Data Quality Control
NGS and Genotype Array Data

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DNA Collection

- Blood samples
  - For unlimited supply of DNA
    - Transformed cell lines
      - Is expensive
    - Whole genome amplification
      - Allows for the creation of large amounts of DNA from initial small DNA sample
        - » Perform WGA on each sample three or more times and use pooled samples
      - Can experience lower call rates and higher genotyping error rates
      - Not recommend for whole genome sequencing or copy number variant (CNV) analysis
- Buccal Swabs
  - Small amounts of DNA
  - DNA not stable
- Saliva (Origene collection kit)

Measurement of DNA Concentrations

- Nanodrop
- Picogreen

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Effect of Genotyping Error – Same Error Rates for Cases and Controls

- For family-based association studies - Trios
  - Can increase both type I and II error
- Population based studies
  - Increases type II error only

Quantitative Traits
If genotyping error is not correlated with trait values type II errors will be increased

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Effects of Genotyping Error – Different Error Rates for Cases and Controls

- Cases and controls are sequenced/genotyped
  - At different times
  - Different institutions
  - Or one group, e.g., case or control, is predominately sequenced/genotyped in the same batch
- Can lead to different genotyping error rates in cases and controls
  - In this situation both type I and II error can be increased
- If sequencing/genotyping cases and controls
  - Randomize cases and controls so they are spread evenly across batches

Quantitative Traits
If genotyping error is correlated with trait values, it will also increase type I and II errors, e.g., individuals with elevated systolic blood pressure are genotyped in one batch and those with systolic blood pressure within the normotensive range in another batch

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Genotype SNPs (~20-96) before Exome or Whole Genome Sequencing

- Genotype markers which can be used as DNA fingerprint
- Allows for Assessment of DNA quality
- Aids in determining the the genetic sex of study subjects
  - To aid in identification of potential sample swaps
- Detects cryptic duplicates
- For family data
  - Aids in determining close familial relationships
    - Non-paternity
    - Sample swaps
    - Cryptic relationships

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Detecting Genotyping Errors

- Duplicate samples genotyped using arrays to detect inconsistencies
  - Can use duplicate samples that are inconsistent to adjust clusters to improve allele calls
    - Will not detect systematic errors
- Usually generated only for genotype array data
  - Due to expense, duplicate samples are usually not generated for exome or whole genome sequencing studies

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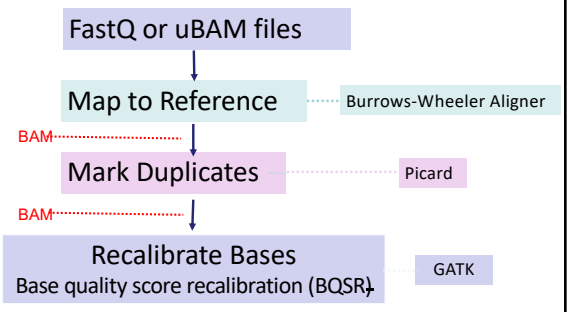
Variant Calling Pipeline -Step 1 Preprocessing



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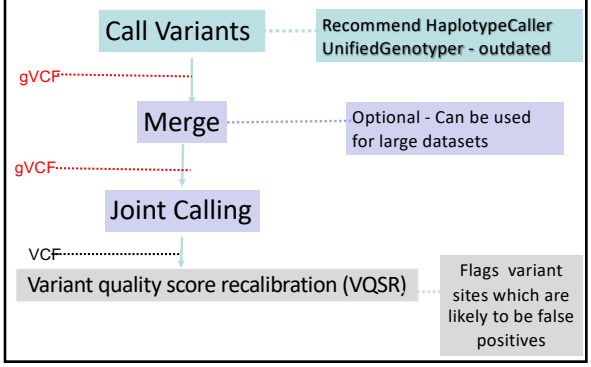
Variant Calling Pipeline-Step 2 Variant Discovery



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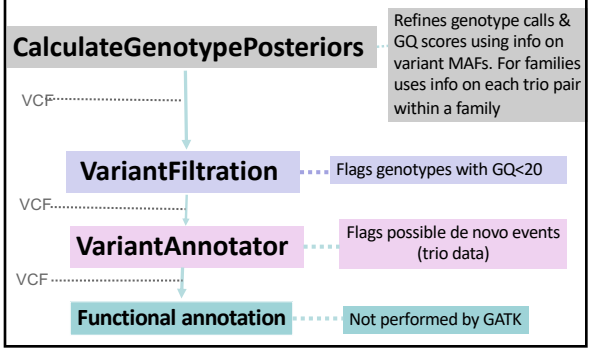
Variant Calling Pipeline - Step 3 Call Set Refinement



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A Short List of Additional Software to Detect Genetic Variation

- Exome data copy number variation
  - CoNIFER (Copy Number Inference From Exome Reads)
    - Krumm et al. 2012
  - XHMM
    - Fromer et al. 2014
- WGS data structural variation
  - MetaSV
    - Mohiyuddin et al. 2015
  - LUMPY
    - Layer et al. 2014

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Variant Calling

- BAM files are large and take considerable resources
  - Storage is expensive
  - One 30x whole genome is ~80-90 gigabytes
  - A small study of 1,000 samples will consume 80 terabytes of disk space
- The cost of cloud computing to call variants
  - (Souilmi et al. 2015)
  - $5 per exome
  - $50 per genome
    - For 1,000 samples
      - $5,000 exome
      - $50,000 genome

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Working with gVCF Files

- Instead of obtaining VCF files
- Can obtain gVCF files to perform joint calling and complete the GATK pipeline
  - A whole genome gVCF
    - ~1 Gigabyte
      - 1/100th the size of a BAM file for one individual

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Influences on Sequence Quality

- DNA quality
  - Age of sample
  - Extraction method
  - Source of sample
    - e.g., blood, skin punch, buccal
- Sequencing machines (read length)
- Median sequencing depth
- Alignment
- Variant calling method used
  - Single nucleotide variants and insertion/deletions
  - Structural variants

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NGS Data Quality Control

- Extremely important to perform before data analysis
  - Poor data quality can increase type I and II errors
  - Due to inclusion of false positive variant sites or incorrect genotype calls
- Protocols for data QC are still in their infancy
  - No set protocols for QC
- QC is data specific
  - Dependent on read depth
  - Batch effects
  - Availability of duplicate samples
  - etc.

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NGS Data Quality – Removal of Genotype Calls and Samples

- Sequence depth of coverage
  - DP_variant
    - High DP could be an indication of copy number variants
      - Which can introduce false positive variant calls
        - » Due to down sampling in GATK maximum DP is 250
  - DP_genotype
    - Concerned if depth is too low or too high
      - Low insufficient reads to call a variant site
    - Remove genotypes with low read depth, e.g., DP≤8
- Genotype quality (GQ) score
  - Removal of sites with low genotype quality core, e.g., GQ≤ 20

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VCF Example

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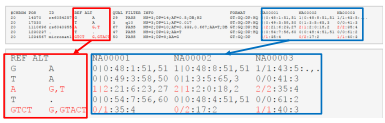
Variants with more than 2 Alleles

- Genetic analysis tools are usually developed to analyze variant sites that are diallelic
- Some sites may have >2 alleles
- The alleles at these sites need to be split
  - New loci are made each multi-allelic site each with only 2 alleles
    - bcftools
- Multiallelic sites can have higher error rates compared to diallelic sites



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NGS Data Quality – Removal of Genotype Calls and Samples

- Removal of sites with missing data
  - e.g., missing > 10% of genotypes
- Removal of "novel" variant sites which only occur in one batch and the alternative allele is observed multiple times or the minor allele frequency (MAF) is high in overall sample
- Removal of sites that deviate from Hardy-Weinberg Equilibrium (HWE)
  - Must be performed by population, e.g., African American and European American
  - Related individuals should be removed from the sample before testing for deviations from HWE

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NGS Data Quality Control

- GATK - Variant Quality Score Recalibration (VQSR)
  - Used to determine variant sites of bad quality
    - Variant site is a false positive call
- However even after this step
  - Concordance of duplicates (when available) and
  - and Ti/Tv ratios are often low
- Additional QC steps needs to be performed

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NGS Data Quality Control

- Values which are used for DP (genotype), GQ, and missing data cut offs are based upon
  - Concordance rates
    - If there are duplicate samples are available
  - Ti/Tv ratios
    - By individual
    - By batch
    - Entire data set
  - Amount of data removed
    - QC can remove substantial amounts of data which should be avoided
      - e.g., >15% of variant sites

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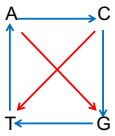
Transition/Transversion (Ti/TV) Ratios

- Transition
  - Purine → Purine
  - Pyrimidine → Pyrimidine
- Transversion
  - Purine → Pyrimidine
  - Pyrimidine → Purine



Transition
Transversion

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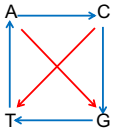
Transition/Transversion (Ti/TV) Ratios

- Ti/Tv Ratios
  - Whole genome ~2.0
  - Exome novel ~2.7
  - Exome known ~3.5
- Ti/Tv ratios can be calculated by
  - Sample or
  - Dataset
- Ti/Tv ratios can be evaluated for subsets of data
  - e.g., by batch



Transition
Transversion

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Sequence Data QC Overview

- Variant and genotype call level
  - Evaluation of batch effects
- Genotype call level – Removal of genotype calls
  - Low or high depth of coverage DP< 8
  - Low genotype quality score GQ< 20
- Removal of individual samples
  - >20% missing data
    - After taking the intersect of capture arrays
  - Samples without phenotype information

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Sequence Data QC Overview

- Variant level – removal of variant sites
  - Low call rate
    - i.e., missing call rate > 10%
  - "Novel" variant sites observed ≥2 only in a single batch
  - Deviation from Hardy-Weinberg-Equilibrium
    - Population specific
    - Unrelated individuals
      - e.g., $p<5 \times 10^{-8}$, $p<5 \times 10^{-15}$

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Data Clean – Assessing Sex Chromosomes

- When data is collected on study subjects they are asked about their gender/sex and not their genetic sex
  - Differences in gender/sex and genetic sex can be due to
    - Sample swaps
    - Study subjects who are not cisgender
- Some study subjects may have neither a XX nor XY karyotype
  - Turner syndrome X0
  - Klinefelter syndrome XXY

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Data Clean – Assessing Chromosomal Sex

- Study subjects labeled as females with an excess of homozygous genotypes on the X chromosome can denote
  - That their genetic sex is male
  - Turner Syndrome

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Data Clean – Assessing Chromosomal Sex

- Study subjects labeled as males with an excess of heterozygous SNPs* on the X chromosome can denote
  - That their genetic sex is female
  - Klinefelter syndrome
- Note: Individuals who are XY will also be heterozygous for markers in the pseudoautosomal regions
- Availability of Y chromosome data
  - Can greatly aid in determining genetic sex and if an individual has Turner or Klinefelter syndrome

*Both genetic males and females have two alleles for each locus on the X chromosome in the datafile, although males are hemizygous

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Data Clean – Assessing Sex Chromosomes

- Individuals whose labeled gender/sex does not match their genetic sex are removed from the analysis
- This observation may be due to a sample swap
  - When samples are swapped
    - Phenotype data will be incorrect
      - e.g., may be a case when labeled as a control

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Checking for Duplicate and Related Individuals

- Duplicate samples are sometimes included in a study as part of quality control to detect inconsistencies
  - Will not detect systematic errors
  - Usually not included in exome and whole genome sequencing studies
  - Intentional duplicates can easily be removed before data quality control
- Cryptic duplicates (unintentional)
  - DNA sample aliquoted more than once
  - Individual ascertained more than once for a study
    - e.g. The same individual undergoes the same operation more than once and is ascertained each time
- Individuals who are related to each other may participate in the same study
  - Unknown to the investigator
  - Or be part of the study design

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Duplicate and Related Individuals Need to be Identified

- For duplicate samples
  - Only one can be retained
- For related individuals
  - PCA is performed first with unrelated individuals and related individuals are then projected onto the PCs of unrelated individuals
  - Mixed-models need to be used to analyze the data if related individuals are included*
    - Case-Control
      - Generalized linear mixed models (GLMM)
    - Quantitative traits
      - Linear mixed models (LMM)
  - If not type I error rates can be increased

*If only a few related individuals in sample, may wish to remove them or use LMM/GLMM to control type I errors. Must use LMM/GLMM if related individuals are included in the dataset. If possible, opt for LMM/GLMM since it can help to control type I error due to other types of structure in the data, even when no closely related individuals are included in the analysis.

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Identifying Duplicate and Related Individuals

- Duplicate and related individuals can be detected
  - By examining **Identity-by-State** (IBS) adjusted for allele frequencies (p-hat) between all pairs of individuals within a sample
  - Identify-by-descent (IBD) sharing can be estimated

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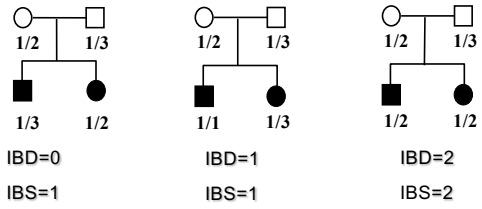
Identity by Descent (IBD)/Identity-by-State (IBS)



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IBD Sharing Estimated Pairwise for all Individuals in a Samples

- PLINK (Purcell et al. 2007)
- Uses sequence (or genotype array) data to check IBD
  - Prune markers to remove those in LD
    - e.g., r^2<0.1
- P-hat is calculated using the "population" allele frequency
- Used to approximates IBD sharing
- IBD is the number of alleles of alleles which are shared between a pair of individuals
  - Can either share 0, 1, and 2 alleles

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Identifying Duplicate and Related Individuals

- Monozygote twins and duplicate samples will share 100% of their alleles IBD
  - IBD=2 is 1.0 (can be lower due to genotyping error)
- Siblings and child-parent pairs will share 50% of their alleles IBD
  - For parent-child IBD=1 is 1.0 (IBD=0 is 0 & IBD=2 is 0)
  - For sibs IBD=1 is ~0.50 (IBD=0 is ~0.25 & IBD=2 is ~0.25)
    - For more distantly related individuals the IBD measure will be lower

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Identifying Duplicate and Related Individuals

- KING [Kinship-based INference for Gwas (*Manichaikul et al. 2010*)] can also be used to identify duplicate and related individuals
  - KING is more robust to population substructure and admixture
    - Prune markers for LD (e.g., r^2<0.1)
  - Provides kinship coefficients
    - Duplicate samples
      - Kinship coefficient equals 0.5
    - Siblings
      - Kinship coefficient equals 0.25

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UK Biobank Related Individuals > Kinship Coefficient 0.0625

White European

# of Relatives	# of relatives
1	86089
2	18491
3	3691
4	707
5	165
6	40
7	9
8	5
9	1
10	11
11	2
12	2
16	1
19	1
25	1
30	1
3985	1

African

# of relatives	# of individuals
1	715
2	153
3	26
4	10
5	3
6	5
7	5
8	4
9	1
10	4
11	2
13	3
17	2
19	3
20	2
21	1
23	1
.	.
.	.
.	.
390	1
391	1
393	1
396	1

Asian

# of relatives	# of individuals
1	743
2	115
3	33
4	4
5	4

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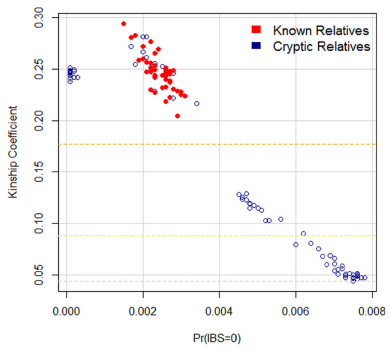
King Graphical Output



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Multiple Individuals observed that are distantly "Related"

- If individuals in sample come from different populations
  - e.g., individuals from the same population within the sample will have inflated p-hat values due to incorrect allele frequencies
    - Incorrectly appear to be related to each other
- "Relatedness" amongst many individuals can also be observed when batches are combined if they have different error rates
  - Individuals from the same batch appear to be related
- DNA contamination can cause "relatedness" between multiple individuals

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Principal Components Analysis (PCA) / Multidimensional Scaling (MDS)

- Can be used to identify outliers
- Population substructure
  - Individuals from different ancestry
    - e.g., African American samples included in samples of European Americans
- Batch effects
- Use a subset of markers which have been LD pruned
  - Only very low levels of LD between marker loci
    - e.g., $r^2<0.1$
  - MAF cutoff dependent on sample size
    - e.g MAF> 0.01
      - Can use lower MAF for large sample sizes

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Principal Components Analysis (PCA) / Multidimensional Scaling (MDS)

- Unrelated individuals are used to generate PC plots
  - Related individuals are projected onto to the PC plots
- Plot 1st component vs. 2nd component
  - Additional PCs should also be plotted
    - e.g.. PCs 1-10
- Mahalanobis distance can be used to determine outliers
  - e.g., <1

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PCA/MDS Can be Used to Identify Outliers

- Individuals of different ancestry
  - e.g., African American samples included with European Americans samples
  - Can use samples from HapMap/1000 genomes to help to determine the ancestry for samples that are outliers
    - Should not include HapMap/1000 genomes samples when calculating components to control for population substructure/admixture
- Batch effects

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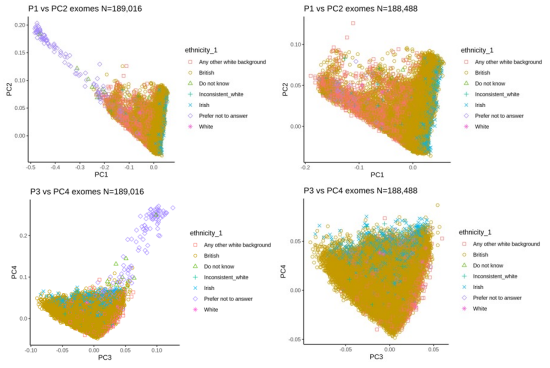
Principal Components Analysis Example



Exclusion of Outliers using Mahalanobis distance (0.997)

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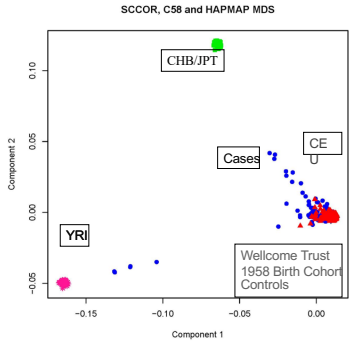
Detecting Outliers Using PCA and HapMap Sample



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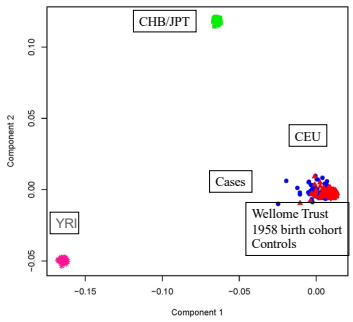
Detecting Outliers Using PCA and HapMap Sample



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Detecting Genotyping Error – Examining HWE

- Testing for deviations from HWE not very powerful to detect genotyping errors
- The power to detect deviations from HWE dependent on:
  - Error rates
  - Underlying error model
    - Random
    - Heterozygous genotypes -> homozygous genotypes
    - Homozygous genotypes ->Heterozygous genotype
  - Minor allele frequencies (MAF)

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Detecting Genotyping Error – Examining HWE

- Controls and Cases are evaluated separately
  - Deviation found only in cases can be due to an association
- Test for deviation from HWE only in samples of the same ancestry
  - Population substructure can introduce deviations from HWE
- Do not include related individuals when testing for deviations from HWE
  - Can cause deviations from HWE

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Detecting Genotyping Error – Examining HWE

- What criterion is used to remove variants due to a deviation from HWE
  - GWAS studies have used 5.0×10^{-7} to 5.0×10^{-15}
- Quantitative Traits
  - Caution should be used removing markers which deviate from HWE may be due to an association
    - Remove markers with extreme deviations from HWE and Flag markers with less extreme deviations from HWE
- When performing imputation need to be more stringent in removing variants which deviate from HWE

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Sequence Data QC Overview

- Remove variant sites that fail VQSR
- Remove genotypes with low DP, GQ scores, etc.
- Remove variant sites with large percent of missing data
- Remove samples with missing large percent of missing data
- Evaluate genetic sex of individuals based upon X and Y chromosomal data
  - Sample mix-ups
  - Individuals with Turner or Klinefelter Syndrome

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Sequence Data QC Overview

- Evaluate samples for cryptically related individuals and duplicates
  - Use variants which have been pruned for LD
    - e.g., $r^2<0.1$
  - King or Plink algorithm
    - Always remove duplicate individuals
      - Retaining only one in the sample
    - If sample includes related samples use linear mix models (LMM)/Generalized LMM (GLMM) to control for relatedness
      - Best to perform even for data without related individuals
    - If only a few related individuals can retain only one individual of a relative group if not using LMM or GLMM

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Sequence Data QC Overview

- Detection of sample outliers
  - Perform principal components analysis (PCA) or multidimensional scaling (MDS) to detect outliers
    - Use variants pruned for LD
      - e.g., $r^2<0.1$
    - Use unrelated individuals and then project related individuals onto the PCs
- Due to population substructure/admixture and batch effects
- Remove effects by
  - Additional QC
  - Removal of outliers (can be determined by Mahalanobis distance) and\or
  - Inclusion of MDS or PCA components in the association analysis

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Sequence Data QC Overview

- Remove/flag variant sites that deviate from HWE in controls
  - HWE should be only be tested in unrelated individuals from the same population
- Post Analysis - Quantile-Quantile (QQ) plots
  - To evaluate uncontrolled batch effects and population substructure/admixture

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QQ Plots - Genome Wide Association Diagnosis

- Thousands of variants/genes are tested simultaneously
- The p-values of neutral markers follow the uniform distribution
- If there are systematic biases, e.g., population substructure, genotyping errors, there will be a deviation from the uniform distribution
- QQ plots offers an intuitive way to visually detect biases
- Observed p-values are ordered from largest to smallest and their $-\log_{10}(p)$ values are plotted on the y axis and the expected $-\log_{10}(p)$ values under the null (uniform distribution) on the x axis

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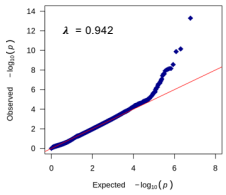
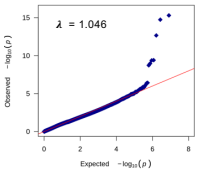
QQ Plot of Exome Wide P Values UK Biobank 200K



Hearing aid users

Case N= 6,436
Controls N= 96,601



Problem hearing with background noise

Cases N=65,660
Controls N= 96,601

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Genomic Inflation Factor to Evaluate Inflation of the Test Statistic

- Genomic Inflation Factor (GIF): ratio of the median of the test statistics to expected median and is usually represented as λ
  - No inflation of the test statistic λ=1
  - Inflation λ>1
  - Deflation λ<1
    - Can be observed when a study is underpowered
- Problematic to examine the mean of the test statistic
  - Can be large if many variants are associated
    - Particularly if they have very small p-values
    - Should not be used

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Phenotype	Covariate	Mean Chi-Square	GIF (λ)
BP		1.23829	1.16932
BP	Age	1.24119	1.18025
BP	**Age-EV1**	1.09471	**1**
BP	Age-EV2	1.0881	1
BP	Age-EV4	1.08385	1
BP	Age-EV10	1.09582	1.00402
BPI		1.14931	1.08921
BPI	Age	1.15139	1.08113
BPI	Age-EV1	1.05079	1.01148
BPI	**Age-EV2**	1.0428	**1**
BPI	Age-EV4	1.04204	1
BPI	Age-EV10	1.05421	1.01724
BPII		1.17283	1.25664
BPII	Age	1.17583	1.26996
BPII	Age-EV1	1.09874	1.15065
BPII	Age-EV2	1.09904	1.16425
BPII	Age-EV4	1.09502	1.14609
BPII	Age-EV10	1.10046	1.1418
BPII	Sex,Age-EV1	1.05958	1.06424
BPII	**Sex,Age-EV4**	1.05817	**1.05323**
BPII	Sex,Age-EV10	1.06338	1.05581

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Example Project Description

- 1,667 Samples
- Seven cohorts
- Two sequencing centers
  - Center 1
    - Two capture arrays
      - NimbleGen V2Refseq 2010 (CA1): 1082
        - » Batch 1 and 3
      - NimbleGen bigexome 2011 (CA2): 234
        - » Batch 2
  - Center 2
    - One capture array
      - Agilent SureSelect
        - » Batch 4
- Four batches
- No intentional duplicate samples

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Example Project Description

- Intersection of the three capture arrays used
  - NimbleGen V2Refseq 2010
    - Batch 1 and 3
  - NimbleGen bigexome 2011
    - Batch 2
  - Agilent Sure Select
    - Batch 4
- Sequencing machine
  - Illumina HiSeq
- Sequence alignment
  - BWA
- Multi-sample variant calling
  - GATK

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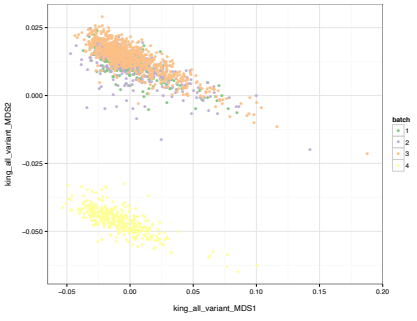
MDS First 2 Components Before QC*



*After VQSR

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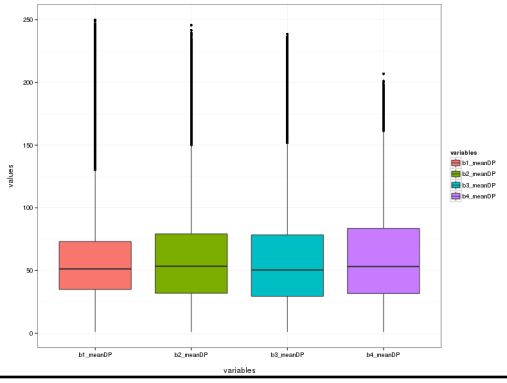
Mean GP (genotype) by Batch



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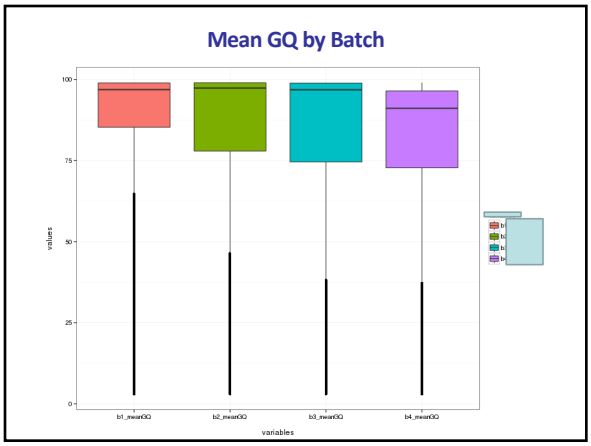
Mean GQ by Batch



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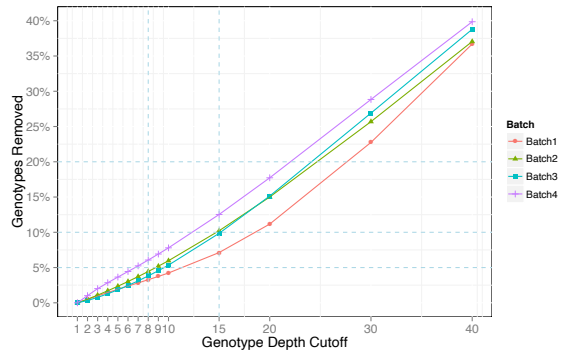
Genotypes Removed by DP (genotype) Cut-off by Batch



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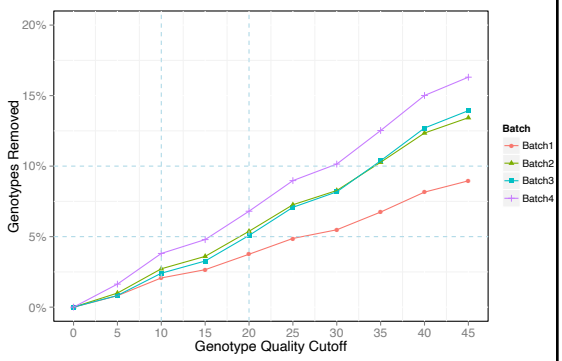
Genotypes Removed by GQ Cut-offs by Batch



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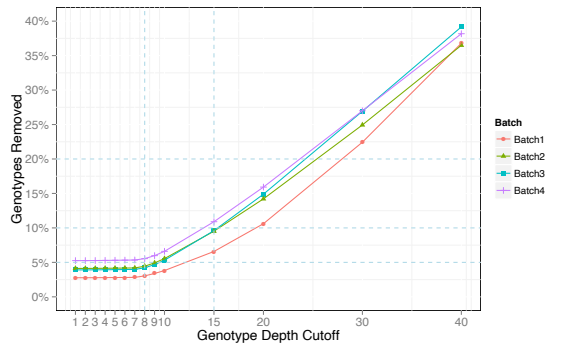
Genotypes Removed by DP (genotype) Cut-off by Batch (First removing genotypes with GQ ≤ 20)



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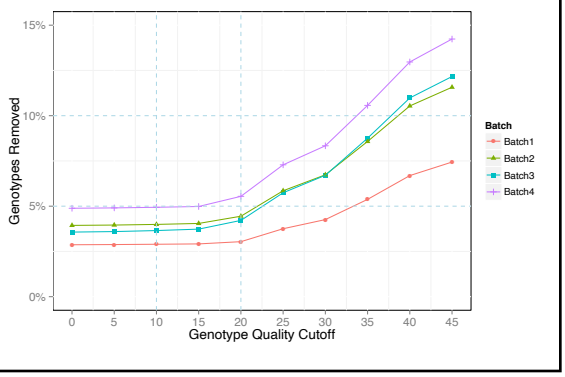
Genotypes Removed by GQ Cut-offs by Batch (First removing genotypes with a DP≤8)



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Missing Rate Criteria & Sites Removed

	Variant sites removed if missing >10% of their genotypes	Variant sites removed if missing >5% of their genotypes
	Percent of genotype data removed	
Before QC*	2.5%	3.9%
After QC	12.9%	18.3%

Variant sites missing >10% of their data were removed

*After VQSR

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Ti/Tv Ratios during QC Process

	Known	Novel	All
Before VQSR	2.95 ± 0.05	1.18 ± 0.29	2.86 ± 0.07
Before additional QC	3.12 ± 0.03	2.01 ± 0.32	3.11 ± 0.03
Genotype QC DP≤8, GQ≤20	3.18 ± 0.04	2.10 ±0.32	3.16 ± 0.03
Remove sites missing >10% genotypes	3.39 ± 0.04	2.42 ± 0.52	3.39 ± 0.04
Remove batch specific novel sites ≥2 N=17,835	3.39 ± 0.04	2.41 ± 0.53	3.39 ± 0.04
Remove sites deviating from HWE $p \leq 5 \times 10^{-8}$ N=4,414	3.41 ± 0.04	2.39 ± 0.54	3.40 ± 0.04

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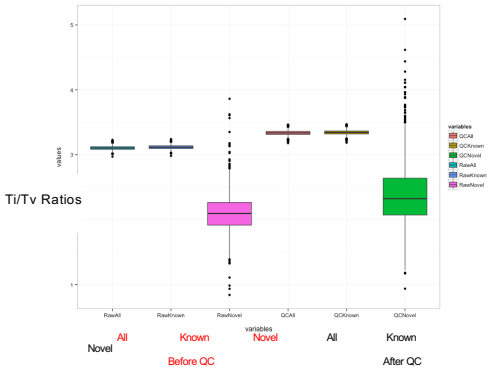
Ti/Tv Ratios by Individual Before and After QC



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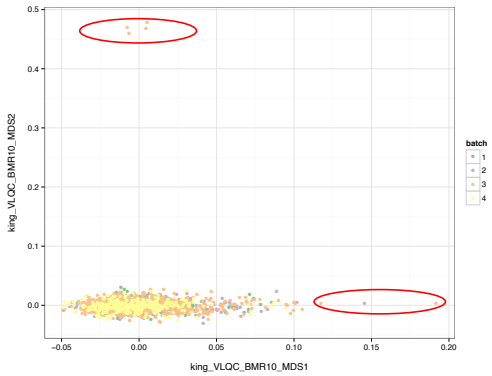
MDS First 2 Components After QC



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Sequence Data QC

- Batch effects can sometimes be removed with additional QC
- Extreme outliers should be removed
- Additionally, MDS\PCA components can be included in the analysis to control for population substructure\admixture and batch effects
  - Unless correlated with the outcome (phenotype)
  - The MDS or PCA components should be recalculated after QC only including those samples included in the analysis
- Batch (dummy coding) may be included as a covariate in the analysis
  - Unless correlated with the outcome (phenotype)

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Convenience Controls

- Can reduce the cost of a study
- Genotype data
- Type I error can be increased
  - Ascertainment from different population
  - Differential genotyping error
    - Even if performed at the same facility
- Proper QC can reduce or remove biases

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Convenience Controls–Sequence Data

- Obtain BAM files and recall cases and control together
  - Can still have differential errors between cases and controls
  - Check variant frequency by variant types in cases and control
    - Synonymous variants should have the same frequencies
    - Would not expect large differences in numbers of variants between cases and controls
- For single variants can compare difference in frequencies with gnomAD but is problematic
  - Differences in frequencies can be due to differences in ancestry and/or sequencing errors
  - Cannot adjust for confounders
    - e.g., sex, population substructure/admixture
- Don't perform an aggregate test using frequency information obtained from databases, e.g., gnomAD, TOPMed Bravo

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Genotype Array Data
Genotype Data QC – Population Based Studies

- Initially remove DNA samples from individuals who are missing >10% or their genotype data
- For variant sites with a minor allele frequency (MAF)≥0.05
  - Remove variants sites missing >5% of their genotype data
- For variant sites with a MAF<5%
  - Remove variant sites missing > 1% of their genotype data
- The genotypes for variant sites with missing data may have higher genotype error rates

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Order of Data Cleaning-Genotype Array Data

- Remove samples missing >10% genotype data
- Remove SNPs with missing genotype data
  - If minor allele frequency >5%
    - Remove markers with >5% missing genotypes
  - If minor allele frequency <5%
    - Remove markers with >1% missing genotypes
- Remove samples missing >3% genotype calls
- Check genetic sex of individuals based on X-chromosome markers & Y chromosome marker data (if available)
  - Remove individual whose reported gender/sex is inconsistent with genetic data
    - Could be due to a sample mix-up
- Check for cryptic duplicates and related individuals
  - Used "trimmed data set of markers which are not in LD
    - e.g. r2<0.1
  - Remove duplicate samples

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Order of Data Cleaning-Genotype Array

- Perform PCA or MDS to check for outliers
  - Use trimmed data set of markers which are not in LD
    - e.g., r2<0.1
  - First with unrelated individuals and then project related individuals on the components
  - Remove outliers from data
    - e.g., Mahalanobis distance
- Check for deviations from HWE
  - Separately in cases and controls
  - Only unrelated individuals
  - If more than one ancestry group
    - Separately for each ancestry group
      - As determined via PCA or MDS
- Examine QQ plots for potential problems with the data
  - e.g., not controlling adequately for population admixture

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