Day 3, AM Session

PLINK

whole genome association analysis toolkit

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What is PLINK?

- An open-source tool for analyzing genotype and phenotype data from large omics datasets

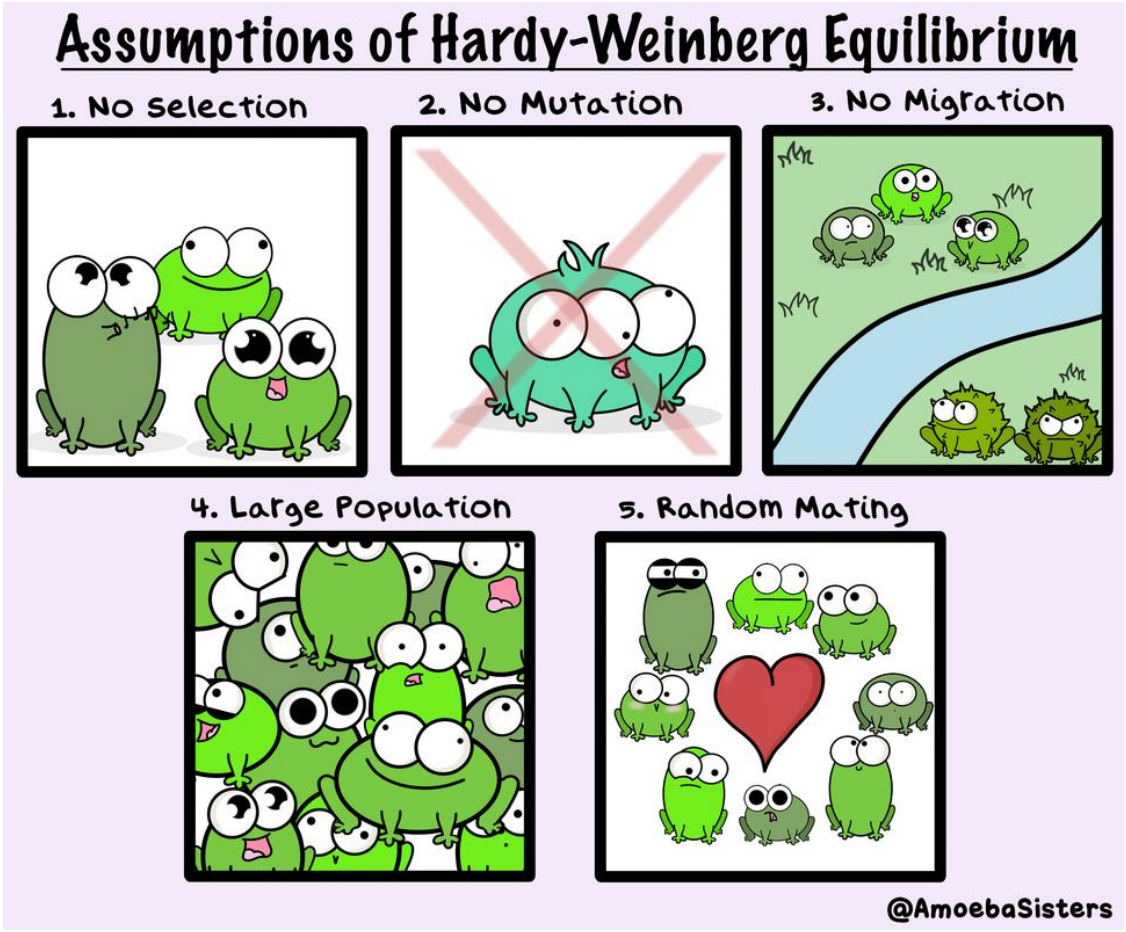
https://zzz.bwh.harvard.edu/plink/index.shtml

- Variant calling must be done beforehand

- Visualization of results has limited support --> we will visualize results using R and associated packages

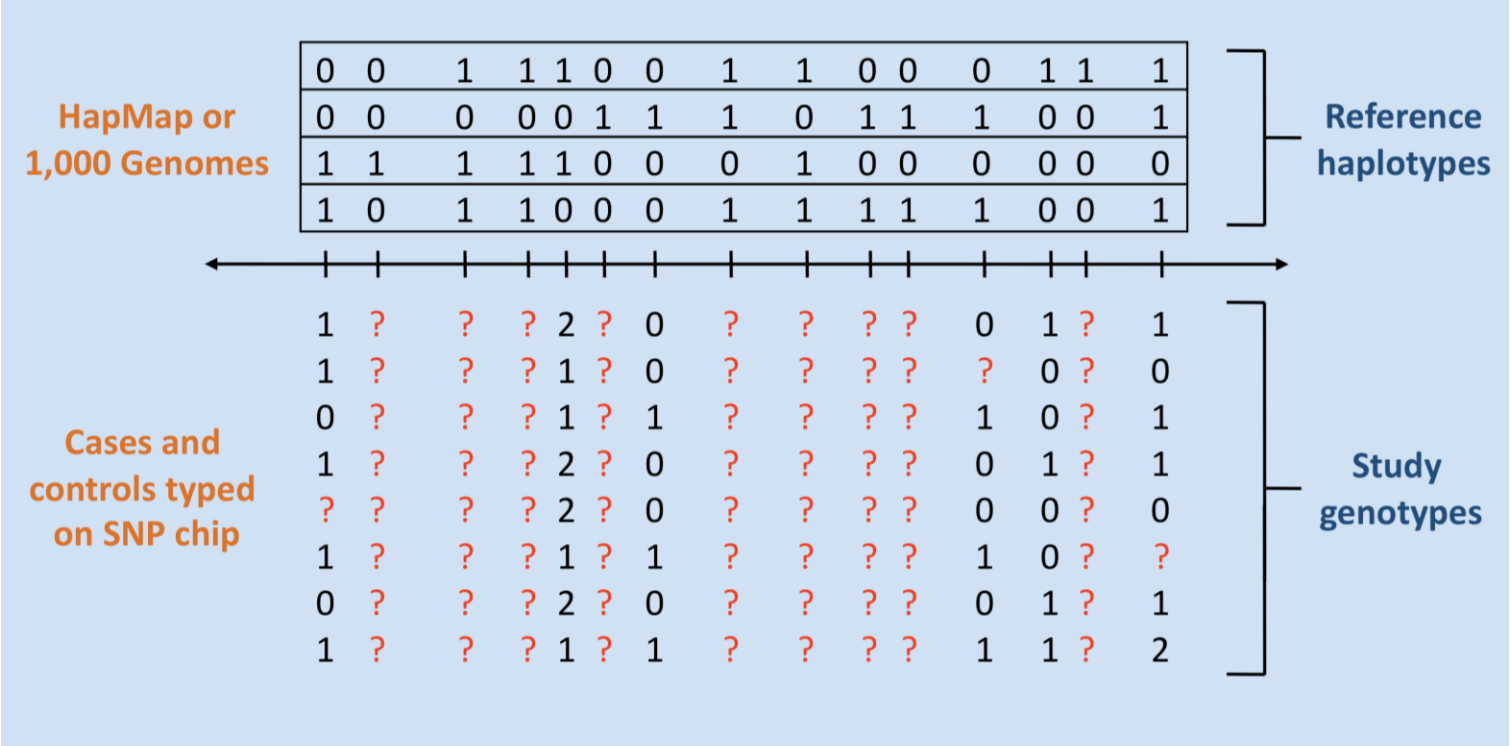
Some PLINK Capabilities

- Data management
  - Read in data in various formats
  - Merge files
  - Extract SNP subset
- Summary statistics
  - Allele frequencies
  - Hardy-Weinberg Equilibrium
  - Missing genotype rates
  - Kinship



More PLINK Capabilities

- Population stratification
  - Significance tests for whether two individuals belong to same population
- Association testing
  - Case/control
    - Fisher's exact test
    - Logistic regression
  - Quantitative traits
    - Linear regression
- Imputation



https://mathgen.stats.ox.ac.uk/impute/impute_v2.html

PED (.ped) File

- Pedigree, genotype, and phenotype information
- No header line allowed

```
  GNU nano 2.0.6                          File: toy.ped

1 1000000000 0 0 1 1 0 0 A A
1 1000000001 0 0 1 2 C C A G
```

https://www.cog-genomics.org/plink/1.9/general_usage

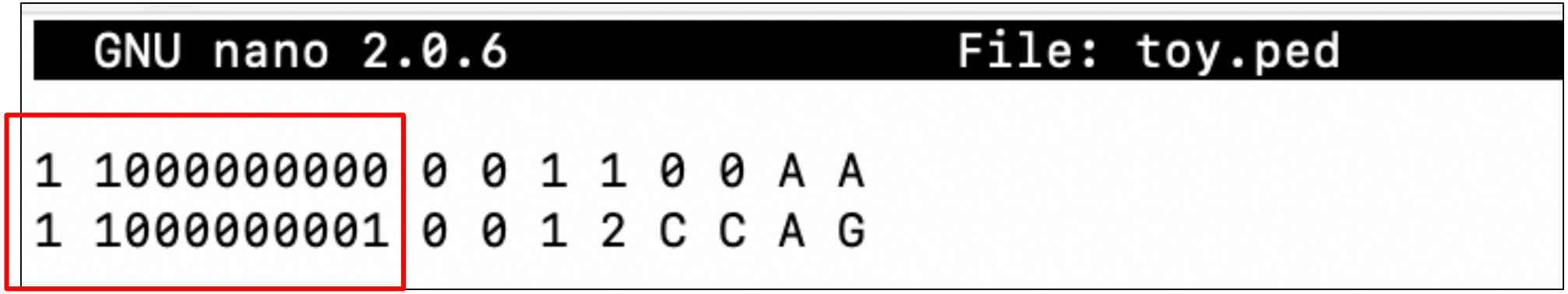
PED: Columns 1 and 2 (FID and IID)

Column 1

- Family ID (FID)
- Can be same as individual ID

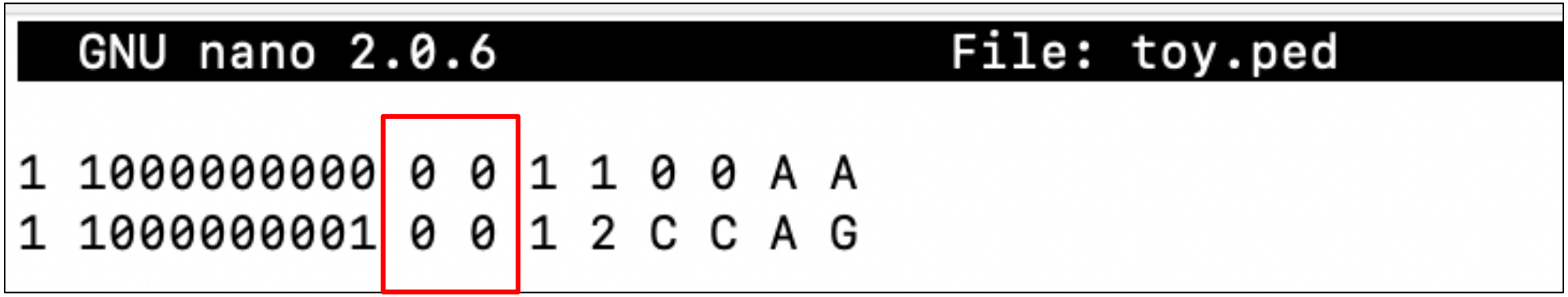
Column 2

- Individual ID (IID)
- This must be unique if FIDs are the same!



PED: Columns 3 (PATID) and 4 (MATID)

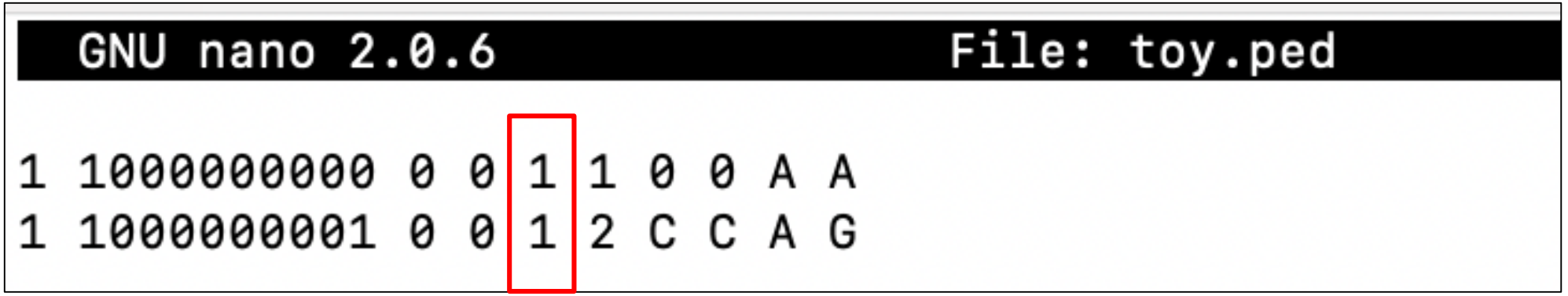
- Required even if pedigree is unknown
- Column 3 = paternal ID (PATID or FATID)
- Column 4 = maternal ID (MATID)
- If pedigree is known, fill in the cell with the appropriate ID
- If the pedigree is not known, fill in the cell with –9 or 0, which code for missing data



PED: Column 5 (Sex)

Column 5 = Sex

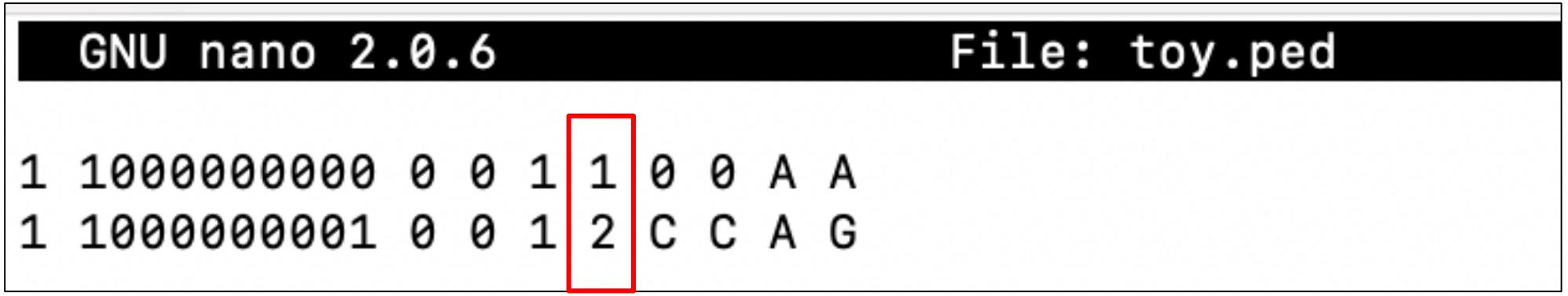
- If male --> 1
- If female --> 2
- If unknown --> 0 or -9



PED: Column 6 (Trait of interest)

Column 6 = Phenotype

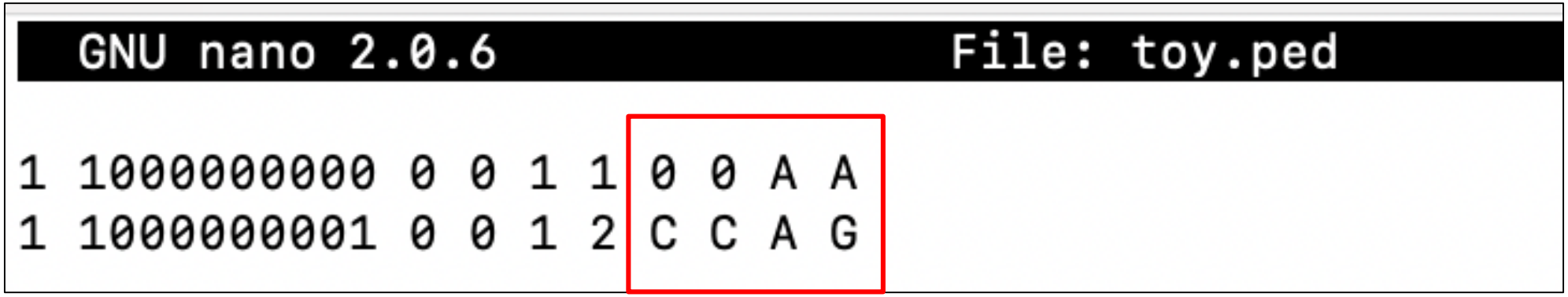
- Quantitative
  - Plant height: 1.12
  - No commas
- Case/control
  - Unaffected = 1
  - Affected = 2
  - Missing = -9 or 0



PED: Columns 7 and Beyond (Genotypes)

Genotype data

- Must be biallelic
- Can code allele as numbers 1,2,3, etc. or letters (A,B,C, etc. or A,T,G,C)
- Missing data
  - Coded as 0 or -9
  - Cannot have one allele present and one allele missing



MAP (.map) File

- Four columns
- Marker information
- Same order as PED file
- Do not need to be in genomic order

```
  GNU nano 2.0.6                          File: toy.map

1         rs0      0         1000
1         rs10     0         1001
```

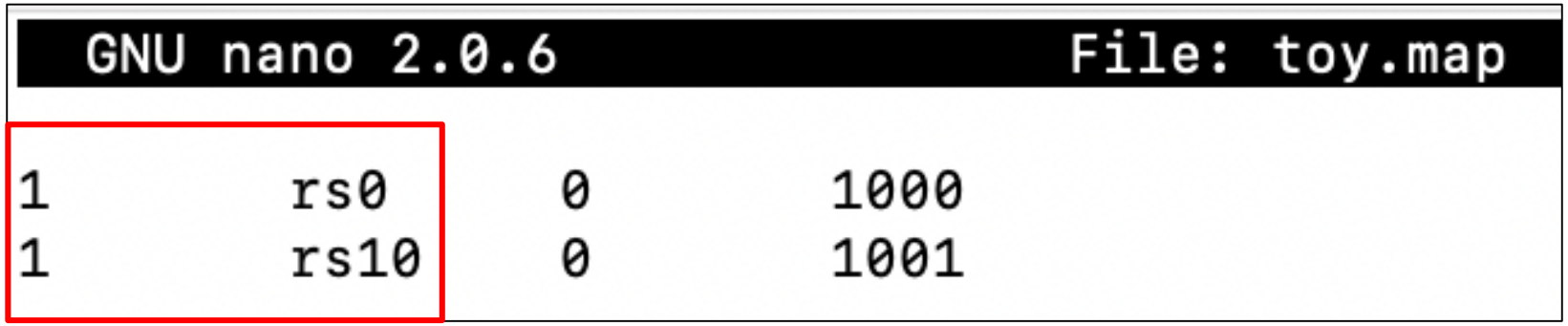
MAP: Columns 1 and 2

Column 1 = Chromosome

- Number (1, 2, 3, etc.)
- If unplaced --> 0
- X = X chromosome
- Y = Y chromosome
- XY = pseudo-autosomal region
- MT = mitochondrion

Column 2 = SNP identifier

- rs# for human SNP ID
- chromosome:position for most other organisms



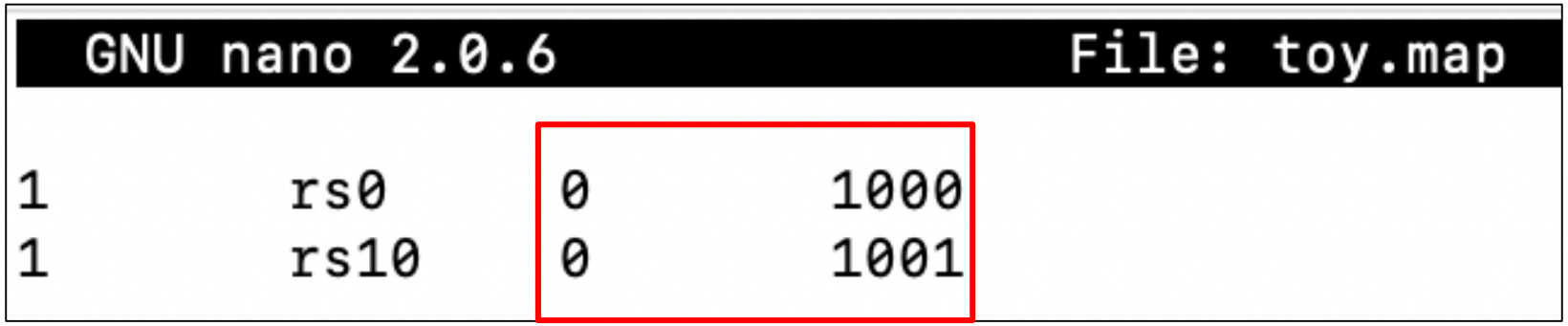
MAP: Columns 3 and 4

Column 3 = Genetic distance (cM)

- Set to 0 for association testing

Column 4 = Physical base-pair position

- If you preface this with a "-", you exclude the SNP from analysis
  - Example: -1562



PLINK Exercise

1. Check PLINK is installed on the system.

   plink

2. Download hapmap1.zip data and unzip. Move this folder to Desktop).

   (Do this manually)

3. Open and observe each file.

   nano hapmap1.ped

   nano hapmap1.map (Exit a nano file with Control X)

4. Identify how many individuals and SNPs are in the initial dataset.

   plink --file hapmap1 --out hapmap1

Note: this implies there are the files hapmap1.ped and hapmap1.map in the directory

Binary PED file

- Compact form that saves time and space
- Produced from command plink --file hapmap1
  - .bed = raw genotype data
    - this is the binary file
    - Cannot be viewed easily
  - .bim = raw genotype data + allele names
    - Can be viewed (nano)
  - .fam = first six columns of .ped file
    - Can be viewed (nano)

Exercise:
ls
nano hapmap1.bim
(Exit a nano file with Control X)
nano hapmap1.fam
(Exit a nano file with Control X)

Summary Statistics

- --missing --> missing data per individual (.imiss) and marker (.lmiss)
- --freq --> minor allele frequencies (.frq)
- --hardy --> Hardy-Weinberg Equilibrium (.hwe)

Adding the --chr modifier (example: --chr 1) provides the statistics for only the chromosome of interest

Adding the --snp modifier (with a SNP ID) provides the statistics for only the SNP of interest

Missing Data Exercise

Determine amount of missing data

1. plink --file hapmap1 --missing --out miss_stat
2. nano miss_stat.lmiss Gives missing data per marker/locus

   nano miss_stat.imiss Gives missing data per individual

Missing data shown as counts and frequencies.

.lmiss

```
 GNU nano 2.0.6                File: miss_stat.lmiss

 CHR          SNP   N_MISS   N_GENO   F_MISS
   1    rs6681049        0       89        0
   1    rs4074137        0       89        0
   1    rs7540009        0       89        0
   1    rs1891905        0       89        0
```

N_MISS = number of missing genotypes/locus
F_MISS = percent of missing data/locus

.imiss

FID	IID	MISS_PHENO	N_MISS	N_GENO	F_MISS
HCB181	1	N	671	83534	0.008033
HCB182	1	N	1156	83534	0.01384
HCB183	1	N	498	83534	0.005962

N_MISS = number of missing genotypes/individual
F_MISS = percent of missing data/individual

Minor Allele Frequency Exercise

Using the complete dataset, no population subdivision

plink --file hapmap1 --freq --out freq_stat

nano freq_stat.frq

.frq

GNU nano 2.0.6 File: freq_stat.frq

CHR	SNP	A1	A2	MAF	NCHROBS
1	rs6681049	1	2	0.2135	178
1	rs4074137	1	2	0.07865	178
1	rs7540009	0	2	0	178
1	rs1891905	1	2	0.4045	178
1	rs9729550	1	2	0.1292	178
1	rs3813196	1	2	0.02809	178
1	rs6704013	0	2	0	174
1	rs307347	0	2	0	154

A1 = minor allele

A2 = major allele

MAF = minor allele frequency

NCHROBS = number of alleles without missing data (actually genotyped)

MAF (population subdivision)

1. Look at the pop.phe file

nano pop.phe

FID, IID, Population

```
HCB222 1 1
HCB223 1 1
HCB224 1 1
HCB225 1 1
JPT226 1 2
JPT227 1 2
JPT228 1 2
```

2. Calculating MAF per subpopulation

plink --file hapmap1 --freq --within pop.phe --out popfreq_stat

nano popfreq_stat.frq.strat

.frq.strat

GNU nano 2.0.6 File: popfreq_stat.frq.strat

CHR	SNP	CLST	A1	A2	MAF	MAC	NCHROBS
1	rs6681049	1	1	2	0.2333	21	90
1	rs6681049	2	1	2	0.1932	17	88
1	rs4074137	1	1	2	0.1	9	90
1	rs4074137	2	1	2	0.05682	5	88
1	rs7540009	1	0	2	0	0	90
1	rs7540009	2	0	2	0	0	88

CLST = cluster
MAC = minor allele count

Filtering Options

- --mind --> allowed missing data/individual to retain individual
  - Example: --mind 0.05 for retaining individuals with 95% data present
- --geno --> allowed missing data/marker to retain marker
  - Example: --geno 0.05 for retaining markers with genotyping call rate of 95%
- --maf --> allow markers with this Minor Allele Frequency and above
  - Example: --maf 0.05 for retaining markers with MAF 0.05 and above
- --hwe --> performs Hardy-Weinberg Exact test at specified threshold
  - Be careful when using this filter
  - Make sure you do this for each population separately
- Syntax example: plink --file hapmap1 --make-bed --mind 0.05 –out filter1

Filtering Options Exercise

Retain markers with a genotype call rate of 85%.

a. How many markers were removed?

b. How many markers were retained?

Filtering Options

- To keep or remove individuals from an analysis
  - Two column text file (family ID, individual ID), no header
  - --keep *textfile* = keep these individuals
  - --remove *textfile* = remove these individuals

- To keep or remove certain SNP_IDs from an analysis
  - One column with SNP_IDs, no header
  - --extract *textfile* = keeps these variants
  - --exclude *textfile* = remove these variants

What type of data is the phenotype? Exercise

1. Open the hapmap1.ped file.

   nano hapmap1.ped

2. Is phenotype continuous, binary, or categorical?

3. What type of regression should we perform?

(Options: linear, binary logistic, multinomial logistic, ordered logistic)

Logistic Regression Exercise

plink --file hapmap1 --make-bed --logistic --covar pop.phe --out withpopA

nano withpopA.assoc.logistic

Output File

GNU nano 2.0.6 File: plink.assoc.logistic

CHR	SNP	BP	A1	TEST	NMISS	OR	STAT	P
1	rs6681049	1	1	ADD	89	0.5781	-1.447	0.1479
1	rs6681049	1	1	COV1	89	9.436	4.486	7.247e-06
1	rs4074137	2	1	ADD	89	1.54	0.6577	0.5107
1	rs4074137	2	1	COV1	89	9.764	4.533	5.809e-06
1	rs7540009	3	0	ADD	89	NA	NA	NA
1	rs7540009	3	0	COV1	89	NA	NA	NA
1	rs1891905	4	1	ADD	89	1.079	0.232	0.8166
1	rs1891905	4	1	COV1	89	9.307	4.533	5.822e-06
1	rs9729550	5	1	ADD	89	2.846	2.007	0.04472
1	rs9729550	5	1	COV1	89	11.23	4.604	4.153e-06
1	rs3813196	6	1	ADD	89	1.352	0.2785	0.7806
1	rs3813196	6	1	COV1	89	9.229	4.519	6.205e-06
1	rs6704013	7	0	ADD	87	NA	NA	NA

NA is present when the SNP was monomorphic

Columns:
1. Chromosome
2. SNP ID
3. Base position
4. Minor Allele
5. Test (ADD = additive effects of allele dosage, COV1 = covariate)
6. NMISS = # of nonmissing genotypes
7. Odds ratio
8. T-statistic
9. P-value

Test Column

- We are interested in the additive effects of allele dosage line.
  - Additive effects of allele dosage = more copies of an allele are more or less strongly correlated with the phenotype.
- This is the significance of the SNP when controlling for the covariate (population).
- We can hide the covariate including the argument --hide-covar

Exercise

```
plink --file hapmap1 --make-bed --logistic --covar pop.phe --hide-covar
        --out withpopB
nano withpopB.assoc.logistic
```

Excluding Covariate Exercise

plink --file hapmap1 --make-bed --logistic --out withoutpop

nano withoutpop.assoc.logistic

```
 GNU nano 2.0.6                                          File: withpopA.assoc.logistic

 CHR          SNP         BP    A1        TEST      NMISS         OR         STAT            P
   1    rs6681049          1     1         ADD         89     0.5781       -1.447       0.1479
   1    rs6681049          1     1        COV1         89      9.436        4.486    7.247e-06
```

```
 GNU nano 2.0.6                                          File: withoutpop.assoc.logistic

 CHR          SNP         BP    A1        TEST      NMISS         OR         STAT            P
   1    rs6681049          1     1         ADD         89      0.592       -1.534       0.1251
```

Adding the covariate, makes the SNP effect less significant.

Correcting for Multiple Testing Exercise

plink --file hapmap1 --make-bed --logistic --covar pop.phe --hide-covar --adjust --out withpopC

nano withpopC.assoc.logistic.adjusted

Correcting for Multiple Testing

GNU nano 2.0.6 File: withpopC.assoc.logistic.adjusted

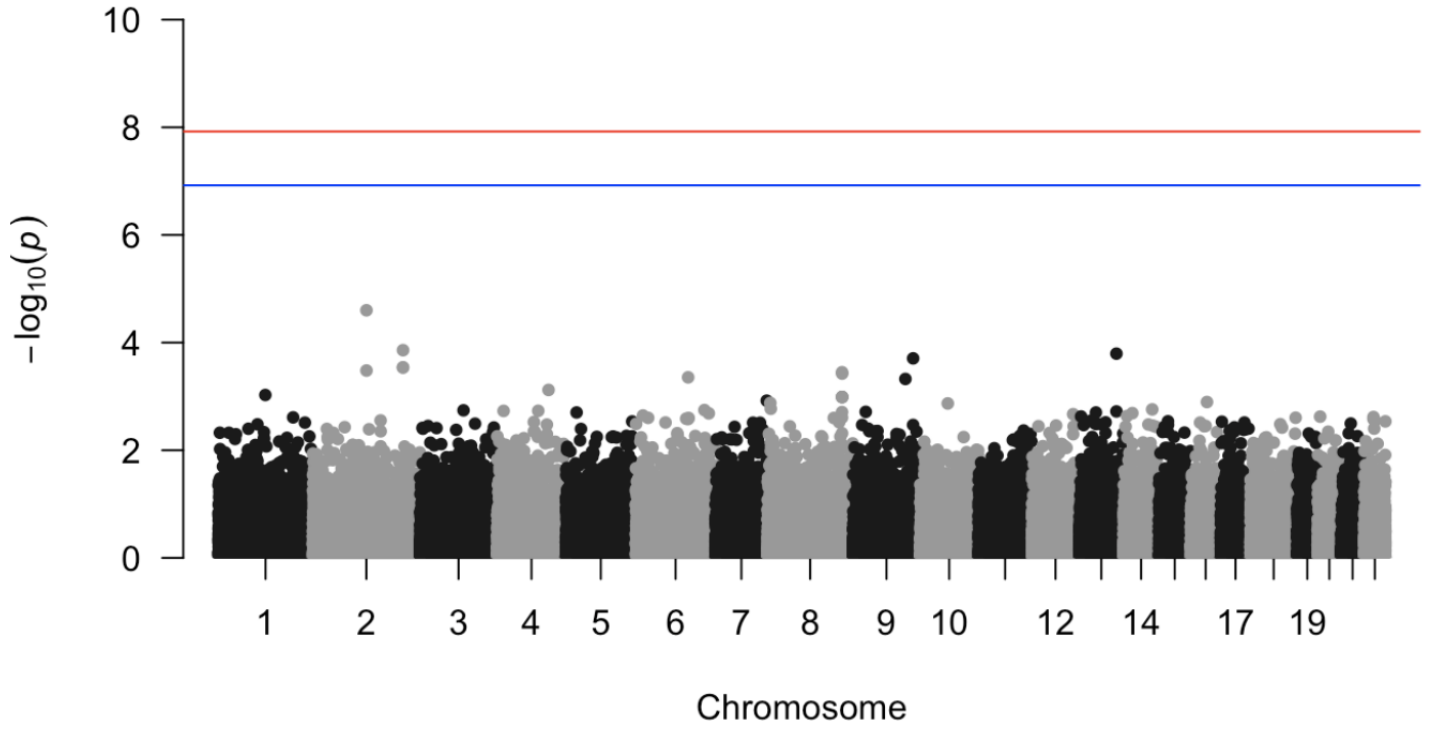
CHR	SNP	UNADJ	GC	BONF	HOLM	SIDAK_SS	SIDAK_SD	FDR_BH	FDR_BY
2	rs2222162	2.507e-05	2.507e-05	1	1	0.8056	0.8056	0.9945	1
2	rs4675607	0.0001382	0.0001382	1	1	0.9999	0.9999	0.9945	1
13	rs9585021	0.0001603	0.0001603	1	1	1	1	0.9945	1
9	rs7046471	0.0001963	0.0001963	1	1	1	1	0.9945	1
2	rs4673349	0.0002892	0.0002892	1	1	1	1	0.9945	1

Manhattan Plot Exercise

- If you remember, we produced a file “withpopB.assoc.logistic” today.
- We are going to use this file, to produce a Manhattan plot.

- Please run the R Markdown named ‘ManhattanPlot.Rmd’ in RStudio.

Manhattan Plot Answer



References

- grunwaldlab.github
- https://bookdown.org/kdonovan125/ibis_data_analysis_r4/documenting-your-results-with-r-markdown.html