Genome-wide Association Studies

Practical 1: genotype cleaning and intro to software

Outline

Importance of data cleaning

Introduction to software - PLINK & GCTA

Typical QC performed on SNP-chip genotypes

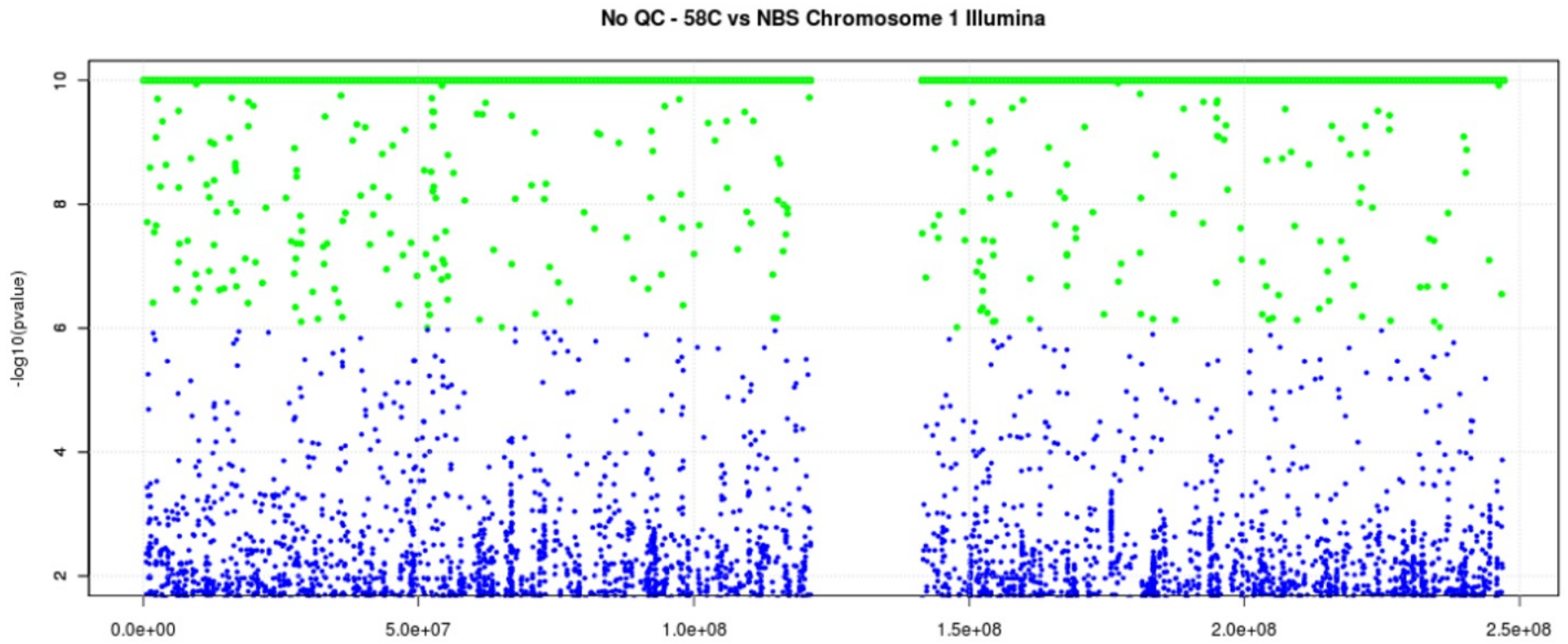
Example: Importance of Good Cleaning

- The WTCCC study used controls from two populations:

  - 1,500 from the 1958 British Birth Cohort (58C)
  - 1,500 from the National Blood Service (NBS)

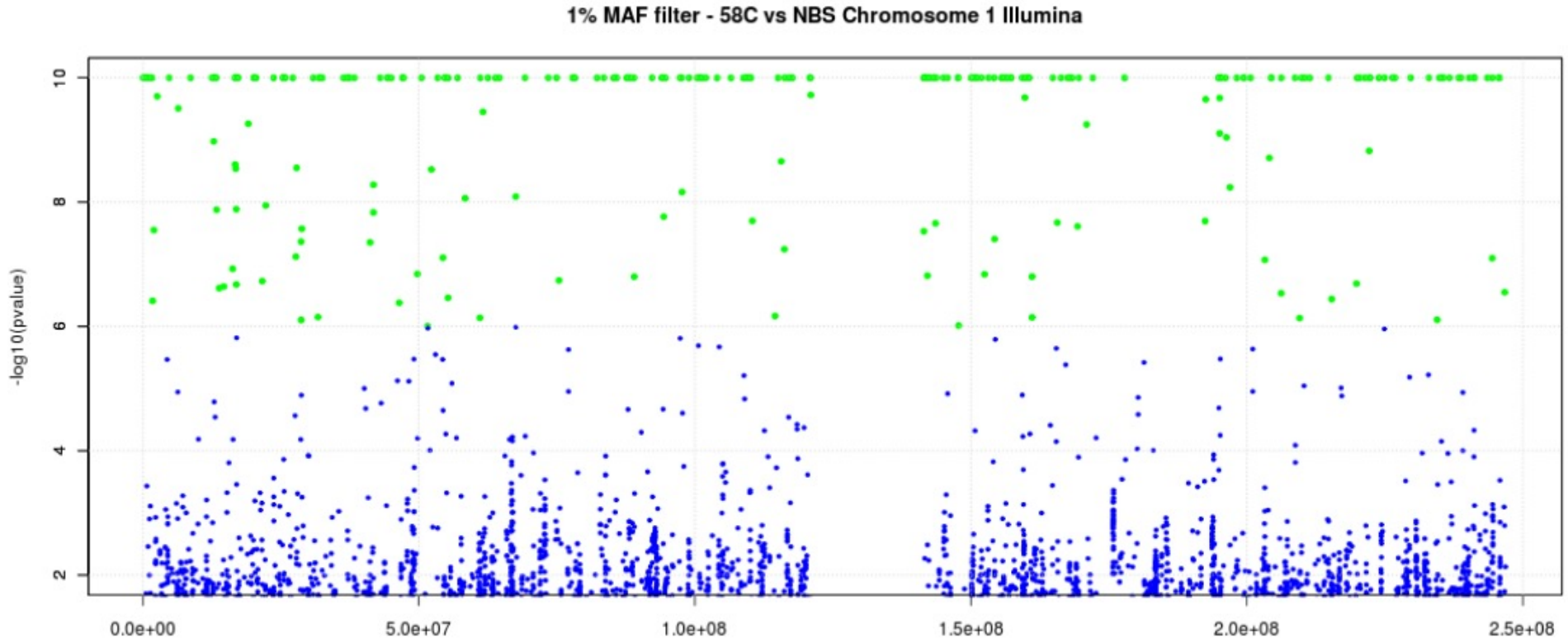
- Both these are unselected population cohorts, so performing a “case-control” study between these populations should find no significant differences

Importance of Good Cleaning



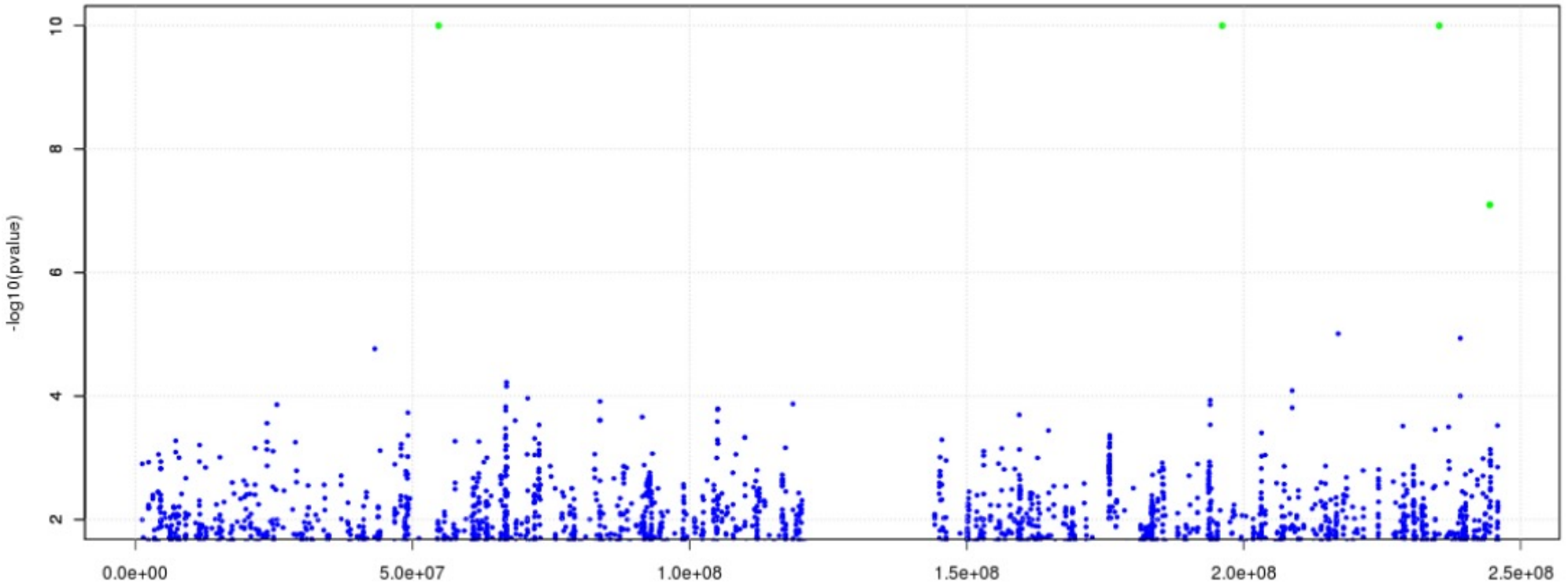
100% of SNPs

Importance of Good Cleaning



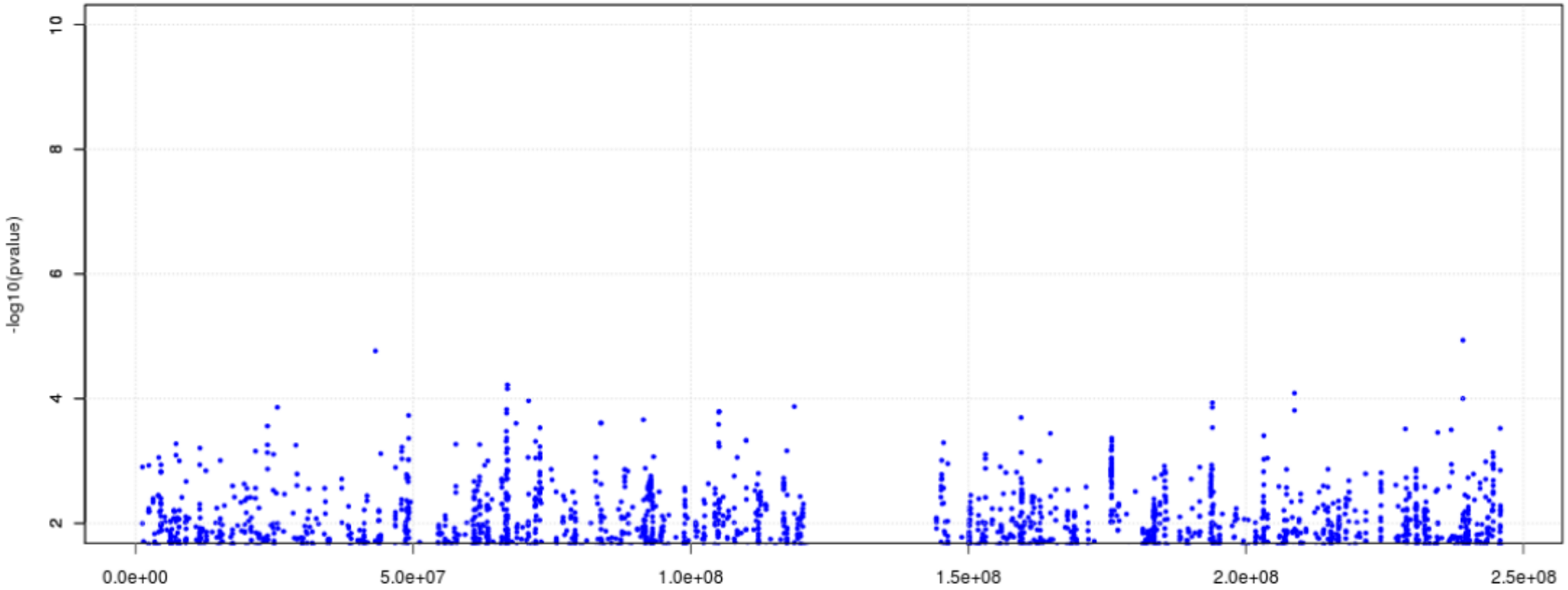
80.69% of SNPS
Filtering: MAF

Importance of Good Cleaning



78.36% of SNPs
Filtering: MAF + HWE

Importance of Good Cleaning

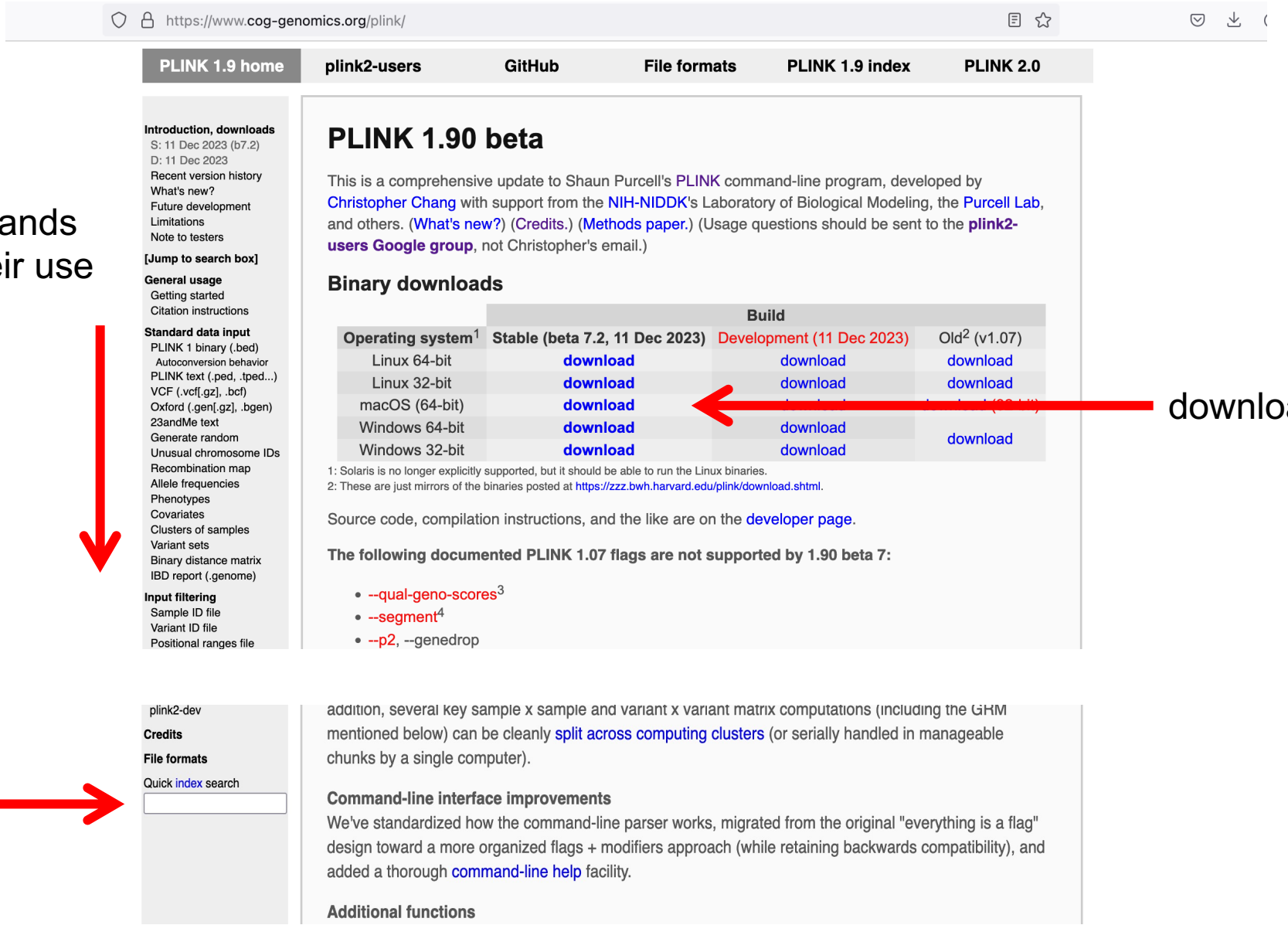


77.92% of SNPs
Filtering: MAF + HWE + Missingness

PLINK

- PLINK is a free, open-source whole genome association analysis toolset
  - Efficiently store, manipulate and analyse large datasets
  - Widely used
- 3 main versions of PLINK
  - PLINK v1.07 2007 https://zzz.bwh.Harvard.edu/plink/ < good website on basics >
  - PLINK v1.90 2015 https://www.cog-genomics.org/plink/1.9/ < major upgrade of v1.07 >
  - PLINK v2.0 2017 https://www.cog-genomics.org/plink/2.0/ < under development? >

PLINK v1.90 website



many commands
& info on their use

download the right version

Index!

PLINK v1.90

- Need to run PLINK via command line, e.g.

```
delta2:~/60days/UQWS_2023$ plink
PLINK v1.90b6.22 64-bit (16 Apr 2021)          www.cog-genomics.org/plink/1.9/
(C) 2005-2021 Shaun Purcell, Christopher Chang   GNU General Public License v3

  plink <input flag(s)...> [command flag(s)...] [other flag(s)...]
  plink --help [flag name(s)...]

Commands include --make-bed, --recode, --flip-scan, --merge-list,
--write-snplist, --list-duplicate-vars, --freqx, --missing, --test-mishap,
--hardy, --mendel, --ibc, --impute-sex, --indep-pairphase, --r2, --show-tags,
--blocks, --distance, --genome, --homozyg, --make-rel, --make-grm-gz,
--rel-cutoff, --cluster, --pca, --neighbour, --ibs-test, --regress-distance,
--model, --bd, --gxe, --logistic, --dosage, --lasso, --test-missing,
--make-perm-pheno, --tdt, --qfam, --annotate, --clump, --gene-report,
--meta-analysis, --epistasis, --fast-epistasis, and --score.

"plink --help | more" describes all functions (warning: long).
```

- if you have downloaded PLINK into your local directory, could be:

```
./plink
```

PLINK data format

- Three files:
  - `gwas.bed` → binary file containing all genotypes
  - `gwas.bim` → information about SNP markers
  - `gwas.fam` → information about individuals

```
[allan@analysis1 ~]$ head /data/module1/gwas/part1/gwas.bim
1       rs3131972       0       752721  1       2
1       rs3115850       0       761147  1       2
1       rs12562034      0       768448  1       2
1       rs4040617       0       779322  2       1
1       rs4970383       0       838555  1       2
1       rs950122        0       846864  1       2
1       rs6657440       0       850780  2       1
1       rs13303101      0       862124  1       2
1       rs1110052       0       873558  2       1
1       rs3748592       0       880238  1       2
```

chromosome

SNP ID

(dummy variable) position, cM

position, bp

allele 1

allele 2

```
[allan@analysis1 ~]$ head /data/module1/gwas/part1/gwas.fam
7653762 7653762 0 0 2 -9
8144519 8144519 0 0 2 -9
2337680 2337680 0 0 2 -9
5219864 5219864 0 0 1 -9
1417721 1417721 0 0 1 -9
2371103 2371103 0 0 2 -9
472262 472262 0 0 1 -9
566177 566177 0 0 2 -9
8097907 8097907 0 0 2 -9
8738370 8738370 0 0 2 -9
```

FID (Family ID)

IID (individual ID)

PID/MID/sex/phenotype
-> not necessary to specify, but “0 0 0 -9” is needed

PLINK data format

- Other files, e.g. quantitative covariates, covariate & phenotype files
  - can have any name/suffix
  - columns must be FID, IID, then data

phenotype file format

> Input file format
> test.phen (no header line; columns are family ID, individual ID and phenotypes)

```
011       0101    0.98
012       0102    -0.76
013       0103    -0.06
......
```

covariate file format

> Input file format
> test.covar (no header line; columns are family ID, individual ID and discrete covariates)

```
01       0101    F       Adult           0
02       0203    M       Adult           0
03       0305    F       Adolescent      1
......
```

quantitative covariate file format

> Input file format
> test.qcovar (no header line; columns are family ID, individual ID and quantitative covariates)

```
01       0101    -0.024    0.012
02       0203    0.032     0.106
03       0305    0.143     -0.056
......
```

GCTA

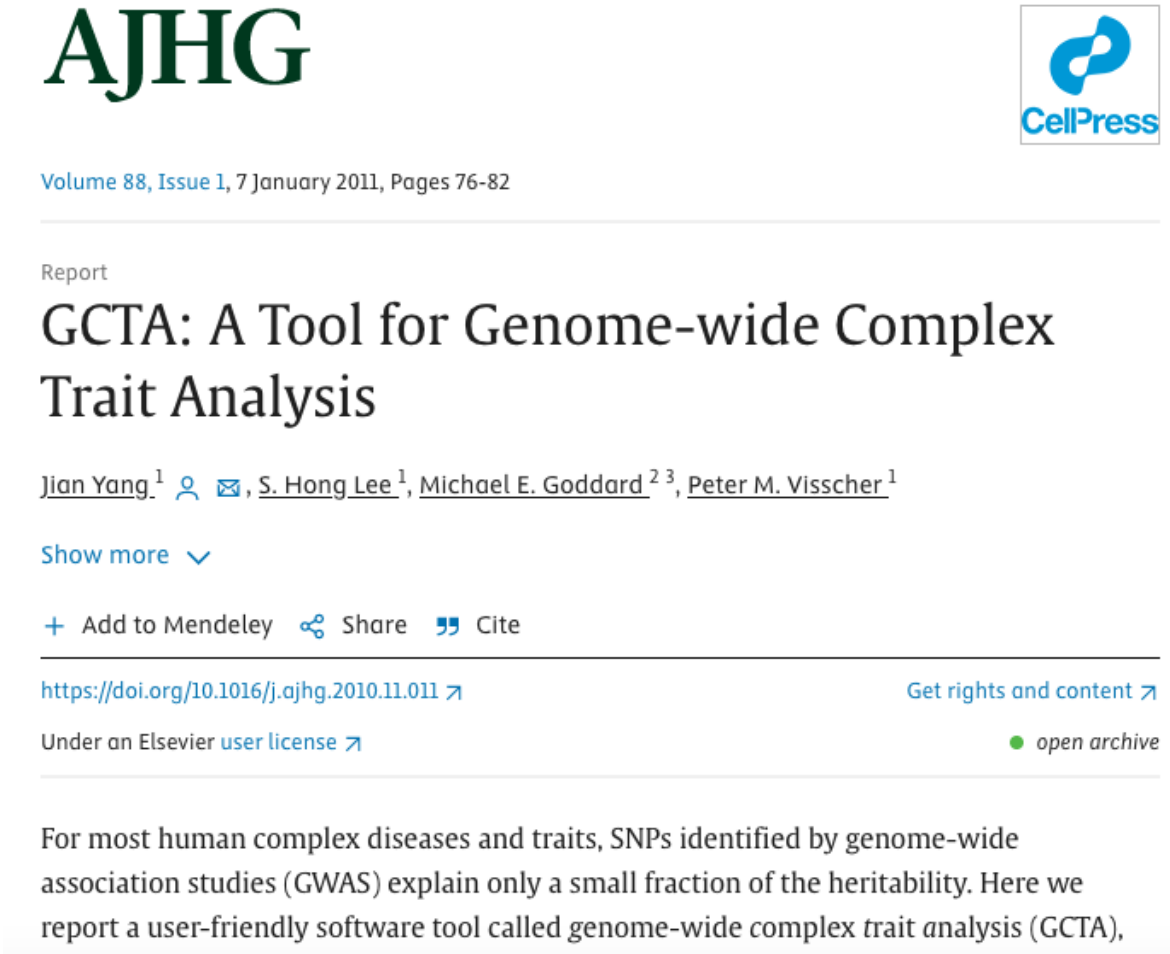
- We will also use GCTA

Comprehensive website:
https://yanglab.westlake.edu.cn/software/gcta/#Overview

- Runs like PLINK, same command format and input format

```
gcta64 --bfile <data prefix> --command
```

- Primarily for variance component estimation via REML (QG2 module) but has expanded to include other useful features



AJHG

CellPress

Volume 88, Issue 1, 7 January 2011, Pages 76-82

Report

GCTA: A Tool for Genome-wide Complex Trait Analysis

Jian Yang [1], S. Hong Lee [1], Michael E. Goddard [2 3], Peter M. Visscher [1]

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For most human complex diseases and traits, SNPs identified by genome-wide association studies (GWAS) explain only a small fraction of the heritability. Here we report a user-friendly software tool called genome-wide complex trait analysis (GCTA),

Preparing Genotype Data

We divide the cleaning of genotype data into two steps

STEP 1) removing any individuals with poor quality data

STEP 2) removing SNP markers that have substandard genotyping performance

- we use on statistical measures to detect bad quality data and remove it

- Performing the per-individual steps first prevents individuals with poor quality genotypes having an undue influence on the removal of SNP markers in the later step.

Per Individual Quality Control

Suggestions for removing individuals with ‘poor quality’ genotypes

1. removal of individuals with excess missing genotypes
2. removal of individuals with outlying homozygosity values
3. remove of samples showing a discordant sex
4. removal of related or duplicate samples, and
5. removal of ancestry outliers

more details in the prac

Per Individual Quality Control - removal of ancestry outliers

1. Download and perform PCA on diverse individuals with known ancestry, e.g. 1000 Genomes
2. Project your samples onto PCs
3. Exclude ‘outliers’ from further analysis

e.g. with GCTA

Example

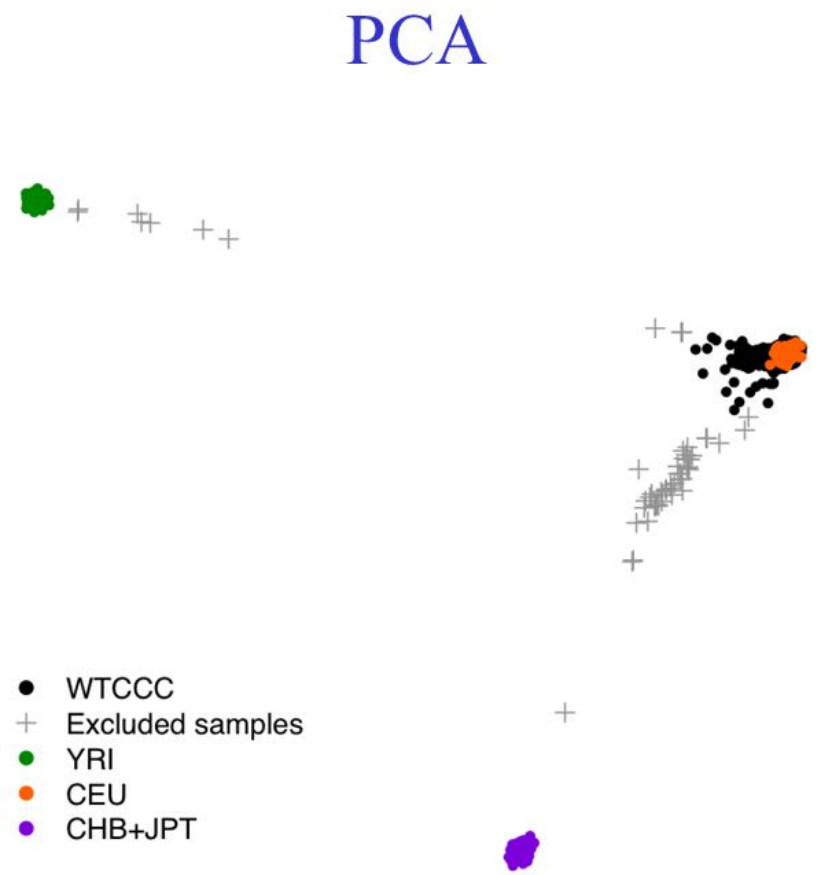
REF: SNP genotype data of the reference sample; TAR: SNP genotype data of the target sample;

```
# To make a GRM
gcta64 --bfile REF --maf 0.01 --autosome --make-grm --out REF
# PCA analysis
gcta64 --grm REF --pca 20 --out REF_pca20

# To use the PCs generated above to produce PC loadings of each SNP
gcta64 --bfile REF --pc-loading REF_pca20 --out REF_snp_loading

# To compute the PCs of the target sample using the PC loading generated above
# Note that the analysis can be performed with one chromosome at a time
gcta64 --bfile TAR --project-loading REF_snp_loading 20 --out TAR_pca20
```

https://yanglab.westlake.edu.cn/software/gcta/#PCloadingandprojection

Per Individual Quality Control - removal of ancestry outliers

1. Perform PCA on GRM of diverse individuals with known ancestry, e.g. 1000 Genomes
2. Project your samples onto PCs
3. Exclude ‘outliers’ from further analysis



Per Marker Quality Control

- Suggestions for removing ‘bad’ SNPs,

e.g. 1) removal of SNPs with excess missing genotypes
2) removal of SNPs that deviate from Hardy-Weinberg equilibrium
3) removal of SNPs with low minor allele frequency
4) comparing allele frequency to known values

In the prac - we will use PLINK to do the QC

- Summary of PLINK commands
  - the commands can be run individually to help visualise what you're doing, and for trouble shooting
  - In practice, they are usually grouped & several commands run in a single step where appropriate

Individual QC	command	SNP QC	command
1) Excess missing genotypes	`--missing`	1) Excess missingness	`--missing`
2) Outlying homozygosity	`--het`	2) Hardy-Weinberg equilibrium	`--hardy`
3) Discordant Sex	`--check-sex`	3) MAF	`--maf`
4) Remove relatives	`--genome` `--rel-cutoff`	4) Compare to known allele freq	`--freq`