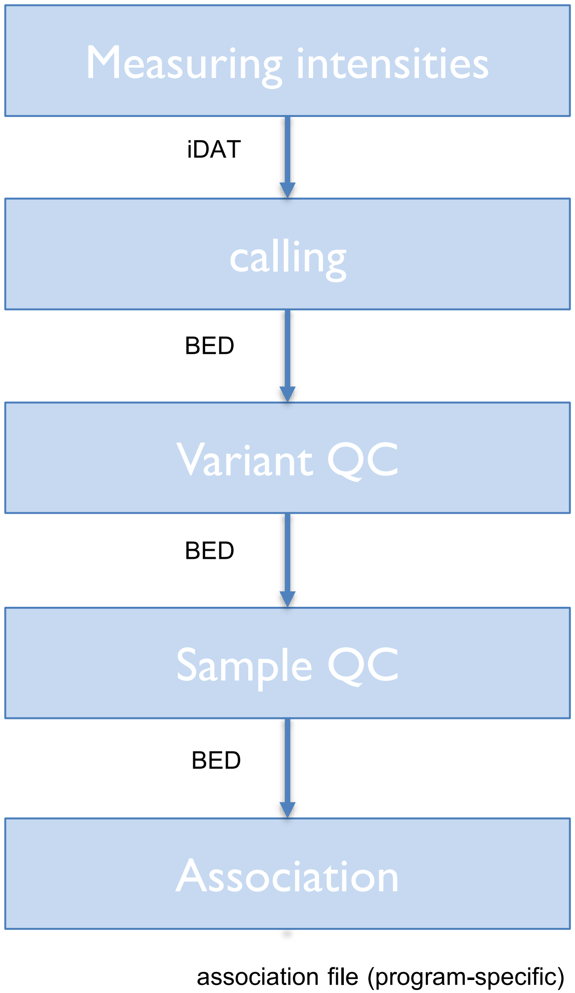
Lecture 2 : Basic tools and formats in bioinformatics

Volos Summer School

21 / 05 / 2018

Arthur Gilly

The GWAS analysis pipeline



Measuring intensities

iDAT

calling

BED

Variant QC

BED

Sample QC

BED

Association

association file (program-specific)

Tools

Genotyping machine software(illumina)

genCall/ zCall

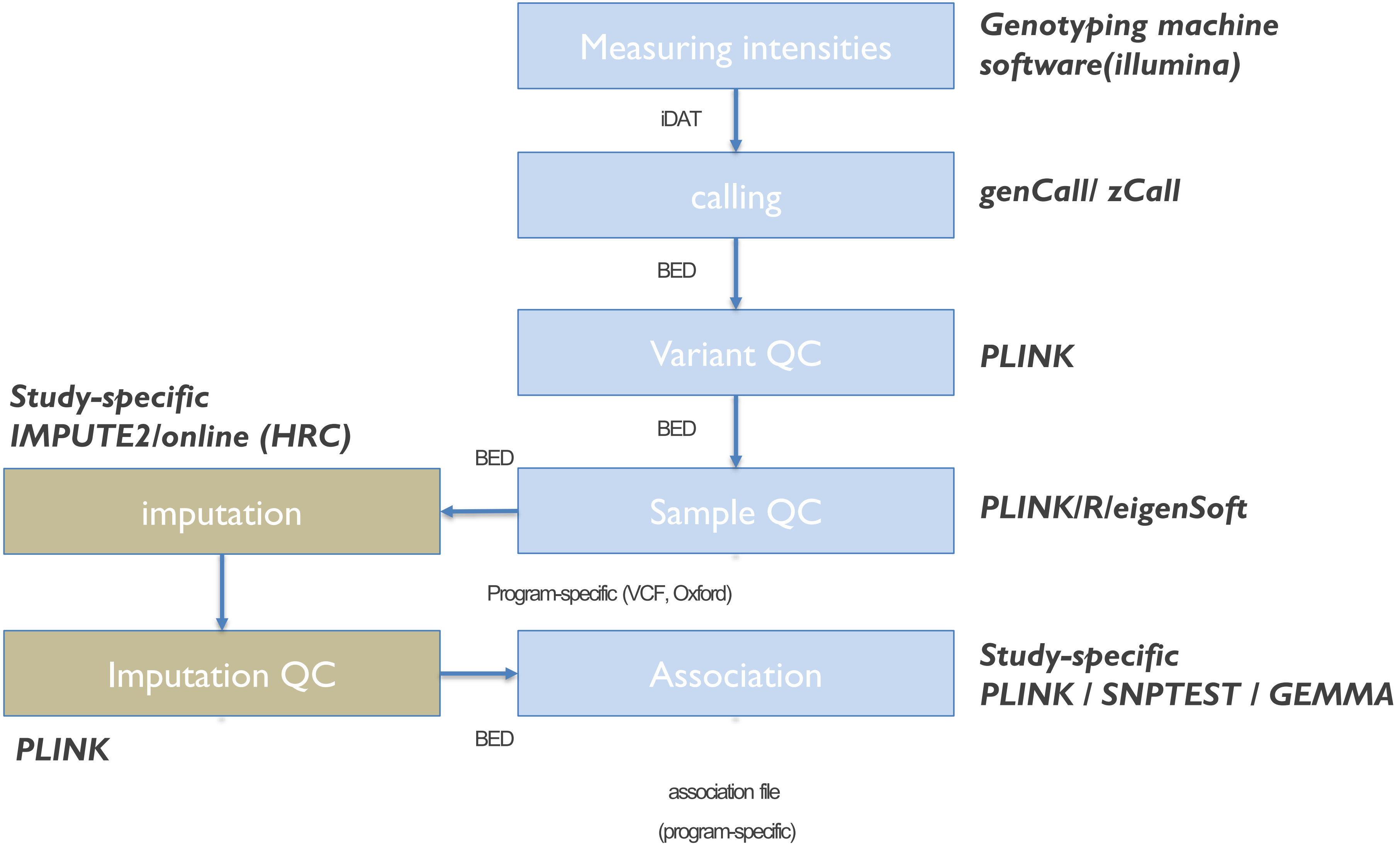
PLINK

PLINK/R/eigenSoft

Study-specific
PLINK / SNPTEST / GEMMA

Data input

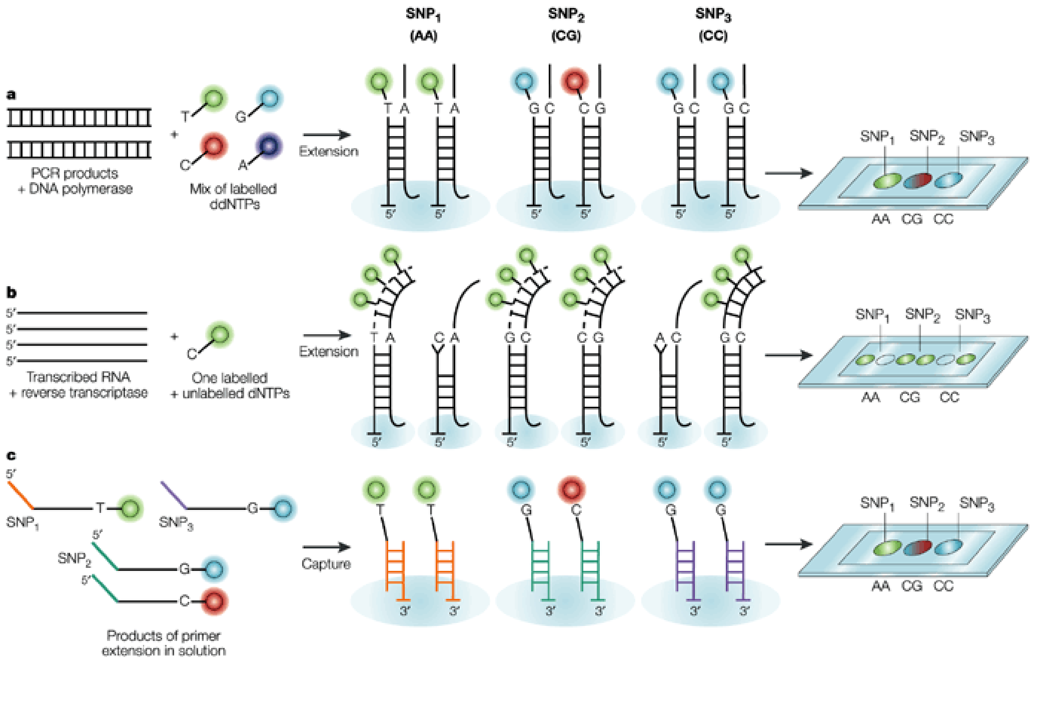
The (imputed) GWAS analysis pipeline



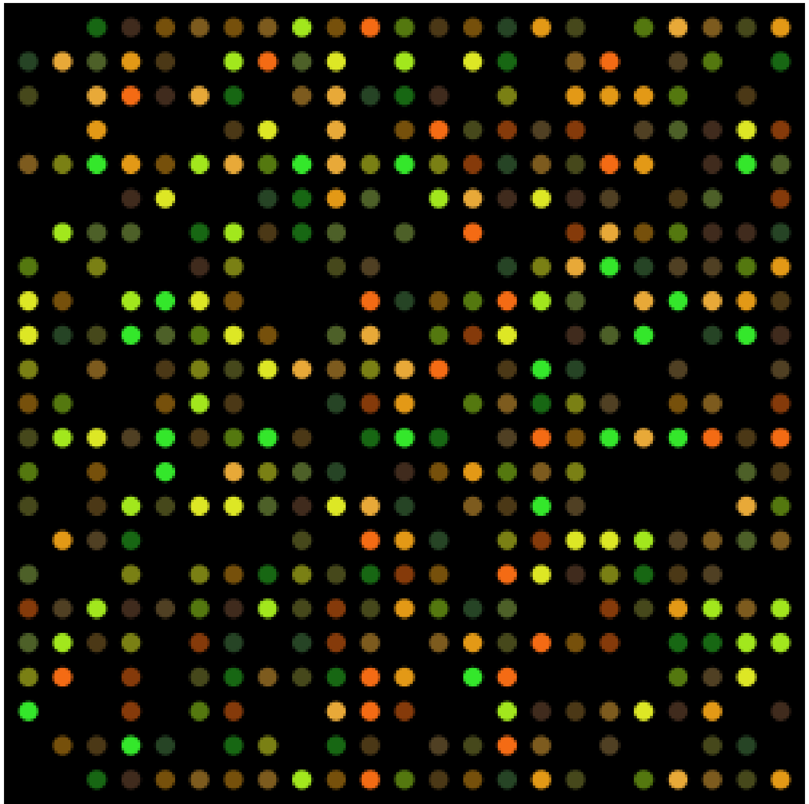
Genotyping data calling

Data input

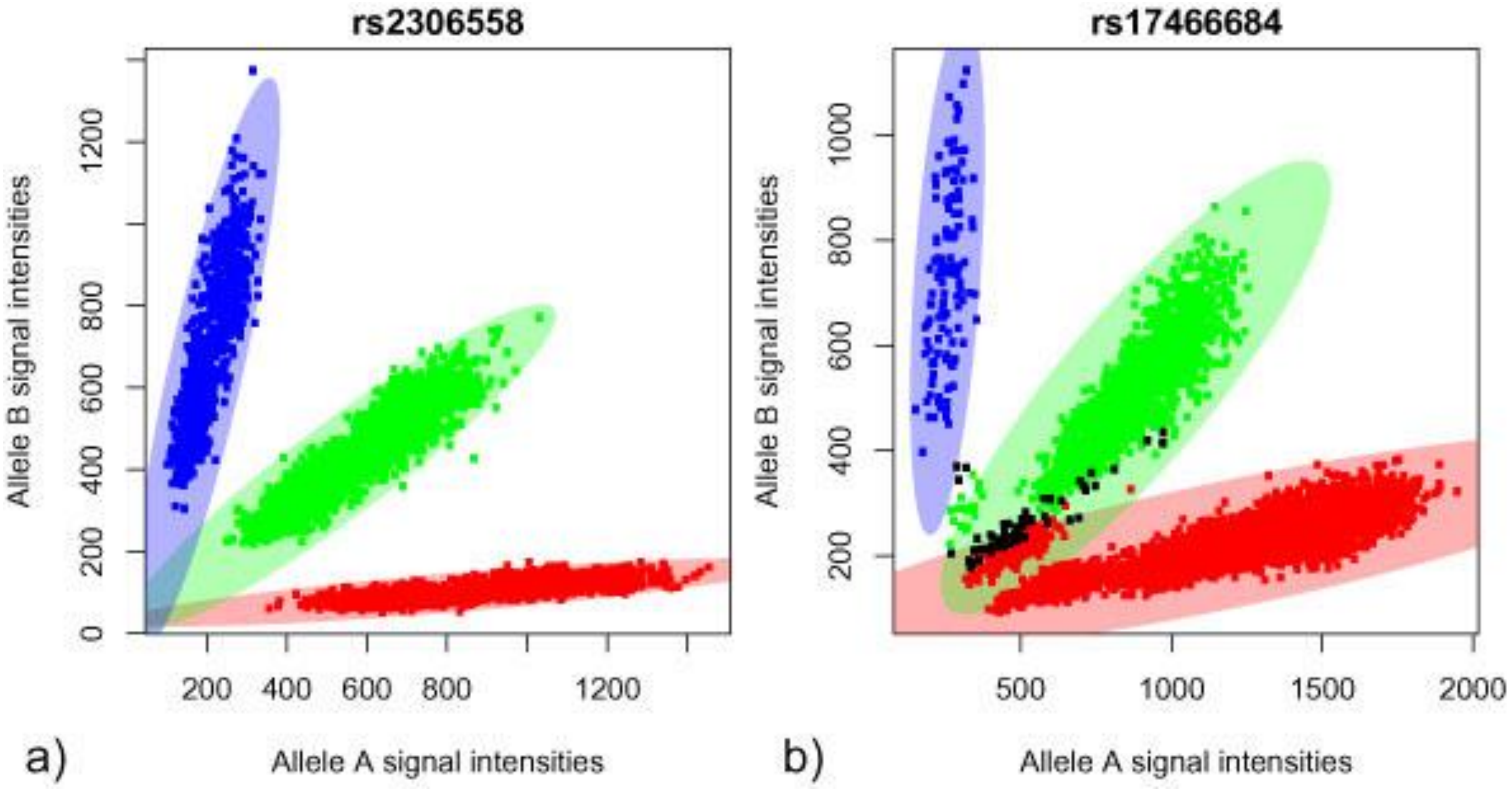
Intensities: what intensities?



Nature Reviews | Genetics



Intensities: the good and the bad



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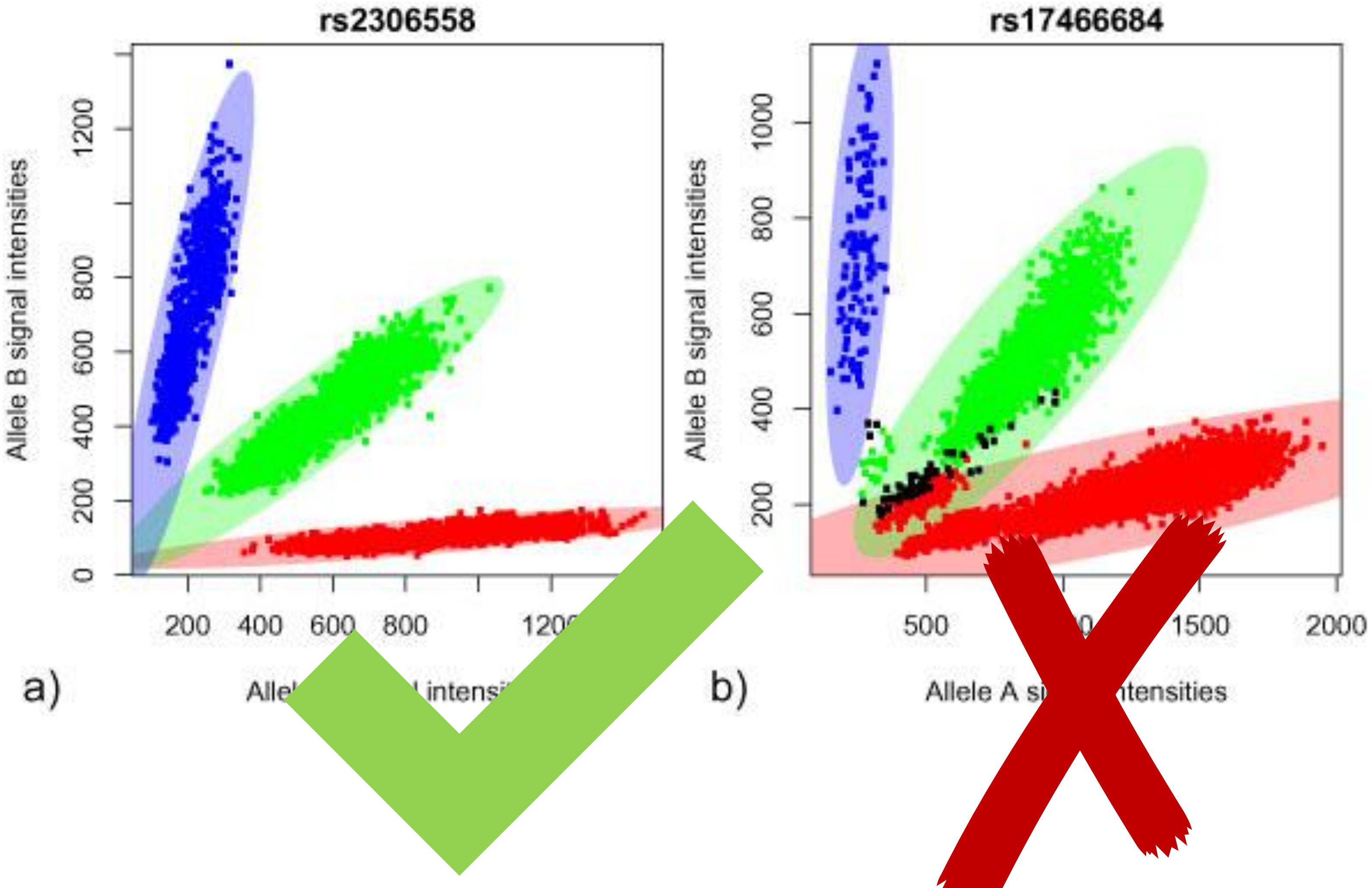

Genotyping data storage

Data input

Which data types do we need?

Data input

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$phenotype \sim$

Data input

Which data types do we need?

$\textit{phenotype} \sim \textit{genotype}$

Data input

Which data types do we need?

$$\textit{phenotype} \sim \textit{genotype} + \textit{covariates}$$

Data input

Which data types do we need?

$$\boldsymbol{phenotype} \sim \boldsymbol{genotype} + \boldsymbol{covariates} + \boldsymbol{structure}$$

Data input

Which data types do we need?

$$phenotype \sim genotype + covariates + structure + \epsilon$$

Data input

Which data types do we need?

$$\boldsymbol{phenotype} \sim \boldsymbol{\beta} \times \boldsymbol{genotype} + \boldsymbol{covariates} + \boldsymbol{structure} + \boldsymbol{\epsilon}$$

Data input

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$$\boldsymbol{phenotype} \sim \boldsymbol{\beta} \times \boldsymbol{genotype} + \boldsymbol{covariates} + \boldsymbol{structure} + \boldsymbol{\epsilon}$$

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

Data input

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As we go from variant to variant…

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These stay constant (they describe the samples)

Data input

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These stay constant (they describe the samples)

This one changes

Our first format: TPED

$$\underbrace{\begin{bmatrix} id_0 & pheno_0 & male & 22\ years \\ \vdots & \vdots & \vdots & \vdots \\ id_n & pheno_n & female & 65\ years \end{bmatrix}}_{\text{FAM/TFAM file}}$$

$$\underbrace{\cancel{\begin{bmatrix} r_{00} & \cdots & r_{0n} \\ \vdots & r_{ij} & \vdots \\ r_{n0} & \cdots & r_{nn} \end{bmatrix}}}_{\substack{\text{Matrix file} \\ \text{(program-specific)}}}$$

$$\underbrace{\begin{array}{c|c} \substack{\textit{all} \\ \textit{variants}} & \underline{\begin{bmatrix} A/T & \cdots & G/C \\ \vdots & \vdots & \vdots \\ T/T & \cdots & G/G \end{bmatrix}} \\ & \substack{\textit{all} \\ \textit{individuals}} \end{array}}_{\text{TPED file}}$$

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$$\underbrace{\begin{bmatrix} id_0 & pheno_0 & male & 22\,years \\ \vdots & \vdots & \vdots & \vdots \\ id_n & pheno_n & female & 65\,years \end{bmatrix}}_{\text{FAM/TFAM file}}$$

```
FAMILY1 SAMPLE1 0 0 1 22 1.5
FAMILY2 SAMPLE2 0 0 2 65 2.1
```

- One of PLINK's traditional formats
  - Not used in practice
  - Convenient for looping over SNPs
  - Input `--tfile`
  - Output `--recode transpose`

$$\underbrace{\textit{all variants}\;\left|\;\underset{\textit{all individuals}}{\underline{\begin{bmatrix} A/T \cdots G/C \\ \vdots \quad \vdots \quad \vdots \\ T/T \cdots G/G \end{bmatrix}}}\right.}_{\text{TPED file}}$$

```
1 rs15933  0 752721 A G G G
1 1:846808 0 846808 C C T C
```

Another format: PED/MAP

```
FAMILY1 SAMPLE1 0 0 1 1.5 A G C C
FAMILY2 SAMPLE2 0 0 2 2.1 T T A A
```

FAM/TFAM file

PED file

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$$\textit{all ind} \; \Big| \; \underbrace{\begin{bmatrix} A/T \cdots G/C \\ \vdots \quad \vdots \quad \vdots \\ T/T \cdots G/G \end{bmatrix}}_{\textit{all individuals}}$$

```
1 rs15933   0 752721
1 1:846808 0 846808
```

MAP file

Exercise 1 : Format conversion

- In `/Workshop_data/Lecture2/Exercise1`
  - Convert `cohort1.tped/tfam` to `cohort1.ped/map`
  - Use the `transpose.sh` script provided or try to d.i.y

```
FAMILY1 SAMPLE1 0 0 1 1.5 A G C C
FAMILY2 SAMPLE2 0 0 2 2.1 G G T C
```

  - Convert the file to PED using PLINK
  - Compare both files using `diff`

Exercise 1 : Solution

- cut -d' ' -f1-4 cohort1.tped | tr ' ' '\t'> cohort1.map
- paste -d' ' cohort1.tfam <(./transpose.sh <(cut -d' ' -f5- cohort1.tped)) > cohort1.ped
- plink --tfile cohort1 --recode --out fortest
- diff cohort1.ped fortest.ped

Exercise 2 : Storage

- Consider 3 different genotyping chips
  - 500,000 SNPs (Illumina OmniExpress)
  - 1,000,000 SNPs (ExomeChip)
  - 2,500,000 SNPs (Illumina Onmi 2.5)

- How large is a PED file containing genetic information for10,000 samples on each of these chips?

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- 1 character = 1 byte
- Each genotype = 2 alleles + 2 spaces = 4 characters

$$n_{SNPs} \times 4 \times n_{individuals} = 19\ Gb, 38Gb, 95Gb$$

Binary formats

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- **Can we make this better?**

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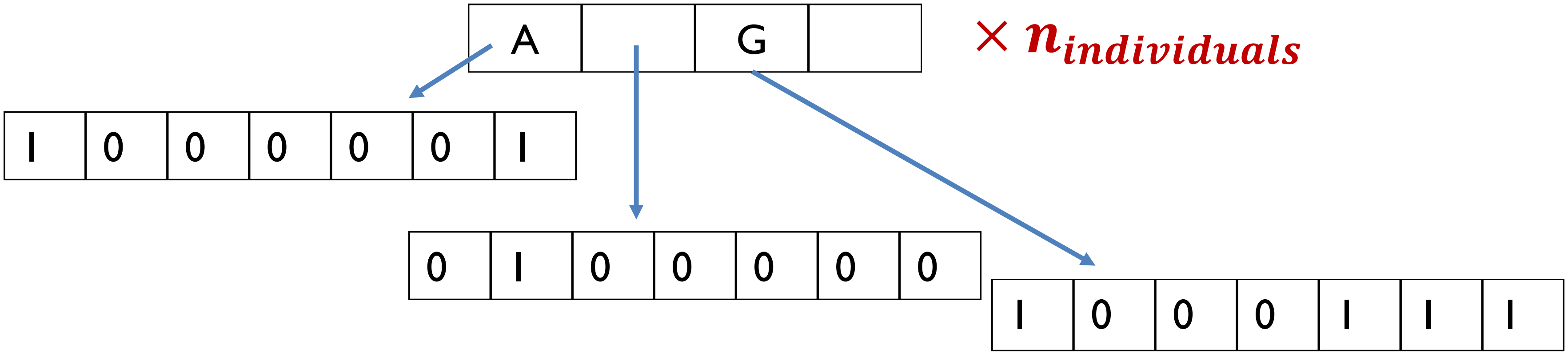
- 2 solutions
    - Compress using ZIP/GZIP
    - Use binary formats



A		G	

$\times\, n_{individuals}$

1	0	0	0	0	0	1

0	1	0	0	0	0	0

1	0	0	0	1	1	1

Binary formats

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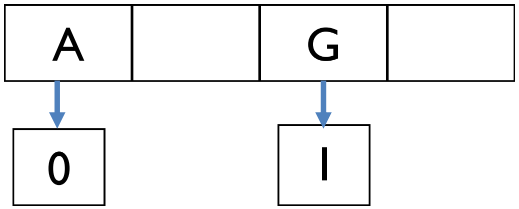

$\times n_{individuals}$

- Question: how smaller is the size now?

Binary formats

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- **Can we make this better?**

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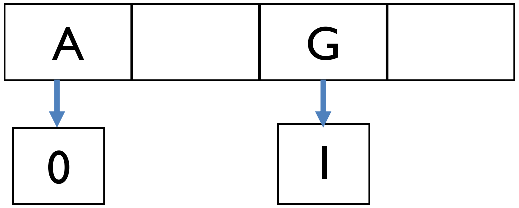


$\times n_{individuals}$

- Question: how smaller is the size now?

$$\frac{n_{SNPs} \times n_{individuals}}{4} = 1.1Gb$$

Data input

One (binary) format to rule them all : BED/BIM/FAM

```
FAMILY1 SAMPLE1 0 0 1 22 1.5
FAMILY2 SAMPLE2 0 0 2 65 2.1
```

FAM/TFAM file

```
1    rs15933   0   564862  C   T
1   1:752566   0   752566  G   A
```

BIM file

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

BED file

- Input: `--bfile`
- Output: `--make-bed`
- Do not open a BED file with less, cat, head, or tail !
- If you absolutely want to look, `xxd –b` or `od -c`

Genotyping data : common operations

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

Common operations

Variant QC

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

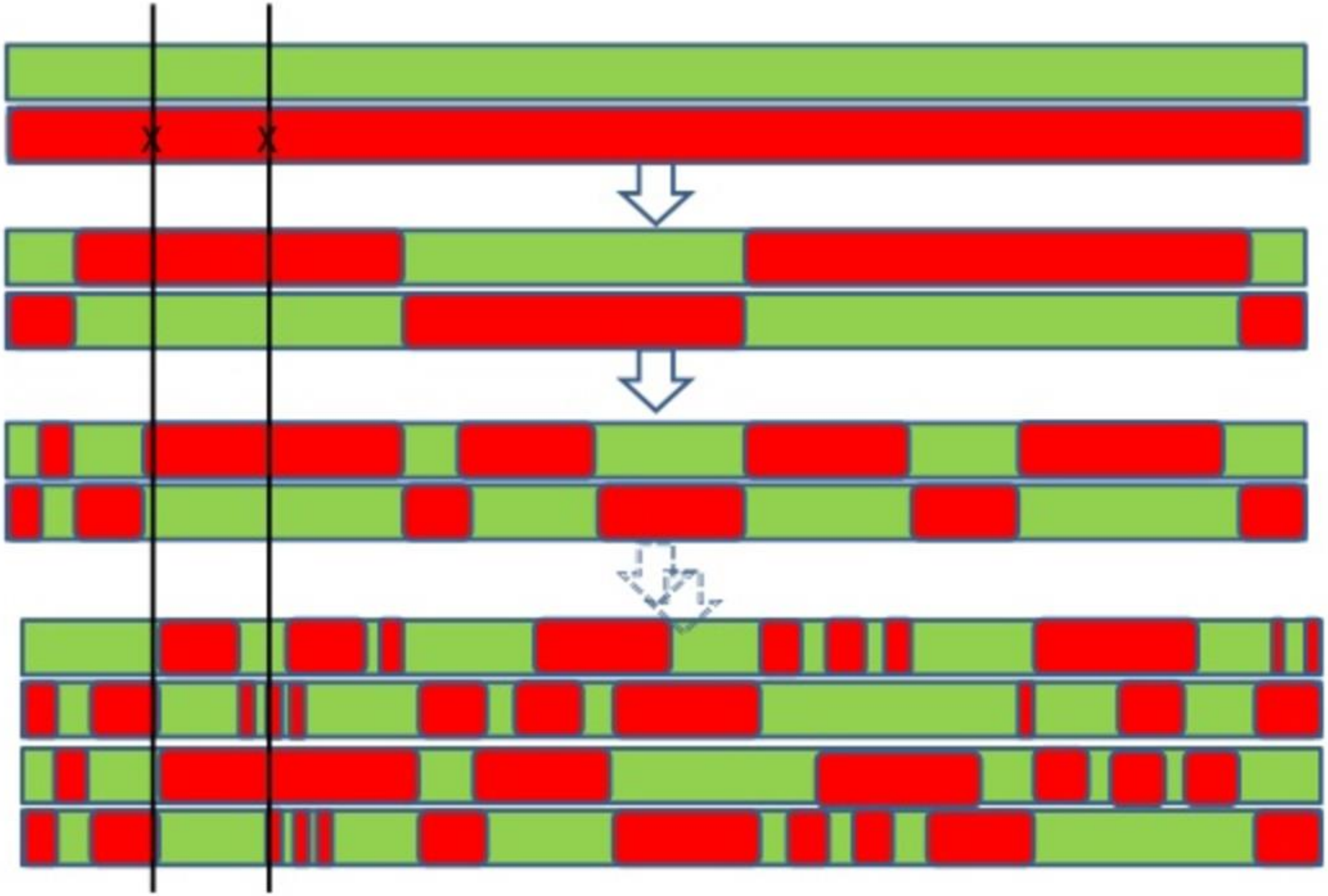
Sample QC

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

Linkage disequilibrium

LD

The strange beautiful world of linkage disequilibrium



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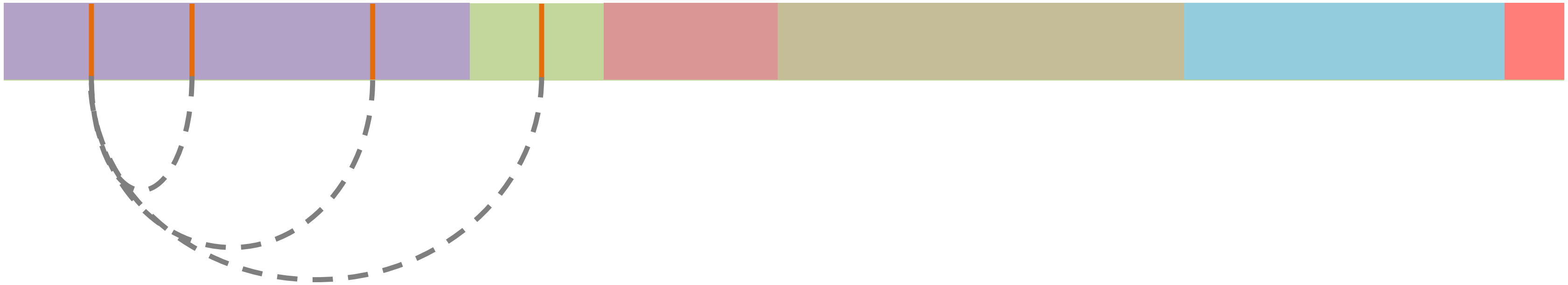

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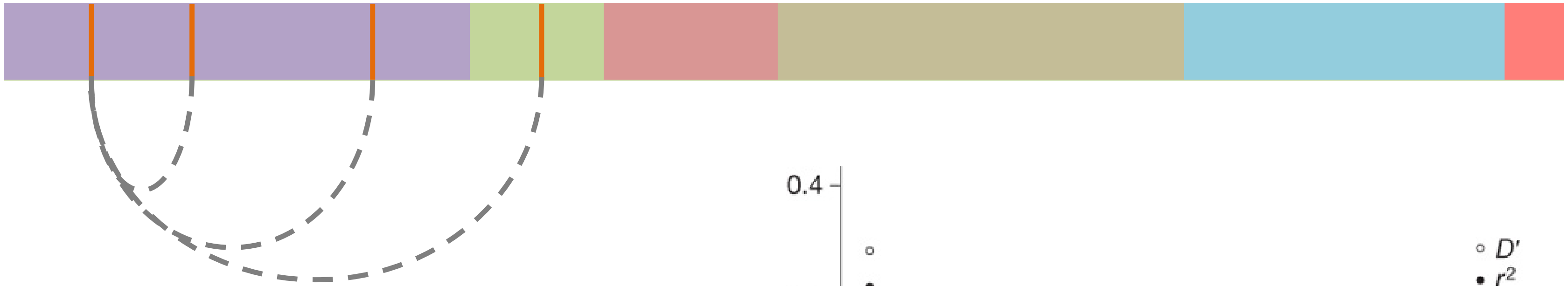
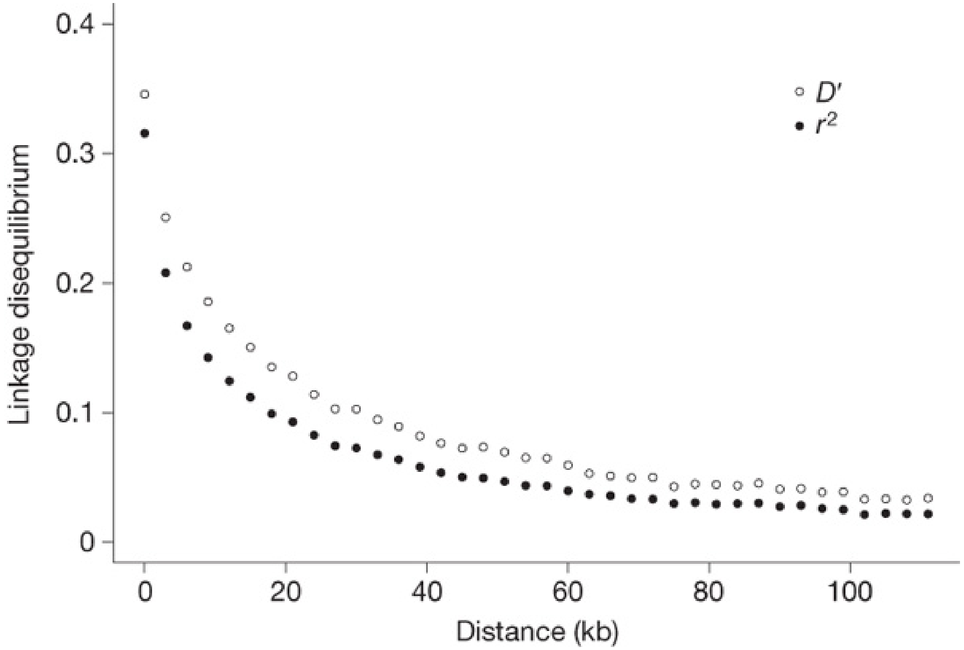




LD

LD between 2 or more SNPs

```
plink --r2 --ld-snps rs1234,rs4567
```

```
plink --r2 --ld-snp-list [file]
```

Pairwise LD in a region

```
plink --r2 --ld-window 10 --ld-window-kb 1000 -
-ld-window-r2 0.2
```

LD-pruning (only independent SNPs)

```
plink --indep 50 5 2
```

```
plink --indep-pairwise 50 5 0.2
```

```
Plink --indep-pairphase 50 5 0.2
```

Exercise 3 : Stretching the PLINK muscle

- In `/Workshop_data/Lecture2/Exercise3`
  - How many common (MAF>5%) variants are there on chromosome 11 in the `cohort1` dataset?
  - How many variants are in LD (r2>0.4) with 21:28759840 on chromosome 21 in a 1Mbp window?

Exercise 3 : Stretching the PLINK muscle

```
plink --bfile cohort1 --maf 0.05 --chr 11 --out
chr11 --make-bed
```

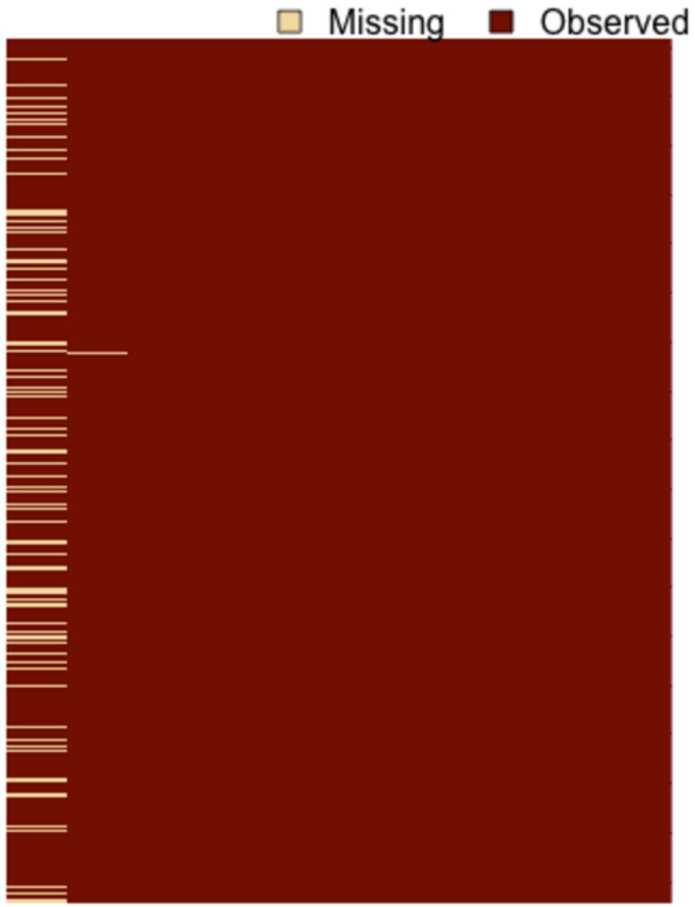
```
wc –l chr11.bim
```

```
plink --bed cohort1.bed --bim cohort1.bim --fam
cohort1.fam --r2 --ld-snp 21:28759840 --ld-window-kb
1000000 --ld-window 1000000 -ld-window-r2 0.4
```

```
wc –l plink.ld
```

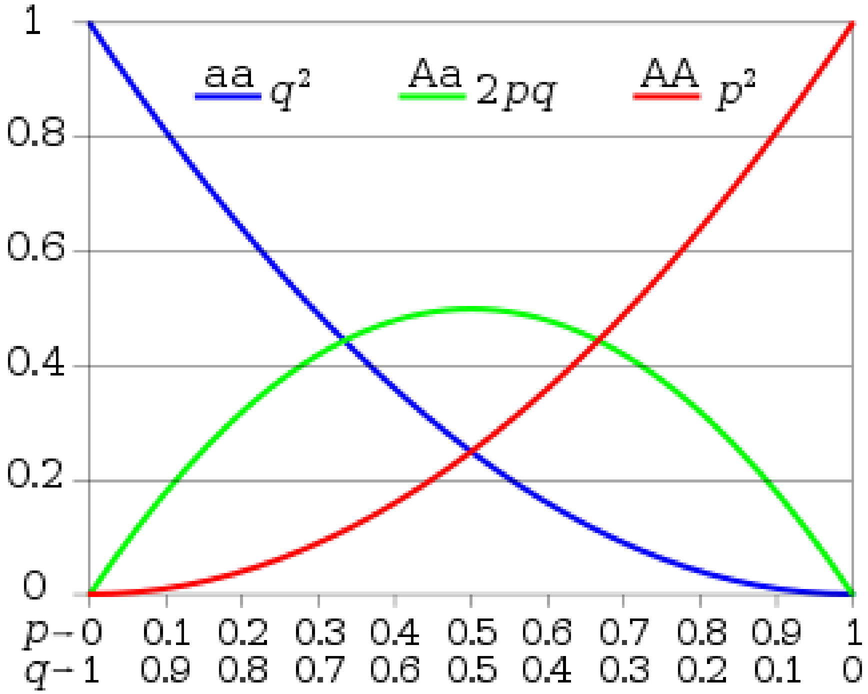
QC steps

Variant QC: which variants do we want to remove?



Variant QC: which variants do we want to remove?

- Calling is not perfect: some genotypes are missing



Variant QC: which variants do we want to remove?

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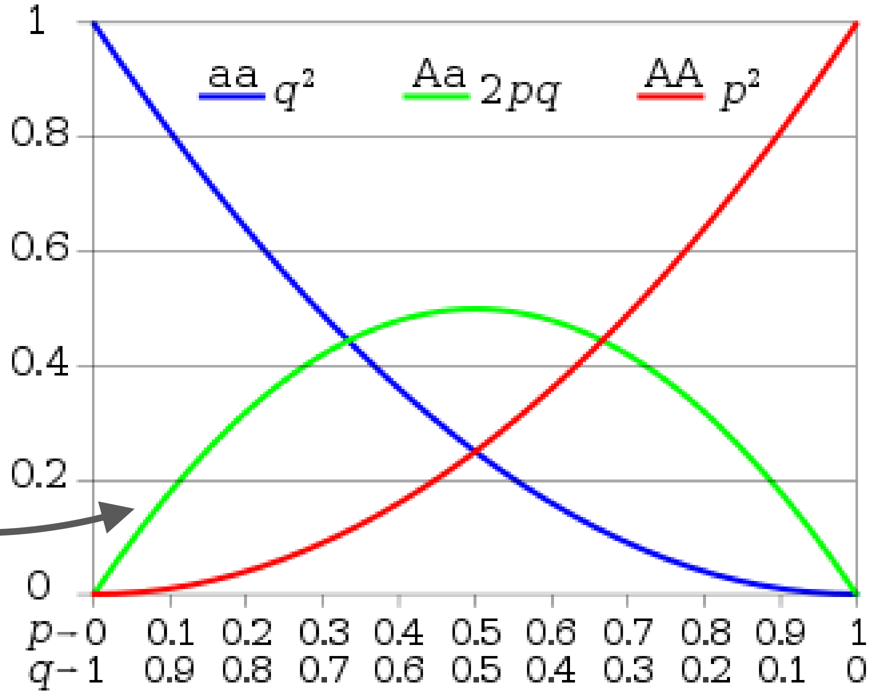
If we see that 40% of all alleles are a, what is the proportion of aa, Aa, AA?



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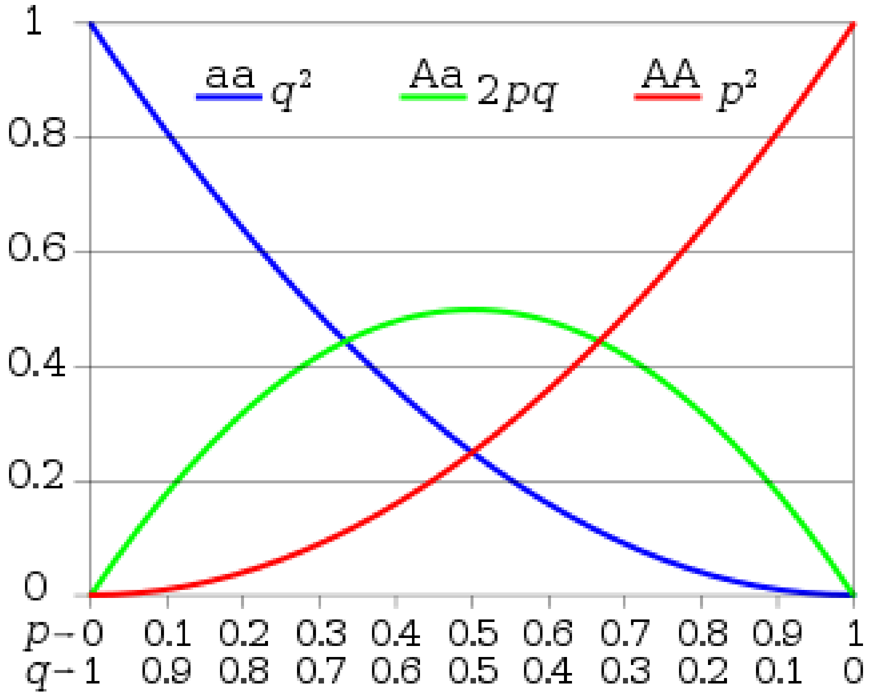

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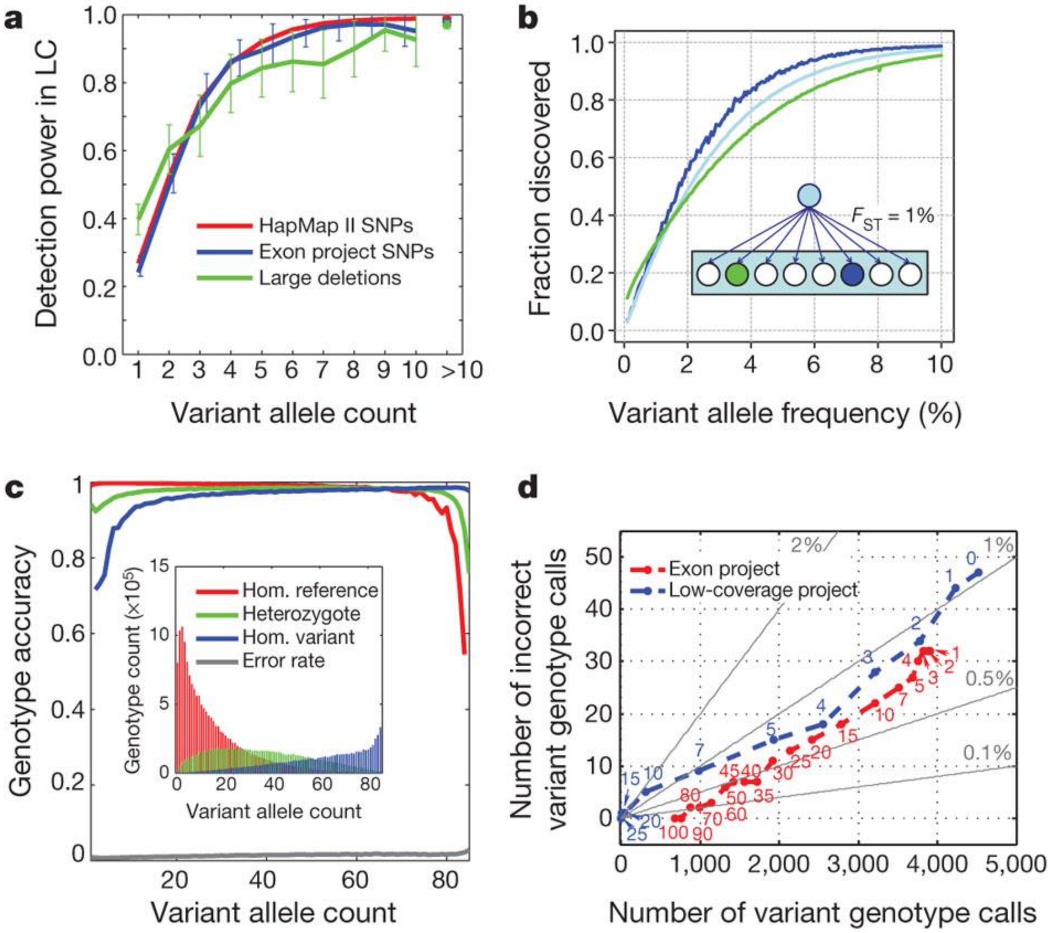
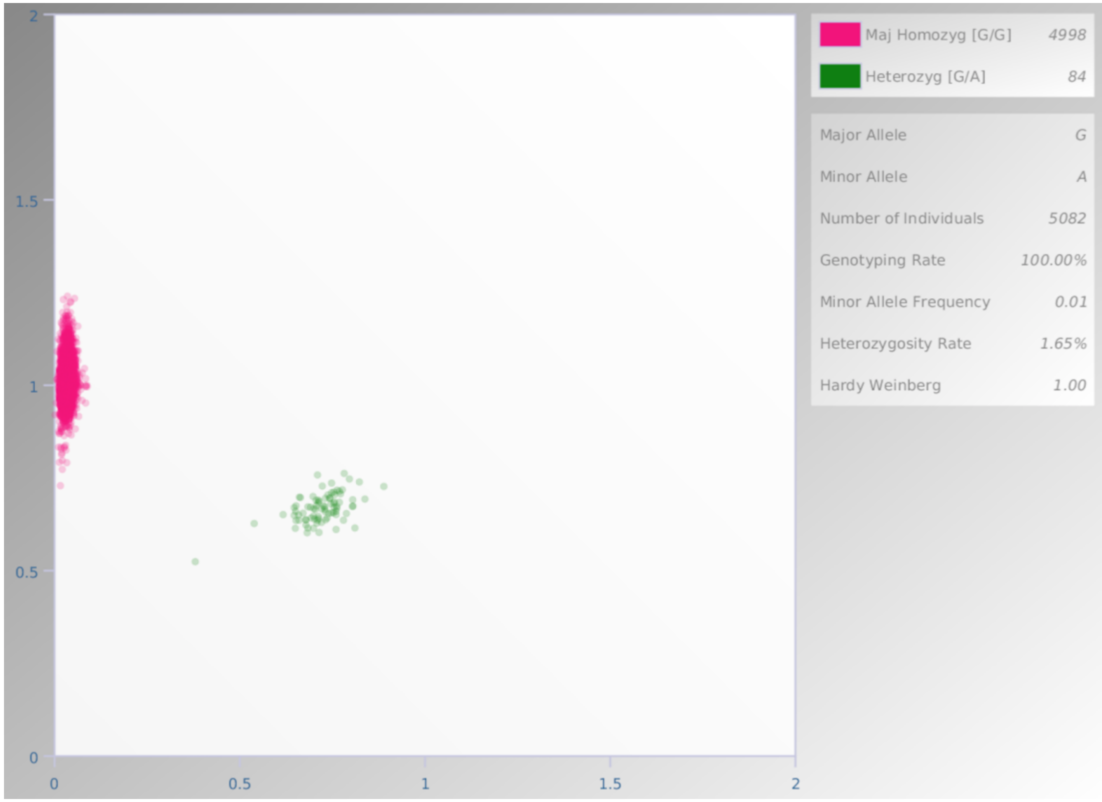


Variant QC: which variants do we want to remove?

- Calling is not perfect: some genotypes are missing
- Variants violating Hardy-Weinberg equilibrium are improbable
- Rare variants are difficult to call



🟥 Maj Homozyg [G/G]	4998
🟩 Heterozyg [G/A]	84

Major Allele	G
Minor Allele	A
Number of Individuals	5082
Genotyping Rate	100.00%
Minor Allele Frequency	0.01
Heterozygosity Rate	1.65%
Hardy Weinberg	1.00

Sample QC: which individuals do we want to remove?

All the different ways in which our samples could be the wrong ones

What are some defining sample characteristics?

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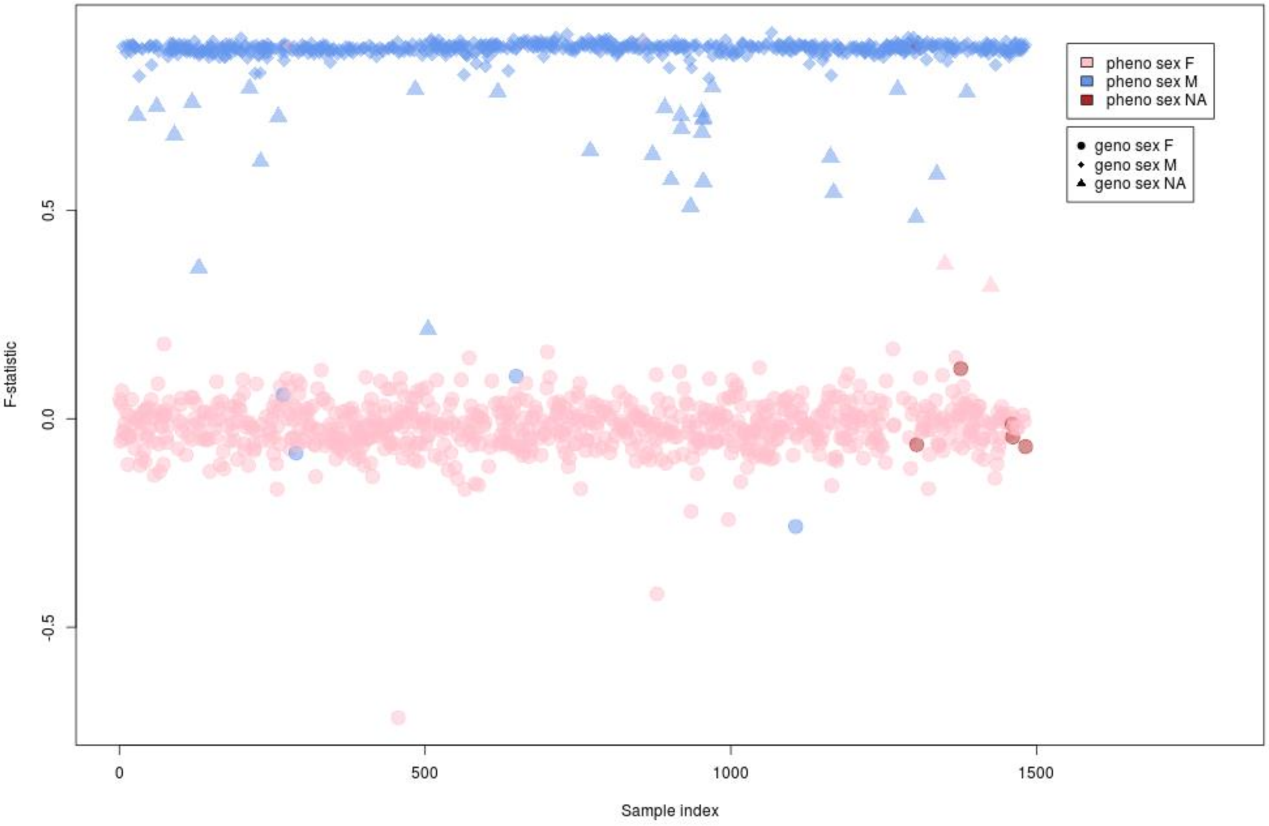
- Sex checks
- Ethnicity checks



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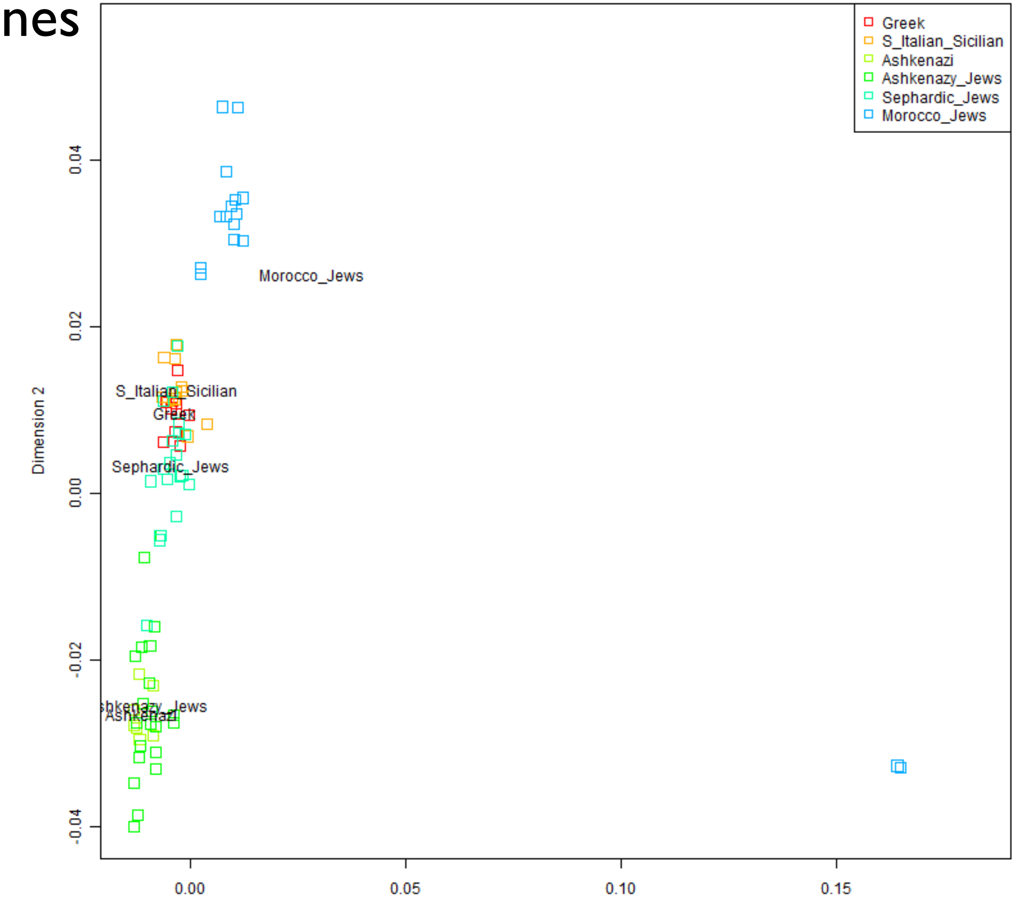

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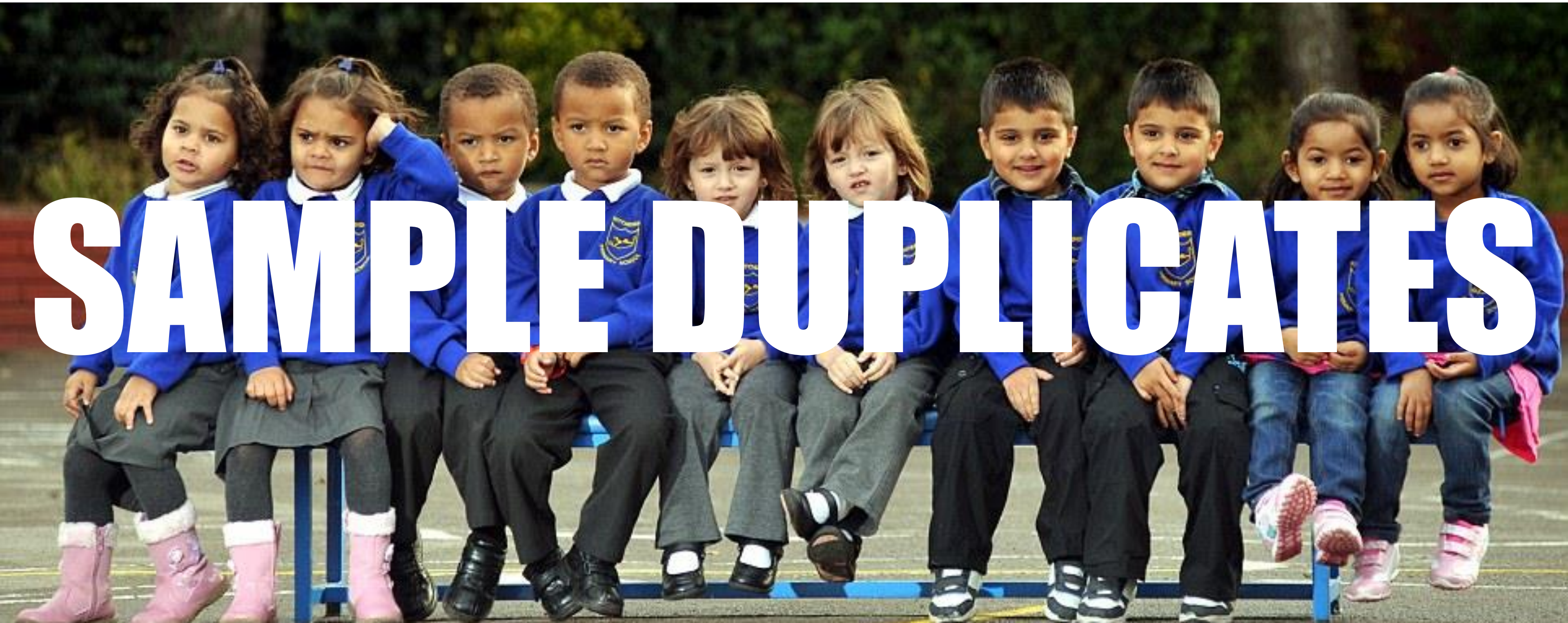

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