

Data Quality Control (QC) in Association Studies

Svetlana (Sarah) Cherlin

Populatin Health Sciences Institute
Faculty of Medical Sciences
Newcastle University, UK

`svetlana.cherlin@newcastle.ac.uk`





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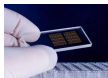




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- **Tutorials**
 - ▶ Anderson et al. Nature Protocols 2010, doi:10.1038/nprot.2010.116
 - ▶ Turner et al. Curr Protoc Hum Genet. 2011. doi:10.1002/0471142905.hg0119s68
 - ▶ Marees et al. Int J Methods Psychiatr Res. 2018. doi: 10.1002/mpr.1608

Genotype Calling

illumina

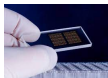


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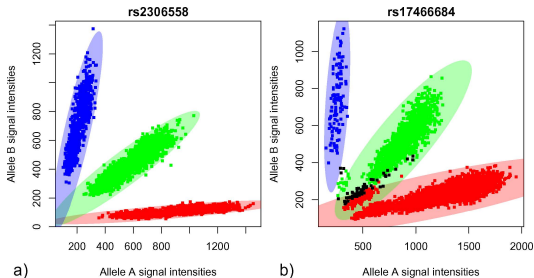


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- Examples of cluster plots for two SNPs. One spot corresponds to one sample.
- Samples with genotypes **AA** and **BB** are red and blue, respectively. **Heterozygous** samples are shown in green; samples with **missing genotypes** are black. The 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.

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

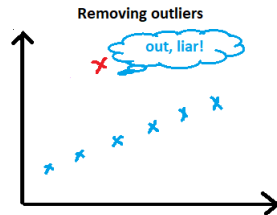


Sample Quality Control



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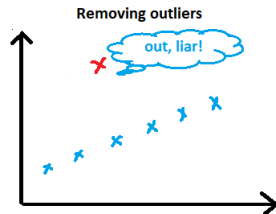
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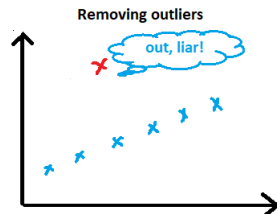
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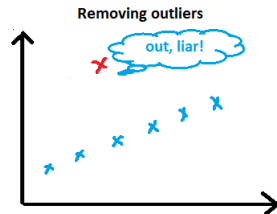
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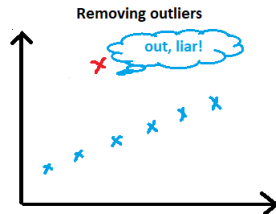
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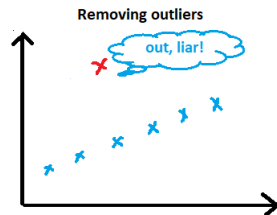
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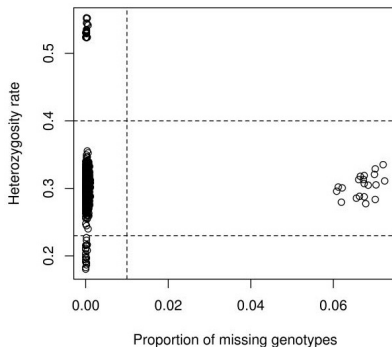
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- Outlying population **ancestry**
 - ▶ confounding due to population structure



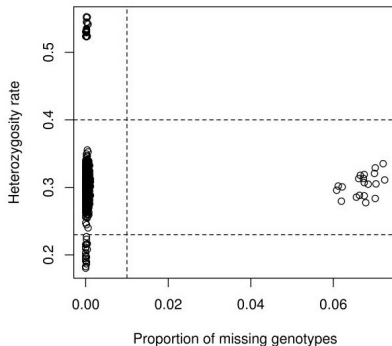
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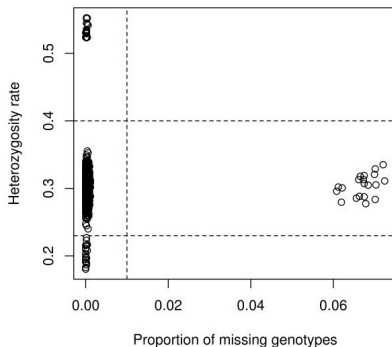


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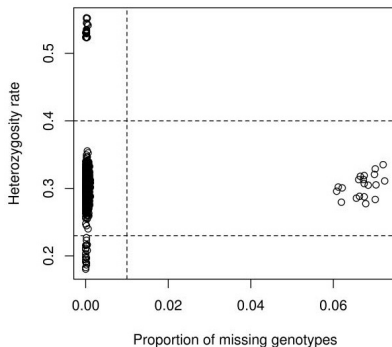


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- Dashed lines denote QC thresholds (exclude samples with missing call rate > 0.1 , and samples with heterozygosity rate < 0.23 and > 0.4)
- Rule of thumb:** remove individuals who deviate ± 3 SD from the samples' heterozygosity rate mean



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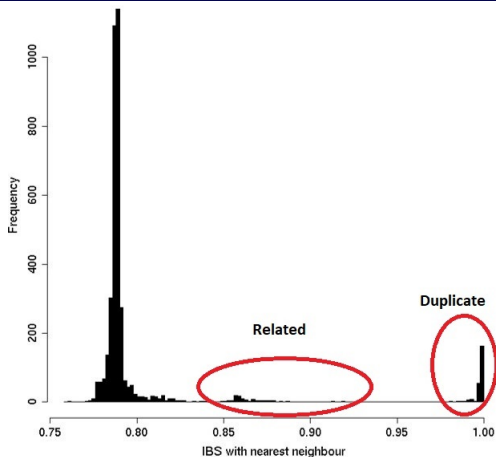
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- Common to **plot histogram** of IBS of each individual with “nearest neighbour”



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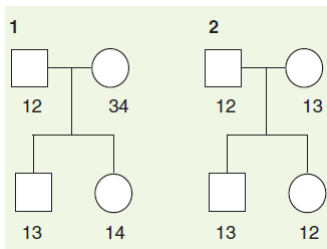
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- Pedigree 1: Siblings share **allele 1 IBD** (inherited from the father)
- Pedigree 2: Siblings share **allele 1 IBS** (inherited from different parents)

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



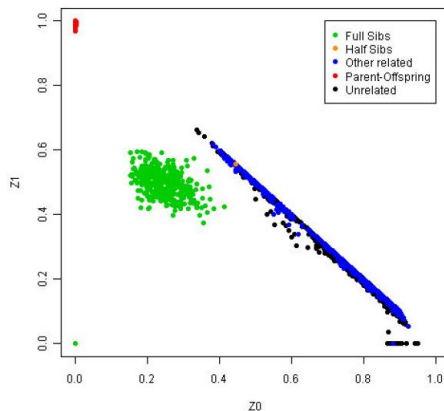
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 - in practice, use $\hat{\pi} > 0.98$
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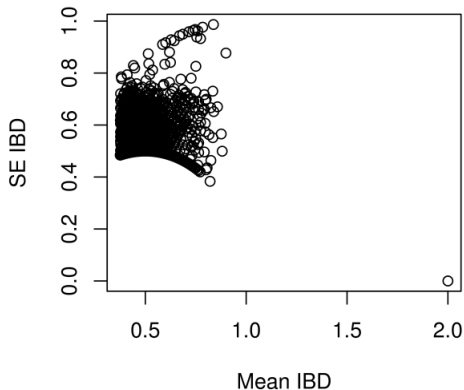
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- Prune the data for LD before assessing IBD
 - ▶ shared region of high LD results in more shared variants than one of low LD, even if the two regions are the same size



IBD Plot

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

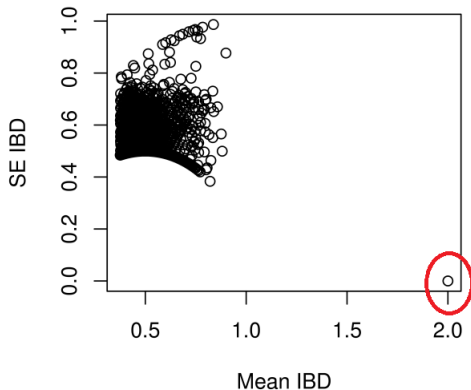


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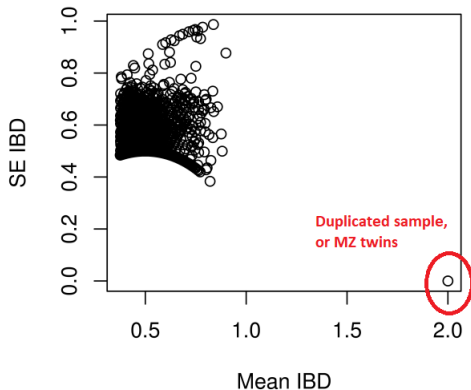


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Duplicated samples



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- Including related individuals in the analysis, without accounting for these relationships, can increase **false positive error rates** and reduce **power**
- **Mixed modelling approaches** account for “relatedness” between individuals (families, cryptic relatedness, population structure) by allowing for kinship



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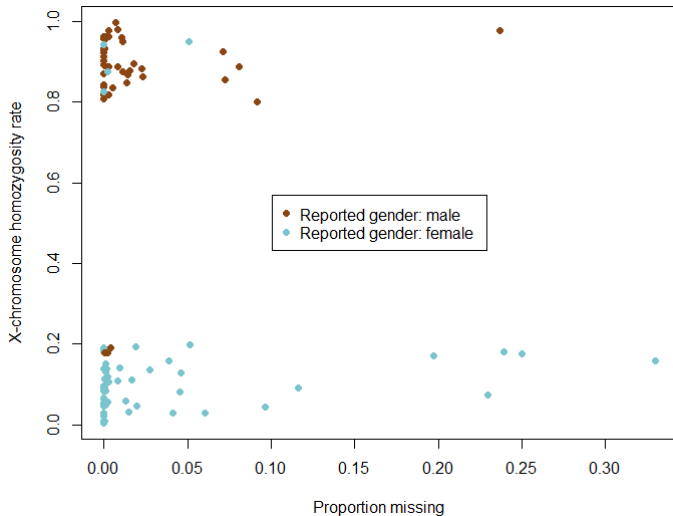
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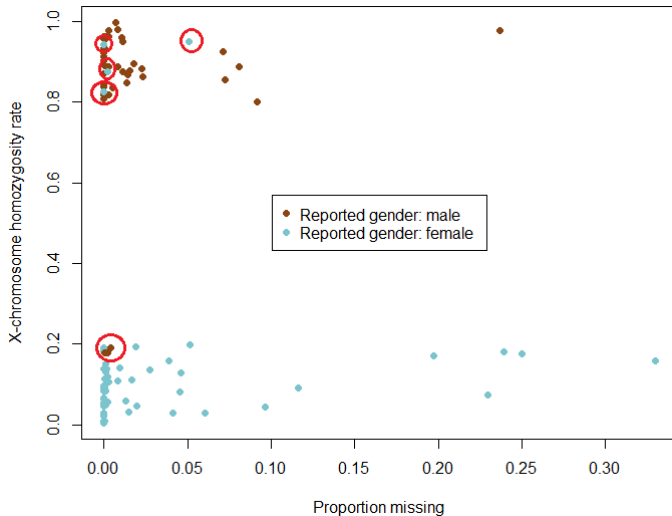
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- Gender error reported for **mismatch in reported and genetic sex**
- **Discrepancies** with external gender information may reflect:
 - ▶ **errors** in external data
 - ▶ sample **mix-up**



Gender Check - Plot



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Spot the discrepancies...



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)

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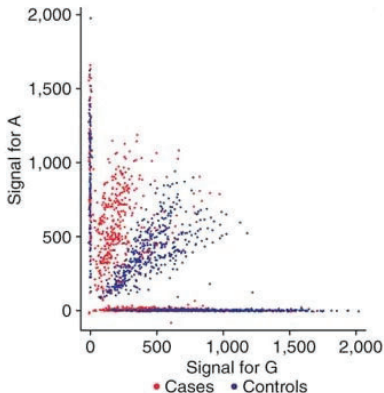
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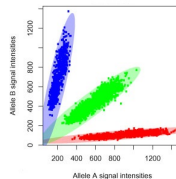
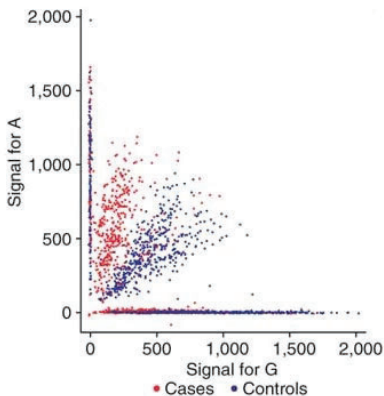
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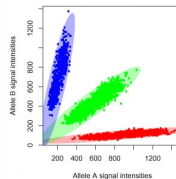
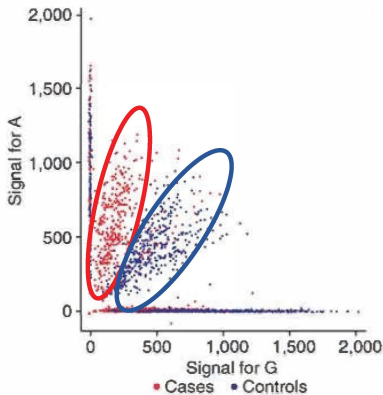
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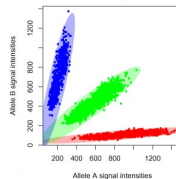
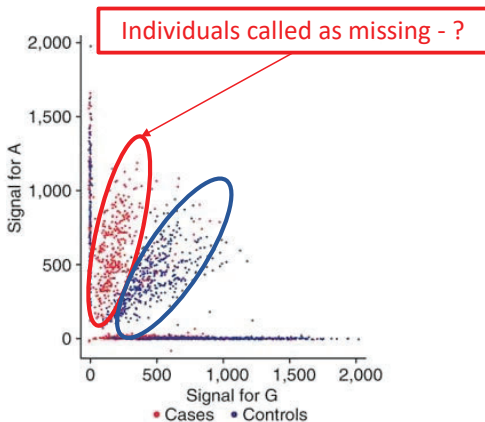
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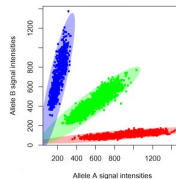
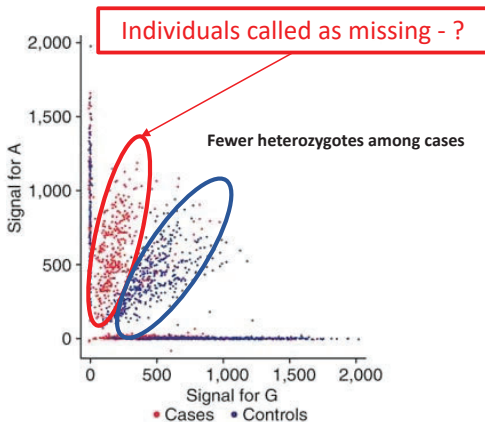
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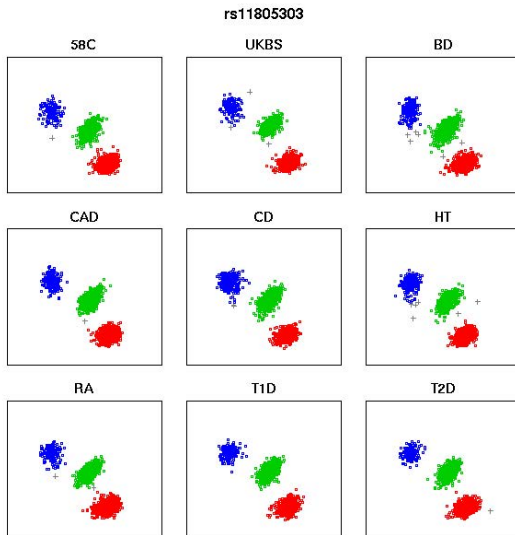
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 - ▶ **Spoiler alert**: PLINK will be used in practicals



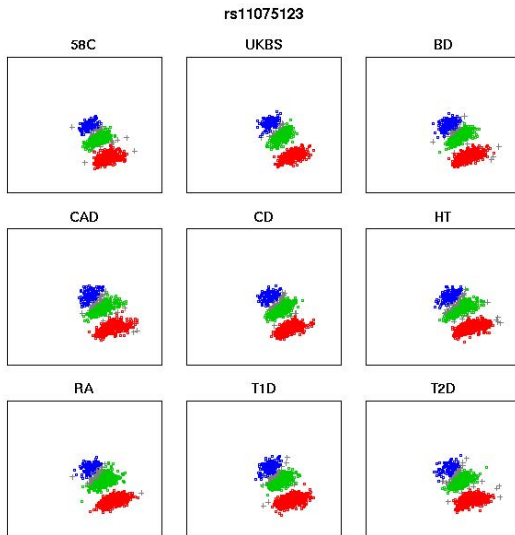
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The Wellcome Trust Case Control Consortium. *Nature* (2007). doi:10.1038/nature05911



Visual Inspection of Cluster Plots - Bad SNP



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Summary



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- QC is an **essential step** of the analysis
- 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
- SNP QC filters should **eliminate the worst quality markers**
- All SNPs demonstrating evidence for association should be followed up with **visual inspection** of cluster plots

