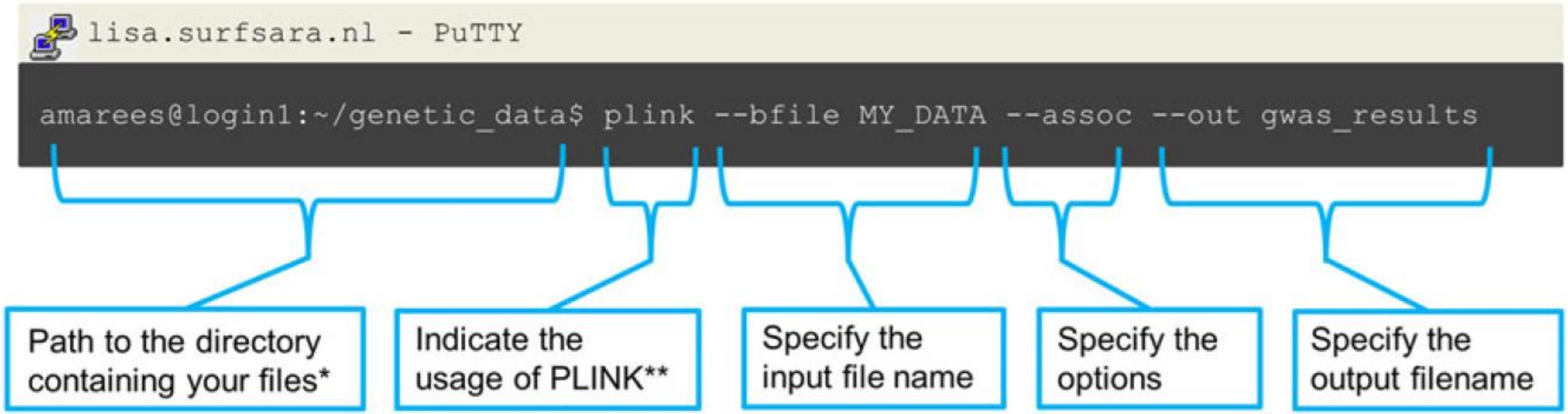
FID	IID	C1	C2	C3
1	1	0.00812835	0.00606235	-0.000871105
2	2	-0.0600943	0.0318994	-0.0827743
3	3	-0.0431903	0.00133068	-0.000276131

Phenotype files have 2 + M columns: Family ID,
Individual ID,
then value for each of M phenotypes

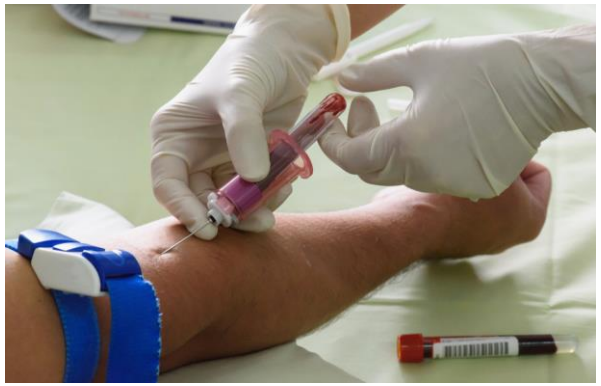
Marees et al., 2017

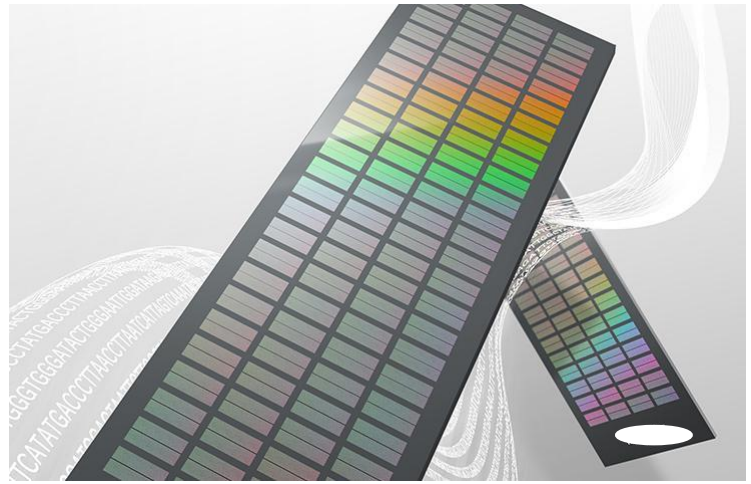
Genotyping data : PLINK common operations

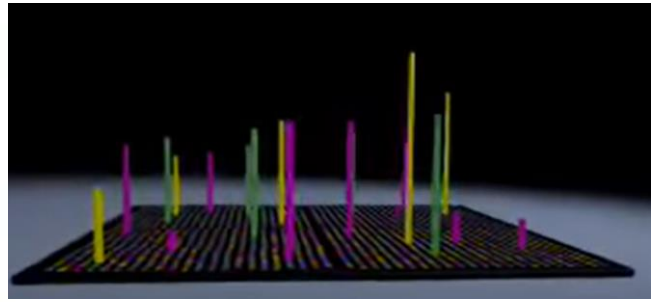
- https://www.cog-genomics.org/plink/1.9/index
- https://www.cog-genomics.org/plink/2.0/index



Why Quality Control?







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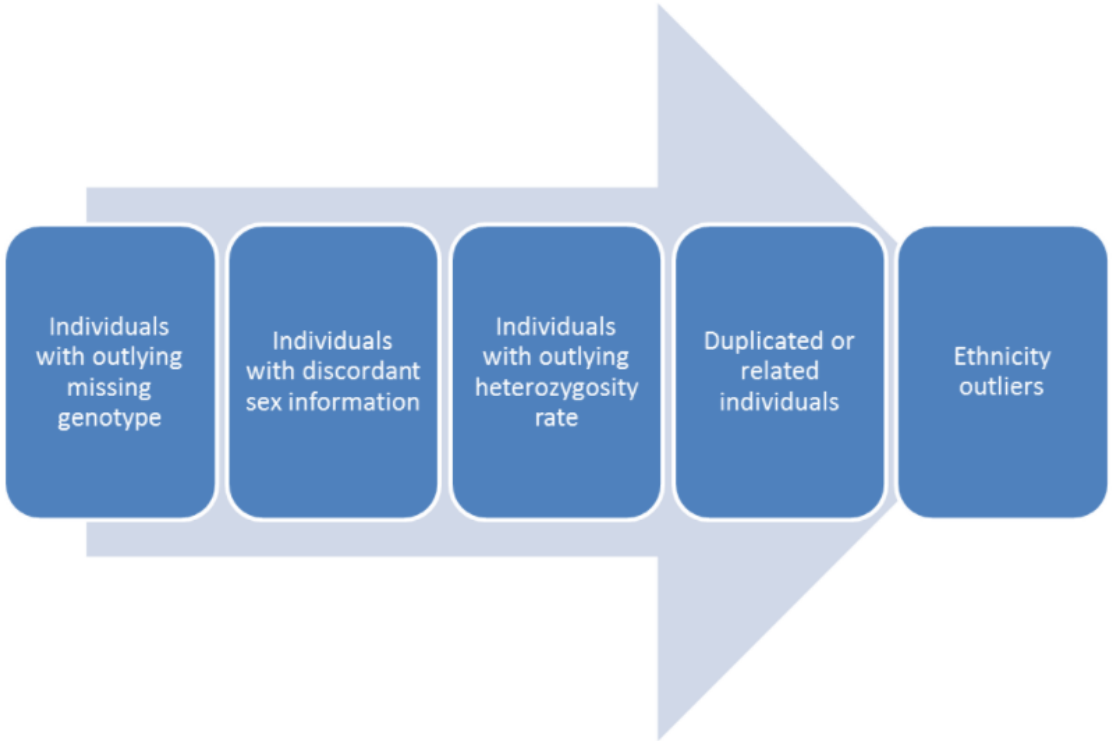
Why Quality Control?

The QC protocol of a GWAS is usually split into two broad categories.

"Sample QC"



"Variant QC"

1. Identification of variants with an **excessive missing genotype**
2. Identification of variants demonstrating a significant deviation from **Hardy-Weinberg equilibrium (HWE)**
3. Removal of all makers with a **very low minor allele frequency**
4. Removal of all makers with **cluster separation score <0.4**
5. **Differential missingness** (case/control studies)

(A generalized) Quality Control process

Missingness

1. Per sample missingness

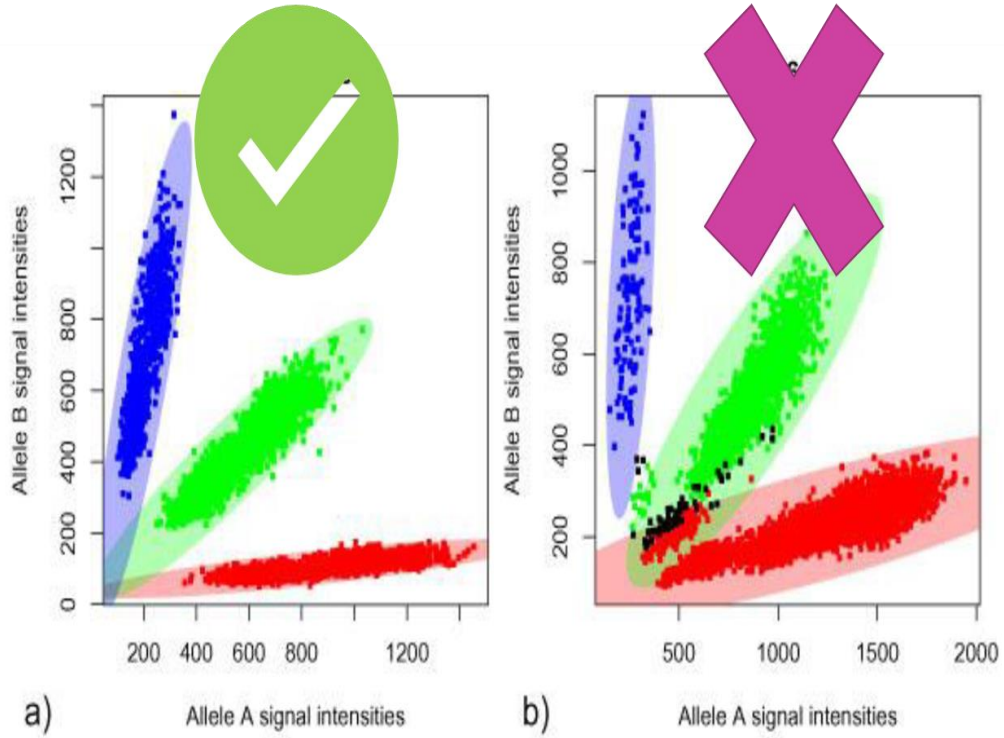
➢ % missing for a sample across your variants

2. Per SNP missingness

➢ % missing for a particular variant among your samples

Quality control step	PLINK summary commands	PLINK filtering commands
Missingness	`--missing`	`--geno`, `--mind`

➢Low genotyping call rate indicates issues with sample DNA (eg low concentration).



(A generalized) Quality Control process

Discordant Sex Check

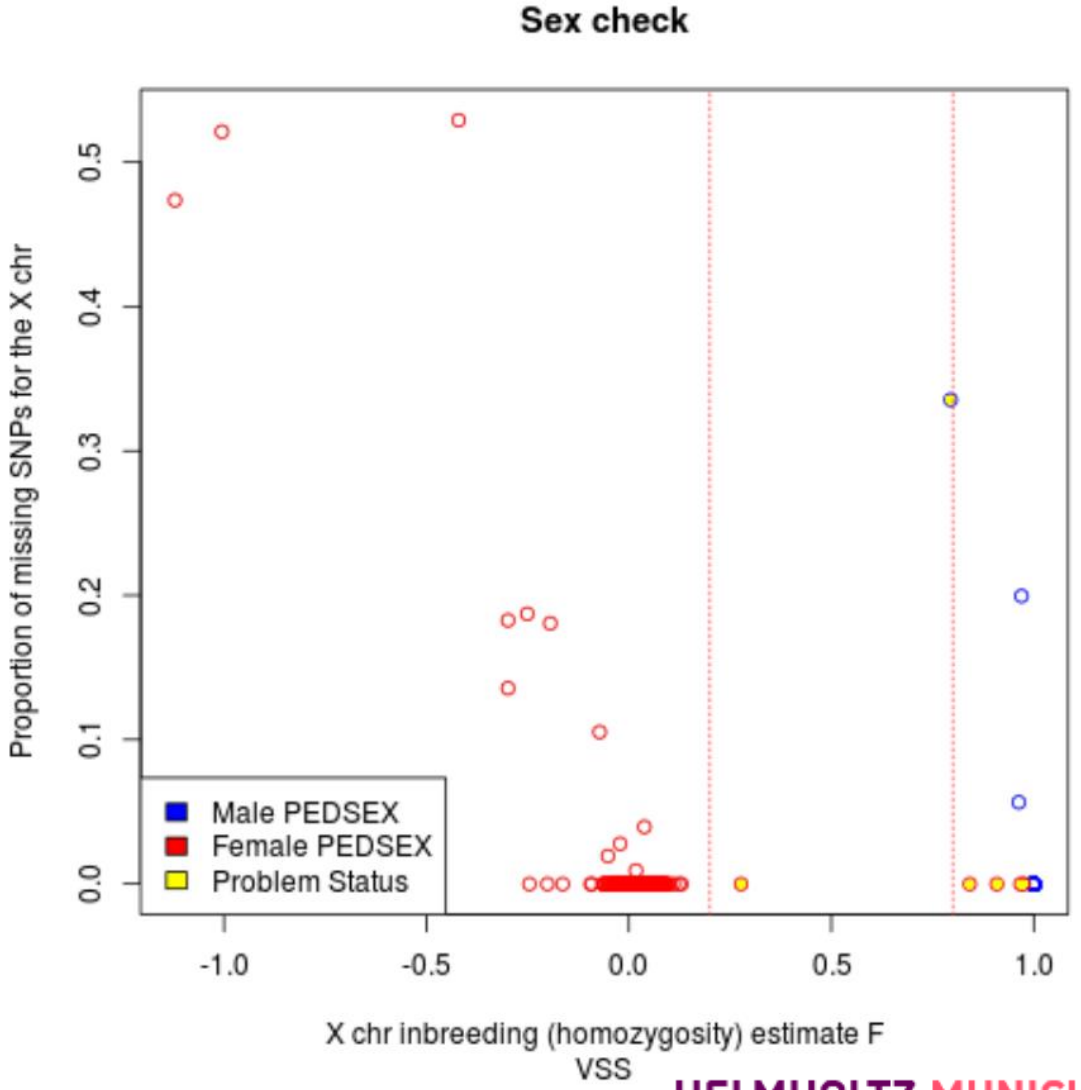
- Men have only one copy of the X chromosome
  - All X chromosome data is expected to be homozygous.

Example Alleles	Female genotypes possible	Male genotypes possible
A,C	A/A, A/C, C,C	A/A or C/C

- X chromosome homozygosity estimate for males (F statistic or inbreeding coefficient) is 1.
- In Plink

`--check-sex`	Check sexes by looking at chrX

- Male (1) : XHE >0.80
- • Female (2) : XHE <0.20
- • No sex (0) : 0.20 <XHE <0.80



(A generalized) Quality Control process

Heterozygosity rate

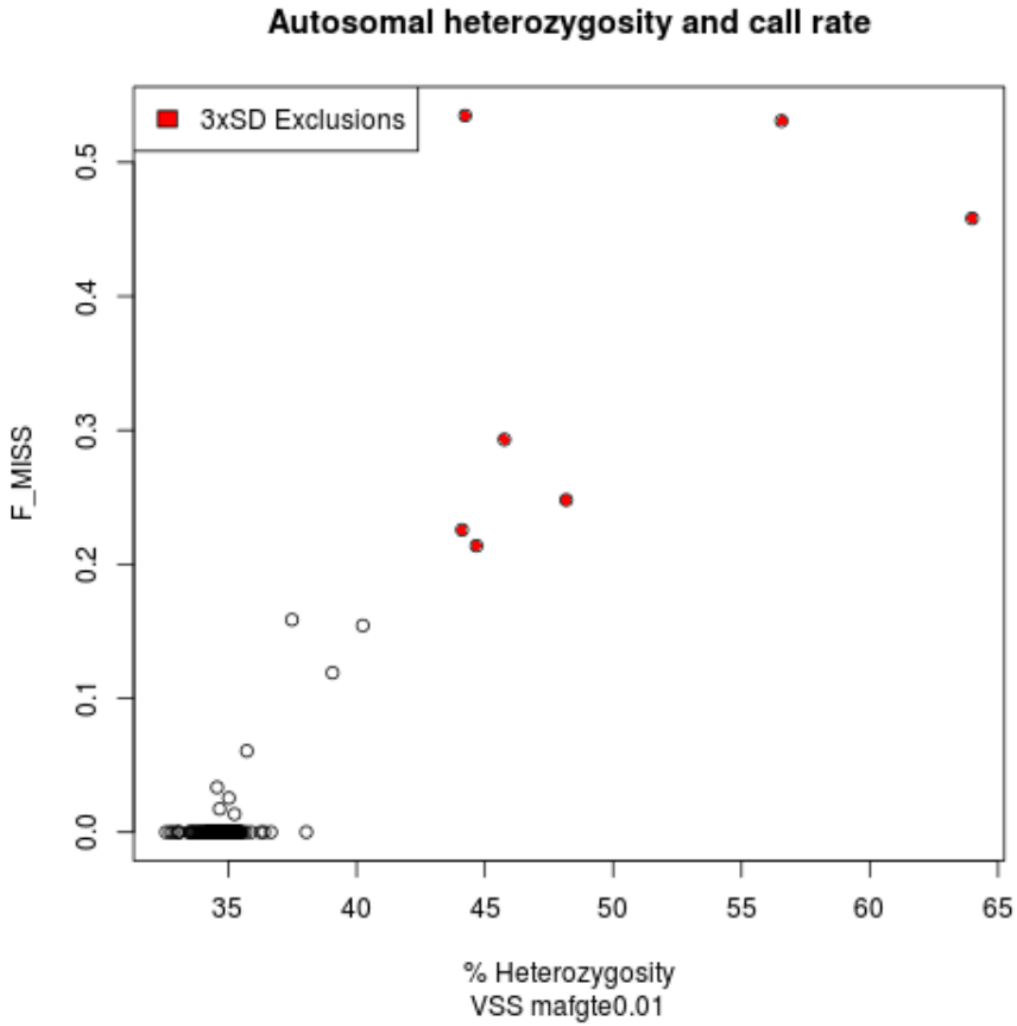
- The proportion of heterozygous genotypes (per sample)
- Various ways of calculating the rate

PLINK: (<observed hom. count> - <expected count>) / (<total observations> - <expected count>))

--het (gives back and F estimate)

<custom scripts>

- Excess heterozygosity -> Possible sample contamination
- Less than expected heterozygosity –> Possibly inbreeding



(A generalized) Quality Control process

Duplicated or related individuals

A basic assumption of GWAS: unrelated individuals

- Either exclude or account for it

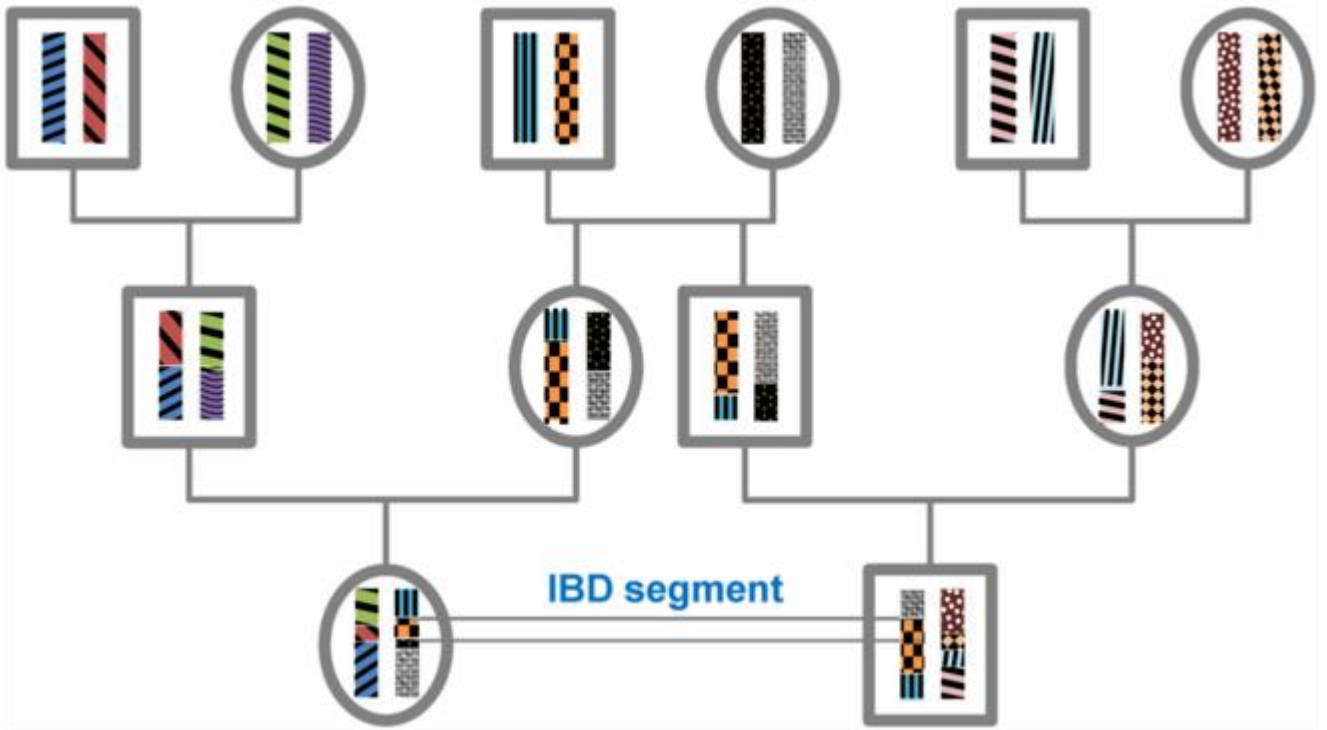
The presence can introduce a bias: genotypes in families to be over-represented

(A generalized) Quality Control process

Duplicated or related individuals

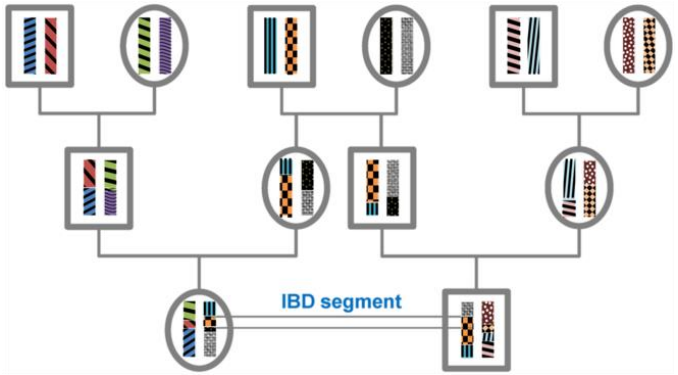
Calculated metrics:

- **Identity by state (IBS): A DNA segment is identical by state (IBS) in two or more individuals if they have identical nucleotide sequences in this segment.**
- **Identity by Descent (IBD): An IBS segment is identical by descent (IBD) in two or more individuals if they have inherited it from a common ancestor without recombination, that is, the segment has the same ancestral origin in these individuals.**



Wikipedia

(A generalized) Quality Control process



Duplicated or related individuals

PLINK calculates identity by descent (IBD) of all sample

```
--genome
```

Approximates the percentage IBD overall, representing pairs as s

- Zero alleles IBD (z0)
- One allele IBD (z1)
- Two alleles IBD (z2)

PI_HAT (the proportion IBD, defined as P(IBD = 2) + 0.5*P(IBD =

Use an independent SNP set before running this command:
1) removing regions of extended Linkage Disequilibrium (LD)
and
2) pruning the remaining regions so that no pair of SNPs within a given window is correlated.

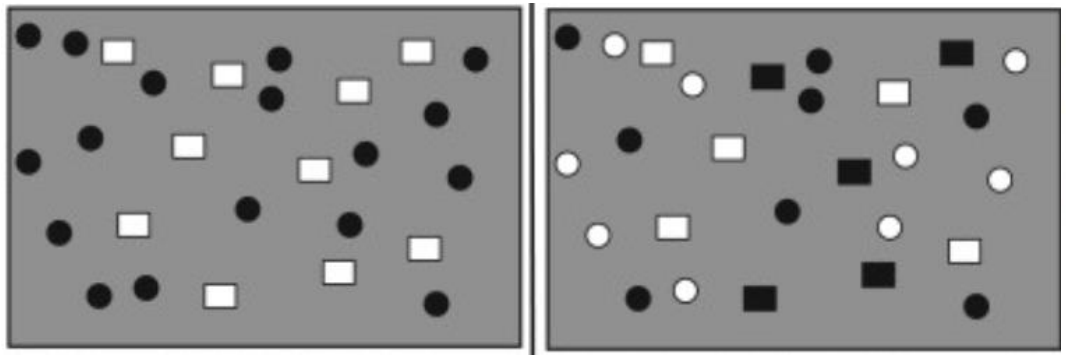
Relationship type	z0	z1	z2	PI_HAT
Unrelated	1	0	0	0
Monozygotic (MZ) twin	0	0	1	1
Full siblings	0.25	0.5	0.25	0.5
Half siblings	0.5	0.5	0	0.25
Parent-offspring	0	1	0	0.5

Slifer, 2018

(A generalized) Quality Control process

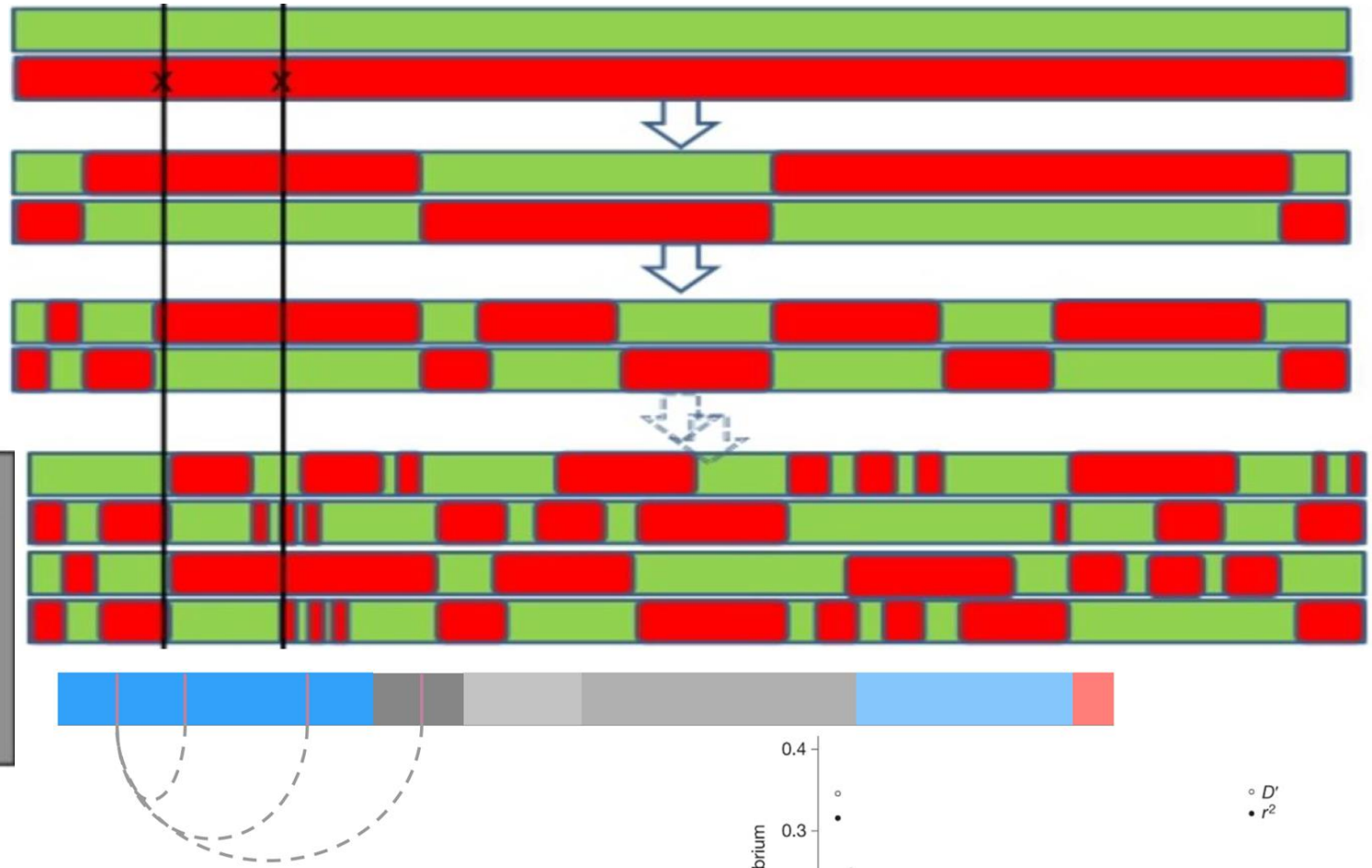
Linkage disequilibrium (LD)

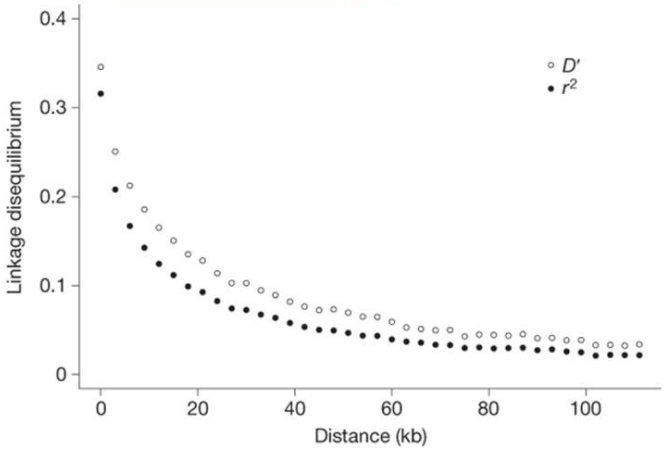
Is the non-random association of alleles at different loci in a given population.



In GWAS we (mainly) use correlation coefficient between pairs of loci, r^2

r^2=1 is perfect LD



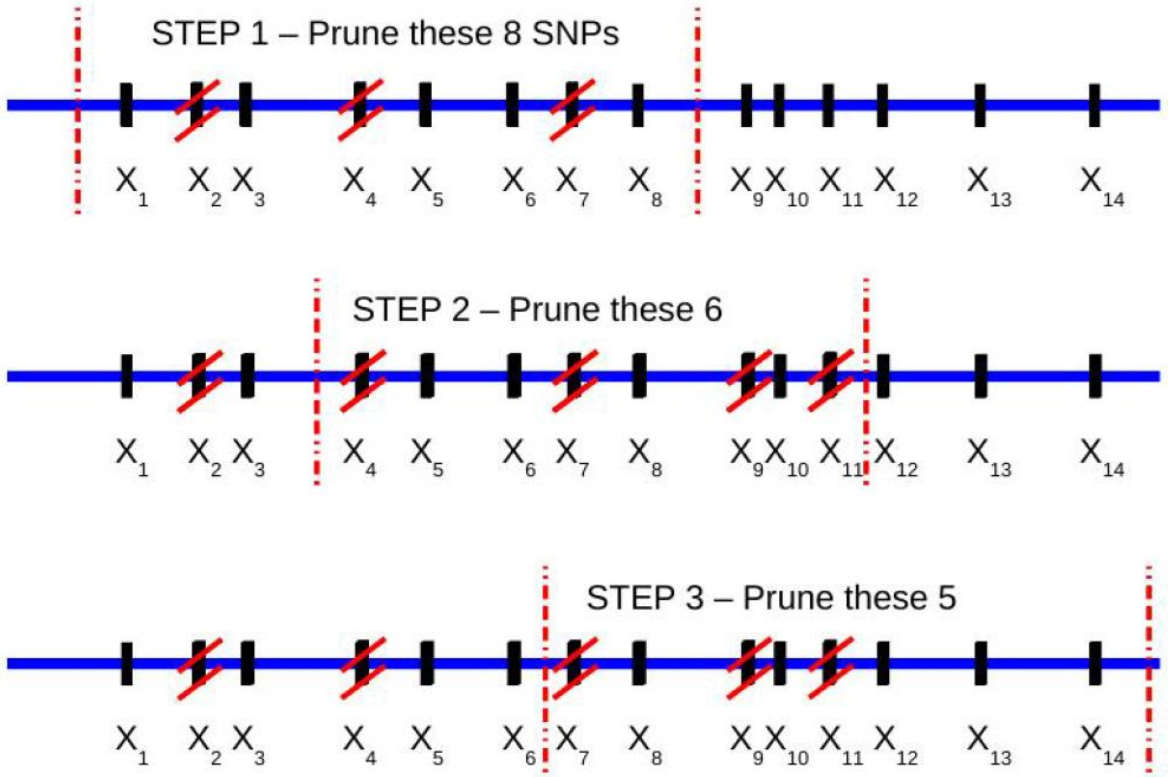


(A generalized) Quality Control process

PLINK: LD-based SNP prunning

```
plink --indep-pairwise <window> <step> <rsq> --bfile <data> --out <output>
```

```
plink --indep-pairwise 8 3 <rsq> --bfile <data> --out <output>
```



(A generalized) Quality Control process

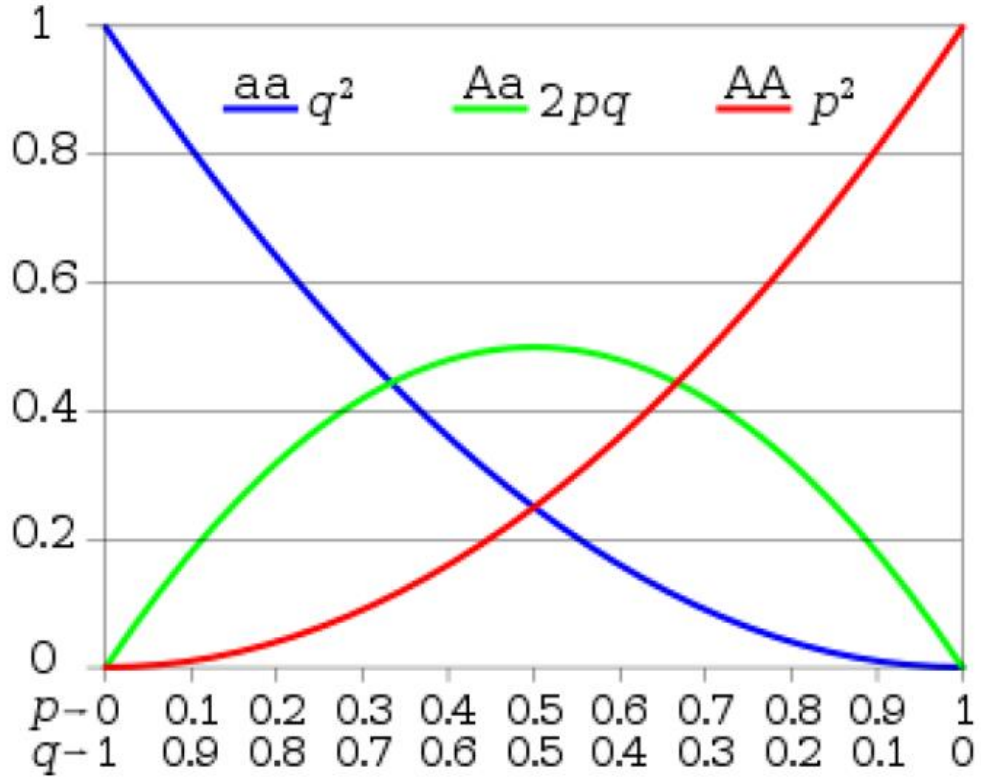
The Hardy–Weinberg (dis)equilibrium (HWE) law:

The genotype and the allele frequencies are constant over generations.

Assumes:

- An indefinitely large population
- With no selection, no Mutation, no Migration ……

Significant deviations indicate genotyping errors



PLINK:

Quality control step	PLINK summary commands	PLINK filtering commands
Hardy-Weinberg equilibrium check	`--hardy`	`--hwe`

Less strict case threshold avoids discarding disease-associated SNPs

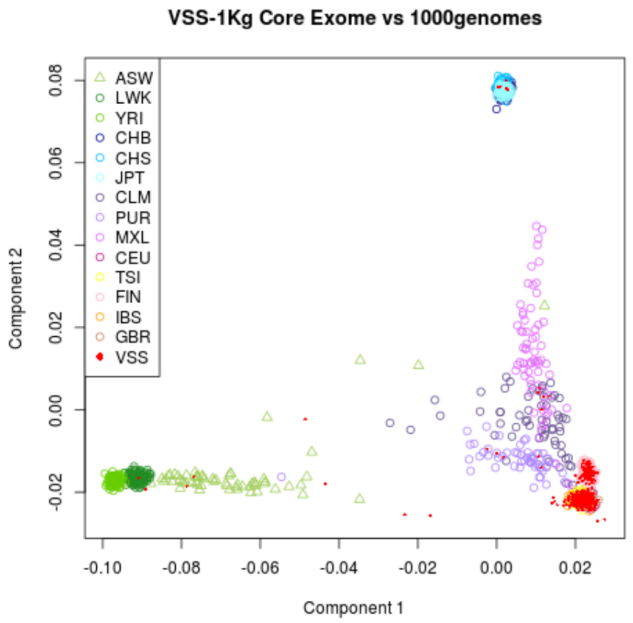
(A generalized) Quality Control process

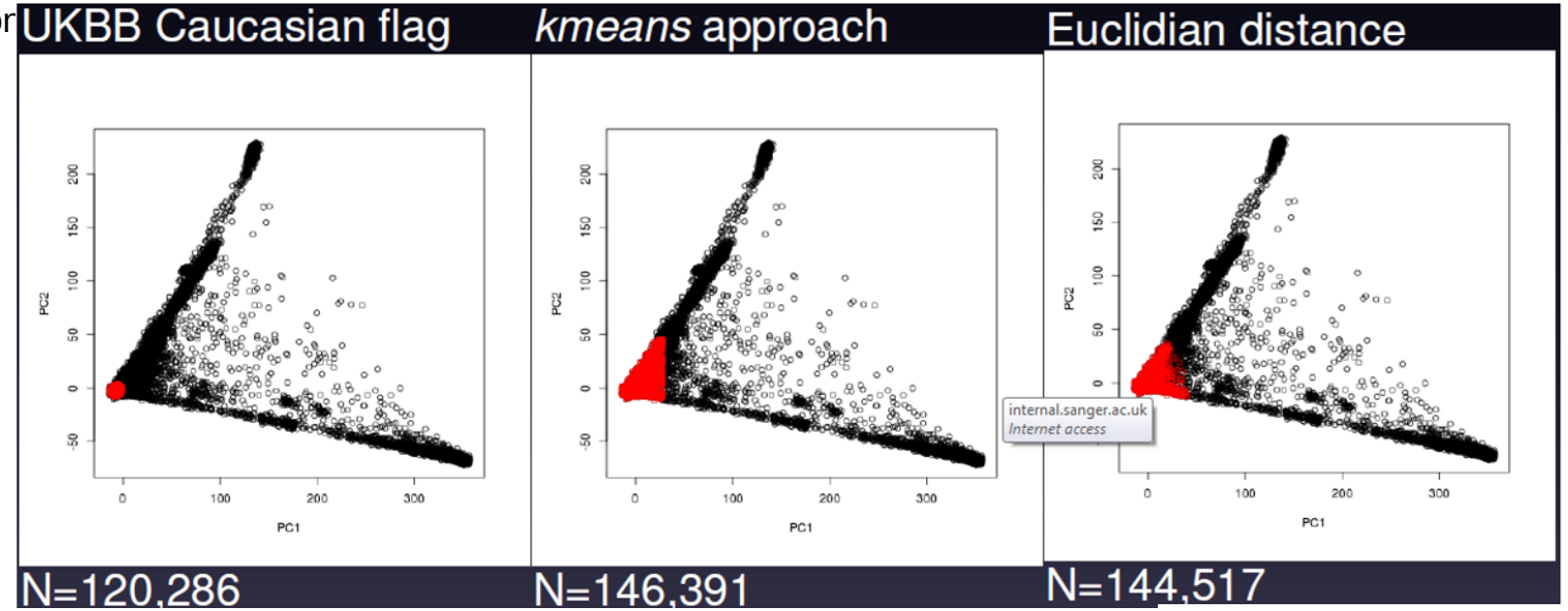
Population structure

Occurs when samples have different genetic ancestries

Allele frequencies can differ between subpopulations and can lead to spurious associations due to differences in ancestry rather than true associations

PLINK: Merge with a population of known ethnic structure (e.g., HapMap/1KG data) and identify outliers through dimension reduction analyses such as Principal Component Analysis and/or MultiDimensior





(A generalized) Quality Control process

Variant QC

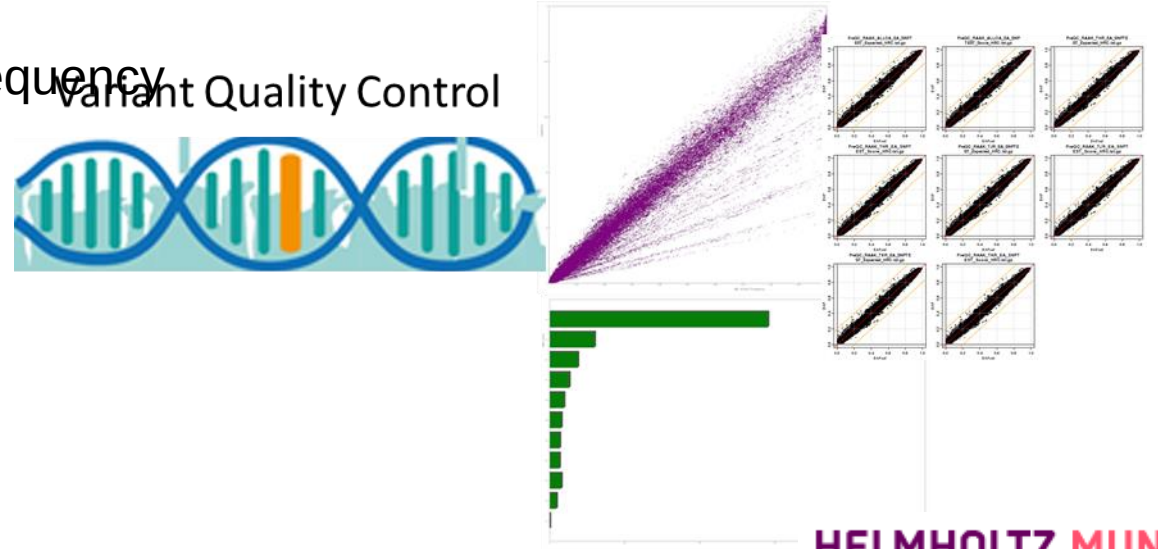
It consists of (at least) four steps:

1. Identification of variants with an excessive missing genotype

2. Identification of variants demonstrating a significant deviation from Hardy-Weinberg equilibrium (HWE)

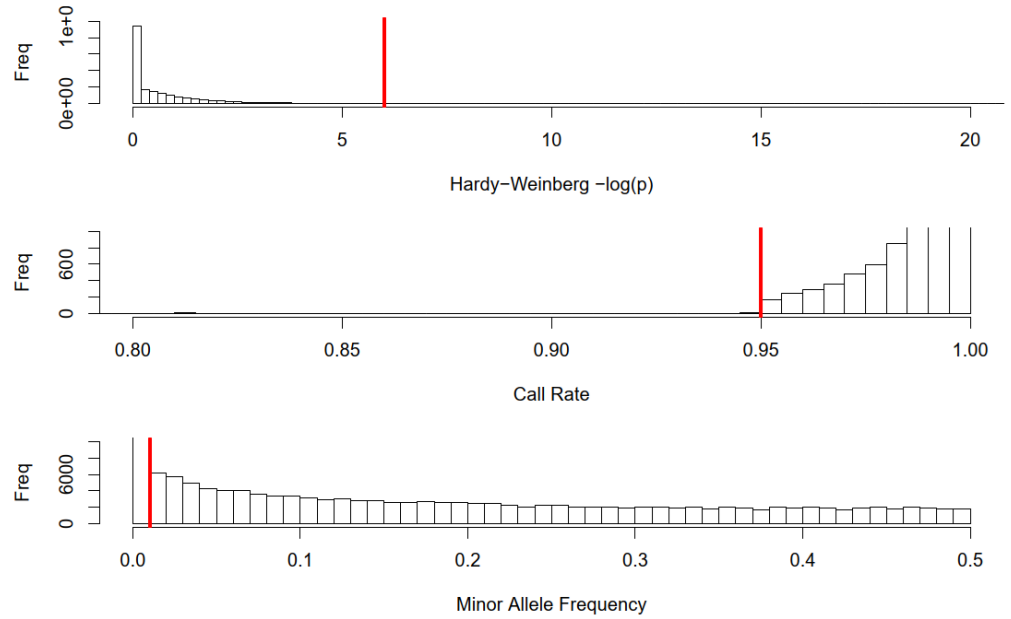
3. Removal of all makers with a very low minor allele frequency

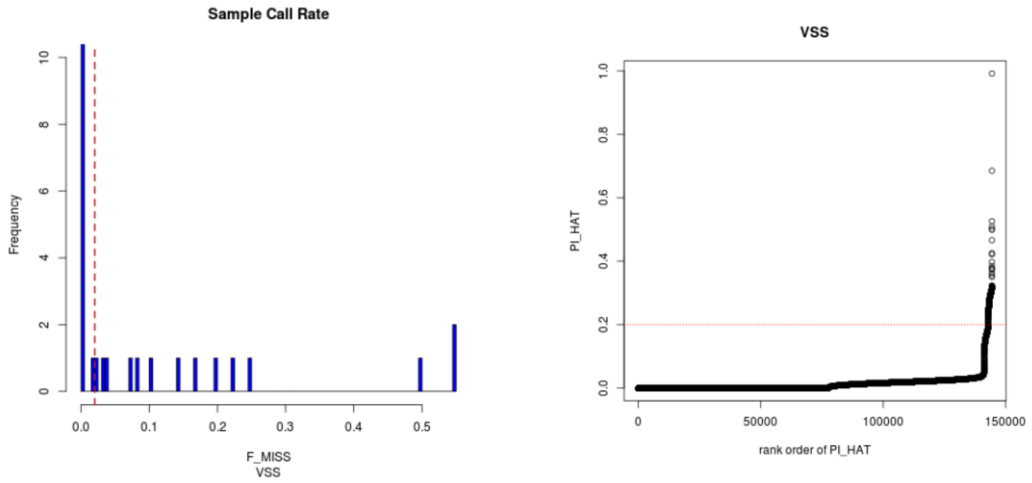
4. Removal of all makers with cluster separation score



(A generalized) Quality Control process

Where to draw the line?





Genotyping data : PLINK common operations

Sample management

`--keep [file]`	Keep samples in file
`--remove [file]`	Remove samples in file

SNP management

`--extract [file]`	Keep SNPs in file
`--exclude [file]`	Remove SNPs in file

Extracting regions

`--chr [name]`	Extract data on specified chromosome
`--from-bp [pos]`	From specified position
`--to-bp [pos]`	To specified position

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Variant QC

`--maf [threshold]`	Keep variants with MAF>threshold
`--hwe midp [threshold]`	Keep variants with HWE p>threshold

Sample QC

`--missing`	Compute per-sample and per-variant missingness
`--check-sex`	Check sexes by looking at chrX
`--genome`	Compute relatedness, check for duplicates

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What is the command for :

- Excluding SNPs that are missing in a large proportion of the subjects (<0.90).
- Excluding individuals who have high rates of genotype missingness (<0.85).
- Keeping autosomal SNPs.
- Extracting the top 20 principal components.
- The association between SNPs and a binary/quantitative outcome.

Thank you.