

Data Quality Control (QC) in Association Studies

Svetlana (Sarah) Cherlin

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- Poor study design and errors in genotype calling can introduce **systematic bias** in association studies



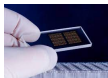
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- **Tutorials**
 - ▶ Anderson et al. Nature Protocols 2010, doi:10.1038/nprot.2010.116
 - ▶ Turner et al. Curr Protoc Hum Genet. 2011. doi:10.1002/0471142905.hg0119s68
 - ▶ Marees et al. Int J Methods Psychiatr Res. 2018. doi: 10.1002/mpr.1608

Genotype Calling

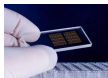
illumina



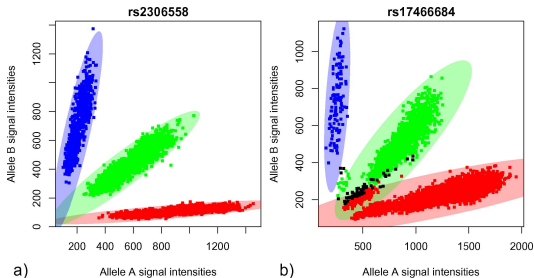
affymetrix

Genotype Calling

illumina



affymetrix



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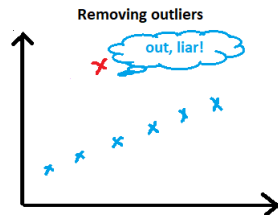
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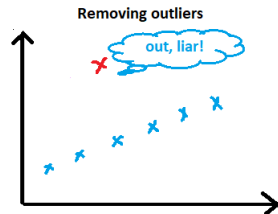
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- **Missing call rate** is not only a measure of data completeness, but is also a measure of **genotype quality**

Sample Quality Control



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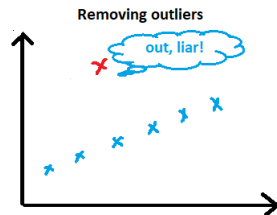
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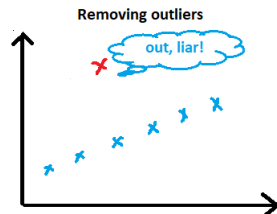
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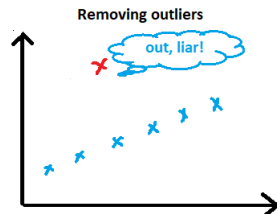
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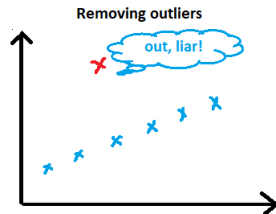
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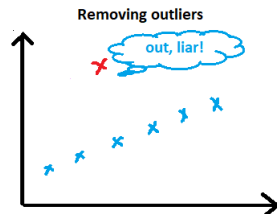
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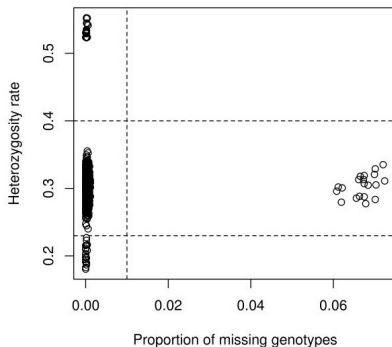
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- Outlying population **ancestry**
 - ▶ confounding due to population structure



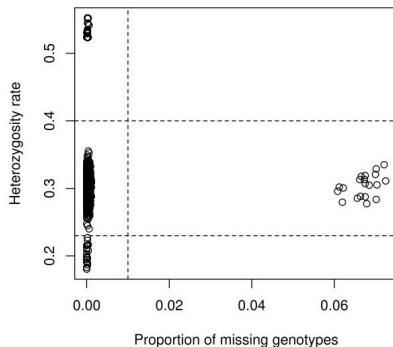
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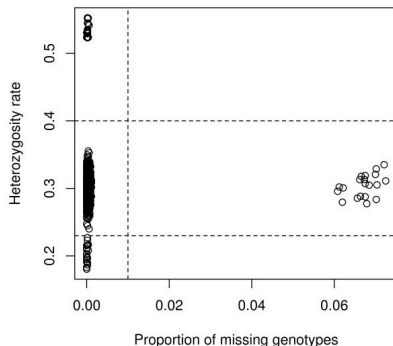
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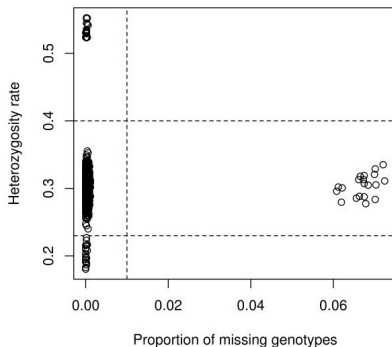
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- Dashed lines denote QC thresholds (exclude samples with missing call rate > 0.1 , and samples with heterozygosity rate < 0.23 and > 0.4)
- Rule of thumb**: remove individuals who deviate ± 3 SD from the samples' heterozygosity rate mean

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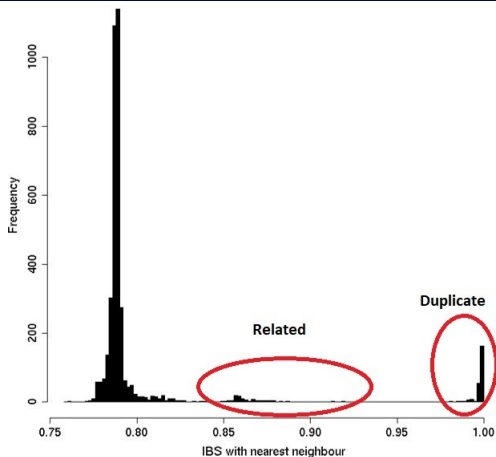
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- Common to **plot histogram** of IBS of each individual with “nearest neighbour”

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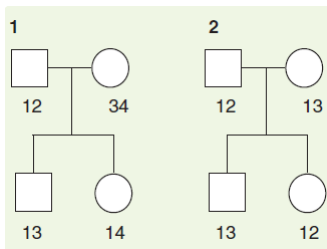
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- Pedigree 1: Siblings share **allele 1 IBD** (inherited from the father)
- Pedigree 2: Siblings share **allele 1 IBS** (inherited from different parents)

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

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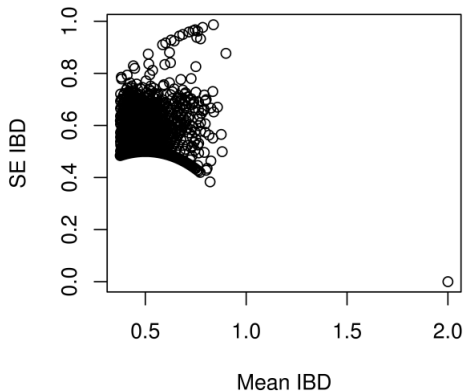
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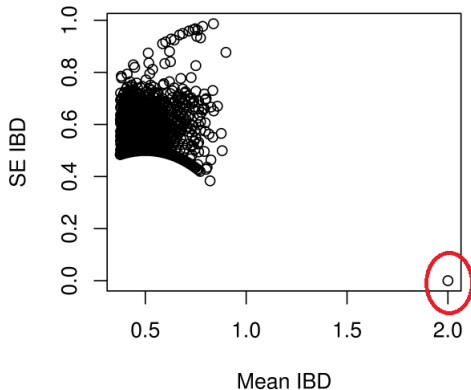
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- **Prune the data for LD** before assessing IBD
 - ▶ shared region of high LD results in more shared variants than one of low LD, even if the two regions are the same size

IBD Plot



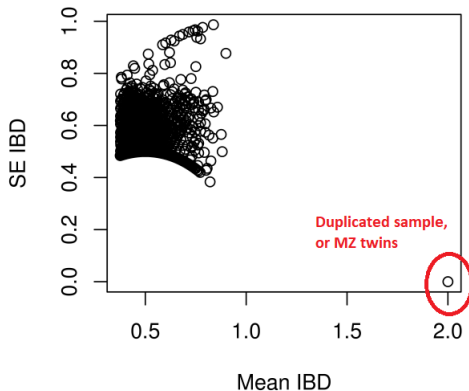
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- Including related individuals in the analysis, without accounting for these relationships, can increase **false positive error rates** and reduce **power**
- **Mixed modelling approaches** account for “relatedness” between individuals (families,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

Gender Check - X Chromosome



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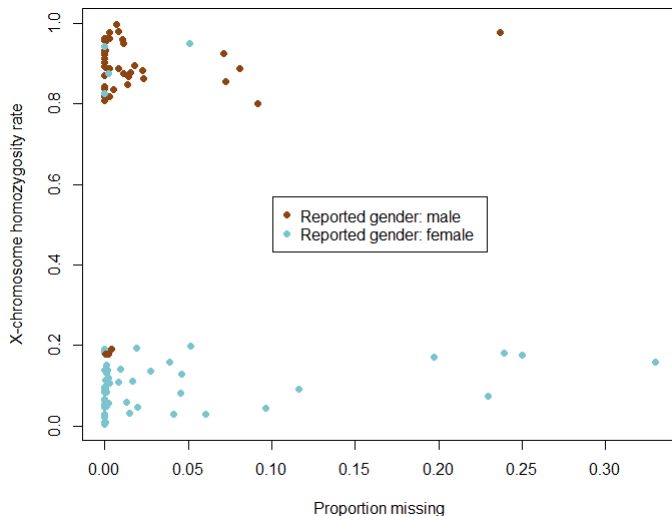
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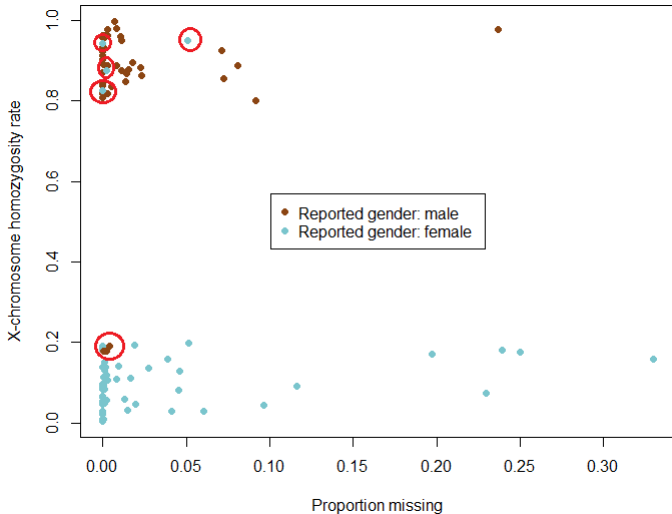


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- Gender error reported for **mismatch in reported and genetic sex**
- **Discrepancies** with external gender information may reflect:
 - ▶ **errors** in external data
 - ▶ sample **mix-up**

Gender Check - Plot



Gender Check - Plot



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

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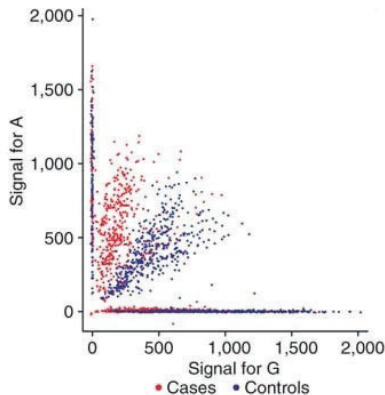
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- Extreme **differential call rates between cases and controls**

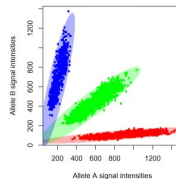
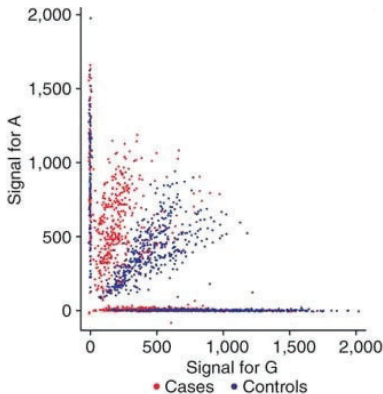
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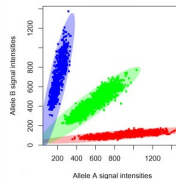
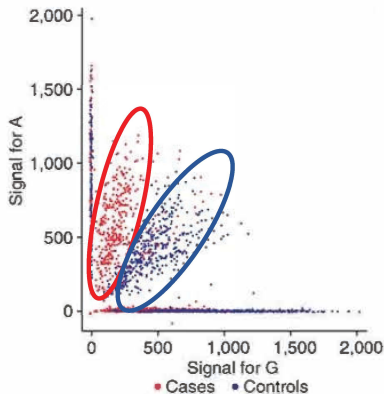
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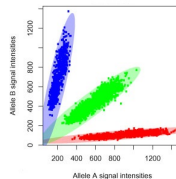
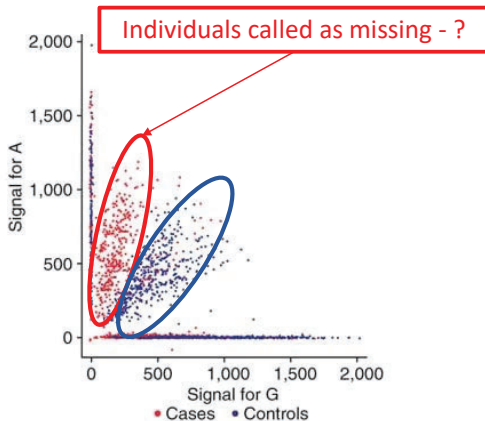
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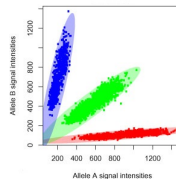
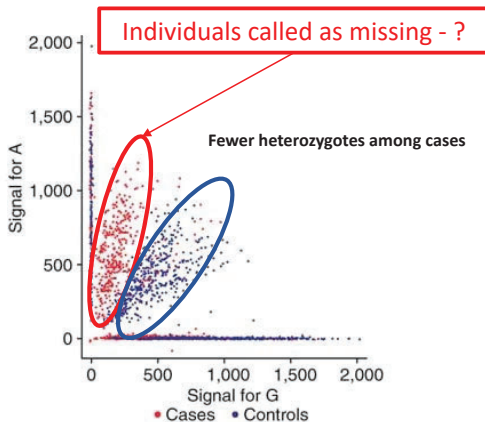
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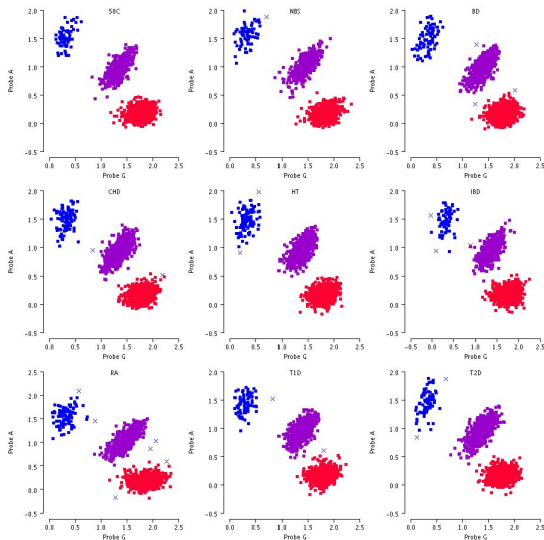
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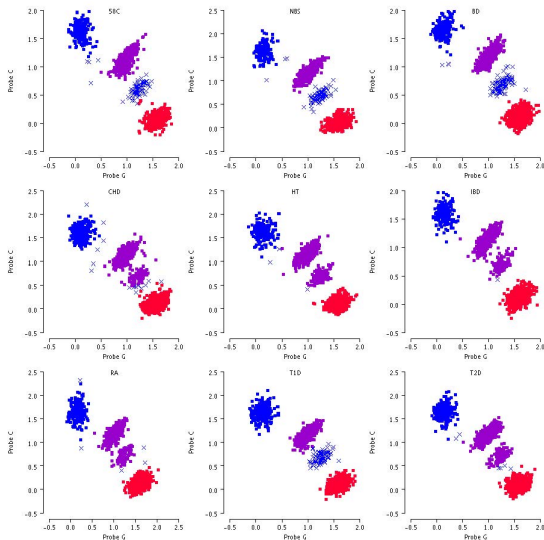


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 - ▶ **Spoiler alert**: PLINK will be used in practicals

Visual Inspection of Cluster Plots - Good SNP



Visual Inspection of Cluster Plots - Bad SNP



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