Lecture 2: Quality Control

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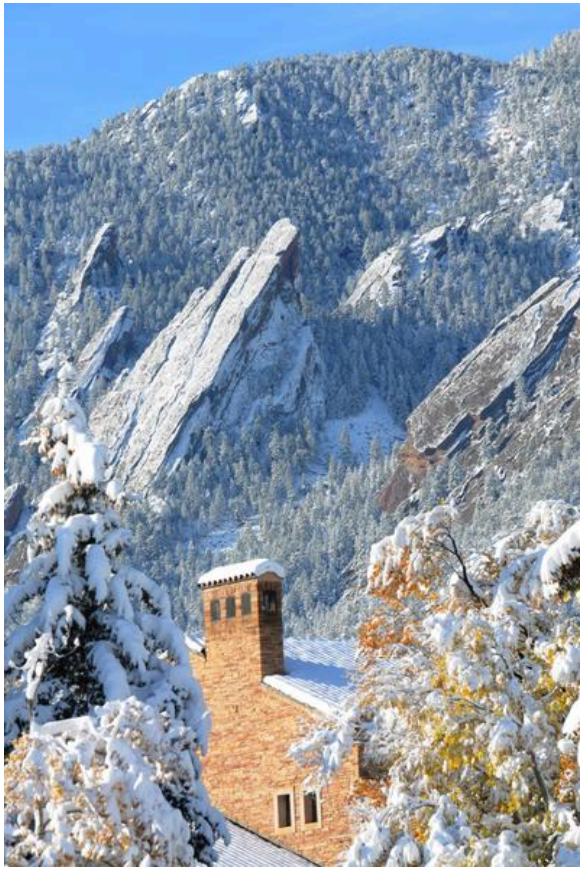
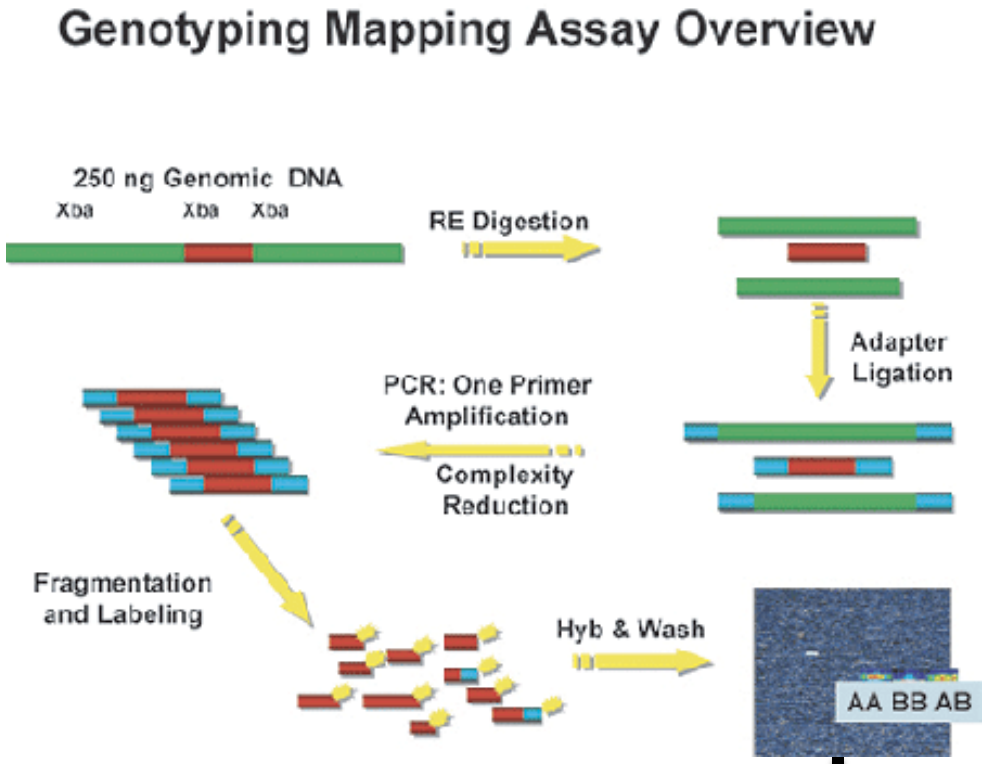


QC Overview

- Genotyping
- Quality Control for Samples
  - Genotyping Call Rate and Heterozygosity
  - Sex Check
  - Relatedness Measures
- Quality Control for Genetic Markers
  - Missing Data Rate
  - Hardy-Weinberg Equilibrium
- Genetic Ancestry

Genotyping

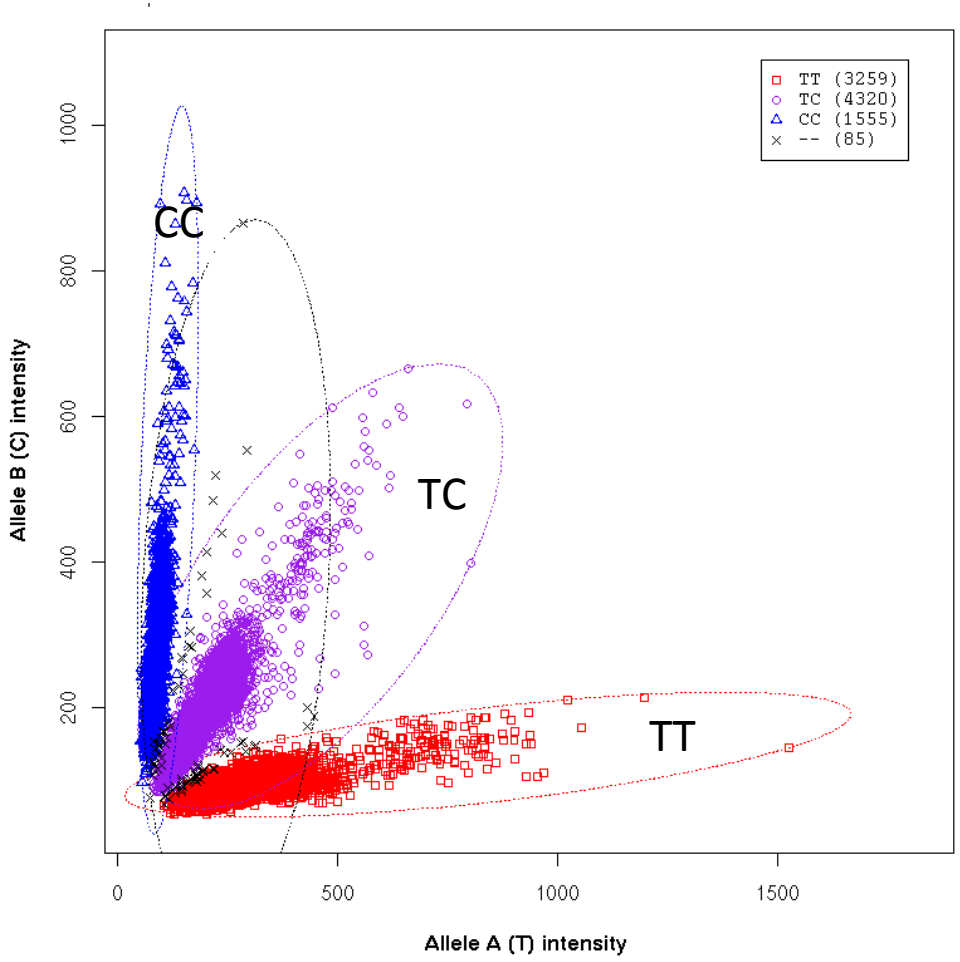
Genotyping



Affymetrix System

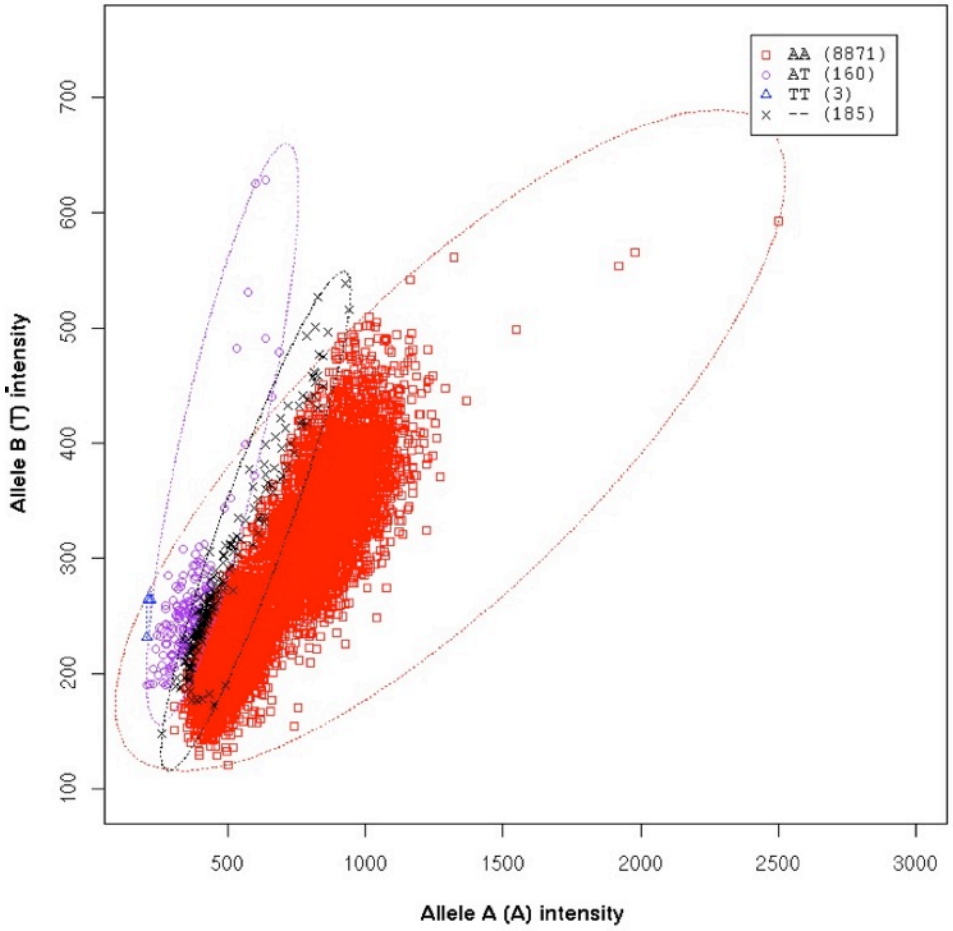
Intensity Files

Intensity to Genotypes



When things go well…

Intensity to Genotypes



When things **don't** go well…

Intensity to Genotypes

- Can’t possibly visibly inspect ALL plots
- Scrutinize any significant or “interesting” associations by going back to the plots
- Just something to be aware of…

Strand Orientation

- Strand unambiguous
  - A/G alleles -> T/C alleles
- Strand ambiguous
  - A/T and C/G

Strand Reporting

- Probe/Target
  - Generically as A/B
  - Illumina/Affymetrix
- Plus (+) / Minus (-)
  - 5’ end at the tip of the short arm = plus
  - 1000 genomes, HapMap
- FWD / REV
  - Based upon submitted flanking DNA sequence
  - dbSNP (NCBI)
- TOP/BOT
  - Based upon flanking sequences
  - Illumina

Strand orientation

http://browser.1000genomes.org

Example:

- rs932402

Raw files (Illumina GenomeStudio)

```
[Header]
GSGT Version    1.6.3
Processing Date 6/28/2011 12:25 PM
Content         HumanOmni1-Quad_v1-0_B.bpm
Num SNPs        1140419
Total SNPs      1140419
Num Samples     672
Total Samples   672
File    1 of 17
[Data]
SNP Name        Sample ID       Allele1 - Top   Allele2 - Top       GC Score
200006  1481-01A_30011425_A01   G                   G               0.8273
200052  1481-01A_30011425_A01   T                   T               0.9487
200053  1481-01A_30011425_A01   A                   A               0.6645
200070  1481-01A_30011425_A01   G                   G               0.9347
200078  1481-01A_30011425_A01   C                   G               0.6885
200087  1481-01A_30011425_A01   A                   A               0.8085
```

GC Score

- GenCall Score
  - Confidence “measure” on the quality of genotypes
  - GC < 0.15 are typically set to “missing”
    - By individual for each SNP

Quality Control for Samples

Genotyping Call Rate

- Per sample (individual) rate
- Number of non-missing genotypes divided by the total number of successfully genotyped markers
- Typical thresholds vary
  - 95-97% call rate

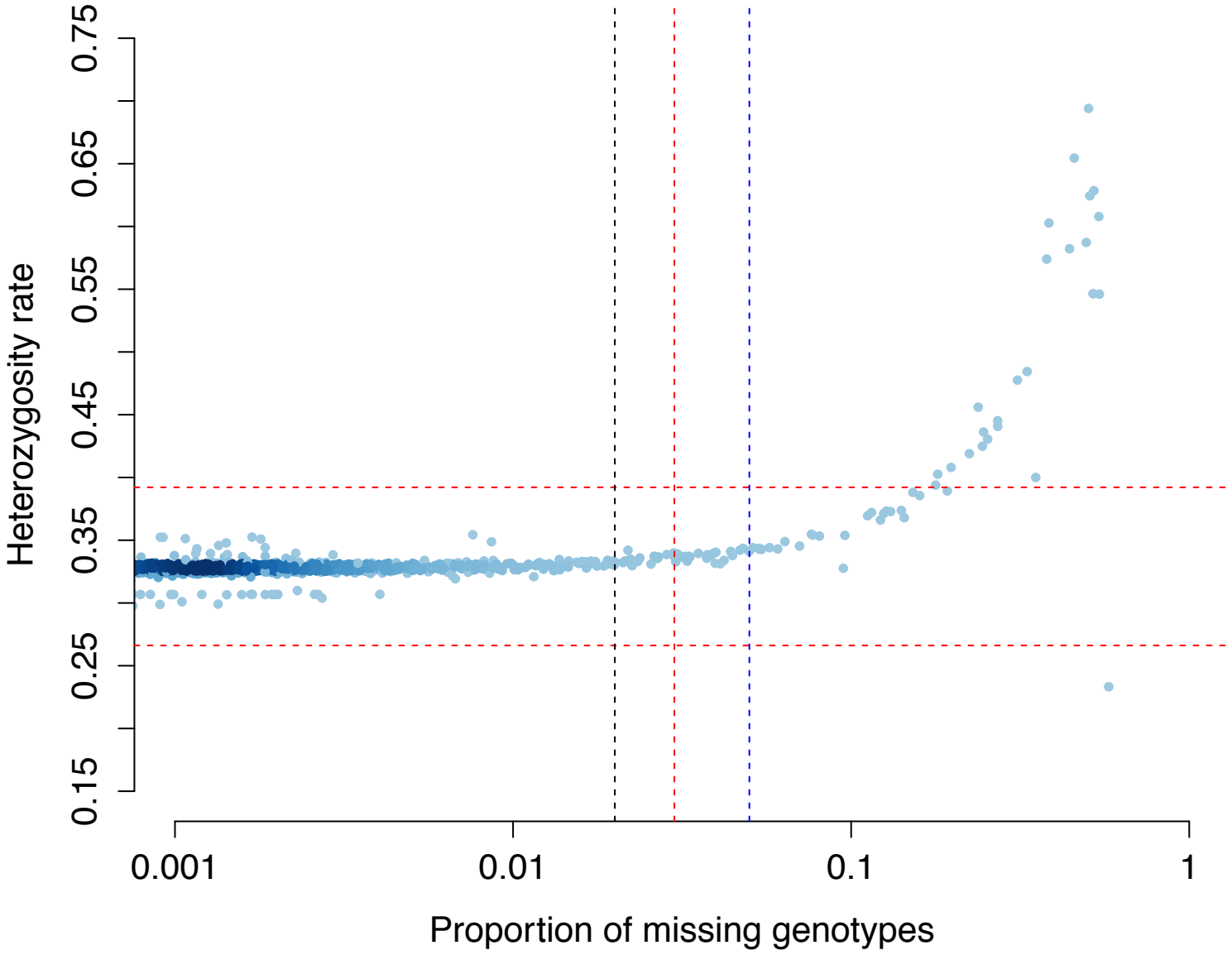
Heterozygosity Rate

- Per sample (individual) rate
- Number of (non-missing – homozygous) genotypes divided by number of homozygous genotypes
- Excess heterozygosity
  - Possible sample contamination
- Less than expected heterozygosity
  - Possibly inbreeding

Graphical inspection

- Often, genotyping call rate and heterozygosity rate are plotted to visualize data

Genotyping Call Rate by Heterozygosity

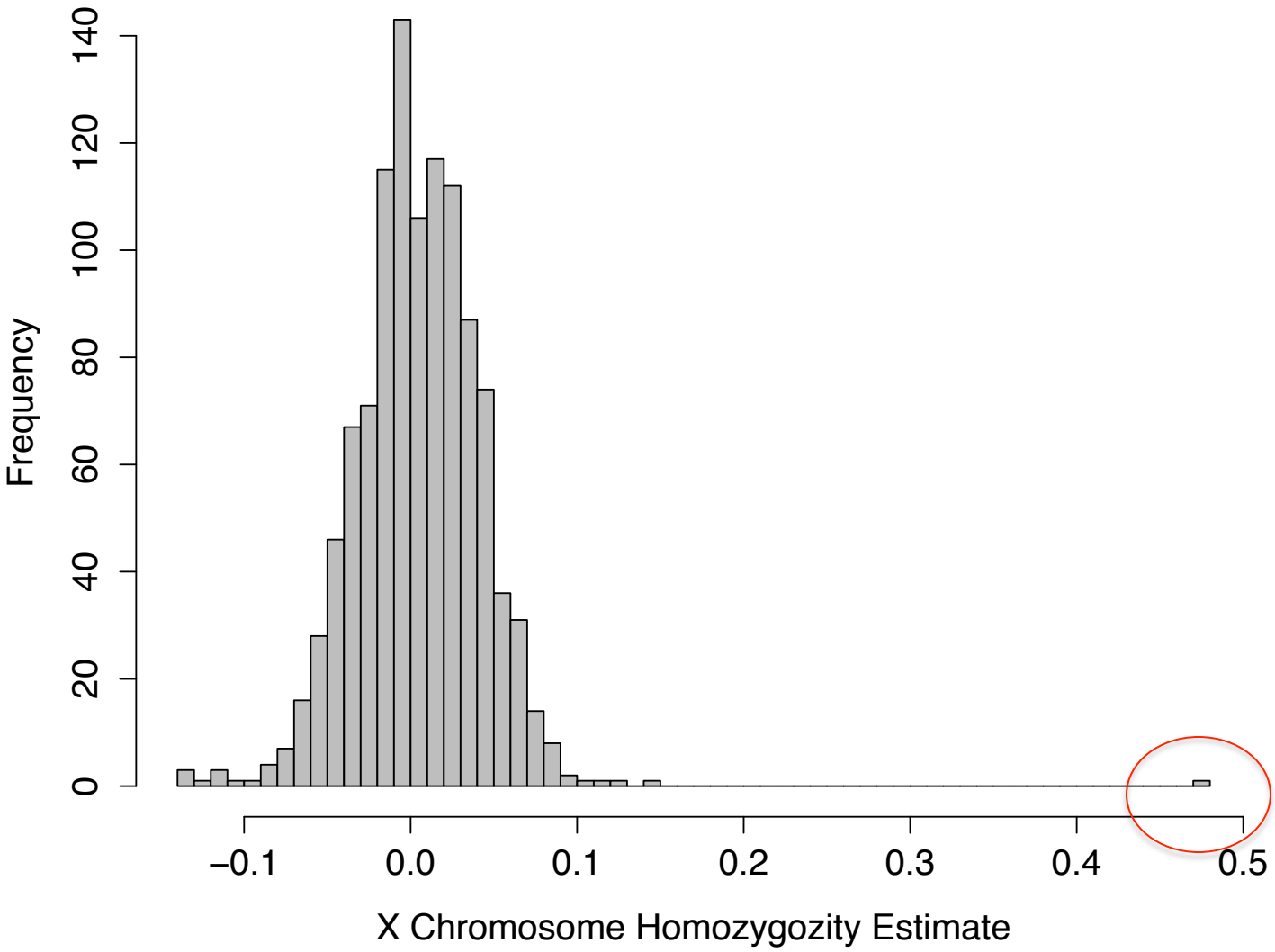


Sex Checks

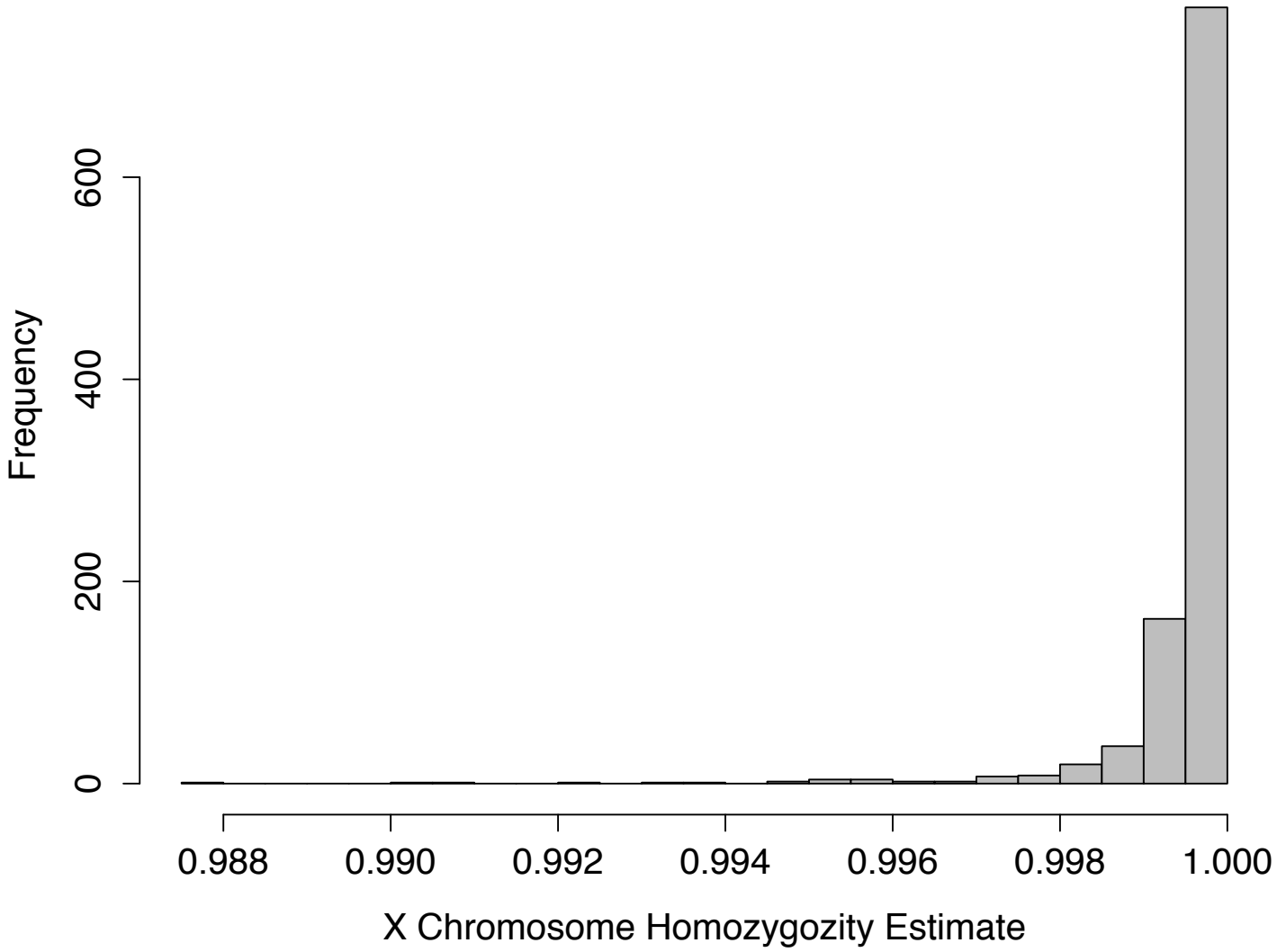
X-Chromosome Homozygosity

- Inbreeding estimate (F)
  - Theoretical range between -1 and 1
- E(Female) ~ 0
  - Two copies of X
- E(Male) ~ 1
  - One copy of X
- Can be used to identify miscoded sex or sample mix-ups, etc.

Females


All Female Samples
Frequency
0
20
40
60
80
100
120
140
−0.1
0.0
0.1
0.2
0.3
0.4
0.5
X Chromosome Homozygozity Estimate

Males


All Male Samples
Frequency
0
200
400
600
0.988
0.990
0.992
0.994
0.996
0.998
1.000
X Chromosome Homozygozity Estimate

Genetic Relatedness

Identity by State (IBS)

ac bd

How many alleles are in common?

IBS = 0

Identity by State (IBS)

ac ad

How many alleles are in common?

IBS = 1

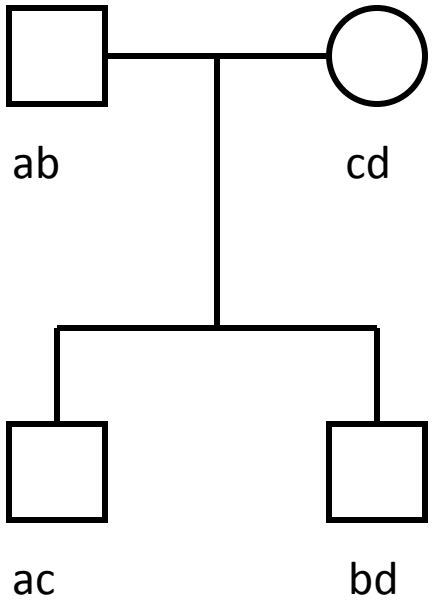
Identity by State (IBS)

ac ac

How many alleles are in common?

IBS = 2

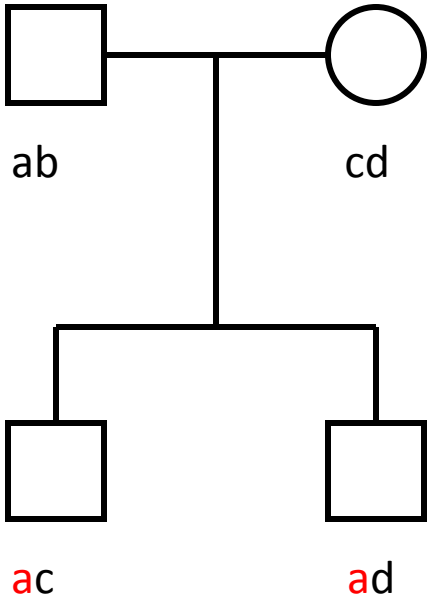
Identity by Descent (IBD)



How many alleles are common by descent?

IBD = 0

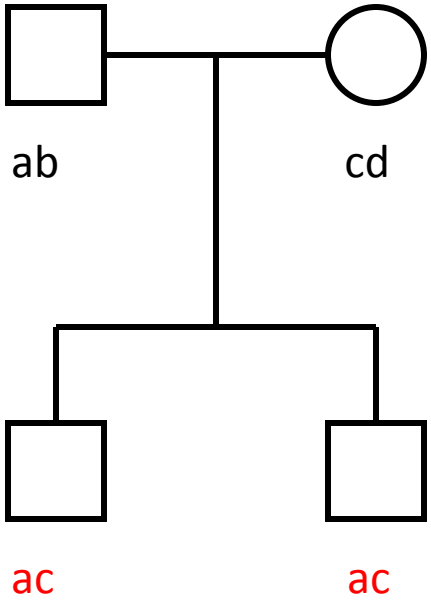
Identity by Descent (IBD)



How many alleles are common by descent?

IBD = 1

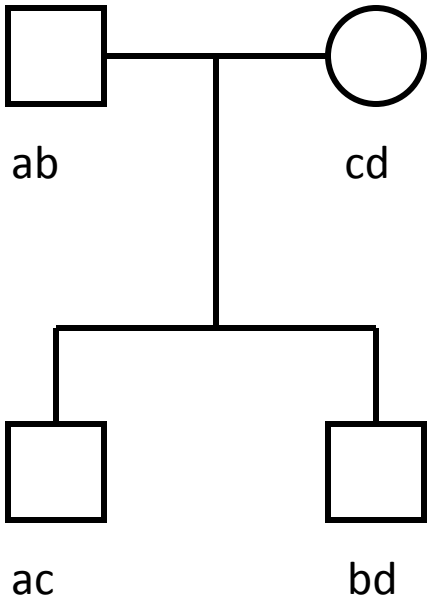
Identity by Descent (IBD)



How many alleles are common by descent?

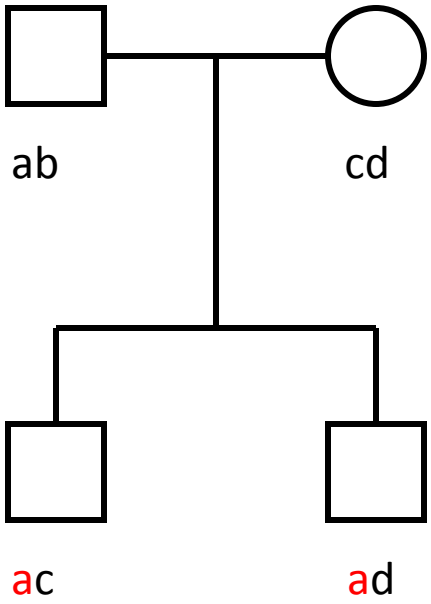
IBD = 2

IBS and IBD



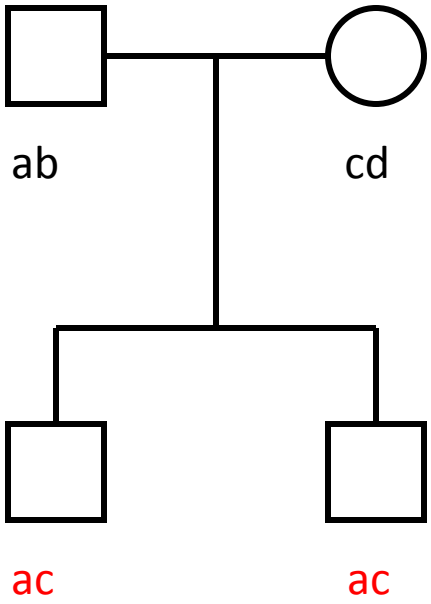
IBS = 0
IBD = 0

IBS and IBD



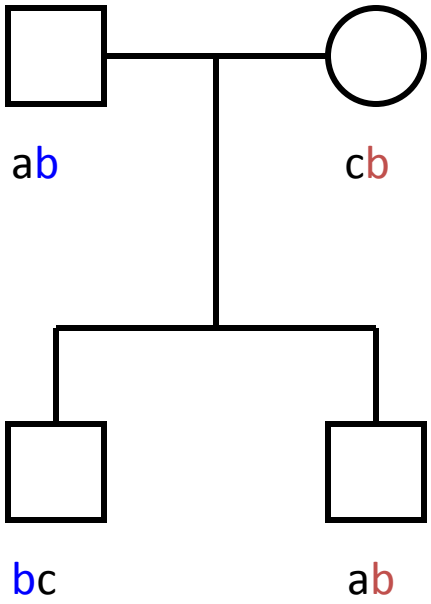
IBS = 1
IBD = 1

IBS and IBD



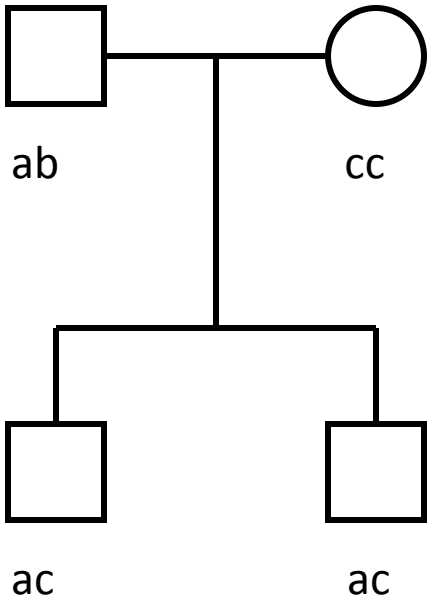
IBS = 2
IBD = 2

Ambiguous IBD



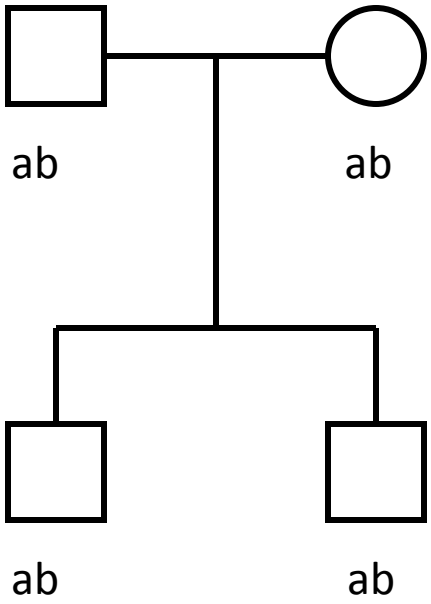
IBS = 1
IBD = 0

Ambiguous IBD



IBS = 2
IBD = ?

Ambiguous IBD



IBS = 2
IBD = ?

IBD Probabilities

	Probability of Sharing IBD Alleles		
Relative Pair	π_0	π_1	π_2
MZ Twins	*0*	*0*	*1*
Full Sibs	*0.25*	*0.50*	*0.25*
Parent-Offspring	*0*	*1*	*0*
First Cousin	*0.75*	*0.25*	*0*
Grandparent-Grandchild	*0.50*	*0.50*	*0*
Half-Sibs	*0.50*	*0.50*	*0*
Avuncular	*0.50*	*0.50*	*0*

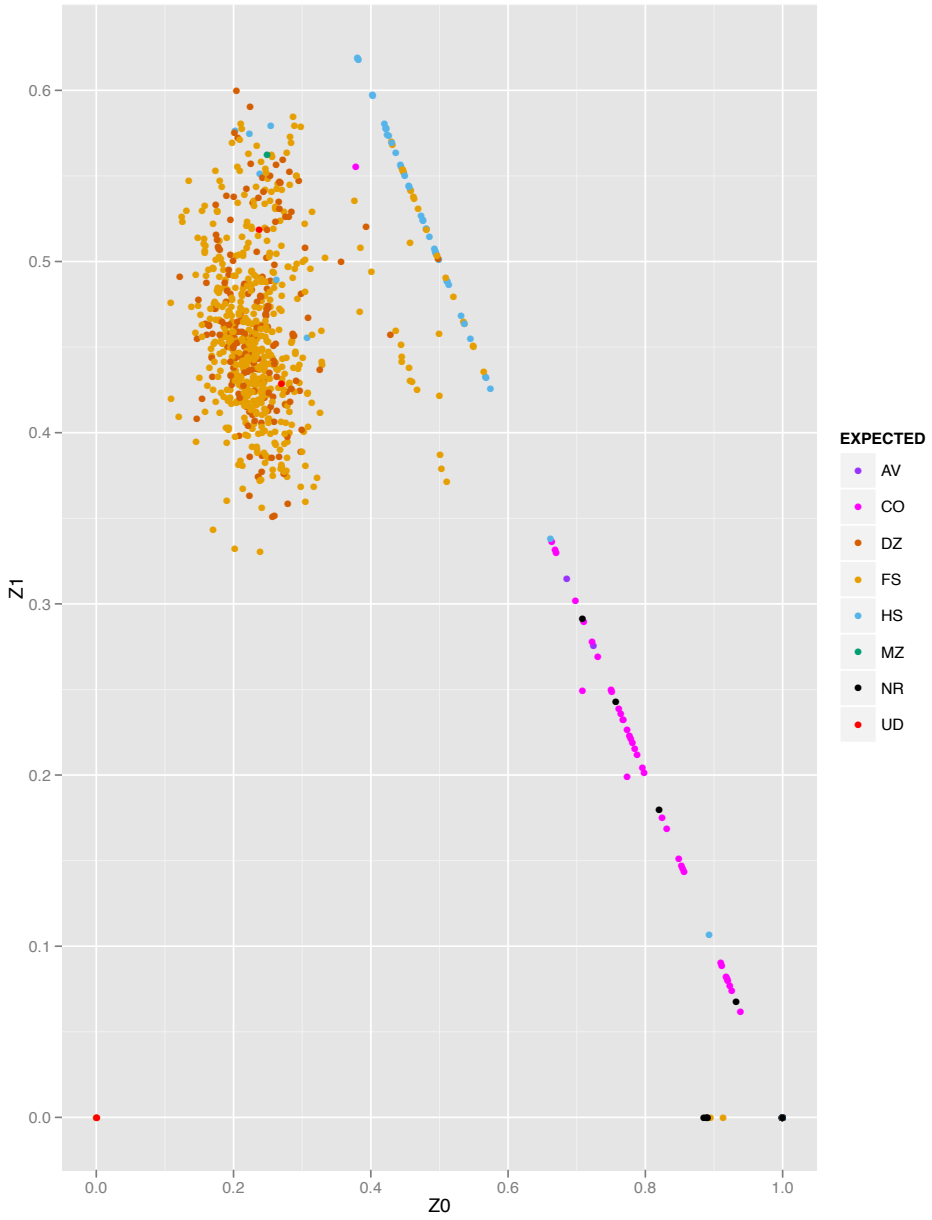
Checking Relationships

- Mean IBD = [(Z1/2) + Z2]
- "PI_HAT" in PLINK
  - PI_HAT = 1 for duplicates/MZ twin pairs
  - PI_HAT = 0.5 for sibling pairs
  - Etc.

Be warned!

- Using PLINK for relationships (PI_HAT) can be biased with a diverse sample
  - Two people may appear related (PI_HAT > 0.10) but are merely of the same ethnic group
- Alternatives
  - Kinship-based Inference for GWAS
    - KING
  - Relatedness Estimation in Admixed Populations
    - REAP

Checking Relationships



Using Relatedness Measures

- Identify related subjects
  - When they should be unrelated
- Identify unrelated subjects
  - When they should be related
- Duplicates
  - IBS/PI_HAT =1

Quality Control for Genetic Markers

Sample Removal

- Low genotyping call rate: < 97%
- High/low heterozygozity rate
  - (visual inspection)
- Unresolved sex-check
- Duplicate samples (relationship measures)

Missing Data Rate (Markers)

- Per marker
- Number of samples missing the marker genotype divided by the total number of successfully genotyped samples at that marker
- Typically, markers with missing data > 5% are removed
  - This is flexible however

Missing Data Rate Patterns

- Chi-square test
  - Cases vs Controls
  - Samples from population X vs population Y
  - Blood vs Saliva
  - Etc.

Hardy Weinberg Assumptions

- Diploid organisms
- Infinite population size
- Non-overlapping generations
- Random mating
- No selection, mutation or migration

Testing for HWE

1. Calculate the allele frequency (p)
   - Using observed genotype counts

2. Calculate the expected genotype counts
   - Using the allele frequency (p)

3. Compare the observed to the expected counts
   - χ^2 test

HWE Example

- Observed Genotypes

Genotype	*GG*	*Gg*	*gg*
Frequency	*12*	*2*	*8*

Step I

- Observed Genotypes

Genotype	*GG*	*Gg*	*gg*
Frequency	*12*	*2*	*8*

1. *Calculate the allele frequency (p):*

$$p = \frac{2(12)+2}{2(22)} = 0.59$$

Step II

- Observed Genotypes

Genotype	*GG*	*Gg*	*gg*
Frequency	*12*	*2*	*8*

2. Calculate the expected genotype counts:

$$E(GG) = np^2 = 22(0.59^2) = 7.66$$

$$E(Gg) = n2pq = 22(0.59)(1-0.59) = 10.64$$

$$E(gg) = nq^2 = 22((1-0.59)^2 = 3.68$$

Step III

- Observed Genotypes

Genotype	*GG*	*Gg*	*gg*
Frequency	*12*	*2*	*8*

3. Compare the observed and expected counts:

$$\chi_1^2 = \frac{(12-7.66)^2}{7.66} + \frac{(2-10.64)^2}{10.64} + \frac{(8-3.69)^2}{3.69} = 14.50$$

REJECT THE NULL!

Reasons for HW Deviations

- Genotyping Error
- Subdivided Population
  - Excess homozygotes = “Wahlund Effect”
- Any violations of the HW assumptions

HWE on the Genome-Wide Scale

- Threshold for significance
  - 10^{-3} to 10^{-6}

Marker Removal

- Missing Data > 5%
- Missing data rate associated with case status
- HWE test p-value < 0.001
- No reliable map location
- Monomorphic (no variation)
- Low frequency
  - For example, < 1% or < 5%

Genetic Ancestry

Genetic Ancestry

- Falls under QC to an extent, but also used in the analysis
- Most ancestry measures are based upon "relatedness" estimation
  - IBS, IBD
- Use pair-wise relationship measures to capture population level information

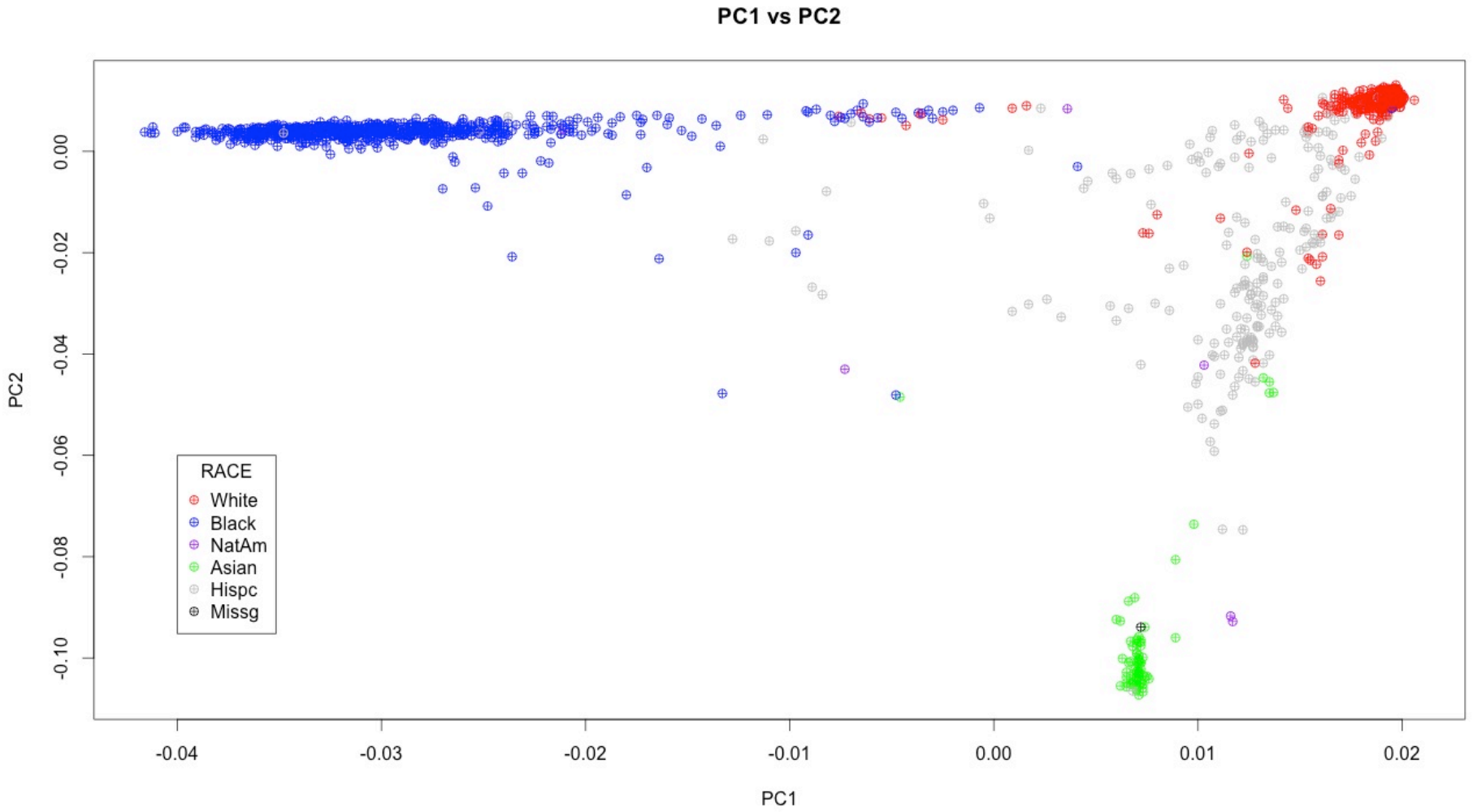
Genetic Ancestry Tools

- PLINK
  - Multidimensional Scaling (MDS)
- Eigenstrat
  - Principal Components
- STRUCTURE
  - Cluster-based algorithm
- ADMIXTURE
  - ML approach (similar to STRUCTURE)

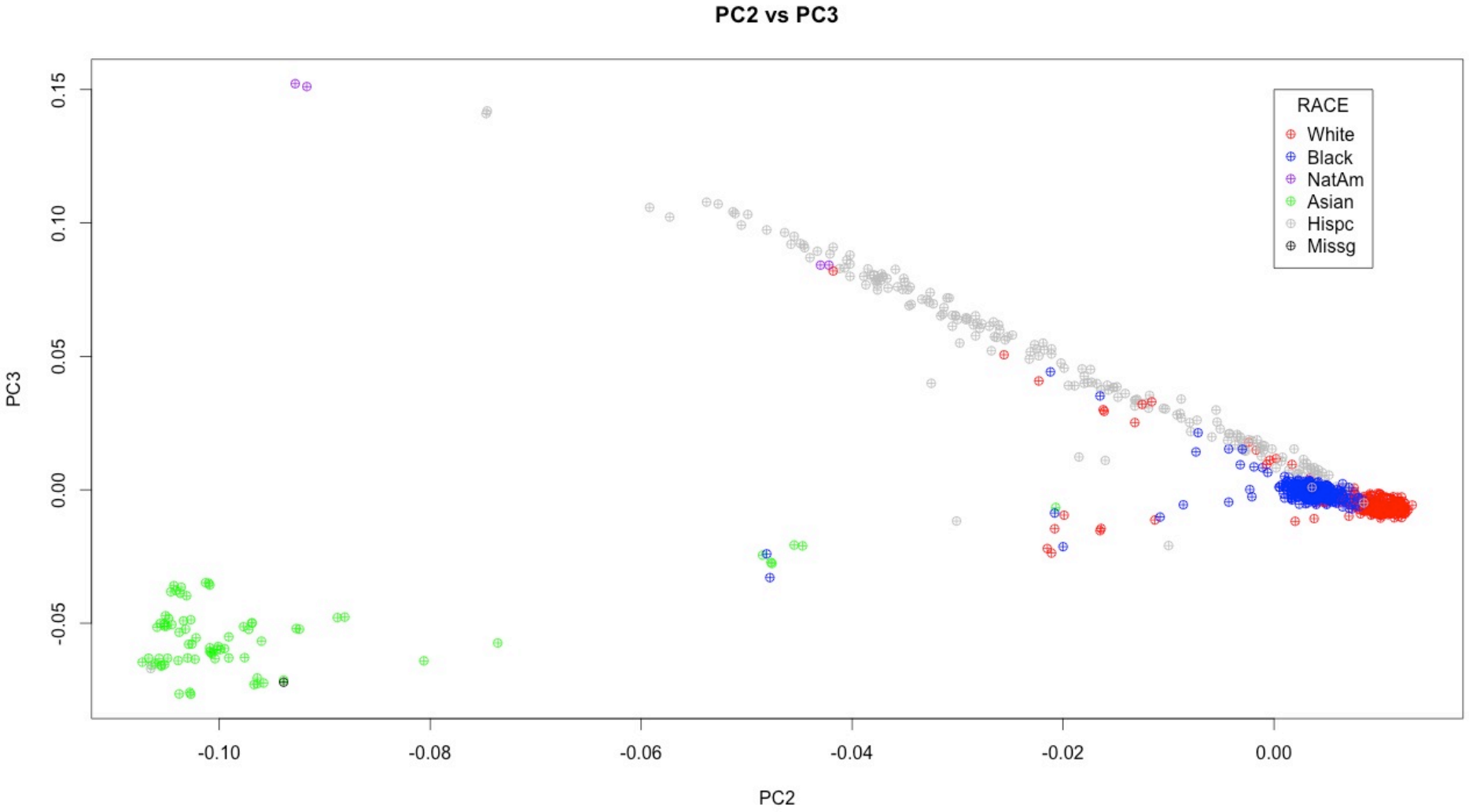
Using PLINK for Ancestry

- MDS Approach
- Using IBS/IBD information
- Generate any number of principal coordinates
  - Typically, at least 10 (some up to 20)

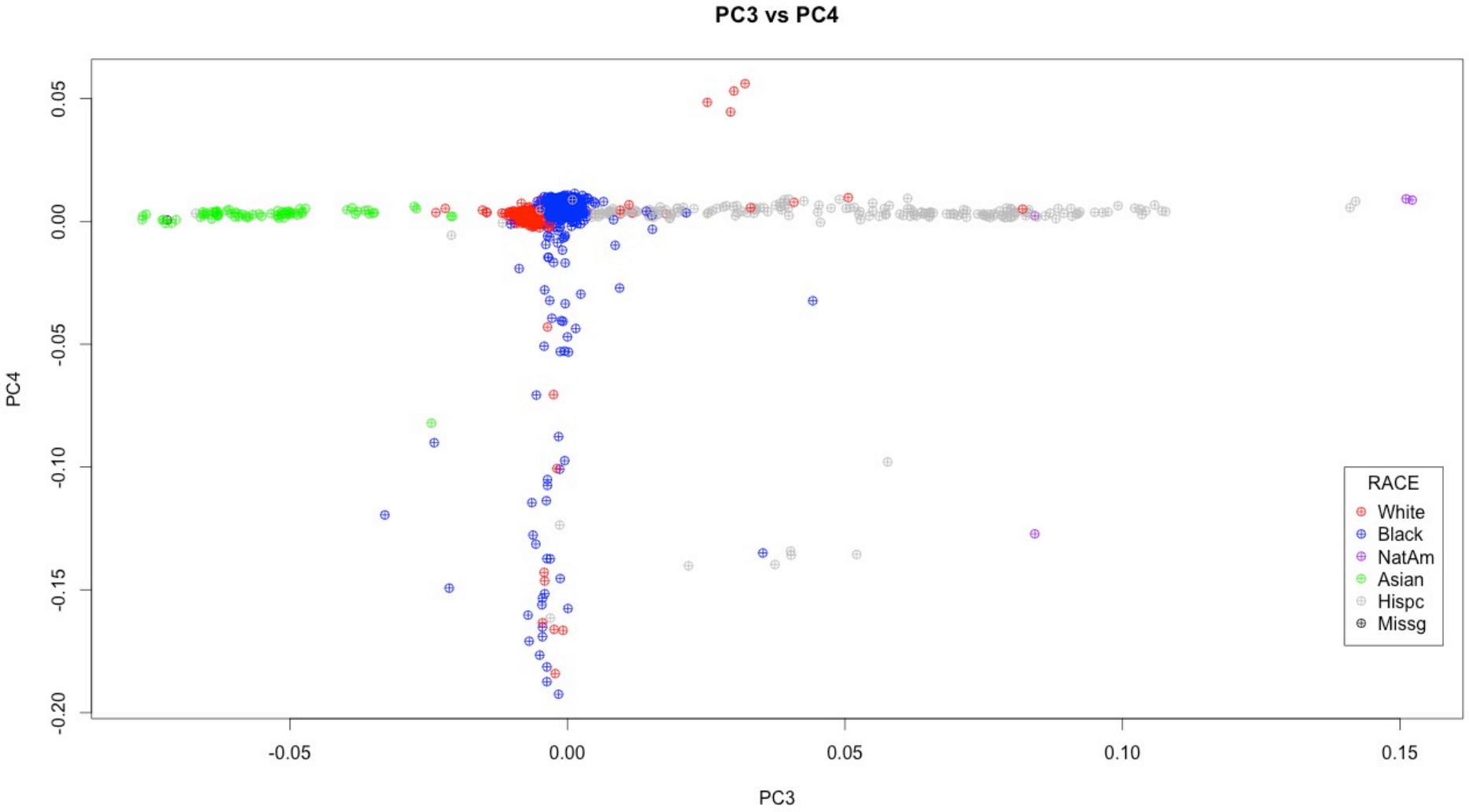
MDS Plot


PC1 vs PC2
PC2
PC1
0.00
-0.02
-0.04
-0.06
-0.08
-0.10
-0.04
-0.03
-0.02
-0.01
0.00
0.01
0.02
RACE
White
Black
NatAm
Asian
Hispc
Missg

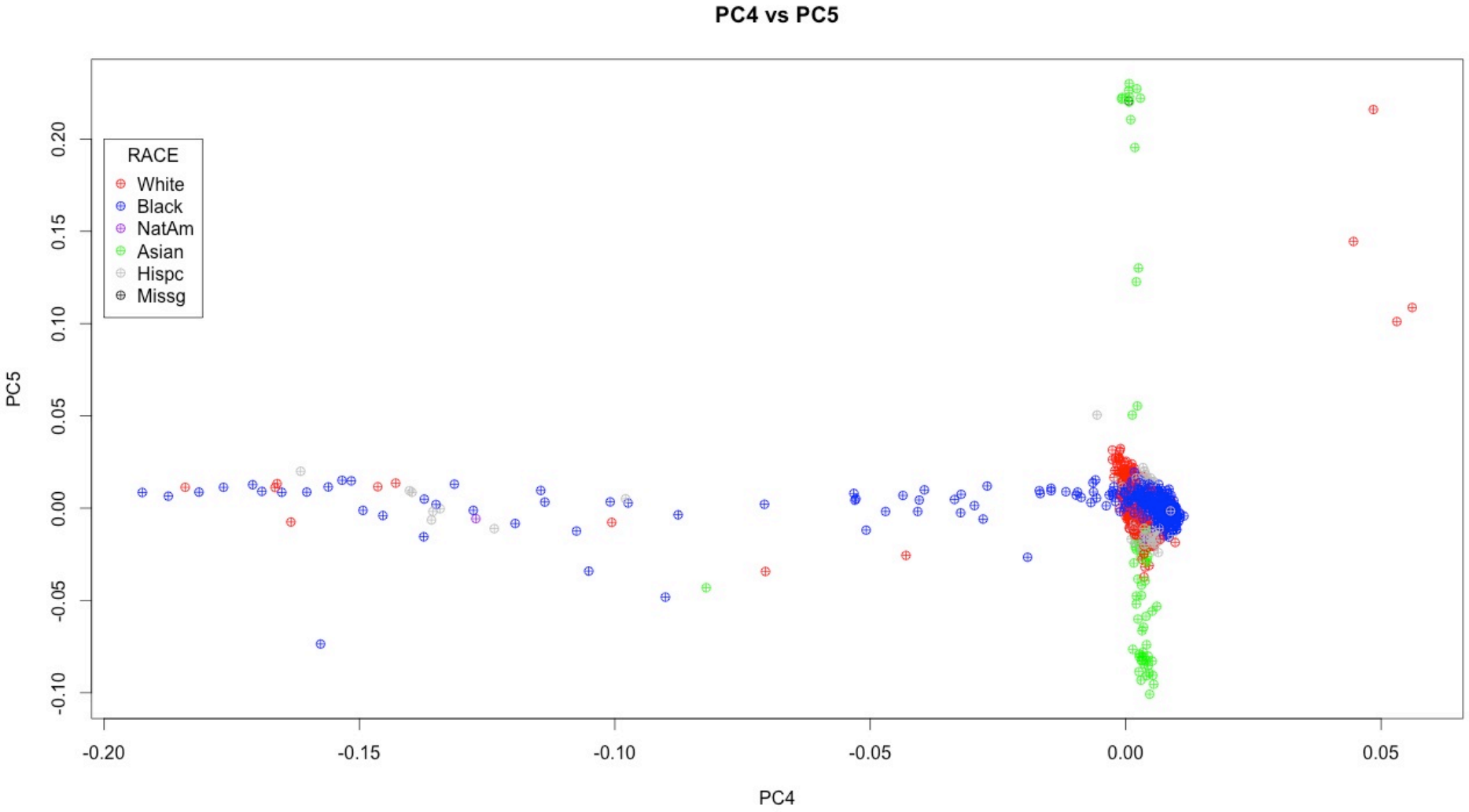
MDS Plot


PC2 vs PC3
RACE
White
Black
NatAm
Asian
Hispc
Missg
PC3
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.08
-0.06
-0.04
-0.02
0.00
PC2

MDS Plot


PC3 vs PC4
PC4
PC3
0.05
0.00
-0.05
-0.10
-0.15
-0.20
-0.05
0.00
0.05
0.10
0.15
RACE
White
Black
NatAm
Asian
Hispc
Missg

MDS Plot

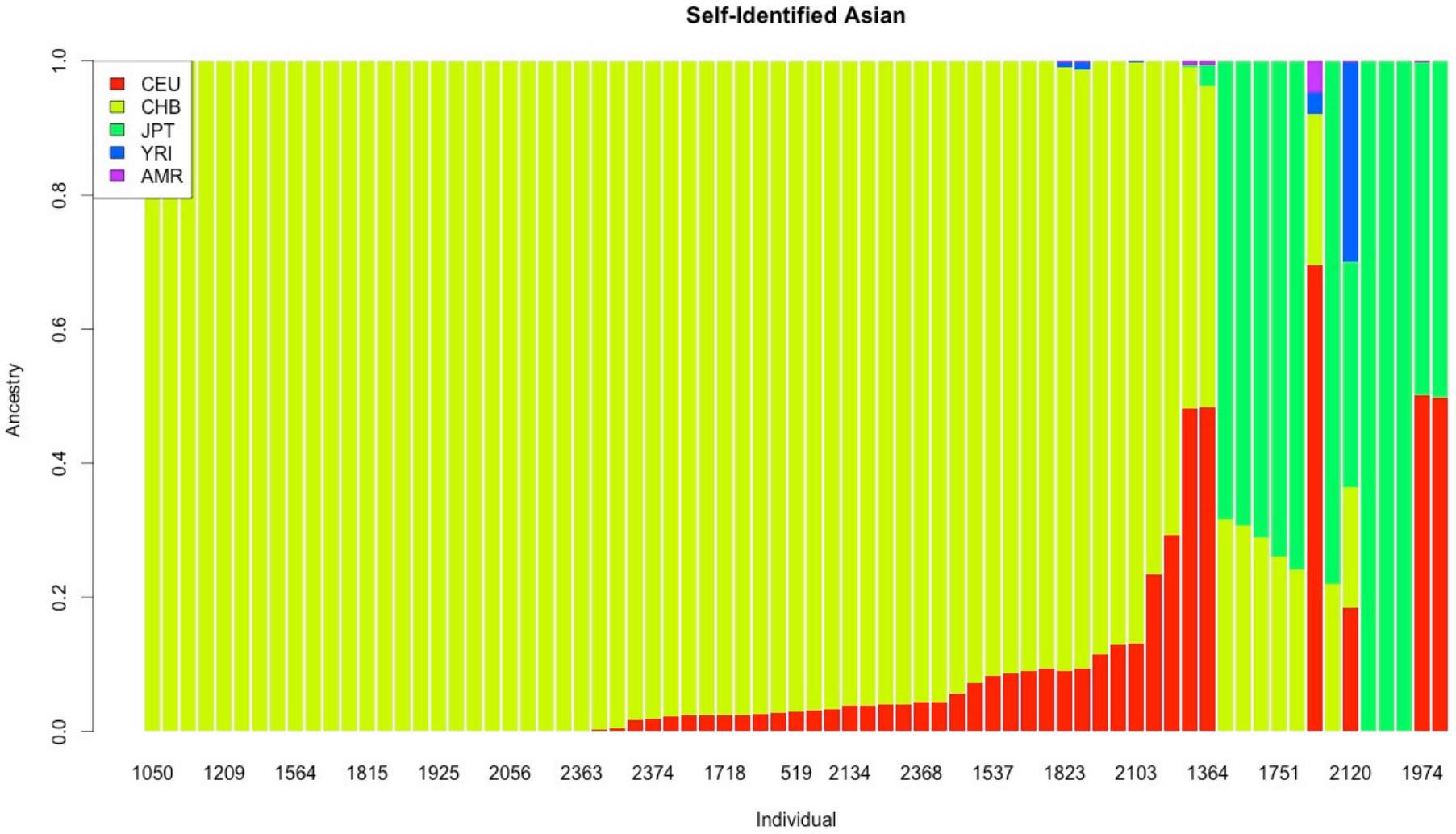

PC4 vs PC5
RACE
White
Black
NatAm
Asian
Hispc
Missg
PC5
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.20
-0.15
-0.10
-0.05
0.00
0.05
PC4

ADMIXTURE

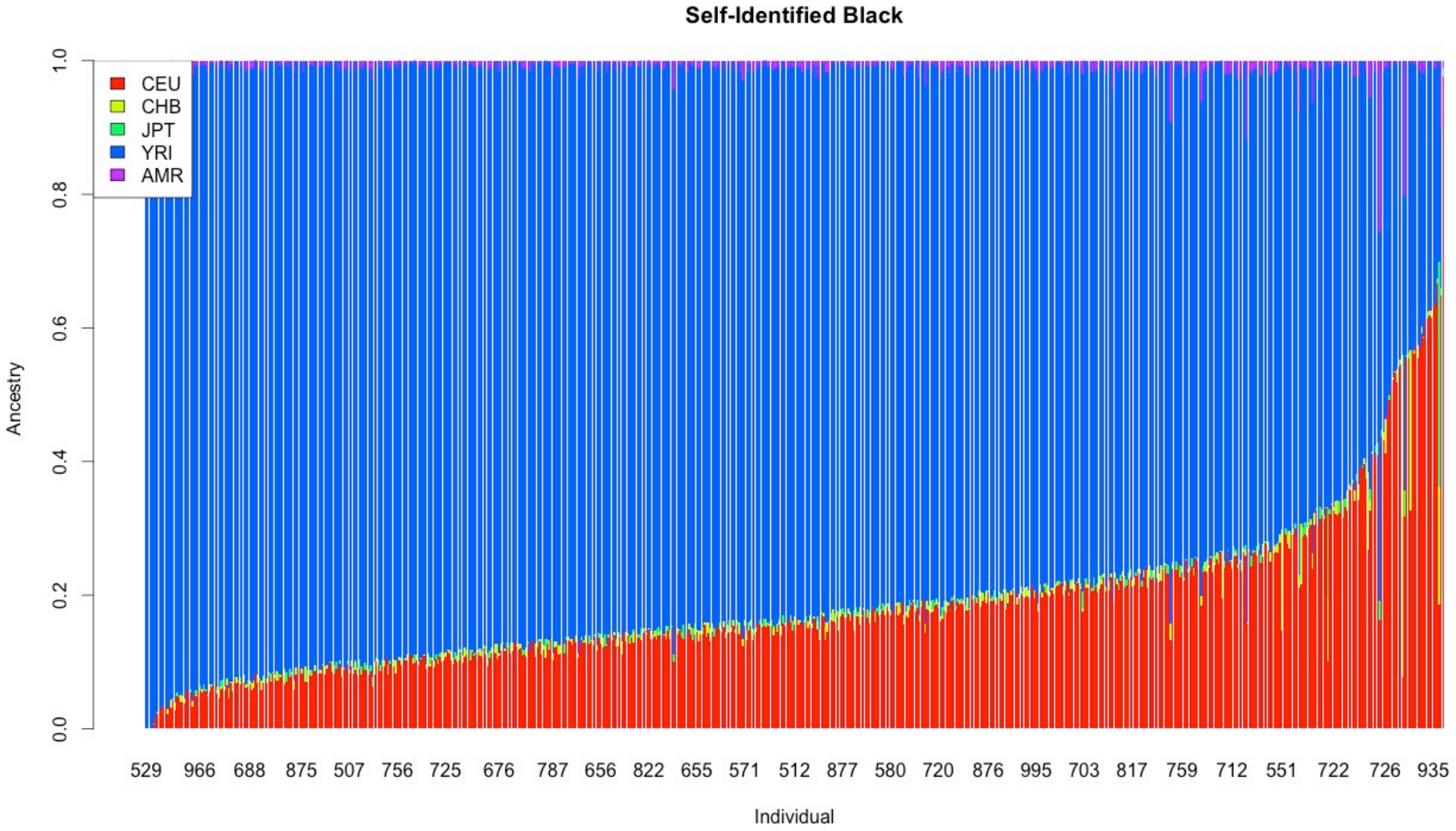
- Similar to STRUCTURE (but faster)
- ML estimation of individual ancestries

https://www.genetics.ucla.edu/software/admixture/

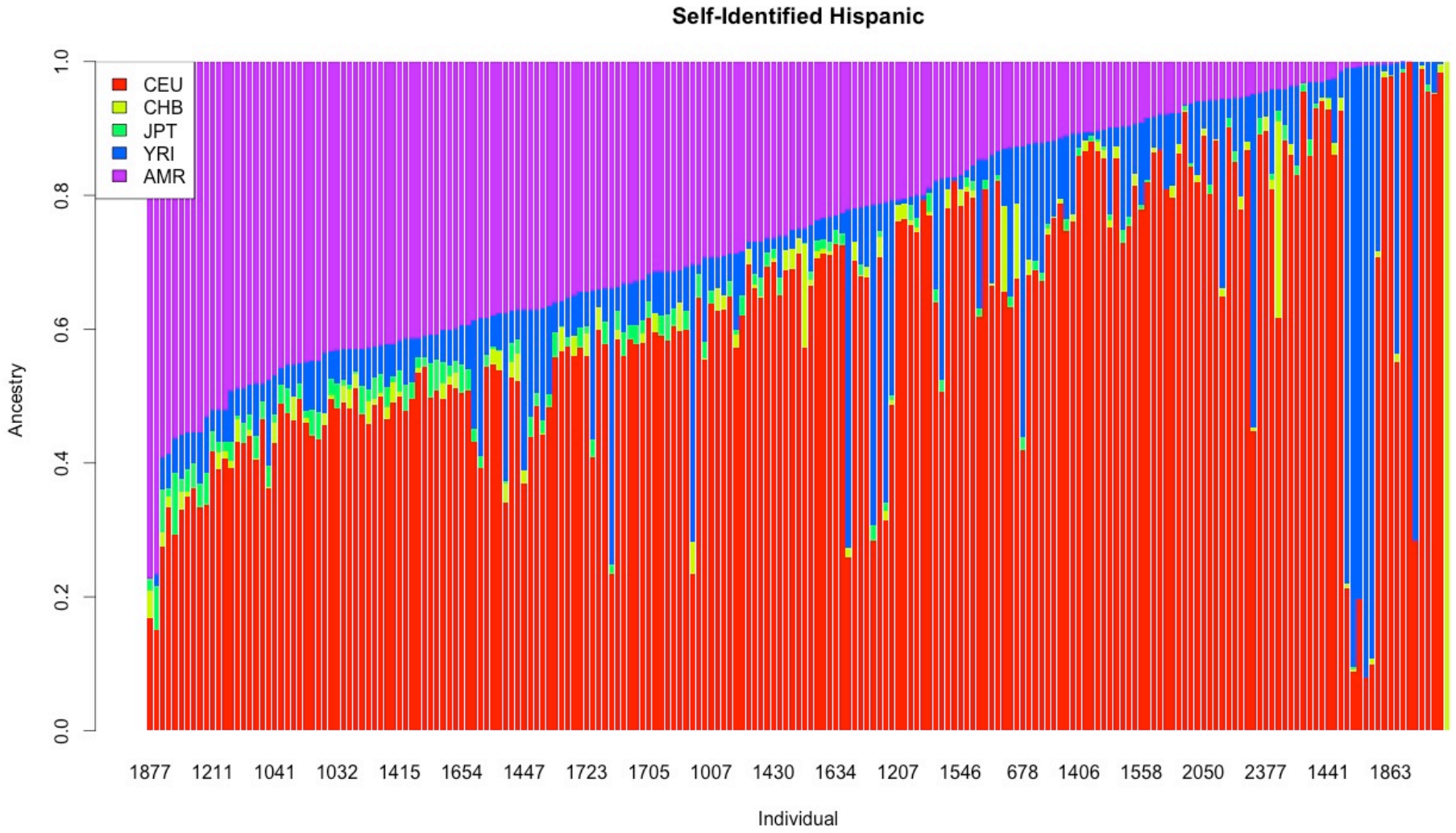
ADMIXTURE - Asian



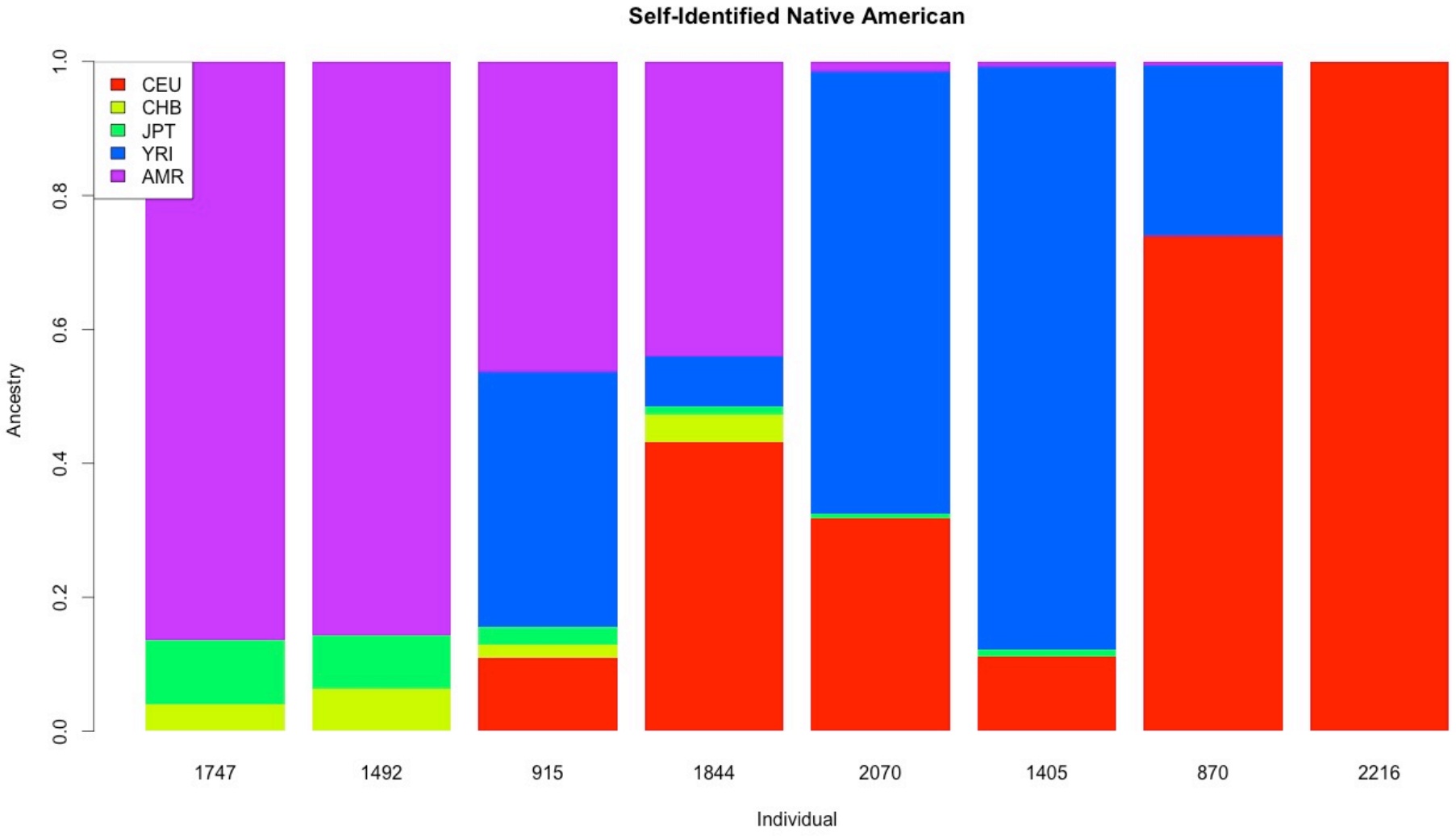
ADMIXTURE – African American



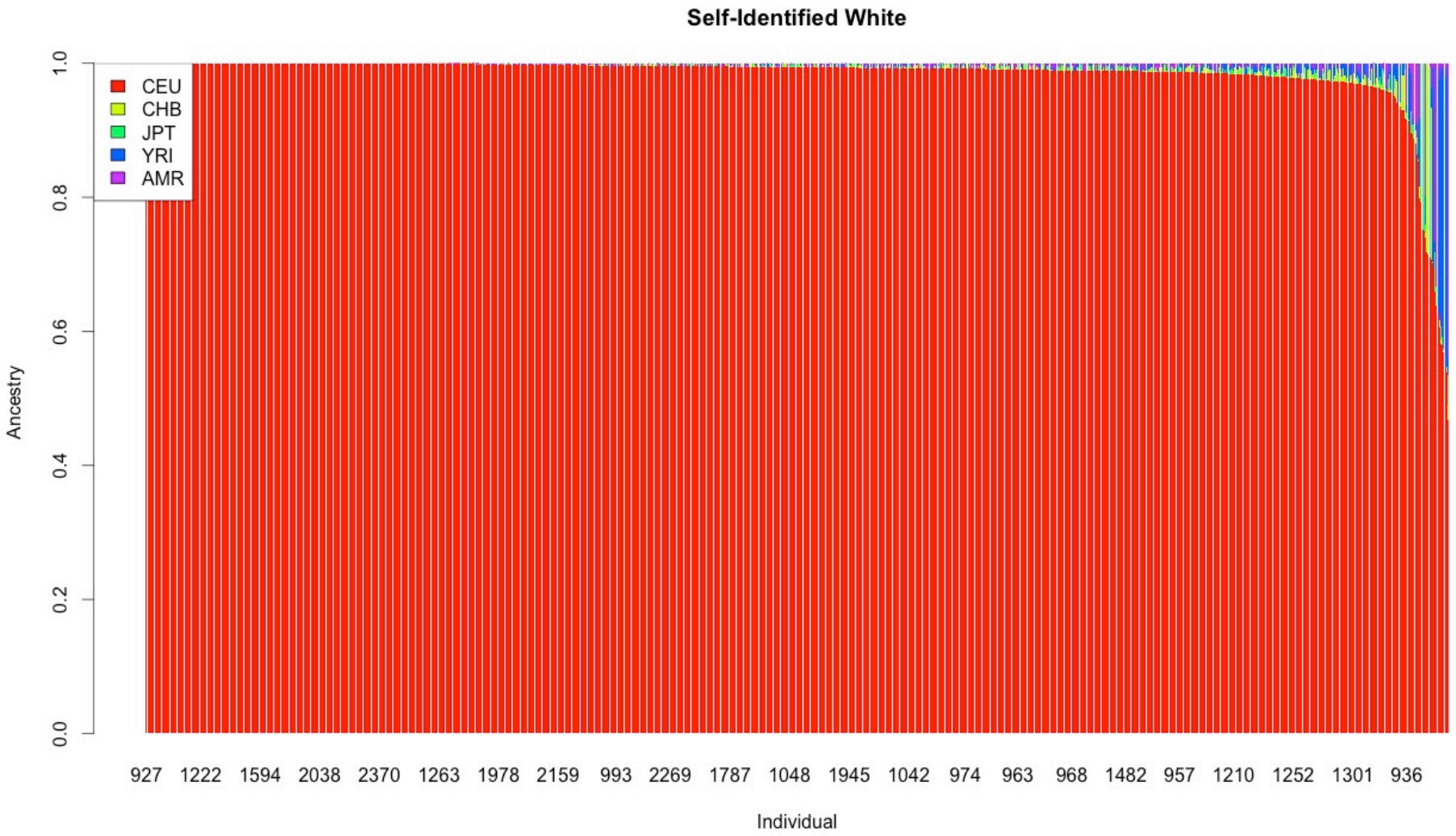
ADMIXTURE - Hispanic



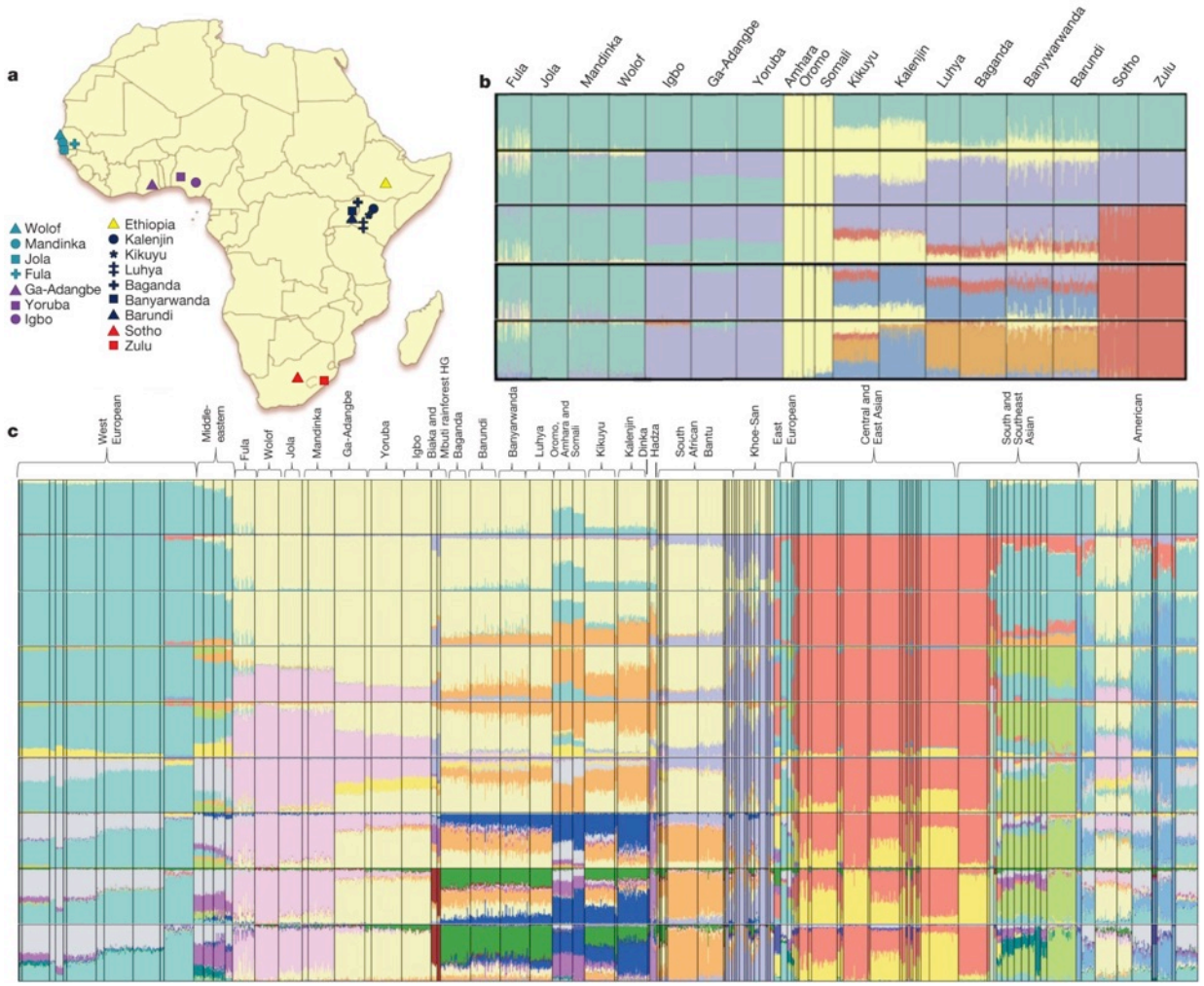
ADMIXTURE – Native American



ADMIXTURE – White



Populations studied in the AGVP.



D Gurdasani *et al. Nature* **000**, 1-6 (2014) doi:10.1038/nature13997

nature

Applications

- Control for population stratification
  - More on this later
- Construct a homogenous sample
  - Beyond self-report ethnicity
- "Control" for the genetic influences
- Identify mislabeled samples

Next up…

- Tutorial 2