Data Quality Control (QC) in Association Studies

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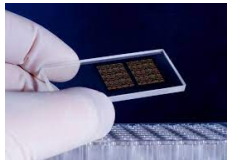
- Tutorials
  - Anderson et al. Nature Protocols 2010, doi:10.1038/nprot.2010.116
  - Turner et al. Curr Protoc Hum Genet. 2011. doi:10.1002/0471142905.hg0119s68
  - Marees et al. Int J Methods Psychiatr Res. 2018. doi: 10.1002/mpr.1608

Genotype Calling

illumina



affymetrix

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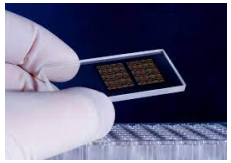

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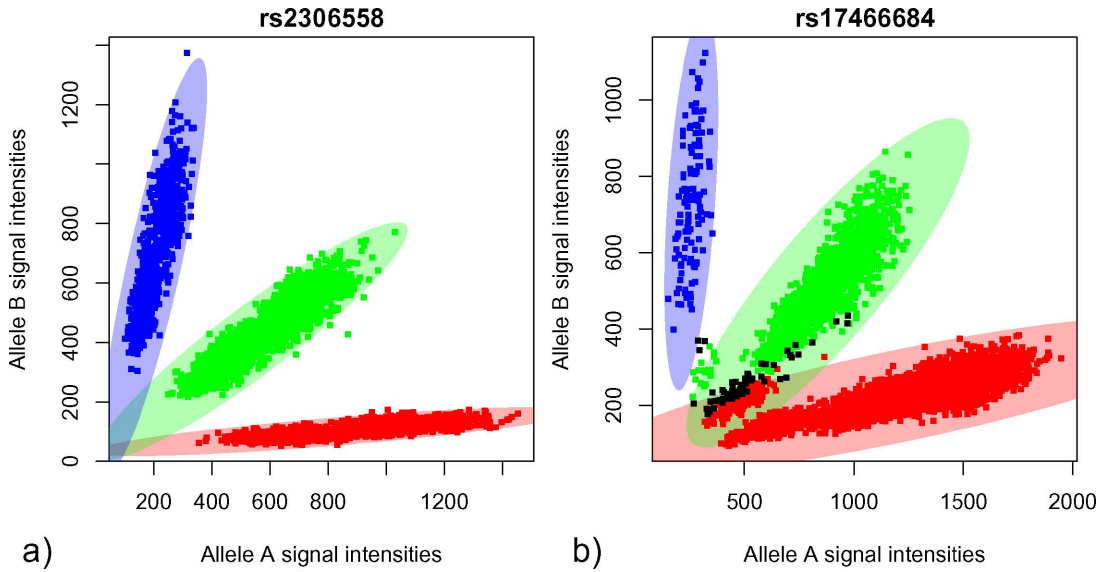

- Examples of cluster plots for two SNPs. One spot corresponds to one sample.
- Samples with genotypes **AA** and **BB** are red and blue, respectively. **Heterozygous** samples are shown in green; samples with **missing genotypes** are shown in black. The ellipses represent the cluster boundaries as computed by ACPA.
- **a)** No samples in overlapping ellipses; **b)** Red samples lie in the green ellipse. At the bottom of the green ellipse, samples have been erroneously classified as red samples.

Schillert et al. BMC Proceedings 2009, 3(Suppl 7):S58 doi: 10.1186/1753-6561-3-S7-S58

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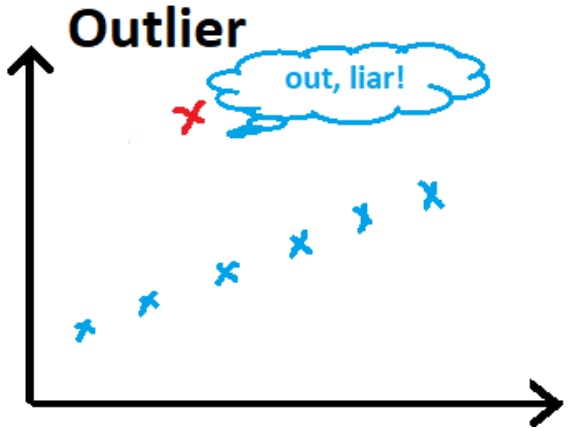
- Missing call rate is not only a measure of data completeness, but is also a measure of genotype quality

Sample Quality Control



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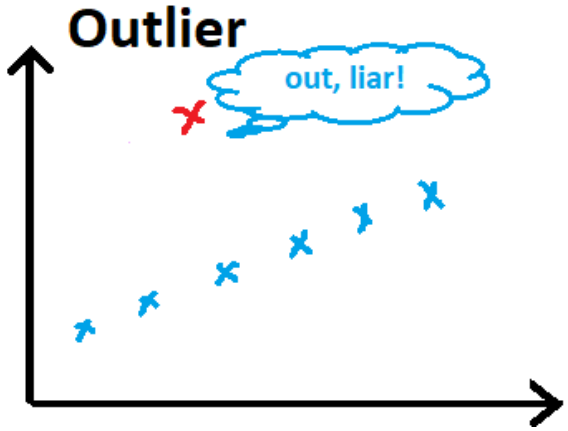

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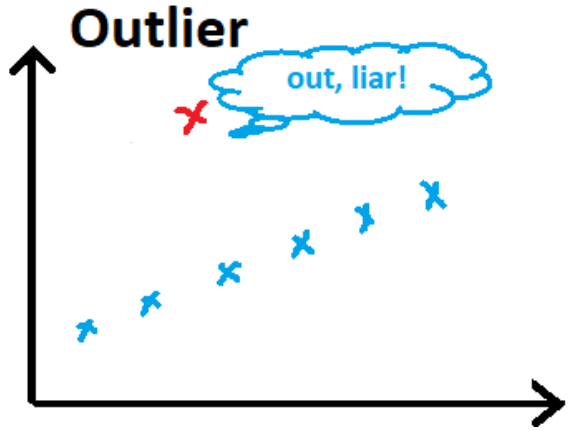

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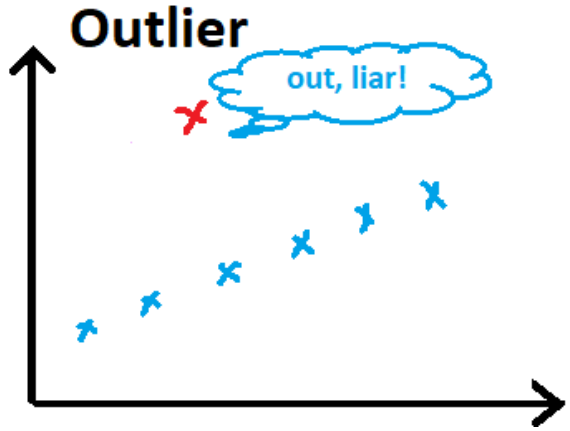

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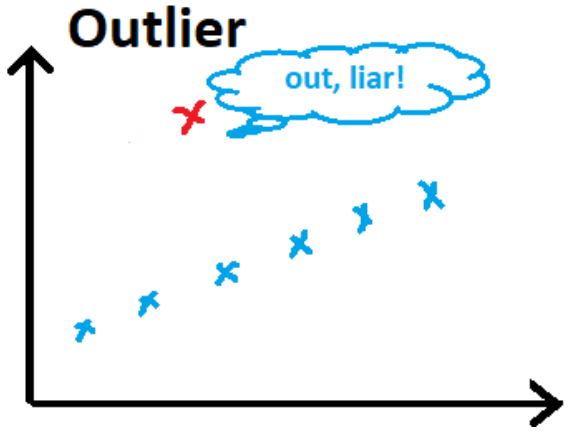

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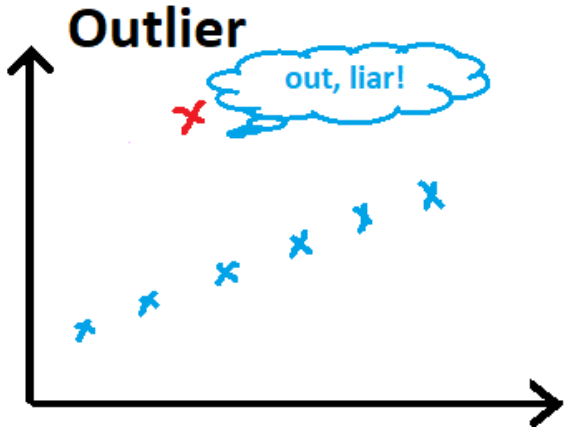

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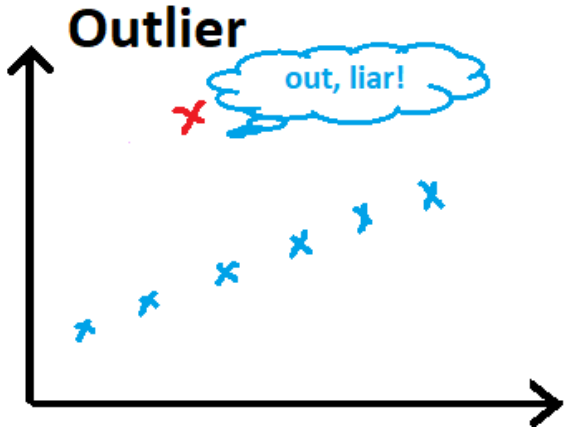

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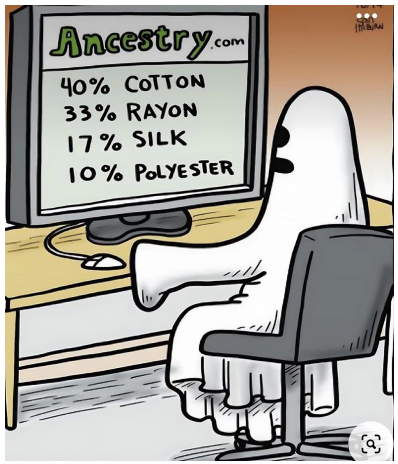
- Outlying population ancestry
  - confounding due to population structure



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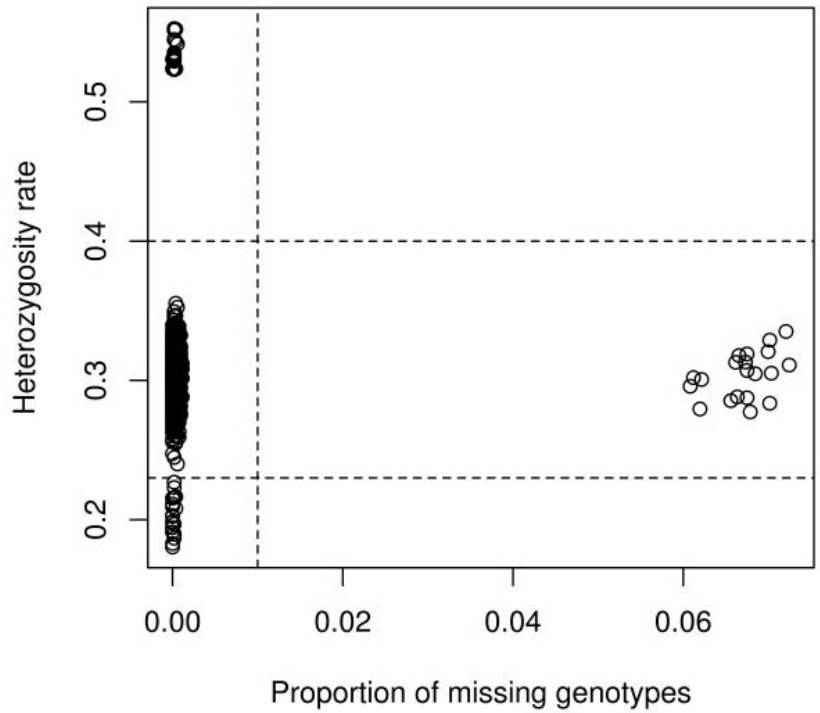

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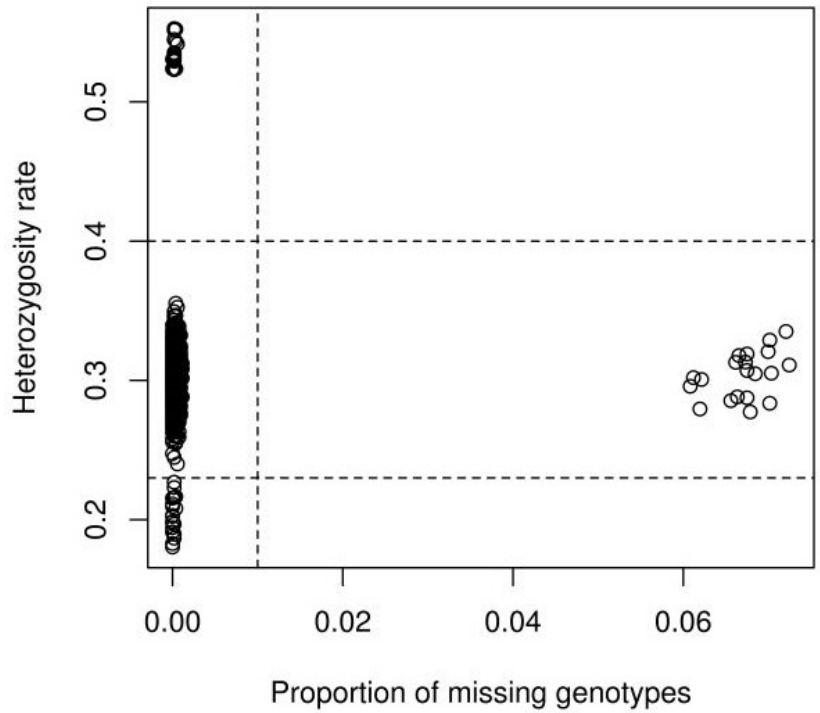

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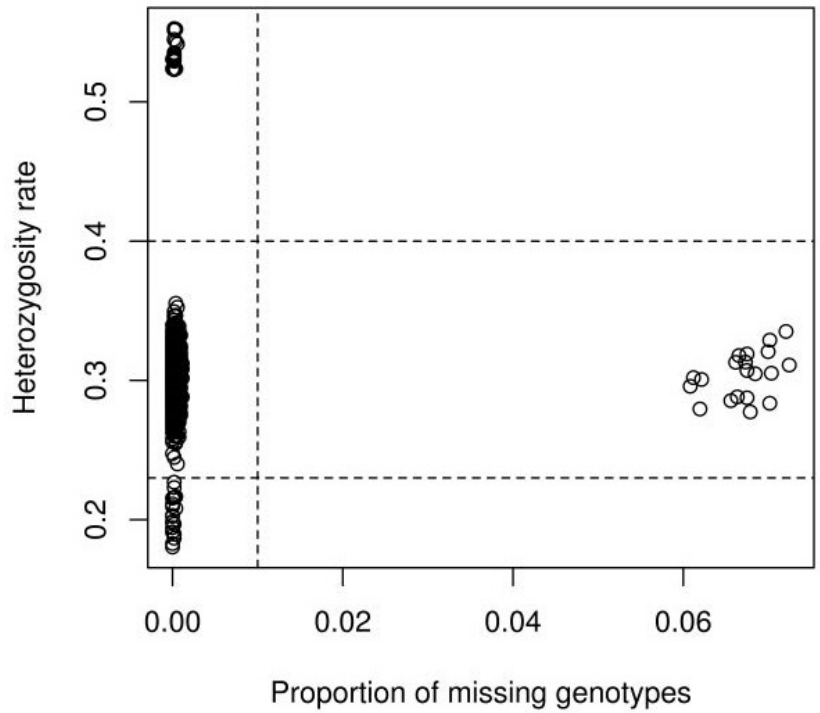

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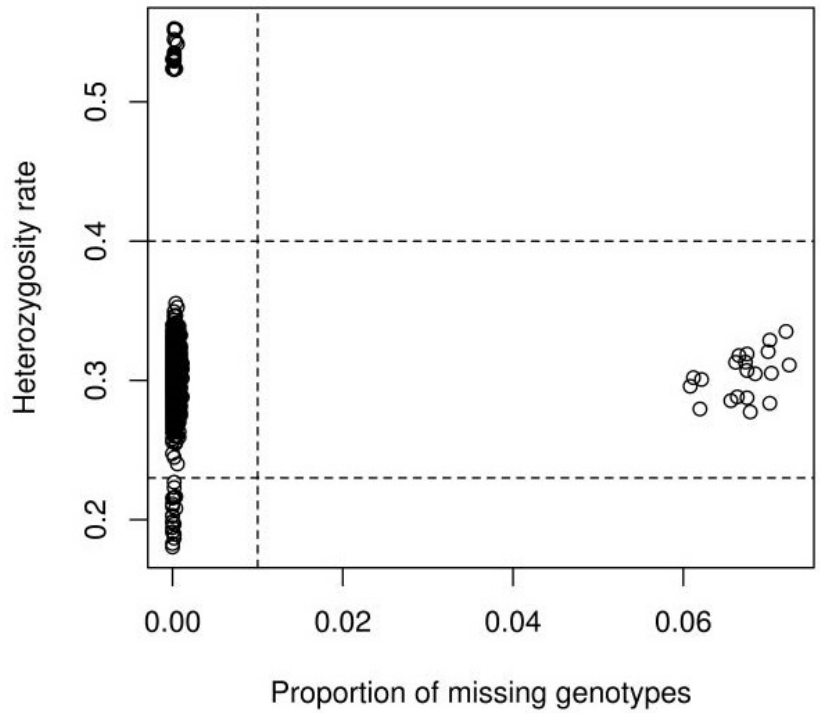

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- Dashed lines denote QC thresholds (exclude samples with missing call rate > 0.1, and samples with heterozygosity rate < 0.23 and > 0.4)
- Rule of thumb: remove individuals who deviate $\pm$ 3 SD from the samples' heterozygosity rate mean

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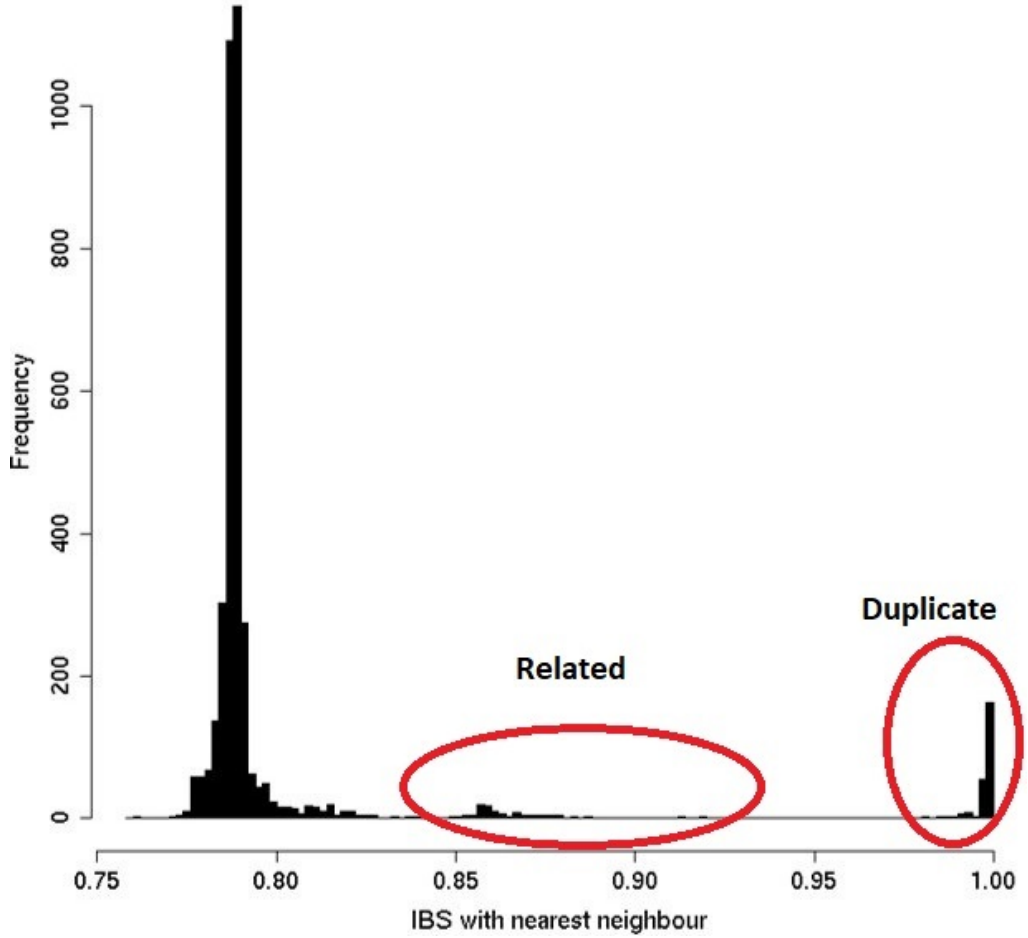
- Common to plot histogram of IBS of each individual with "nearest neighbour"

IBS Distribution



- For each individual, the distance to its nearest neighbour is calculated
- Remove one sample from each duplicate or related pair (usually one with lowest call rate)
- Alternative: take account of relatedness in analysis
- The absolute amount of IBS sharing depends on allele frequencies in the population
- Methods that estimate kinship or relatedness coefficients typically aim for estimating identity-by-descent (IBD)

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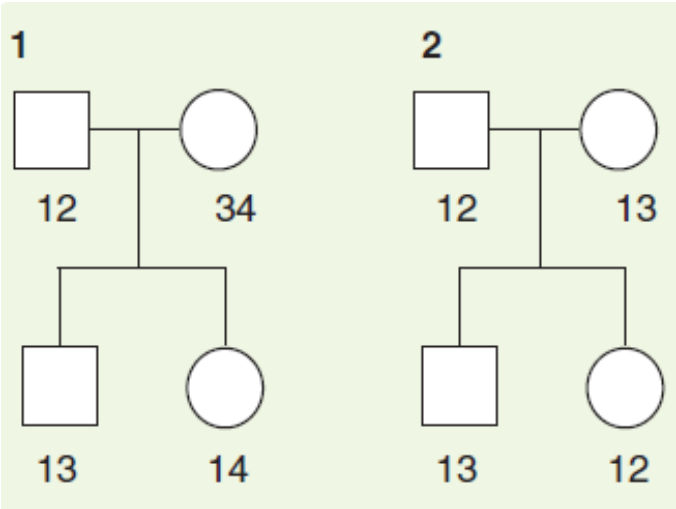

- Pedigree 1: Siblings share allele 1 IBD (inherited from the father)
- Pedigree 2: Siblings share allele 1 IBS (inherited from different parents)

Forabosco et al. Expert Rev. Mol. Diagn. 5(5), (2005). doi: 10.1586/14737159.5.5.781

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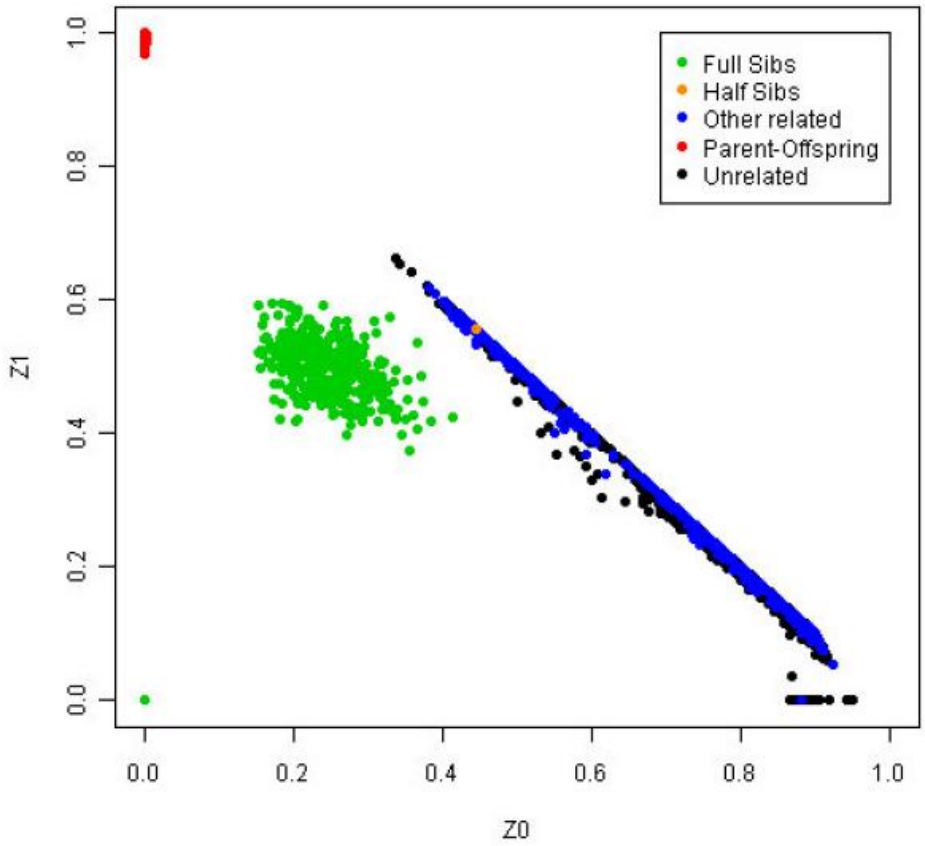

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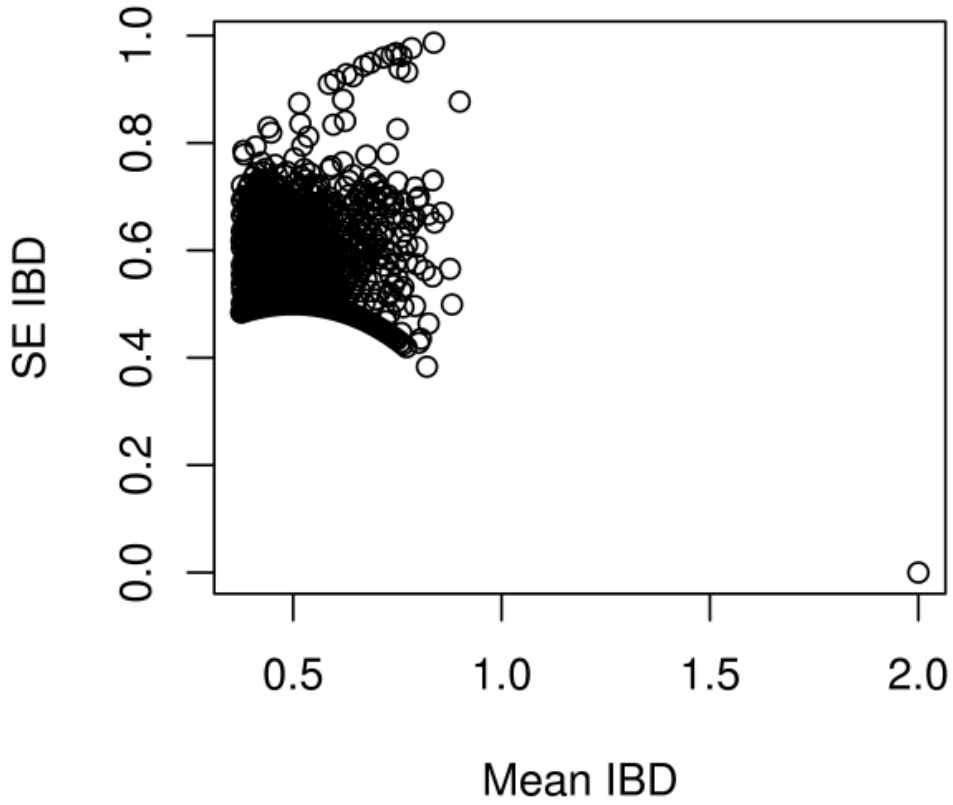
  - halfway between the expected IBD for third- and second-degree relatives
- Prune the data for LD before assessing IBD
  - shared region of high LD results in more shared variants than one of low LD, even if the two regions are the same size

IBD Plot

Mean IBD per pair of individuals ($\hat{\pi} \times 2$)



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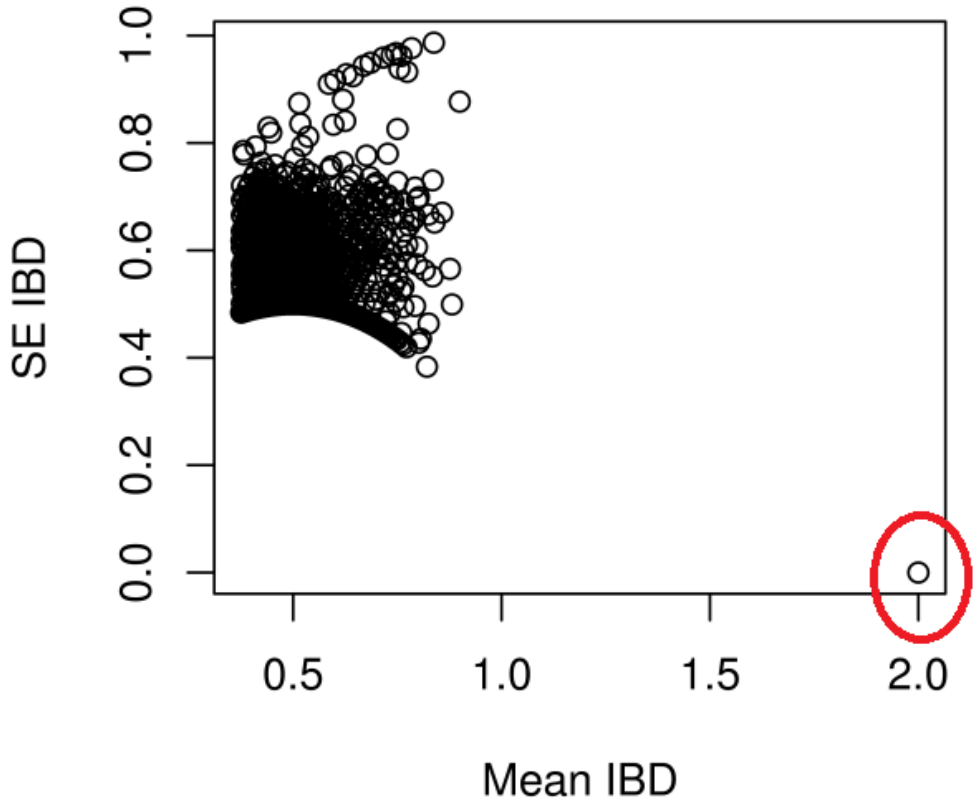

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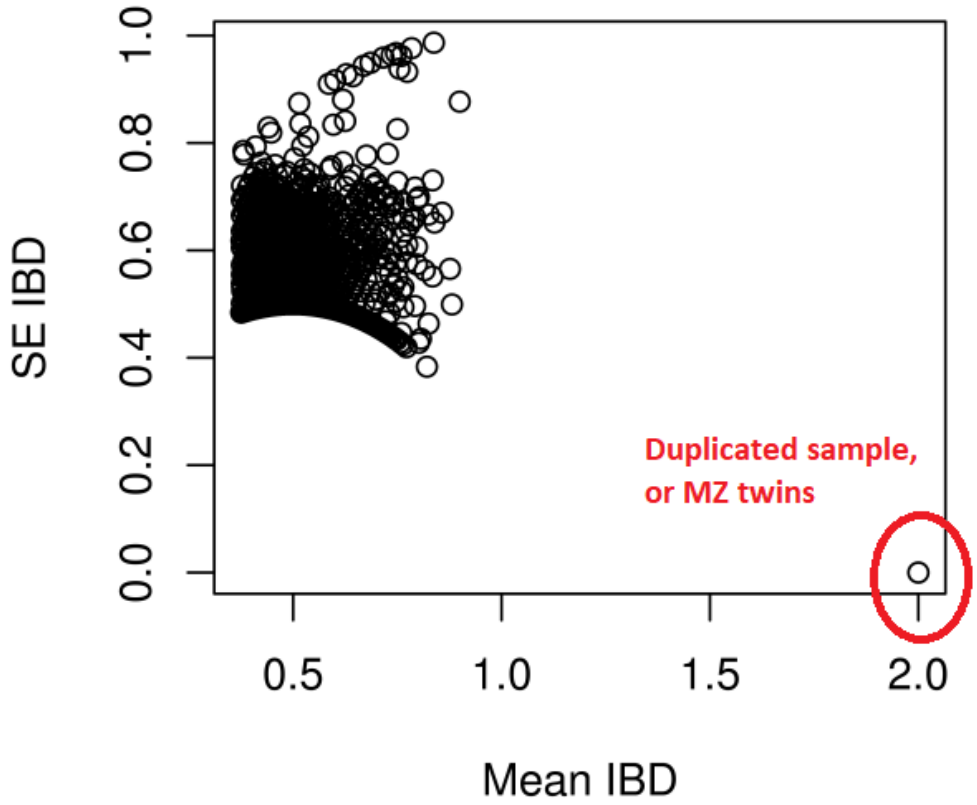

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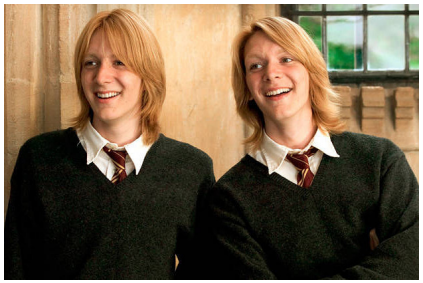

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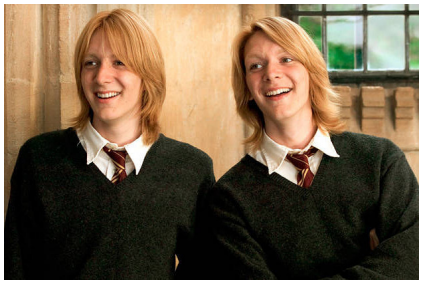


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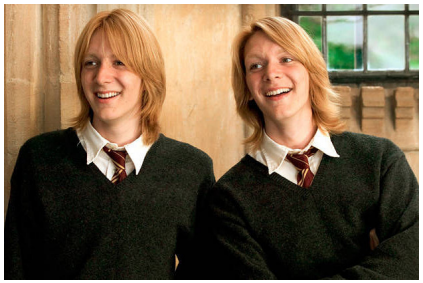


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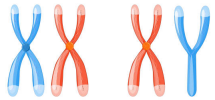
- Mixed modelling approaches account for "relatedness" between individuals (families, cryptic relatedness, population structure) by allowing for kinship

Gender Check - X Chromosome



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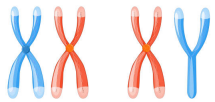

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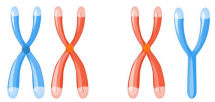
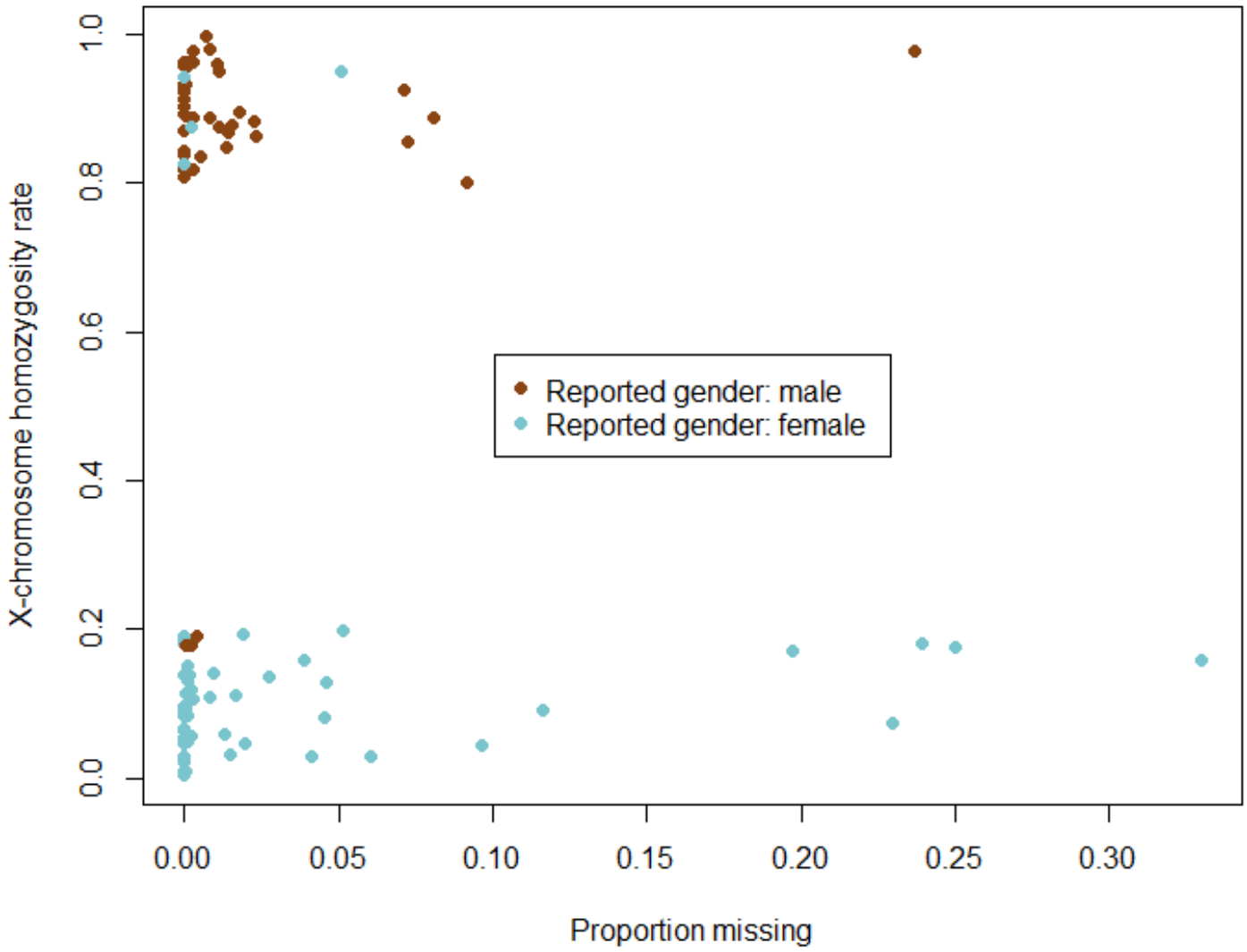
- Expected homozygosity rates
  - $F > 0.8$ for male samples and $F < 0.2$ for female samples
  - males have only one X - cannot be heterozygous
    - expect some genotyping errors

Gender Check - X Chromosome



- Calculate the homozygosity rate F across all X-chromosome SNPs for each individual in the sample and compare these to the expected rate
- Expected homozygosity rates
  - $F > 0.8$ for male samples and $F < 0.2$ for female samples
  - males have only one X - cannot be heterozygous
    - expect some genotyping errors
- Gender error reported for mismatch in reported and genetic sex
- Discrepancies with external gender information may reflect:
  - errors in external data
  - sample mix-up

Gender Check - Plot



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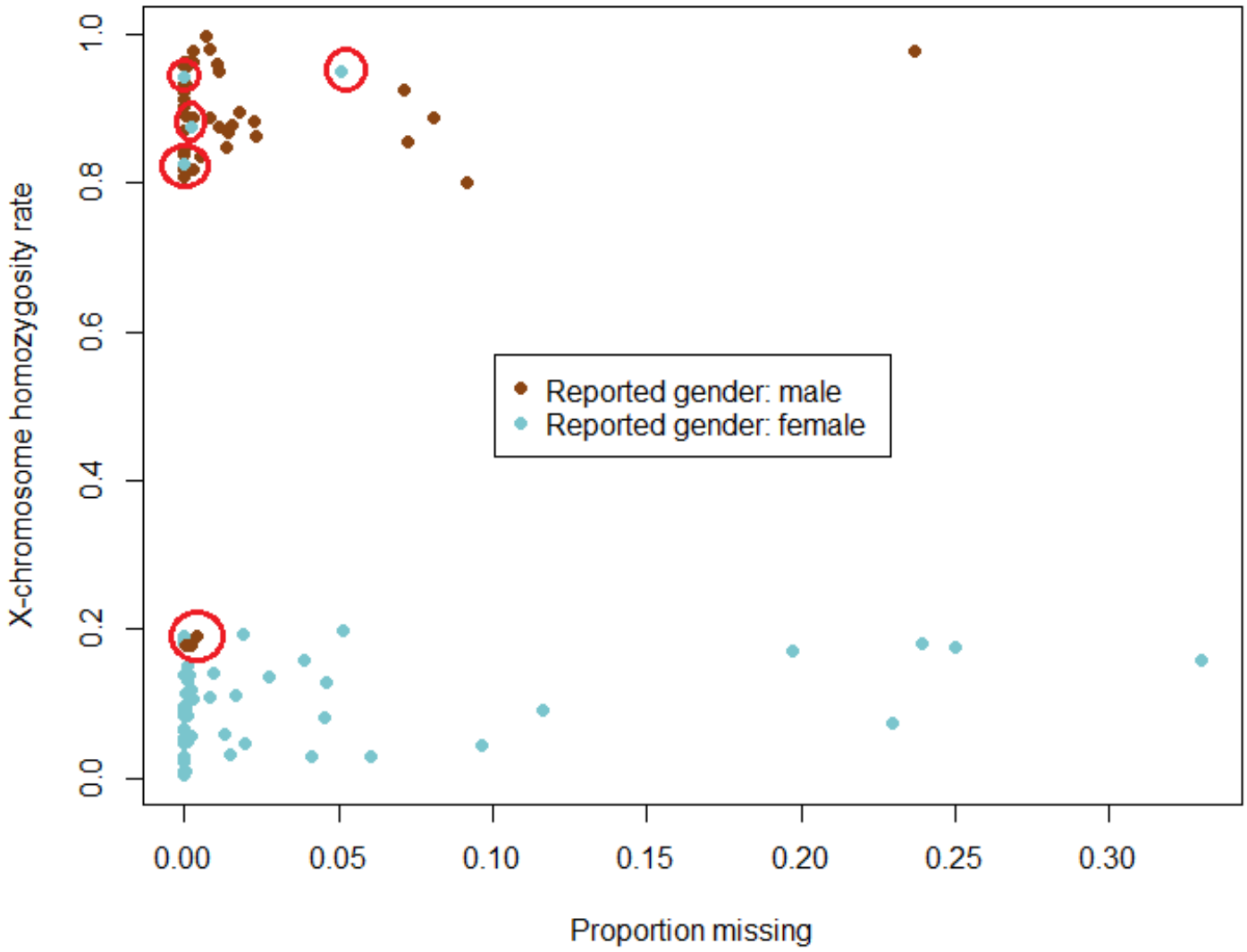

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Gender Check - Examples

Reported gender	Homozygosity rate	Gender according to SNPs
Male	0.98	Male
Female	0.03	Female
Female	0.99	Male
Female	0.28	Unknown*
Female	0.35	Unknown**

* Likely a female with sex chromosome anomaly (e.g. XX/XO mosaic, loss-of-heterozygosity on X)
** Likely a male with sex chromosome anomaly (e.g. XXY or XX/XY mosaic)

Adapted from Turner et al. Curr Protoc Hum Genet (2011). doi:10.1002/0471142905.hg0119s68

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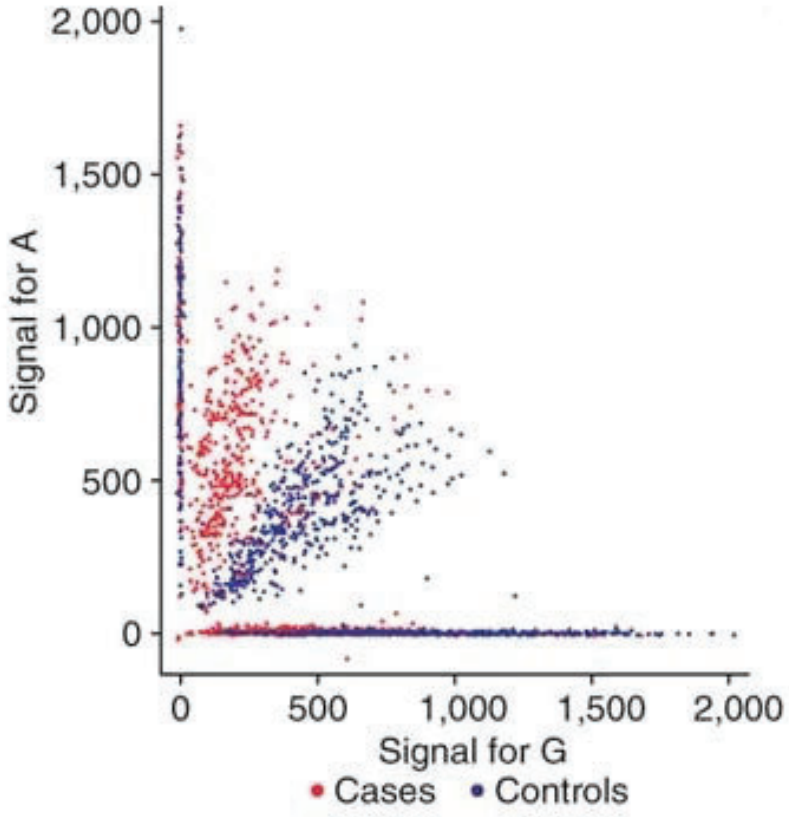
- Extreme differential call rates between cases and controls

Effect of Differential Call Rate

Clayton et al. Nature Genetics (2005). doi:10.1038/ng1653



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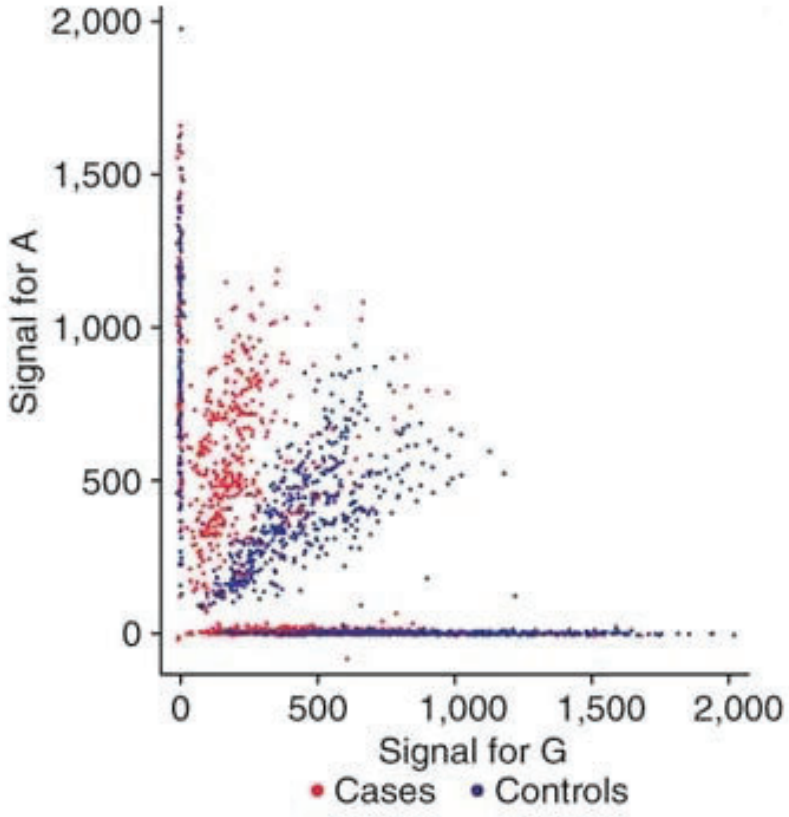

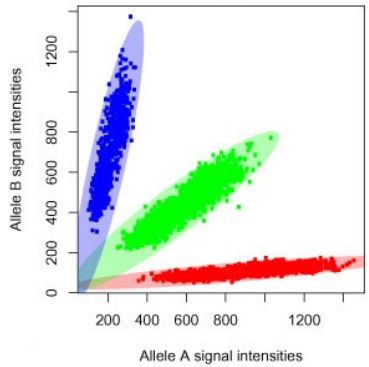

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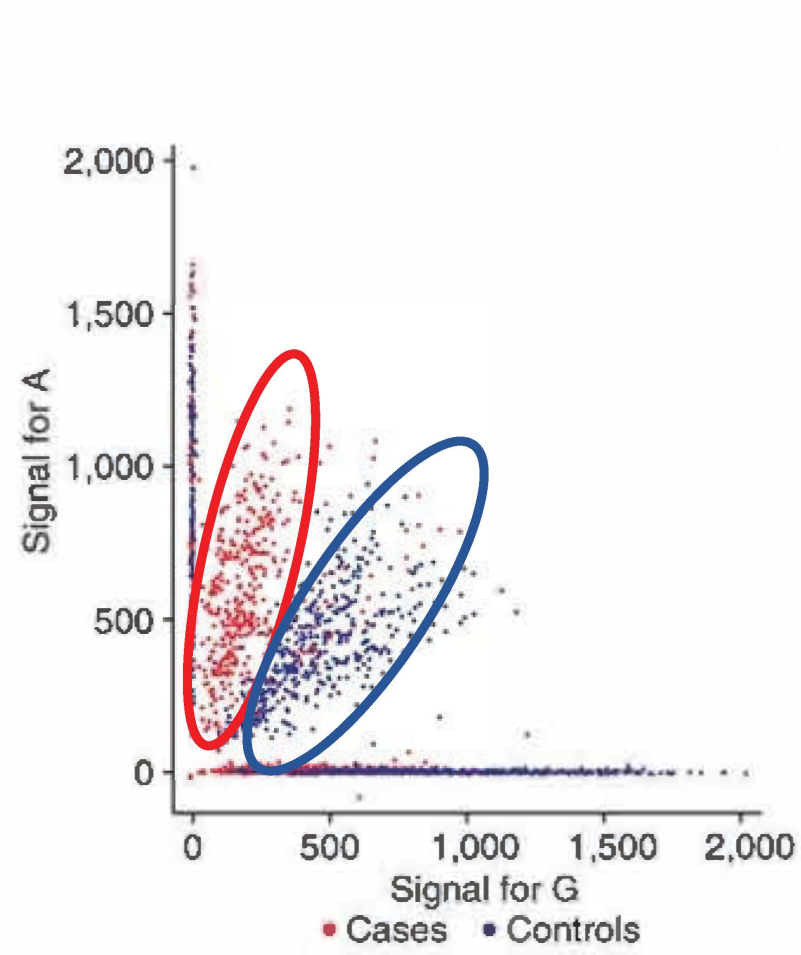

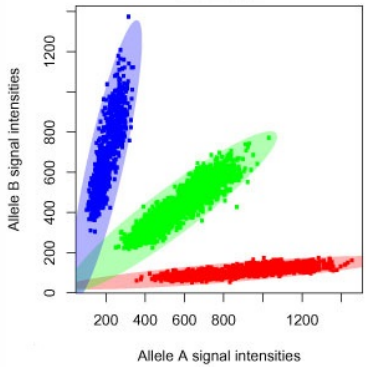

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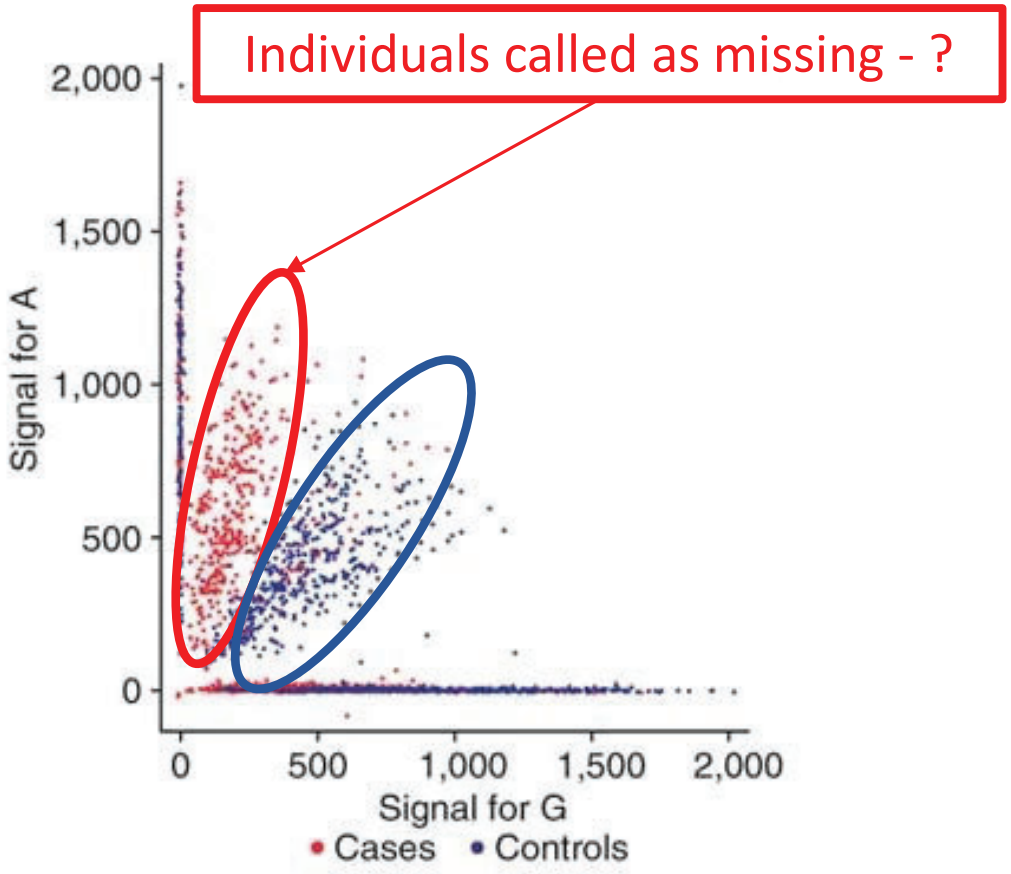

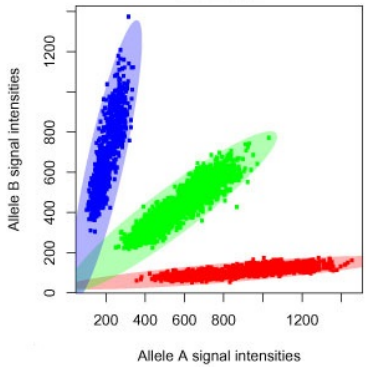

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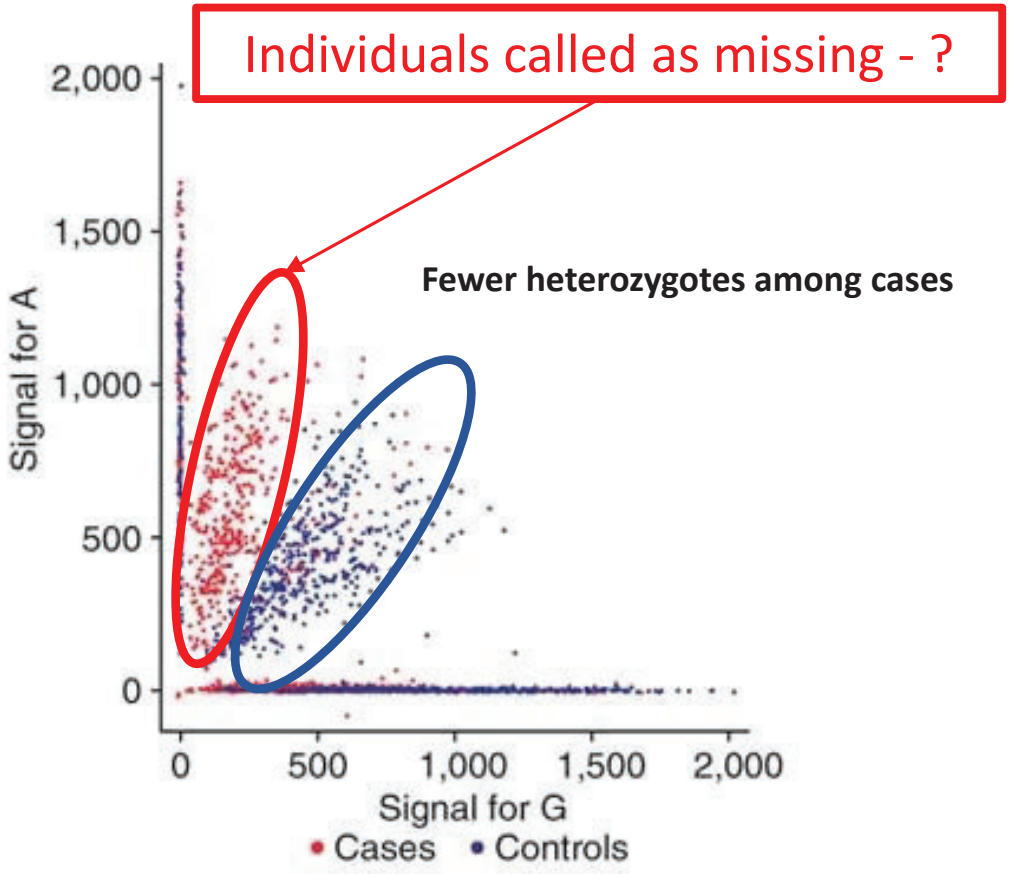

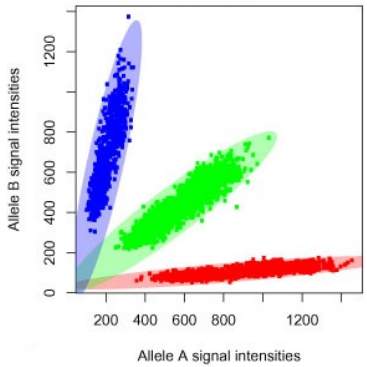

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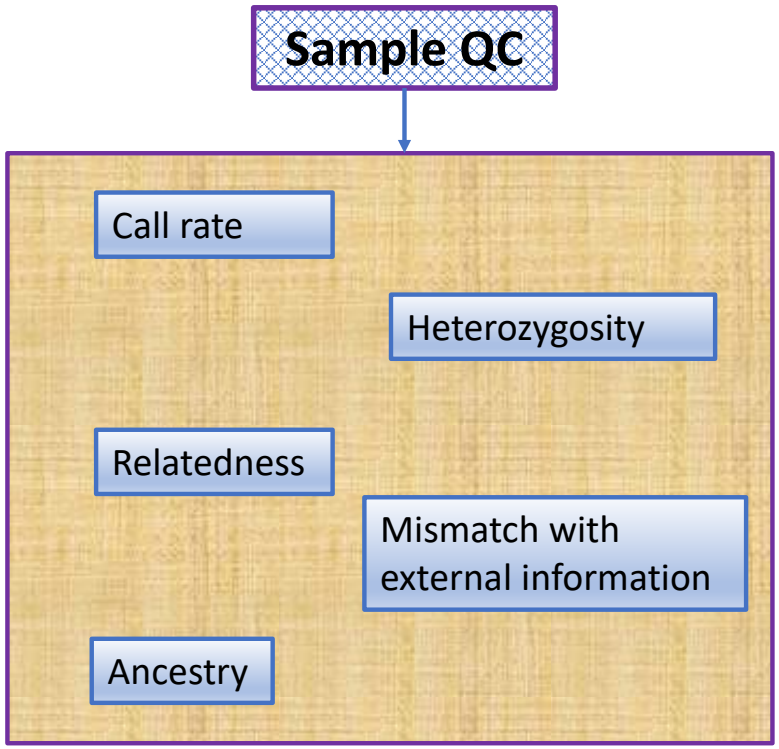
  - **Spoiler alert**: PLINK will be used in practicals

Summary of QC Steps



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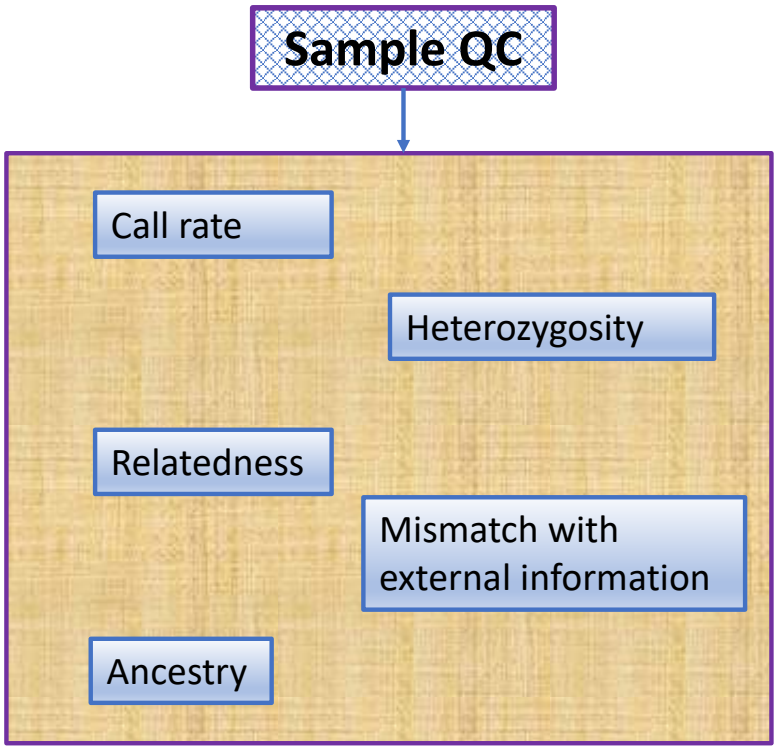

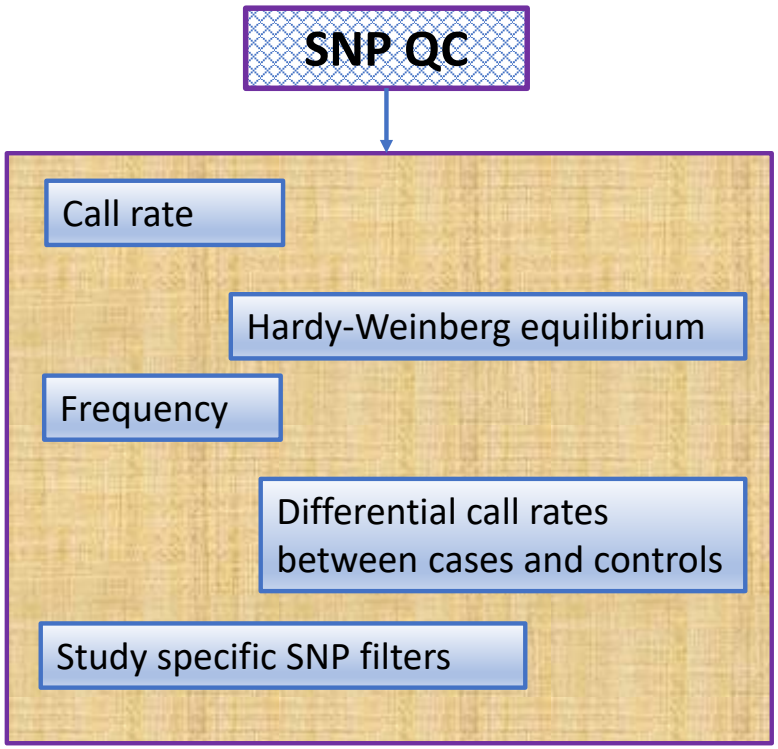

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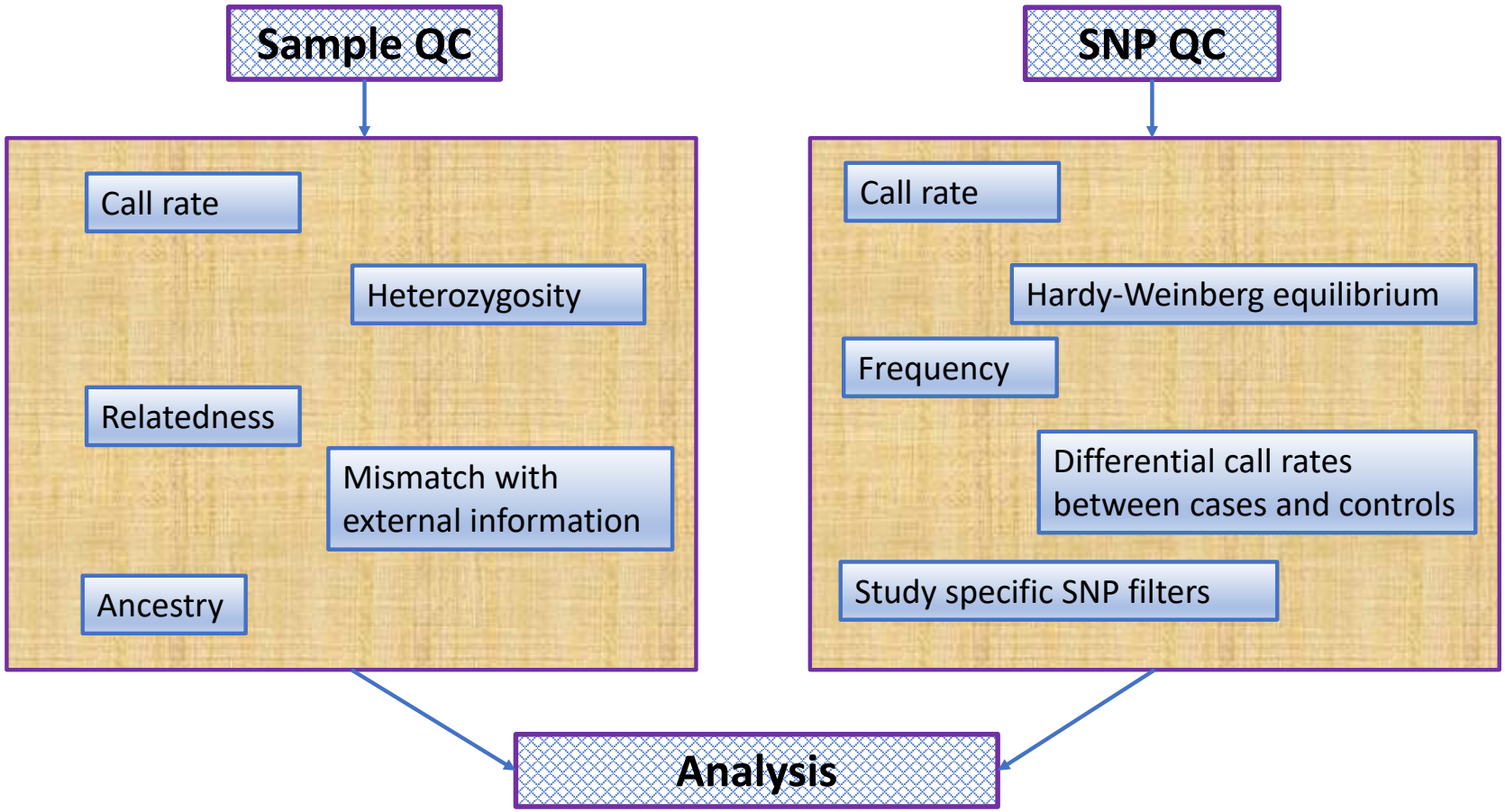

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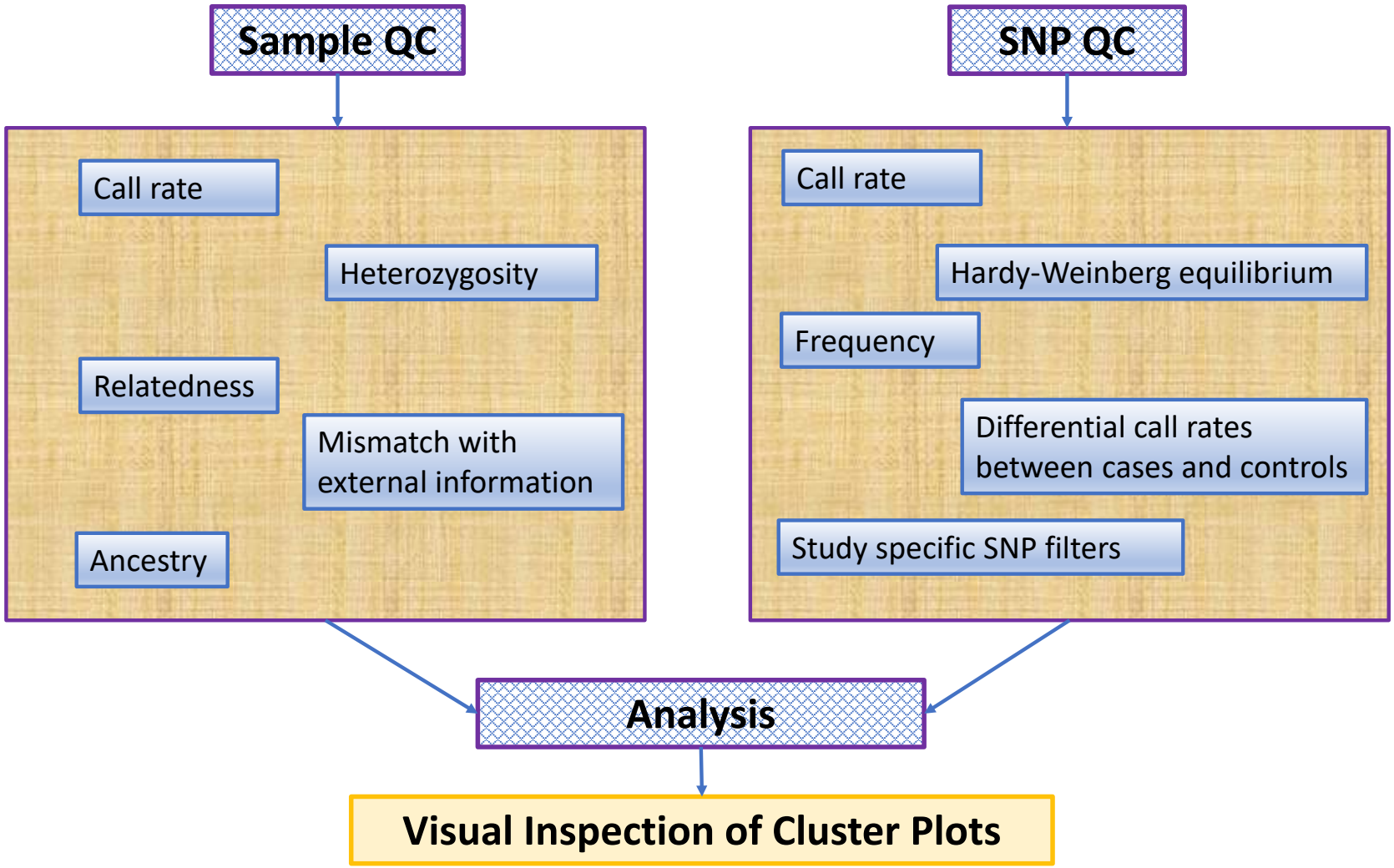
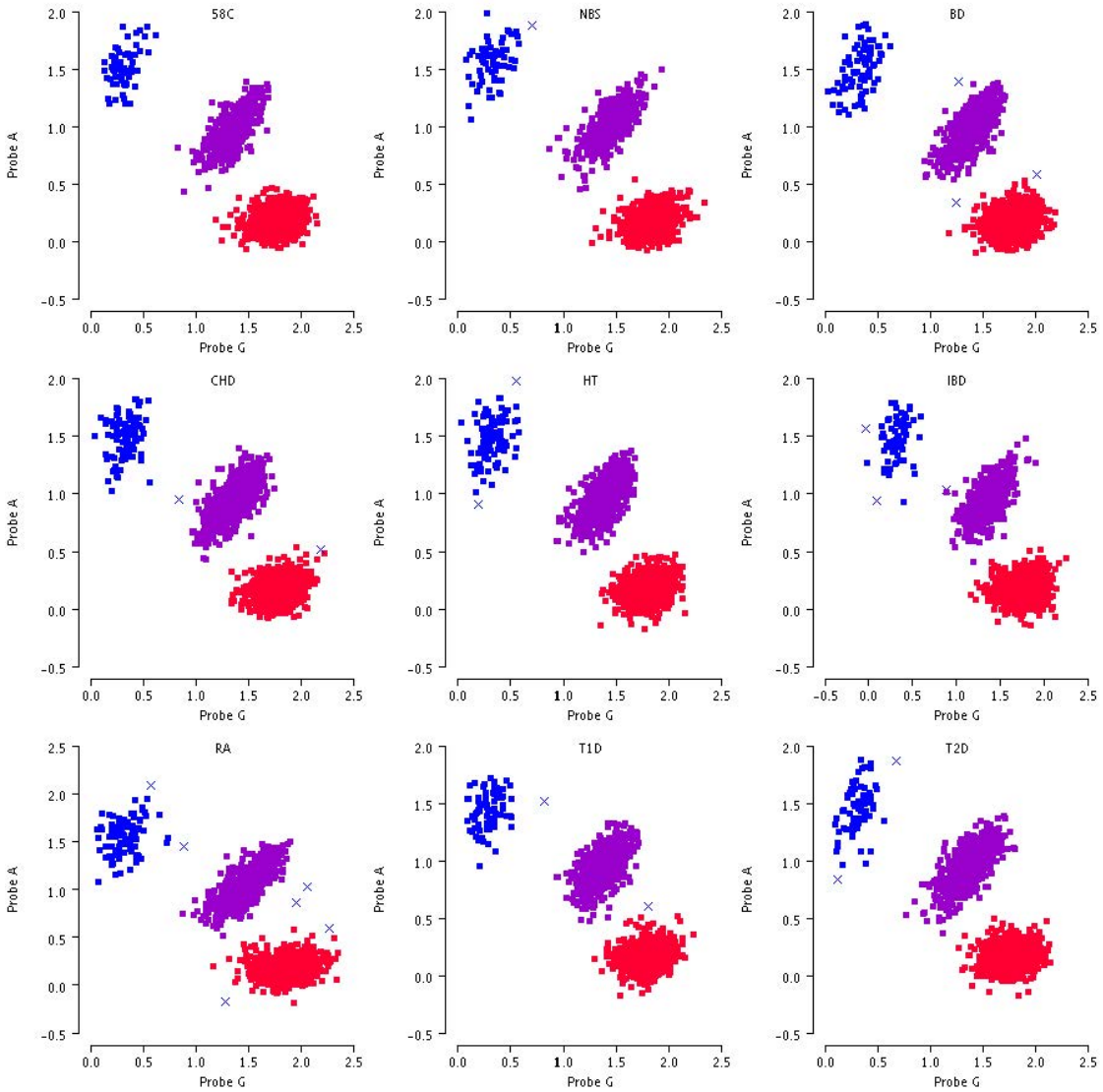


Visual Inspection of Cluster Plots - Good SNP



The Wellcome Trust Case Control Consortium. Nature (2007). doi:10.1038/nature05911

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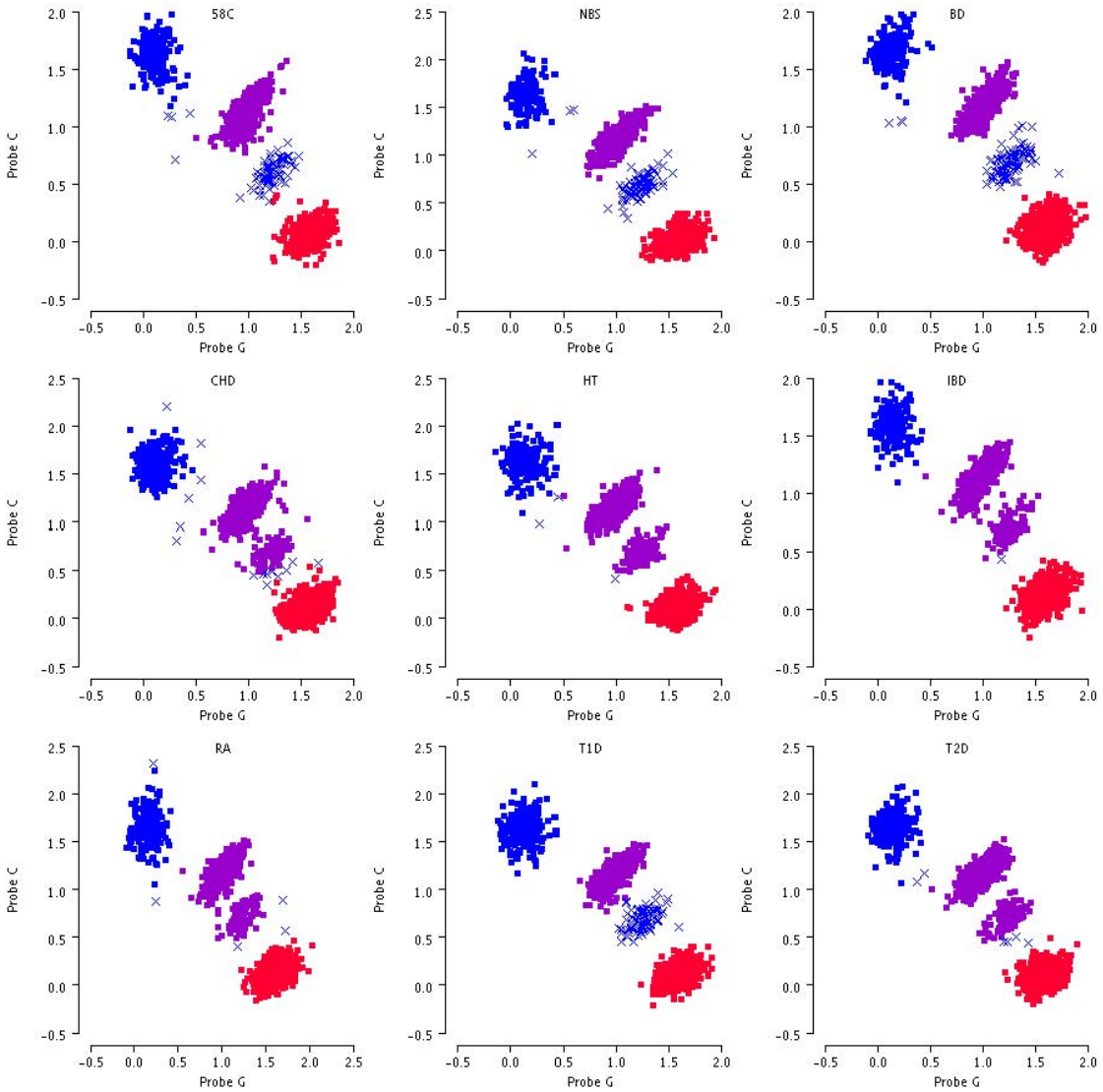

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- QC criteria are subjective and vary from one study to another
- Sample QC filters should not be so stringent as to remove the majority of the analysis cohort
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- All SNPs demonstrating evidence for association should be followed up with visual inspection of cluster plots