BIOL 350: Bioinformatics

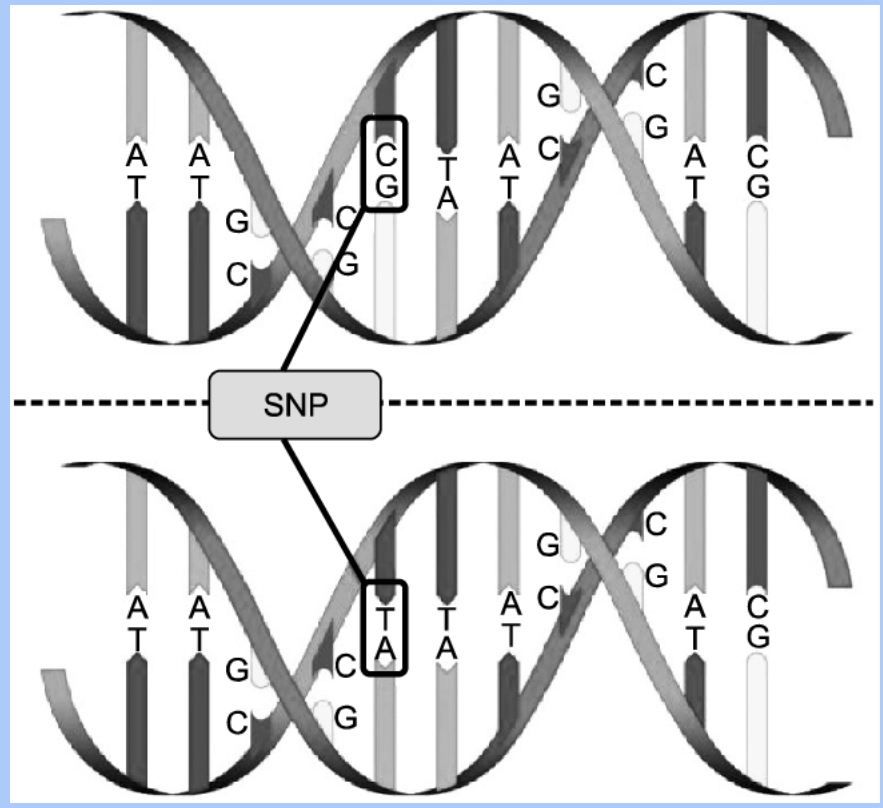
Introduction to genetic association studies

What is a SNP?

Polymorphisms and their role in genetics

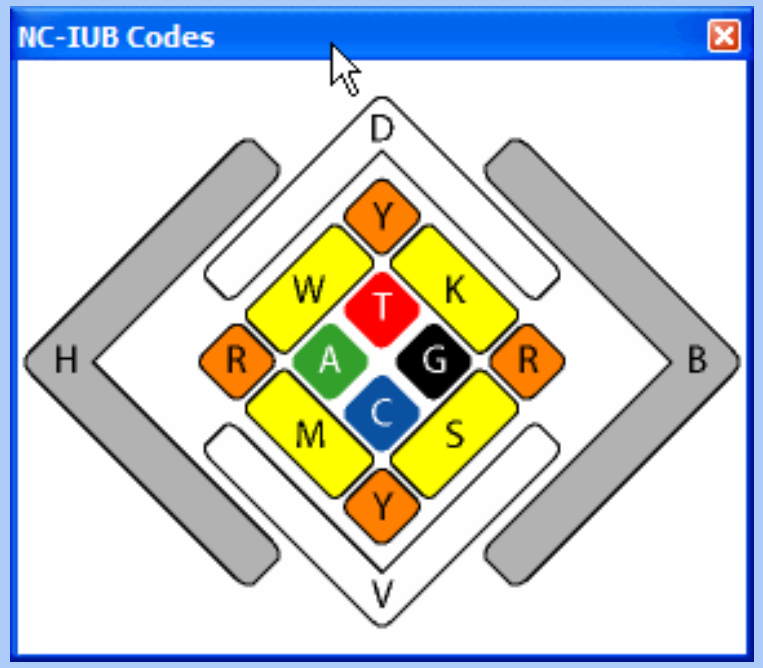
Single-nucleotide polymorphisms

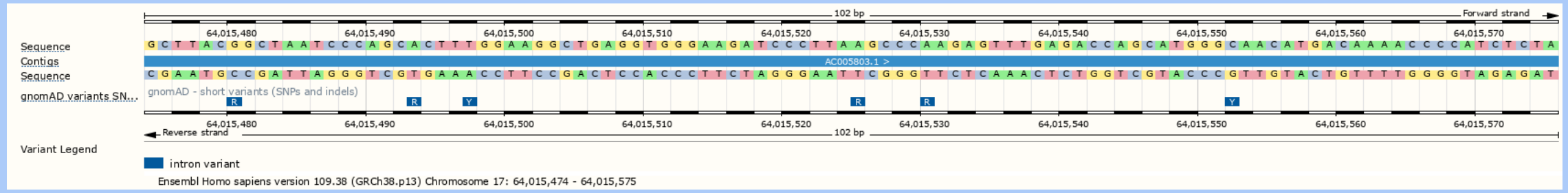
- **Polymorphism** is the tendency of DNA to admit different nucleotide pairs at a single locus
- Of 3.2 billion bases, any individual is polymorphic at 4–5 million sites
- The more common allele is called the **major allele**; the less common allele is called the **minor allele**



IUPAC-IUB SNP codes

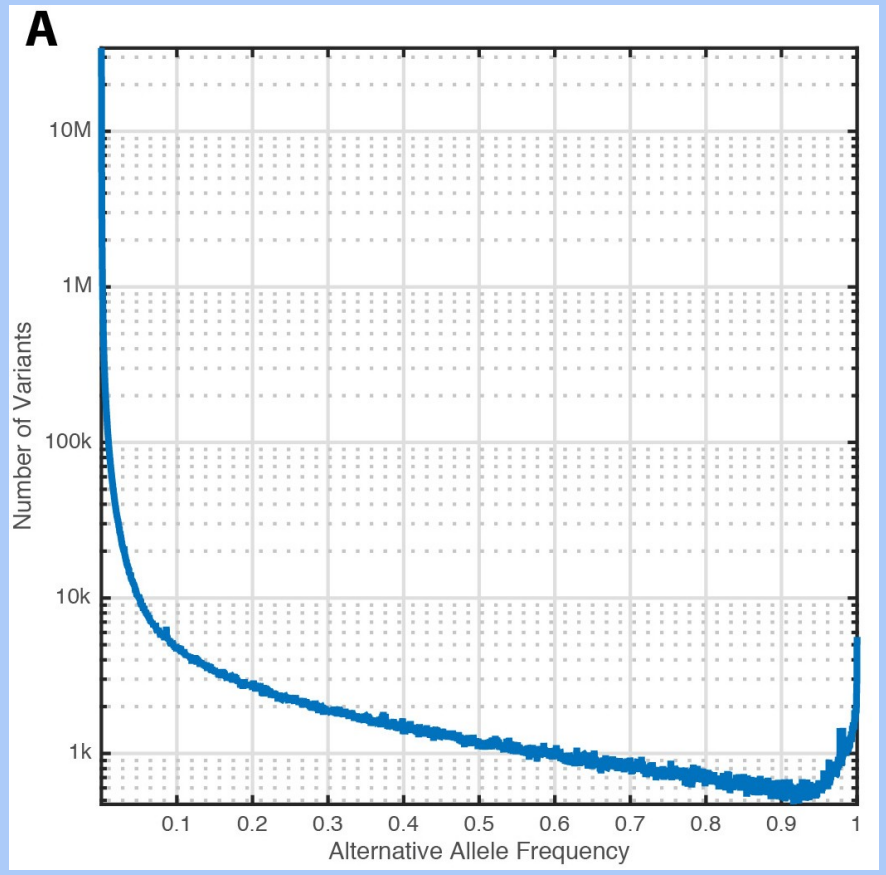
- More than just A, T, G, and C?
- Each polymorphism is coded by its possible alleles





Many rare SNPs

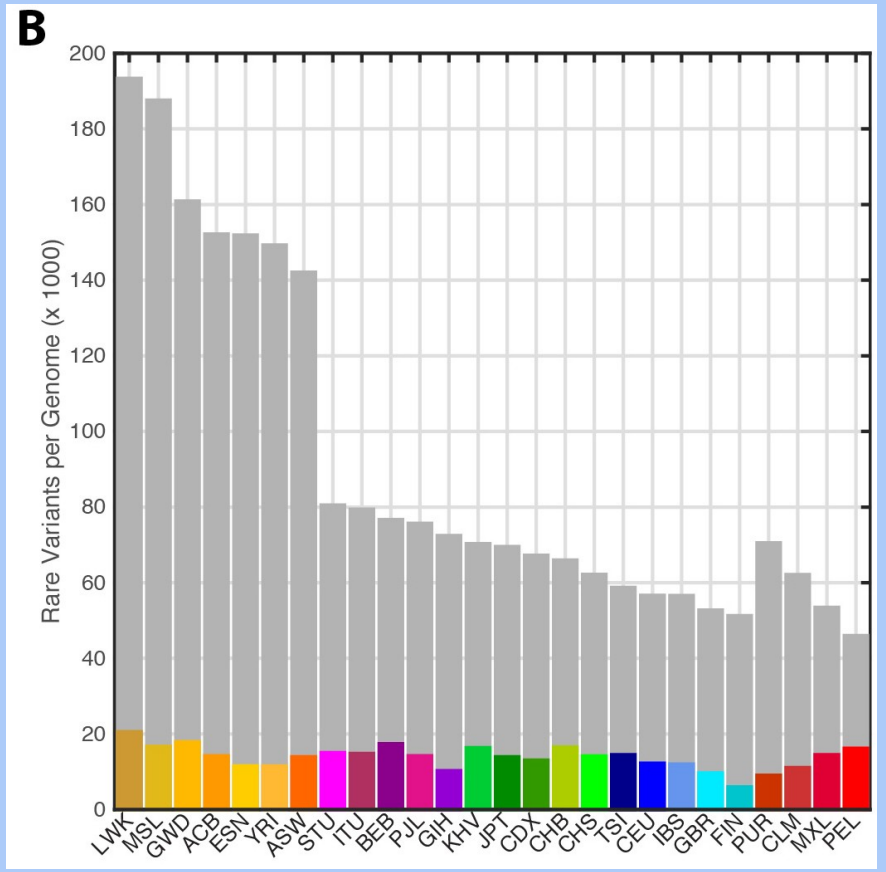
- Most SNPs of the >600 million known SNPs are very rare (frequency < 0.5%), but <5% of an individual's genome consists of rare SNPs



https://www.nature.com/articles/nature15393

Few rare SNPs per genome

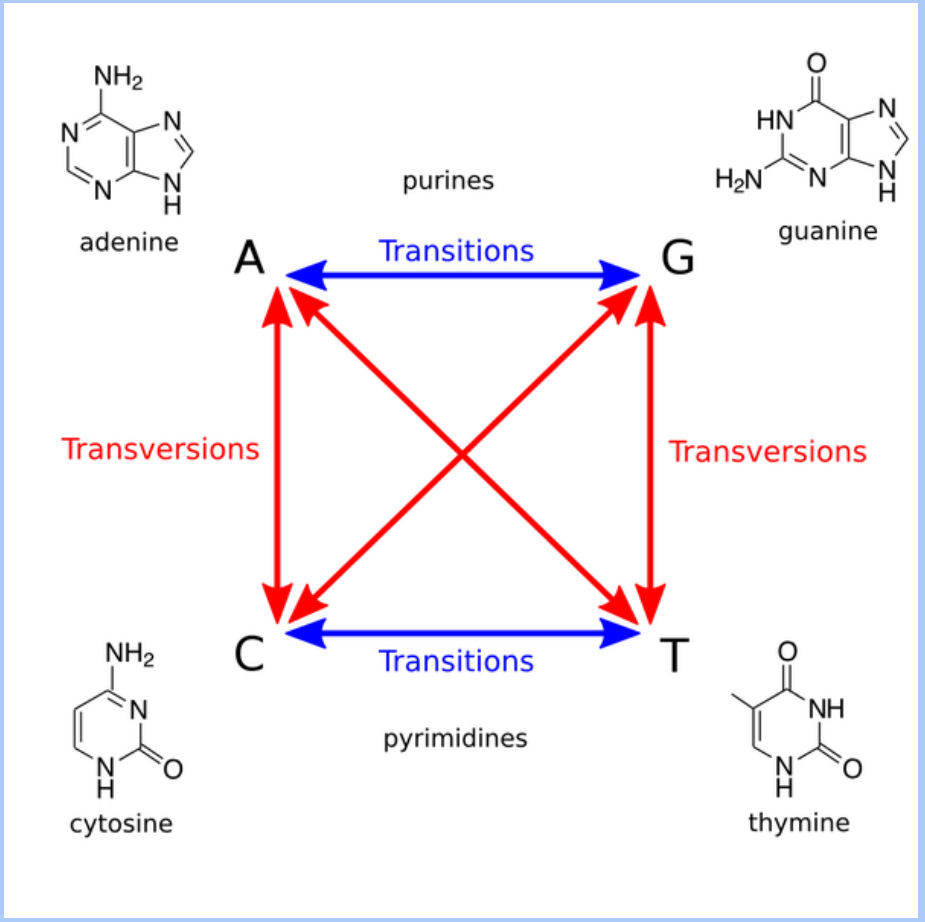
- Most SNPs of the >600 million known SNPs are very rare (frequency < 0.5%), but <5% of an individual's genome consists of rare SNPs
- Common SNPs have **minor allele frequency (MAF)** >5%



https://www.nature.com/articles/nature15393

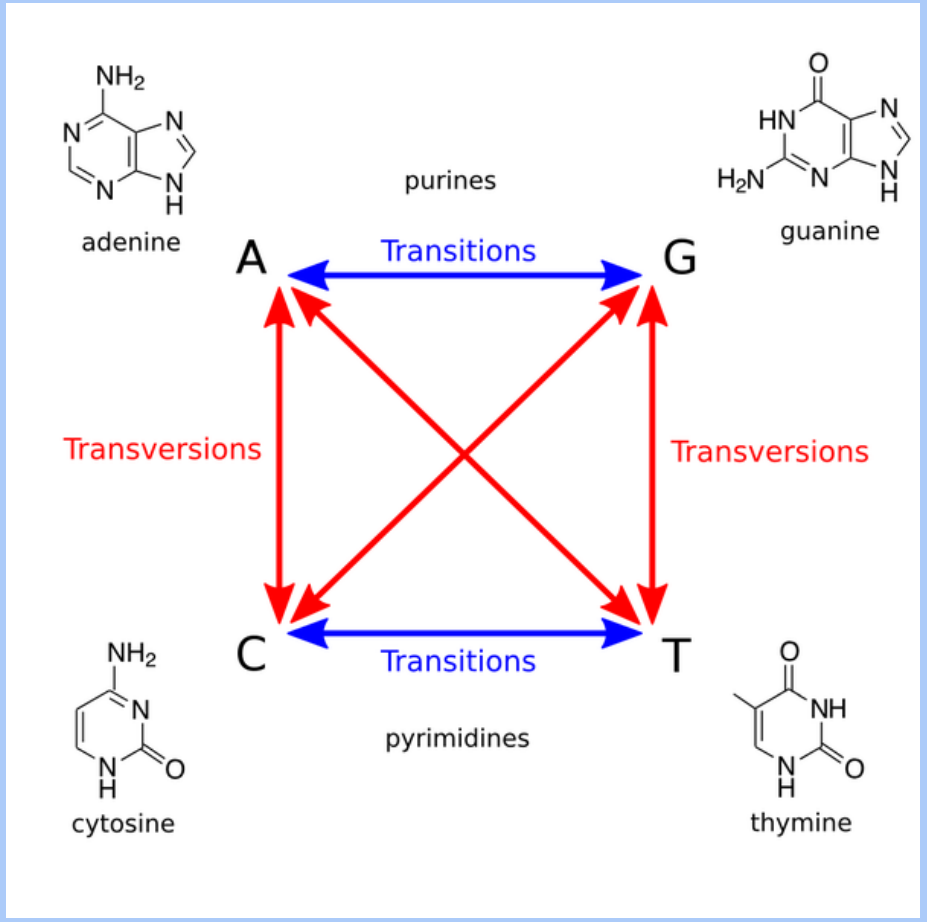
Transitions and transversions

- **Transitions** occur between nucleotides of the same type (purines or pyrimidines)
- **Transversions** occur between nucleotides of opposite type (between purines and pyrimidine)



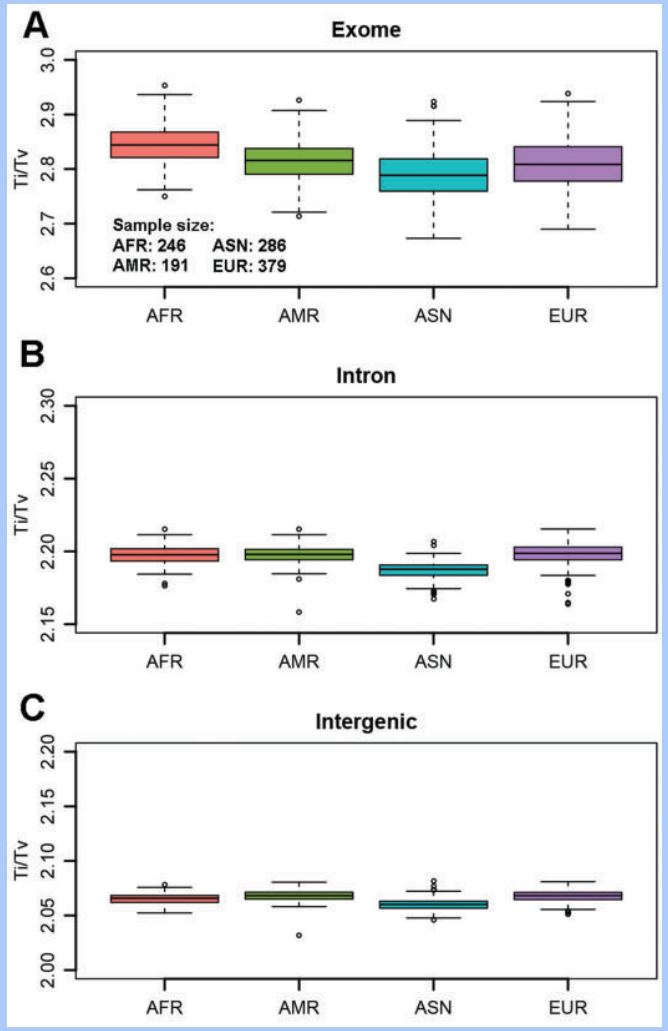
How many polymorphisms are there?

- If there are n nucleotide pairs, there are n symmetric conversions: A/T → T/A and C/G → G/C transversions
- If there are n nucleotide pairs, there are $n(n-1)$ asymmetric conversions: A/T → C/G transversions and A/T → G/C transitions
- A total of $n + n(n-1) = n^2$ polymorphims



Transition-transversion ratio

- Even though there are three times as many transversions possible as transitions, in humans the ratio of transitions to transversions is approximately 2, genome-wide
- In coding regions, the ratio is as high as 3



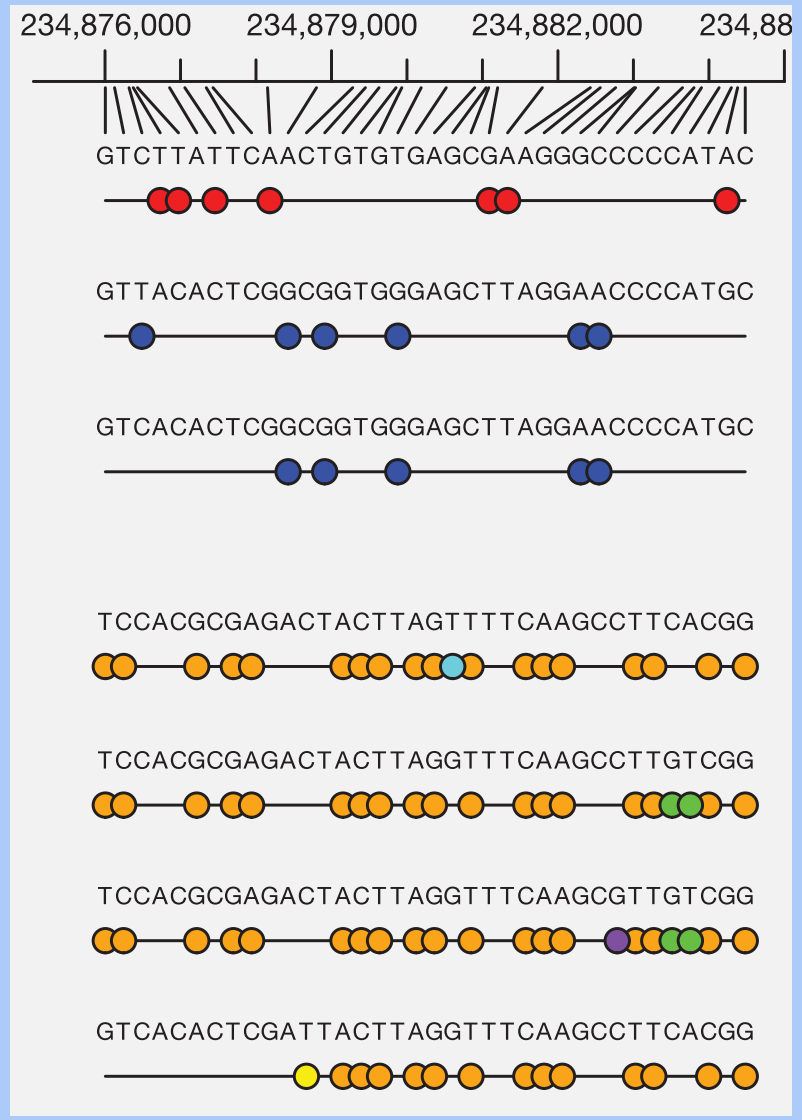
https://pubmed.ncbi.nlm.nih.gov/25297068/

Generation of sequencing data

Sequencing projects and technologies

The HapMap Project

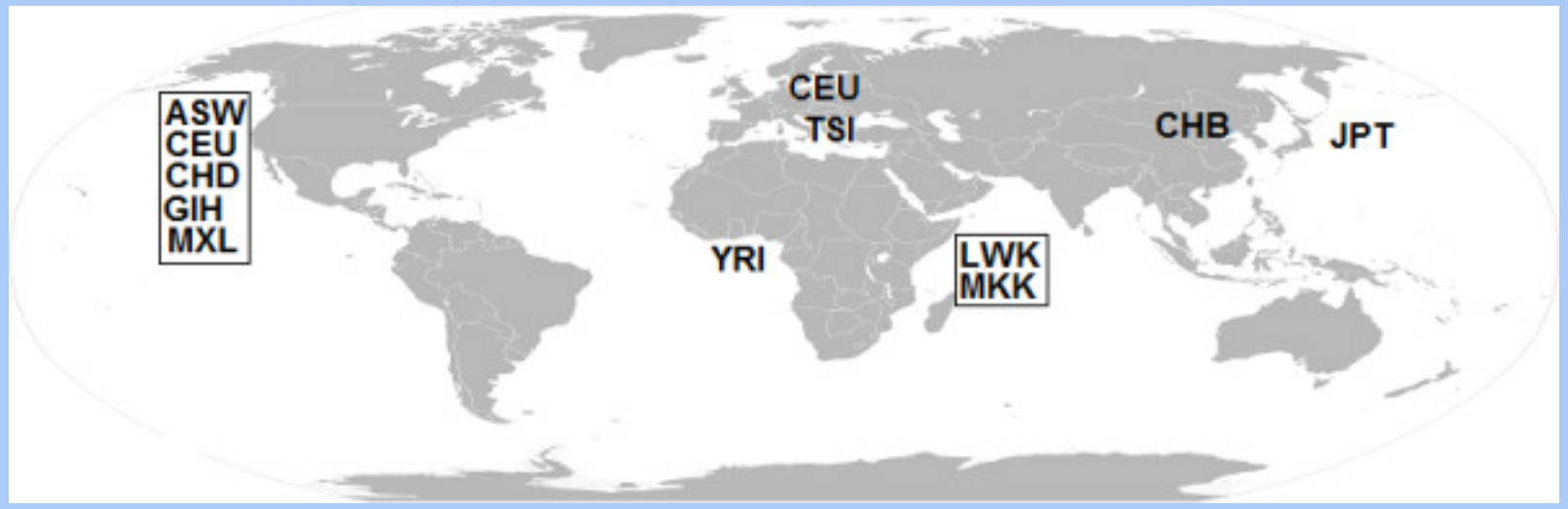
- International genotyping consortium launched in 2002 to find common polymorphisms linked to rare disease loci
- Variants occur together on a small number of **haplotypes**



https://pubmed.ncbi.nlm.nih.gov/16255080/

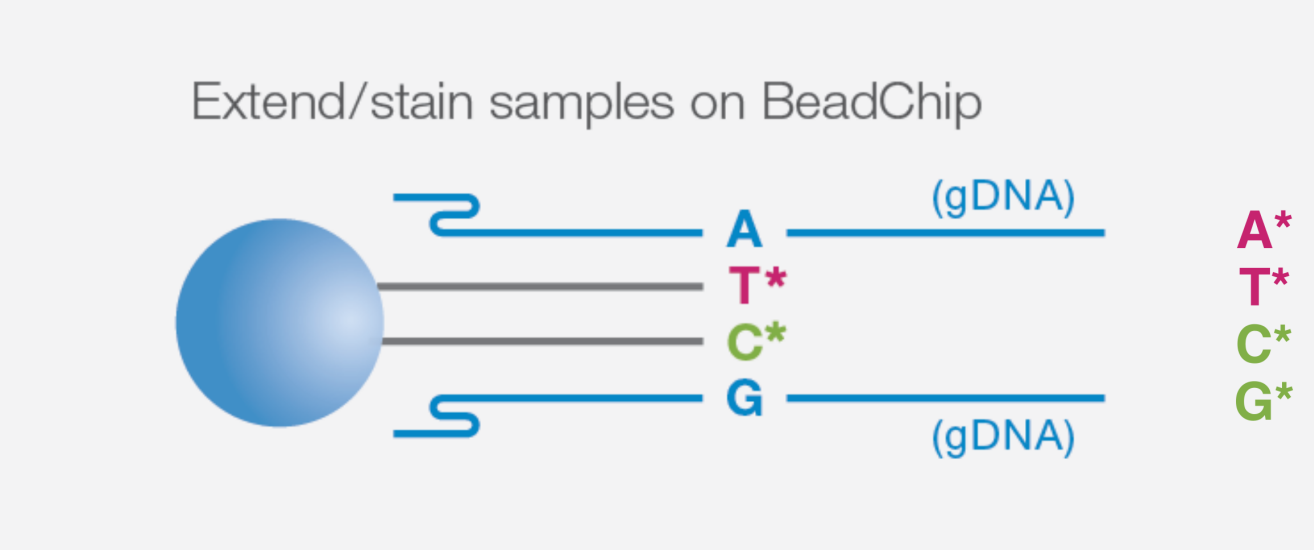
The HapMap Project

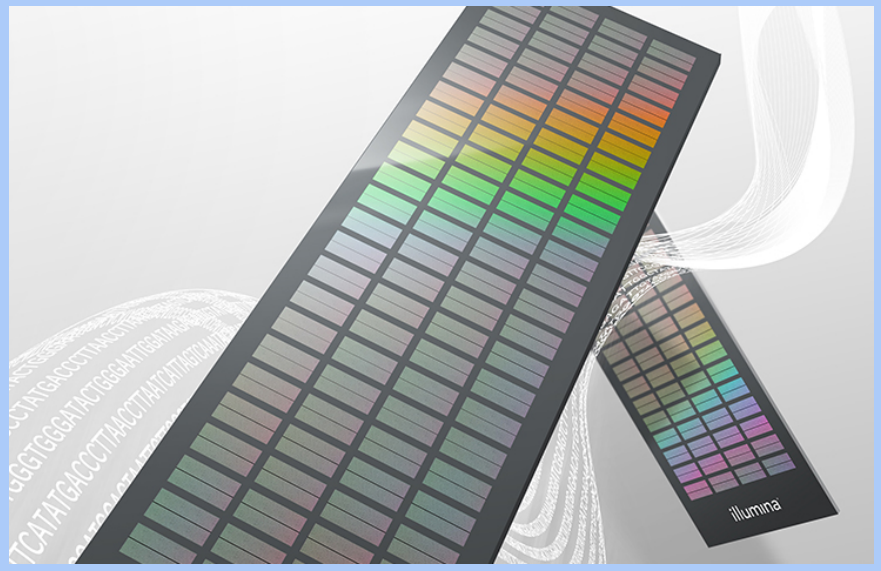
- Phase 3 (2010): genotyping and PCR resequencing of 1.6 million SNPs from 1,184 human samples from different parts of the world



SNP Genotyping

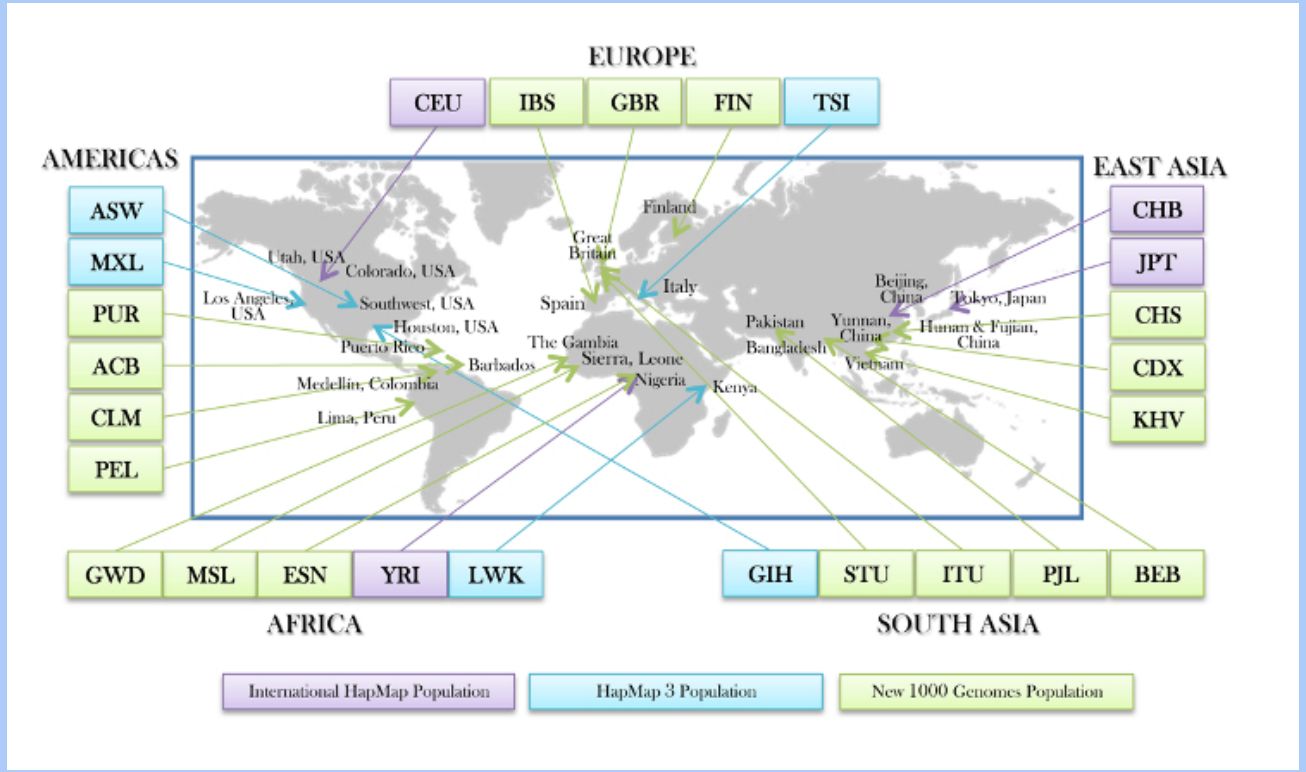
- Genomic DNA with binds to a complementary sequence and incorporates a fluorescently labelled nucleotide
- The ratio of red to green at a spot identifies the sample allele





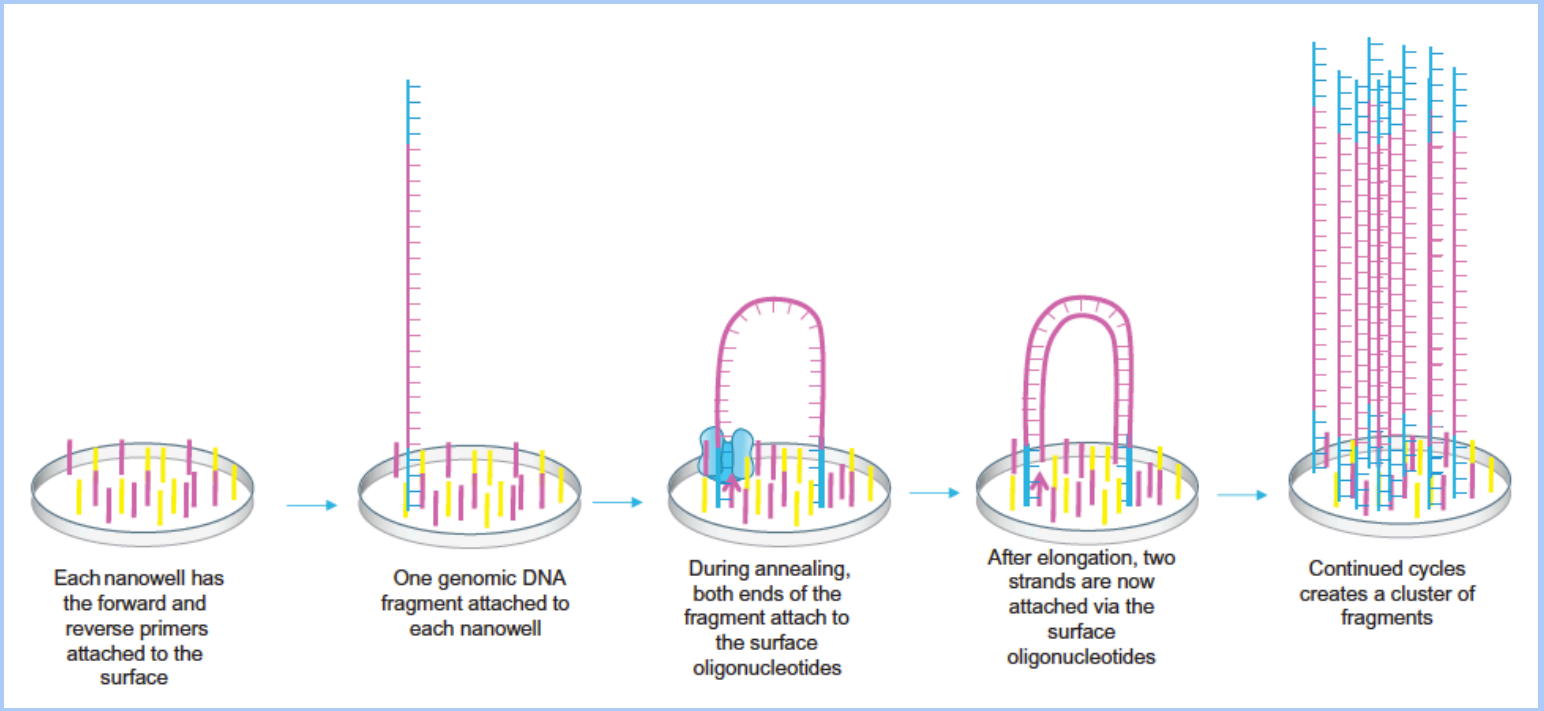
The 1000 Genomes Project

- An international consortium launched in 2008 to catalog rare variants (frequency < 1%) taking advantage of new sequencing technologies
- Phase 3 release (2015) contained data from 2,504 individuals representing 26 populations across the globe and identified 85 million new SNPs



Whole-genome sequencing (WGS)

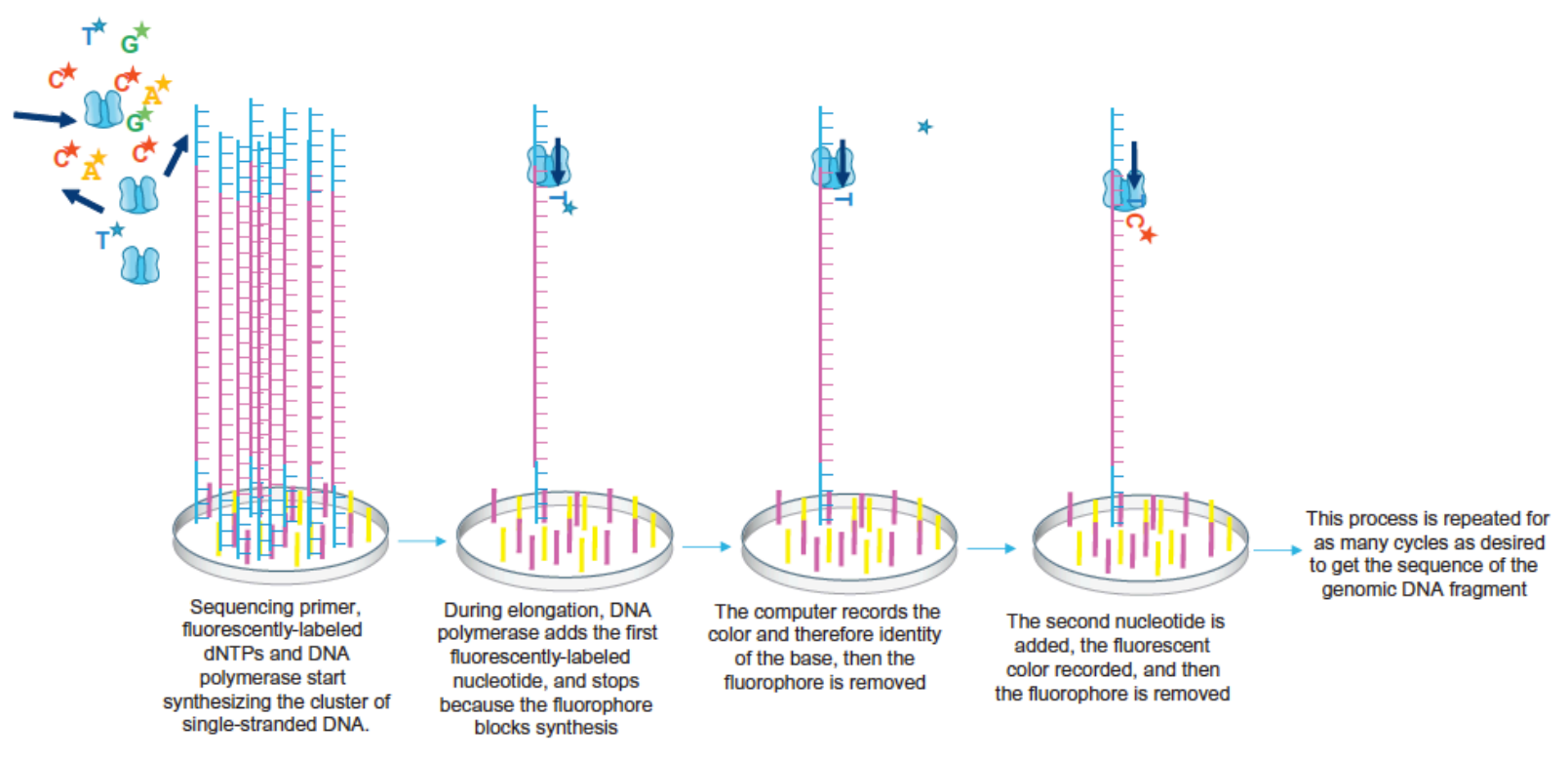
- DNA fragments from a sample are attached to a flow cell and amplified



Clark et al. *Molecular Biology (3rd Edition)*. Ch. 8: DNA Sequencing, 240-269 (2019)

Whole-genome sequencing (WGS)

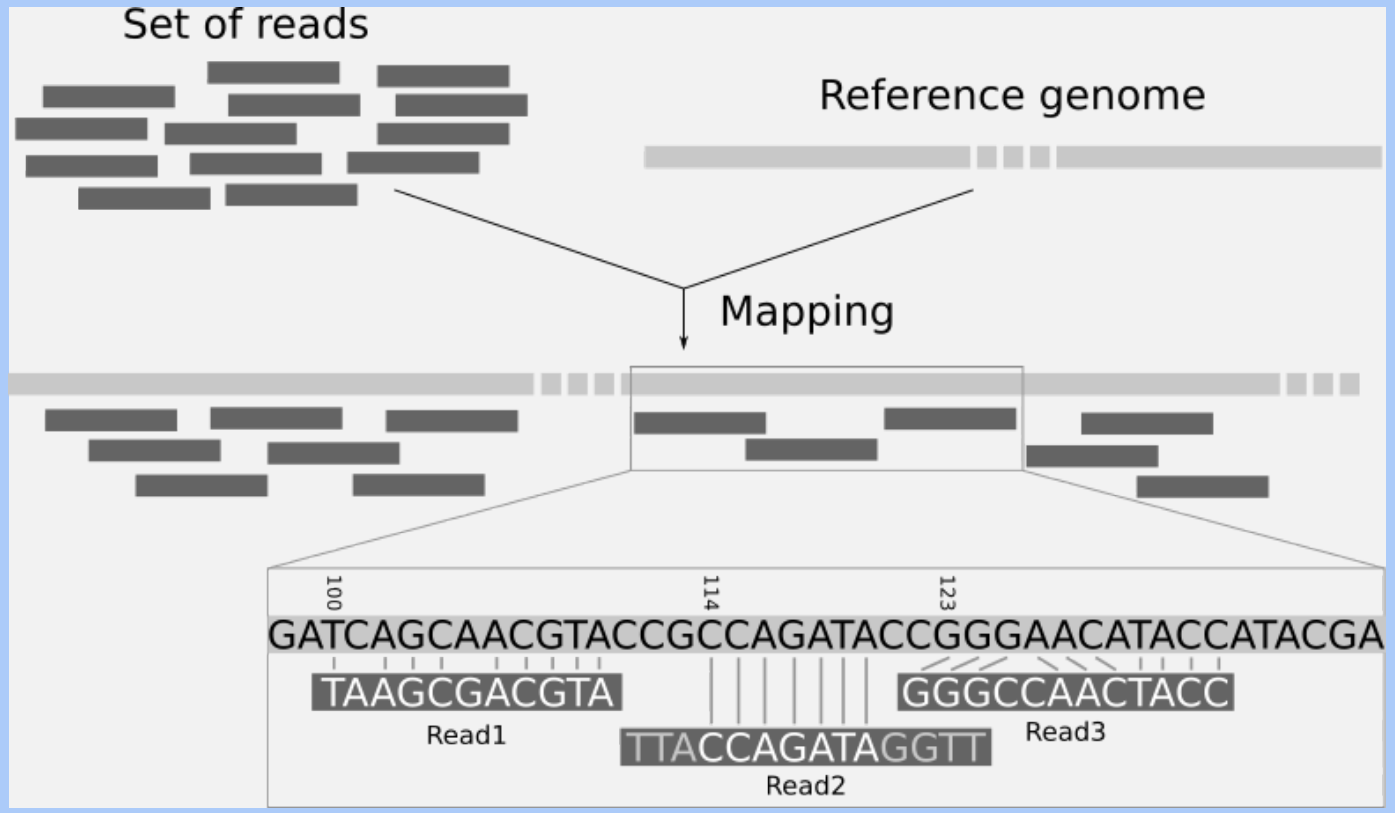
- Sequencing by synthesis: Short reads are produced as fluorescent nucleotides are incorporated one base at a time



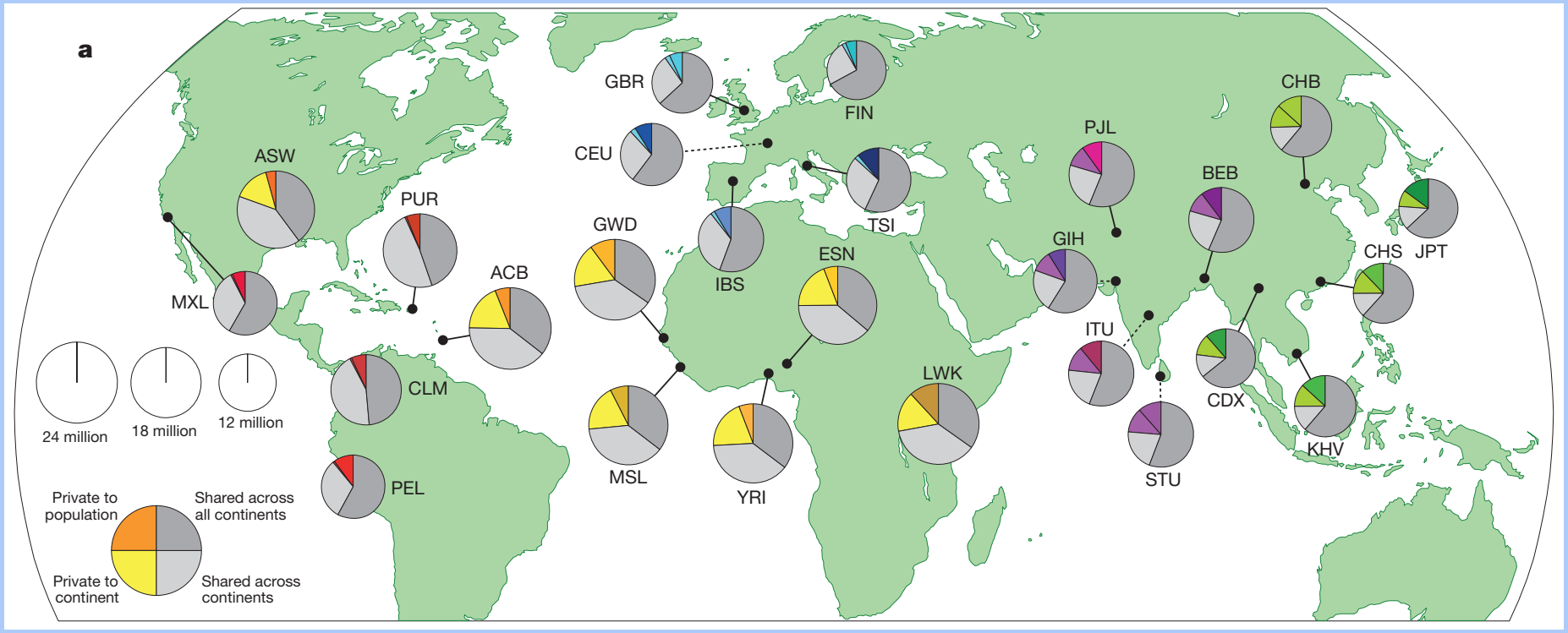
Clark et al. *Molecular Biology (3rd Edition)*. Ch. 8: DNA Sequencing, 240-269 (2019)

Mapping to the reference genome

- Locate from where in the genome the reads came
- Detect single-nucleotide differences from the reference sequence



Global genetic variation



- Most SNPs are shared across continents, and the majority of variation (~85%) is within rather than between populations

Statistical variation of an allele

- Variation of the counts x_i of an allele about the group mean $\overline{x}_j$ and the population mean $\overline{x}$

$$\sum_i (x_i - \overline{x})^2 = \sum_i (x_i - \overline{x}_{j(i)})^2 + \sum_i (\overline{x}_{j(i)} - \overline{x})^2$$

Total variation | Within-population variation | Between-population variation

- Most SNPs are shared across continents, and the majority of variation (~85%) is within rather than between populations

Principal components analysis

The concept of genetic ancestry

The same yet different?

- Most variation is within-populations rather than between-populations
- Yet regional differences in allele frequencies lead to noticeable differences in phenotypes

Example: lactase nonpersistence (lactose intolerance)

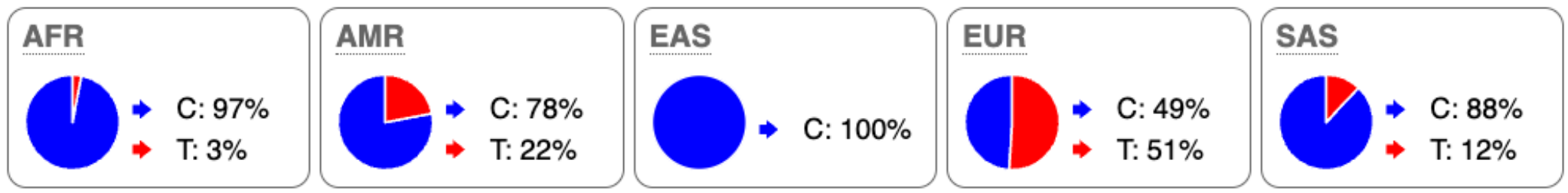
- The T allele of rs182549 is completely associated with the ability to digest lactose in Europeans

	CC	CT	TT
Non-persistence	59	0	0
Persistence	0	63	74

https://pubmed.ncbi.nlm.nih.gov/11788828/

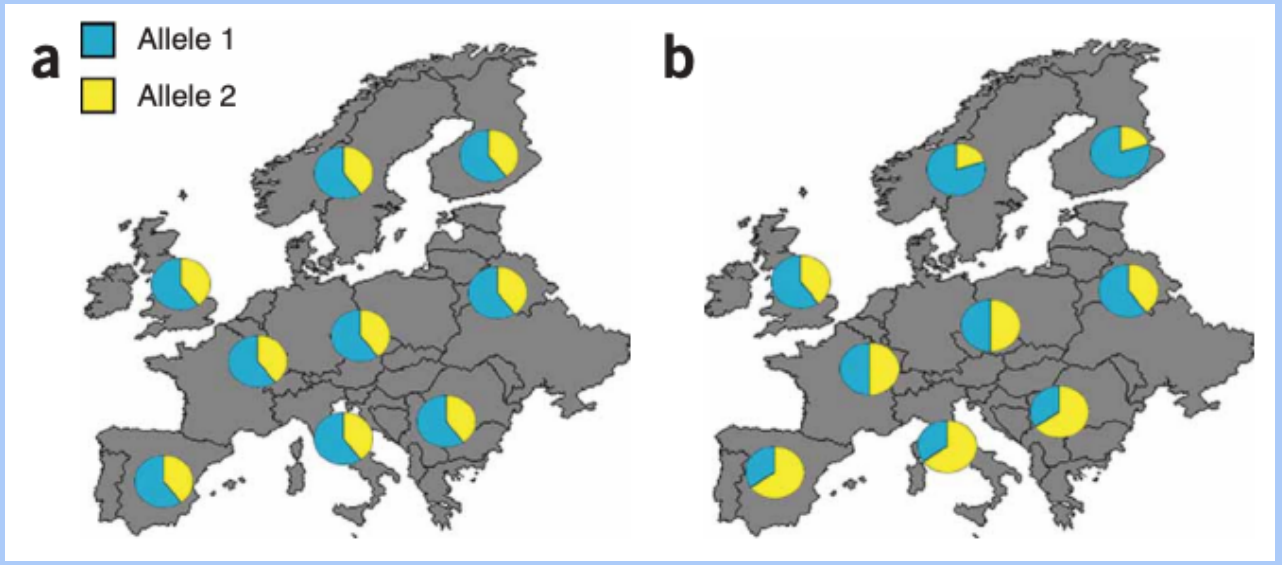
Example: lactase nonpersistence (lactose intolerance)

- Yet the polymorphism is almost absent in the African population, despite the presence of lactase persistence https://pubmed.ncbi.nlm.nih.gov/15106124/



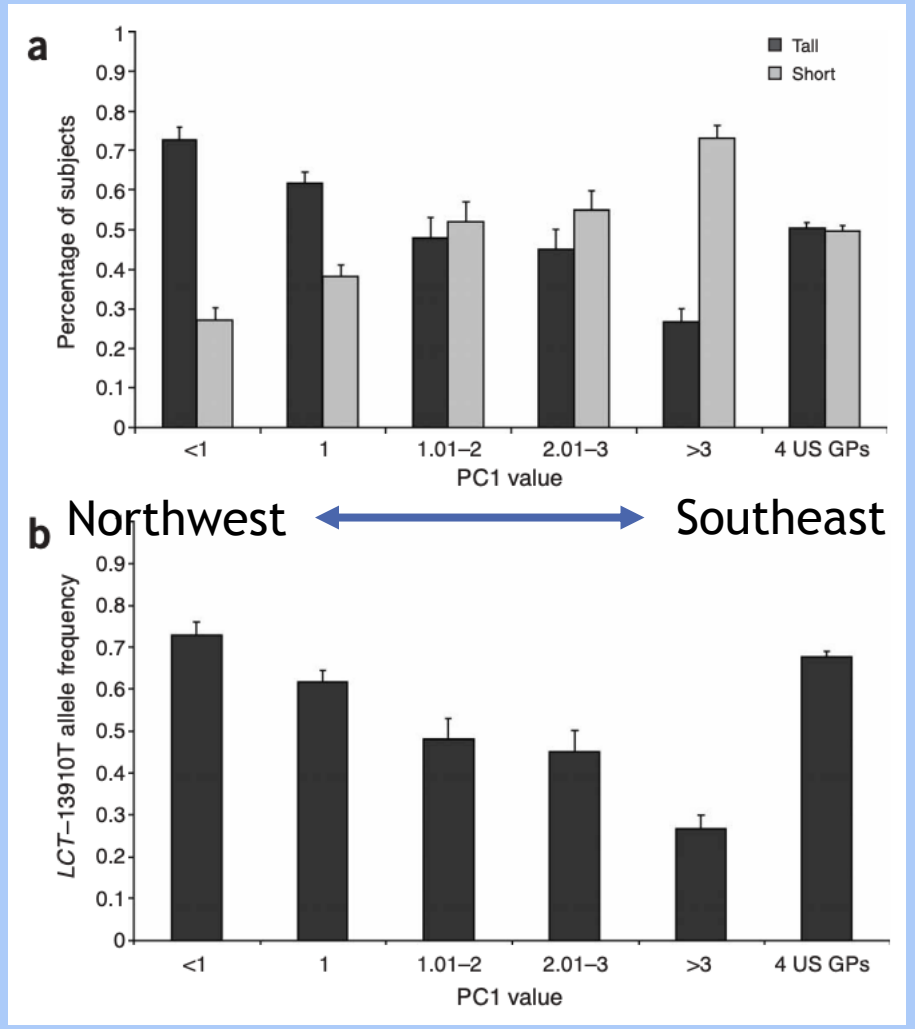
Population stratification

- An allele may appear associated with a phenotype when in fact it is associated with geographic origin (genetic ancestry)



Spurious association

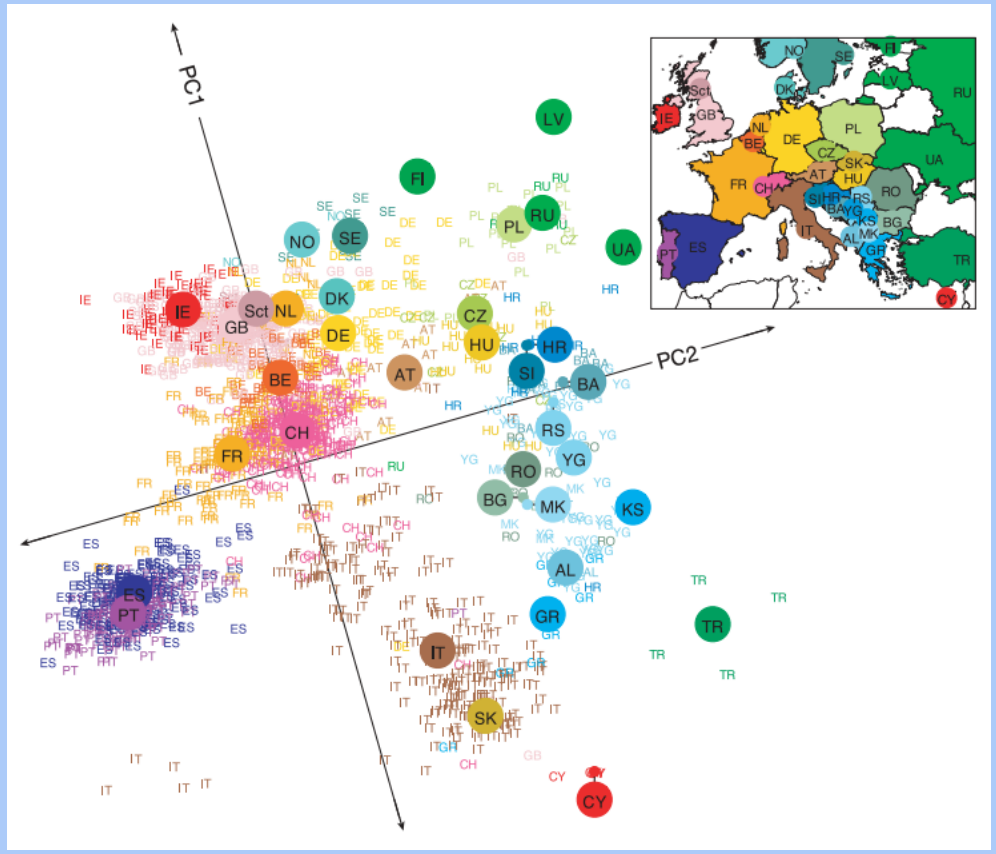
- An allele of the lactase-persistence SNP is spuriously associated with height, as its frequency is higher in individuals with Northern European ancestry vs. Southern



https://pubmed.ncbi.nlm.nih.gov/16041375/

Principal components analysis

- Genotypes can distinguish population groups
- Looking a **which variants segregate together** can tell us about an individual's likely genetic ancestry



https://pubmed.ncbi.nlm.nih.gov/18758442/

Genotype matrix

- n individuals are genotyped at m SNPs
- The number of alternate alleles is 0, 1, or 2
- “Standardize” each genotype by subtracting the mean allele (column) frequency and dividing by its standard error

$$\mathbf{X} = \begin{pmatrix} x_{11} & \cdots & x_{1m} \\ x_{21} & \cdots & x_{2m} \\ \vdots & & \vdots \\ x_{n1} & \cdots & x_{nm} \end{pmatrix}$$

“Idealized” individuals

- An “idealized” subject of a particular genetic ancestry has genotypes v at m SNPs
- The position of individual 1 on PC1 is the “amount” of idealized person 1 in individual 1

$$\mathbf{XV}^T = \begin{pmatrix} x_{11} & \cdots & x_{1m} \\ x_{21} & \cdots & x_{2m} \\ \vdots & & \vdots \\ x_{n1} & \cdots & x_{nm} \end{pmatrix} \begin{pmatrix} v_{11} & v_{21} & v_{31} \\ \vdots & \vdots & \vdots \\ v_{1m} & v_{2m} & v_{3m} \end{pmatrix}$$

Genomic relationship matrix (GRM)

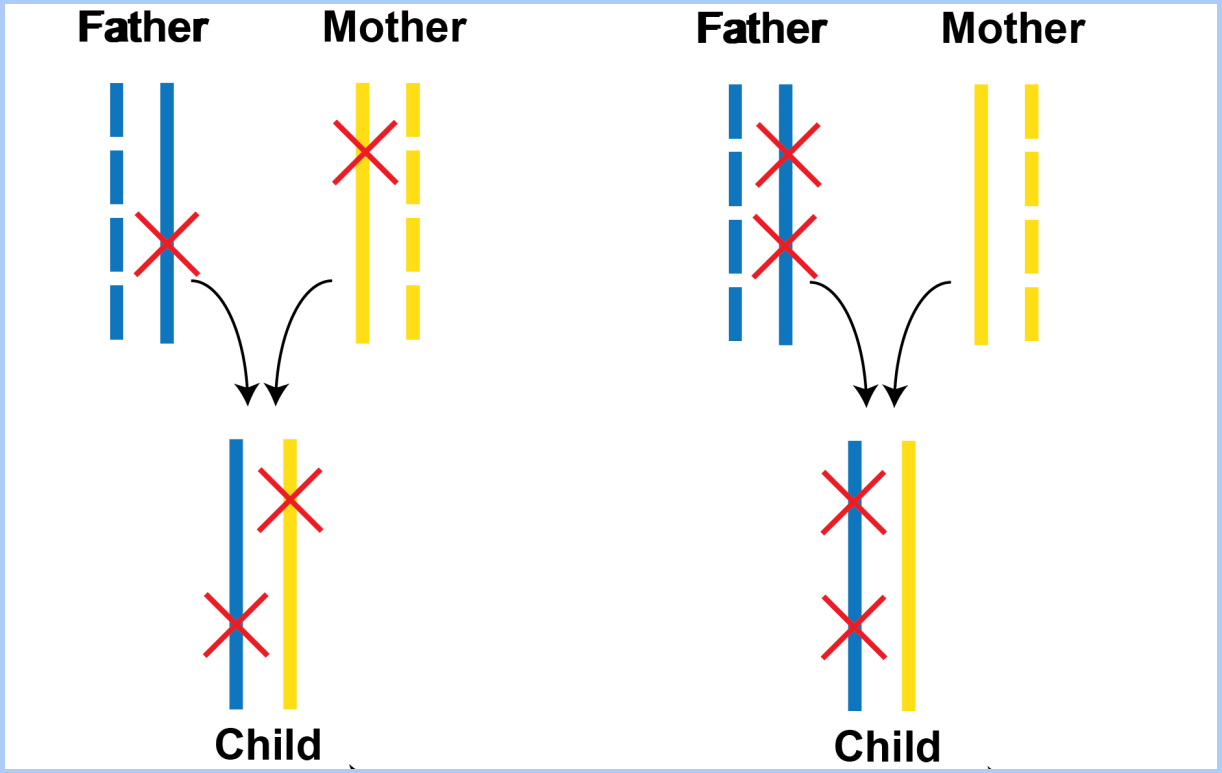
- The idea of PCA is to find the amount of each idealized individual in each actual individual
- The **eigenvectors** of the GRM contain the ancestry components
- The GRM is computed by comparing how similar any subject is to any other

$$\mathbf{X}\mathbf{X}^T = \begin{pmatrix} \mathbf{x}_1 \cdot \mathbf{x}_1 & \cdots & \mathbf{x}_1 \cdot \mathbf{x}_n \\ \mathbf{x}_2 \cdot \mathbf{x}_1 & \cdots & \mathbf{x}_2 \cdot \mathbf{x}_n \\ \vdots & & \vdots \\ \mathbf{x}_n \cdot \mathbf{x}_1 & \cdots & \mathbf{x}_n \cdot \mathbf{x}_n \end{pmatrix}$$

Linkage disequilibrium

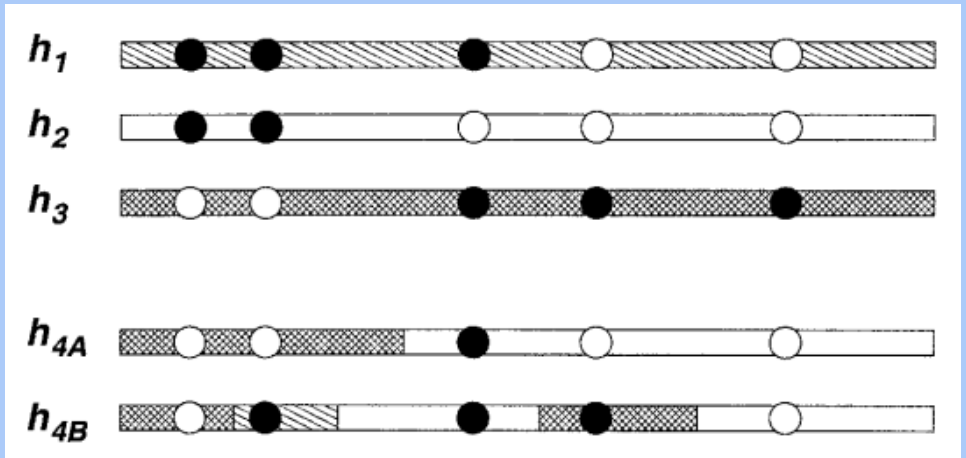
Determining a set of independent SNPs

SNPs can occur on either of two chromosomes



- Genotype data do not tell us which chromosomes carry the polymorphism
- When at least one parent is homozygous at each SNP, **haplotype phase** can be unambiguously assigned

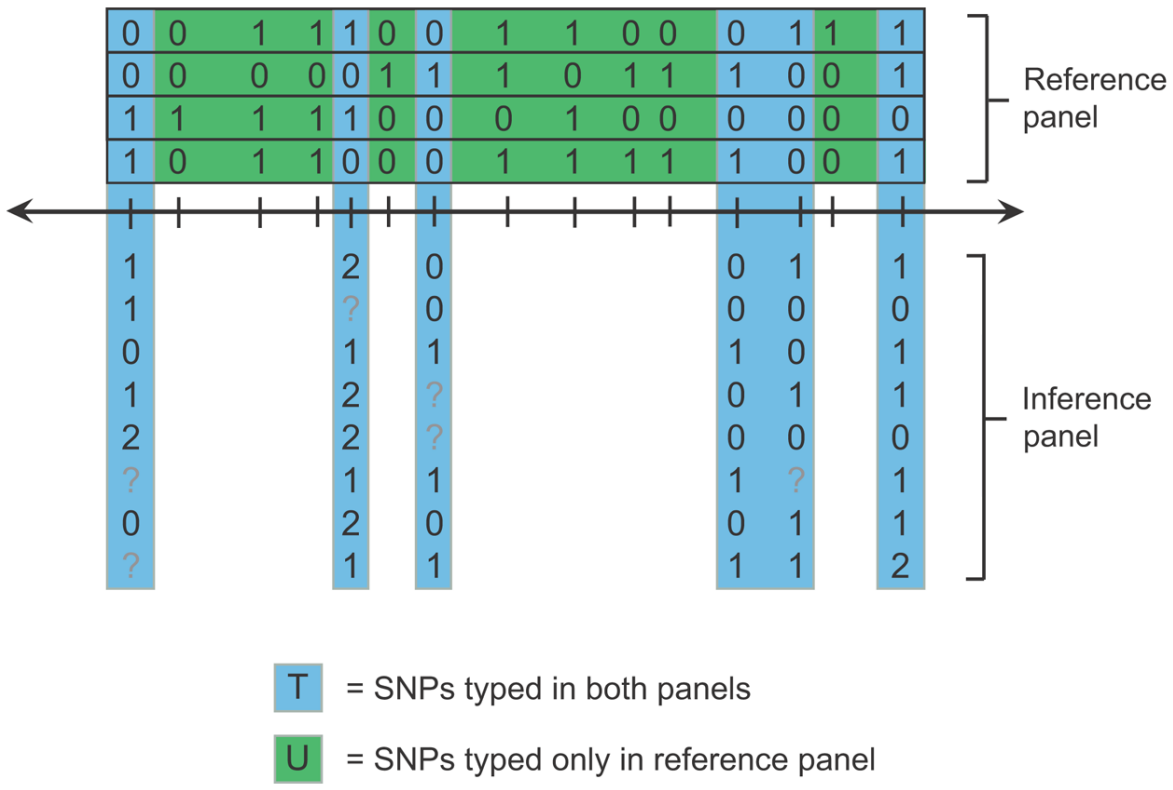
Statistical phasing and imputation



https://pubmed.ncbi.nlm.nih.gov/14704198/

- Genotyped individuals can be computationally **phased** by modelling each chromosome as an imperfect **mosaic** of chromosomes from a reference panel

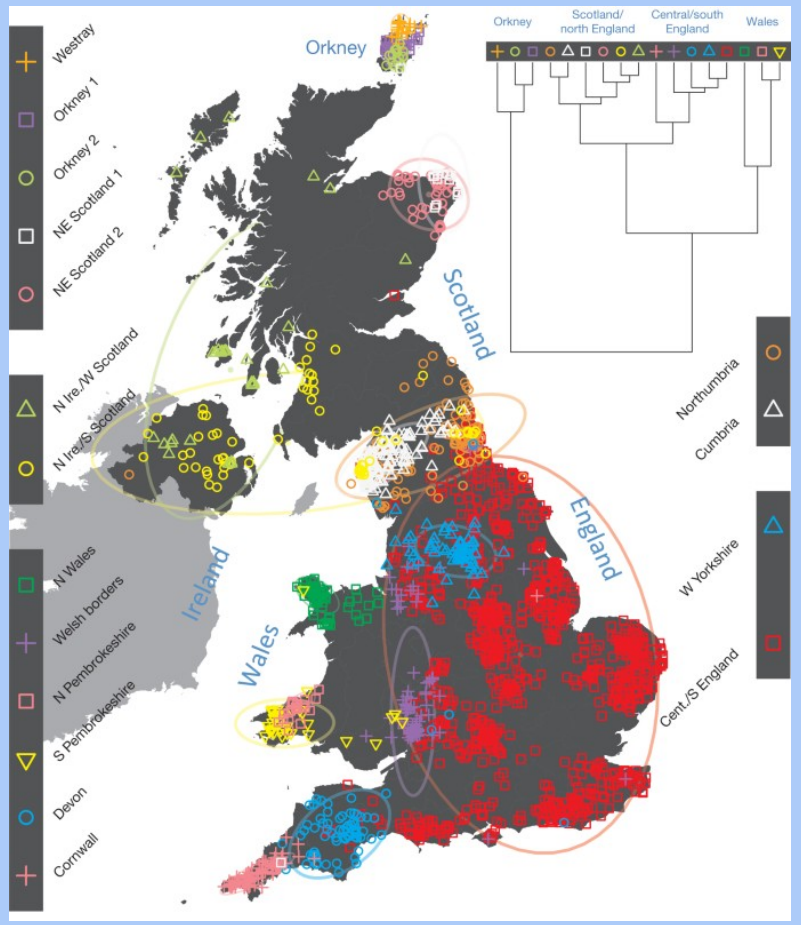
Statistical phasing and imputation



https://pubmed.ncbi.nlm.nih.gov/19543373/

- Variants that have not been typed can be **imputed** into the inference sample
- Imputation accuracy depends on the inference and reference samples being of similar genetic ancestry

Different haplotypes distinguish different populations



https://pubmed.ncbi.nlm.nih.gov/25788095/

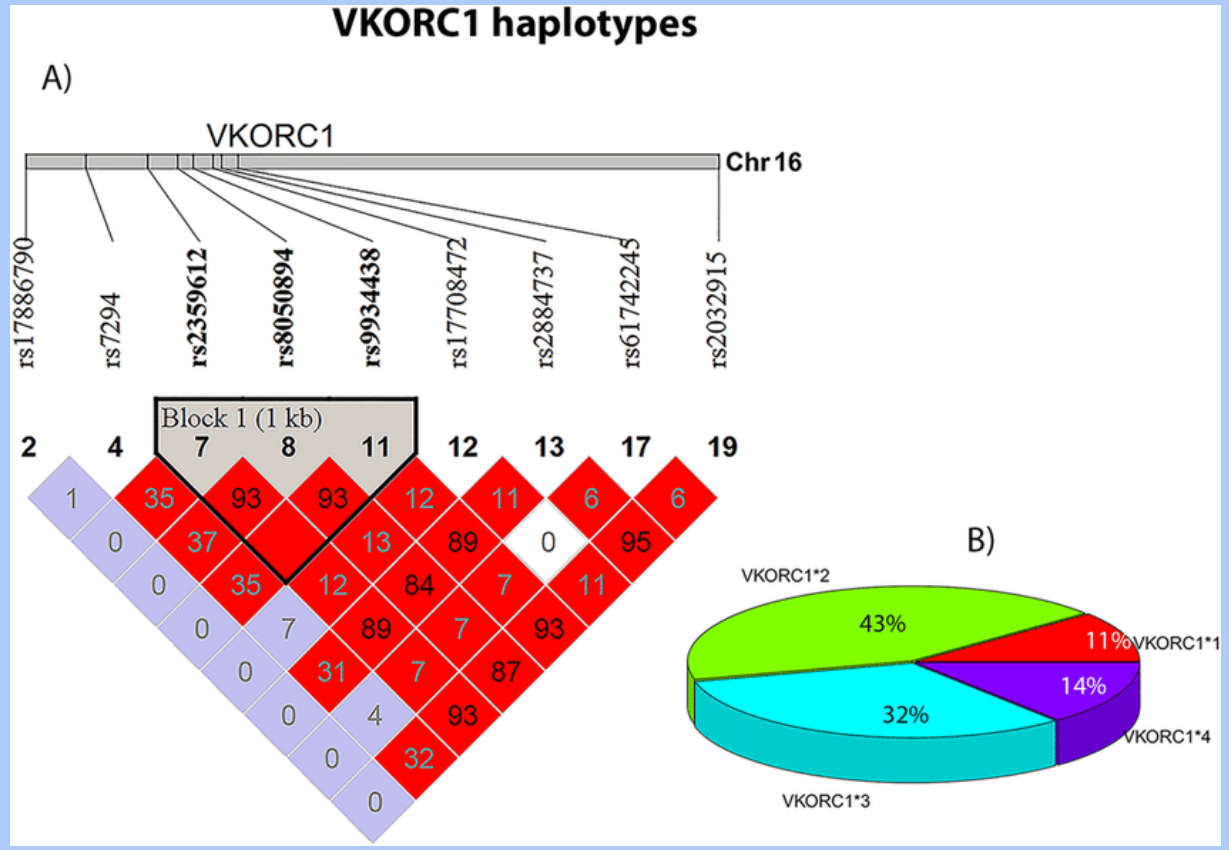
- Individuals can be grouped into populations with which they have the most haplotype-sharing

Linkage disequilibrium

- Linkage disequilibrium is the population tendency of alleles to be inherited on a single chromosome and is measure using a correlation coefficient between the alleles of different SNPs

$$r_{A,B} = \frac{p_{A,B} - p_A p_B}{\sqrt{p_A (1 - p_A) p_B (1 - p_B)}}$$

LD blocks and haplotype structure



https://pubmed.ncbi.nlm.nih.gov/32221414/

- Plots of pairwise r^2 values show which SNPs are inherited together in the population as common haplotypes

Haplotype simulation using LD

- Simulate a haplotype by ensuring that the frequencies and LD between any two alleles match the reference data
- No assumptions about phylogenies or knowledge of evolutionary theory is required

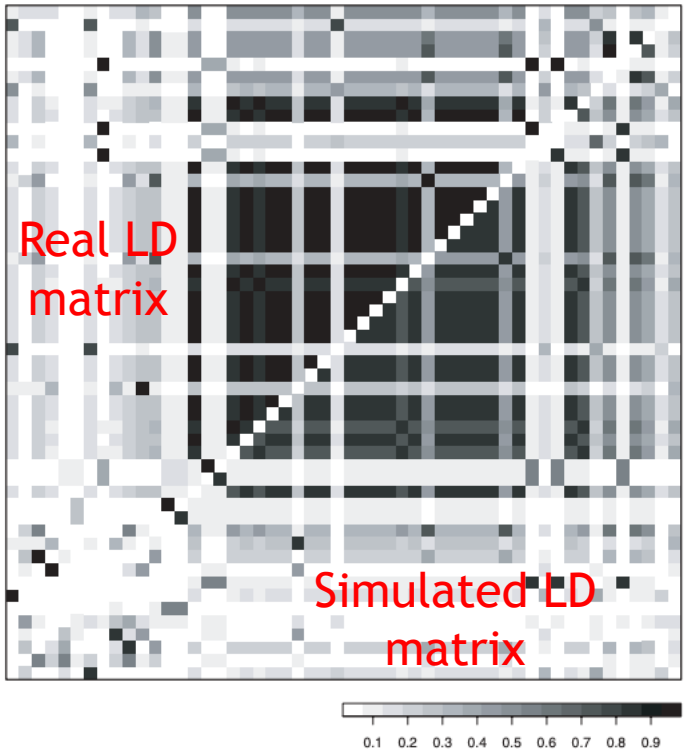


Fig. 2. ACE data: hybrid LD matrix obtained from real and artificial samples of 11 individuals each.

https://pubmed.ncbi.nlm.nih.gov/16188927/

sim1000G: simulate haplotypes from an input vcf

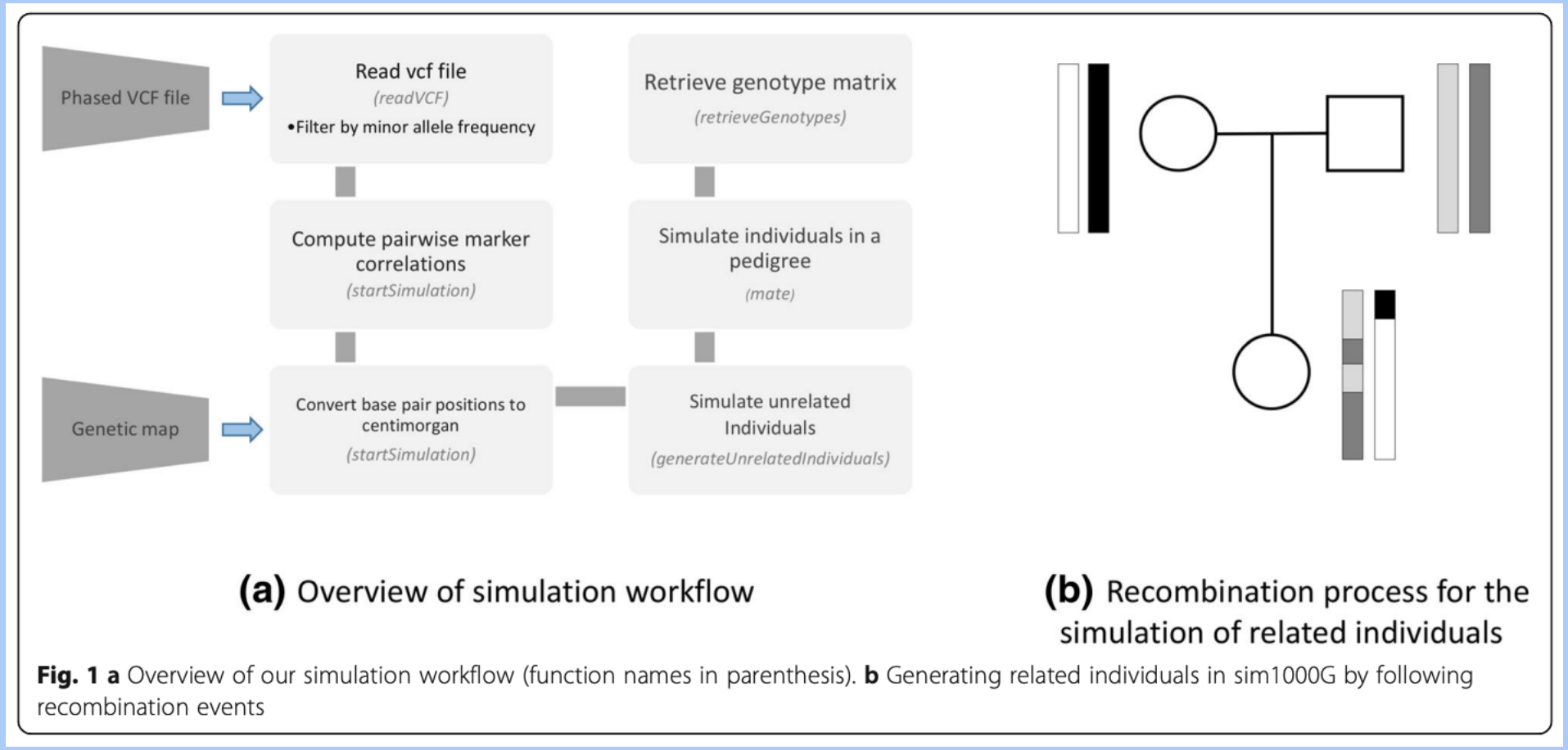


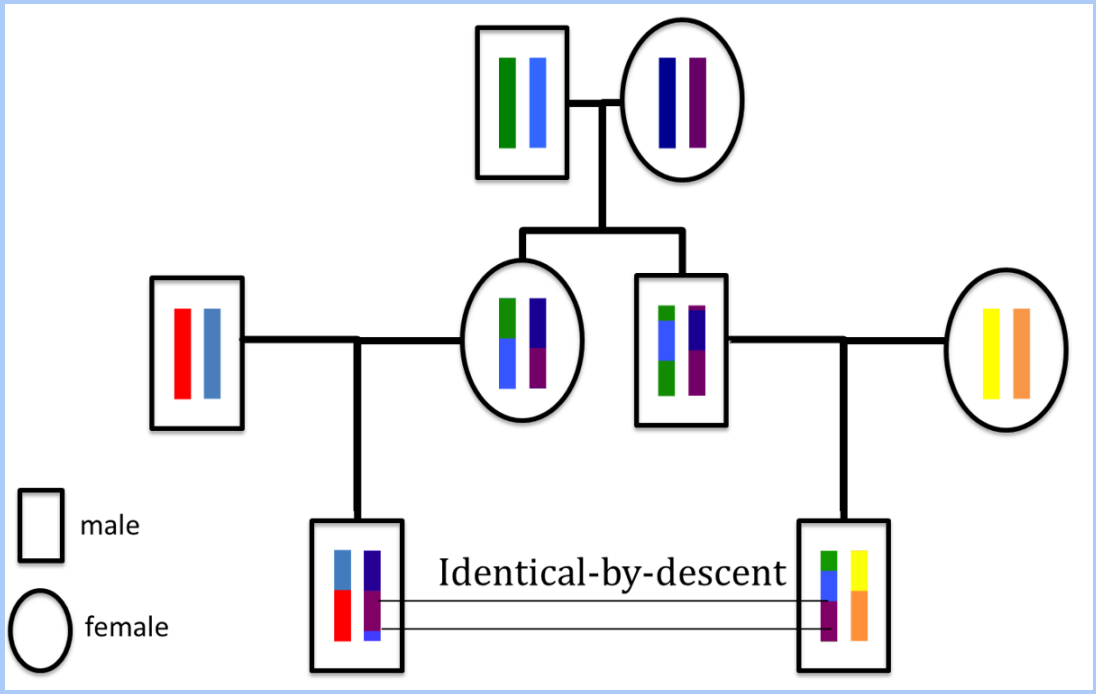
Fig. 1 a Overview of our simulation workflow (function names in parenthesis). **b** Generating related individuals in sim1000G by following recombination events

https://pubmed.ncbi.nlm.nih.gov/30646839/

Kinship analysis

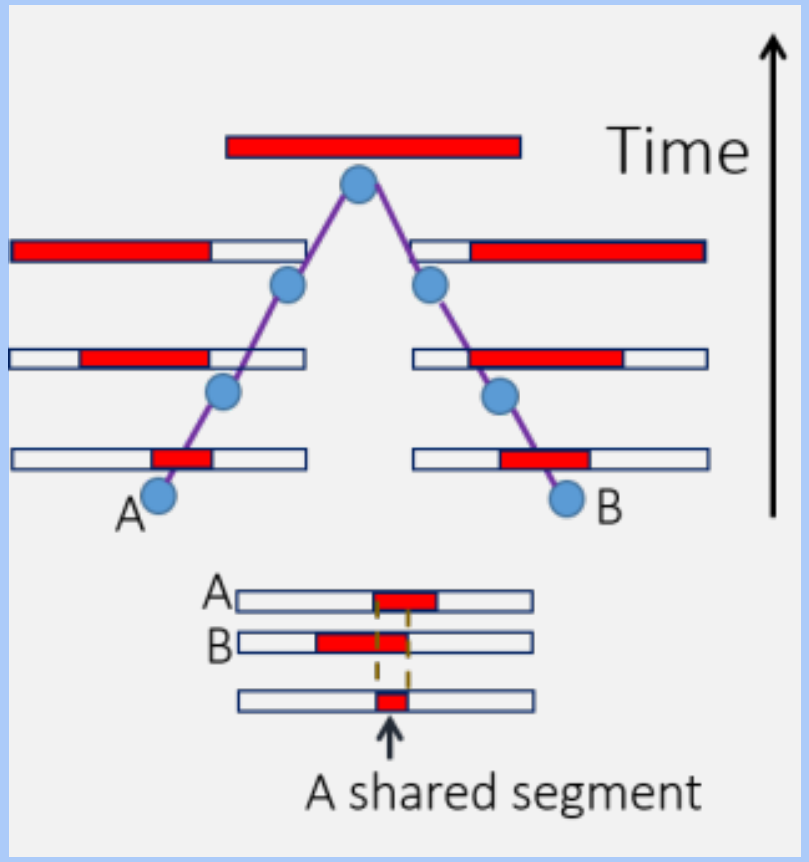
The concept of genetic relatedness

Relatives share haplotypes IBD



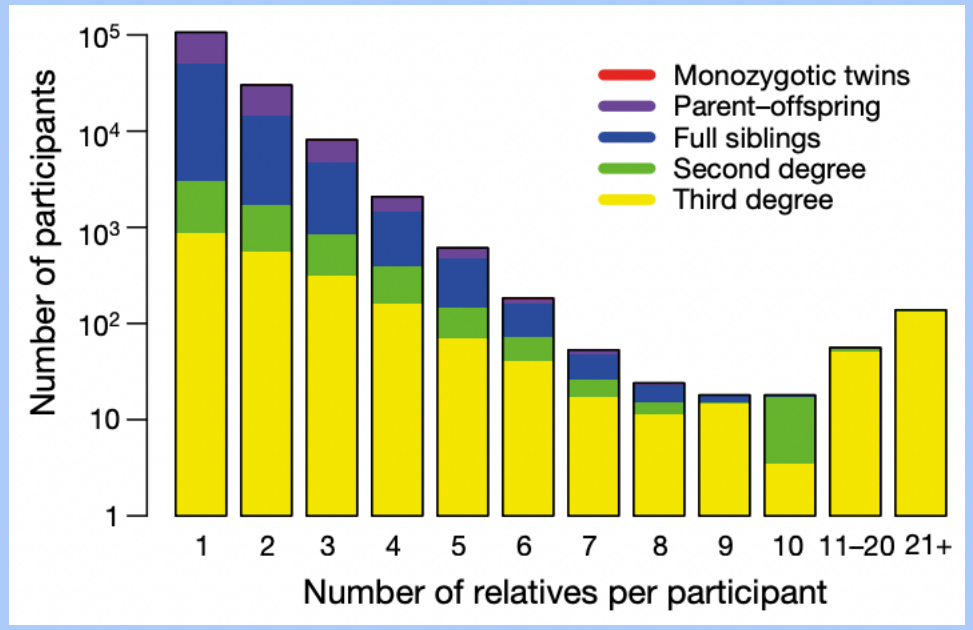
- Segments of DNA inherited from a common ancestor are said to be **identical by descent**
- DNA that just happens to be the same is **identical by state**

Haplotype sharing decays over time



- The longer the IBD segment, the more closely related are the two individuals

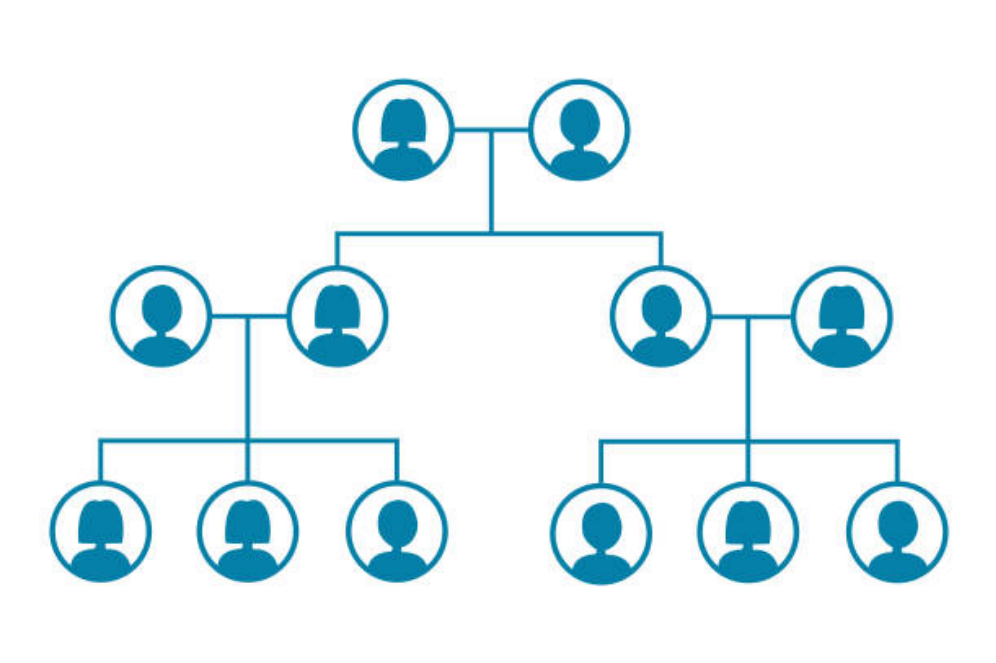
Kinship in genetic association studies



https://pubmed.ncbi.nlm.nih.gov/30305743/

- Genomic datasets, such as the UK Biobank, contain related individuals
- Sometimes there is even "cryptic" relatedness
- Because of IBD sharing, not all the observations are independent, and genotype-phenotype associations may be **confounded**

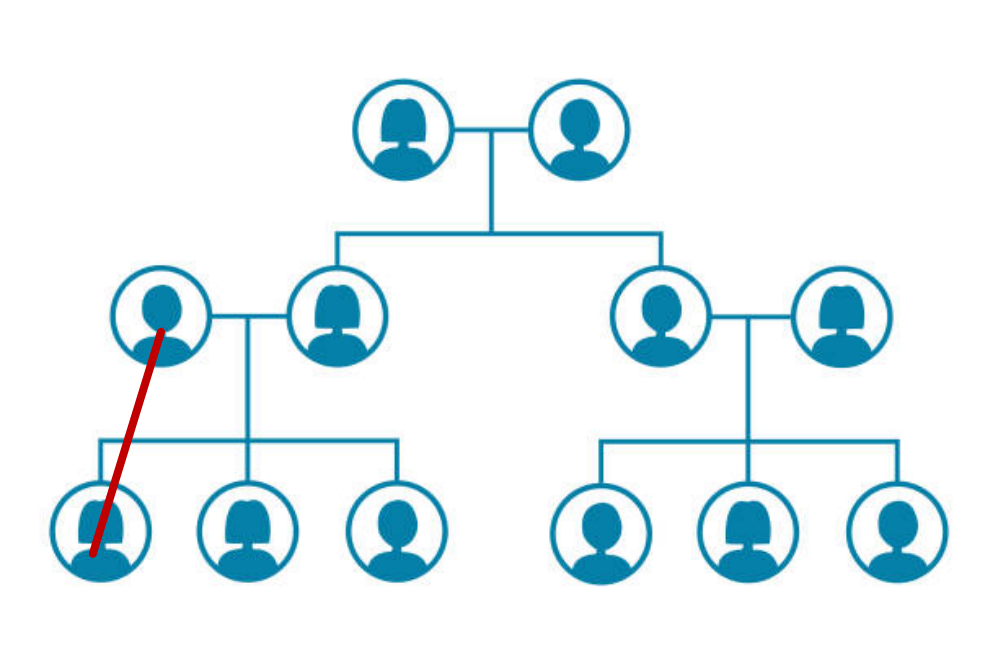
Degree of relatedness



- R is the effective number of meioses separating two individuals through their two parents 1 and 2
- R → ∞ for unrelated individuals

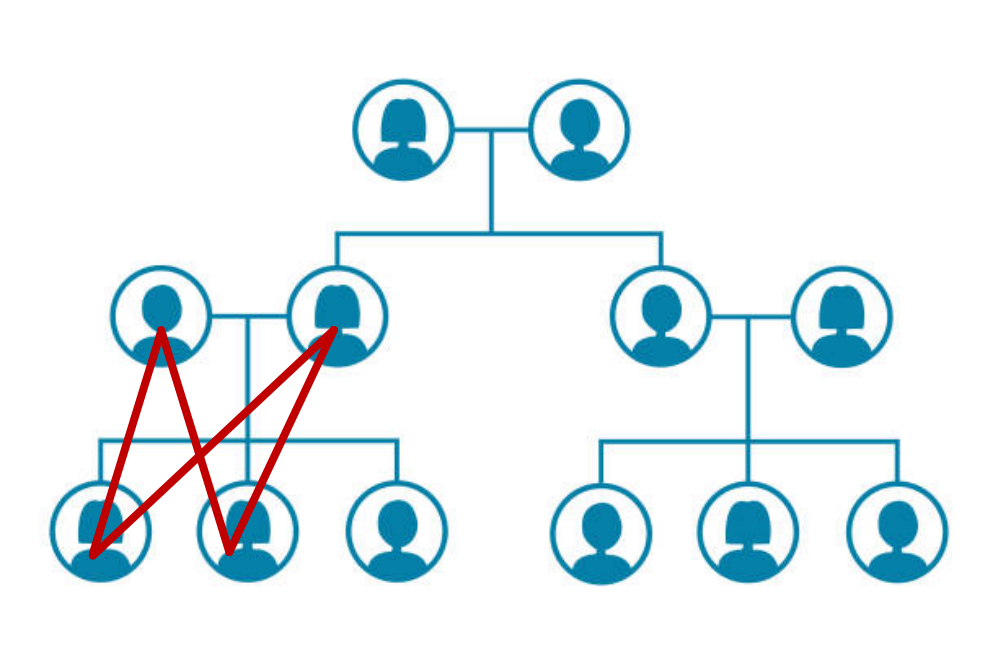
$$\frac{1}{2^R} = \frac{1}{2^{R_1}} + \frac{1}{2^{R_2}}$$

Degree of relatedness



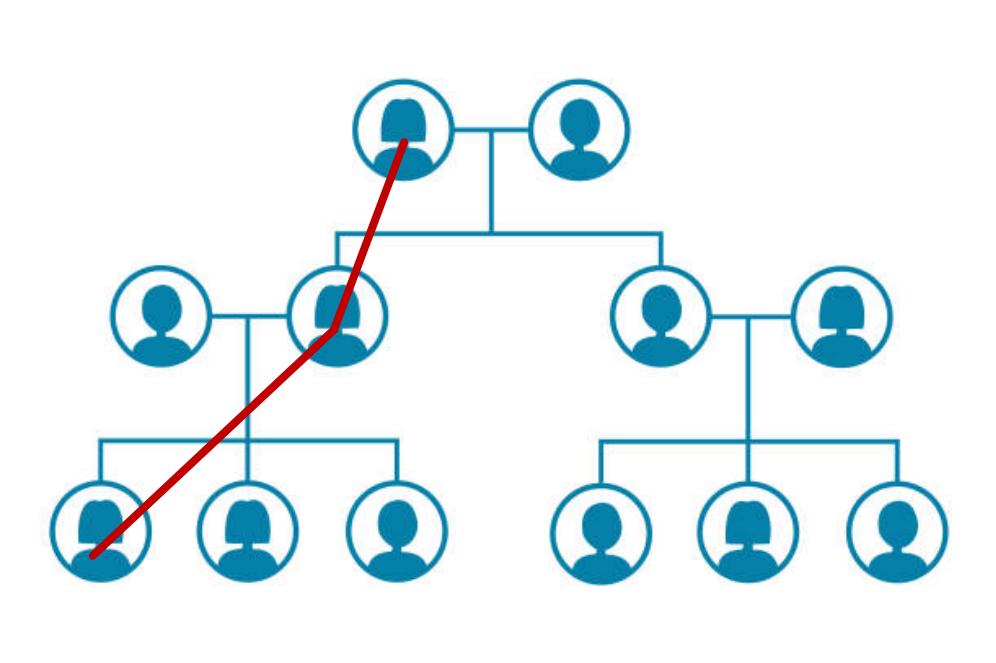
- Parent-child: R = 1 meiosis

Degree of relatedness



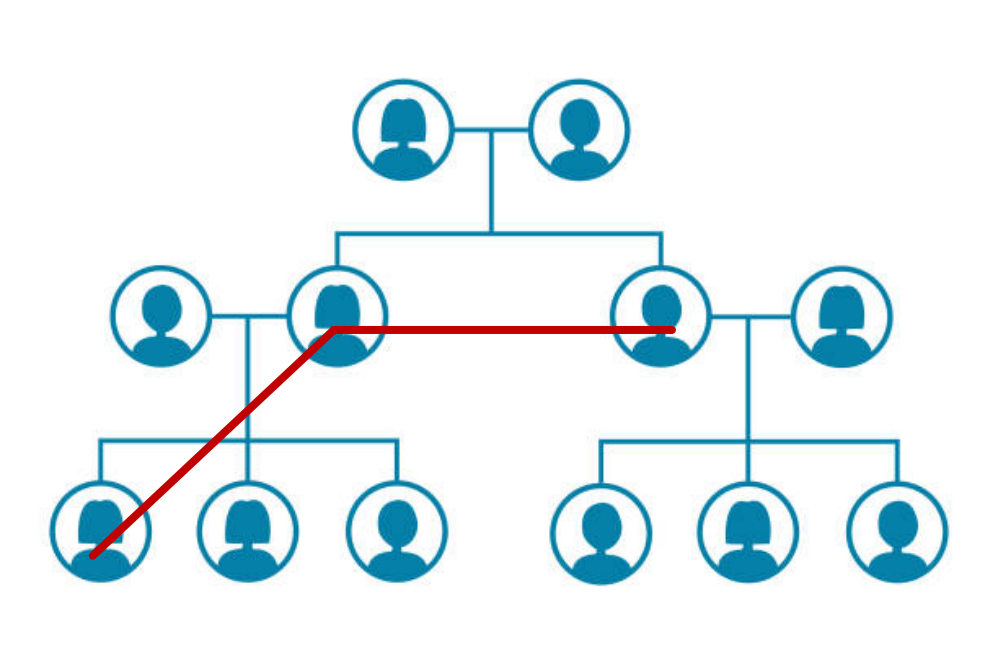
- Siblings: R = 1 “effective” meiosis:
  - $1 / 2^1 = 1 / 2^2 + 1 / 2^2$

Degree of relatedness



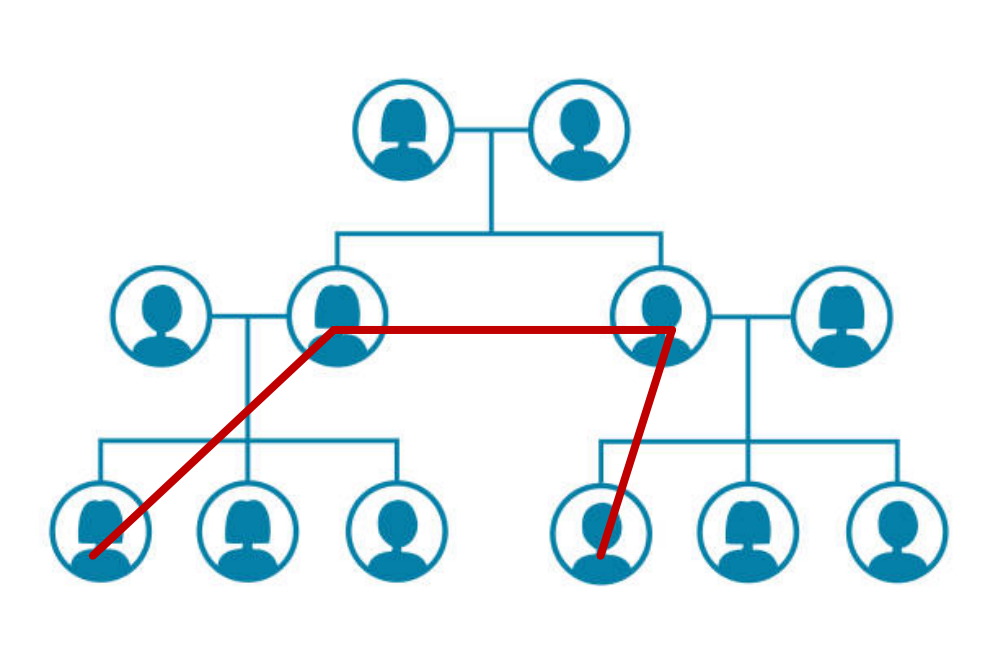
- Grandparent-grandchild: R = 2 meioses

Degree of relatedness



- Avuncular: R = 2 meioses

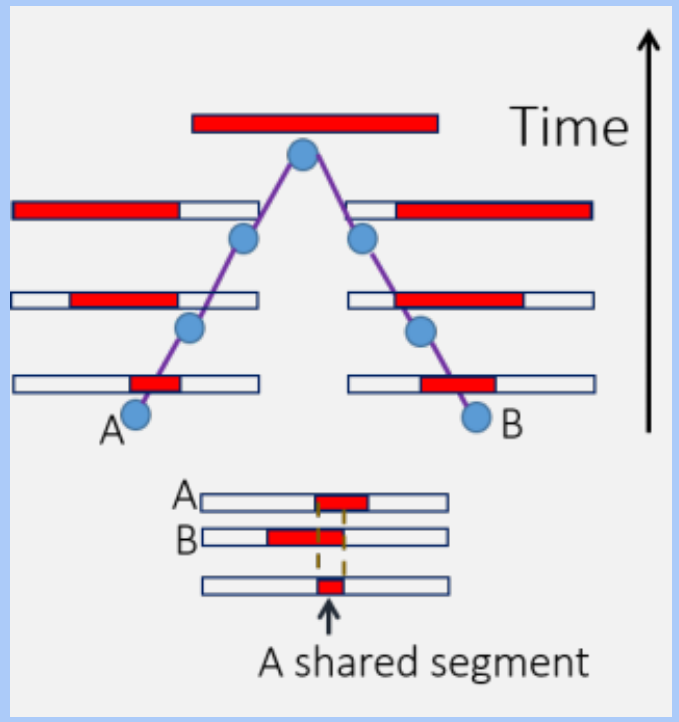
Degree of relatedness



- Cousins: R = 3 meioses

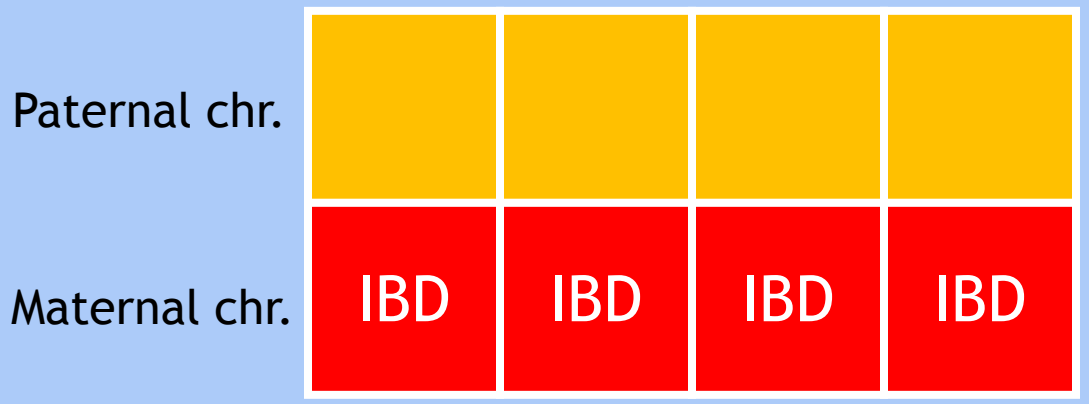
Degree of relatedness and the fraction of the genome shared IBD

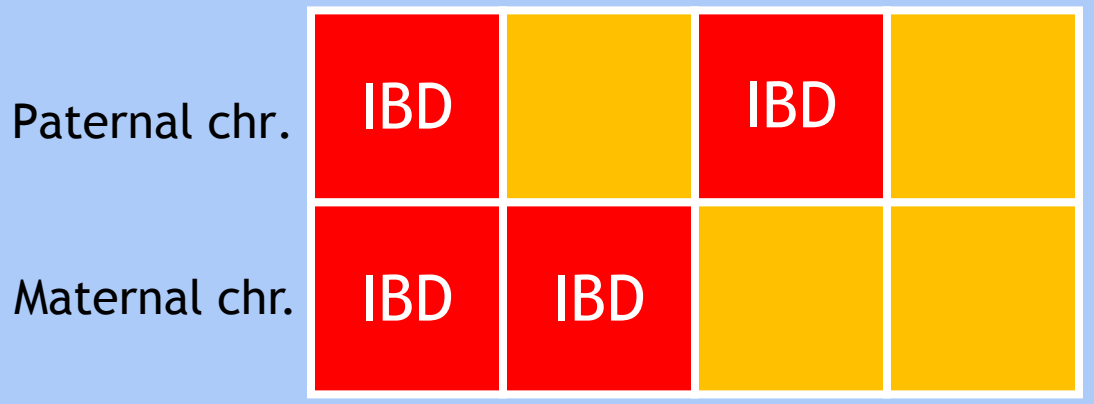
- $r = 1 / 2^R$ is the fraction of the genome shared IBD, because there is a ½ probability that the gene is passed on in each of R meioses



Degree of relatedness and the fraction of the genome shared IBD

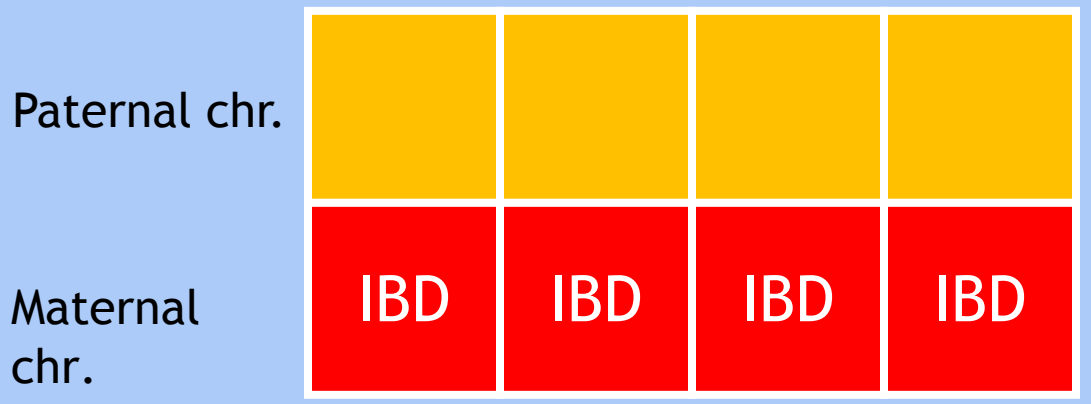
- A child shares **half** of its DNA with its parent
- A child shares (a different) **half** its DNA with its full sib

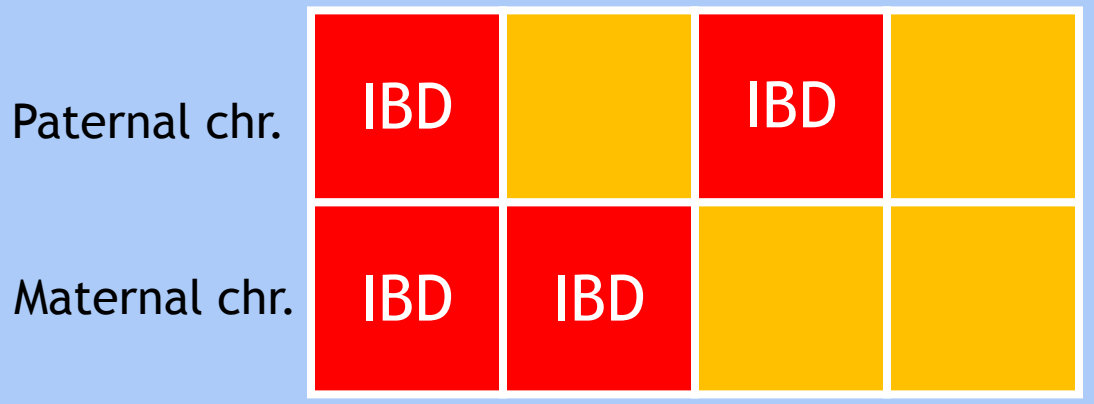




Degree of relatedness and the fraction of the genome shared IBD

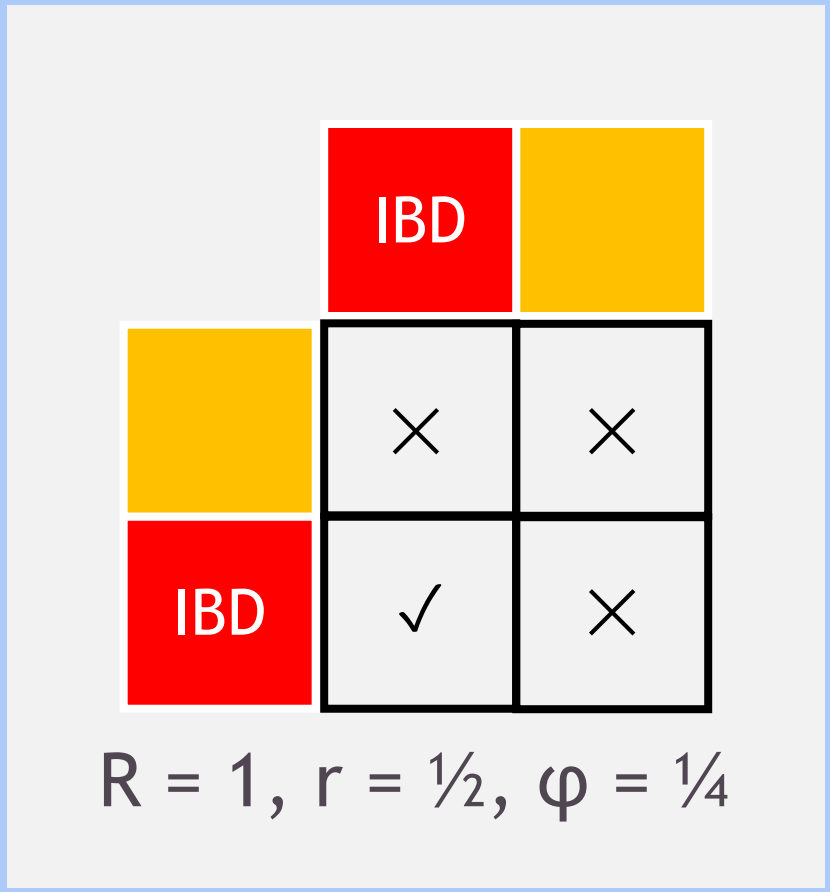
- A child has 0 probability of IBD = 0 with its parent
- A child has 0.25 probability of IBD = 0 with its sib





The coefficient of relatedness φ

- φ is the probability that any two alleles at a single locus chosen from two individuals are shared IBD
- φ is equal to half of $r = 1 / 2^R$



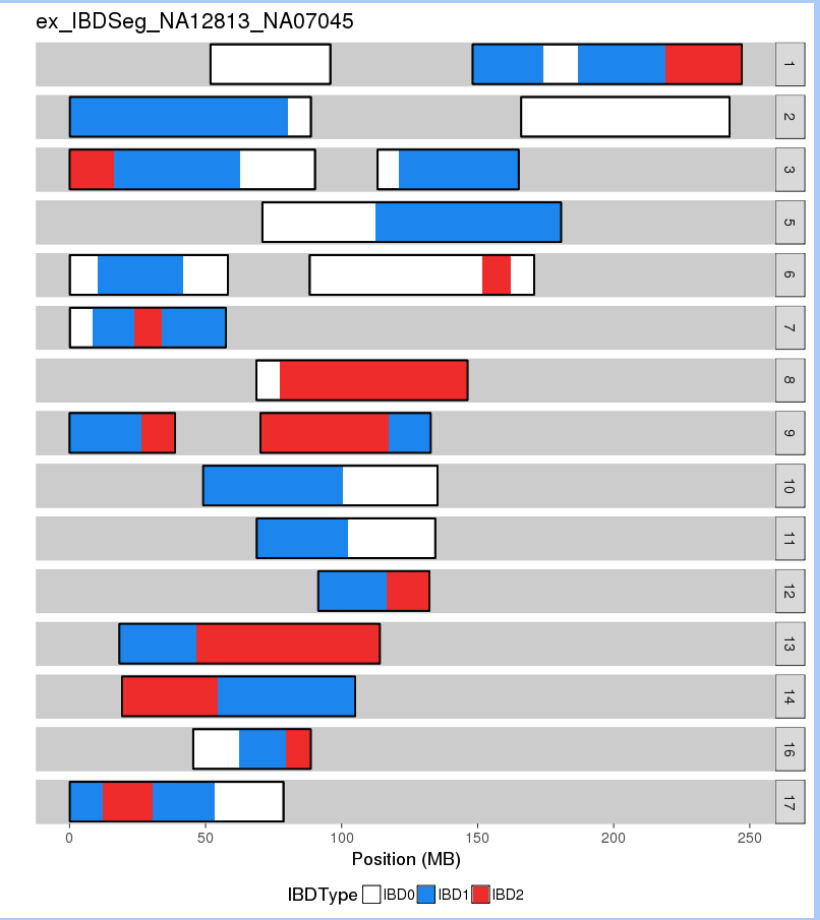
Coefficient of relatedness and IBD = 0

- φ decreases as the probability that a pair of individuals should be IBD = 0 increases

Relationship	R	φ	IBD = 0
Monozygotic twins	0	0.5	0
Parent-child	1	0.25	0
Full sibs	1	0.25	0.25
2nd degree	2	0.125	0.5
3rd degree	3	0.0625	0.75
Unrelated	∞	0	1

Kinship-based Inference for GWAS (KING)

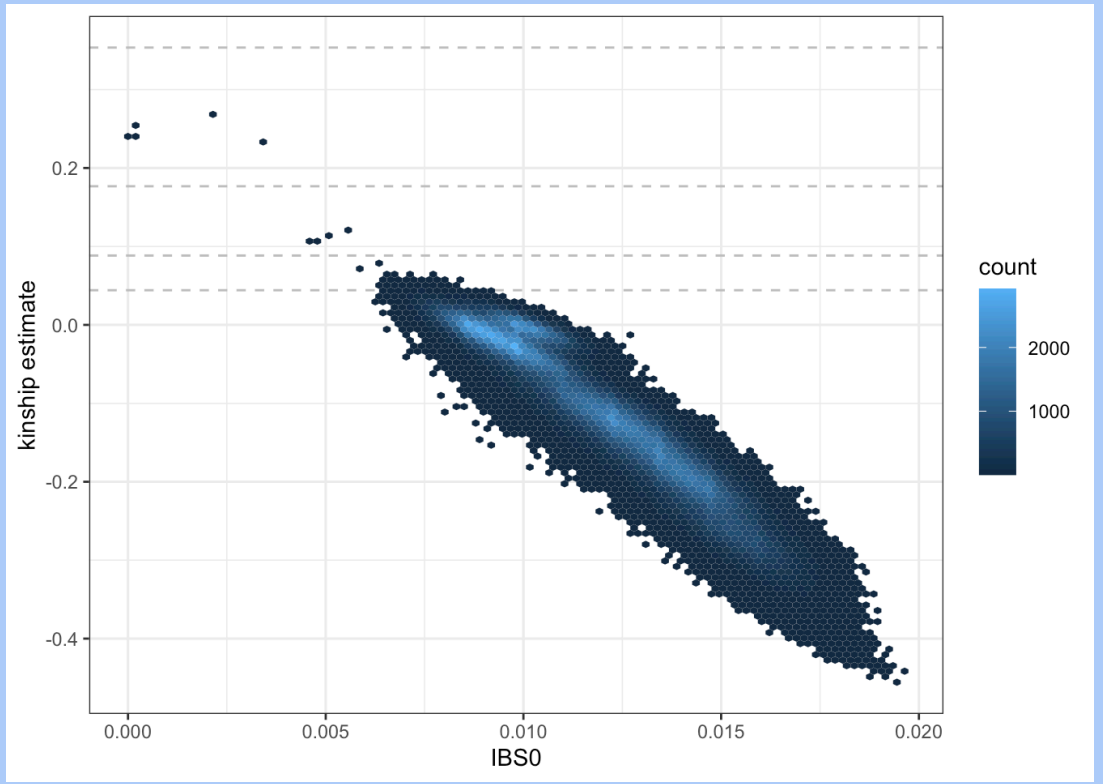
- Estimate φ and IBD sharing from the number of sites at which two individuals are both heterozygotes (Aa,Aa) or opposite homozygotes (AA,aa)
- Avoids estimating population allele fractions, just focuses on pairs



https://www.kingrelatedness.com/manual.shtml

Kinship-based Inference for GWAS (KING)

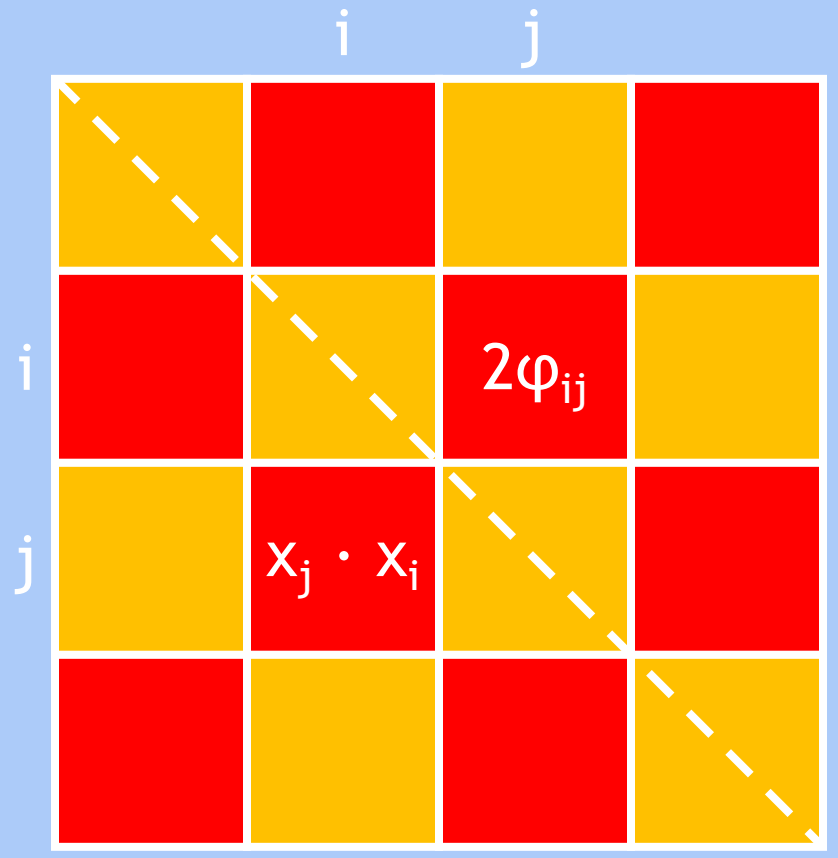
- φ is plotted vs. the fraction of IBS = 0 sites (AA,aa)
- Negative estimates indicate unrelated individuals from different populations



https://uw-gac.github.io/SISG_2021/ancestry-and-relatedness-inference.html

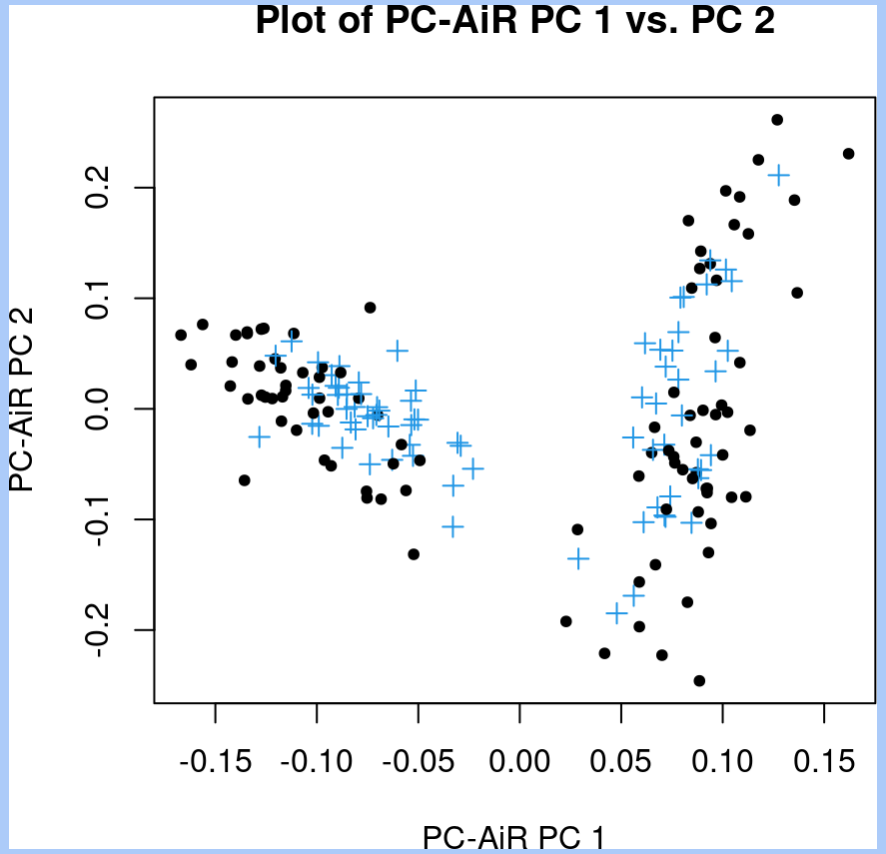
Updating the GRM

- The KING kinship coefficients 2φ are approximately equal to the GRM, but the estimate may be biased by population structure



PC-AiR: PCA in Related Samples

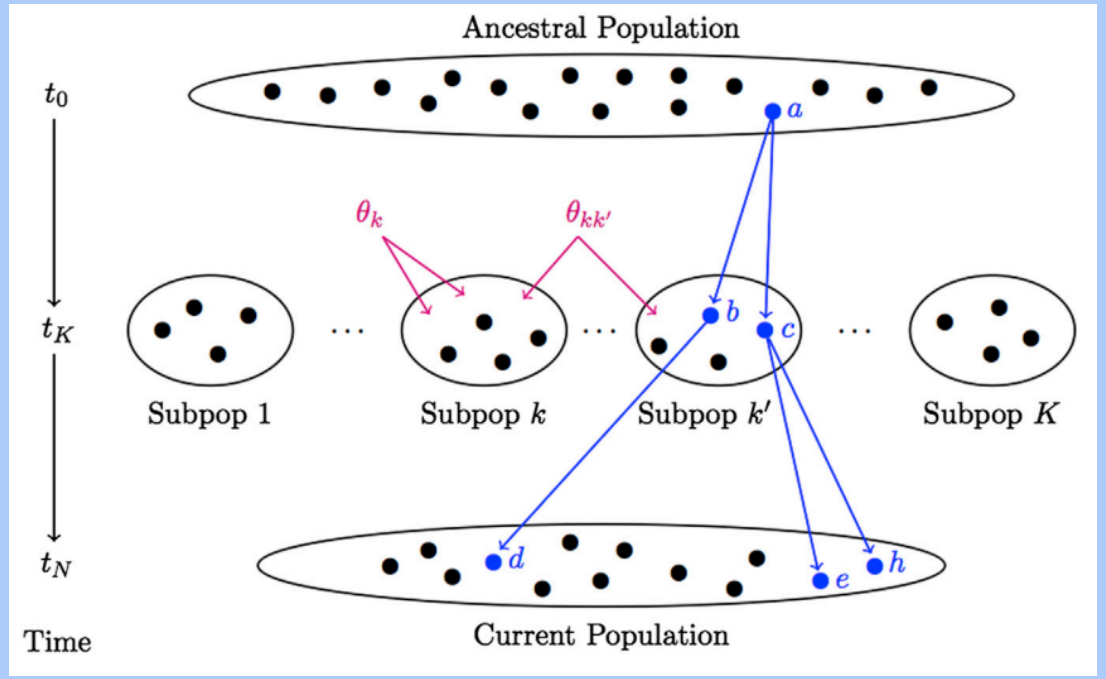
- Based on the KING estimates, PC-AiR computes PCs for a set of unrelated individuals (black)
- PCs for the remaining samples (blue) are estimated from their similarity to the unrelated subset



https://bioconductor.org/packages/devel/bioc/vignettes/GENESIS/inst/doc/pcair.html

PC-Relate

- PC-Relate uses the updated PCs to correct the GRM for population structure
- The updated GRM reflects recent kinship only



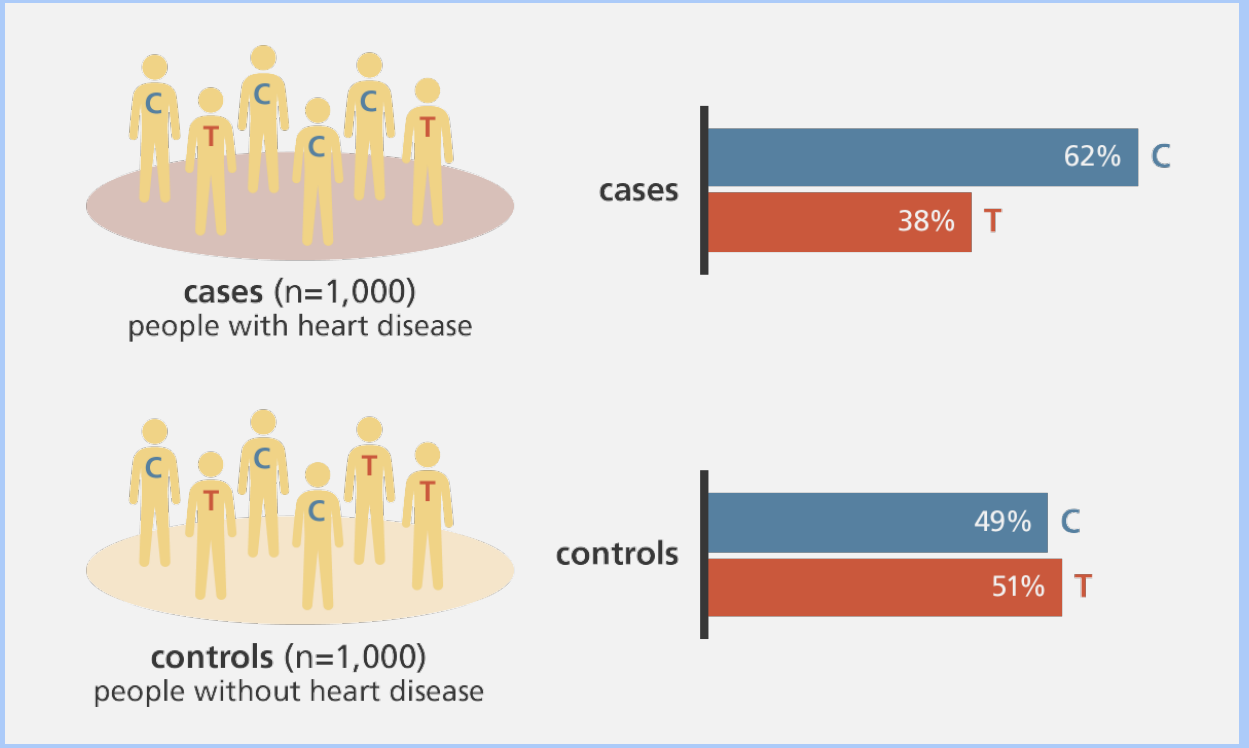
https://pubmed.ncbi.nlm.nih.gov/26748516/

Association testing

Logistic regression and linear mixed models

Case-control studies

- Is a genetic variant associated with disease?
- Is a genetic variant enriched in people with disease compared to people without?
- To find out, collect many people with disease (Cases) and many healthy individuals (Controls) from the same population



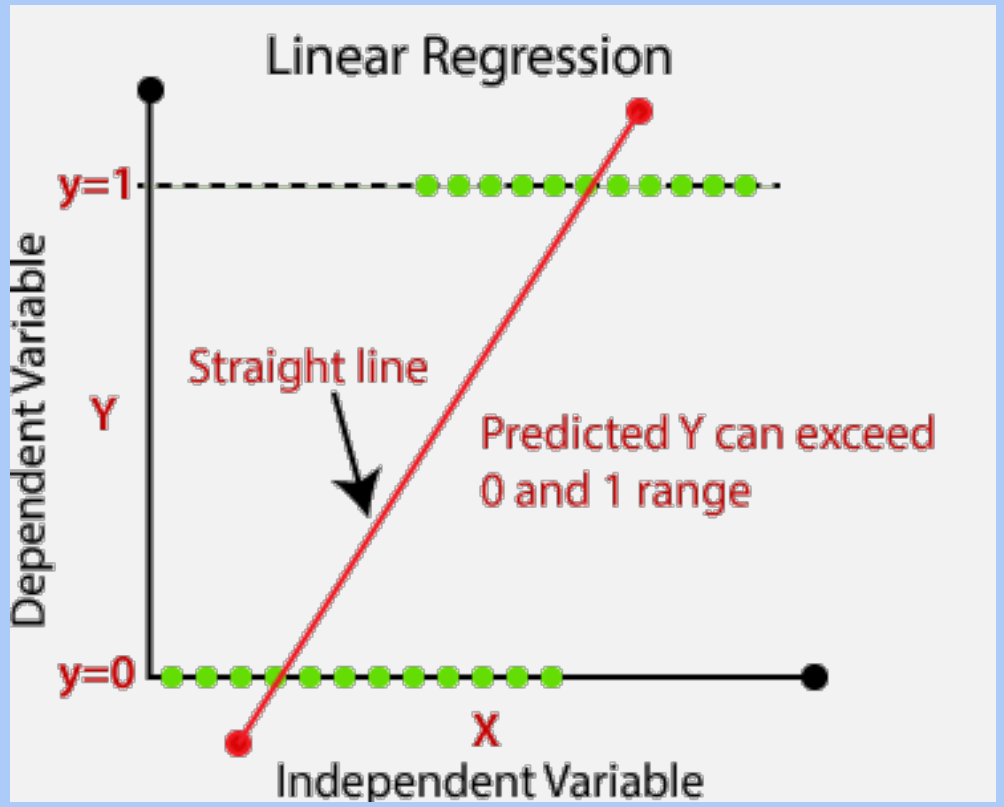
The odds ratio

- The OR is the ratio of the odds that Cases have the risk allele (620 / 380) to the odds that Controls have the risk allele (490 / 510)
- The OR is a **crude** measure of association that is not **adjusted** for other covariates (age, sex, ethnicity, etc.) that may also be associated with disease

	Cases	Controls
C	620	490
T	380	510

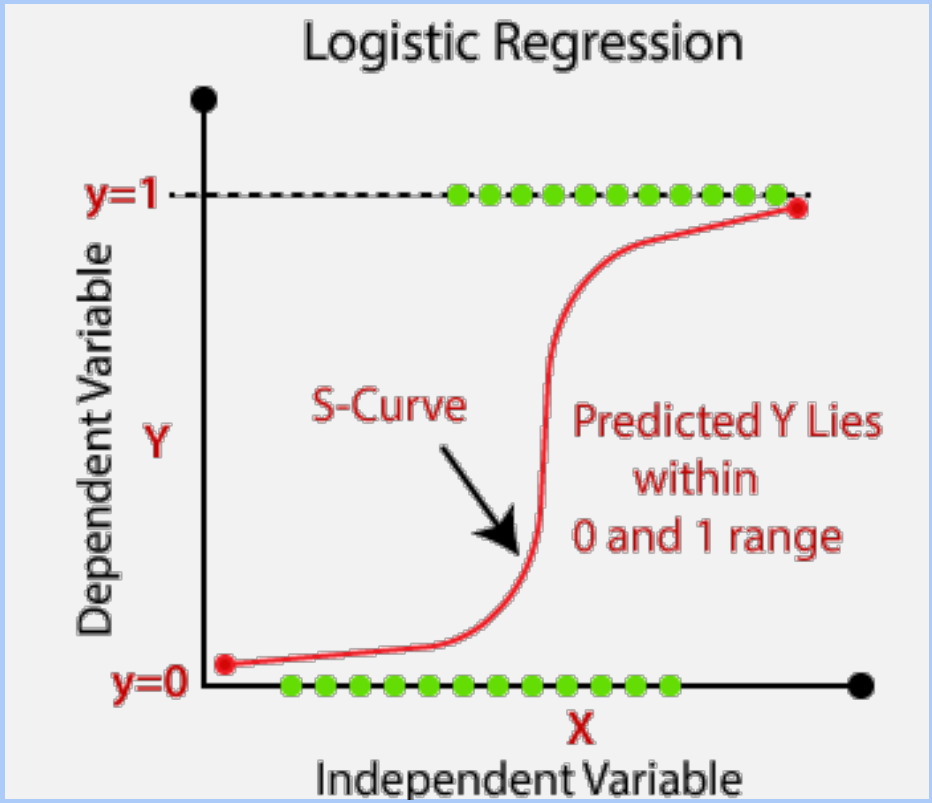
OR = (620 × 510) / (490 × 380) = 1.70

Linear vs. logistic regression



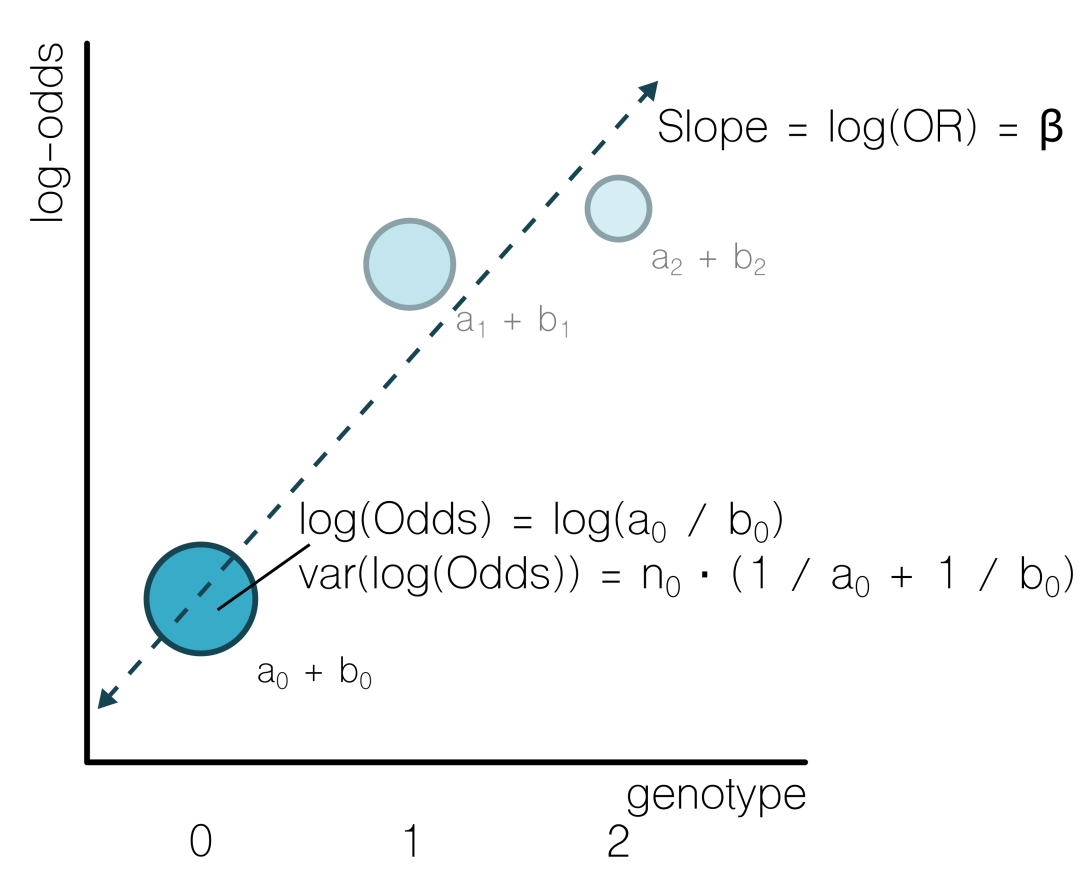
- In linear regression, we can find the association of a **continuous variate** Y with a predictor X_1 and other covariates X_2, X_3, etc.

Linear vs. logistic regression



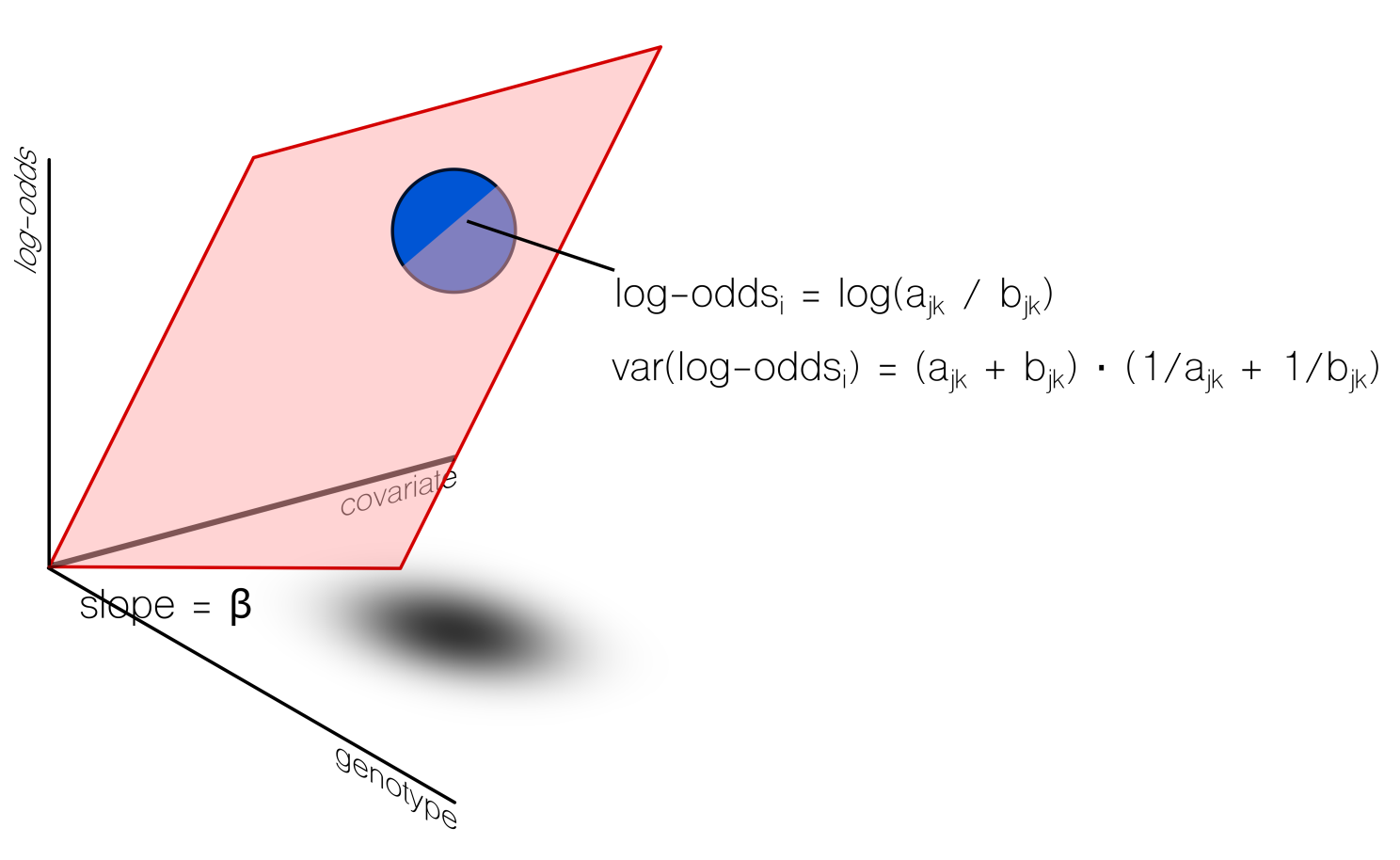
- In logistic regression, we can find the association of a **binary variate** Y with a predictor X_1 and other covariates X_2, X_3, etc.
- The sigmoid curve is an individual's probability of developing disease

Linear vs. logistic regression



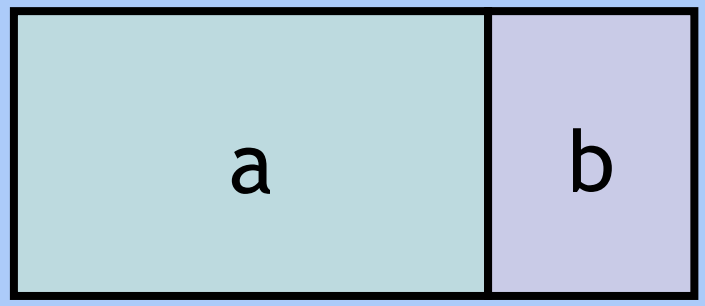
- Logistic regression can be thought of like linear regression is we transform the OR into the log(OR) and regress vs. SNP genotype

Linear vs. logistic regression



- Other covariates can be accounted for as additional independent variables
- The model is actually fit using the principle of **maximum-likelihood**

Simulating a binary phenotype

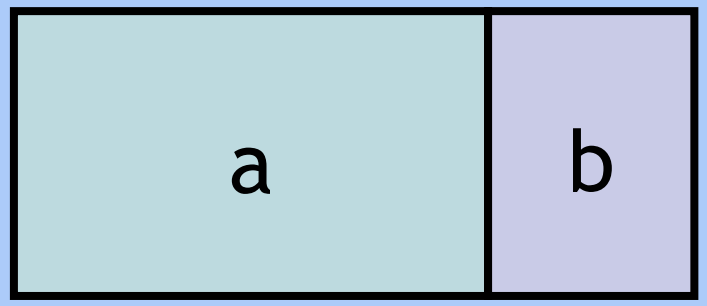


- If odds = a / b, then prob = a / (a + b) = odds / (1 + odds)

Simulating a binary phenotype

$$\log(\text{odds}) = \beta_0 + X_1\beta_1$$

- β_0 is the baseline odds
- β_1 is the log-OR
- X_1 is the SNP genotype

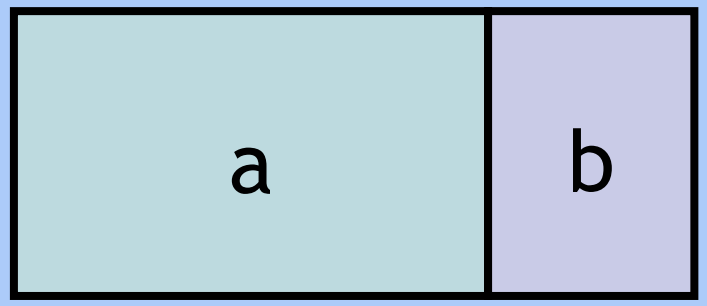


- If odds = a / b, then prob = a / (a + b) = odds / (1 + odds)

Simulating a binary phenotype

$$\text{prob} = \frac{e^{\beta_0 + X_1\beta_1}}{1 + e^{\beta_0 + X_1\beta_1}}$$

- prob is the probability of developing disease (being a Case in the study)

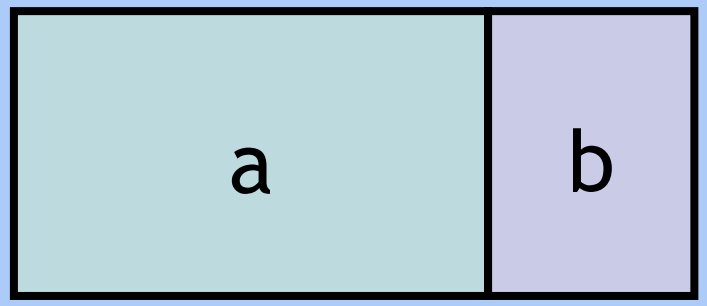


- If odds = a / b, then prob = a / (a + b) = odds / (1 + odds)

Simulating a binary phenotype

$$\text{prob} = \frac{e^{(X_1 - \overline{X}_1)\beta_1}}{1 + e^{(X_1 - \overline{X}_1)\beta_1}}$$

- β_0 becomes the mean log-odds so that the mean odds of disease is 1 (50% Cases, 50% Controls)

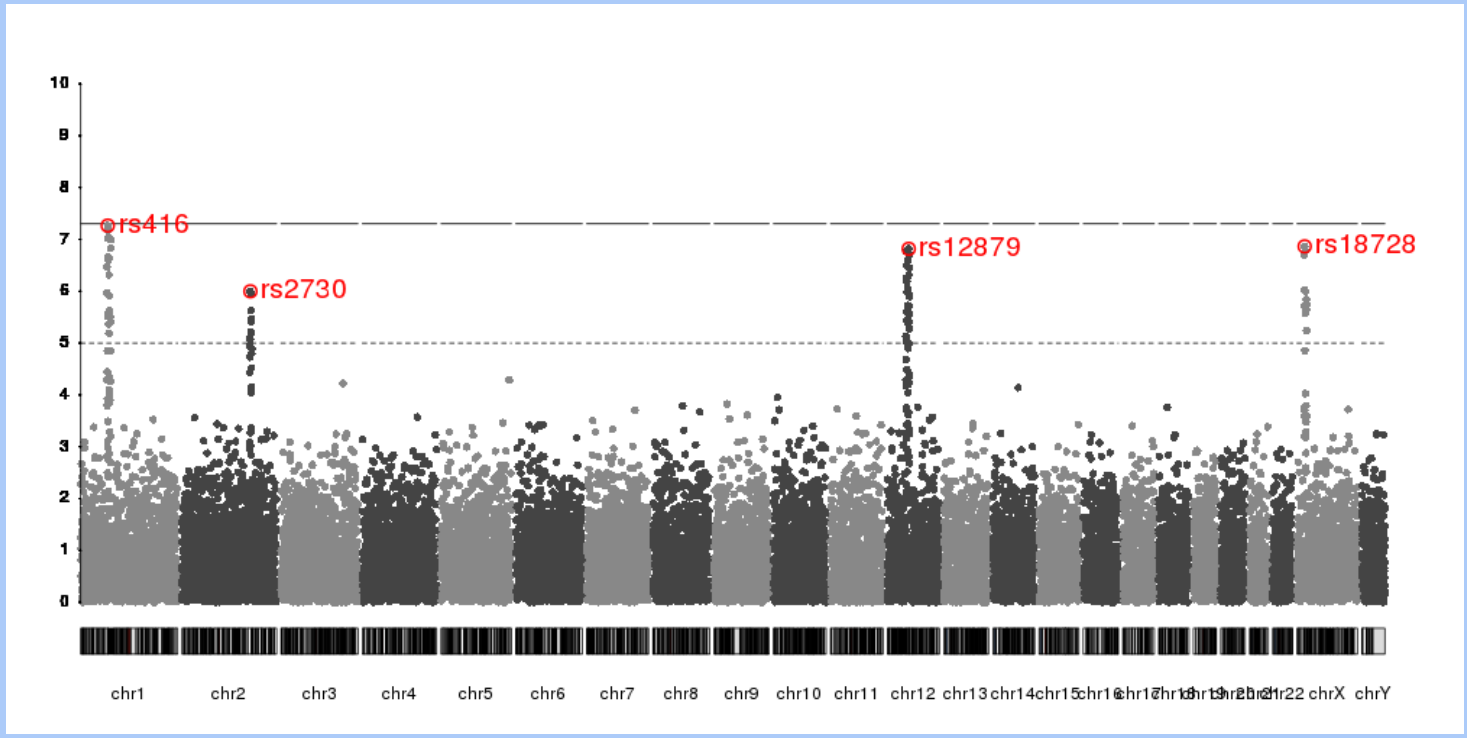


- If odds = a / b, then prob = a / (a + b) = odds / (1 + odds)

Estimating the SNP effect

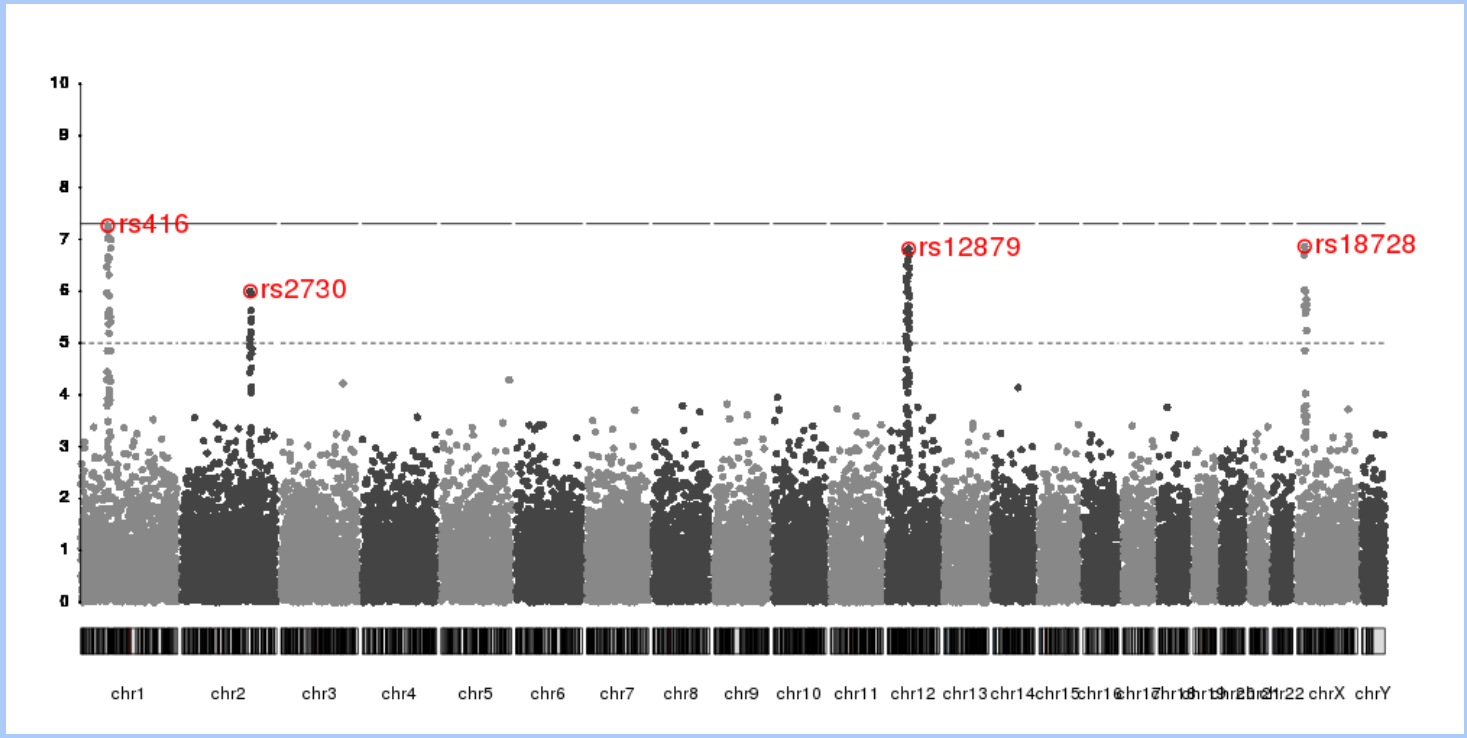
- We want to be able to **detect** the association of one SNP with disease by fitting the model $Y = \beta_0 + \beta_1 X_1 + \cdots$ and finding a slope β_1 significantly different from 0

Estimating the SNP effect



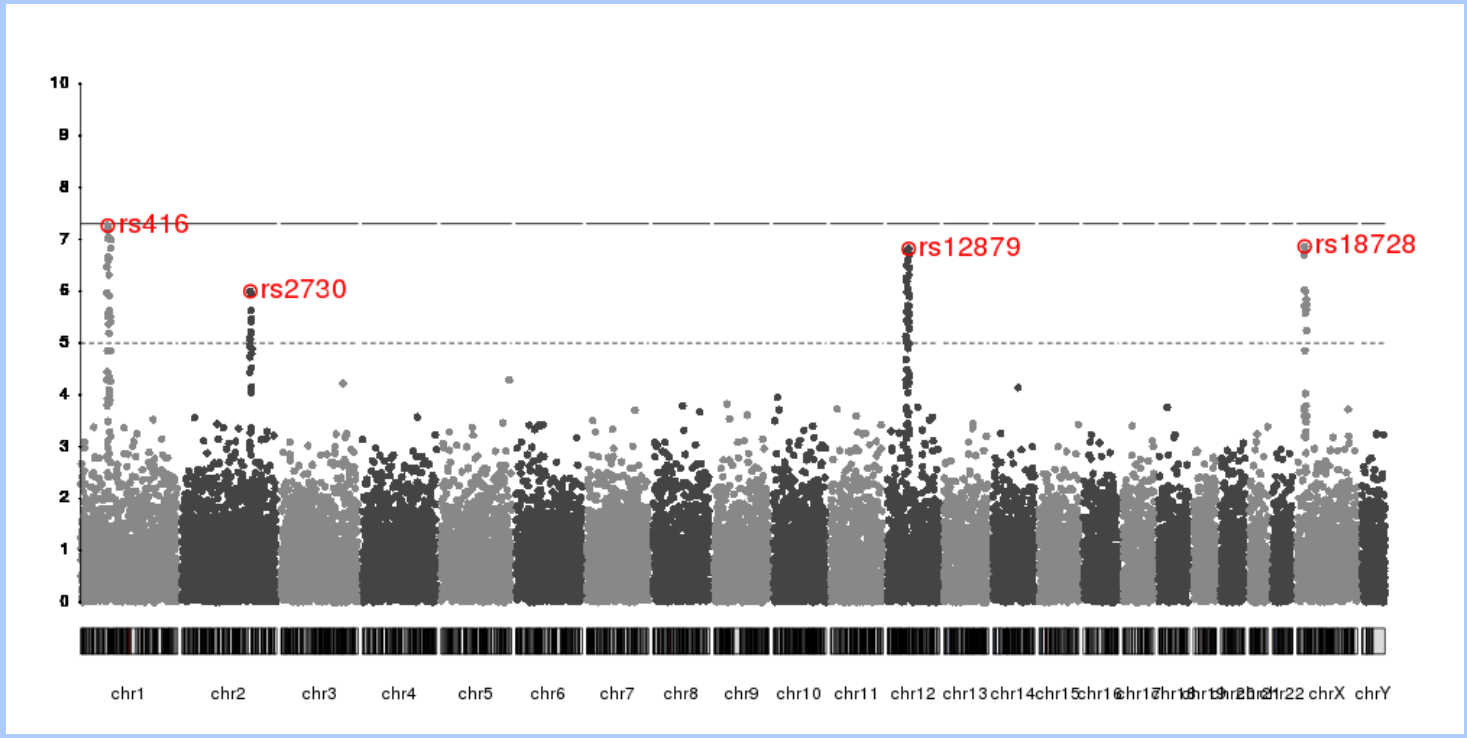
- A **Manhattan plot** gives the p-value of the log-OR estimate for each SNP

Estimating the SNP effect



- Because there are more SNPs than subjects, we cannot fit all SNPs at once

Estimating the SNP effect

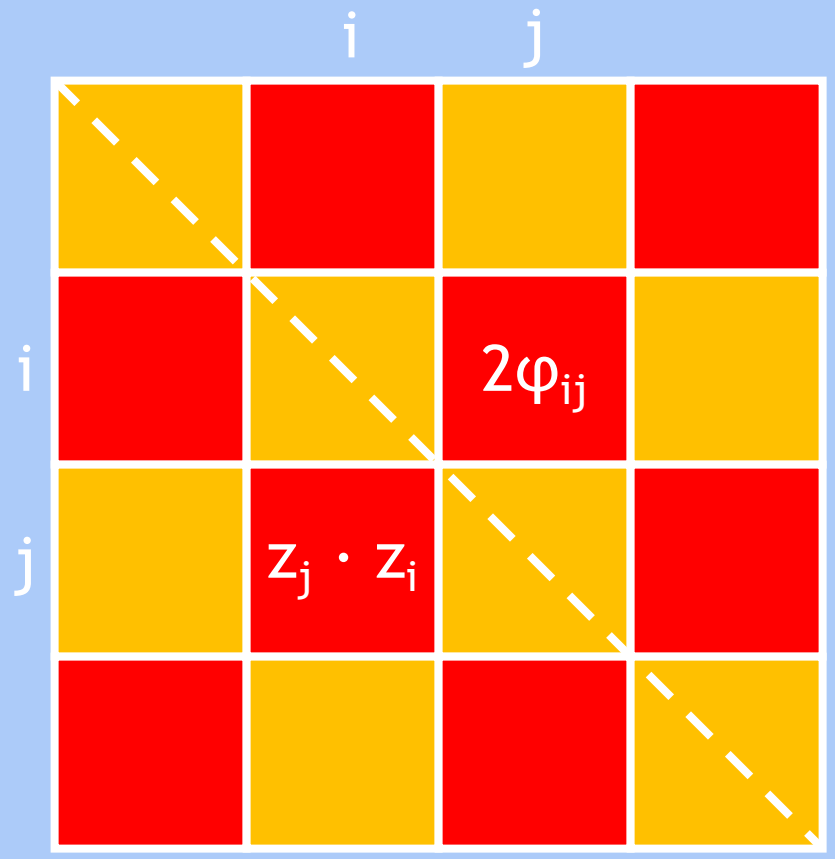


- But we can fit one SNP plus the "average" effect of all the remaining SNPs

Linear mixed models

- The solution for the **best estimate** of the SNP effect β_1 in the presence of all the remaining SNPs involves the GRM ZZ^T (from PC-Relate)

$$\mathbf{X}^T \left(\mathbf{I} + \mathbf{Z}\mathbf{Z}^T\right)^{-1} \hat{\beta} = \mathbf{X}^T \left(\mathbf{I} + \mathbf{Z}\mathbf{Z}^T\right)^{-1} \mathbf{Y}$$



Linear mixed models

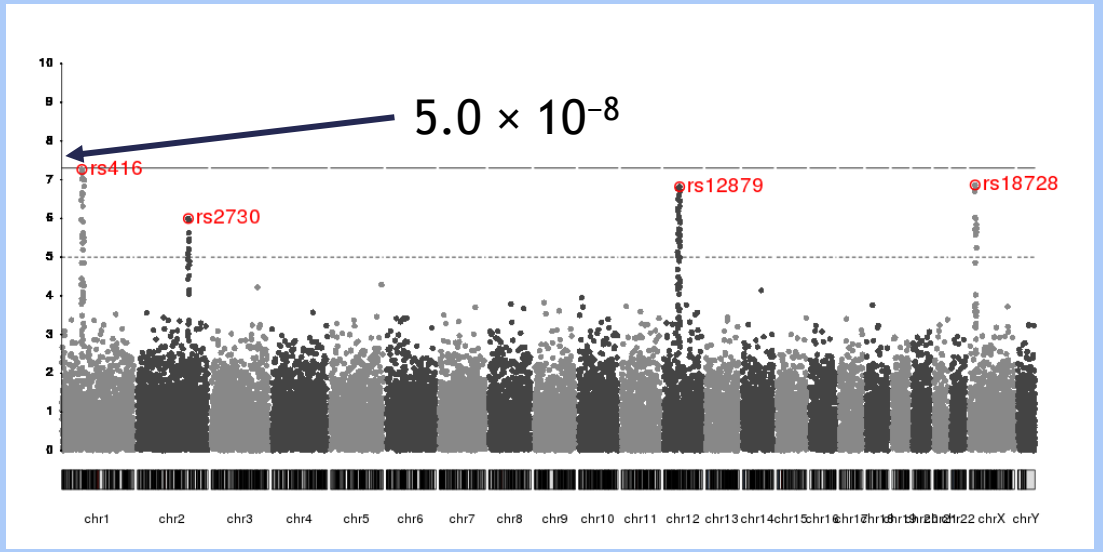
- Other covariates commonly included in the model are age, sex, and the **first few genotype principal components** (from PC-AiR)

Linear mixed models

- If the model including the SNP represents a significant improvement over the **null model** (the model without the SNP), we can reject the null hypothesis that the OR = 1

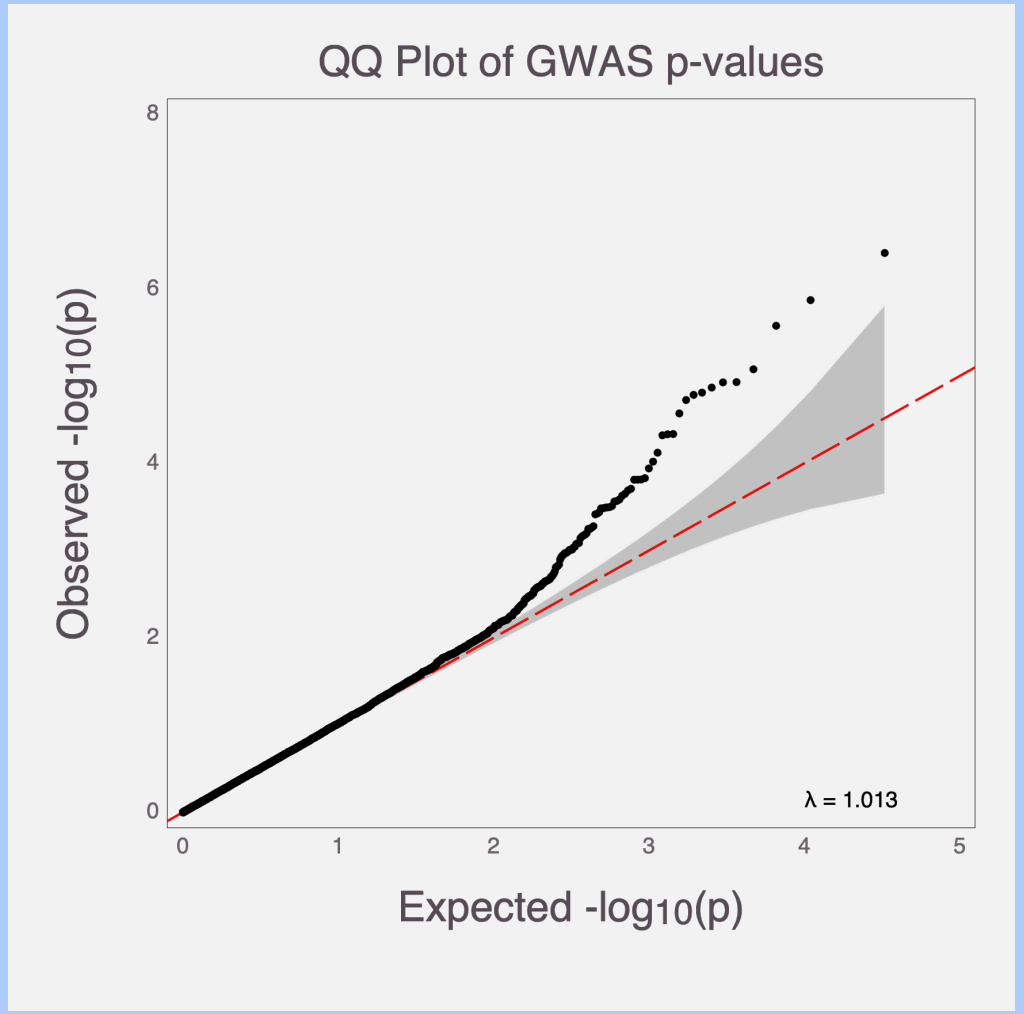
Linear mixed models

- But because of **multiple-testing**, our p-value threshold is $0.05 / 10^6$ (i.e., you perform the same test 10^6 times)
- SNPs with $p < 5.0 \times 10^{-8}$ are said to achieve **genome-wide significance**



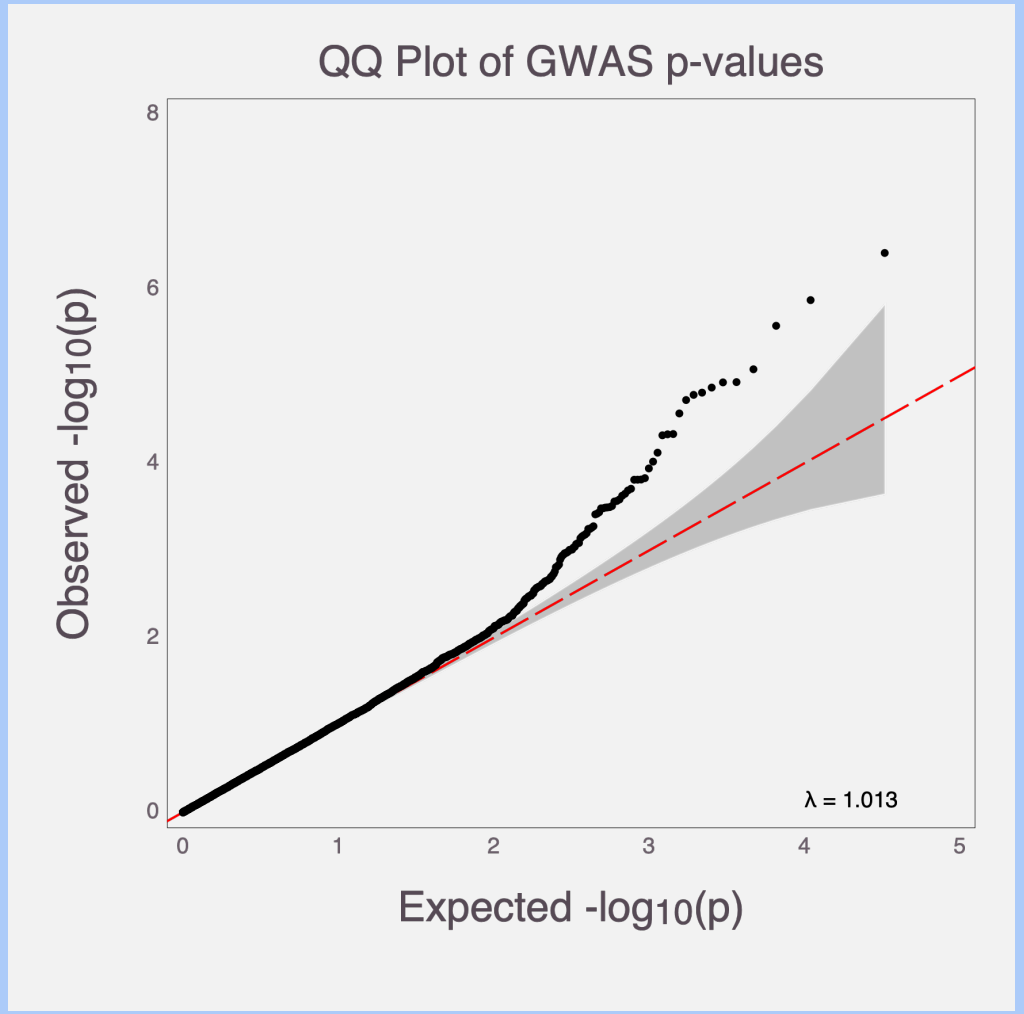
QQ plots

- To assess if the distribution of SNP effects is significantly different from that expected by chance, we make a quantile or **QQ plot**



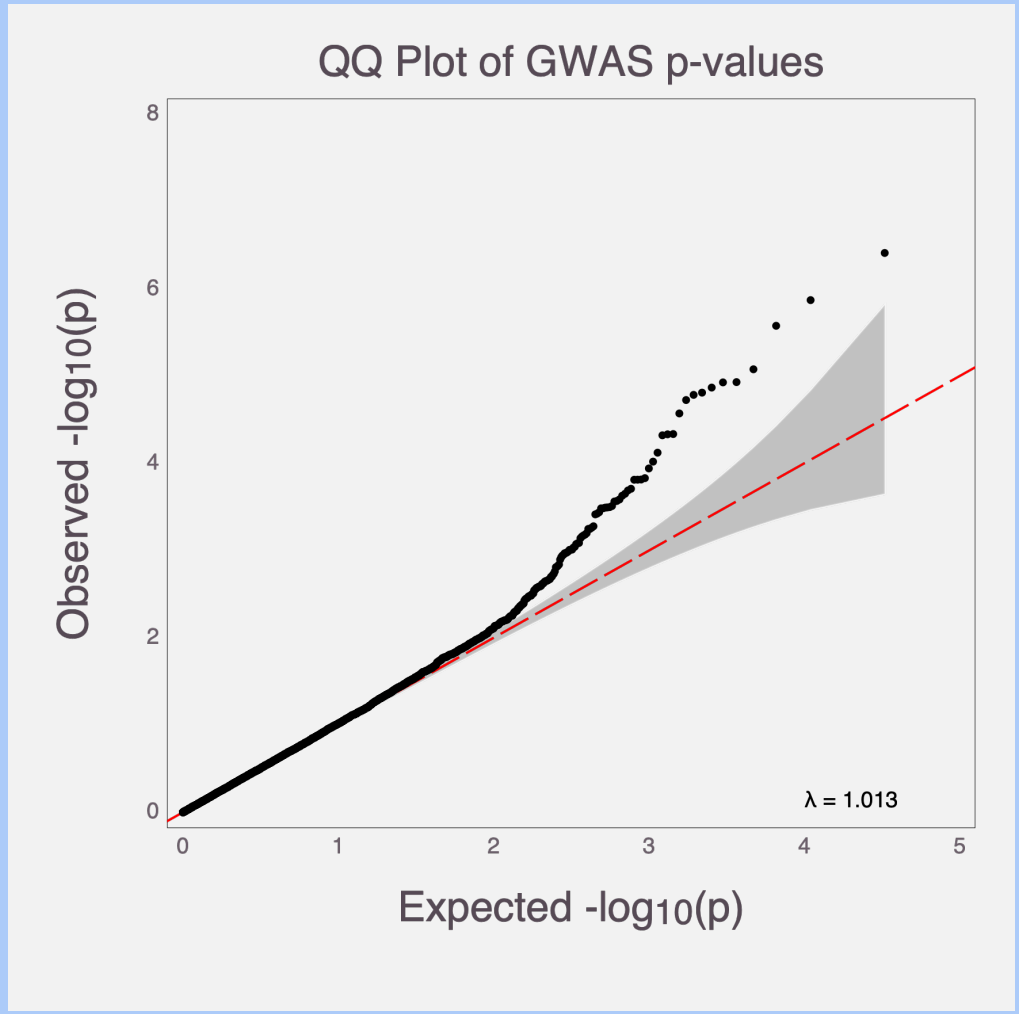
QQ plots

- Put the **observed** p-value (negative log-10) in order from smallest to biggest



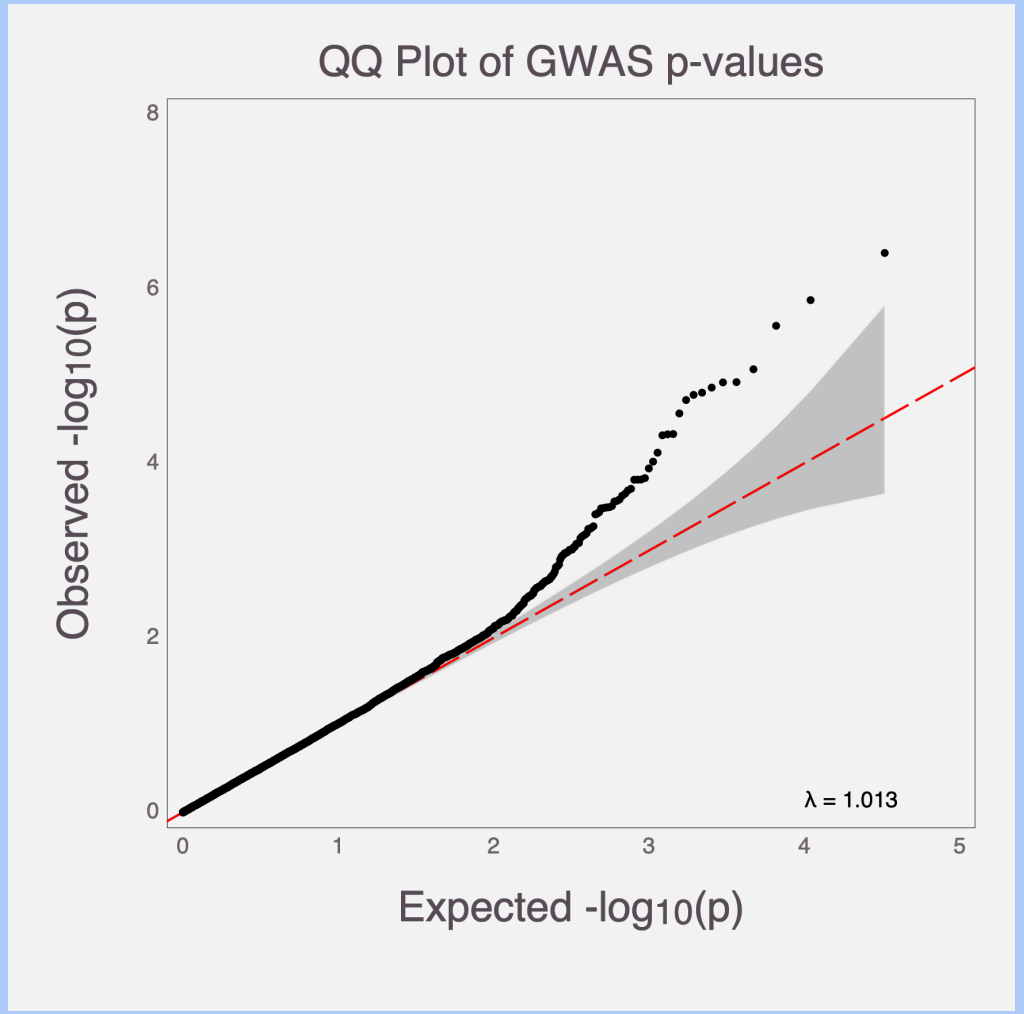
QQ plots

- The **expected** p-values for the quantiles of m SNPs, are 1/m, 2/m,..., 1
- Take the negative log-10 and put in order from smallest to biggest



QQ plots

- SNPs falling above the line of identity indicate an excess of quantiles (ß's) with small p-values



Common data formats

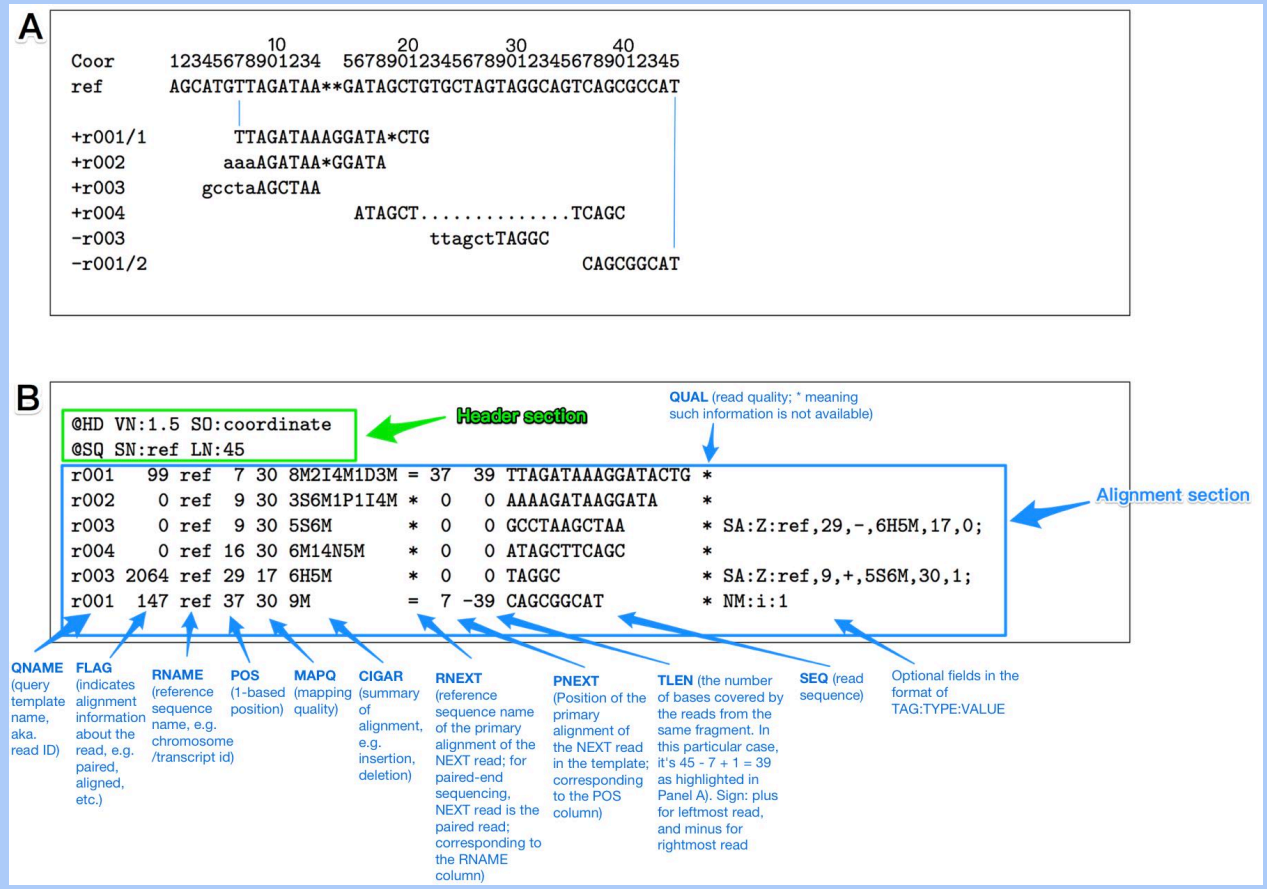
How genotype data are stored

FASTQ

- Contains raw sequence reads and their quality scores
- Meant to be aligned to a reference genome (FASTA)

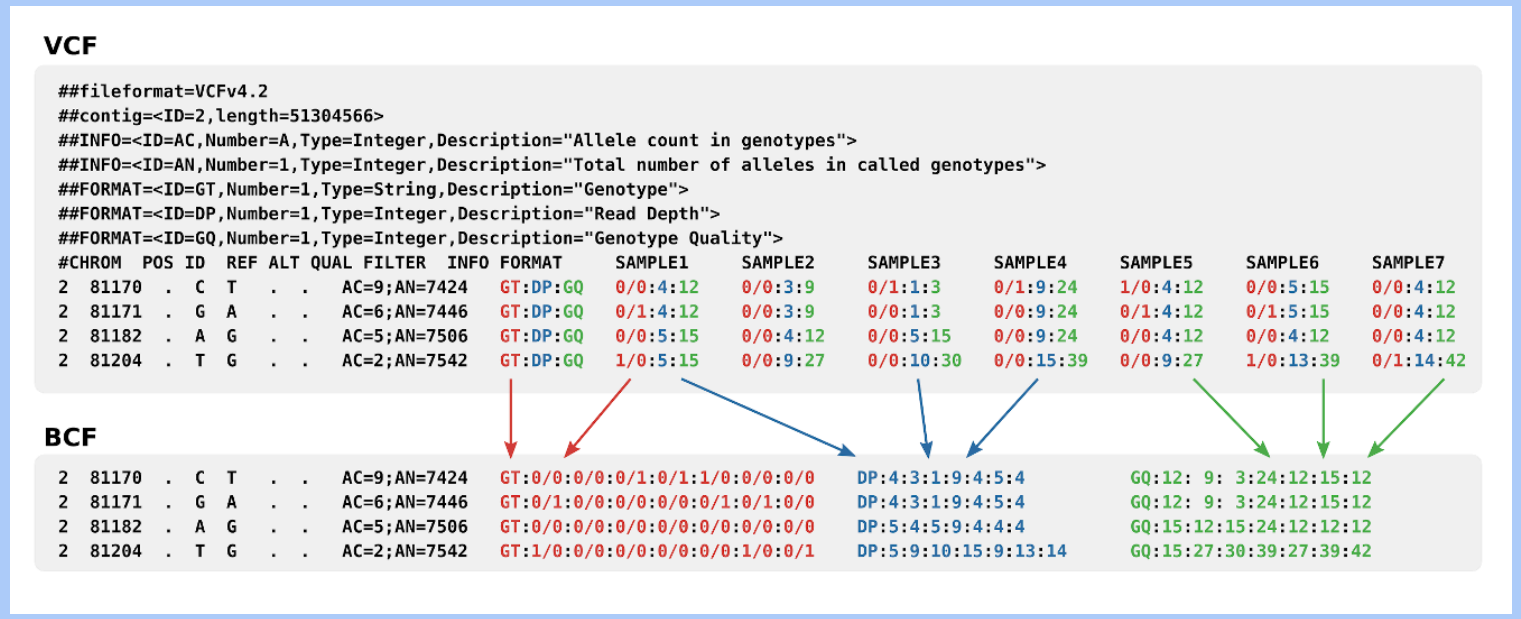
SAM (BAM)

- Sequence alignment map and binary alignment map
- Contains alignments to reference genome



VCF

- Variant call format
- Locations and types of variation at a number of different samples
- May be phased (A|T) or unphased (A/T)



MAP

- PLINK file containing the locations and names of SNPs

```
1       rs12562034      0       758311  A       G
1       rs12124819      0       766409  G       A
1       rs4475691       0       836671  T       C
1       rs3748597       0       878522  T       C
1       rs28705211      0       890368  C       G
1       rs13303118      0       908247  G       T
1       rs9777703       0       918699  C       T
1       rs3121567       0       933331  A       G
1       rs3934834       0       995669  T       C
1       rs9442372       0       1008567 A       G
1       rs3737728       0       1011278 T       C
1       rs6687776       0       1020428 T       C
1       rs9651273       0       1021403 A       G
1       rs4970405       0       1038818 G       A
```

PED

- PLINK file containing genotypes and phenotypes of individuals in different families

FID	IID	FATID	MATID	SEX	PHENO	rs1		rs2		rs3		rs4		rs5	
FAM1	1	0	0	1	1	G	G	A	A	A	A	C	C	G	G
FAM1	2	0	0	1	2	G	G	A	A	A	A	C	C	G	G
FAM1	3	0	0	1	2	G	G	A	A	A	A	C	C	G	G
FAM2	1	0	0	1	2	G	G	A	A	A	A	C	C	G	G
FAM2	2	0	0	1	2	G	G	A	A	A	A	C	C	G	G
FAM2	3	0	0	1	2	G	G	A	A	A	A	C	C	G	G
FAM3	1	0	0	1	2	G	G	A	A	A	A	C	C	G	G
FAM3	2	0	0	1	2	G	G	A	A	A	A	C	C	G	G
FAM3	3	0	0	1	2	A	A	G	G	G	G	C	C	G	G

GDS

- Genomic data structure used for conducting GWAS in R

```
# Open a GDS file
(genofile <- snpgdsOpen(snpgdsExampleFileName()))
```

```
## File: /tmp/RtmpfdOhhS/Rinst4d3a91a981738/SNPRelate/extdata/hapmap_geno.gds (709.6K)
## +    [  ] *
## |--+ sample.id   { VStr8 279 ZIP(29.9%), 679B }
## |--+ snp.id   { Int32 9088 ZIP(34.8%), 12.3K }
## |--+ snp.rs.id   { VStr8 9088 ZIP(40.1%), 36.2K }
## |--+ snp.position   { Int32 9088 ZIP(94.7%), 33.6K }
## |--+ snp.chromosome   { UInt8 9088 ZIP(0.94%), 85B } *
## |--+ snp.allele   { VStr8 9088 ZIP(11.3%), 4.0K }
## |--+ genotype   { Bit2 279x9088, 619.0K } *
## \--+ sample.annot   [ data.frame ] *
##    |--+ family.id   { VStr8 279 ZIP(34.4%), 514B }
##    |--+ father.id   { VStr8 279 ZIP(31.5%), 220B }
##    |--+ mother.id   { VStr8 279 ZIP(30.9%), 214B }
##    |--+ sex   { VStr8 279 ZIP(17.0%), 95B }
##    \--+ pop.group   { VStr8 279 ZIP(6.18%), 69B }
```