# PLINK (1.06) Documentation

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# Contents

# Chapter 1

# Getting started with PLINK

This page contains some important information on learning to use PLINK and how to handle any problems you encounter.

We suggest that after downloading PLINK you first try the tutorial. This will familiarize you with the basic PLINK commands.

## 1.1 Citing PLINK

If you use PLINK in any published work, please cite both the software (as an electronic resource/URL) and the manuscript describing the methods.

```
        Package:     PLINK (including version number)
        Author:      Shaun Purcell
        URL:         http://pngu.mgh.harvard.edu/purcell/plink/
        Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR,
   Bender D, Maller J, Sklar P, de Bakker PIW, Daly MJ & Sham PC (2007)
        PLINK: a toolset for whole-genome association and population-based
   linkage analysis. American Journal of Human Genetics, 81.
```

## 1.2 Reporting problems, bugs and questions

If you have any problems with PLINK or would like to report a bug, please follow these steps:

**PLEASE READ THIS SECTION BEFORE E-MAILING!**

When an analysis does not report the results you expect, or when PLINK seemingly gives different answers to previous versions or to other software packages, or the last time you ran it, etc, please feel me to e-mail me

```
plink AT chgr DOT mgh DOT harvard DOT edu
```

***but*** also please consider the following before doing so:

- Please first check the Frequently Asked Questions list to see if your question has already been answered
- Please check the LOG file, it often contains important information. For example, did it filter out some individuals based on genotyping rate or missing phenotype/sex information which you were not expecting?
- Please check the format of your data: is it plain text? does each file have the correct number of rows, etc. Are the missing value codes appropriate?

- Please recheck the web-documentation: sometimes the syntax of an option may change.
- If the above steps do not resolve your problem, then please e-mail me `plink AT chgr dot mgh dot harvard dot edu` (this is different from the mailing list – i.e. your e-mail will only be sent to me, not the whole list). The more specific your e-mail, the easier it will be for me to diagnose any problem or error. Please include:
  - The whole LOG file(s)
  - The type of machine you were using
  - Ideally, please try to make some reduced dataset that replicates the problem that you are able to send to me in a ZIP file, so that I will be able to recreate the problem; any data sent to me for these purposes will be immediately deleted after I have resolved the problem.

**HINT** The more of the above steps you follow, the more likely you are to receive a timely, useful response! If you haven't heard within a week or so, please feel free to send a reminder e-mail...

**IMPORTANT** I am willing and able to advise on the use of specific features implemented in PLINK: to diagnose whether they are working as intended and to give a generic description of a procedure or method, if it is unclear from the web documentation. I'm afraid I will not necessarily be able to give specific advice on any one particular dataset, why you should use one method over another, what it all means, etc...

This page contains some important information regarding how to set up and use `PLINK`. Individuals familiar with using command line programs can probably skip most of this page.

## 1.3 Download

**PLINK** is now available for free download. Below are links to ZIP files containing binaries compilied on various platforms as well as the C/C++ source code. Linux/Unix users should download the source code and compile (see notes below).

These downloads also contain a version of gPLINK, an (optional) GUI for PLINK. Please see these pages for instructions on use of gPLINK.

**Remember** This release is considered a *stable* release, although please remember that we cannot guarantee that it, just like most computer programs, does not contain bugs...

| Platform | File | Version |
|---|---|---|
| Linux (x86_64) | plink-1.06-x86_64.zip `http://pngu.mgh.harvard.edu/~purcell/dist/plink-1.06-x86\_64.zip` | v1.06 |
| Linux (i686) | plink-1.06-i686.zip `http://pngu.mgh.harvard.edu/~purcell/dist/plink-1.06-i686.zip` | v1.06 |
| MS-DOS | plink-1.06-dos.zip `http://pngu.mgh.harvard.edu/~purcell/dist/plink-1.06-dos.zip` | v1.06 |
| Apple Mac (PPC) | plink-1.06-mac.zip `http://pngu.mgh.harvard.edu/~purcell/dist/plink-1.06-mac.zip` | v1.06 |
| Apple Mac (Intel) | plink-1.06-mac-intel.zip `http://pngu.mgh.harvard.edu/~purcell/dist/plink-1.06-mac-intel.zip` | v1.06 |
| C/C++ source (.zip) | plink-1.06-src.zip `http://pngu.mgh.harvard.edu/~purcell/dist/plink-1.06-src.zip` | v1.06 |

**One more thing...** If you download `PLINK` please either join the very low-volume e-mail list (link from Introduction page) or drop an e-mail to **`plink AT chgr dot mgh dot harvard dot edu`** letting me know you've downloaded a copy.

For old versions of `PLINK` please visit the archive.

**Debian users** PLINK is available as a Debian package, see these notes `http://packages.debian.org/sid/plink`. Note, the executable is named `snplink` in the Debian `plink` package.

## 1.4 Development version source code

You can download the very latest development source code in this ZIP file `http://pngu.mgh.harvard.edu/~purcell/dist/plink-latest.zip`. This is **really, strongly not recommended** for most users. The code posted here could change on a daily basis and is not versioned.

Development source code versions have a `p` suffix, meaning pre-release. For example, if the current release is `1.04`, the next stable release will be `1.05` and the development code will be `1.05p`. Note that `1.05` may differ from `1.05p` and as noted before, from day-to-day the `1.05` development code may change in any case.

The principle reason for including the source code here is to allow access for specific users to specific, new features. These features are described here.

## 1.5 General installation notes

The `PLINK` executable file should be placed in either the current working directory or somewhere in the command path. This means that typing

```
plink
```

or

```
./plink
```

at the command line prompt will run `PLINK`, no matter which current directory you happen to be in. `PLINK` is a command line program – **clicking on an icon with the mouse will get you nowhere**.

Below, on this page, is a general overview of how to use the command line to run PLINK. The next sections give details about how to install PLINK on different platforms.

## 1.6 Windows/MS-DOS notes

Unzipping the downloaded ZIP file should reveal a single executable program `plink.exe`. The Windows/MS-DOS version of `PLINK` is also a command line program, and is run by typing

```
plink options...
```

not by clicking on the icon with the mouse. Open a DOS windows by selecting "Command Prompt" from the start menu, or entering "command" or "cmd" in the "Run..." option of the start menu.

The folders `c:\windows\` or `c:\winnt\` are typically in the path, so these are good places to copy the file `plink.exe` to. You can copy the `plink.exe` file using Windows, as you would copy-and-paste any file (e.g. using the right-button menu or the keyboard shortcuts control-C (paste) and control-V (paste).

Alternatively, if you know that you will only ever run `PLINK` on files in a single folder, then you can paste `plink.exe` into that folder, e.g. `C:\work\genetics\`. The disadvantage of this approach is that `PLINK` will not be available from the command line if you are in a folder other than this one.

Once you have copied `plink.exe` to the correct location, you can test whether or not `PLINK` is available (i.e. in your command path) by simply typing

```
plink
```

at the command line. You should see something like the following message:

```
Microsoft Windows XP [Version 5.1.2600]
(C) Copyright 1985-2001 Microsoft Corp.
C:\>plink
@----------------------------------------------------------@
|         PLINK!       |     v0.991     |   27/Jul/2006     |
|----------------------------------------------------------|
|  (C) 2006 Shaun Purcell, GNU General Public License, v2  |
|----------------------------------------------------------|
|       http://pngu.mgh.harvard.edu/purcell/plink/         |
@----------------------------------------------------------@
Web-based version check ( --noweb to skip )
Connecting to web...  OK, v0.991 is current
*** Pre-Release Testing Version ***
Writing this text to log file [ plink.log ]
Analysis started: Fri Jul 28 10:07:57 2006
Options in effect:
ERROR: No file [ plink.ped ] exists.
```

Do not worry about this *error message* – normally you would specify your own PED/MAP file names to analyse (i.e. the default input filename is `plink.ped`).

Please ask your system administrator for help if you do not understand this.

**HINT** In MS-DOS, you can to increase the width of the window to avoid output lines wrapping around and being hard to read. To do this under Windows XP DOS: right click on the top title/menu bar of the window and select Properties / Layout / Window Size / Width – increse the width value to a larger value (e.g. 120, or as large as possible without the window getting too big to fit on your screen!).

## 1.7 UNIX/Linux notes

If you are not familiar with the concept of the path variable, ask your system administrator to help. In a UNIX/Linux environment, this would mean either copying the `PLINK` executable to a folder such as

```
/usr/local/bin/
```

or

```
    ~/bin/
```

assuming these directories exist and are in the path. To see which directories are in the path, typing

```
$PATH
```

at the command prompt will often work. To create a directory, say called `bin` in your home directory and add it to the path, try

```
mkdir ~/bin

export PATH=$PATH:~/bin/
```

although this will depend on which shell you are using. Some shells do not include the current directory in the path: in this case, you might need to prefix all `PLINK` commands with the characters `./`, e.g.

```
./plink --file mydata --assoc
```

## 1.8 Source code compilation

`PLINK` is also distributed as C/C++ source code, which you can compile for your particular system using any standard C/C++ compile. Download the `.zip` or `.tar.gz` files and perform the following steps:

```
tar -xzvf plink-0.99s-src.tar.gz
```

or

```
unzip plink-0.99s-src.zip
```

or use a graphical tool such as WinZip to extract the contents of the archive. This should create a directory called

```
    plink-0.99s-src
```

(the exact version number might be different, of course). On the command line, move to that dirctory and simply type `make` :

```
cd plink-0.99s
```

You will need a C/C++ compiler installed on your system for the next step. Linux distributions will include `gcc/g++` by default. Ask your system administrator about installing a C/C++ compiler if you do not have one already (Windows, MS-DOS users).

**Hint** `PLINK` has not been exhaustively tested on different compilers. We sugest you use a recent download of MinGW for Windows, or at least gcc 4.1.

**WARNING** We suggest using the most recent stable release of the compiler available on your platform to avoid compilation problems. For most platforms this means gcc 4.2 as of writing this. Some issues with specific older compiler and specific platforms have been detected, e.g. gcc 3.3.3 on a SGI Altix 3700 system.

Use a standard text editor such as emacs, pico or WordPad to edit the Makefile to suit your particular platform: the top of the Makefile should look like this:

```
# ---------------------------------------------------------------------
#
#   Makefile for PLINK
#
#   Supported platforms
#       Unix / Linux                LINUX
#       Windows                     WIN
#       Mac                         MAC
#       Solaris                     SOLARIS
#
#   Compilation options
#       R plugins                   WITH_R_PLUGINS
#       Web-based version check     WITH_WEBCHECK
#       Ensure 32-bit binary        FORCE_32BIT
#       (Ignored)                   WITH_ZLIB
#       Link to LAPACK              WITH_LAPACK
#       Force dynamic linking       FORCE_DYNAMIC
#
# ---------------------------------------------------------------------
# Set this variable to either UNIX, MAC or WIN
SYS = UNIX
# Leave blank after "=" to disable; put "= 1" to enable
WITH_R_PLUGINS = 1
WITH_WEBCHECK = 1
FORCE_32BIT =
WITH_ZLIB =
WITH_LAPACK =
FORCE_DYNAMIC =
# Put C++ compiler here; Windows has it's own specific version
CXX_UNIX = g++
CXX_WIN = c:\bin\mingw\bin\mingw32-g++.exe
# Any other compiler flags here ( -Wall, -g, etc)
CXXFLAGS =
# Misc
LIB_LAPACK = /usr/lib/liblapack.so.3
# --------------------------------------------------------------------
# Do not edit below this line
# --------------------------------------------------------------------
```

The steps to edit this:

- Change the `SYS` variable to your platform, e.g. `WIN` for Windows
- For the next set of options, put either a `1` or leave blank to turn on or off these options, respectively.
  - `WITH_R_PLUGINS` This enables support for R plugins using `Rserve` as described here. Currently this only works for Unix-based machines.
  - If you want to disable the web-based version check option (not recommended) or if compilation fails with this on, you might try removing the `1` after `WITH_WEBCHECK`
  - When compiling on a 64-bit machine, this option, `FORCE_32BIT`, can force (when set) a 32 bit binary (assumes all necessary libraries, etc) are in place
  - Other options listed here are described below.
- Edit the `CXX_*` variable to point to the C/C++ compiler you wish to use
- To pass any extra commands to the compiler (e.g. location of libraries, etc), you can edit `CXX_FLAGS`

### 1.8.1 LAPACK support

As described here, linking to the LAPACK library can greatly speed up MDS analysis of population stratificaiton. This may take a little tweaking:

- Obtain and compile LAPACK, here `http://www.netlib.org/lapack/`. This requires the gfortran `http://gcc.gnu.org/fortran/` compiler. I cannot assist in any technical difficulties you have with this: ask you IT staff. It is quite possible that LAPACK is already installed somewhere in your institution.

- Determine where the LAPACK library file is located, and whether it is a shared (e.g. `liblapack.so.3`) or static (e.g. `lapack_LINUX.a`) library. (Libraries ending `.a` are static; libraries ending `.so.*` are shared, or dynamically linked. If the LAPACK libraries are shared libraries, then set the `FORCE_DYNAMIC` flag to have `1` after it in the PLINK Makefile.

- Set the variable `LIB_LAPACK` to point to the LAPACK libraries. This may vary by machine and the precise installation of LAPACK. For example, on one machine, I have three static LAPACK libraries in the directory I compiled LAPACK in:

  ```
  ~/src/plink> ls ../lapack-3.2/*a
  ../lapack-3.2/blas_LINUX.a  ../lapack-3.2/lapack_LINUX.a  ../lapack-3.2/tmglib_LINUX.a
  ```

  In this case, set (all one line)

  ```
  LIB_LAPACK = ../lapack-3.2/lapack_LINUX.a
  LIB_LAPACK += ../lapack-3.2/blas_LINUX.a
  LIB_LAPACK += ../lapack-3.2/tmglib_LINUX.a
  ```

  On this machine, it was also necessary to add

  ```
  LIB_LAPACK += -lgfortran
  ```

  On a different (Linux) machine, the LAPACK library was a shared one, in `/usr/lib/liblapack.so.3`, that worked as a single file. In this case, the necessary changes were to set the `WITH_LAPACK` and `FORCE_DYNAMIC` flags, then set

  ```
  LIB_LAPACK = /usr/lib/liblapack.so.3
  ```

Doubtless there is a better way to configure this, but for now I present the above as a quick-fix way of achieving LAPACK support. A little tweaking by somebody who knows what they are doing should suffice. I will not be able to provide detailed help for platforms I am unfamiliar with: you are on your own I'm afraid! You are likely to see some linker errors when compiling if things are not right.

### 1.8.2 Starting compilation

You should then just type

```
make
```

and `PLINK` should (hopefully) start compiling. You should use GNU version, which is sometimes called `gmake` on some platforms (e.g. FreeBSD). It is also possible that you have installed `make` but it is not in your path and/or your version of `make.exe` is called something slightly different, in which case use the full path, e.g. change the following to suit your system:

```
c:\mingw\bin\mingw32-make
```

**NOTE** Often problems in compilation will reflect system-specific / compiler-specific problems: unfortunately, we are not able to give detailed advice on how to do this. If things do not work and you are unsure, you will need to enlist the help of your systems/IT department.

You should see something like the following output (abbreviated)

```
g++ -O3 -I. -DUNIX -static -c plink.cpp
g++ -O3 -I. -DUNIX -static -c options.cpp
g++ -O3 -I. -DUNIX -static -c input.cpp
...
g++ -O3 -static -o plink plink.o options.o input.o binput.o
helper.o genome.o snpfilter.o indfilter.o locus.o multi.o
regress.o crandom.o cluster.o output.o informative.o affpair.o
assoc.o bins.o epi.o phase.o trio.o sharing.o genepi.o sets.o
perm.o mh.o genedrop.o gxe.o merge.o hotel.o multiple.o
```

After a minute or so, this will have created an executable binary file called `plink` (or `plink.exe` for Windows/MSDOS users).

## 1.9 Running PLINK from the command line

A typical session might involve running several commands, e.g. to produce summary statistics on missing data, to exclude some SNPs based on these results, to run an association analysis. Each command involves a separate instantiation of plink – note that `PLINK` does not remember any parameter settings between different runs or store any other information. In otherwords, if you want to perform two association tests with different PED files, but only including SNPs that are above a certain minor allele frequency in both runs, you would use the following:

```
plink --ped file1.ped --map file1.map --maf 0.05 --assoc

plink --ped file2.ped --map file2.map --maf 0.05 --assoc
```

In otherwords, **the following sequence would not work**:

```
plink --ped file1.ped --map file1.map --maf 0.05

plink --ped file1.ped --map file1.map --assoc
```

*MAF returns to default 0.01*

```
plink --ped file2.ped --map file2.map --assoc
```

*As above*

## 1.10 Viewing PLINK output files

**UPDATE** We are developing the tool gPLINK to integrate `PLINK` with Haploview `http://www.broad.mit.edu/mpg/haploview/`. Haploview 4.0 provides a number of features for viewing, filtering and plotting PLINK results files. This is intended to supplant the methods suggested below.

All the output files that `PLINK` generates are plain-text, space-delimited files. Most files will have the same number of fields per line and will have the field names in the first line, facilitating use of a spreadsheet or statistics package to view and process the results.

For small results files, simply printing the files to the terminal or viewing in a text-editor should work well. In Windows/MS-DOS use the `type` command, e.g.

```
type mydata.assoc
```

to view a results file. Alternatively, you can call up WordPad from the command line as follows:

```
write mydata.assoc
```

If you are using a Unix/Linux system, then commands such as `cat`, `more` or `less` can be used to display the results; alternatively text-editors such as `pico`, `emacs` or `vi`.

Of course, Unix/Linux users also have available the entire range of text-processing tools (`grep`, `gawk`, `perl`, `sort`, `head`, etc) and shell-scripting tools, as well as powerful text-editors (`emacs`, etc) that are ideal for processing very large result files. Another alternative is to use a statistics package such as the R package `www.r-project.org` which will provide powerful visualisation tools also.

Windows/MS-DOS users have fewer options for handling very large results files: For moderate size files (e.g. up to 50K SNPs), you could use Excel. For larger files, you can either install cygwin `http://www.cygwin.com/` to provide a Linux-like environment, or use a statistics package such as the R package `www.r-project.org`.

**Personal opinion...** Although a MS-DOS version of `PLINK` is supported, we would, in general, advise any any researchers planning on performing many large-scale analyses to look into adopting a Linux environment, if they are not already using this.

# Chapter 2

# A PLINK tutorial

In this tutorial, we will consider using PLINK to analyse example data: randomly selected genotypes (approximately 80,000 autosomal SNPs) from the 89 Asian HapMap individuals. A phenotype has been simulated based on the genotype at one SNP. In this tutorial, we will walk through using PLINK to work with the data, using a range of features: data management, summary statistics, population stratification and basic association analysis.

**NOTE** These data do not, of course, represent a realistic study design or a realistic disease model. The point of this exercise is simply to get used to running PLINK.

## 2.1 89 HapMap samples and 80K random SNPs

The first step is to obtain a working copy of PLINK and of the example data files.

- Make sure you have PLINK installed on your machine (see these instructions).
- Download the example data archive file which contains the genotypes, map files and two extra phenotype files, described below (zipped, approximately 2.8M)
- Create a new folder/directory on your machine, and unzip the file you downloaded (called `hapmap1.zip`) into this folder.

**HINT!** If you are a Windows user who is unsure how to do this, follow this link
¡hr width=25%¿ Two phenotypes were generated: a quantitative triat and a disease trait (affection status, coded 1=unaffected, 2=affected), based on a median split of the quantitative trait. The quantitative trait was generated as a function of three simple components:

- A random component
- Chinese versus Japanese identity
- A variant on chromosome 2, `rs2222162`

Remember, this model is not intended to be realistic. The following contingency table shows the joint distribution of disease and subpopulation:

| | **Chinese** | **Japanese** |
|---|---|---|
| **Control** | 34 | 11 |
| **Case** | 11 | 33 |

which shows a strong relationship between these two variables. The next table shows the association between the variant `rs2222162` and disease:

|  | **Genotype** |  |  |
|---|---|---|---|
|  | **11** | **12** | **22** |
| **Control** | 17 | 22 | 6 |
| **Case** | 3 | 19 | 22 |

Again, the strong association is clear. Note that the alleles have been recoded as `1` and `2` (this is not necessary for `PLINK` to work, however – it can accept any coding for SNPs).

In summary, we have a single causal variant that is associated with disease. Complicating factors are that this variant is one of 83534 SNPs and also that there might be some degree of confounding of the SNP-disease associations due to the subpopulation-disease association – i.e. a possibility that population stratification effects will exist. Even though we might expect the two subpopulations to be fairly similar from an overall genetic perspective, and even though the sample size is small, this might still lead to an increase in false positive rates if not controlled for.

We will use the affection status variable as the default variable for analysis (i.e. the sixth column in the PED file). The quantitative trait is in a separate alternate phenotype file, `qt.phe`. The file `pop.phe` contains a dummy phenotype that is coded 1 for Chinese individuals and 2 for Japanese individuals. We will use this in investigating between-population differences. You can view these alternate phenotype files in any text editor.

In this tutorial dataset we focus on autosomal SNPs for simplicity, although `PLINK` does provide support for X and Y chromosome SNPs for a number of analyses. See the main documentation for further information.

## 2.2 Using `PLINK` to analyse these data

This tutorial is intended to introduce some of `PLINK`'s features rather than provide exhaustive coverage of them. Futhermore, it is not intended as an analysis plan for whole genome data, or to represent anything close to 'best practice'.

These hyperlinks show an overview of topics:

- Getting started
- Making a binary PED file
- Working with the binary PED file
- Summary statistics: missing rates
- Summary statistics: allele frequencies
- Basic association analysis
- Genotypic association models
- Stratification analysis
- Association analysis, accounting for clusters
- Quantitative trait association analysis
- Extracting a SNP of interest

***Getting started***

Just typing `plink` and specifying a file with no further options is a good way to check that the file is intact, and to get some basic summary statistics about the file.

```
plink --file hapmap1
```

The `--file` option takes a single parameter, the root of the input file names, and will look for two files: a PED file and a MAP file with this root name. In otherwords, `--file hapmap1` implies `hapmap1.ped` and `hapmap1.map` should exist in the current directory.

**HINT!** It is possible to specify files outside of the current directory, and to have the PED and MAP files have different root names, or not end in `.ped` and `.map`, by using the `--ped` and `--map` options.

PED and MAP files are plain text files; PED files contain genotype information (one person per row) and MAP files contain information on the name and position of the markers in the PED file. If you are not familiar with the file formats required for PED and MAP files, please consult this page.

The above command should generate something like the following output in the console window. It will also save this information to a file called `plink.log`.

```
@----------------------------------------------------------@
|        PLINK!       |     v0.99l     |   27/Jul/2006     |
|----------------------------------------------------------|
|  (C) 2006 Shaun Purcell, GNU General Public License, v2  |
|----------------------------------------------------------|
|       http://pngu.mgh.harvard.edu/purcell/plink/         |
@----------------------------------------------------------@
Web-based version check ( --noweb to skip )
Connecting to web...  OK, v0.99l is current
*** Pre-Release Testing Version ***
Writing this text to log file [ plink.log ]
Analysis started: Mon Jul 31 09:00:11 2006
Options in effect:
   --file hapmap1
83534 (of 83534) markers to be included from [ hapmap1.map ]
89 individuals read from [ hapmap1.ped ]
89 individuals with nonmissing phenotypes
Assuming a binary trait (1=unaff, 2=aff, 0=miss)
Missing phenotype value is also -9
Before frequency and genotyping pruning, there are 83534 SNPs
Applying filters (SNP-major mode)
89 founders and 0 non-founders found
0 of 89 individuals removed for low genotyping ( MIND > 0.1 )
859 SNPs failed missingness test ( GENO > 0.1 )
16994 SNPs failed frequency test ( MAF < 0.01 )
After frequency and genotyping pruning, there are 65803 SNPs
Analysis finished: Mon Jul 31 09:00:19 2006
```

The information contained here can be summarized as follows:

- A banner showing copyright information and the version number – the web-based version check shows that this is an up-to-date version of `PLINK` and displays a message that v0.99l is a pre-release testing version.
- A message indicating that the log file will be saved in `plink.log`. The name of the output file can be changed with the `--out` option – e.g. specifying `--out anal1` will generate a log file called `anal1.log` instead.
- A list of the command options specified is given next: in this case it is only a single option, `--file hapmap1`. By keeping track of log files, and naming each analysis with its own `--out` name, it makes it easier to keep track of when and how the different output files were generated.

- Next is some information on the number of markers and individuals read from the MAP and PED file. In total, just over 80,000 SNPs were read in from the MAP file. It is written `"...83534 (of 83534)..."` because some SNPs might be excluded (by making the physical position a negative number in the MAP file), in which case the first number would indicate how many SNPs are included. In this case, all SNPs are read in from the PED file. We also see that 89 individuals were read in from the PED file, and that all these individuals had valid phenotype information.

- Next, `PLINK` tells us that the phenotype is an affection status variable, as opposed to a quantitative trait, and lets us know what the missing values are.

- The next stage is the filtering stage – individuals and/or SNPs are removed on the basis of thresholds. Please see this page for more information on setting thresholds. In this case we see that no individuals were removed, but almost 20,000 SNPs were removed, based on missingness (859) and frequency (16994). This particularly high proportion of removed SNPs is based on the fact that these are random HapMap SNPs in the Chinese and Japanese samples, rather than pre-selected markers on a whole-genome association product: there will be many more rare and monomorphic markers here than one would normally expect.

- Finally, a line is given that indicates when this analysis finished. You can see that it took 8 seconds (on my machine at least) to read in the file and apply the filters.

If other analyses had been requsted, then the other output files generated would have been indicated in the log file. All output files that `PLINK` generates have the same format: `root.extension` where `root` is, by default, "plink" but can be changed with the `--out` option, and the extension will depend on the type of output file it is (a complete list of extensions is given here).

***Making a binary PED file***

The first thing we will do is to make a binary PED file. This more compact representation of the data saves space and speeds up subsequent analysis. To make a binary PED file, use the following command.

```
plink --file hapmap1 --make-bed --out hapmap1
```

If it runs correctly on your machine, you should see the following in your output:

```
above as before
...
Before frequency and genotyping pruning, there are 83534 SNPs
Applying filters (SNP-major mode)
89 founders and 0 non-founders found
0 SNPs failed missingness test ( GENO > 1 )
0 SNPs failed frequency test ( MAF < 0 )
After frequency and genotyping pruning, there are 83534 SNPs
Writing pedigree information to [ hapmap1.fam ]
Writing map (extended format) information to [ hapmap1.bim ]
Writing genotype bitfile to [ hapmap1.bed ]
Using (default) SNP-major mode
Analysis finished: Mon Jul 31 09:10:05 2006
```

There are several things to note:

- When using the `--make-bed` option, the threshold filters for missing rates and allele frequency were automatically set to exclude nobody. Although these filters can be specified manually (using `--mind`, `--geno` and `--maf`) to exclude people, this default tends to be wanted when creating a new PED or binary PED file. The commands `--extract` / `--exclude` and `--keep` / `--remove` can also be applied at this stage.

- Three files are created with this command – the binary file that contains the raw genotype data `hapmap1.bed` but also a revsied map file `hapmap1.bim` which contains two extra columns that give the allele names for each SNP, and `hapmap1.fam` which is just the first six columns of `hapmap1.ped`. You can view the `.bim` and `.fam` files – but do not try to view the `.bed` file. None of these three files should be manually editted.

If, for example, you wanted to create a new file that only includes individuals with high genotyping (at least 95% complete), you would run:

```
plink --file hapmap1 --make-bed --mind 0.05 --out highgeno
```

which would create files

```
highgeno.bed
highgeno.bim
highgeno.fam
```

***Working with the binary PED file***

To specify that the input data are in binary format, as opposed to the normal text PED/MAP format, just use the `--bfile` option instead of `--file`. To repeat the first command we ran (which just loads the data and prints some basic summary statistics):

```
plink --bfile hapmap1

    Writing this text to log file [ plink.log ]
    Analysis started: Mon Jul 31 09:12:08 2006
    Options in effect:
            --bfile hapmap1
    Reading map (extended format) from [ hapmap1.bim ]
    83534 markers to be included from [ hapmap1.bim ]
    Reading pedigree information from [ hapmap1.fam ]
    89 individuals read from [ hapmap1.fam ]
    89 individuals with nonmissing phenotypes
    Reading genotype bitfile from [ hapmap1.bed ]
    Detected that binary PED file is v1.00 SNP-major mode
    Before frequency and genotyping pruning, there are 83534 SNPs
    Applying filters (SNP-major mode)
    89 founders and 0 non-founders found
    0 of 89 individuals removed for low genotyping ( MIND > 0.1 )
    859 SNPs failed missingness test ( GENO > 0.1 )
    16994 SNPs failed frequency test ( MAF < 0.01 )
    After frequency and genotyping pruning, there are 65803 SNPs
    Analysis finished: Mon Jul 31 09:12:10 2006
```

The things to note here:

- That three files `hapmap1.bim`, `hapmap1.fam` and `hapmap1.bed` were loaded instead of the usual two files. That is, `hapmap1.ped` and `hapmap1.map` are not used in this analysis, and could in fact be deleted now.
- The data are loaded in much more quickly – based on the timestamp at the beginning and end of the log output, this took 2 seconds instead of 10.

***Summary statistics: missing rates***

Next, we shall generate some simple summary statistics on rates of missing data in the file, using the `--missing` option:

```
plink --bfile hapmap1 --missing --out miss_stat
```

which should generate the following output:

```
...
0 of 89 individuals removed for low genotyping ( MIND > 0.1 )
Writing individual missingness information to [ miss_stat.imiss ]
Writing locus missingness information to [ miss_stat.lmiss ]
...
```

Here we see that no individuals were removed for low genotypes (`MIND > 0.1` implies that we accept people with less than 10 percent missingness).

The per individual and per SNP (after excluding individuals on the basis of low genotyping) rates are then output to the files `miss_stat.imiss` and `miss_stat.lmiss` respectively. If we had not specified an `--out` option, the root output filename would have defaulted to "plink".

These output files are standard, plain text files that can be viewed in any text editor, pager, spreadsheet or statistics package (albeit one that can handle large files). Taking a look at the file `miss_stat.lmiss`, for example using the `more` command which is present on most systems:

```
more miss_stat.lmiss
```

we see

```
CHR         SNP   N_MISS     F_MISS
  1   rs6681049        0          0
  1   rs4074137        0          0
  1   rs7540009        0          0
  1   rs1891905        0          0
  1   rs9729550        0          0
  1   rs3813196        0          0
  1   rs6704013        2  0.0224719
  1    rs307347       12   0.134831
  1   rs9439440        2  0.0224719
...
```

That is, for each SNP, we see the number of missing individuals (`N_MISS`) and the proportion of individuals missing (`F_MISS`). Similarly:

```
more miss_stat.imiss
```

we see

```
   FID         IID MISS_PHENO     N_MISS     F_MISS
HCB181           1          N        671 0.00803266
HCB182           1          N       1156  0.0138387
HCB183           1          N        498 0.00596164
HCB184           1          N        412 0.00493212
HCB185           1          N        329 0.00393852
HCB186           1          N       1233  0.0147605
HCB187           1          N        258 0.00308856
...
```

The final column is the actual genotyping rate for that individual – we see the genotyping rate is very high here.

**HINT** If you are using a spreadsheet package that can only display a limited number of rows (some popular packages can handle just over 65,000 rows) then it might be desirable to ask `PLINK` to analyse the data by chromosome, using the `--chr` option. For example, to perform the above analysis for chromosome 1:

```
plink --bfile hapmap1 --chr 1 --out res1 --missing
```

then for chromosome 2:

```
plink --bfile hapmap1 --chr 2 --out res2 --missing
```

and so on.

***Summary statistics: allele frequencies***

Next we perform a similar analysis, except requesting allele frequencies instead of genotyping rates. The following command generates a file called `freq_stat.frq` which contains the minor allele frequency and allele codes for each SNP.

```
plink --bfile hapmap1 --freq --out freq_stat
```

It is also possible to perform this frequency analysis (and the missingness analysis) stratified by a categorical, cluster variable. In this case, we shall use the file that indicates whether the individual is from the Chinese or the Japanese sample, `pop.phe`. This cluster file contains three columns; each row is an individual. The format is described more fully in the main documentation.

To perform a stratified analysis, use the `--within` option.

```
plink --bfile hapmap1 --freq --within pop.phe --out freq_stat
```

The output will now indicate that a file called `freq_stat.frq.strat`. has been generated instead of `freq_stat.frq`. If we view this file:

```
more freq_stat.frq.strat
```

we see each row is now the allele frequency for each SNP stratifed by subpopulation:

```
CHR          SNP     CLST    A1    A2          MAF
  1    rs6681049        1     1     2     0.233333
  1    rs6681049        2     1     2     0.193182
  1    rs4074137        1     1     2          0.1
  1    rs4074137        2     1     2    0.0568182
  1    rs7540009        1     0     2            0
  1    rs7540009        2     0     2            0
  1    rs1891905        1     1     2     0.411111
  1    rs1891905        2     1     2     0.397727
...
```

Here we see that each SNP is represented twice - the `CLST` column indicates whether the frequency is from the Chinese or Japanese populations, coded as per the `pop.phe` file.

If you were just interested in a specific SNP, and wanted to know what the frequency was in the two populations, you can use the `--snp` option to select this SNP:

```
plink --bfile hapmap1 --snp rs1891905 --freq --within pop.phe --out snp1_frq_stat
```

would generate a file `snp1_frq_stat.frq.strat` containing only the population-specific frequencies for this single SNP. You can also specify a range of SNPs by adding the `--window` *kb* option or using the options `--from` and `--to`, following each with a different SNP (they must be in the correct order and be on the same chromosome). Follow this link for more details.

***Basic association analysis***

Let's now perform a basic association analysis on the disease trait for all single SNPs. The basic command is

```
plink --bfile hapmap1 --assoc --out as1
```

which generates an output file `as1.assoc` which contains the following fields

```
     CHR         SNP   A1      F_A      F_U   A2       CHISQ            P           OR
       1   rs6681049    1   0.1591   0.2667    2       3.067      0.07991       0.5203
       1   rs4074137    1  0.07955  0.07778    2    0.001919       0.9651        1.025
       1   rs1891905    1   0.4091      0.4    2     0.01527       0.9017        1.038
       1   rs9729550    1   0.1705  0.08889    2       2.631       0.1048        2.106
       1   rs3813196    1  0.03409  0.02222    2      0.2296       0.6318        1.553
       1  rs12044597    1      0.5   0.4889    2     0.02198       0.8822        1.045
       1  rs10907185    1   0.3068   0.2667    2      0.3509       0.5536        1.217
       1  rs11260616    1   0.2326      0.2    2      0.2754       0.5998        1.212
       1    rs745910    1   0.1395   0.1932    2      0.9013       0.3424       0.6773
  ...
```

where each row is a single SNP association result. The fields are:

- Chromosome
- SNP identifier
- Code for allele 1 (the minor, rare allele based on the entire sample frequencies)
- The frequency of this variant in cases
- The frequency of this variant in controls
- Code for the other allele
- The chi-squared statistic for this test (1 df)
- The asymptotic significance value for this test
- The odds ratio for this test

If a test is not defined (for example, if the variant is monomorphic but was not excluded by the filters) then values of `NA` for *not applicable* will be given (as these are read by the package `R` to indicate missing data, which is convenient if using `R` to analyse the set of results).

In a Unix/Linux environment, one could simply use the available command line tools to sort the list of association statistics and print out the top ten, for example:

```
sort --key=7 -nr as1.assoc | head
```

would give

```
      13   rs9585021    1    0.625   0.2841    2       20.62    5.586e-06          4.2
       2   rs2222162    1   0.2841   0.6222    2       20.51    5.918e-06       0.2409
       9  rs10810856    1   0.2955  0.04444    2       20.01    7.723e-06        9.016
       2   rs4675607    1   0.1628   0.4778    2       19.93     8.05e-06       0.2125
       2   rs4673349    1   0.1818      0.5    2       19.83    8.485e-06       0.2222
       2   rs1375352    1   0.1818      0.5    2       19.83    8.485e-06       0.2222
      21    rs219746    1      0.5   0.1889    2       19.12    1.228e-05        4.294
       1   rs4078404    2      0.5      0.2    1       17.64    2.667e-05            4
      14   rs1152431    2   0.2727   0.5795    1       16.94    3.862e-05       0.2721
      14   rs4899962    2   0.3023   0.6111    1       16.88    3.983e-05       0.2758
```

Here we see that the simulated disease variant `rs2222162` is actually the second most significant SNP in the list, with a large difference in allele frequencies of 0.28 in cases versus 0.62 in controls. However, we also see that, just by chance, a second SNP on chromosome 13 shows a slightly higher test result, with coincidentally similar allele frequencies in cases and controls. (Whether this result is due to chance alone or perhaps represents some confounding due to the population structure in this sample, we will investigate below). This highlights the important point that when performing so many tests, particularly in a small sample, we often expect the distribution of true positive results to be virtually indistinguishable from the best false positive results. That our variant appears in the top ten list is reassuring however.

To get a sorted list of association results, that also includes a range of significance values that are adjusted for multiple testing, use the `--adjust` flag:

```
plink --bfile hapmap1 --assoc --adjust --out as2
```

This generates the file `as2.assoc.adjust` in addition to the basic `as2.assoc` output file. Using `more`, one can easily look at one's most significant associations:

```
more as2.assoc.adjusted
```

```
 CHR         SNP      UNADJ          GC      BONF      HOLM   SIDAK_SS   SIDAK_SD     FDR_BH  FDR_BY
  13   rs9585021  5.586e-06  3.076e-05    0.3676    0.3676     0.3076     0.3076    0.09306
1
   2   rs2222162  5.918e-06  3.231e-05    0.3894    0.3894     0.3226     0.3226    0.09306
1
   9  rs10810856  7.723e-06  4.049e-05    0.5082    0.5082     0.3984     0.3984    0.09306
1
   2   rs4675607   8.05e-06  4.195e-05    0.5297    0.5297     0.4112     0.4112    0.09306
1
   2   rs1375352  8.485e-06  4.386e-05    0.5584    0.5583     0.4279     0.4278    0.09306
1
   2   rs4673349  8.485e-06  4.386e-05    0.5584    0.5583     0.4279     0.4278    0.09306
1
  21    rs219746  1.228e-05  6.003e-05    0.8083    0.8082     0.5544     0.5543     0.1155
1
   1   rs4078404  2.667e-05  0.0001159         1         1     0.8271      0.827     0.2194
1
  14   rs1152431  3.862e-05  0.0001588         1         1     0.9213     0.9212     0.2621
1
  14   rs4899962  3.983e-05   0.000163         1         1     0.9273     0.9272     0.2621
1
   8   rs2470048  4.487e-05  0.0001804         1         1     0.9478     0.9478     0.2684
1
```

Here we see a pre-sorted list of association results. The fields are as follows:

- Chromosome
- SNP identifier
- Unadjusted, asymptotic significance value
- Genomic control adjusted significance value. This is based on a simple estimation of the inflation factor based on median chi-square statistic. These values do not control for multiple testing therefore.
- Bonferroni adjusted significance value
- Holm step-down adjusted significance value
- Sidak single-step adjusted significance value
- Sidak step-down adjusted significance value

- Benjamini & Hochberg (1995) step-up FDR control
- Benjamini & Yekutieli (2001) step-up FDR control

In this particular case, we see that no single variant is significant at the 0.05 level after genome-wide correction. Different correction measures have different properties which are beyond the scope of this tutorial to discuss: it is up to the investigator to decide which to use and how to interpret them.

When the `--adjust` command is used, the log file records the inflation factor calculated for the genomic control analysis, and the mean chi-squared statistic (that should be 1 under the null):

```
Genomic inflation factor (based on median chi-squared) is 1.18739
Mean chi-squared statistic is 1.14813
```

These values would actually suggest that although no very strong stratification exists, there is perhaps a hint of an increased false positive rate, as both values are greater than 1.00.

**HINT** The adjusted significance values that control for multiple testing are, by default, based on the unadjusted significance values. If the flag `--gc` is specified as well as `--adjust` then these adjusted values will be based on the genomic-control significance value instead.

In this particular instance, where we already know about the Chinese/Japanese subpopulations, it might be of interest to directly look at the inflation factor that results from having population membership as the phenotype in a case/control analysis, just to provide extra information about the sample. That is, running the command using the alternate phenotype option (i.e. replacing the disease phenotype with the one in `pop.phe`, which is actually subpopulation membership):

```
plink --bfile hapmap1 --pheno pop.phe --assoc --adjust --out as3
```

we see that testing for frequency differences between Chinese and Japanese individuals, we do see some departure from the null distribution:

```
Genomic inflation factor (based on median chi-squared) is 1.72519
Mean chi-squared statistic is 1.58537
```

That is, the inflation factor of 1.7 represents the maximum possible inflation factor if the disease were perfectly correlated with subpopulation that could arise from the Chinese/Japanese split in the sample (this does not account for any possible within-subpopulation structure, of course, that might also increase SNP-disease false positive rates).

We will return to this issue below, when we consider using the whole genome data to detect stratification more directly.

***Genotypic and other association models***

We can calculate association statistics based on the 2-by-3 genotype table as well as the standard allelic test; we can also calculate tests that assume dominant or recessive action of the minor allele; finally, we can perform the Cochran-Armitage trend test instead of the basic allelic test. All these tests are performed with the single command `--model`. Just as the `--assoc` command, this can be easily applied to all SNPs. In this case, let's just run it for our SNP of interest, `rs2222162`

```
plink --bfile hapmap1 --model --snp rs2222162 --out mod1
```

This generates the file `mod1.model` which has more than one row per SNP, representing the different tests performed for each SNP. The format of this file is described here. The tests are the basic allelic test, the Cochran-Armitage trend test, dominant and recessive models and a genotypic test. All test statistics are distributed as chi-squared with 1 df under the null, with the exception of the genotypic test which has 2 df.

But there is a problem here: in this particular case, running the basic model command will not produce values for the genotypic tests. This is because, by default, every cell in the 2-by-3 table is required to have at least 5 observations, which does not hold here. This default can be changed with the `--cell` option. This option is followed by the minimum number of counts in each cell of the 2-by-3 table required before these extended analyses are performed. For example, to force the genotypic tests for this particular SNP just for illustrative purposes, we need to run:

```
plink --bfile hapmap1 --model --cell 0 --snp rs2222162 --out mod2
```

and now the genotypic tests will also be calculated, as we set the minimum number in each cell to 0. We see that the genotype counts in affected and unaffected individuals are

```
CHR         SNP     TEST         AFF       UNAFF    CHISQ   DF           P
  2   rs2222162     GENO     3/19/22     17/22/6    19.15    2   6.932e-05
  2   rs2222162    TREND       25/63       56/34    19.15    1   1.207e-05
  2   rs2222162  ALLELIC       25/63       56/34    20.51    1   5.918e-06
  2   rs2222162      DOM       22/22        39/6    13.87    1   0.0001958
  2   rs2222162      REC        3/41       17/28    12.24    1   0.0004679
```

which, reassuringly, match the values presented in the table above, which were generated when the trait was simulated. Looking at the other test statistics, we see all are highly significant (as would be expected for a strong, common, allelic effect) although the allelic test has the most significant p-value. This makes sense, as the data were essentially simulated under an allelic (dosage) model.

***Stratification analysis***

The analyses so far have ignored the fact that our sample consists of two similar, but distinct subpopulations, the Chinese and Japanese samples. In this particular case, we already know that the sample consists of these two groups; we also know that the disease is more prevalent in one of the groups. More generally, we might not know anything of potential population substructure in our sample upfront. One way to address such issues is to use whole genome data to cluster individuals into homogeneous groups. There are a number of options and different ways of performing this kind of analysis in PLINK and we will not cover them all here. For illustrative purposes, we shall perform a cluster analysis that pairs up individuals on the basis of genetic identity. The command, which may take a number of minutes to run, is:

```
plink --bfile hapmap1 --cluster --mc 2 --ppc 0.05 --out str1
```

which requests IBS clustering (`--cluster`) but with the constraints that each cluster has no more than two individuals (`--mc 2`) and that any pair of individuals who have a significance value of less than 0.05 for the test of whether or not the two individuals belong to the same population based on the available SNP data are not merged. These options and tests are described in further detail in the relevant main documentation.

We see the following output in the log file and on the console:

```
...
Clustering individuals based on genome-wide IBS
Merge distance p-value constraint = 0.05
Of these, 3578 are pairable based on constraints
Writing cluster progress to [ plink.cluster0 ]
Cannot make clusters that satisfy constraints at step 45
Writing cluster solution (1) [ str1.cluster1 ]
Writing cluster solution (2) [ str1.cluster2 ]
Writing cluster solution (3) [ str1.cluster3 ]
...
```

which indicate that IBS-clustering has been performed. These files are described in the main documentation. The file `str1.cluster1` contains the results of clustering in a format that is easy to read:

```
more str1.cluster1

   SOL-0    HCB181_1 JPT260_1
   SOL-1    HCB182_1 HCB225_1
   SOL-2    HCB183_1 HCB193_1
   SOL-3    HCB184_1 HCB202_1
   SOL-4    HCB185_1 HCB217_1
   SOL-5    HCB186_1 HCB196_1
   SOL-6    HCB187_1 HCB213_1
```

```
SOL-7    HCB188_1 HCB194_1
SOL-8    HCB189_1 HCB192_1
SOL-9    HCB190_1 HCB224_1
SOL-10   HCB191_1 HCB220_1
SOL-11   HCB195_1 HCB204_1
SOL-12   HCB197_1 HCB211_1
SOL-13   HCB198_1 HCB210_1
SOL-14   HCB199_1 HCB221_1
SOL-15   HCB200_1 HCB218_1
SOL-16   HCB201_1 HCB206_1
SOL-17   HCB203_1 HCB222_1
SOL-18   HCB205_1 HCB208_1
SOL-19   HCB207_1 HCB223_1
SOL-20   HCB209_1 HCB219_1
SOL-21   HCB212_1 HCB214_1
SOL-22   HCB215_1 HCB216_1
SOL-23   JPT226_1 JPT242_1
SOL-24   JPT227_1 JPT240_1
SOL-25   JPT228_1 JPT244_1
SOL-26   JPT229_1 JPT243_1
SOL-27   JPT230_1 JPT267_1
SOL-28   JPT231_1 JPT236_1
SOL-29   JPT232_1 JPT247_1
SOL-30   JPT233_1 JPT248_1
SOL-31   JPT234_1 JPT255_1
SOL-32   JPT235_1 JPT264_1
SOL-33   JPT237_1 JPT250_1
SOL-34   JPT238_1 JPT241_1
SOL-35   JPT239_1 JPT253_1
SOL-36   JPT245_1 JPT262_1
SOL-37   JPT246_1 JPT263_1
SOL-38   JPT249_1 JPT258_1
SOL-39   JPT251_1 JPT252_1
SOL-40   JPT254_1 JPT261_1
SOL-41   JPT256_1 JPT265_1
SOL-42   JPT257_1
SOL-43   JPT259_1 JPT269_1
SOL-44   JPT266_1 JPT268_1
```

Here we see that all but one pair are concordant for being Chinese or Japanese (the IDs for each individual are in this case coded to represent which HapMap subpopulation they belong to: HCB and JPT. Note, these do not represent any official HapMap coding/ID schemes – I've used them purely to make it clear which population each individual belongs to). We see that one individual was not paired with anybody else, as there is an odd numbered of subjects overall. This individual would not contribute to any subsequent association testing that conditions on this cluster solution. We also see that the Japanese individual `JPT260_1` paired with a Chinese individual `HCB181_1` rather than `JPT257_1`. Clearly, this means that `HCB181_1` and `JPT260_1` do not differ significantly based on the test we performed: this test will have limited power to distinguish individuals from very similar subpopulations, alternatively, one of these individuals could be of mixed ancestry. In any case, it is interesting that `JPT260_1` was not paired with `JPT257_1` instead. Further inspection of the data actually reveal that `JPT257_1` is somewhat unusual, having very long stretches of homozygous genotypes (use the `--homozyg-kb` and `--homozyg-snp` options) are a high inbreeding coefficient, which probably explain why this individual was not considered similar to the other Japanese individuals by this algorithm.

**Note** By using the `--genome` option, it is possible to examine the significance tests for all pairs of individuals, as described in the main documentation.

***Association analysis, accounting for clusters***

After having performed the above matching based on genome-wide IBS, we can now perform the association test conditional on the matching. For this, the relevant file is the `str1.cluster2` file, which contains the same information as `str1.cluster1` but in the format of a cluster variable file, that can be used in conjunction with the `--within` option.

For this matched analysis, we shall use the Cochran-Mantel-Haenszel (CMH) association statistic, which tests for SNP-disease association conditional on the clustering supplied by the cluster file; we will also include the `--adjust` option to get a sorted list of CMH association results:

```
plink --bfile hapmap1 --mh --within str1.cluster2 --adjust --out aac1
```

The relevant lines from the log are:

```
...
Reading clusters from [ str1.cluster2 ]
89 of 89 individuals assigned to 45 cluster(s)
...
Cochran-Mantel-Haenszel 2x2xK test, K = 45
Writing results to [ aac1.cmh ]
Computing corrected significance values (FDR, Sidak, etc)
Genomic inflation factor (based on median chi-squared) is 1.03878
Mean chi-squared statistic is 0.988748
Writing multiple-test corrected significance values to [ aac1.cmh.adjusted ]
...
```

We see that `PLINK` has correctly assigned the individuals to 45 clusters (i.e. one of these clusters is of size 1, all others are pairs) and then performs the CMH test. The genomic control inflation factors are now reduced to essentially 1.00, which is consistent with the idea that there was some substructure inflating the distribution of test statistics in the previous analysis.

Looking at the adjusted results file:

```
more aac1.cmh.adjusted
```

```
CHR        SNP      UNADJ         GC    BONF    HOLM  SIDAK_SS  SIDAK_SD  FDR_BH  FDR_BY
  2  rs2222162  1.906e-06  2.963e-06  0.1241  0.1241    0.1167    0.1167  0.1241       1
  2  rs4673349  6.436e-06  9.577e-06  0.4192  0.4191    0.3424    0.3424  0.1261       1
  2  rs1375352  6.436e-06  9.577e-06  0.4192  0.4191    0.3424    0.3424  0.1261       1
 13  rs9585021  7.744e-06  1.145e-05  0.5043  0.5043    0.3961    0.3961  0.1261       1
  2  rs4675607  5.699e-05  7.845e-05       1       1    0.9756    0.9756  0.7423       1
 13  rs9520572  0.0002386  0.0003122       1       1         1         1  0.9017       1
 11   rs992564    0.00026  0.0003392       1       1         1         1  0.9017       1
 12  rs1290910  0.0002738  0.0003566       1       1         1         1  0.9017       1
  4  rs1380899  0.0002747  0.0003577       1       1         1         1  0.9017       1
 14  rs1190968  0.0002747  0.0003577       1       1         1         1  0.9017       1
  8   rs951702  0.0003216  0.0004164       1       1         1         1  0.9017       1
  ...
```

Here we see that the "disease" variant, `rs2222162` has moved from being number 2 in the list to number 1, although it is still not significant after genome-wide correction.

In this last example, we specifically requested that `PLINK` pair up the most similar individuals. We can also perform the clustering, but with fewer, or different, constraints on the final solution. For example, here we do not impose a maximum cluster size: rather we request that each cluster contains at least 1 case and 1 control (i.e. so that it is informative for association) with the `--cc` option, and specify a threshold of 0.01 for `--ppc`:

```
plink --bfile hapmap1 --cluster --cc --ppc 0.01 --out version2
```

which generates the following final solution (`version2.cluster1`):

```
SOL-0   HCB181_1(1) HCB189_1(1) HCB198_1(1) HCB210_1(2) HCB222_1(1) HCB203_1(1) HCB196_1(1)

        HCB183_1(2) HCB195_1(1) HCB185_1(1) HCB187_1(1) HCB215_1(2) HCB216_1(1)
SOL-1   HCB182_1(1) HCB186_1(1) HCB207_1(2) HCB223_1(1) HCB194_1(1) HCB188_1(1) HCB199_1(1)
        HCB221_1(2) HCB225_1(1) HCB217_1(1) HCB190_1(1) HCB202_1(1) HCB224_1(2) HCB201_1(2)
        HCB206_1(1) HCB208_1(1) HCB209_1(1) HCB213_1(1) HCB212_1(1) HCB214_1(2)
SOL-2   HCB184_1(1) HCB219_1(2) HCB218_1(1) HCB200_1(1) HCB191_1(2) HCB220_1(1) HCB197_1(1)
        HCB211_1(2) HCB192_1(1) HCB204_1(1) JPT255_1(2) HCB193_1(1) JPT245_1(2)
SOL-3   HCB205_1(1) JPT264_1(2) JPT253_1(1) JPT258_1(2) JPT228_1(1) JPT244_1(2) JPT238_1(2)
        JPT269_1(2) JPT242_1(2) JPT234_1(2) JPT265_1(1) JPT230_1(2) JPT262_1(1) JPT267_1(2)
        JPT231_1(1) JPT239_1(2) JPT263_1(2) JPT260_1(2)
SOL-4   JPT226_1(1) JPT251_1(2) JPT240_1(2) JPT227_1(2) JPT232_1(2) JPT235_1(2) JPT237_1(2)
        JPT250_1(1) JPT246_1(2) JPT229_1(2) JPT243_1(1) JPT266_1(2) JPT252_1(2) JPT249_1(2)
        JPT233_1(1) JPT248_1(2) JPT241_1(2) JPT254_1(1) JPT261_1(2) JPT259_1(2) JPT236_1(2)
        JPT256_1(1) JPT247_1(2) JPT268_1(2) JPT257_1(2)
```

The lines have been wrapped for clarity of reading here: normally, an entire cluster file is on a single line. Also note that the phenotype has been added in parentheses after each family/individual ID (as the `--cc` option was used). Here we see that the resulting clusters have largely separated Chinese and Japanese individuals into different clusters. The clustering results in a five class solution based on the `--ppc` constraint – i.e. clearly, to merge any of these five clusters would have involved merging two individuals who are different at the 0.01 level, and this is why the clustering stopped at this point (as opposed to merging everybody, ultimately arriving at a 1-class solution).

Based on this alternate clustering scheme, we can repeat our association analysis.

**Note** This is not necessarily how actual analysis of real data should be conducted, of course, i.e. by trying different analyses, clusters, etc, until one finds the most significant result... The point of this is just to show what options are available.

```
plink --bfile hapmap1 --mh --within version2.cluster2 --adjust --out aac2
```

Now the log file records that five clusters were found, and a low inflation factor:

```
...
Cochran-Mantel-Haenszel 2x2xK test, K = 5
Writing results to [ aac2.cmh ]
Computing corrected significance values (FDR, Sidak, etc)
...
Genomic inflation factor (based on median chi-squared) is 1.01489
Mean chi-squared statistic is 0.990643
...
```

Looking at `aac2.cmh.adjusted`, we now see that the disease SNP is genome-wide significant:

```
 CHR        SNP      UNADJ         GC       BONF       HOLM   SIDAK_SS   SIDAK_SD     FDR_BH     FDR_BY
   2  rs2222162  8.313e-10  1.104e-09  5.47e-05  5.47e-05  5.47e-05  5.47e-05  5.47e-05  0.0006384
  13  rs9585021  2.432e-06  2.882e-06      0.16      0.16    0.1479    0.1479   0.06931      0.809
   2  rs4675607   3.16e-06  3.731e-06    0.2079    0.2079    0.1877    0.1877   0.06931      0.809
   2  rs4673349   5.63e-06  6.594e-06    0.3705    0.3705    0.3096    0.3096    0.0741     0.8648
   2  rs1375352   5.63e-06  6.594e-06    0.3705    0.3705    0.3096    0.3096    0.0741     0.8648
 ...
```

That is, rs2222162 now has a significance value of 5.47e-05 even if we use Bonferroni adjustment for multiple comparisons.

A third way to perform the stratification analysis is to specify the number of clusters one wants in the final solution. Here we will specify two clusters, using the `--K` option, and remove the significance test constraint by setting `--ppc` to 0 (by omitting that option):

```
plink --bfile hapmap1 --cluster --K 2 --out version3
```

This analysis results in the following two-class solution:

```
SOL-0 HCB181_1 HCB182_1 HCB225_1 HCB189_1 HCB188_1 HCB194_1 HCB205_1
      HCB208_1 HCB199_1 HCB221_1 HCB201_1 HCB206_1 HCB196_1 JPT253_1
      HCB202_1 HCB203_1 HCB191_1 HCB220_1 HCB197_1 HCB211_1 HCB215_1
      HCB216_1 HCB212_1 HCB213_1 HCB183_1 HCB195_1 HCB193_1 HCB186_1
      HCB207_1 HCB223_1 HCB187_1 HCB209_1 HCB214_1 HCB184_1 HCB219_1
      HCB218_1 HCB200_1 HCB185_1 HCB217_1 HCB198_1 HCB210_1 HCB222_1
      HCB192_1 HCB190_1 HCB224_1
SOL-1 HCB204_1 JPT255_1 JPT257_1 JPT226_1 JPT242_1 JPT228_1 JPT244_1
      JPT238_1 JPT269_1 JPT232_1 JPT247_1 JPT231_1 JPT239_1 JPT229_1
      JPT243_1 JPT236_1 JPT256_1 JPT265_1 JPT227_1 JPT266_1 JPT268_1
      JPT263_1 JPT235_1 JPT237_1 JPT250_1 JPT246_1 JPT240_1 JPT251_1
      JPT259_1 JPT252_1 JPT233_1 JPT248_1 JPT241_1 JPT254_1 JPT261_1
      JPT245_1 JPT264_1 JPT249_1 JPT258_1 JPT230_1 JPT267_1 JPT262_1
      JPT234_1 JPT260_1
```

Here we see that the solution has assigned all Chinese and all Japanese two separate groups except for two individuals. If we use this cluster solution in the association analysis, we obtain the following results, again obtaining genome-wide significance:

```
 CHR       SNP     UNADJ        GC      BONF      HOLM SIDAK_SS SIDAK_SD   FDR_BH    FDR_BY
   2 rs2222162 8.951e-10 1.493e-09 5.89e-05 5.89e-05 5.89e-05 5.89e-05 5.89e-05 0.0006875
   2 rs4675607 9.255e-06 1.217e-05    0.609     0.609   0.4561   0.4561   0.2679         1
  13 rs9585021 1.222e-05 1.594e-05   0.8038    0.8038   0.5524   0.5524   0.2679         1
   2 rs1375352 2.753e-05 3.519e-05        1         1   0.8366   0.8365   0.3505         1
   2 rs4673349 2.753e-05 3.519e-05        1         1   0.8366   0.8365   0.3505         1
   9 rs7046471 3.196e-05 4.071e-05        1         1   0.8779   0.8779   0.3505         1
   6 rs9488062 4.481e-05 5.659e-05        1         1   0.9476   0.9476   0.4213         1
   ...
```

with similarly low inflation factors:

```
Genomic inflation factor (based on median chi-squared) is 1.02729
Mean chi-squared statistic is 0.982804
```

Finally, given that the actual ancestry of each individual is known in this particular sample, we can always use this external clustering in the analysis:

```
plink --bfile hapmap1 --mh --within pop.phe --adjust --out aac3
```

Unsurprisingly, this gives very similar results to the two-class solution derived from cluster analysis.
***In summary,***

- We have seen that simple IBS-based clustering approaches seem to work well, at least in terms of differentiating between Chinese and Japanese individuals, with this number of SNPs
- We have seen that accounting for this population substructure can lower false positive rates and increase power also - the disease variant is only genome-wide significant after performing a stratified analysis
- We have seen a number of different approaches to clustering applied. Which to use in practice is perhaps not a straightforward question. In general, when a small number of discrete subpopulations exist in the sample, then a cluster solution that most closely resembles this structure might be expected

to work well. In contrast, if, instead of a small number of discrete, homogeneous clusters, the sample actually contains a complex mixture of individuals from across a range of clines of ancestry, then we might expect the approaches that form a large number of smaller classes (e.g. matching pairs) to perform better.

Finally, it is possible to generate a visualisation of the substructure in the sample by creating a matrix of pairwsie IBS distances, then using a statistical package such as `R` to generate a multidimensional scaling plot, for example: use

```
plink --bfile hapmap1 --cluster --matrix --out ibd_view
```

which generates a file `ibd_view.mdist`. Then, in `R`, perform the following commands: (**note:** obviously, you need `R` installed for to perform these next actions – it can be freely downloaded here `http://www.r-project.org/`)

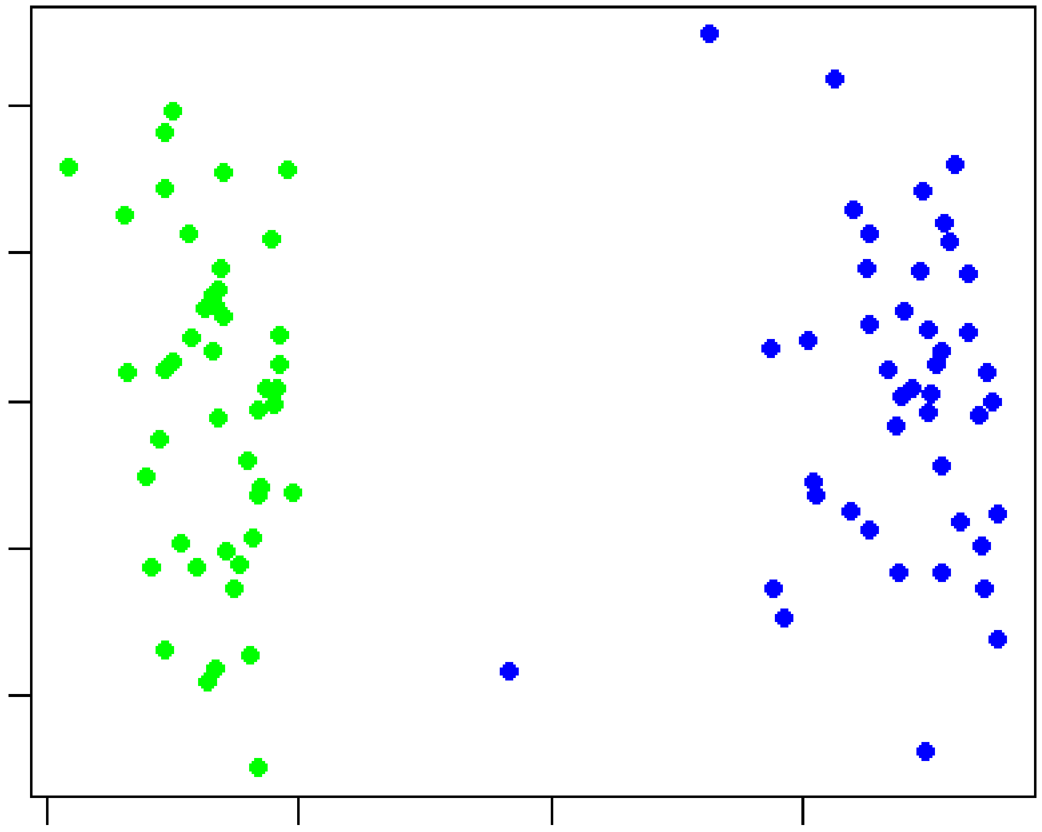
```
m <- as.matrix(read.table("ibd_view.mdist"))

mds <- cmdscale(as.dist(1-m))

k <- c( rep("green",45) , rep("blue",44) )

plot(mds,pch=20,col=k)
```

which should generate a plot like this: (green represents Chinese individuals, blue represents Japanese individuals).



This plot certainly seems to suggest that at least two quite distinct clusters exist in the sample. Based on viewing this kind of plot, one would be in a better position to determine which approach to stratification to subsequently take.

**NEW** This plot can now be automatically generated with the `--mds-plot` option – see this page.

***Quantitative trait association analysis***

At the beginning of this tutorial, we mentioned that the disease trait was based on a simple median split of a quantitative trait. Let's now analyse this quantitative trait directly. The basic analytic options are largely unchanged, except that the `--mh` approach is no longer available (this applies only to case/control samples). The `--assoc` flag will detect whether or not the phenotype is an affection status code or a quantitative trait and use the appropriate analysis: for quantitative traits, this is ordinary least squares regression. We simply need to tell `PLINK` to use the quantitative trait (which is in the file `qt.phe` instead of the default phenotype (i.e. column six of the `.ped` or `.fam` file):

```
plink --bfile hapmap1 --assoc --pheno qt.phe --out quant1
```

This analysis generates a file `quant1.qassoc` which has the following fields:

```
 CHR         SNP  NMISS       BETA      SE        R2        T        P
   1   rs6681049     89    -0.2266  0.3626  0.004469  -0.6249   0.5336
   1   rs4074137     89    -0.2949  0.6005  0.002765  -0.4911   0.6246
   1   rs1891905     89    -0.1053  0.3165  0.001272  -0.3328   0.7401
   1   rs9729550     89     0.5402  0.4616    0.0155     1.17   0.2451
   1   rs3813196     89     0.8053   1.025   0.00705   0.7859    0.434
   1  rs12044597     89    0.01658  0.3776 2.217e-05  0.04392   0.9651
   1  rs10907185     89      0.171   0.373   0.00241   0.4584   0.6478
   1  rs11260616     88    0.03574   0.444 7.533e-05  0.08049    0.936
   1    rs745910     87    -0.3093  0.4458  0.005632  -0.6938   0.4897
   1    rs262688     89      0.411  0.4467  0.009637   0.9201   0.3601
   1   rs2460000     89   -0.03558  0.3821 9.969e-05 -0.09314    0.926
   1    rs260509     89     -0.551   0.438   0.01787   -1.258   0.2118
   ...
```

The fields in this file represent:

- Chromosome
- SNP identifier
- Number of non-missing individuals for this analysis
- Regression coefficient
- Standard error of the coefficient
- The regression r-squared (multiple correlation coefficient)
- t-statistic for regression of phenotype on allele count
- Asymptotic significance value for coefficient

If we were to add the `--adjust` option, then a file `quant1.qassoc.adjust` would be created:

```
 CHR        SNP      UNADJ         GC       BONF       HOLM   SIDAK_SS   SIDAK_SD     FDR_BH     FDR_BY
   2  rs2222162  9.083e-11  3.198e-09  5.977e-06  5.977e-06  5.977e-06  5.977e-06  5.977e-06  6.976e-05
  21   rs219746  1.581e-07  1.672e-06    0.01041     0.0104    0.01035    0.01035   0.005203    0.06072
   7  rs1922519  4.988e-06  3.038e-05     0.3283     0.3282     0.2798     0.2798     0.1094          1
   2  rs2969348  1.008e-05  5.493e-05     0.6636     0.6636      0.485      0.485     0.1122          1
   3  rs6773558  1.313e-05  6.857e-05     0.8638     0.8638     0.5785     0.5784     0.1122          1
  10  rs3862003  1.374e-05  7.123e-05    0.9038    0.9038     0.595     0.595    0.1122         1
   8   rs660416  1.554e-05  7.905e-05         1         1    0.6405    0.6404    0.1122         1
  14  rs2526935  1.611e-05  8.146e-05         1         1    0.6536    0.6535    0.1122         1
  ...
```

Here we see that the disease variant is significant after genome-wide correction. However, these tests do not take into account the clustering in the sample in the same way we did before. The genomic control inflation factor estimate is now:

```
Genomic inflation factor (based on median chi-squared) is 1.19824
Mean chi-squared statistic is 1.21478
```

Instead of performing a stratified analysis or including covariates, one approach is to use permutation: specifically, it is possible to permute (i.e. label-swap phenotypes between individuals) but only within cluster. This controls for any between-cluster association, as this will be constant under all permuted datasets. We request clustered permutation as follows, using the original pairing approach to matching:

```
plink --bfile hapmap1 --assoc --pheno qt.phe --perm --within str1.cluster2 --out
quant2
```

In this case we are using adaptive permutation. See the section of the main documentation that describes permutation testing for more details. The output will show:

```
...
89 of 89 individuals assigned to 45 cluster(s)
...
Set to permute within 45 cluster(s)
Writing QT association results to [ quant2.qassoc ]
Adaptive permutation: 1000000 of (max) 1000000 : 25 SNPs left
```

This analysis will take some time depending on how fast your computer is, probably at least 1 hour. The last line shown above will change, counting the number of permutations performed, and the number of SNPs left in the analysis at any given stage. Here it reaches the default maximum of 1 million permutations and 25 SNPs remain still (see the link above for more details on this procedure).

The adaptive permutation procedure results in a file `quant2.qassoc.perm`. Sorting this file by the emprical p-value (`EMP1`, the fourth column) we see that the disease variant `rs2222162` is top of the list, with an empirical significance value of 1e-6 (essentially indicating that no permuted datasets had a statistic for `rs2222162` that exceeded this).

```
 CHR         SNP      STAT          EMP1         NP
   2   rs2222162     42.01         1e-06    1000000
   6   rs1606447     5.869     5.206e-05      38415
  10   rs1393829     16.34     0.0001896      10549
  21    rs219746     27.49     0.0001896      10549
   2   rs2304287     9.021     0.0001896      10549
   6   rs2326873     6.659     0.0001896      10549
   2   rs1385855      7.59     0.0002227      13468
   2   rs6543704     8.131     0.0002227      13468
  ...
```

**IMPORTANT** When using the `--within` option along with permutaion, the empirical significance values `EMP1` will appropriately reflect that we have controlled for the clustering variable. In contrast, the standard chi-squared statistics (`STAT` in this file) will not reflect the within-cluster analysis. That is, the test used is the same identical test as used in standard analysis – the only thing that changes is the way we permute the sample. The `STAT` values will be identical to the standard, non-clustered analysis therefore.

The `NP` field shows how many permutations were conducted for each SNP. For the SNPs at the bottom of the list, `PLINK` may well have given up after only 6 permutations (i.e. these were SNPs that were clearly not going to be highly significant if after 6 permutations they were exceeded more than a couple of times).

Naturally, this approach speeds up permutation analysis but does not provide a means for controlling for multiple testing (i.e. by comparing each observed test statistic against the maximum of all permuted statistics in each replicate). This can be achieved with the `--mperm` option:

```
plink --bfile hapmap1 --assoc --pheno qt.phe --mperm 1000 --within str1.cluster2
--out quant3
```

With `--mperm` you must also specify the number of replicates – this number can be fairly low, as one is primarily interested in the corrected p-values being less than some reasonably high nominal value such as 0.05, rather than accurately estimating the point-wise empirical significance, which might be very small.

Finally, we might want to test whether the association with the continuous phenotype differs between the two populations: for this we can use the `--gxe` option, along with population membership (which is currently limited to the dichotomous case) being specified as a covariate with the `--covar` option (same format as cluster files). Let's just perform this analysis for the main SNP of interest rather than all SNPs:

```
plink --bfile hapmap1 --pheno qt.phe --gxe --covar pop.phe --snp rs2222162 --out
quant3
```

The output will show that a file `quant3.qassoc.gxe` has been created, which contains the following fields:

```
 CHR         SNP NMISS1    BETA1       SE1   NMISS2   BETA2      SE2    Z_GXE    P_GXE
   2  rs2222162     45   -2.271   0.2245       44  -1.997  0.1722  -0.9677   0.3332
```

which show the number of non-missing individuals in each category along with the regression coefficient and standard error, followed by a test of whether these two regression coefficients are significantly different (`Z_GXE`) and an asymptotic significance value (`P_GXE`). In this case, we see the similar effect in both populations (regression coefficients around -2) and the test for interaction of SNP x population interaction is not significant.

***Extracting a SNP of interest***

Finally, given you've identified a SNP, set of SNPs or region of interest, you might want to extract those SNPs as a separate, smaller, more manageable file. In particular, for other applications to analyse the data, you will need to convert from the binary PED file format to a standard PED format. This is done using the `--recode` options (fully described here). There are a few forms of this option: we will use the `--recodeAD` that codes the genotypes in a manner that is convenient for subsequent analysis in `R` or any other non-genetic statistical package. To extract only this single SNP, use:

```
plink --bfile hapmap1 --snp rs2222162 --recodeAD --out rec_snp1
```

(to select a region, use the `--to` and `--from` options instead, or use `--window 100` with `--snp` to select a 100kb region surrounding that SNP, for example). This particular recode feature codes genotypes as additive (0,1,2) and dominance (0,1,0) components, in a file called `rec_snp1.recode.raw`. We can then load this file into our statistics package and easily perform other analyses: for example, to repeat the main analysis as a simple logistic regression using the `R` package (not controlling for clusters):

```
d <- read.table("rec_snp1.recode.raw" , header=T)

summary(glm(PHENOTYPE-1 ~rs2222162_A, data=d, family="binomial"))


   Coefficients:
               Estimate Std. Error z value Pr(>|z|)
   (Intercept)  -1.6795     0.4827  -3.479 0.000503 ***
   rs2222162_A   1.5047     0.3765   3.997 6.42e-05 ***
```

which confirms the original analysis. Naturally, things such as survival analysis or other models not implemented in `PLINK` can now be performed.

***Other areas...***

That's all for this tutorial. We've seen how to use `PLINK` to analyse a dummy dataset. Hopefully things went smoothly and you are now more familiar with using `PLINK` and can start applying it to your own datasets. There are a large number of areas that we have not even touched here:

- Using haplotype-based tests, or other multi-locus tests (Hotelling's T(2) test, etc)
- Analysing family-based samples
- Other summary statistic measures such as Hardy-Weinberg and Mendel errors
- Estimating IBD between pairs of individuals
- Tests of epistasis
- Data-management options such as merging files
- etc

but this is enough for now. In time, a second tutorial might appear that covers some of these things...

# Chapter 3

# Basic usage / data formats

PLINK is a command line program written in C/C++. All commands involve typing `plink` at the command prompt (e.g. DOS window or Unix terminal) followed by a number of options (all starting with `--`*`option`*) to specify the data files / methods to be used. All results are written to files with various extensions. The name of the file is by default `plink.ext` where `.ext` will change depending on the content of the file. Often these files will be large: using a package such as R is suggested for visualising and tabulating output. The majority of output files are in a standard plain text 'rectangular' format, with one header row and a fixed number of columns per line. A complete list of all options and output file types is given in the reference section

## 3.1 Running PLINK

PLINK is a command-line program: clicking on an icon will get you nowhere: please consult these notes on downloading and installing PLINK. Open up a command prompt or terminal window and perform all analyses by typing commands as described below.

```
plink --file mydata
```

where we expect two files: in this case, `mydata.ped` and `mydata.map`.

When PLINK starts it will attempt to contact the web, to check whether there is a more up-to-date version available or not. After checking, PLINK writes a file called `.pversion` to the working directory and use this cached information for the rest of the day. This option can be disabled with the `--noweb` option on the command line. When using PLINK on a machine with no, or a very slow, web connection, it may be desirable to turn this feature off. This feature is turned on by default so that users are aware of new versions that may contain important new features or bug fixes. If your current version of PLINK is out of date, then a warning message will be displayed, suggesting that you download and install the current version. (This is the only reason the web connection is made – no other data is transmitted to the server.) If the current version is up-to-date, you will see something like the following:

```
Web-based version check ( --noweb to skip )
Connecting to web...  OK, v1.04 is current
```

whereas, if the current version is not up-to-date, you will see something like the following:

```
Web-based version check ( --noweb to skip )
Connecting to web...
          *** UPDATE REQUIRED ***
        This version        : 1.03
        Most recent version : 1.04
Please upgrade your version of PLINK as soon as possible!
```

```
        (visit the above website for free download)
      Old versions of PLINK (<1.04) contain bugs fixed in 1.04
```

The web-based version check will also produce warning if an command used was found to have some issue discovered since that version was released (the warning will contain a link to a web page describing the issue).

To re-run a previous job, use the `--rerun` option, which takes a PLINK LOG file as the parameter. This option will scan the LOG file, extract the previous PLINK commands and re-execute them. If new commands are added to the command line, they will also be included; if the command also appeared in the original file, any parameters will be taken from the newer version. For example, if the original command was

```
plink --file mydata --pheno pheno.raw --assoc --maf 0.05 --out run1
```

then the command

```
plink --rerun run1.log --maf 0.1
```

would repeat the analysis but with the new minor allele frequency threshold of 0.1, not 0.05. Note that commands in the old LOG file can be overwritten but not removed with the rerun command.

**Note** By default, the `--out` statement would also be copied, and so the new output would overwrite any old results (i.e. with the `run1` fileroot). It is often a good idea to also add a new `--out` command, therefore:

```
plink --rerun run1.log --maf 0.1 --out run2
```

For very long a complex commands, `--rerun` can save typing and help reduce mistakes.

**HINT** MS-DOS only allows command lines to be 127 characters in length – sometimes, `PLINK` command lines can grow longer than this. In this case, use the `--script` option, where the remaining options will be read from a text file. For example,

```
plink --script myscript1.txt
```

where the file `myscript1.txt` is a plain text file containing

```
--ped ..\data\version1\50K\allsamples.ped
--map ..\data\allmapfiles\finalversion\autosomal.map
--out ..\results\working\sample-missingness-v1.22
--from rs66537222
--to rs8837323
--geno 0.25
--maf 0.02
--missing
```

would be the same as typing all these options in at the command line (note that the commands do not need to be all on the same line now). Another advantage of using script files is that it aids attempts at making one's research reproducible.

## 3.2 PED files

As well as the `--file` command described above, PED and MAP files can be specified separately, if they have different names:

```
plink --ped mydata.ped --map autosomal.map
```

**Note** Loading a large file (100K+ SNPs) can take a while (which is why we suggest converting to binary format). `PLINK` will give an error message in most circumstances when something has gone wrong.

The PED file is a white-space (space or tab) delimited file: the first six columns are mandatory:

```
Family ID
Individual ID
Paternal ID
Maternal ID
Sex (1=male; 2=female; other=unknown)
Phenotype
```

The IDs are alphanumeric: the combination of family and individual ID should uniquely identify a person. **A PED file must have 1 and only 1 phenotype in the sixth column.** The phenotype can be either a quantitative trait or an affection status column: `PLINK` will automatically detect which type (i.e. based on whether a value other than 0, 1, 2 or the missing genotype code is observed).

**NOTE** Quantitative traits with decimal points must be coded with a period/full-stop character and not a comma, i.e. `2.394` not `2,394`

If an individual's sex is unknown, then any character other than 1 or 2 can be used. When new files are created (PED, FAM, or other which contain sex) then the original coding will be preserved. However, these individuals will be dropped from any analyses (i.e. phenotype set to missing also) and an error message will arise if an analysis that uses family information is requested and an individual of 'unknown' sex is specified as a father or mother.

**HINT** To disable the automatic setting of the phenotype to missing if the individual has an ambiguous sex code, add the `--allow-no-sex` option. When using a data generation command (e.g. `--make-bed`, `--recode`, etc) as opposed to an analysis command, then by default the phenotype is not set to missing is sex is missing. This behaviour can be changed by adding the flag `--must-have-sex`.

**HINT** You can add a comment to a PED or MAP file by starting the line with a `#` character. The rest of that line will be ignored. Do not start any family IDs with this character therefore.

Affection status, by default, should be coded:

```
-9 missing
 0 missing
 1 unaffected
 2 affected
```

If your file is coded 0/1 to represent unaffected/affected, then use the `--1` flag:

```
plink --file mydata --1
```

which will specify a disease phenotype coded:

```
-9 missing
 0 unaffected
 1 affected
```

The missing phenotype value for quantitative traits is, by default, -9 (this can also be used for disease traits as well as 0). It can be reset by including the `--missing-phenotype` option:

```
plink --file mydata --missing-phenotype 99
```

Other phenotypes can be swapped in by using the `--pheno` (and possibly `--mpheno`) option, which specify an alternate phenotype is to be used, described below.

Genotypes (column 7 onwards) should also be white-space delimited; they can be any character (e.g. 1,2,3,4 or A,C,G,T or anything else) except `0` which is, by default, the missing genotype character. **All markers should be biallelic**. All SNPs (whether haploid or not) must have two alleles specified. Either Both alleles should be missing (i.e. `0`) or neither. No header row should be given. For example, here are two individuals typed for 3 SNPs (one row = one person):

```
FAM001  1  0 0  1  2  A A  G G  A C
FAM001  2  0 0  1  2  A A  A G  0 0
```

...

The default missing genotype character can be changed with the `--missing-genotype` option, for example:

```
plink --file mydata --missing-genotype N
```

**NOTE** Different values to the missing phenotype or genotype code can be specified for output datasets created, with `--output-missing-phenotype` and `--output-missing-genotype`.

### 3.2.1 Different PED file formats: missing fields

Sometimes data arrive in a number of different formats: for example, where the genotype information just has a single ID column followed by all the SNP data, with the other family and phenotype information residing in a separate file. Rather than have to recreate new files, it is sometimes possible to read in such files directly. The standard behavior of PLINK when reading a PED file with `--file` or `--ped` can be modified to allow for the fact that one or more of the normally obligatory 6 fields are missing:

```
--no-fid
```

indicates there is no Family ID column: here the first field is taken to be individual ID, and the family ID is automatically set to be the same as the individual ID (i.e. obviously, all individuals would be treated as unrelated). In other files that require family and individual ID (e.g. alternate phenotype file and cluster files, for which this flag has no effect), the individual ID would need to be entered also as the family ID therefore.

```
--no-parents
```

indicates that there are no paternal and maternal ID codes; all individuals would be assumed to be founders in this case

```
--no-sex
```

indicates that there is no sex field; all individuals set to have a missing sex code (which also sets that individual to missing unless the `allow-no-sex` option is also used)

```
--no-pheno
```

indicates that there is no phenotype filed; all individuals are set to missing unless an alternate phenotype file is specified.

It is possible to use these flags together, so using all of them would specify the most simple kind of file mentioned above: a single, unique ID code followed by all genotype data.

**IMPORTANT** These options only work for the basic PED file (i.e. specified by `--file` or `--ped`. They do not work for transposed files, when merging in a file with `--merge`, or with binary filesets or covariate, cluster or alternate phentype files.

If the genotype codes in a PED file are in the form `AG` rather than `A G`, for example, such that every genotype is exactly two characters long, then then flag

```
./plink --file mydata --compound-genotypes </tt>
```

can be added. Note that this only works for input for PED files (not TPED or LGEN files, and not for any output options, e.g. `--recode`, etc).

**Note** To load the PED file from the standard input stream instead of a file, use the `-` symbol as the file name, e.g.

```
perl retrieve_data.pl | ./plink --ped - --map mymap.map --make-bed
```

The MAP file still needs to be a normal file; this currently only works for `--ped` files.

## 3.3 MAP files

By default, each line of the MAP file describes a single marker and must contain exactly 4 columns:

```
chromosome (1-22, X, Y or 0 if unplaced)
rs# or snp identifier
Genetic distance (morgans)
Base-pair position (bp units)
```

Genetic distance can be specified in centimorgans with the `--cm` flag. Alternatively, you can use a MAP file with the genetic distance excluded by adding the flag `--map3`, i.e.

```
plink --file mydata --map3
```

In this case, the *three* columns are expected to be

```
chromosome (1-22, X, Y or 0 if unplaced)
rs# or snp identifier
Base-pair position (bp units)
```

**Note** Most analyses do not require a genetic map to be specified in any case; specifying a genetic (cM) map is most crucial for a set of analyses that look for shared segments between individuals. For basic association testing, the genetic distance column can be set at 0.

SNP identifers can contain any characters except spaces or tabs; also, you should avoid `*` symbols in names also.

To exclude a SNP from analysis, set the 4th column (physical base-pair position) to any negative value (this will only work for MAP files, not for binary BIM files).

```
1  rs123456  0  1234555
1  rs234567  0  1237793
1  rs224534  0  -1237697        <-- exclude this SNP
1  rs233556  0  1337456
...
```

The MAP file must therefore contain as many markers as are in the PED file. The markers in the PED file do not need to be in genomic order: (i.e. the order MAP file should align with the order of the PED file markers).

### 3.3.1 Chromosome codes

The autosomes should be coded 1 through 22. The following other codes can be used to specify other chromosome types:

```
X    X chromosome                    -> 23
Y    Y chromosome                    -> 24
XY   Pseudo-autosomal region of X    -> 25
MT   Mitochondrial                   -> 26
```

The numbers on the right represent PLINK's internal numeric coding of these chromosomes: these will appear in all output rather than the original chromosome codes.

For haploid chromosomes, genotypes should be specified as homozygotes: for most analyses, `PLINK` will treat these appropriately. For example, consider the following example PED file, containing two males (1 and 2) and two females (3 and 4):

```
1 1 0 0 1   1   A A    A A    A A    A A    A A
2 1 0 0 1   1   A C    A C    A C    A C    A C
3 1 0 0 2   1   A A    A A    A A    A A    A A
4 1 0 0 2   1   A C    A C    A C    A C    A C
```

and MAP file

```
1    snp1   0   1000
X    snp2   0   1000
Y    snp3   0   1000
XY   snp4   0   1000
MT   snp5   0   1000
```

Generating frequencies for these SNPs,

```
plink --file test --freq
```

we see `plink.frq` is

```
 CHR         SNP   A1   A2          MAF        NM
   1        snp1    C    A         0.25         8
  23        snp2    C    A          0.2         5
  24        snp3    C    A            0         1
  25        snp4    C    A         0.25         8
  26        snp5    C    A            0         2
```

There are several things to note. First, the numeric chromosome codes are used in the output to represent X, Y, XY and MT. Second, haploid chromosomes are only counted once (i.e. male X and Y chromosome SNPs and all MT SNPs). Third, several genotypes have been set to missing if they are not valid (female Y genotype, heterozygous haploid chromosome). The `NM` field represents the number of non-missing alleles for each SNP – this is because invalid genotypes are automatically set to missing.

We can see which genotypes have been set to missing by running the `--recode` command; however, usually PLINK preserves all genotypes when generating a new file (i.e. if one is just reformatting a file, say from text to binary format, it is not necessarily desirable to change any of the content; as above, summary statistic and analysis commands do set these genotypes missing automatically still). However, if we also add the `--set-hh-missing` flag, any invalid genotypes will be set to missing in the new file:

```
plink --file test --set-hh-missing
```

which creates the new PED file `plink.recode.ped`

```
1 1 0 0 1 1 A A A A A A A A A A
2 1 0 0 1 1 C A 0 0 0 0 C A 0 0
3 1 0 0 2 1 A A A A 0 0 A A A A
4 1 0 0 2 1 C A C A 0 0 C A 0 0
```

In other words, the actual alleles that PLINK pays attention to are shown in **bold**, all non-bold alleles are ignored.

1 1 0 0 1 1 **A A** **A** A **A** A **A A** **A** A
2 1 0 0 1 1 **A C** A C A C **A C** A C
3 1 0 0 2 1 **A A** **A A** A A **A A** **A** A
4 1 0 0 2 1 **A C** **A C** A C **A C** A C

### 3.3.2 Allele codes

By default, the minor allele is coded `A1` and the major allele is coded `A2` (this is used in many output files, e.g. from `--freq` or `--assoc`). By default this is based on all founders (unless `--nonfounders` is added) with sex-codes specified (unless `--allow-no-sex` is added). This coding is applied *after* any other filters have been applied. It is sometimes desirable to prevent this automatic flipping of `A1` and `A2` alleles, by use of the `--keep-allele-order` option. For example, if one wishes to dump the genotype counts by use of the `--model` command, for two groups of individuals (using the `--filter` command), this ensures that the same minor allele will always be used in `grp1.model` as `grp2.model` (which can facilitate downstream processing of these files, for instance).

```
plink --bfile --filter pop.dat POP1 --model --keep-allele-order --out pop-1-genotypes

plink --bfile --filter pop.dat POP2 --model --keep-allele-order --out pop-2-genotypes
```

That is, for any SNP that happens to have a different minor allele in `POP1` versus `POP2`, the output in the two `.model` files will still line up in an easy manner.

## 3.4 Transposed filesets

Another possible file-format called a *transposed* fileset, containing two text files: one (TPED) containing SNP and genotype information where one row is a SNP; one (TFAM) containing individual and family information, where one row is an individual.

The first 4 columns of a TPED file are the same as a standard 4-column MAP file. Then all genotypes are listed for all individuals for each particular SNP on each line. The TFAM file is just the first six columns of a standard PED file. In otherwords, we have just taken the standard PED/MAP file format, but swapped all the genotype information between files, after rotating it 90 degrees. For each, the above example PED/MAP fileset

```
<---- normal.ped ---->                  <--- normal.map --->
1 1 0 0 1  1  A A  G T                  1  snp1   0  5000650
2 1 0 0 1  1  A C  T G                  1  snp2   0  5000830
3 1 0 0 1  1  C C  G G
4 1 0 0 1  2  A C  T T
5 1 0 0 1  2  C C  G T
6 1 0 0 1  2  C C  T T
```

would be represented as TPED/TFAM files:

```
<------------- trans.tped ------------->      <- trans.tfam ->
1 snp1 0 5000650 A A A C C C A C C C C C      1  1  0  0  1  1
1 snp2 0 5000830 G T G T G G T T G T T T      2  1  0  0  1  1
                                              3  1  0  0  1  1
                                              4  1  0  0  1  2
                                              5  1  0  0  1  2
                                              6  1  0  0  1  2
```

This kind of format can be convenient to work with when there are very many more SNPs than individuals (i.e. WGAS data). In this case, the TPED file will be very long (as opposed to the PED file being very wide).

To read a transposed fileset, use the command

```
plink --tfile mydata
```

which implies `mydata.tped` and `mydata.tfam` exists; alternatively, if the files are differently named, they can be individually, fully specified:

```
plink --tped mydata.tped --tfam pedinfo.txt
```

**HINT** You can generate transposed filesets with the `--transpose` option, described in the data management section

## 3.5 Long-format filesets

Another possible file-format called a *long-format* fileset, containing three text files:

- a LGEN file containing genotypes (5 columns, one row per genotype)

- a MAP file containing SNPs (4 columns, one row per SNP)
- a FAM file containing individuals (6 columns, one row per person)

The MAP and FAM/PED files are described elsewhere this page. Consider the following example: A MAP file `test.map`

```
1 snp2 0 2
2 snp4 0 4
1 snp1 0 1
1 snp3 0 3
5 snp5 0 1
```

as described above. A FAM file `test.fam`

```
1 1 0 0 1 2
2 1 0 0 2 2
2 2 0 0 1 1
9 1 1 2 0 0
```

as described below. Finally, an LGEN file, `test.lgen`

```
1 1 snp1 A A
1 1 snp2 A C
1 1 snp3 0 0
2 1 snp1 A A
2 1 snp2 A C
2 1 snp3 0 0
2 1 snp4 A A
2 2 snp1 A A
2 2 snp2 A C
2 2 snp3 0 0
2 2 snp4 A A
```

The columns in the LGEN file are

```
family ID
individual ID
snp ID
allele 1 of this genotype
allele 2 of this genotype
```

Not all entries need to be present in the LGEN file (e.g. snp5 or person 9/1) or snp4 for person 1/1. These genotypes will be set to missing internally. The order also need not be the same in the LGEN file as for the MAP or FAM files. If a genotype is listed more than once, the final version of it will be used.

LGEN file can be reformatted as a standard PED file using the following command:

```
plink --lfile test --recode
```

which creates these two files: a PED file, `plink.recode.map`

```
1 1 0 0 1  2   A A  A C  0 0  0 0  0 0
2 1 0 0 2  2   A A  A C  0 0  A A  0 0
2 2 0 0 1  1   A A  A C  0 0  A A  0 0
9 1 1 2 0  0   0 0  0 0  0 0  0 0  0 0
```

and the MAP file, `plink.recode.map` (note: it has been put in genomic order)

```
1       snp1    0       1
1       snp2    0       2
1       snp3    0       3
2       snp4    0       4
```

```
     5        snp5    0        1
```

**NOTE** All individuals must be uniquely identified by the combination of the family and individual IDs.

To read a long-format fileset, use the command

```
plink --lfile mydata
```

which implies `mydata.lgen`, `mydata.map` and `mydata.map` exist.

**NOTE** Currently, you cannot output a fileset in this format in PLINK.

### 3.5.1 Additional options for long-format files

If the LGEN file has specific allele codes, but as `TG` instead of `T G` (i.e. no spaces between the two alleles), add the flag

```
    --compound-genotypes
```

It is possible to specify the reference allele with the `--reference` command when using long-format file input. This might be appropriate, for example, if the data file contains calls for rare variants from a resequencing study. In this case, the majority of alleles will be the reference, and so need not be repeated here. For example, consider this FAM file `f1.fam`

```
1 1 0 0 1 1
2 1 0 0 1 1
3 1 0 0 1 1
4 1 0 0 1 1
5 1 0 0 1 1
6 1 0 0 1 1
```

and MAP file `f1.map`

```
1       rs0001    0       1000001
1       rs0002    0       1000002
1       rs0003    0       1000003
```

and LGEN file `f1.lgen`

```
1 1 rs0001 C C
2 1 rs0001 0 0
6 1 rs0003 C C
1 1 rs0002 G T
4 1 rs0002 T T
5 1 rs0002 G T
```

then

```
plink --lfile f1 --recode
```

would yield a file `plink.ped` that is as follows:

```
1 1 0 0 1 1  C C  G T  0 0
2 1 0 0 1 1  0 0  0 0  0 0
3 1 0 0 1 1  0 0  0 0  0 0
4 1 0 0 1 1  0 0  T T  0 0
5 1 0 0 1 1  0 0  G T  0 0
6 1 0 0 1 1  0 0  0 0  C C
```

If the reference all for each variant was set, e.g. with the following command

```
plink --lfile f1 --reference ref.txt --recode
```

and the file `ref.txt` is

```
rs0001 A
rs0002 G
rs0009 T
```

then the output `plink.ped` will instead read:

```
1 1 0 0 1 1  C C  T G  0 0
2 1 0 0 1 1  0 0  G G  0 0
3 1 0 0 1 1  A A  G G  0 0
4 1 0 0 1 1  A A  T T  0 0
5 1 0 0 1 1  A A  T G  0 0
6 1 0 0 1 1  A A  G G  C C
```

That is, the non-specified genotypes for the first two SNPs are now homozygous for the reference allele. Note: the word *reference* is used in the context of the human genome reference allele, rather than for the calculation of an odds ratio. The command to set the latter is `--reference-allele` ***`file`***

Also note in this example, that a) when an individual is set as explicitly missing in the LGEN file, they stay missing, b) that when a reference allele is not set, then non-specified genotypes are missing (e.g. the third SNP, rs0003), c) that SNPs in the reference file that are not present in the dataset (e.g. rs0009) are ignored.

When reading a long-format file, the command

```
--allele-count
```

when specified along with `--reference` allows the data to be in the form of the number of non-reference alleles. For example, if input LGEN file were

```
1 1 rs0001 0
2 1 rs0001 1
3 1 rs0001 2
4 1 rs0001 -1
5 1 rs0001 9
6 1 rs0001 X
```

this should translate into the first three individuals having the reference homozygote (0 non-reference alleles), the heterozygote (1 non-reference allele) and the non-reference homozygote (2 non-reference alleles). The final three individuals (FID 4 to 6) are all set to missing: this just indicates that any value other than a 0, 1 or 2 under this scheme is set to a missing genotype. If the reference file only contains a single allele for that SNP, then the non-reference allele is coded as whatever is in the reference allele plus a `v` character appended, e.g. just considering this one SNP:

```
1 1 0 0 1 1   A  A
2 1 0 0 1 1   A  Av
3 1 0 0 1 1   Av Av
4 1 0 0 1 1   0  0
5 1 0 0 1 1   0  0
6 1 0 0 1 1   0  0
```

However, if the reference file contains two alleles, then the second is taken to be the non-reference allele, e.g. if `ref.txt` is

```
rs0001 A  G
```

then the output will read

```
1 1 0 0 1 1 A A
2 1 0 0 1 1 A G
3 1 0 0 1 1 G G
4 1 0 0 1 1 0 0
5 1 0 0 1 1 0 0
```

```
6 1 0 0 1 1 0 0
```

## 3.6 Binary PED files

To save space and time, you can make a binary ped file (*.bed). This will store the pedigree/phenotype information in separate file (*.fam) and create an extended MAP file (*.bim) (which contains information about the allele names, which would otherwise be lost in the BED file). To create these files use the command:

```
plink --file mydata --make-bed
```

which creates (by default)

```
plink.bed      ( binary file, genotype information )
plink.fam      ( first six columns of mydata.ped )
plink.bim      ( extended MAP file: two extra cols = allele names)
```

The `.fam` and `.bim` files are still plain text files: these can be viewed with a standard text editor. Do not try to view the `.bed` file however: it is a compressed file and you'll only see lots of strange characters on the screen...

**NOTE** ***Do not make any changes any of these three files; e.g. setting the position to a negative value will not work to exclude a SNP for binary files***

You can specify a different output root file name (i.e. different to "plink") by using the `--out` option:

```
plink --file mydata --out mydata --make-bed
```

which will create

```
mydata.bed
mydata.fam
mydata.bim
```

To subsequently load a binary file, just use `--bfile` instead of `--file`

```
plink --bfile mydata
```

When creating a binary ped file, the MAF and missingness filters are set to include everybody and all SNPs. If you want to change these, use `--maf`, `--geno`, etc, to manually specify these options: for example,

```
plink --file mydata --make-bed --maf 0.02 --geno 0.1
```

**More information...** If you want to write your own software that uses the BED file format, please follow this link for more information of the specification.

## 3.7 Alternate phenotype files

To specify an alternate phenotype for analysis, i.e. other than the one in the `*.ped` file (or, if using a binary fileset, the `*.fam` file), use the `--pheno` option:

```
plink --file mydata --pheno pheno.txt
```

where `pheno.txt` is a file that contains 3 columns (one row per individual):

```
Family ID
Individual ID
Phenotype
```

The original PED file must still contain a phenotype in column 6 (even if this is a dummy phenotype, e.g. all missing), unless the `--no-pheno` flag is given.

If an individual is in the original file but not listed in the alternate phenotype file, that person's phenotype will be set to missing. If a person is in the alternate phenotype file but not in the original file, that entry will be ignored. The order of the alternate phenotype file need not be the same as for the original file. If the phenotype file contains more than one phenotype, then use the `--mpheno N` option to specify the *Nth* phenotype is the one to be used:

```
plink --file mydata --pheno pheno2.txt --mpheno 4
```

where `pheno2.txt` contains 5 different phenotypes (i.e. 7 columns in total), this command will use the 4th for analysis (phenotype D):

```
Family ID
Individual ID
Phenotype A
Phenotype B
Phenotype C
Phenotype D
Phenotype E
```

Alternatively, your alternate phenotype file can have a header row, in which case you can use variable names to specify which phenotype to use. If you have a header row, the first two variables **must** be labelled `FID` and `IID`. All subsequent variable names cannot have any whitespace in them. For example,

```
FID    IID     qt1   bmi    site
F1     1110    2.3   22.22  2
F2     2202    34.12 18.23  1
...
```

then

```
plink --file mydata --pheno pheno2.txt --pheno-name bmi --assoc
```

will select the second phenotype labelled "bmi", for analysis

Finally, if there is more than one phenotype, then for basic association tests, it is possible to specify that all phenotypes be tested, sequentially, with the output sent to different files: e.g. if `bigpheno.raw` contains 10,000 phenotypes, then

```
plink --bfile mydata --assoc --pheno bigpheno.raw --all-pheno
```

will loop over all of these, one at a time testing for association with SNP, generating a lot of output. You might want to use the `--pfilter` command in this case, to only report results with a p-value less than a certain value, e.g. `--pfilter 1e-3`.

**WARNING Currently, all phenotypes must be numerically coded, including missing values, in the alternate phenotype file. The default missing value is `-9`, change this with `--missing-phenotype`, but it must be a numeric value still (in contrast to the main phenotype in the PED/FAM file).**

### 3.7.1 Creating a new binary phenotype automatically

To automatically form a one-versus-others binary phenotype (note: binary meaning dichotomous here, rather than a BED/binary-PED file) from a categorical covariate/phenotype file, use the command

```
plink --bfile mydata --make-pheno site.cov SITE3 --assoc
```

which assumes the file

```
site.cov
```

contains exactly three fields

```
Family ID
```

```
Individual ID
Code from which phenotype is created
```

For example, if it were

```
A1  1  SITE1
B1  1  SITE1
C1  1  SITE2
D1  1  SITE3
E1  1  SITE3
F1  1  SITE4
G2  1  SITE4
```

then the above command would make individuals `D1` and `E1` as *cases* and everybody else as *controls*. However, if individuals present in `mydata` were not specified in `site.cov`, then these people would be set to have a missing phenotype.

An alternate specification is to use the `*` symbol instead of a value, e.g.

```
plink --bfile mydata --make-pheno p1.list * --assoc
```

which assumes the file

```
p1.list
```

contains exactly two fields

```
Family ID
Individual ID
```

In this case, anybody in the file `p1.list` would be made a case; all other individuals in `mydata` but not in `p1.list` would be set as a control.

### 3.7.2 "Loop association": automatically testing each group versus all others

You may have a categorical factor that groups individuals (e.g. which plate they were genotyped on, or which sample they come from) and want to test whether there are allele frequency differences between each group and all others. This can be accomplished with the `--loop-assoc` command, e.g.

```
./plink --bfile mydata --loop-assoc plate.lst --assoc
```

The file `plate.lst` should be in the same format as a cluster file, although it is only allowed to have a single variable (i.e. 3 columns, FID, IID and the cluster variable). If this were

```
10001  1   P1
10002  1   P1
10003  1   P2
10004  1   P2
10005  1   P3
10006  1   P3
...
```

This command would test all `P1` individuals against all others, then all `P2` individuals against all others, etc. Any of the main single SNP association tests for diseases can be supplied instead of `--assoc` (e.g. `--fisher`, `--test-missing`, `--logistic`, etc). The output is written to different files for each group, e.g. in the format `outputname.label.extension`

```
plink.P1.assoc
plink.P2.assoc
plink.P3.assoc
...
```

## 3.8 Covariate files

Certain PLINK commands support the inclusion of one or more covariates. Note that for stratified analyses, namely using the CMH (`--mh`) options, the strata are specified using the `--within` option to define clusters, rather than `--covar`.

To load a covariate use the option:

```
plink --file mydata --covar c.txt
```

The covariate file should be formatted in a similar manner to the phenotype file. If an individual is not present in the covariate file, or if the individual has a missing phenotype value (i.e. -9 by default) for the covariate, then that individual is set to missing (i.e. will be excluded from association analysis).

To select a particular subset of covariates, use one of the following commands, which either use numbers or names (i.e. if a header row exists in the file),

```
plink --file mydata --covar c.txt --covar-number 2,4-6,8
```

or

```
plink --file mydata --covar c.txt --covar-name AGE,BMI-SMOKE,ALC
```

Note that ranges can be used in both cases, with the `-` hyphen symbol, e.g. if the first row were

```
FID IID SITE AGE DOB BMI ETH SMOKE STATUS ALC
```

then both the above commands would have the same effect, i.e. selecting `AGE`, `BMI`, `ETH`, `SMOKE`, `ALC`.

To output a new covariate file, possibly with categorical variables downcoded to binary dummy variables use the `--write-covar` option as described here

**Exception** If the `--gxe` command is used, that selects only a single covariate, then use the command `--mcovar`, that works similarly to `--mpheno` to select which single covariate to use: with the `--gxe` command, the `--covar-name` and `--covar-number` options will not work.

**NOTE** Not all commands accept covariates, and PLINK will not always give you an error or warning. The basic association (`--assoc`, `--mh`, `--model`, `--tdt`, `--dfam`, and `--qfam`) do **not** accept covariates, neither do the basic haplotype association methods (`--hap-assoc`, `--hap-tdt`). Among the commands that do are `--linear`, `--logistic`, `--chap` and `--proxy-glm`. Also `--gxe` accepts a single covariate only (the others listed here accept multiple covariates).

## 3.9 Cluster files

To load a cluster solution, or indeed any categorical grouping of the sample, use the `--within` option:

```
plink --file mydata --within f.txt
```

If this option is used, then permutation procedures will permute within-cluster only, effectively controlling for any effect of cluster membership. Similarly, tests that perform stratified analyses, such as the Cochran-Mantel-Haenszel, this option is used to define the strata.

This file should have a similar structure to the alternate phenotype file. The clusters can be coded either numerically or as strings:

```
F1 I1  A
F2 I1  B
F3 I1  B
F4 I1  C1
F5 I1  A
F6 I1  C2
F7 I1  C2
```

```
...
```

Here, individuals would be grouped in four groups:

```
Cluster A:  F1/I1  F5/I1
Cluster B:  F2/I1  F3/I1
Cluster C1: F4/I1
Cluster C2: F6/I1  F7/I1
...
```

All individuals in the file should be assigned to a single cluster in the cluster file.

## 3.10 Set files

Certain analyses (e.g. set based tests) require sets of SNPs to be specified. This is performed by including the `--set` option on the command line, followed by a filename that defines the sets. The file `mydata.set` should be in the following format:

```
SET_A
rs10101
rs20234
rs29993
END
GENE-B
rs2344
rs888833
END
```

That is, each set must start with a *set name* (e.g. `SET_A`), which might be a gene name, for example. **This name can not have any spaces in it.** The name is followed by a list of SNPs in that set. The keyword `END` specifies the end of that particular set. **Do not name any SNPs to have the name `END`!**

Sets can be overlapping. Any SNPs specified in the set that do not appear in the actual data, or that have been excluded due to filters used, will be ignored.

The format is flexible in terms of whether each item appears on one line: the set file only needs to be whitespace delimited. For example, the file above could be specified as:

```
SET_A    rs10101 rs20234 rs29993 END
GENE-B   rs2344 rs888833 END
```

**HINT** It is possible to automatically create a set-file, given a list of genomic co-ordinates, using the `--make-set` command, described here.

To extract a subset of sets from a set file, use the `--subset` command in addition to `--set`. For example,

```
--set mydata.set --subset extract.txt
```

where `extract.txt` is a text file with the set names you wish to extract, e.g. `SET_A` or `GENE-B` in this example.

# Chapter 4

# Data management tools

PLINK provides a simple interface for recoding, reordering, merging, flipping DNA-strand and extracting subsets of data.

## 4.1 Recode and reorder a sample

A basic, but often useful feature, is to output a dataset:

- with the PED file markers reordered for physical position,
- with excluded SNPs (negative values in the MAP file) excluded from the new PED file
- possibly excluding other SNPs based on filters such as genotyping rate
- possibly recoding the SNPs to a 1/2 coding
- possibly recoding the SNPs between letters and numbers (A,C,G,T / 1,2,3,4)
- possibly transposing the genotype file (SNPs as rows)
- possibly recoding the SNP to an additive and dominant pair of components
- possibly listing the data with each specific genotype as a distinct row

The basic option to generate a new dataset is the `--recode` option:

```
plink --file data --recode
```

which will output the allele labels as they appear in the original; also, the missing genotype code is preserved if this is different from `0`. Also, if `--output-missing-genotype` is specified (which can be as well as `--missing-genotype`) then this value will be used instead (i.e. so that input and output files can have different missing codes; this also applies to the phenotype with `--output-missing-phenotype` and `--missing-phenotype`).

The `--make-bed` option does the same as `--recode` but creates binary files; these can also be filtered, etc, as described below.

In contrast,

```
plink --file data --recode12
```

will recode the alleles as `1` and `2` (and the missing genotype will always be `0`).

Both these commands will create two new files

```
plink.ped
plink.map
```

(where, as usual, "plink" would be replaced by any specified –out filename ).

Unless manually specified, for all these options, the usual filters for missingness and allele frequency will be set so as not to exclude any SNPs or individuals. By explicitly including an option, e.g. `--maf 0.05` on the command line, this behaviour is overriden (see this page).

By default, any `--recode` option, and also `--make-bed` will preserve all genotypes exactly as they are. To set to missing Mendel errors or heterozygous haploid calls, use the options `--set-me-missing` and `--set-hh-missing` respectively. For the former, you will also need to specify `--me 1 1` (i.e. to invole an evalation of Mendel errors, which does not occur by default, by not excluding any individuals or SNPs based on the results, i.e. if you only want to zero-out certain genotypes).

To recode SNP alleles from A,C,G,T to 1,2,3,4 or vice versa, use `--allele1234` (to go from letters to numbers) and `--alleleACGT` (to go from numbers to letters). These flags should be used in conjunction with a data generation command (e.g. `--make-bed`), or any other analysis or summary statistic option. Alleles other than A,C,G,T or 1,2,3,4 will be left unchanged. It is sometimes useful to have a PED file that is tab-delimited, except that between alleles of the same genotype a space instead of a tab is used. A file formatted in this way can load into Excel, for example, as a tab-delimited file, but with one genotype per column instead of one allele per column. Use the option `--tab` as well as `--recode` or `--recode12` to achieve this effect.

To make a new file in which non-founders without both parents also in the same fileset are recoded as founders (i.e. pat and mat codes set both to 0), add the `--make-founders` flag.

### 4.1.1 Transposed genotype files

When using either `--recode` or `--recode12`, you can obtain a transposed text genotype file by adding the `--transpose` option. This generates two files:

```
plink.tped
plink.fam
```

The first contains the genotype data, with SNPs as rows and individuals as columns, for example: if the original file was

```
1 1 0 0 1  1  1 1  G G
1 2 0 0 2  1  0 0  A G
1 3 0 0 1  1  1 1  A G
1 4 0 0 2  1  2 1  A A
```

then this would generate

```
1 snp1 0 10001  1 1  0 0  1 1  2 1
1 snp2 0 20001  G G  G A  G A  A A
```

The first four columns are from the MAP file (chromosome, SNP ID, genetic position, physical position), followed by the genotype data. The `plink.fam` gives the ID, sex and phenotype information for each individual. The order of individuals in this file is the same as the order across the columns of the TPED file. The FAM file is just the first six columns of the PED file (or literally the same FAM file if the input where a binary fileset).

### 4.1.2 Additive and dominance components

The following format is often useful if one wants to use a standard, non-genetic statistical package to analyse the data, as here genotypes are coded as a single allele dosage number. To create a file with SNP genotypes recoded in terms of additive and dominant components, use the option:

```
plink --file data --recodeAD
```

which, assuming `C` is the minor allele, will recode genotypes as follows:

```
SNP       SNP_A ,  SNP_HET
---       -----     -----
A A   ->    0   ,   0
A C   ->    1   ,   1
C C   ->    2   ,   0
0 0   ->   NA   ,  NA
```

In otherwords, the default for the additive recoding is to count the number of minor alleles per person. The `--recodeAD` option produces both an additive and dominance coding: use `--recodeA` instead to skip the `SNP_HET` coding.

The `--recodeAD` option saves the data to a single file

```
plink.raw
```

which has a header row indicating the SNP names (with `_A` and `_HET` appended to the SNP names to represent additive and dominant components, respectively).

For example, consider the following PED file, which has two SNPs:

```
1 1 0 0 1  1  1 1  G G
1 2 0 0 2  1  0 0  A G
1 3 0 0 1  1  1 1  A G
1 4 0 0 2  1  2 1  A A
```

Using the `--recodeAD` option generates the file `plink-recode.raw`:

```
FID IID PAT MAT SEX PHENOTYPE snp1_2 snp1_HET snp2_G snp2_HET
1 1 0 0 1 1  0  0   2 0
1 2 0 0 2 1  NA NA  1 1
1 3 0 0 1 1  0  0   1 1
1 4 0 0 2 1  1  1   0 0
```

The column labels reflect the snp name (e.g. `snp1`) with the name of the minor allele appended (i.e. `snp1_2` in the first instance, as `2` is the minor allele) for the additive component. The dominant component ( a dummy variable reflecting heterozygote state) is coded with the `_HET` suffix.

This file can be easily loaded into `R`: for example:

```
d <- read.table("plink.raw",header=T)
```

For example, for the first SNP, the individuals are coded `1/1`, `0/0`, `1/1` and `2/1`. The additive count of the number of common (`1`) alleles is therefore: `2`, `NA`, `2` and `1`, which is reflected in the field `snp1_2`. The field `snp1_HET` is coded `1` for the fourth individual who is heterozygous – this field can be used to model dominance effect of the allele.

The behavior of the `--recodeA` and `--recodeAD` commands can be changed with the `--recode-allele` command. This allows for the 0, 1, 2 count to reflect the number of a pre-specified allele type per SNP, rather than the number of the minor allele. This command takes as a single argument the name of a file that lists SNP name and allele to report, e.g. if the file `recode.txt` contained

```
snp1   1
snp2   A
```

then

```
plink --file data --recodeAD --recode-allele recode.txt
```

would now report in the LOG file

```
Reading allele coding list from [ recode.txt ]
Read allele codes for 2 SNPs
```

and the `plink.raw` file would read

```
FID IID PAT MAT SEX PHENOTYPE snp1_1 snp1_HET snp2_A snp2_HET
```

```
1 1 0 0 1 1   2  0   0 0
1 2 0 0 2 1   NA NA  1 1
1 3 0 0 1 1   2  0   1 1
1 4 0 0 2 1   1  1   2 0
```

If the SNP is monomorphic, by default the allele code out will be `0` and all individuals will have a count of 0 (or `NA`). If an allele is specified in `--recode-allele` that is not seen in the data, similarly all individuals will receive a 0 count (i.e. rather than an error being given).

**NOTE** For alleles that have exactly 0.50 minor allele frequency, as for the second SNP in the example above, then which allele is labelled as minor will depend on which was first encountered in the PED file.

### 4.1.3 Listing by genotype

Another format that might sometimes be useful is the `--list` option which genetes a file

```
plink.list
```

that is ordered one genotype per row, listing all family and individual IDs of people with that genotype. For example, if we have a file with two SNPs `rs1001` and `rs2002` (both on chromosome 1):

```
A 1 0 0 1  2  A A  1 1
B 2 0 0 1  2  A C  0 0
C 3 0 0 1  1  A C  1 2
D 4 0 0 1  1  C C  1 2
```

then then option

```
plink --file mydata --list
```

will generate the file `plink.list`

```
1 rs1001 AA A 1
1 rs1001 AC B 2 C 3
1 rs1001 CC D 4
1 rs1001 00
1 rs2002 22
1 rs2002 21 C 3 D 4
1 rs2002 11 A 1
1 rs2002 00 B 2
```

which has columns

```
Chromosome
SNP identifier
Genotype
Family ID, Individual ID for 1st person
Family ID, Individual ID for 2nd person
...
Family ID, Individual ID for final person
```

Obviously, different rows will have a different number of columns. Here, we see that individual `A 1` has the `A/A` genotype for `rs1001`, etc. This option is often useful in conjunction with `--snp`, if you want an easy breakdown of which individuals have which genotypes.

## 4.2 Write SNP list files

To output just the list of SNPs that remain after all filtering, etc, use the `--write-snplist` command, e.g. to get a list of all high frequency, high genotyping-rate SNPs:

```
plink --bfile mydata --maf 0.05 --geno 0.05 --write-snplist
```

which generates a file

```
plink.snplist
```

This file is simply a list of included SNP names, i.e. the same SNPs that a `--recode` or `--make-bed` statement would have produced in the corresponding MAP or BIM files.

## 4.3 Update SNP information

To automatically update either the genetic or physical positions for some or all SNPs in a dataset, use the `--update-map` command, which takes a single parameter of a filename, e.g.

```
plink --bfile mydata --update-map build36.txt --make-bed --out mydata2
```

where, for example, the file `build36.txt` contains new physical positions for SNPs, based on dbSNP126/build 36, in the simple format of SNP/position per line, e.g.

```
rs100001  1000202
rs100002  6252678
rs100003  7635353
...
```

To change genetic position (3rd column in map file) add the flag `--update-cm` *as well as* `--update-map`. There is no way to change chromosome codes using this command. Normally, one would want to save the new file with the changed positions, as in the example above, although one could combine other commands instead (e.g. association testing, etc) although the updated positions would then be lost (i.e. the changes are not automatically saved).

The file with new SNP information does not need to feature all of the SNPs in the current dataset: SNPs not in this file will be left unchanged. If a SNP is listed more than once in the file, an error will be reported.

**NOTE** When updating the map positions, it is possible that the implied ordering of SNPs in the dataset might change. If this is the case, a message will be written to the LOG file. Although the positions are updated, the order is not changed internally: as SNPs might be out of order, it is important to correct this by saving and reloading the file. For example, the if the original contains

```
...
rs10001   500000
rs10002   520000
rs10003   540000
rs10004   560000
...
```

but we update `rs10002` to position 580000, the data will be

```
...
rs10001   500000
rs10002   580000
rs10003   540000
rs10004   560000
...
```

Only after saving and reloading (e.g. `--make-bed` / `--bfile` ) will the file be in the correct order

```
...
rs10001   500000
rs10003   540000
rs10004   560000
rs10002   580000
```

```
...
```

This will only be an issue for commands which rely on relative SNP positions (e.g. –hap-window, –homozyg, etc). If the LOG file does not show a message that the order of SNPs has changed after using `--update-map`, one need not worry.

The name and chromosome code of a SNP can also be changed, by adding the modifiers `--update-name` or `--update-chr`, e.g.

```
./plink --bfile mydata --update-map rsID.lst --update-name --make-bed --out mydata2
```

or

```
./plink --bfile mydata --update-map chr-codes.txt --update-chr --make-bed --out mydata2
```

In both case, the format of the input file should be two columns per line, e.g.

```
SNP_A-1919191   rs123456
SNP_A-64646464  rs222222
...
```

or, for chromosome codes (use numeric values and codes X, Y, etc)

```
rs123456     1
rs987654     18
rs678678     X
..
```

You cannot update more than one attribute at a time for SNPs.

## 4.4 Update allele information

To recode alleles, for example from A,B allele coding to A,C,G,T coding, use the command `--update-alleles`, for example

```
./plink --bfile mydata --update-alleles mylist.txt --make-bed --out newfile
```

where the file `mylist.txt` contains five columns per row listing,

```
SNP identifier
Old allele code for one allele
Old allele code for other allele
New allele code for first allele
New allele code for other allele
```

For example,

```
rs10001  A B   G T
rs10002  A B   A C
...
```

will change allele A to G and allele B to T for rs10001, etc.

## 4.5 Force a specific reference allele

It is possible to manually specify which allele is the `A1` allele and which is `A2`. By default, the minor allele is assigned to be `A1`. All odds ratios, etc, are calculated with respect to the `A1` allele (i.e. an odds ratio greater than 1 implies that the `A1` allele increases risk).

To set a particular allele as `A1`, which might not be the minor allele, use the command `--reference-allele`, which can be used with any other analysis or data generation command, e.g.

```
./plink --bfile mydata --reference-allele mylist.txt --assoc
```

where the file `mylist.txt` contains a list of SNP IDs and the allele to be set as `A1`, e.g.

```
rs10001 A
rs10002 T
rs10003 T
...
```

This command can make comparing results across studies easier, so that odds ratios reported can be made to be in the same direction as the other study, for example.

## 4.6 Update individual information

Rather than try to manually edit PED or FAM files (which is not advised), use these functions to change ID codes, sex and parental information for individuals in a fileset. The command

```
plink --bfile mydata --update-ids recoded.txt --make-bed --out mydata2
```

changes ID codes for individuals specified in `recoded.txt`, which should be in the format of four columnds per row: old FID, old IID, new FID, new IID, e.g.

```
FA 1001        F0001   I0001
FA 1002.dup    F0002   I0002
...
```

will, for example find the person `FA/1001` and change their FID/IID values to `F0001/I0001`. Not all people need be listed in the file (they will not be changed; the order of the file need not match the original dataset.

Two simular commands (but that cannot be run at the same time as `--update-ids`) are

```
--update-sex myfile1.txt
```

that expects 3 columns per row:

```
FID
IID
SEX    Coded 1/2/0 for M/F/missing
```

and

```
--update-parents myfile2.txt
```

that expects 4 columns per row:

```
FID
IID
PAT   New paternal IID code
MAT   New maternal IID code
```

PLINK does not check see whether the new parents actually exist in the current file.

With all of these commands, you need to issue a data output command (`--make-bed`, `--recode`, etc) for the changes to be preserved.

## 4.7 Write covariate files

If a covariate file is specified along with any of the above `--recode` options or with `--make-bed`, then that covariate file will also be written, as `plink.cov` by default. This option is useful if the covariate file has a different number of individuals, or is ordered differently, to produce a set of covariate values that line up more easily with the newly-created genotype and phenotype files.

```
plink --file data --covar myfile.txt --recode
```

creates also `plink.cov`. If you want just to create a revised version of the covariate file, but without creating a new set of genotype files, then use the `--write-covar` option. This can be used in conjunction with filters, etc, to output, for example, only covariates for high-genotyping (99%) cases, as in this example:

```
plink --file data --write-covar myfile.txt --filter-cases --mind 0.01
```

will output just the relevant lines of `myfile.txt` to `plink.cov`, sorted to match the order of `data.ped`.

To also include phenotype information in the `plink.cov` file add the flag `--with-phenotype`. This can be useful, for example, when used in conjunction with `--recodeA` to generate the files needed to replicate an analysis in R (e.g. extracting the appropriate genotype data, and applying filters, etc).

To recode a categorical variable to a set of binary dummy variables, add the command

```
--dummy-coding
```

for example

```
./plink --bfile mydate --covar cdata.raw --write-covar --dummy-coding
```

If the original covariate had two fields, a categorical variable with 8 levels (coded 0 to 7, although it could have any numeric coding, e.g. 100, 150, 200, 250, etc), and a second variable that was continuous, e.g.

```
A8504 1   5  0.606218
A8008 1   1  0.442154
A8542 1   7  0.388042
A8022 1   2  0.286125
A8024 1   3  0.903004
A8026 1   4  0.790778
A8524 1   -9 0.713952
A8556 1   0  0.814292
A8562 1   1  0.803336
...
```

then the command above will create `mynewfile.cov`, with added header row, with the fields:

```
FID      Family ID
IID      Individual ID
COV1_2   Dummy variable for first covariate, coded  1/0 for 2/other
COV1_3   Dummy variable for first covariate, coded  1/0 for 3/other
COV1_4   etc
COV1_5
COV1_6
COV1_7
COV1_0
COV2    Unchanged continuous covariate
```

Thus `mynewfile.cov` is as follows (spaces added for clarity):

```
FID IID   COV1_2 COV1_3 COV1_4 COV1_5 COV1_6 COV1_7 COV1_0 COV2
A8504 1   0 0 0 1 0 0 0          0.606218
A8008 1   0 0 0 0 0 0 0          0.442154
A8542 1   0 0 0 0 0 1 0          0.388042
A8022 1   1 0 0 0 0 0 0          0.286125
A8024 1   0 1 0 0 0 0 0          0.903004
A8026 1   0 0 1 0 0 0 0          0.790778
A8524 1   -9 -9 -9 -9 -9 -9 -9   0.713952
A8556 1   0 0 0 0 0 0 1          0.814292
A8562 1   0 0 0 0 0 0 0          0.803336
```

That is, for a variable with *K* categories, *K-1* new dummy variables are created. This new file can be used with `--linear` and `--logistic`, and a coefficient for each level would now be estimated for the first covariate (otherwise PLINK would have incorrectly treated the first covariate as an ordinal/ratio measure). For covariate `Y`, each new dummy variable for level `X` is named `Y_X`, e.g. `COV1_2`, etc.

Note that one level is automatically excluded (1 in this case, i.e. there is no `COV1_1`), which implicitly makes 1 the reference category in subsequent analysis. If PLINK detects more than 50 levels, it assumes the variable is not categorical (i.e. like `COV2`) and so leaves it unchanged. The command can operate on multiple covariates in a single file at the same time. Note that missing values are correctly handled (i.e. left as missing).

**NOTE** Note that, unlike cluster files (see below) PLINK cannot handle any string information in covariate files.

## 4.8 Write cluster files

Similar to `--write-covar`, the `--write-cluster` will output the *single* selected cluster from the file specified by `--within`. Unlike covariate files, this allows string labels to be used.

```
plink --bfile mydata --within clst.dat --write-cluster --out mynewfile
```

which writes a file

```
mynewfile.clst
```

Use `--mwithin` to select which of multiple clusters is selected. The `--dummy-coding` can not currently be used with `--write-cluster` however.

## 4.9 Flip DNA strand for SNPs

This command will read the list of SNPs in the file `list.txt` and flip the strand for these SNPs, then save a new PED or BED fileset (i.e. by using either the `--recode` or `--make-bed` commands):

```
plink --file data --flip list.txt --recode
```

The `list.txt` should just be a simple list of SNP IDs, one SNP per line.

Flipping strand means changing alleles

```
A -> T
C -> G
G -> C
T -> A
```

so, for example, a `A/C` SNP will become a `T/G`; alternatively, a `A/T` SNP will become a `T/A` SNP (i.e. in this case, the labels remain the same, but whether the minor allele is `A` or `T` will still depend on strand).

To flip strand for just a subset of the sample (e.g. if two samples have already been merged, and subsequently a strand issue has been identified for one of those samples) use the option `--flip-subset`, for example

```
plink --file data --flip list.txt --flip-subset mylist.txt --recode
```

where `mylist.txt` is a text file containing the individuals (family ID, individual ID) to be flipped.

**HINT** When merging two datasets, it is clearly very important that the two sets of SNPs are concordant in terms of positive or negative strand. Whereas some mismatches will be easy to spot as more than two alleles will be observed in the merged dataset, other instances will not be so easy to spot, i.e. for `A/T` and `C/G` SNPs.

## 4.10 Using LD to identify incorrect strand assignment in a subset of the sample

If cases and controls have been genotyped separately and then the data merged, it is always possible that strand has been incorrectly or incompletely assigned to each SNP, meaning that the merged data may contain a number of SNPs for which the allele coding differs between cases and controls (or between any other grouping, such as collection site, etc).

If the two mis-matched groups correspond to cases and controls exactly, then rare SNPs will show a very strong association with disease (e.g. 5% MAF in cases, 95% in controls) and be easy to spot as potential problems. More common SNPs could show intermediate levels of association that might be easier to confuse with a real signal.

A simple approach to detect some proportion of such SNPs uses differential patterns of LD in cases versus controls: the command `--flip-scan` will query each SNP, and calculate the signed correlation between it and a set of nearby SNPs in cases and controls separately (of course, with the `--pheno` command, *case* and *control* status can be set to represent any binary split of the sample).

For each index SNP, PLINK identifies other SNPs in which the absolute value of the genotypic correlation is above some threshold. For these SNP pairs, it counts the number of times the signed correlation is different in sign between cases and controls (a *negative* LD pair) versus the same (a *positive* LD pair). For example, the command

```
plink --bfile mydata --flip-scan
```

produces the output file

```
plink.flipscan
```

with the fields

| | |
|---|---|
| CHR | Chromosome |
| SNP | SNP identifier for index SNP |
| BP | Base-pair position |
| A1 | Minor allele code |
| A2 | Major allele code |
| F | Allele frequency (A1 allele) |
| POS | Number of positive LD matches |
| R_POS | Average correlation of these |
| NEG | Number of negative LD matches |
| R_NEG | Average correlation of these |
| NEGSNPS | The SNPs showing negative correlation |

For example, the majority of this file should show SNPs have a `NEG` value of 0; the value of `POS` will be zero or greater, depending on the extent of LD. For example:

```
CHR        SNP       BP  A1  A2      F  POS  R_POS  NEG  R_NEG  NEGSNPS
  1  rs9439462  1452629   T   C      0    0     NA    0     NA (NONE)
  1  rs1987191  1457348   C   T      0    0     NA    0     NA (NONE)
  1  rs3766180  1468016   C   T  0.285    2  0.893    0     NA (NONE)
```

However, occasionally one might observe different patterns of results. Of particular interest is when one SNP shows a large number of `NEG` SNPs. For example, here we show `rs2240344` and nearby SNPs, all of which have at least one `NEG` SNP (lines truncated)

```
CHR         SNP        BP  A1  A2      F  POS    R_POS  NEG    R_NEG  NEGSNPS
 14  rs12434442  72158039   T   C  0.249    5    0.515    1     0.46  rs2240344
 14   rs4899437  72190986   G   C  0.394    5    0.802    1    0.987  rs2240344
 14   rs2803980  72196284   G   A   0.41    5    0.808    1     0.95  rs2240344
 14   rs2240344  72197893   C   G  0.489    0       NA    7    0.807  rs12434442|rs4899437|...
 14   rs2286068  72198107   C   T  0.407    7    0.741    1    0.962  rs2240344
```

```
        14    rs7160830    72209491    T    C    0.414    6    0.801    1    0.922  rs2240344
        14   rs10129954    72220454    T    C    0.413    6    0.729    1     0.73  rs2240344
        14    rs7140455    72240734    T    C    0.469    4     0.72    1     0.64  rs2240344
```

This pattern of results quite clearly points to `rs2240344` as being the odd man out: for 7 other SNPs, there is strong LD ($r$ above 0.5) in either cases or controls, *but* with a SNP-SNP correlation in the other phenotype class that has the opposite direction. In contrast, there is not a single SNP for which both cases and controls have a consistent pattern of LD. For the nearby SNPs, all of which have only 1 `NEG` SNP, it is with rs2240344. So, in this particular case, it would suggest that stand is flipped in either cases or controls.

To display the specific sets of correlations in cases and controls for each SNP, add the option

```
--flip-scan-verbose
```

which generates a file

```
plink.flipscan.verbose
```

which lists for any SNP with at least one `NEG` pair of LD values, the correlations between the index SNP and the other flanking SNPs, showing the correlation in cases (`R_A`) and controls (`R_U`):

```
CHR_INDX    SNP_INDX     BP_INDX  A1_INDX    SNP_PAIR     BP_PAIR  A1_PAIR      R_A       R_U
      14   rs2240344    72197893        C  rs12434442    72158039        T   -0.504     0.416
      14   rs2240344    72197893        C   rs4899437    72190986        G   -0.99      0.983
      14   rs2240344    72197893        C   rs2803980    72196284        G   -0.969     0.931
      14   rs2240344    72197893        C   rs2286068    72198107        C   -0.971     0.952
      14   rs2240344    72197893        C   rs7160830    72209491        T   -0.935     0.91
      14   rs2240344    72197893        C  rs10129954    72220454        T   -0.782     0.679
      14   rs2240344    72197893        C   rs7140455    72240734        T   -0.671     0.609
```

Here we see a clear pattern in which the correlation is similar between cases and controls in magnitude but has the opposite direction, strongly suggestive of a strand flip problem for this C/G SNP. In this case, the allele frequency turns out to be quite different between cases and controls (60% versus 40%) but the LD approach would have clearly detected this particular SNP being flipped in either cases or controls even if the true allele frequency were exactly 50%. This latter class of SNP would not cause problems of spurious association in single SNP analysis, but it could cause severe problems in haplotype and imputation analysis.

Naturally, if a SNP does not show strong LD with nearby SNPs, then this approach will not be able to resolve strand issues. Also, if more than one SNP in a region shows strand flips, or if there is a higher level of mis-coding alleles in general, then this approach may indicate that there are problems (many `NEG` scores above 0) but it might be less clear how to remedy them.

To know which to resolve (cases or controls) one would need to look at the frequency in other panels, or even the correlations, e.g. in HapMap. Ideally, one would only need to do this for a small number of SNPs if any. The `--flip` and `--flip-subset` commands described above can then be used to flip the appropriate genotypes.

Finally, the default threshold for counting can be changed by the following command:

```
--flip-scan-threshold 0.8
```

The default is set at 0.5 (i.e. the pair needs to have a correlation of 0.5 or greater in either cases or controls). The number of flanking SNPs with are considered for each index SNP can be modified with the commands

```
--ld-window 10
```

to set the number of SNPs considered upstream and downstream; the maximum physical distance away from the index SNP (1Mb by default) is specified in kb with the command:

```
--ld-window-kb 500
```

## 4.11 Merge two filesets

To merge two PED/MAP files:

```
plink --file data1 --merge data2.ped data2.map --recode --out merge
```

The `--merge` option must be followed by 2 arguments: the name of the second PED file and the name of the second MAP file. A `--recode` (or `--make-bed`, etc) option is necessary to output the newly merged file; in this case, `--out` option will create the files `merge-recode.ped` and `merge-recode.map`.

The `--merge` option can also be used with binary PED files, either as input or output, but not as the second file: i.e.

```
plink --bfile data1 --merge data2.ped data2.map --make-bed --out merge
```

will create `merge.bed`, `merge.fam` and `merge.bim`, as the `--make-bed` option was used instead of the `--recode` option. Likewise, the `data1.*` files point to a binary PED file set.

If the second fileset (`data2.*`) were in binary format, then you must use `--bmerge` instead of `--merge`

```
plink --bfile data1 --bmerge data2.bed data2.bim data2.fam --make-bed --out merge
```

which takes 3 parameters (the names of the BED, BIM and FAM files, in that order).

The two filesets can either overlap completely, partially, or not at all both in terms of markers and individuals. Imputed genotypes will be set to missing (i.e. if `SNP_B` is not measured in the first file, but it is in the second, then any individuals in the first file who are not also present in the second file will be set to missing for `SNP_B`.

By default, any existing genotype data (i.e. in `data1.ped`) will not be over-written by data in the second file (`data2.ped`). By specifying a `--merge-mode` this default behavior can be changed. The modes are:

```
1    Consensus call (default)
2    Only overwrite calls which are missing in original PED file
3    Only overwrite calls which are not missing in new PED file
4    Never overwrite
5    Always overwrite mode
6    Report all mismatching calls (diff mode -- do not merge)
7    Report mismatching non-missing calls (diff mode -- do not merge)
```

The default (mode 1) behaviour is to call the merged genotype as missing if the original and new files contain different, non-missing calls; otherwise: i.e.

```
                                Merge mode
data1.ped ,  data2.ped  ->  1    2    3    4    5
---------    ---------      ----------------------
 0/0      ,   0/0       ->  0/0  0/0  0/0  0/0  0/0
 0/0      ,   A/A       ->  A/A  A/A  A/A  0/0  A/A
 A/A      ,   0/0       ->  A/A  A/A  A/A  A/A  0/0
 A/A      ,   A/T       ->  0/0  A/A  A/T  A/A  A/T
```

Modes 6 and 7 effectively provide a means for comparing two PED files – no merging is performed in these cases; rather, a list of mismatching SNPs is written to the file

```
plink.diff
```

They should also report the concordance rate in the LOG file, based on all SNPs that feature in both sets.

A warning will be given if the chromosome and/or physical position differ between the two MAP files.

**NOTE** Alleles must be exactly coded to match: that is, PLINK will not assume that a `1,2,3,4` SNP coding maps onto a `A,C,G,T` coding. You can use the `--allele1234` and `--alleleACGT` commands *prior* to merging to convert datasets and then merge these consistently coded files (you cannot convert and merge on the fly, i.e. simply do putting `--allele1234` on the command line along with `--merge` will not work: you need to use `--allele1234` and `--make-bed` first).

## 4.12 Merge multiple filesets

To merge more than two standard and/or binary filesets, it is often more convenient to specify a single file that contains a list of PED/MAP and/or BED/BIM/FAM files and use the `--merge-list` option. Consider, for an extreme example, the case where each fileset contains only a single SNP, and that there are thousands of these files – this option would help build a single fileset, in this case.

For example, consider we had 4 PED/MAP filesets (labelled `fA.*` through `fD.*`) and 4 binary filesets, labelled `fE.*` through `fH.*`). Then using the command

```
plink --file fA --merge-list allfiles.txt --make-bed --out mynewdata
```

would create the binary fileset

```
mynewdata.bed
mynewdata.bim
mynewdata.fam
```

(alternatively, the `--recode` option could have been used instead of `--make-bed` to generate a standard ASCII PED/MAP fileset). In this case, the file `allfiles.txt` was a list of the to-be-merged files, one set per row:

```
fB.ped fB.map
fC.ped fC.map
fD.ped fD.map
fE.bed fE.bim fE.fam
fF.bed fF.bim fF.fam
fG.bed fG.bim fG.fam
fH.bed fH.bim fH.fam
```

**Important** Each fileset must be on a line by itself: lines with two files are interpreted as PED/MAP filesets; lines with three files are interpreted as binary BED/BIM/FAM filesets. The files on a line must always be in this order (PED then MAP; BED then BIM then FAM)

**Note** In this case the first of the 8 files must be the starting file, i.e. associated with `--file` on the command line; this file only contains the 8-1 remaining files therefore. The final `mynewdata.*` files will contain information from all 8 files.

The `--merge-mode` option can also be used with the `--merge-list` option, as described above: however, it is not possible to specify the "diff" features (i.e. modes 6 and 7).

## 4.13 Extract a subset of SNPs: command line options

There are multiple ways to extract just specific SNPs for analysis; this section describes options that use the command-line directly; the next section describes other methods that read a file containing the information.

### 4.13.1 Based on a single chromosome (`--chr`)

To analyse only a specific chromosome use

```
plink --file data --chr 6
```

### 4.13.2 Based on a range of SNPs (`--from` and `--to`)

To select a specific range of markers (that must all fall on the same chromosome) use, for example:

```
plink --bfile mydata --from rs273744 --to rs89883
```

### 4.13.3 Based on single SNP (and window) (`--snp` and `--window`)

Alternatively, you can specify a single SNP and, optionally, also ask for all SNPs in the surrounding region, with the `--window` option:

```
plink --bfile mydata --snp rs652423 --window 20
```

which extracts only SNPs within +/- 20kb of rs652423.

### 4.13.4 Based on multiple SNPs and ranges (`--snps`)

Alternatively, the newer `--snps` command is more flexible but slower than the previously described `--snp` and `--from`/`--to` commands. The `--snps` command will accept a comma-delimited list of SNPs, including ranges based on physical position. For example,

```
plink --bfile mydata --snps rs273744-rs89883,rs12345-rs67890,rs999,rs222
```

selects the same range as above (`rs273744` to `rs89883`) but also the separate range `rs273744` to `rs89883` as well as the two individual SNPs `rs999` and `rs222`. Note that SNPs need not be on the same chromosome; also, a range can span multiple chromosomes (the range is defined based on chromosome code order in that case, as well as physical position, i.e. a range from a SNP on chromosome 4 to one on chromosome 6 includes all SNPs on chromosome 5). No spaces are allowed between SNP names or ranges, i.e. it is

```
--snps rs1111-rs2222,rs3333,rs4444
```

and **not**

```
--snps rs1111 - rs2222, rs3333 ,rs4444
```

**Hint** As mentioned above, unlike other methods mentioned above, `--snps` will load in all the data before extracting what it needs, whereas `--snp` only loads in what it needs, as so is a much faster way to extract a region from a very large dataset: as a result, if you really do want only a single SNP or a single range, use `--snp` (with `--window`) or some variant of the `from/--to` commands.

### 4.13.5 Based on physical position (`--from-kb`, etc)

One can also select regions based on a window defined in terms of physical distance rather than SNP ID, using the command: e.g.

```
plink --bfile mydata --chr 2 --from-kb 5000 --to-kb 10000
```

to select all SNPs within this 5000kb region on chromosome 2 (when using `--from-kb` and `--to-kb` you always need to specify the chromosome with the `--chr` option).

**HINT** Two alternate forms of the `--from-kb` command are `--from-bp` and `--from-mb` that take a parameter in terms of base-pair position or megabase position, instead of kilobase (to be used with the corresponding `--to-bp` and `--to-mb` options).

### 4.13.6 Based on a random sampling (`--thin`)

To keep only a random 20% of SNPs, for example, add the flag

```
--thin 0.2
```

All the above options can be used either with standard pedigree files (i.e. using `--ped` or `--file`) or with binary format pedigree (BED) files (i.e. using `--bfile`). One must combine this option with the desired analytic (e.g. `--assoc`), summary statistic (e.g. `--freq`) or data-generation (e.g. `--make-bed`) option.

## 4.14 Extract a subset of SNPs: file-list options

To extract only a subset of SNPs, it is possible to specify a list of required SNPs and make a new file, or perform an analysis on this subset, by using the command

```
plink --file data --extract mysnps.txt
```

where the file is just a list of SNPs, one per line, e.g.

```
snp005
snp008
snp101
```

Alternatively, you can use the command `--range` to modify the behavior of `--extract` and `--exclude`. If the `--range` flag is added, then instead of a list of SNPs, PLINK will expect a list of chromosomal ranges to be given instead, one per line.

```
plink --file data --extract myrange.txt --range
```

All SNPs within that range will then be excluded or extracted. The format of `myrange.txt` should be, one range per line, whitespace-separated:

```
CHR     Chromosome code (1-22, X, Y, XY, MT, 0)
BP1     Start of range, physical position in base units
BP2     End of range, as above
```

For example,

```
2 30000000 35000000
2 60000000 62000000
X 10000000 20000000
```

would extract/exclude all SNPs in these three regions (5Mb and 2Mb on chromosome 2 and 10Mb on chromosome X).

### 4.14.1 Based on an attribute file (`--attrib`)

See below

### 4.14.2 Based on a set file (`--gene`)

Finally, if a SET file is also specified, you can use the `--gene` option to extract all SNPs in that gene/region. For example, if the SET file `genes.set` contains two genes:

```
GENE1
rs123456
rs10912
rs66222
END
GENE2
rs929292
rs288222
rs110191
END
```

then

```
plink --file mydata --set genes.set --gene GENE2 --recode
```

would, for example, create a new dataset with only the 3 SNPs in `GENE2`. One must combine these options with the desired analytic (e.g. `--assoc`), summary statistic (e.g. `--freq`) or data-generation (e.g. `--make-bed`) option.

## 4.15 Remove a subset of SNPs

To re-write the PED/MAP files, but with certain SNPs excluded, use the option

```
plink --file data --exclude mysnps.txt
```

where the file `mysnps.txt` is, as for the `--extract` command, just a list of SNPs, one per line. As described above, the `--range` command can modify the behaviour of `--exclude` in the same manner as for `--extract`.

One must combine this option with the desired analytic (e.g. `--assoc`), summary statistic (e.g. `--freq`) or data-generation (e.g. `--make-bed`) option.

**NOTE** Another way of removing SNPs is to make the physical position negative in the MAP file (this can not be done for binary filesets (e.g. the `*.bim` file).

## 4.16 Make missing a specific set of genotypes

To blank out a specific set of genotypes, use the following commands, e.g.

```
--zero-cluster test.zero  --within test.clst
```

in conjunction with other data analysis, file generation or summary statistic commands, where the file `test.zero` is a list of SNPs and clusters, and `test.clust` is a standard cluster file.

If the original PED file is

```
1  1 0 0 1 1  A A  C C  A A
2  1 0 0 1 1  C C  A A  C C
3  1 0 0 1 1  A C  A A  A C
4  1 0 0 1 1  A A  C C  A A
5  1 0 0 1 1  C C  A A  C C
6  1 0 0 1 1  A C  A A  A C
1b 1 0 0 1 1  A A  C C  A A
2b 1 0 0 1 1  C C  A A  C C
3b 1 0 0 1 1  A C  A A  A C
4b 1 0 0 1 1  A A  C C  A A
5b 1 0 0 1 1  C C  A A  C C
6b 1 0 0 1 1  A C  A A  A C
```

and the MAP file is

```
1 snp1 0 1000
1 snp2 0 2000
1 snp3 0 3000
```

and the list of SNPs/clusters to zero out in `test.zero` is

```
snp2   C1
snp3   C1
snp1   C2
```

and the cluster file `test.clst` is

```
1b 1 C1
2b 1 C1
3b 1 C1
4b 1 C1
5b 1 C1
6b 1 C1
2  1 C2
3  1 C2
```

then the command

```
plink --file test --zero-cluster test.zero --within test.clst --recode
```

results in a new PED file, `plink.ped`,

```
1  1 0 0 1  1  A A C C A A
2  1 0 0 1  1  0 0 A A C C
3  1 0 0 1  1  0 0 A A A C
4  1 0 0 1  1  A A C C A A
5  1 0 0 1  1  C C A A C C
6  1 0 0 1  1  A C A A A C
1b 1 0 0 1  1  A A 0 0 0 0
2b 1 0 0 1  1  C C 0 0 0 0
3b 1 0 0 1  1  A C 0 0 0 0
4b 1 0 0 1  1  A A 0 0 0 0
5b 1 0 0 1  1  C C 0 0 0 0
6b 1 0 0 1  1  A C 0 0 0 0
```

i.e. with the appropriate genotypes zeroed out.

**HINT** See the section on handling obligatory missing genotype data, which can often be useful in this context.

## 4.17 Extract a subset of individuals

To keep only certain individuals in a file, use the option:

```
plink --file data --keep mylist.txt
```

where the file `mylist.txt` is, as for the `--remove` command, just a list of Family ID / Individual ID pairs, one set per line, i.e. one person per line. (fields can occur after the 2nd column but they will be ignored – i.e. you could use a FAM file as the parameter of the `--keep` command, or have comments in the file. For example

```
F101   1
F1001  2_B
F3033  1_A  Drop this individual because of consent issues
F4442  22
```

would be fine.

One must combine this option with the desired analytic (e.g. `--assoc`), summary statistic (e.g. `--freq`) or data-generation (e.g. `--make-bed`) option.

## 4.18 Remove a subset of individuals

To remove certain individuals from a file

```
plink --file data --remove mylist.txt
```

where the file `mylist.txt` is, as for the `--keep` command, just a list of Family ID / Individual ID pairs, one set per line, i.e. one person per line (although, as for `--keep`, fields after the 2nd column are allowed but they will be ignored).

One must combine this option with the desired analytic (e.g. `--assoc`), summary statistic (e.g. `--freq`) or data-generation (e.g. `--make-bed`) option.

## 4.19 Filter out a subset of individuals

Whereas the options to keep or remove individuals are based on files containing lists, it is also possible to specify a filter to include only certain individuals based on phenotype, sex or some other variable.

The basic form of the command is `--filter` which takes two arguments, a filename and a value to filter on, for example:

```
plink --file data --filter myfile.raw 1 --freq
```

implies a file `myfile.raw` exists which has a similar format to phenotype and cluster files: that is, the first two columns are family and individual IDs; the third column is expected to be a numeric value (although the file can have more than 3 columns), and only individuals who have a value of `1` for this would be included in any subsequent analysis or file generation procedure. e.g. if `myfile.raw` were

```
F1  I1   2
F2  I1   7
F3  I1   1
F3  I2   1
F3  I3   3
```

then only two individuals (`F3 I1` and `F3 I2`) would be included based on this filter for the calculation of allele frequencies. The filter can be any integer numeric value.

As with `--pheno` and `--within`, you can specify an offset to read the filter from a column other than the first after the obligatory ID columns. Use the `--mfilter` option for this. For example, if you have a binary fileset, and so the FAM file contains phenotype as the sixth column, then you could specify

```
plink --bfile data --filter data.fam 2 --mfilter 4
```

to select cases only; i.e. cases have the value `2`, and this is the 4th variable in the file (i.e. the first two columns are ignored, as these are the ID columns).

Because filtering on cases or controls, or on sex, or on position within the family, will be common operations, there are some shortcut options that can be used instead of `--filter`. These are

```
--filter-cases
--filter-controls
--filter-males
--filter-females
--filter-founders
--filter-nonfounders
```

These flags can be used in any circumstances, e.g. to make a file of control founders,

```
plink --bfile data --filter-controls --filter-founders --make-bed --out newfile
```

or to analyse only males

```
plink --bfile data --assoc --filter-males
```

**IMPORTANT** Take care when using these with options to merge filesets: the merging occurs **before** these filters.

## 4.20 Attribute filters for markers and individuals

One can define an attribute file for SNPs (or for individuals, see below) that is simply a list of user-defined attributes for SNPs. For example, this might be a file

```
snps.txt
```

which contains

```
rs0001   exonic
rs0007   candidate
rs0010   failed exonic
rs0012   nssnp
```

These codes can be whatever you like, as is appropriate for your study; a SNP can have multiple, white-space delimited attributes. Not all SNPs need appear in this file; SNPs not in the dataset are allowed to appear (they are just ignored); the order does not need to be the same. Each SNP should only be listed once however. A SNP can be listed by itself without any attributes (for example, to ensure it is not excluded when filtering to exclude SNPs with a certain attribute, see below).

To filter SNPs on these, use the command (combined with some other data generation or analysis option)

```
--attrib snps.txt exonic
```

for example, to extract only *exonic* SNPs (rs0001 and rs0010 in this example, assuming they've been coded this way).

To *exclude* SNPs that match the attribute, preface the attribute with a minus sign on the command line, e.g.

```
--attrib snps.txt -failed
```

to extract only non-failed SNPs. Finally, multiple filters can be combined in a comma-delimited list

```
--attrib snps.txt exonic,-failed
```

would select exonic SNPs that did not fail. If a SNP does not feature in the attribute file, it will always be excluded.

**NOTE** Within match type, multiple matches are treated as logical ORs; between positive and negative matches as AND. For example, matching on `A,B,-C,-D` implies *individuals with ( A or B )* **and** *not ( C or D )*

This approach works similarly for individuals, except the command is now `--attrib-indiv`, e.g.

```
--attrib-indiv inddat.txt  sample1,fullinfo
```

and the attribute file starts with family ID and individual ID before listing any attributes, e.g.

```
F1  1   sample2
F2  1   sample1
F3  1   sample2 fullinfo
...
```

## 4.21 Create a SET file based on a list of ranges

Given a list of ranges in the following format (4 columns per row; no header file)

```
Chromosome
Start base-pair position
End base-pair position
Set/range/gene name
```

then the command

```
plink --file mydata --make-set gene.list --write-set
```

will generate the file

```
plink.set
```

in the standard set file format. The command `--make-set-border` takes a single integer argument, allowing for a certain kb window before and after the gene to be included, e.g. for 20kb upstream and downstream:

```
plink --file mydata --make-set gene.list --make-set-border 20 --write-set
```

**HINT** The `--make-set` command doesn't necessarily have to be used with `--write-set`. Rather, it can be used anywhere that `--set` can be used, to make sets on the fly. Similar, `--set` and `--write-set` can be combined, e.g. to create a new, filtered set file.

### 4.21.1 Options for `--make-set`

To collapse all ranges into a single set (i.e. to generate one set that corresponds to all SNPs in a gene, from a list of gene co-ordinates, for example), use

```
--make-set-collapse-all SETNAME
```

along with `--make-set`, where *SETNAME* is any single word that you use to name to newly created set. To make a set file of all SNPs **not** in the specified ranges, add

```
--make-set-complement-all SETNAME
```

Optionally, the range file can contain a fifth column, to specify groups of ranges. Sets can be constructed which collapse over these groups. That is, the input for `--make-set` is now

```
Chromosome
Start base-pair position
End base-pair position
Set/range/gene name
Group label
```

e.g.

```
1   10001   20003  GENE1  PWAY-A
8   80001   99995  GENE2  PWAY-A
12  1001    10001  GENE3  PWAY-B
5   110001 127362  GENE4  PWAY-B
...
```

Normally, the fifth column will just be ignored, unless the command

```
--make-set-collapse-group
```

is added, which creates sets of SNPs that correspond to each group (i.e. `PWAY-A`, `PWAY-B`, etc, in this example) rather than each gene/region (i.e. `GENE1`, etc). The command

```
--make-set-complement-group
```

works in a similar manner, except now forming sets of all SNPs **not** in the given group of ranges.

**HINT** See the resources page for pre-compiled RefSeq gene-lists that can be used here.

## 4.22 Tabulate set membership for all SNPs

It is possible to create a table that maps SNPs to sets, given a `--set` file has been specified, with the `--set-table` command, e.g.

```
./plink --bfile mydata --set mydata.set --set-table
```

which generates a file

```
plink.set.table
```

which contains the fields

```
SNP               SNP identifier
CHR               Chromosome code
BP                Base-pair physical position
```

```
First set name     Membership of first set
Second set name    Membership of second set
...
```

For each row, a series of 0s and 1s indicate whether or not each SNP in the dataset is in a given SET. This format can be useful for subsequent analyses (i.e. it can be easily lined up with other result files, e.g. from `--assoc`).

## 4.23 SNP-based quality scores

PLINK supports quality scores for SNPs and, described in the next section, genotypes. These can be used to filter on user-defined thresholds. The command `--qual-scores` indicates the file containing the scores. Scores are assumed to be numbers between 0 and 1, a higher number representing better quality. The threshold at which SNPs are selected can be set with the command `--qual-threshold`. For example,

```
./plink --bfile mydata --qual-scores myscores.txt --qual-threshold 0.8 --make-bed
--out qc-data
```

where `myscores.txt` is a text file of SNPs and scores, e.g.

```
rs10001 0.87
rs10002 0.46
rs10003 1.00
...
```

will remove SNPs with scores less than 0.8. The additional flag `--qual-max-threshold` can be used to specify a maximum threshold also (i.e. to select low-quality SNPs only). Not all SNPs need be in the file (the SNP is left in, in this case; the order can be different, it can contain SNPs not in the data).

## 4.24 Genotype-based quality scores

Quality scores for each genotype, rather than each SNP, can also be applied to PLINK datasets, using the `--qual-geno-scores` command, e.g.

```
./plink --bfile mydata --qual-geno-scores gqual.txt --qual-geno-threshold 0.99 --assoc
```

(with a similar `--qual-geno-max-threshold` command as well).
The file containing the genotype quality scores should have the following format:

```
Q FID IID SNPID score
```

e.g.

```
Q fam1 ind1 rs10001 0.873
Q fam1 ind1 rs10002 0.998
...
```

Not all genotypes need be in this file. Rather than have a very large file, one could only list genotype scores that are below some threshold, for example, assuming most genotypes are of very good quality. Genotypes not in the this file will be untouched. This format is designed to accept wildcards, as follows. Every item should start with a `Q` character, to allow PLINK to check the correctness of the file format. Consider this example file,

```
Q  A 1  rs1234 0.986
Q  B 1  rs1234 0.923
Q  A 1  rs5678 0.323
Q  B 1  rs5678 0.97
```

that lists two genotypes for people with FID/IID A/1 and B/1 for SNPs rs1234 and rs5678. If a score if below threshold, it is set to missing in the data. The order of this file is arbitrary; not all individuals/SNPs need appear.

PLINK accepts *wildcards* in this file, to allow for different data formats to be specified. With a *person wild-card*, PLINK expects all quality scores for that SNP, in order as in the FAM or PED file, e.g.

```
Q * rs1234 0.986 0.923
Q * rs5678 0.323 0.97
```

With a *SNP wildcard*, PLINK exects all SNPs for a given person:

```
Q A 1 * 0.986 0.323
Q B 1 * 0.923 0.97
```

All these formats can be mixed together in a single file. These can be combined (in which case, PLINK expects all individuals for the first SNP, all for the second SNP, etc)

```
Q * * 0.986 0.923 0.323 0.97
```

**WARNING** This option is recently added in beta-stage of development. Currently, a wild card looks to the current data to get the list of individuals and SNPs to loop over. This could cause a problem if the file has been filtered, etc. The next release will include commands to specify the order of individuals and SNPs, e.g.

```
--qual-people-list mysamples.lst
```

where `mysamples.lst` is a file with 2 columns (FID/IID), and

```
--qual-geno-snp-list mysnp.lst
```

where `mysnp.lst` is list of SNPs. This way if somebody is in the quality score file but they have been removed from the actual genotype dataset (or added), then this can be handled properly without needing to change the whole quality score file.

# Chapter 5

# Summary statistics

PLINK will generate a number of standard summary statistics that are useful for quality control (e.g. missing genotype rate, minor allele frequency, Hardy-Weinberg equilibrium failures and non-Mendelian transmission rates). These can also be used as thresholds for subsequent analyses (described in the next section).

All the per-SNP summary statistics described below are conducted after removing individuals with high missing genotype rates, as defined by the `--mind` option. The default value of which is 0 however, i.e. do not exclude any individuals.

**NOTE** Regarding the calculation of genotype rates for sex chromosomes: for the Y, females are ignored completely. For the males, heterozygous X and heterozygous Y genotypes are treated as missing. Having the correct designation of gender is therefore important to obtain accurate genotype rate estimates, or avoid incorrectly removing samples, etc.

## 5.1 Missing genotypes

To generate a list genotyping/missingness rate statistics:

```
plink --file data --missing
```

This option creates two files:

```
plink.imiss
plink.lmiss
```

which detail missingness by individual and by SNP (**l**ocus), respectively. For individuals, the format is:

| | |
|---|---|
| FID | Family ID |
| IID | Individual ID |
| MISS_PHENO | Missing phenotype? (Y/N) |
| N_MISS | Number of missing SNPs |
| N_GENO | Number of non-obligatory missing genotypes |
| F_MISS | Proportion of missing SNPs |

For each SNP, the format is:

| | |
|---|---|
| SNP | SNP identifier |
| CHR | Chromosome number |
| N_MISS | Number of individuals missing this SNP |
| N_GENO | Number of non-obligatory missing genotypes |
| F_MISS | Proportion of sample missing for this SNP |

**HINT** To test for case/control differences in missingness, see the `--test-missing` option.

**HINT** To produce summary of missingness that is stratified by a categorical cluster variable, use the `--within` *filename* option as well as `--missing`. In this way, the missing rates will be given separately for each level of the categorical variable. For example, the categorical variable could be which plate that sample was on in the genotyping. Details on the format of a cluster file can be found here.

## 5.2 Obligatory missing genotypes

Often genotypes might be missing obligatorarily rather than because of genotyping failure. For example, some proportion of the sample might only have been genotyped on a subset of the SNPs. In these cases, one might not want to filter out SNPs and individuals based on this type of missing data. Alternatively, genotypes for specific plates (sets of SNPs/individuals) might have been blanked out with the `--zero-cluster` option, but you still might want to be able to sensibly set missing data thresholds.

**HINT** See the section on data management to see how to make missing certain sets of genotypes.

Two functions allow these 'obligatory missing' values to be identified and subsequently handled specially during the filtering steps:

```
plink --bfile mydata --oblig-missing myfile.zero --oblig-clusters myfile.clst --assoc
```

This command applies the default genotyping thresholds (90% per individual and per SNP) but accounting for the fact that certain SNPs are obligatory missing (with the 90% only refers to those SNPs actually attempted, for example). The file specified by `--oblig-clusters` has the same format as a cluster file (except only a single cluster field is allowed here, i.e. only 3 columns). For example,

```
1  1 0 0 1 1   A A  C C  A A
2  1 0 0 1 1   C C  A A  C C
3  1 0 0 1 1   A C  A A  A C
4  1 0 0 1 1   A A  C C  A A
5  1 0 0 1 1   C C  A A  C C
6  1 0 0 1 1   A C  A A  A C
1b 1 0 0 1 1   A A  0 0  0 0
2b 1 0 0 1 1   C C  0 0  0 0
3b 1 0 0 1 1   A C  0 0  0 0
4b 1 0 0 1 1   A A  0 0  0 0
5b 1 0 0 1 1   C C  0 0  0 0
6b 1 0 0 1 1   A C  0 0  0 0
```

and MAP file `test.map`

```
1 snp1 0 1000
1 snp2 0 2000
1 snp3 0 3000
```

If the obligatory missing file, `test.oblig` is

```
snp2   C1
snp3   C1
```

it implies that SNPs `snp2` and `snp3` are obligatory missing for all individuals belonging to cluster `C1`. The corresponding cluster file is `test.clst`

```
1b 1 C1
2b 1 C1
3b 1 C1
4b 1 C1
5b 1 C1
6b 1 C1
```

indicating that the last six individuals belong to cluster `C1`. (Not all individuals need be specified in this file.)

**NOTE** You can have more than one cluster category specified in these files (i.e. implying different patterns of obligatory missing data for different sets of individuals).

Running a `--missing` command on the basic fileset, ignoring the obligatory missing nature of some of the data, results in the following:

```
plink --file test --missing
```

which shows in the LOG file that 6 individuals were removed because of missing data

```
...
6 of 12 individuals removed for low genotyping ( MIND > 0.1 )
...
```

and the corresponding output files (`plink.imiss` and `plink.lmiss`) indicate no missing data (purely because the six individuals with 2 of 3 genotypes missing were already filtered out and everybody else left happens to have complete genotyping).

```
 FID  IID MISS_PHENO   N_MISS   F_MISS
   1    1          N        0        0
   2    1          N        0        0
   3    1          N        0        0
   4    1          N        0        0
   5    1          N        0        0
   6    1          N        0        0
```

and

```
 CHR  SNP   N_MISS   F_MISS
   1 snp1        0        0
   1 snp2        0        0
   1 snp3        0        0
```

In contrast, if the obligatory missing data are specified as follows:

```
plink --file test --missing --oblig-missing test.oblig --oblig-clusters test.clst
```

we now see

```
...
0 of 12 individuals removed for low genotyping ( MIND > 0.1 )
...
```

and the corresponding output files now include an extra field, `N_GENO`, which indicates the number of non-obligatory missing genotypes, which is the denominator for the genotyping rate calculations

```
 FID  IID MISS_PHENO   N_MISS   N_GENO   F_MISS
   1    1          N        0        3        0
   2    1          N        0        3        0
   3    1          N        0        3        0
   4    1          N        0        3        0
   5    1          N        0        3        0
   6    1          N        0        3        0
  1b    1          N        0        1        0
  2b    1          N        0        1        0
  3b    1          N        0        1        0
  4b    1          N        0        1        0
  5b    1          N        0        1        0
  6b    1          N        0        1        0
```

and

```
     CHR  SNP   N_MISS   N_GENO   F_MISS
       1 snp1        0       12        0
       1 snp2        0        6        0
       1 snp3        0        6        0
```

Seen another way, if one specified `--mind 1` to include all individuals (i.e. not apply the default 90% genotyping rate threshold for each individual before this step), then the results would not change with the obligatory missing specification in place, as expected; in contrast, without the specification of obligatory missing data, we would see

```
     FID  IID MISS_PHENO   N_MISS   F_MISS
       1    1          N        0        0
       2    1          N        0        0
       3    1          N        0        0
       4    1          N        0        0
       5    1          N        0        0
       6    1          N        0        0
      1b    1          N        2 0.666667
      2b    1          N        2 0.666667
      3b    1          N        2 0.666667
      4b    1          N        2 0.666667
      5b    1          N        2 0.666667
      6b    1          N        2 0.666667
```

and

```
     CHR  SNP   N_MISS   F_MISS
       1 snp1        0        0
       1 snp2        6      0.5
       1 snp3        6      0.5
```

In this not particularly exciting example, there are no missing genotypes that are non-obligatory missing (i.e. that not specified by the two files) – if there were, it would counted appropriately in the above files, and used to filter appropriately also.

**NOTE** All subsequent analyses do not distingush whether genotypes were missing due to failure or were obligatory missing – that is, this option only effects the behavior of the `--mind` and `--geno` filters.

**NOTE** If a genotype is set to be obligatory missing but actually in the genotype file it is not missing, then it will be set to missing and treated as if missing.

## 5.3 Cluster individuals based on missing genotypes

Systematic batch effects that induce missingness in parts of the sample will induce correlation between the patterns of missing data that different individuals display. One approach to detecting correlation in these patterns, that might possibly idenity such biases, is to cluster individuals based on their *identity-by-missingness* (IBM). This approach use exactly the same procedure as the IBS clustering for population stratification, except the distance between two individuals is based not on which (non-missing) allele they have at each site, but rather the proportion of sites for which two individuals are both missing the same genotype.

To use this option:

```
plink --file data --cluster-missing
```

which creates the files:

```
plink.matrix.missing
plink.cluster3.missing
```

which have similar formats to the corresponding IBS clustering files. Specifically, the `plink.mdist.missing` file can be subjected to a visualisation technique such as multidimensinoal scaling to reveal any strong systematic patterns of missingness.

**Note** The values in the `.mdist` file are distances rather than similarities, unlike for standard IBS clustering. That is, a value of 0 means that two individuals have the same profile of missing genotypes. The exact value represents the proportion of all SNPs that are discordantly missing (i.e. where one member of the pair is missing that SNP but the other individual is not).

The other constraints (significance test, phenotype, cluster size and external matching criteria) are not used during IBM clustering. Also, by default, all individuals and all SNPs are included in an IBM clustering analysis, unlike IBS clustering, i.e. even individuals or SNPs with very low genotyping, or monomorphic alleles. By explicitly specifying `--mind` or `--geno` or `--maf` certain individuals or SNPs can be excluded (although the default is probably what is usually required for quality control procedures).

## 5.4 Test of missingness by case/control status

To obtain a *missing chi-sq* test (i.e. does, for each SNP, missingness differ between cases and controls?), use the option:

```
plink --file mydata --test-missing
```

which generates a file

```
plink.missing
```

which contains the fields

```
CHR        Chromosome number
SNP        SNP identifier
F_MISS_A   Missing rate in cases
F_MISS_U   Missing rate in controls
P          Asymptotic p-value (Fisher's exact test)
```

The actual counts of missing genotypes are available in the `plink.lmiss` file, which is generated by the `--missing` option.

**Note** This test is only applicable to case/control data.

## 5.5 Haplotype-based test for non-random missing genotype data

The previous test asks whether genotypes are missing at random or not with respect to phenotype. This test asks whether or not genotypes are missing at random with respect to the true (unobserved) genotype, based on the observed genotypes of nearby SNPs.

**Note** This test assumes dense SNP genotyping such that flanking SNPs are typically in LD with each other. Also bear in mind that a negative result on this test may simply reflect the fact that there is little LD in the region.

This test works by taking a SNP at a time (the 'reference' SNP) and asking whether haplotype formed by the two flanking SNPs can predict whether or not the individual is missing at the reference SNP. The test is a simple haplotypic case/control test, where the phenotype is missing status at the reference SNP. If missingness at the reference is not random with respect to the true (unobserved) genotype, we may often expect to see an association between missingness and flanking haplotypes.

**Note** Again, just because we might not see such an association does not necessarily mean that genotypes are missing at random – this test has higher specificity than sensitivity. That is, this test will miss a lot; but, when used as a QC screening tool, one should pay attention to SNPs that show highly significant patterns of non-random missingness.

This option is run with the command:

```
plink --file data --test-mishap
```

which generates an output file called

```
plink.missing.hap
```

which has the fields

|  |  |
|---|---|
| LOCUS | Reference SNP |
| HAPLOTYPE | Flanking haplotype, or heterozygosity |
| F_0 | Frequency of HAPLOTYPE if missing reference SNP |
| F_1 | Frequency of HAPLOTYPE if not missing reference SNP |
| M_H1 | N missing/not missing for HAPLOTYPE |
| M_H2 | N missing/not missing for not-HAPLOTYPE |
| CHISQ | Chisquare test for non-random missingness |
| P | Asymptotic p-value |
| SNPS | Identifier for flanking SNPs |

The `HAPLOTYPE` typically represents each two-SNP flanking haplotype (i.e. not including the reference SNP itself); each reference SNP will also have a row labelled `HETERO` in this column, which means we are testing whether or not being heterozygous for the flanking haplotypes (which would, under many sets of haplotype frequencies, increase the chance of being heterozygous for the reference SNP). SNPs with no or very little missing genotype data are skipped. Only haplotypes above the `--maf` threshold are used in analysis.

Here is an example from real data (rows split into two sets for clarity):

```
LOCUS         HAPLOTYPE        F_0       F_1         M_H1         M_H2
rs17012390           CT     0.5238   0.01949       55/104      50/5233
rs17012390           TC     0.4762    0.9805      50/5233       55/104
rs17012390       HETERO          1   0.04252       56/114       0/2567
LOCUS         HAPLOTYPE      CHISQ         P   SNPS
rs17012390           CT      923.4         0   rs17012387|rs17012393
rs17012390           TC      923.4         0   rs17012387|rs17012393
rs17012390       HETERO      863.3         0   rs17012387|rs17012393
```

This clearly shows a huge chi-square (the sample is large, N of over 2500 individuals). We see that of 56 missing genotypes for this reference SNP, all occur when the flanking haplotypic background is heterozygous (i.e. `M_H1` shows `56/114`, indicating that there are 114 other instances of a heterozygous haplotypic background when the reference SNP is not missing) whereas we see not a single missing call when the flanking SNP background is homozygous, of which we see 2567 observations. This is clearly indicative of non-random association between the unobserved genotype and missing status.

Looking at the same data a different way, `F_1` indicates that the majority of the sample (people not missing at the reference SNP) have haplotype frequencies of `CT` and `TC` haplotypes at approximately 0.02 and 0.98 respectively). In contrast, because all people missing this SNP are on heterozygous backgrounds, these frequencies become approximately 50:50 in this group (shown in `F_0`).

In the particular dataset this example comes from, this SNP would have passed a standard quality control test. The `--hardy` command shows that this SNP does not failure the HWE test; also, it does not show excessive amounts of missing data (the `--missing` command indicates a missing rate of 0.021). The genotype counts (obtained by the `--hardy` option) are, for the whole sample, `0/104/2584`.

In contrast, here are the same results for a different SNP that does not show any evidence of non-random missingness.

```
     LOCUS    HAPLOTYPE        F_0        F_1          M_H1         M_H2
 rs3912752           CC    0.07692    0.06507         2/354      24/5086
 rs3912752           TT     0.1154      0.205        3/1115      23/4325
 rs3912752           CT     0.8077       0.73       21/3971       5/1469
 rs3912752       HETERO     0.2308     0.4279        3/1164      10/1556
     LOCUS    HAPLOTYPE      CHISQ          P   SNPS
 rs3912752           CC    0.05967      0.807   rs3912751|rs351596
 rs3912752           TT      1.276     0.2586   rs3912751|rs351596
 rs3912752           CT     0.7938     0.3729   rs3912751|rs351596
 rs3912752       HETERO      2.056     0.1516   rs3912751|rs351596
```

Here we do not see any deviation between the flanking haplotype frequencies between people missing versus genotyped for the reference SNP. Of course, there is less missingness for this SNP (26 missing genotypes) so we might expect power is lower, even if there were non-random missingness. This only highlights the point made above that, in general, significant results are more interpretable than non-signficant results for this test. But more importantly, if there are only a handful of missing genotypes, we do not particular care whether or not they are missing at random, as they would not bias the association with disease in any case. Of course, whether there is non-random genotyping *error* is another question...

By default, we currently just select exactly two flanking SNPs. This can be changed with the option `--mishap-window`. For example,

```
plink --bfile mydata --test-mishap --mishap-window 4
```

Future releases will feature a more intelligent selection of flanking markers.

**Note** This routine currently skips the SNPs on the X and Y chromosomes.

## 5.6 Hardy-Weinberg Equilibrium

To generate a list of genotype counts and Hardy-Weinberg test statistics for each SNP, use the option:

```
plink --file data --hardy
```

which creates a file:

```
plink.hwe
```

This file has the following format

```
SNP            SNP identifier
TEST           Code indicating sample
A1             Minor allele code
A2             Major allele code
GENO           Genotype counts: 11/12/22
O(HET)         Observed heterozygosity
E(HET)         Expected heterozygosity
P              H-W p-value
```

For case/control samples, each SNP will have three entries (rows) in this file, with `TEST` being either `ALL`, `AFF` (cases only) or `UNAFF` (controls only). For quantitative traits, only a single row will appear for each SNP, labelled `ALL(QT)`.

Only founders are considered for the Hardy-Weinberg calculations – ie. for family data, any offspring are ignored.

**WARNING** By default, this procedure only considers founders, so no HW results would be given for sibling-only datasets (i.e. if no parents exist). To perform a rough, somewhat biased test, use the `--nonfounders` option which means that all individuals will be included. Alternatively, manually extract one person per family for this calculation and recode these individuals as founders (see the `--keep` option to facilitate this).

The default test is an exact one, described and implemented by Wigginton *et al* (see reference below), which is more accurate for rare genotypes. You can still perform the standard asymptotic test with the `--hardy2` option.

```
A Note on Exact Tests of Hardy-Weinberg Equilibrium.
Wigginton JE, Cutler DJ and Abecasis GR
Am J Hum Genet (2005) 76: 887-93
```

## 5.7 Allele frequency

To generate a list of minor allele frequencies (MAF) for each SNP, based on all founders in the sample:

```
plink --file data --freq
```

will create a file:

```
plink.frq
```

with five columns:

```
CHR       Chromosome
SNP       SNP identifier
A1        Allele 1 code (minor allele)
A2        Allele 2 code (major allele)
MAF       Minor allele frequency
NCHROBS   Non-missing allele count
```

**HINT** To produce summary of allele frequencies that is stratified by a categorical cluster variable, use the `--within` *filename* option as well as `--missing`. In this way, the frequencies will be given separately for each level of the categorical variable. Details on the format of a cluster file can be found here.

**NOTE** If a SNP fails the genotyping rate threshold (as set by the `--geno` value, which is by default 0.0, i.e. no SNPs will fail) the frequency will appear as `NA` in the `plink.frq` output file. To obtain frequencies on all SNPs irrespective of genotyping rate, set `--mind 1`.

## 5.8 Linkage disequilibrium based SNP pruning

Sometimes it is useful to generate a pruned subset of SNPs that are in approximate linkage equilibrium with each other. This can be achieved via two commands: `--indep` which prunes based on the *variance inflation factor* (VIF), which recursively removes SNPs within a sliding window; second, `--indep-pairwise` which is similar, except it is based only on pairwise genotypic correlation.

**Hint** The output of either of these commands is two lists of SNPs: those that are pruned out and those that are not. A separate command using the `--extract` or `--exclude` option is necessary to actually perform the pruning.

The VIF pruning routine is performed:

```
plink --file data --indep 50 5 2
```

will create files

```
plink.prune.in
plink.prune.out
```

Each is a simlpe list of SNP IDs; both these files can subsequently be specified as the argument for a `--extract` or `--exclude` command.

The parameters for `--indep` are: window size in SNPs (e.g. 50), the number of SNPs to shift the window at each step (e.g. 5), the VIF threshold. The VIF is `1/(1-R^2)` where `R^2` is the multiple correlation coefficient for a SNP being regressed on all other SNPs simultaneously. That is, this considers the correlations between SNPs but also between linear combinations of SNPs. A VIF of 10 is often taken to represent near collinearity problems in standard multiple regression analyses (i.e. implies R2̂ of 0.9). A VIF of 1 would imply that the SNP is completely independent of all other SNPs. Practically, values between 1.5 and 2 should probably be used; particularly in small samples, if this threshold is too low and/or the window size is too large, too many SNPs may be removed.

The second procedure is performed:

```
plink --file data --indep-pairwise 50 5 0.5
```

This generates the same output files as the first version; the only difference is that a simple pairwise threshold is used. The first two parameters (50 and 5) are the same as above (window size and step); the third parameter represents the r2̂ threshold. Note: this represents the pairwise SNP-SNP metric now, not the multiple correlation coefficient; also note, this is based on the genotypic correlation, i.e. it does not involve phasing.

To give a concrete example: the command above that specifies `50 5 0.5` would a) consider a window of 50 SNPs, b) calculate LD between each pair of SNPs in the window, b) remove one of a pair of SNPs if the LD is greater than 0.5, c) shift the window 5 SNPs forward and repeat the procedure.

To make a new, pruned file, then use something like (in this example, we also convert the standard PED fileset to a binary one):

```
plink --file data --extract plink.prune.in --make-bed --out pruneddata
```

## 5.9 Mendel errors

To generate a list of Mendel errors for SNPs and families, use the option:

```
plink --file data --mendel
```

which will create files:

```
plink.mendel
plink.imendel
plink.fmendel
plink.lmendel
```

The `*.mendel` file contains all Mendel errors (i.e. one line per error); the `*.imendel` file contains a summary of per-individual error rates; the `*.fmendel` file contains a summary of per-family error rates; the `*.lmendel` file contains a summary of per-SNP error rates.

The `*.mendel` file has the following columns:

| | |
|---|---|
| FID | Family ID |
| KID | Child individual ID |
| CHR | Chromosome |
| SNP | SNP ID |
| CODE | A numerical code indicating the type of error (see below) |
| ERROR | Description of the actual error |

The error codes are as follows:

| Code | Pat | , | Mat | -¿ | Offspring |
|---|---|---|---|---|---|
| 1 | AA | , | AA | -> | AB |
| 2 | BB | , | BB | -> | AB |
| 3 | BB | , | ** | -> | AA |
| 4 | ** | , | BB | -> | AA |
| 5 | BB | , | BB | -> | AA |
| 6 | AA | , | ** | -> | BB |

```
7      **   ,   AA    ->    BB
8      AA   ,   AA    ->    BB
9      **   ,   AA    ->    BB    (X chromosome male offspring)
10     **   ,   BB    ->    AA    (X chromosome male offspring)
```

The `*.lmendel` file has the following columns:

```
CHR            Chromosome
SNP            SNP ID
N              Number of Mendel errors for this SNP
```

The `*.imendel` file has the following columns:

```
FID            Family ID
IID            Individual ID
N              Number of errors this individual was implicated in
```

The following heurtistic is used to provide a rough estimate of Mendel error rare 'per individual': error types 1 and 2 count for all 3 individuals (child, father, mother); error types 5 and 8 count only for the child (i.e. otherwise requires two errors, one in each parent); error types 3 and 6 count for the child and the father; all other types (4, 7, 9 and 10) count for the offspring and the mother. This metric might indicate that, for example, in a nuclear family with two parents and two offspring, many more Mendel errors can be associated with the first sibling; the remaining trio might not show any increased rate.

Currently, PLINK only scans full trios for Mendel errors. Families with fewer than 2 parents in the dataset will not be tested.

Finally, the `*.fmendel` file has the following columns:

```
FID            Family ID
PAT            Paternal individual ID
MAT            Maternal individual ID
CHLD           Number of offspring in this (nuclear) family
N              Number of Mendel errors for this (nuclear) family
```

## 5.10 Sex check

This option uses X chromosome data to determine sex (i.e. based on heterozygosity rates) and flags individuals for whom the reported sex in the PED file does not match the estimated sex (given genomic data). To run this analysis, use the flag:

```
plink --bfile data --check-sex
```

which generates a file

```
plink.sexcheck
```

which contains the fields

```
FID     Family ID
IID     Individual ID
PEDSEX  Sex as determined in pedigree file (1=male, 2=female)
SNPSEX  Sex as determined by X chromosome
STATUS  Displays "PROBLEM" or "OK" for each individual
F       The actual X chromosome inbreeding (homozygosity) estimate
```

A `PROBLEM` arises if the two sexes do not match, or if the SNP data or pedigree data are ambiguous with regard to sex. A male call is made if `F` is more than 0.8; a femle call is made if `F` is less than 0.2.

The command

```
plink --bfile data --impute-sex --make-bed --out newfile
```

will impute the sex codes based on the SNP data, and create a new file with the revised assignments, in this case a new binary fileset.

## 5.11 Pedigree errors

`PLINK` can accept multigenerational family data for family-based tests and Mendel error checks. It will break multigenerational families down into nuclear family units where appropriate. Extended family information is not used in an optimal manner, however (e.g. to help find Mendel errors using grandparental genotypes if parental genotypes are missing).

Unless `PLINK` is explicitly told to perform a family-based analysis, it will ignore any pedigree structure in the sample and analyse the data as if all individuals are unrelated (i.e. the `--assoc` option, for example, will ignore family structure). It is therefore the responsibility of the user to ensure that the data are appropriate for the type of test (e.g. if performing a standard association test with `--assoc`, this implies that all individuals should be unrelated for asymptotic significance values to be correct). The exception to this general rule is that certain summary statistics are based only on founders.

`PLINK` will spot most pedigree errors (e.g. if an individual has two fathers, for example). For a more comprehensive evaluation of pedigree errors (invalid or incompletely specified pedigree structures) please use a different software package such as PEDSTATS or famtypes `http://pngu.mgh.harvard.edu/purcell/famtypes/`.

# Chapter 6

# Inclusion thresholds

This secion describes options that can be used to filter out individuals or SNPs on the basis of the summary statistic measures described in the previous summary statistics page.

### 6.0.1 Summary statistics versus inclusion criteria

The following table summarizes the relationship between the commands to generate summary statistics (as described on the previous page, versus the commands to exclude individuals and/or markers, which are described on this page.

| **Feature** | **As summary statistic** | **As inclusion criteria** |
|---|---|---|
| Missingness per individual | `--missing` | `--mind` *N* |
| Missingness per marker | `--missing` | `--geno` *N* |
| Allele frequency | `--freq` | `--maf` *N* |
| Hardy-Weinberg equilibrium | `--hardy` | `--hwe` *N* |
| Mendel error rates | `--mendel` | `--me` *N M* |

### 6.0.2 Default threshold values

By default, `PLINK` does not impose any filters on minor allele frequency or genotyping rate. (Note that versions prior to 1.04 use to have thresholds of 0.01 for frequency and 0.1 for individual and SNP missing rate – this is no longer the case, i.e. it is as if the `--all` keyword is always specified).

To perform an analysis, or generate a new dataset, with filters applied, add the `--mind`, `--geno` or `--maf` options are to the command line, for example, when the `--remove` command is given.

## 6.1 Missing rate per person

The initial step in all data analysis is to exclude individuals with too much missing genotype data. This option is set as follows:

```
plink --file mydata --mind 0.1
```

which means exclude with more than 10% missing genotypes (this is the defalt value). A line in the terminal output will appear, indicating how many individuals were removed due to low genotyping. If any individuals were removed, a file called

```
plink.irem
```

will be created, listing the Family and Individual IDs of these removed individuals. Any subsequent analysis also specifeid on the same command line will be performed without these individuals.

One might instead wish to create a new PED file with these individuals permanently removed, simply add an option to generate a new fileset: for example,

```
plink --file data --mind 0.1 --recode --out cleaned
```

will generate files

```
cleaned.ped
cleaned.map
```

with the high-missing-rate individuals removed; alternatively, to create a binary fileset with these individuals removed:

```
plink --file data --mind 0.1 --make-bed --out cleaned
```

which results in the files

```
cleaned.bed
cleaned.bim
cleaned.fam
```

**HINT** You can specify that certain genotypes were never attempted, i.e. that they are obligatory missing, and these will be handled appropriately by these genotyping rate filters. See the summary statistics page for more details.

## 6.2 Allele frequency

Once individuals with too much missing genotype data have been excluded, subsequent analyses can be set to automatically exclude SNPs on the basis of MAF (minor allele frequency):

```
plink --file mydata --maf 0.05
```

means only include SNPs with MAF ¿= 0.05. The default value is 0.01. This quantity is based only on founders (i.e. individuals for whom the paternal and maternal individual codes and both 0).

This option is appropriately counts alleles for X and Y chromosome SNPs.

## 6.3 Missing rate per SNP

Subsequent analyses can be set to automatically exclude SNPs on the basis of missing genotype rate, with the `--geno` option: the default is to include all SNPS (i.e. `--geno 1`). To include only SNPs with a 90% genotyping rate (10% missing) use

```
plink --file mydata --geno 0.1
```

As with the `--maf` option, these counts are calculated after removing individuals with high missing genotype rates.

## 6.4 Hardy-Weinberg Equilibrium

To exclude markers that failure the Hardy-Weinberg test at a specified significance threshold, use the option:

```
plink --file mydata --hwe 0.001
```

By default this filter uses an exact test (see this section). The standard asymptotic (1 df genotypic chi-squared test) can be requested with the `--hwe2` option instead of `--hwe`.

The following output will appear in the console window and in `plink.log`, detailing how many SNPs failed the Hardy-Weinberg test, for the sample as a whole, and (when `PLINK` has detected a disease phenotype) for cases and controls separately:

```
Writing Hardy-Weinberg tests (founders-only) to [ plink.hwe ]
30 markers failed HWE test ( p <= 0.05 ) and have been excluded
        34 markers failed HWE test in cases
```

```
          30 markers failed HWE test in controls
```

This test will only be based on founders (if family-based data are being analysed) unless the `--nonfounders` option is also specified. In case/control samples, this test will be based on controls only, unless the `--hwe-all` option is specified, in which case the phenotype will be ignored. This can be important if parents are coded as missing in an affected offspring trio sample.

Please refer to the `--hardy` option for more details on producing summary statistics of all HWE rates.

## 6.5 Mendel error rate

For family-based data only, to exclude individuals and/or markers on the basis on Mendel error rate, use the option:

```
plink --file mydata --me 0.05 0.1
```

where the two parameters are:

- the first parameter determines that families with more than 5% Mendel errors (considering all SNPs) will be discarded.
- the second parameter indicates that SNPs with more than 10% Mendel error rate will be excluded (i.e. based on the number of trios);

Please refer to the summary statistics page for more details on generating summary statistics for Mendel error rates.

**Note** Currently, `PLINK` calculates the per SNP Mendel error rates at the same time as the per family error rates. In future releases, this may change such that the per family error rate is calculated *after* SNPs failing this test have been removed. Also, using this command currently removes entire nuclear families on the basis of high Mendel error rates: it will often be more appropriate to remove particular individuals (e.g. if a second sibling shows no Mendel errors). For this more fine-grained procedure, use the `--mendel` option to generate a complete enumeration of error rates by family and individual and exclude individuals as desired. Finally, it is possible to zero out specific Mendelian inconsistencies with the option `--set-me-missing`. This should be used in conjunction with a data generation command and the `--me` option. Specifically, the `--me` parameters should be both to 1, in order not to exclude any particular SNP or individual/family, but instead to zero out only specific genotypes with Mendel errors and save the dataset as a new file. (Both parental and offspring genotypes will be set to missing.)

```
plink --bfile mydata --me 1 1 --set-me-missing --make-bed --out newdata
```

# Chapter 7

# Population stratification

PLINK offers a simple but potentially powerful approach to population stratification, that can use whole genome SNP data (the number of individuals is a greater determinant of how long it will take to run). We use complete linkage agglomerative clustering, based on pairwise identity-by-state (IBS) distance, but with some modifications to the clustering process: restrictions based on a significance test for whether two individuals belong to the same population (i.e. do not merge clusters that contain significantly different individuals) , a phenotype criterion (i.e. all pairs must contain at least one case and one control) and cluster size restrictions (i.e. such that, with a cluster size of 2, for example, the subsequent association test would implicitly match every case with its nearest control, as long as the case and control do not show evidence of belonging to different populations). In addition, external matching criteria can be specified, to match on age and sex, for example, as well as genetic information. Any evidence of population substructure (from this or any other analysis) can be incorporated in subsequent association tests via the specification of clusters.

**All these analyses require genome-wide coverage of autosomal SNPs!**

## 7.1 IBS clustering

To perform complete linkage clustering of individuals on the basis of autosomal genome-wide SNP data, the basic command is:

```
plink --file mydata --cluster
```

which generates four output files:

```
plink.cluster0
plink.cluster1
plink.cluster2
plink.cluster3
```

that contain similar information but in different formats. The

The `*.cluster0` file contains some information on the clustering process. This file can be safely ignored by most users.

The `*.cluster1` file contains information on the final solution, listed by cluster: e.g. for 4 individuals with the following Family and Individual IDs

```
A 1
B 1
C 1
D 1
```

we see 3 clusters, one line of output per cluster:

```
0   A_1
```

```
1   B_1 C_1
2   D_1
```

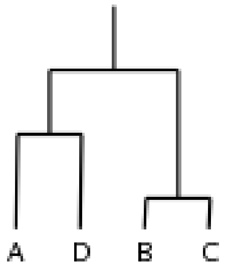
(note how family and individuals IDs are concatenated with the underscore character in the output)

The `*.cluster2` file contains the same information but listed one line per individual: the three columns are family ID, individual ID and assigned cluster:

```
A 1 0
B 1 1
C 1 1
D 1 2
```

The `*.cluster3` file is in the same format as `cluster2` (one line per individual) but contains all solutions (i.e. every step of the clustering from moving from N clusters each of 1 individual (leftmost column after family and individual ID) to 1 cluster (labelled 0) containing all N individuals (the final, rightmost column): also shown is the dendrogram this represents: e.g.

```
A 1 0 0 0 0
B 1 1 1 1 0
C 1 2 1 1 0
D 1 3 2 0 0
```



**NOTE** If any constraints have been placed upon the clustering, then solutions represented in the `*.cluster3` file may not go as far as 1 cluster with all N individuals: in this case, the file `*.cluster2` will contain the final solution (i.e. as far as the clustering could go before running up against constraints, e.g. based on maximum cluster size, etc).

**HINT!** In large samples, cluster analyses can be very slow. Often the most time consuming step is calculating the pairwise IBS metrics: these only need to be calculated once however, even if you want to run the cluster analysis multiple times (e.g. with different constraints). This is achieved with the `--read-genome` option, assuming you have previously run the `--genome` command. It is a good idea to not impose a threshold of the `--genome` output in this case. For example:

```
plink --bfile mydata --genome --out mydata
```

followed by multiple clustering commands (see below for descriptions of the cluster constraint parameters used here)

```
plink --bfile mydata --read-genome mydata.genome --cluster --ppc 0.01
```

and

```
plink --bfile mydata --read-genome mydata.genome --cluster --mc 2 --ibm 0.01
```

etc.

**ADVANCED HINT!** In very large samples, cluster analyses can be very, very slow. When calculating the `plink.genome` file (as described above), if you have access to a cluster of computers for parallel computing, you can use the following approach to greatly reduce the time this step takes. In this case, we will assume you are familiar with and using a Linux operating system, and that the `bsub` prefix is used to send a job to the cluster – obviously, change the script below as appropriate. This uses the `--genome-lists` option to calculate IBS statistics for only a subset of the sample at a time. If the binary fileset is `data.*` then create multiple lists of, for example, 100 individuals per list

```
gawk 'print $1,$2' data.fam | split -d -a 3 -l 100 - tmp.list
```

If this creates, for example, 39 separate files (labelled 0 to 38), then run these in all unqiue pairwise combinations in parallel with something like the following script: (i.e. edit the first line as appropriate)

```
let i=0 a=38
let j=0
while [ $i -le $a ]
do
 while [ $j -le $a ]
 do
    bsub -o /dev/null -e /dev/null ./plink --bfile data \
            --read-freq plink.frq \
            --genome \
            --genome-lists tmp.list`printf "%03i\n" $i` \
                           tmp.list`printf "%03i\n" $j` \
            --out data.sub.$i.$j
 let j=$j+1
 done
 let i=$i+1
 let j=$i
done
```

**NOTE** If you use this approach to calculate the IBD probabilities, then you should first perform `--freq` on the whole dataset, then add the line `--read-freq plink.frq` (obviously replacing the filename with your file) to make sure that everybody has the sample frequencies used in the IBD calculations.

The finally, concatenate these individual files back into one, taking care to get only a single header line (assuming you have no individuals with `FID1` in their ID...)

```
head -n1 data.sub.0.0.genome > header
cat data.sub*genome | fgrep -v FID1 | cat header - > data.genome
rm tmp.list*
rm data.sub.*
```

## 7.2 Permutation test for between group IBS differences

Given that pairwise IBS distances between all individuals have been calculated, we can asked whether or not there are group differences in this metric, with respect to a binary phenotype. The command

```
./plink --bfile mydata --ibs-test
```

or, if an appropriate `plink.genome` file has already been created,

```
./plink --bfile mydata --read-genome plink.genome --ibs-test
```

will permute case/control label, and then recalculate several between-group metrics based on average IBS within that group. This command uses a fixed 10,000 permutations.

All results are written to the LOG file. First, the observed means and standard deviation of each of the 3 groups (case/control, case/case and control/control, in that order) will be displayed: e.g.

```
Between-group IBS (mean, SD) = 0.782377, 0.00203459
In-group (2) IBS (mean, SD) = 0.782101, 0.00232296
In-group (1) IBS (mean, SD) = 0.78273, 0.00170816
```

Then 12 separate tests are presented, which have self-explanatory names. If the label does not explicitly mention a comparison pair-type, it implies that the first pair type is being compared to the other two pair-types.

```
      T1: Case/control less similar                    p = 0.97674
      T2: Case/control more similar                    p = 0.0232698
      T3: Case/case less similar than control/control   p = 0.00285997
      T4: Case/case more similar than control/control   p = 0.99715
      T5: Case/case less similar                       p = 0.00430996
      T6: Case/case more similar                       p = 0.9957
      T7: Control/control less similar                 p = 0.99883
      T8: Control/control more similar                 p = 0.00117999
      T9: Case/case less similar than case/control      p = 0.00726993
     T10: Case/case more similar than case/control      p = 0.99274
     T11: Control/control less similar than case/control p = 1
     T12: Control/control more similar than case/control p = 9.9999e-06
```

For the purpose of stratification effects between cases and conrtols, the test `T1` is probably most appropriate, as it directly asks whether or not, on average, an individual is less similar to another phenotypically-discordant individual than would be expected by chance (i.e. if we randomized phenotype labels). That is, to the extent that cases and controls are from two separate populations, you would expect pairs within a phenotype group to be more similar than pairs across the two groups, i.e. `T1`. Of course, the opposite could also be true (tested by `T2`), which would probably represent certain ascertainment procedures (i.e. taking this to an extreme, imagine a discordant sibling pair design: case/control pairs would on average be more similar than case/case and control/control pairs).

The other tests are provided for completeness and give a more general description of the variability between and within each group. The general pattern shown above would suggest that there is relatively more variability within the case sample than the control sample. Bear in mind when interpreting the empirical p-values that the relative sizes of case and control samples will have an impact on the exact p-value (i.e. these significance tests should not be taken to directly represent the magnitude of differences between groups).

**Note** This test assumes that individuals have a disease phenotype; obviously, one could swap in other labels (e.g. site of collection) via the `--pheno` command, as long as they are dichotomous.

## 7.3 Constraints on clustering

This section describes the extra constraints that can be placed on the clustering procedure, specified via other options in addition to the `--cluster` option. As further described in the association analysis and permutation sections, these options can be used to set up various types of analyses that control for potential stratification in the sample.

**1) Based on pairwise population concordance (PPC) test:**

This is a simple significance test for whether two individuals belong to the same random-mating population. To only merge clusters that do not contain individuals differing at a certain p-value:

```
   --ppc 0.0001
```

**NOTE** This command has been changed from `--pmerge` in older versions of PLINK (pre 0.99n).

This test is based on the observed binomial proportion of IBS 0 loci pairs to IBS 2 het/het pairs: counts of these two types should be in the ratio of 1:2 if the two individuals come from the same population. The significant p-value indicates fewer IBS2 het/het loci than expected (based on normal approximation to binomial). These tests are also given by the `--genome` command.

**WARNING!** Unlike the basic IBS clustering, which places no restrictions on the SNPs that can be used in the analysis, this test assumes that the SNPs used are in linkage equilibrium. By default, this test will only count an 'informative' SNP pair (i.e. one that, for a particular pair of individuals, has two of each allele) as either an IBS 0 or IBS 2 count for this test (the `HOMHOM` and `HETHET` counts from the `--genome` option) if it is more than 500 kb more the previous informative pair of SNPs, for that particular pair of individuals. This gap parameter can be changed with the option

```
--ppc-gap 100
```

which would, in this case, reduce that gap to 100kb. (Note: all SNPs will still be used to calculate the main IBS distance metric, upon which the clustering is based).

**HINT** Also, this test is susceptible to non-random missingness in genotypes, particularly if heterozygotes are more likely to be dropped. It is therefore good practice to set the `--geno` very low for this analysis, i.e. so only SNPs with virtually complete genotyping are included.

**2) Based on phenotype:**

To ensure that every cluster has at least one case and one control:

```
--cc
```

**3) Based on maximum cluster size:**

To set the maximum cluster size to a certain value, e.g. 2:

```
--mc 2
```

Alternatively, to specify a maximum number of cases and a maximum number of controls per cluster, use the option:

```
--mcc 1 3
```

which, in this case, specifies that each cluster can have up to 1 case and 3 controls. Note the different syntax: `-mcc` as opposed to `--mc`. Using this in conjunction with the `--cc` constraint (that ensures at least 1 case and 1 control per cluster) this is an easy way to achieve a certain matching scheme, say 1 case to 3 controls, or 2 cases to 2 controls, etc.

**4) Based on fixed number of clusters:**

To request that the clustering process stops at a certain fixed number of clusters, for example, a 2 cluster solution, use:

```
--K 2
```

**Note** If other clustering constraints are in place, it is possible that clustering may stop *before* reaching the specified number of clusters with the `--K` option; if other constraints are specified, you can think of this as stating the *minimum* number of clusters possible.

**5) Based on pattern of missing genotype data:**

To only cluster individuals with sufficiently similar profiles of missing genotype data (genome-wide) use the option:

```
--ibm 0.02
```

which would only match people if they are discordantly missing (i.e. one person is missing a particular SNP but the other person is not) for 2 percent of the genome or less. Another way to incorporate missingness would be by defining overall call rate per individual as an external quantitative matching criteria (see below); this approach is preferrable however (as it does not match just on average rate, but also on whether it tends to be the same SNPs that are missing).

**6) Based on user-specified external matching criteria:**

To use external matching criteria: for categorical matching criteria, use the option:

```
--match mydata.match
```

where the file `mydata.match` contains the following columns: family and individual ID and the one or more matching variables, one row per person:

```
Family ID
Individual ID
Matching criteria 1
Matching criteria 2
...
Matching criteria N
```

The default behavior is that only individuals with the same matching criteria across all the measures will be paired to make clusters. For example, if the file were:

```
F1 I1   1  1  1
F2 I2   1  2  1
F3 I3   2  2  2
F4 I4   1  2  1
F5 I5   1  1  1
...
```

then only F1/I1 and F5/I5 could be paired; also F2/I2 and F4/I4 could be paired. No other combinations of pairings would be possible. Therefore, no cluster would ever be formed that contained both individuals F1/I1 and F2/I2, for example.

One application of this option would be to ensure that individuals are sex-matched, or matched on some relevant environmental exposure, in addition to the genetic IBS matching.

It is possible to adjust the default behaviour to consider two individuals as potentially 'pairable' is they *differ* on a particular categorical criteria. This is achieved with the optional command:

```
--match-type mydata.bt
```

where `mydata.bt` is the name of a file that contains a series of 0s and 1s (or "-" and "+" characters), whitespace delimited, that indicate whether a criteria should be a "postive match" (i.e. two individuals are potentially pairable only if they have the *same* values for this variable) or a "negative match" (i.e. two individuals are potentially pairable only if they have *different values* for this variable). In the above example, if the file `mydata.bt` were

```
+ - +
```

then the following pairs are potentially pairable:

```
F1/I1 and F2/I2
F1/I1 and F4/I4
F5/I5 and F2/I2
F5/I5 and F4/I4
```

i.e. `F1/I1` can no longer be paired with `F5/I5` because they have the same value for the second matching variable, which is now a negative match criteria.

**Note** In this example, the matching variables only took two values: in practice, one can have any number of categories per matching variable.

**Note** Missing variables can be specified for matching variables – this means that the criteria will be ignored. As all pairs start out as potentially pairable, this means that missing matching criteria data will never be used to make a pair unpairable.

A second form a matching is based on quantitative traits – in this case, a maximum difference threshold is specified for each measure, such that individuals will not be matched if they differ beyond the threshold on the quantitative traits. This is achieved by the following options:

```
--qmatch mydata.match --qt mydata.qt
```

Note that a second `--qt` option is necessary as well as the `--qmatch` option. The `--qt` specifies a file that contains the thresholds, e.g. for 3 external quantitative criteria, this should contain 3 values:

```
5
0.333
120
```

The `--qmatch` should then contain the same number of quantitative matching criteria per person (again, one row per person):

```
F1 I1   27  -0.23   1003
F2 I2   34   2.22   1038
F3 I3   45   1.99    987
F4 I4   19  -9      2374
F5 I5   18  -0.45    996
...
```

In this case, for example, for the first measure only F4/I4 and F5/I5 are pairable, as —19-18— is not more than 5. This measure might represent age, for example. This pair is not matchable on the basis on the third metric, however, as —2374-996— ¿ 120. As such, no pairs could be formed between any of these five individuals, in this particular case. Note that individual is actually missing (default `--missing-phenotype` value is -9) for the second criterion: see below for a description of how missing data are handled in this context.

The `.match` and `.qmatch` files do not need to contain all individuals and do not need to be in the same order as the original PED files. Any individuals in these files who are not in the original files will be ignored.

Missing phenotypes are simply ignored (i.e. two individuals would not be called non-matching if either one or both had missing matching criteria). That is, the default for two individuals is that they are pairable – only non-missing, non-matching external criteria (as well as the p-value test based on genetic data, described above) will make a pair unpairable.

## 7.4 IBS similarity matrix

For the *N* individuals in a sample, to create a *N x N* matrix of genome-wide average IBS pairwise identities:

```
plink --file mydata --cluster --matrix
```

creates the file

```
plink.mibs
```

which contains a square, symmetric matrix of the IBS distances for all pairs of individuals. These values range, in theory, from 0 to 1. In practice, one would never expect to observe values near 0 – even completely unrelated individuals would be expected to share a very large proportion of the genome identical by state by chance alone (i.e. as opposed to identity by descent). A value of 1 would indicate a MZ twin pair, or a sample duplication. More details on pairwise relatedness can be obtained by using the `--genome` command.

The default behavior of `--matrix` to to output similarities (proportions of alleles IBS). To generate a distance matrix (1-IBS) then use the command

```
plink --file mydata --cluster --distance-matrix
```

instead. This will generate a file

```
plink.mdist
```

**HINT** See the FAQ page for instructions on using using R to visualise these results; alternatively, use the `--mds-plot` option described below.

**NOTE** In versions prior to v1.00, there is no `--distance-matrix` command and `--matrix` outputs a file called `plink.mdist` rather than `plink.mibs` – these are still similarities, not distances.

## 7.5 Multidimensional scaling plots

To perform multidimensional scaling analysis on the $N$ $x$ $N$ matrix of genome-wide IBS pairwise distances, use the `--mds-plot` option in conjunction with `--cluster`. This command takes a single parameter, the number of dimensions to be extracted. For example, assuming we have already calculated the `plink.genome` file,

```
plink --file mydata --read-genome plink.genome --cluster --mds-plot 4
```

creates the file

```
plink.mds
```

which contains one row per individual, with the fields

```
FID    Family ID
IID    Individual ID
SOL    Assigned solution code (from --cluster)
C1     Position on first dimension
C2     Position on second dimension
C3     Position on third dimension
C4     Position on fourth dimension
```

Plotting the `C1` values against `C2`, for example, will give a scatter plot in which each point is an individual; the two axes correspond to a reduced representation of the data in two dimensions, which can be useful for identifying any clustering. Standard classical (metric) multidimensional scaling is used.

Instead of using each individual as the unit of analysis, you can make each point a cluster from the final solution (as determined by `--cluster` along with whatever constraints were imposed) and the distances between clusters are the average distances of all individuals in those clusters. Use the `--mds-cluster` flag (as well as `--cluster --mds-plot` *K*) for this.

### 7.5.1 Speeding up MDS plots: 1. Use the LAPACK library

If you compile PLINK to use the LAPACK library `http://www.netlib.org/lapack/` to perform the SVD used in the MDS analysis, this can significantly speed things up. This involves, LAPACK being available on your system, and compiling PLINK from source, with a flag set to use that library. Otherwise, no changes are made to the command: the same `--mds-plot` command is used. A line will be written the LOG file file indicating that the LAPACK library was used

```
Writing MDS solution to [ v2.mds ]
MDS plot of individuals (not clusters)
Using LAPACK SVD library function...
```

**NOTE** This should give similar results, although it is possible that the sign of various components might be flipped: this is expected and of no concern.

See these notes for help on how to compile PLINK to use LAPACK. Please note that I cannot provide support on how to compile LAPACK on your specific system. LAPACK is a set of FORTRAN programs (and requires `gfortran` to compile) – ask your IT people for help if needed.

### 7.5.2 Speeding up MDS plots: 2. pre-cluster individuals

With large samples (over 10,000 individuals say) MDS plots can become very slow. One possible way to speed things up slightly is to first group individuals into groups of fairly similar individuals, and then perform the MDS analysis on the groups rather than the individuals (i.e. based on the mean distances between groups). PLINK will output a file in which each individual in the same group has the identical MDS components therefore. To use this option, add `--mds-cluster` and `--within`, for example

```
plink --bfile mydata
      --read-genome mydata.genome
      --mds-plot 4
      --mds-cluster
      --within clst.cluster2
```

This would be appropriate, for example, if the `clst.cluster2` file resulted from a prior cluster analysis (using `--cluster`) with a setting such as

```
--mc 10
```

to create a fairly large number of small groups (max 10 per group). Obviously, `--mds-cluster` will not give sensible results if there are too few clusters, or if the clusters are too big.

## 7.6 Outlier detecion diagnostics

Sometimes it can be useful to detect a handful of individuals who do not cluster with an otherwise fairly homogeneous sample. It is possible to generate some metrics describing much of an 'outlier' an individual is with respect to the other individuals in that sample, based on the genome-wide IBS information, as decribed above.

For any one individual, we can rank order all other individuals on the basis of how similar (in IBS terms) they are to this particular proband individual. We can then ask, is the proband's closest neighbour significantly more distant to the proband than all other individuals' nearest neighbour is to them. In otherwords, from the distribution of 'nearest neighbour' scores, one for each individual, we can calculate a sample mean and variance and transform this measure into a Z score. If an individual has an extreme low Z score, say less than 4 standard deviation units, this would indicate that this individual is an outlier with respect to the rest of the sample (i.e. this individual's nearest neighbour is a lot less near than the average nearest neighbour). As well as performing this test with the nearest neighbour, we can also perform it with the distribution of second-closest neighbours for each individual; then third closest neighbours, etc. It might sometimes be more informative to look at these 'second-closest' and 'third-closest' measures, to detect, for instance, a pair of individuals who are very similar to each other, but very distant from the rest of the sample – they would score normally on the 'first-closest' neighbour test, but not on the 'second-closest', 'third-closest' tests, etc. It might sometimes be informative to look at the whole distribution of these 'neighbour' metrics, going to 1st nearest to the Nth nearest.

Another metric which can be used to identify potential outliers is, for each individual, to calculate the proportion of binomial IBS tests (described in the constaints section above), for each individual, that showed a significant difference at the `--ppc` threshold.

The basic option is, for example:

```
plink --file data --cluster --neighbour 1 5
```

This command always takes two arguments, specifying, in this case, to consider from the 1st nearest neighbour to the 5th nearest neighbour; this option generates the output file:

```
plink.nearest
```

which contains the fields:

```
FID        Family ID
IID        Individual ID
NN         Nearest neighbour level (see below)
MIN_DST    IBS distance of nth nearest neighbour (see below)
Z          MIN_DST converted to a Z score (see below)
FID2       Family ID of the nth nearest neighbour
IID2       Individual ID of the nth nearest neighbour
```

```
PROP_DIFF    Proportion of significantly different others (see below)
```

Looking at some example output, in this case for two individuals from the Asian HapMap samples, measured on around 50K random SNPs, for nearest neighbours 1 to 5, we see:

| ***FID*** | ***IID*** | ***NN*** | ***MIN_DST*** | ***Z*** | ***FID2*** | ***IID2*** | ***PROP_DIFF*** |
|---|---|---|---|---|---|---|---|
| JPT256 | 1 | 1 | 0.7422 | 0.8897 | JPT265 | 1 | 0.01136 |
| JPT256 | 1 | 2 | 0.742 | 1.223 | JPT236 | 1 | 0.01136 |
| JPT256 | 1 | 3 | 0.7408 | 0.6503 | JPT261 | 1 | 0.01136 |
| JPT256 | 1 | 4 | 0.7405 | 0.7285 | JPT250 | 1 | 0.01136 |
| JPT256 | 1 | 5 | 0.7402 | 0.6204 | JPT269 | 1 | 0.01136 |
| JPT257 | 1 | 1 | 0.7368 | -3.701 | JPT242 | 1 | 0.9318 |
| JPT257 | 1 | 2 | 0.7364 | -3.463 | JPT238 | 1 | 0.9318 |
| JPT257 | 1 | 3 | 0.7359 | -3.832 | JPT244 | 1 | 0.9318 |
| JPT257 | 1 | 4 | 0.7356 | -3.974 | JPT245 | 1 | 0.9318 |
| JPT257 | 1 | 5 | 0.7353 | -4.046 | JPT228 | 1 | 0.9318 |

Here we clearly see that the individual coded as JPT257 seems to be an outlier, with these first five measures being around 4 standard deviations below the group mean. In contrast, individual JPT256 does not appear to be an outlier, as the Z scores are above the mean (greater than 0). Plotting the Z scores for the entire sample makes it clear that JPT257 is indeed an outlier, as does the result for the IBS test – JPT257 is significant different from 93% of the rest of the sample (the threshold for the IBS test is set to be quite stringent here, 0.0005 – this is changed with the `--ppc` option as described above). At this fairly strict level, the subtle differences between Japanese and Han Chinese individuals are not detected – using a threshold at 0.05, for example, one would see that many individuals show greater than the expected 0.05 in the `PROP_DIFF` field, as it is now picking up this group difference.

# Chapter 8

# IBS/IBD estimation

As well as the standard summary statistics described above, PLINK offers some alternative measures such as estimated inbreeding coefficients for each individual and genome-wide identity-by-state and identity-by-descent estimates for all pairs of individuals. The latter can be used to detect sample contaminations, swaps and duplications as well as pedigree errors and unknown familial relationships (e.g. sibling pairs in a case/control population-based sample). PLINK also has functions to detect specific segments shared between distantly-related individuals.

**All these analyses require a large number of SNPs!**

## 8.1 Pairwise IBD estimation

The pairwise clustering based on IBS, as outlined in the previous section is useful for detecting pairs of individuals who look more different from each other than you'd expect in a random, homogeneous sample. In this section, we consider using the same genotype data to provide a complementary analysis: using estimates of pairwise IBD to find pairs of individuals who look *too similar* to eachother, i.e. more than we would expect by chance in a random sample.

***In a homogeneous sample,*** it is possible to calculate genome-wide IBD given IBS information, as long as a large number of SNPs are available (probably 1000 independent SNPs at a bare minimum; ideally 100K or more).

```
plink --file mydata --genome
```

which creates the file

```
plink.genome
```

which has the following fields:

```
FID1      Family ID for first individual
IID1      Individual ID for first individual
FID2      Family ID for second individual
IID2      Individual ID for second individual
RT        Relationship type given PED file
EZ        Expected IBD sharing given PED file
Z0        P(IBD=0)
Z1        P(IBD=1)
Z2        P(IBD=2)
PI_HAT    P(IBD=2)+0.5*P(IBD=1) ( proportion IBD )
PHE       Pairwise phenotypic code (1,0,-1 = AA, AU and UU pairs)
DST       IBS distance (IBS2 + 0.5*IBS1) / ( N SNP pairs )
```

```
     PPC       IBS binomial test
     RATIO     Of HETHET : IBS 0 SNPs (expected value is 2)
```

This file will have as many rows as there are unique pairs of individuals in the sample – for large samples with thousands of individuals, this file can be very large (and take considerable time to generate).

To calculate IBD only for members of the same family (as designated by the PED file), add the command

```
     --rel-check
```

(i.e. this greatly speeds up analysis by not considering all possible pairs of individuals, if the goal is to validate known relationships with genetic data).

To create a more verbose version of this file, add the extra command

```
     --genome-full
```

which will appended the following extra fields to the normal genome file create a file with the following fields

```
     IBS0      Number of IBS 0 nonmissing loci
     IBS1      Number of IBS 1 nonmissing loci
     IBS2      Number of IBS 2 nonmissing loci
     HOMHOM    Number of IBS 0 SNP pairs used in PPC test
     HETHET    Number of IBS 2 het/het SNP pairs in PPC test
```

**HINT** To produce a smaller version of this file use the command `--genome-minimal` instead; however, this is only useful if the purpose is to subsequently merge the data using `--read-genome-minimal` (i.e. when running `--cluster` or `--segment`. A disadvantage is that multiple `plink.genome.min` files cannot be concatenated in the same manner for normal `plink.genome` files; this will be remedied in future releases of PLINK (i.e. to allow parallel computation of the genome file.

**HINT** In 1.05 onwards, the genome files are indexed by the header row, which must be present. When using `--read-genome`, the only fields extracted are the four ID fields and DST and PPC when using the `--cluster` or `--mds-plot` options. You can therefore extract just these columns, if you do not need the other fields,e.g.

```
   gawk '  print $1,$2,$3,$4,$12,$13  ' plink.genome > new.genome
```

As mentioned above, the IBD estimation part of this analysis relies on the sample being reasonably homogeneous – otherwise, the estimates will be biased (i.e. individuals within the same strata will show too much apparent IBD). It is therefore important to run the other population stratification measures provided by `plink` and other packages before estimating pairwise IBD. In addition, see the notes on the IBS test in the previous section where it is introduced as a constrain on clustering.

**HINT** To reduce the file size, use the `--min`*X* option to only output to `plink.genome` pairs where `PI_HAT` is greater than *X*. That is,

```
  plink --file mydata --genome --min 0.05
```

will only display the pairs of individuals showing reasonably high levels of IBD sharing (i.e. typically it will be these pairs that are of interest, rather than the vast majority of pairs that show no excess sharing).

**Hint** Calculating the average pi-hat for each individual and looking for outliers is also useful (in particular, sample contamination will lead to too many heterozygote calls, which leads to fewer IBS 0 calls, which leads to over-estimated IBD with all other people in the sample). Be sure to set `--min 0` and `--max 1` in this case to obtain pairs for all individuals.

**Advanced hint** If you have access to a cluster, use the `--genome-lists` option to facilitate parallelization, as described in the IBS clustering section.

## 8.2 Inbreeding coefficients

Given a large number of SNPs, in a homogeneous sample, it is possible to calculate inbreeding coefficients (i.e. based on the observed versus expected number of homozygous genotypes).

```
plink --file mydata --het
```

which will create the output file:

```
plink.het
```

which contains the fields, one row per person in the file:

|  |  |
|---|---|
| FID | Family ID |
| IID | Individual ID |
| O(HOM) | Observed number of homozygotes |
| E(HOM) | Expected number of homozygotes |
| N(NM) | Number of non-missing genotypes |
| F | F inbreeding coefficient estimate |

This analysis will automatically skip haploid markers (male X and Y chromosome markers).

**Note** With whole genome data, it is probably best to apply this analysis to a subset that are pruned to be in approximate linkage equilibrium, say on the order of 50,000 autosomal SNPs. Use the `--indep-pairwise` and `--indep` commands to achieve this, described here.

**Note** The estimate of F can sometimes be negative. Often this will just reflect random sampling error, but a result that is strongly negative (i.e. an individual has *fewer* homozygotes than one would expect by chance at the genome-wide level) can reflect other factors, e.g. sample contamination events perhaps.

## 8.3 Runs of homozygosity

A simple screen for runs of homozygous genotypes within any one individual is provided by the commands `--homozyg-snp` and `--homozyg-kb` which define the run in terms of the required number of homozygous SNPs spanning a certain kb distance, e.g.

The algorithm is as follows: Take a window of *X* SNPs and slide this across the genome. At each window position determine whether this window looks 'homozygous' enough (yes/no) (i.e. allowing for some number of hets or missing calls). Then, for each SNP, calculate the proportion of 'homozygous' windows that overlap that position. Call segments based on this metric, e.g. based on a threshold for the average.

The exact window size and thresholds, relative to the SNP density and expected size of homozygous segments, etc, is obviously important: sensible default values are supplied for the context of dense SNP maps, scanning for large segments. In general, this approach will ensure that otherwise long runs of homozygosity are not broken by the occassional heterozygote. (For more accurate detection of smaller segments, one might consider approaches that also take population parameters such as allele frequency and recombination rate into account, in a HMM approach for example: but for now, PLINK only supports this basic detection of long, homozygous segments).

To run this option with default values, use the command

```
plink --bfile mydata --homozyg
```

which generates a file

```
plink.hom
```

The `plink.hom` file has the following format, one row per identified homozygous region:

|  |  |
|---|---|
| FID | Family ID |
| IID | Individual ID |
| CHR | Chromosome |

|  |  |
|---|---|
| SNP1 | SNP at start of region |
| SNP2 | SNP at end of region |
| POS1 | Physical position (bp) of SNP1 |
| POS2 | Physical position (bp) of SNP2 |
| KB | Length of region (kb) |
| NSNP | Number of SNPs in run |
| DENSITY | Average SNP density (1 SNP per kb) |
| PHOM | Proportion of sites homozygous |
| PHET | Proportion of sites heterozygous |

The options to change the behavior of this function (along with the default values as parameters) are given below.

To change the definition of the sliding 'window', use the options

```
--homozyg-window-kb 5000
--homozyg-window-snp 50
```

To change the number of heterozygotes allowed in a window

```
--homozyg-window-het 1
```

To change the number of missing calls allowed in window, e.g.

```
--homozyg-window-missing 5
```

To change the proportion of overlapping windows that must be called homozygous to define any given SNP as 'in a homozygous segment', use

```
--homozyg-window-threshold 0.05
```

(i.e. this number is relatively low, so that SNPs at the edge of a true segment will be called, as long as the windows are sufficiently large, such that the probability of a window being homozygous by chance is sufficiently small).

The above options define the 'window' that slides across the genome; the options below relate to the final segments that are called as homozygous or not:

```
--homozyg-snp 100
--homozyg-kb 1000
```

You can also specify the required minimum density (in kb, i.e. 1 SNP per 50 kb)

```
--homozyg-density 50
```

Finally, if two SNPs within a segments are too far apart (measured in kb), that segment can be split in two:

```
--homozyg-gap 1000
```

**HINT** As is, this analysis should be performed on sets of SNPs that have been pruned for strong local LD (if the goal is to find long segments that are more likely to represent homozygosity by descent (i.e. autozygosity) rather than simply by chance).

To obtain pools of overlapping and potentially matching segments, we can use `--homozyg-group` in addition to the above, which generates the file

```
plink.hom.overlap
```

which contains the fields

|  |  |
|---|---|
| FID | Family ID |
| IID | Individual ID |
| PHE | Phenotype of individual |
| CHR | Chromosome |
| SNP1 | SNP at start of segment |
| SNP2 | SNP at end of segment |
| BP1 | Physical position of start of segment |

```
BP2     Physical position of end of segment
KB      Physical size of segment
NS      Number of segments in the pool that match this one
GRP     Allelic-match grouping of each segment
```

For example, the command

```
plink --file test --homozyg --homozyg-group
```

might result in the file `plink.hom.overlap` containing:

```
 FID  IID   PHE  CHR SNP1 SNP2        BP1       BP2    KB   NS     GRP
   1    1     2    1 snp1 snp7   1000000   7000000  6000   1      1
   6    1     1    1 snp1 snp5   1000000   5000000  4000   1      1*
   2    1     1    1 snp2 snp7   2000000   7000000  5000   0      2*
 CON    3   1:2    1 snp2 snp5   2000000   5000000  3000
```

This implies one pool (i.e. each pool is separated by a CON (consensus row) and a space. `CON` is the consensus region; the ratio is the case:control segment ratio; under `IID` we have the number of individuals.

When there is more than one pool, they are ordered by the number of segments in the pool, then physical position. To output only pools of a particular size, use the `--pool-size` *N* option (e.g. `--pool-size 10` to only display pools with at least 10 segments).

A pool contains overlapping segments, which may or may not also allelically match. For allelic matching, segments are compared pairwise, and a match is declared if at least 0.95 of jointly non-missing, jointly homozygous sites are identical. This threshold can be changed with the option

```
--homozyg-match 0.99
```

The number of other segments in the pool that allelically match each segment is shown in the `NS` field. The `GRP` field shows how `PLINK` attempts to group allelically-matching segments within the pool of overlapping segments. It works as follows:

- For each segment, find the number of other segments that match (`NS`).
- Find segment with largest NS, denote as group 1, and put a * to indicate this is the index for this group.
- Denote all other segments that match this index as being in GRP 1 (i.e. but without the *)
- Continue to next ungrouped segment (2*, etc)

By default, we compare all segments pairwise when asking if they match; if the `--consensus-match` flag is given, then for a pool of overlapping segments, matches are defined only on the basis of the consensus region (i.e. the overlapping region shared by all segments). This is probably not very sensible in many cases, as the consensus region can often be small (i.e. a single SNP).

The `NS` field can suggest any intransitivity in matching: e.g. if B matches A and C but A does not match C, then if B has already been grouped with A, C would not be added to this group as being an allelic match. In this case C would have NS ¿ 0 but belong to a `GRP` of its own.

Internally, all pools are formed but then pruned if, for instance, a smaller pool is included in a larger pool completely. That means that in certain circumstances you will see a segment in more than one pool. For example, imagine a grid with three people A, B and C along the columns, each row representing physical position, and the presence of a letter representing a homozygous run:

```
. . .
A . .
A B .
A B C
A B C
A . C
```

```
A . .
. . .
```

In this case, A,B and A,C and B,C pools will not be displayed, as they appear in the super-pool A,B,C. However, if we instead had:

```
. . .
A . .
A B .
A B .
A . .
A . C
A . C
A . .
. . .
```

Then you will see A,B and A,C (i.e. with A shown twice) as we have two distinct consensus regions here.

Finally, if the `--homozyg-verbose` option is added, the `plink.hom.overlap` file will then display the actual segments underneath each pool. Here each individual is listed across the page, so the 3 columns refers to the 3 segments in the pool. For example:

```
plink --file test --homozyg-snp 2 --homozyg-group --homozyg-verbose
```

now generates `plink.hom.overlap` as follows (with annotation added in *italics*):

```
  FID  IID      PHE  CHR SNP1 SNP2    BP1    BP2       KB    NS    GRP
    1    1        2    1 snp1 snp7      1      7    0.006   1     1
    6    1        1    1 snp1 snp5      1      5    0.004   1     1*
    2    1        1    1 snp2 snp7      2      7    0.005   0     2*
  CON    3      1:2    1 snp2 snp5      2      5    0.003
  SNP    1    6    2          <-- Family ID
         1    1    1          <-- Individual ID
         1    1    2          <-- GRP code
snp1 [A/A] [A/A]  C/A         <-- now SNPs are listed down the page
snp2 [A/A] [A/A] [C/C]        <-- start of consensus region
snp3 [A/A] [A/A] [C/C]
snp4 [A/A] [A/A] [C/C]
snp5 [A/A] [A/A] [C/C]        <-- end of consensus region
snp6 [A/A]  A/C  [C/C]
snp7 [A/A]  A/C  [C/C]
```

A bracket indicates that that genotype is part of the homozygous segment: the consensus region is the intersection. The entire union of SNPs is displayed and the consensus region is indicated by spaces before and after. i.e. the consensus region is that where all genotypes are in [brackets].

Obviously, this file can get quite large (+wide) with real data and it is not very machine-readable.

## 8.4 Segmental sharing: detection of extended haplotypes shared IBD

**WARNING** This analysis is still in the *beta* development stage and is considerably more involved than many others provided by this package: currently, you should only perform these analyses if you consider yourself something of *an analytic expert* and are confident you will be able to interpret the output! Over time, we expect that the documentation and features supporting this analysis will improve.

There are five important steps to this analysis:

- Obtain a homogeneous sample
- Remove very closely related individuals
- Prune SNP set
- Detect segments
- Associate with disease

### 8.4.1 Check for a homogenous sample

This analysis requires that all individuals belong to a single, homogeneous population. To ensure this assumption is reasonable: as described here, first run

```
plink --bfile mydata1 --genome
```

to generate a `plink.genome` file. This will be used subsequently in a number of steps.

Then, using the available tools, such as listed here and described more fully in the section on stratification, obtain a relatively homogeneous dataset. Some relevant options are listed here:

```
--cluster    (cluster individuals)
--matrix     (generate .mdist file, used to generate MDS plots)
--ppc        (threshold for PPC test, not to cluster individuals)
--mds-plot   (generate a multidimensional scaling plot)
--ibs-test   (as case/control less similar on average?)
--neighbour  (option to find individual outliers)
```

Also, remove individuals who appear to have higher levels of inbreeding than expected (see above). If you have a set of individuals you want to exclude from analysis based on these steps, for example, listed in the file `outliers.txt` (FID, IID) then use:

```
./plink --bfile mydata1 --remove outliers.txt --make-bed --out mydata2
```

### 8.4.2 Remove very closely related individuals

The focus of this analysis is to look for extended haplotypes shared between distantly related individuals: having very closely related individuals (siblings, first cousins, etc) will likely swamp the results of the analysis. Scan the `plink.genome` file for any individuals with high `PIHAT` values (e.g. greater than 0.05). Optionally, remove one member of the pair if you find close relatives. (Alternatively, to keep them in but just exclude this *pair* from the segmental analysis, see below).

### 8.4.3 Prune the set of SNPs

The segmental sharing analysis requires approximately independent SNPs (i.e. linkage equilibrium). Two options to prune are documented here.

A reasonable strategy might be as follows:

```
plink --bfile mydata2 --mind 1 --geno 0.01 --maf 0.05 --make-bed --out mydata3
```

followed by

```
plink --bfile mydata3 --indep-pairwise 100 25 0.2
```

followed by

```
plink --bfile mydata3 --extract plink.prune.in --make-bed --out mydata4
```

### 8.4.4 Detecting shared segments (extended, shared haplotypes)

With a newly pruned fileset, ideally containing only independent, high quality SNPs in individuals who are not very closely related but are from the same population, run the command

```
plink --bfile mydata4 --read-genome plink.genome --segment
```

PLINK expects the 3rd column the MAP/BIM file to contain genetic distances in Morgan units. A reasonable approximation is to scale from physical position (i.e. column 4) at 1cM=1Mb. If the genetic distances are in cM instead of Morgans, add the `--cm` flag.

To set threshold on who to include/exclude based on genome wide IBD use

```
--min 0.01
--max 0.10
```

For example, this would exclude pairs who share ¿10% of their genomes. Alternatively, to include all pairs, irrespective of whether we estimate any genome-wide sharing or not, add the option

```
--all-pairs
```

instead. This will use all pairs, allowing for a small prior probability of sharing for pairs that otherwise are at the boundary of IBD sharing (i.e. sharing 0% IBD). Naturally, for a large sample, it may become prohibitive to consider all possible pairs.

The `--segment` option generates a file

```
plink.segment
```

which has the fields:

| | |
|---|---|
| FID1 | Family ID of first individual |
| IID1 | Individual ID of first individual |
| FID2 | Family ID of second individual |
| IID2 | Individual ID of second individual |
| PHE | Phenotype concordance: -1,0,1 |
| CHR | Chromosome code |
| BP1 | Start physical position of segment (bp) |
| BP2 | End physical position of segment (bp) |
| SNP1 | Start SNP of segment |
| SNP2 | End SNP of segment |
| NSNP | Number of SNPs in this segment |
| KB | Physical length of segment (kb) |

Here one row represents one segment. The PHE field is coded -1,0,1 for control/control, case/control, or case/case pairs respectively.

The option

```
--segment-length 2000
```

means to only select segments that are at least 2000 kb in length, for example. The option

```
--segment-snp 100
```

means only to select segments that contain at least 100 SNPs, for example.

For ease of interpretation, and to increase the probably that the segments are truly shared IBD and thus tags shared rare variation between two individuals, it makes sense to restrict ones focus to very extended segments (e.g. over 1Mb in size, for example).

Another option is the `--segment-group` option, which generates output similar to `--homozyg-group`, described above; similarly, `--segment-verbose` prints out the actual genotypes for the individuals that overlap. However, these can be large files that are not necessarily easy to interpret.

### 8.4.5 Association with disease

Along with the `--segment` option, as above, if you also add:

```
--mperm N
```

then, for case/control data, this performs a test of whether segments stack up more in case/case pairs versus non-case/case pairs at any position, performing *N* permutations. Equivalently, you can use an already-created segment file:

```
./plink --bfile mydata4 --read-segment plink.segment --mperm 10000
```

This will generate two files:

```
plink.segment.summary
```

which contains one row corresponding to one SNP; there are five fields:

```
CHR    Chromosome code
SNP    SNP identifier
CONU   Number of control/control segments over this SNP
DISC   Case/control segments spanning this position
CONA   Case/case segment count
```

The file

```
plink.segment.summary.mperm
```

contains empirical significance values for each position, asking whether there is a higher rate of case/case sharing than expected. It is important to note that the test statistic is still under developemtn: in this current release, it should merely be interpreted as a rough guide to the data. Naturally, the thresholds for declaring significance will be much lower than for genome-wide association analysis; precise guidelines will be put in place presently.

# Chapter 9

# Association analysis

The basic association test is for a disease trait and is based on comparing allele frequencies between cases and controls (asymptotic and empirical p-values are available). Also implemented are the Cochran-Armitage trend test, Fisher's exact test, different genetic models (dominant, recessive and general), tests for stratified samples (e.g. Cochran-Mantel-Haenszel, Breslow-Day tests), a test for a quantitative trait; a test for differences in missing genotype rate between cases and controls; multilocus tests, using either Hotelling's T(2) statistic or a sum-statistic approach (evaluated by permutation) as well as haplotype tests. The basic tests can be performed with permutation, described in the following section to provide empirical p-values, and allow for different designs (e.g. by use of structured, within-cluster permutation). Family-based tests are described in the next section

**HINT** The basic association commands (`--assoc`, `--model`, `--fisher`, `--linear` and `--logistic`) will test only a single phenotype. If your alternate phenotype file contains more than one phenotype, then adding the `--all-pheno` flag will make PLINK cycle over each phenotype, e.g. instead of a single `plink.assoc` output file, if there are 100 phenotypes, PLINK will now show

```
plink.P1.assoc
plink.P2.assoc
...
plink.P100.assoc
```

Naturally, it will take 100 times longer... If you are testing a very large number of phenotypes, it might be worth specifying `--pfilter` also, to reduce the amount of amount (e.g. only outputing tests significant at p=1e-4 if `--pfilter 1e-4` is specified).

## 9.1 Basic case/control association test

To perform a standard case/control association analysis, use the option:

```
plink --file mydata --assoc
```

which generates a file

```
plink.assoc
```

which contains the fields:

```
CHR    Chromosome
SNP    SNP ID
BP     Physical position (base-pair)
A1     Minor allele name (based on whole sample)
F_A    Frequency of this allele in cases
F_U    Frequency of this allele in controls
```

|  |  |
|---|---|
| A2 | Major allele name |
| CHISQ | Basic allelic test chi-square (1df) |
| P | Asymptotic p-value for this test |
| OR | Estimated odds ratio (for A1) |

**Hint** In addition, if the optional command `--ci` *X* (where *X* is the desired coverage for a confidence interval, e.g. 0.95 or 0.99) is included, then two extra fields are appended to this output:

|  |  |
|---|---|
| L95 | Lower bound of 95% confidence interval for odds ratio |
| U95 | Upper bound of 95% confidence interval for odds ratio |

(where 95 would change if a different value was used with the `--ci` option, naturally).

See the next section on permutation to learn how to generate empirical p-values and use other aspects of permutation-based testing.

See the section on multimarker tests to learn how to perform haplotype-based tests of association.

This analysis should appropriately handle X/Y chromosome SNPs automatically.

## 9.2 Fisher's Exact test (allelic association)

To perform a standard case/control association analysis using Fisher's exact test to generate significance, use the option:

```
plink --file mydata --fisher
```

which generates a file

```
plink.fisher
```

which contains the fields:

|  |  |
|---|---|
| CHR | Chromosome |
| SNP | SNP ID |
| BP | Physical position (base-pair) |
| A1 | Minor allele name (based on whole sample) |
| F_A | Frequency of this allele in cases |
| F_U | Frequency of this allele in controls |
| A2 | Major allele name |
| P | Exact p-value for this test |
| OR | Estimated odds ratio (for A1) |

As described below, if `--fisher` is specified with `--model` as well, `PLINK` will perform genotypic tests using Fisher's exact test.

**Note** You can also use permutation to generate exact, empirical significance values that would also be valid in small samples, etc.

## 9.3 Alternate / full model association tests

It is possible to perform tests of association between a disease and a variant other than the basic allelic test (which compares frequencies of alleles in cases versus controls), by using the `--model` option. The tests offered here are (in addition to the basic allelic test):

- Cochran-Armitage trend test
- Genotypic (2 df) test
- Dominant gene action (1df) test

- Recessive gene action (1df) test

One advantage of the Cochran-Armitage test is that it does not assume Hardy-Weinberg equilibrium, as the individual, not the allele, is the unit of analysis (although the permutation-based empirical p-values from the basic allelic test also have this property). It is important to remember that SNPs showing severe deviations from Hardy-Weinberg are often likely to be bad SNPs, or reflect stratification in the sample, however, and so are probably best excluded in many cases.

The genotypic test provides a general test of association in the 2-by-3 table of disease-by-genotype. The dominant and recessive models are tests for the minor allele (which is the minor allele can be found in the output of either the `--assoc` or the `--freq` commands. That is, if `D` is the minor allele (and `d` is the major allele):

```
Allelic:          D        versus      d
Dominant:     (DD, Dd)     versus      dd
Recessive:       DD        versus   (Dd, dd)
Genotypic:       DD        versus      Dd         versus    dd
```

As mentioned above, these tests are generated with option:

```
plink --file mydata --model
```

which generates a file

```
plink.model
```

which contains the following fields:

| | |
|---|---|
| CHR | Chromosome number |
| SNP | SNP identifier |
| TEST | Type of test |
| AFF | Genotypes/alleles in cases |
| UNAFF | Genotypes/alleles in controls |
| CHISQ | Chi-squated statistic |
| DF | Degrees of freedom for test |
| P | Asymptotic p-value |

Each SNP will feature on five rows of the output, correspondnig to the five tests applied. The column `TEST` refers to either `ALLELIC`, `TREND`, `GENO`, `DOM` or `REC`, refering to the different types of test mentioned above. The genotypic or allelic counts are given for cases and controls separately. For recessive and dominant tests, the counts represent the genotypes, with two of the classes pooled.

These tests only consider diploid genotypes: that is, for the X chromosome males will be excluded even from the ALLELIC test. This way the same data are used for the five tests presented here. Note that, in contrast, the basic association commands (`--assoc` and `--linear`, etc) include single male X chromosomes, and so the results may differ.

The genotypic and dominant/recessive tests will only be conducted if there is a minimum number of observations per cell in the 2-by-3 table: by default, if at least one of the cells has a frequency less than 5, then we skip the alternate tests (`NA` is written in the results file). The Cochran-Armitage and allelic tests are performed in all cases. This threshold can be altered with the `--cell` option:

```
plink --file mydata --model --cell 20
```

If permutation (with the `--mperm` or `--perm` options) is specified, the `-model` option will by default perform a permutation test based on the most significant result of `ALLELIC`, `DOM` and `REC` models. That is, for each SNP, the best original result will be compared against the best of these three tests for that SNP for every replicate. In max(T) permutation mode, this will also be compared against the best result from all SNPs for the `EMP2` field. This procedure controls for the fact that we have selected the best out of three correlated tests for each SNP. The output will be generated in the file

```
plink.model.best.perm
```

or

```
plink.model.best.mperm
```

depending on whether adaptive or max(T) permutation was used.

The behavior of the `--model` command can be changed by adding the `--model-gen`, `--model-trend`, `--model-dom` or `--model-rec` flags to make the permutation use the genotypic, the Cochram-Armitage trend test, the dominant test or the recessive test as the basis for permutation instead. In this case, one of the the following files will be generated:

```
plink.model.gen.perm        plink.model.gen.mperm
plink.model.trend.perm      plink.model.trend.mperm
plink.model.dom.perm        plink.model.dom.mperm
plink.model.rec.perm        plink.model.rec.mperm
```

It is also possible to add the `--fisher` flag to obtain exact p-values:

```
./plink --bfile mydata --model --fisher
```

in which case the `CHISQ` field does not appear. Note that the genotypic, allelic, dominant and recessive models use the Fisher's exact; the trend-test does not and will give the same p-value as without the `--fisher` flag. Also, by default, when `--fisher` is added, the `--cell` field is set to 0, i.e. to include all SNPs.

## 9.4 Stratified analyses

When a cluster variable has been specified, by pointing to a file that contains this information, with the `--within` command, it is possible to perform a number of tests of case/control association that take this clustering into account, or explicitly test for homogeneity of effect between clusters.

**Note** In many cases, permutation procedures can also be used to account for clusters in the data. See the next section for more details. The tests presented below are only applicable for case/control data, so permutation might be useful for quantitative trait outcomes, etc.

There are two basic classes of test:

- Testing for overall disease/gene association, controlling for clusters
- Testing for heterogeneity of the disease/gene assocation between different clusters

The type of cluster structure will vary in terms of how many clusters there are in the sample, and how many people belong to each cluster. At one extreme, we might have two only 2 clusters in the sample, each with a large number of cases and controls. At the other extreme, we might have a very large number of clusters, such that each cluster only has 2 individuals. These factors will influence the choice of stratified analysis.

The tests offered are:

- Cochran-Mantel-Haenszel test for 2x2xK stratified tables
- Cochran-Mantel-Haenszel test for IxJxK stratified tables
- Breslow-Day test of homogeneity of odds ratio
- Partitioning the total association chi-square to perform between and within cluster association, and a test of homogeneity of effect

The Cochran-Mantel-Haenszel (CMH) tests are valid with both a large number of small clusters and a small number of large clusters. These tests provide a test based on an ”average” odds ratio that controls for the potential confounding due to the cluster variable.

The Breslow-Day test asks whether different clusters have different disease/gene odds ratios: this test assumes a moderate sample size within each cluster. The partitioning total association test, which is conceptually similar to the Breslow-Day test, also makes the same assumption.

As mentioned above, the CMH test comes in two flavours: 2x2xK and IxJxK. Currently, the 2x2xK test represents a `disease x SNP | cluster` test. The generalized form, the IxJxK, represents a test of `cluster x SNP | disease` , i.e. does the SNP vary between clusters, controlling for any possible true SNP/disease association. This latter test might be useful in interpreting significant associations in stratified samples. Typically, the first form of the test will be of more interest, however. These two tests are run by using the options:

```
plink --file mydata --mh --within mycluster.dat
```

for the basic CMH test, or

```
plink --file mydata --mh2 --within mycluster.dat
```

for the IxJxK test.
The `--mh` option generates the file

```
plink.cmh
```

which contains the fields

|  |  |
|---|---|
| CHR | Chromosome number |
| SNP | SNP identifier |
| A1 | Minor allele code |
| A2 | Major allele code |
| BP | Physical position (base-pair) |
| CHISQ | Cochran-Mantel-Haenszel statistic (1df) |
| P | Asymptotic p-value for CMH test |
| OR | CMH odds ratio |
| L95 | Lower bound on confidence interval for CMH odds ratio |
| U95 | Upper bound on confidence interval for CMH odds ratio |

The range of the confidence interval with the `--mh` option can be changed with the `--ci` option:

```
plink --file mydata --mh --within mycluster.dat --ci 0.99
```

The `--mh2` option generates the file

```
plink.cmh2
```

which contains the fields:

|  |  |
|---|---|
| CHR | Chromosome |
| SNP | SNP identifier |
| CHISQ_CMH2 | Cochran-Mantel-Haenszel test for IxJxK tables |
| P_CMH2 | Asymptotic p-value for this test |

It is not possible to obtain confidence intervals or odds ratios for `--mh2` tests.

**Hint** A trick to analyse phenotypes with more two categories (but only with nominal, not ordinal outcomes) is to use the `--mh2` option with the phenotype in the cluster file and the phenotype in the PED file set all to a single value.

## 9.5 Testing for heterogeneous association

As mentioned in the previous section, two methods are provided to test for between-cluster differences in association when using a case/control design. The Breslow-Day test is specified with the option:

```
plink --file mydata --bd --within myclst.txt
```

which runs and generates the same files as the `--mh` option, described above, but with two extra fields appended:

| | |
|---|---|
| `CHISQ_BD` | `Breslow-Day test` |
| `P_BD` | `Asymptotic p-value` |

where a significant value indicates between-cluster heterogeneoty in the odds ratios for the disease/SNP association.

A similar test of the homogeneity of odds ratio tests based on partitioning the chi-square statistic is given by:

```
plink --file mydata --homog --within myclst.txt
```

which generates the file

```
plink.homog
```

which contains the fields

| | |
|---|---|
| `CHR` | `Chromosome number` |
| `SNP` | `SNP identifier` |
| `A1` | `Minor allele code` |
| `A2` | `Major allele code` |
| `F_A` | `Case allele frequency` |
| `F_U` | `Control allele frequency` |
| `N_A` | `Case allele count` |
| `N_U` | `Control allele count` |
| `TEST` | `Type of test` |
| `CHISQ` | `Chi-squared association statistic` |
| `DF` | `Degrees of freedom` |
| `P` | `Asymptotic p-value` |
| `OR` | `Odds ratio` |

The `TEST` type is either

| | |
|---|---|
| `TOTAL` | `Total SNP & strata association` |
| `ASSOC` | `SNP association controlling for strata` |
| `HOMOG` | `Between-strata heterogeneity test` |
| `X_1` | `Association in first stratum` |
| `X_2` | `Association in second stratum` |
| `...` | |

## 9.6 Hotelling's T(2) multilocus association test

**IMPORTANT** This command has been temporarily disabled

For disease-traits, `PLINK` provides support for a multilocus, genotype-based test using Hotelling's T2 (T-squared) statistic. The `--set` option should be used to specify which SNPs are to be grouped, as follows:

```
plink --file data --set mydata.set --T2
```

where `mydata.set` defines which SNPs are in which set (see this section for more information on defining sets).

This command will generate a file

```
plink.T2
```

which contains the fields

| | |
|---|---|
| `SET` | `Set name` |
| `SIZE` | `Number of SNPs in this set` |

| | |
|---|---|
| F | F-statistic from Hotelling's test |
| DF1 | Degrees of freedom 1 |
| DF2 | Degrees of freedom 2 |
| P_HOTEL | Asymptotic p-value |

**HINT** Use the `--genedrop` permutation to perform a family-based application of the Hotelling's T2 test. This command can be used with all permutation methods (label-swapping or gene-dropping, adaptive or max(T)). In fact, the permutation test is based on 1-p in order to make the between set comparisons for the max(T) statistic more meaningful (as different sized sets would have F-statistics with different degrees of freedom otherwise). Using permutation will generate one of the following files:

```
plink.T2.perm
```

which contain the fields

| | |
|---|---|
| SET | Set name |
| SIZE | Number of SNPs in this set |
| EMP1 | Empirical p-value |
| NR | Number of permutation replicates |

or, if `--mperm` was used,

```
plink.T2.mperm
```

which contain the fields

| | |
|---|---|
| SET | Set name |
| SIZE | Number of SNPs in this set |
| EMP1 | Empirical p-value |
| EMP2 | max(T) empirical p-value |

Note that this test uses a simple approach to missing data: rather than case-wise deletion (removing an individual if they have at least one missing observation) we impute the mean allelic value. Although this retains power under most scenarios, it can also cause some bias when there are lots of missing data points. Using permutation is a good way around this issue.

## 9.7 Quantitative trait association

Quantitative traits can be tested for association also, using either asymptotic (likelihood ratio test and Wald test) or empirical significance values. If the phenotype (column 6 of the PED file or the phenotype as specified with the `--pheno` option) is quantitative (i.e. contains values other than 1, 2, 0 or missing) then `PLINK` will automatically treat the analysis as a quantitative trait analysis. That is, the same command as for disease-trait association:

```
plink --file mydata --assoc
```

will generate the file

```
plink.qassoc
```

with fields as follows:

| | |
|---|---|
| CHR | Chromosome number |
| SNP | SNP identifier |
| BP | Physical position (base-pair) |
| NMISS | Number of non-missing genotypes |
| BETA | Regression coefficient |
| SE | Standard error |
| R2 | Regression r-squared |
| T | Wald test (based on t-distribtion) |
| P | Wald test asymptotic p-value |

If permutations were also requested, then an extra file, either

```
plink.assoc.perm
```

or

```
plink.assoc.mperm
```

will be generated, depending on whether adaptive or max(T) permutation was used (see the next section for more details). The empirical p-values are based on the Wald statistic.

## 9.8 Genotype means for quantitative traits

Adding the flag `--qt-means` along with the `--assoc` command, when run with a quantitative trait, will produce an additional file with a list of means and standard deviations stratified by genotype, called

```
plink.qassoc.means
```

and format

```
CHR     Chromosome code
SNP     SNP identifier
VALUE   Description of next three fields
G11     Value for first genotype
G12     Value for second genotype
G22     Value for third genotype
```

where `VALUE` is one of `GENO`, `COUNTS`, `FREQ`, `MEAN` or `SD` (standard deviation). For example:

```
 CHR         SNP  VALUE      G11      G12      G22
   5  hCV26311749   GENO      2/2      2/1      1/1
   5  hCV26311749 COUNTS        1       60      597
   5  hCV26311749   FREQ  0.00152  0.09119   0.9073
   5  hCV26311749   MEAN   0.9367   0.4955   0.5074
   5  hCV26311749     SD        0    0.273   0.2902
   5    hCV918000   GENO      2/2      2/1      1/1
   5    hCV918000 COUNTS       47      237      359
   5    hCV918000   FREQ  0.07309   0.3686   0.5583
   5    hCV918000   MEAN    0.505   0.5091   0.5074
   5    hCV918000     SD   0.2867   0.3064   0.2797
```

i.e. each SNP takes up 5 rows.

## 9.9 Quantitative trait interaction (GxE)

`PLINK` provides the ability to test for a difference in association with a quantitative trait between two environments (or, more generally, two groups). This test is simply based on comparing the difference between two regression coefficients. To perform this test:

```
plink --file mydata --gxe --covar mycov.dat
```

where `mycovar.txt` is a file containing the following fields:

```
Family ID
Individual ID
Covariate value
```

See the notes on covariate files for more details.
This option will generate the file

```
plink.qassoc.gxe
```

which contains the fields:

|  |  |
|---|---|
| CHR | Chromosome number |
| SNP | SNP identifier |
| NMISS1 | Number of non-missing genotypes in first group (1) |
| BETA1 | Regression coefficient in first group |
| SE1 | Standard error of coefficient in first group |
| NMISS2 | As above, second group |
| BETA2 | As above, second group |
| SE2 | As above, second group |
| Z_GXE | Z score, test for interaction |
| P_GXE | Asymptotic p-value for this test |

**IMPORTANT!** The covariate must be coded as an affection status variable, i.e. 1 or 2 representing the first or second group. Values of 0 or -9 can be used to indicate missing covariate values, in which case that individual will be excluded from analysis.

## 9.10 Linear and logistic models

These two features allow for multiple covariates when testing for both quantitative trait and disease trait SNP association, and for interactions with those covariates. The covariates can either be continuous or binary (i.e. for categorical covariates, you must first make a set of binary dummy variables).

**WARNING!** These commands are in some ways more flexible than the standard `--assoc` command, but this comes with a price: namely, these run more slowly...

In this section we consider:

- Basic uasge
- Covariate and interactions
- Flexibly specifying the precise model
- Flexibly specifying joint tests

### 9.10.1 Basic usage

For quantitative traits, use

```
plink --bfile mydata --linear
```

For disease traits, specify logistic regression with

```
plink --bfile mydaya --logistic
```

instead. All other commands in this section apply equally to both these models.

These commands will either generate the output file

```
plink.assoc.linear
```

or

```
plink.assoc.logistic
```

depending on the phenotype/command used. The basic format is:

|  |  |
|---|---|
| CHR | Chromosome |
| SNP | SNP identifier |
| BP | Physical position (base-pair) |
| A1 | Reference allele |

```
TEST      Code for the test (see below)
NMISS     Number of non-missing individuals included in analysis
BETA/OR   Regression coefficient (--linear) or odds ratio (--logistic)
STAT      Coefficient t-statistic
P         Asymptotic p-value for t-statistic
```

For the additive effects of SNPs, the direction of the regression coefficient represents the effect of each extra **minor allele** (i.e. a positive regression coefficient means that the minor allele increases risk/phenotype mean). If the `--beta` command is added along with `--logistic`, then the regression coefficients rather than the odds ratios will be returned.

**HINT** Adding the `--ci 0.95`, for example, option will given 95% confidence intervals for the estimated parameters, in additional `L95` and `U95` fields in the output files.

By itself, the `--linear` command will give identical results to the Wald test from the `--assoc` command when applied to quantitative traits.The `--logistic` command may give slightly different results to the `--assoc` command for disease traits, but this is because a different test/model is being applied (i.e. logistic regression rather than allele counting). The difference may be particularly large for very rare alleles (i.e. if the SNP is monomorphic in cases or controls, then the logistic regression model is not well-defined and asymptotic results might not hold for the basic test either).

The `TEST` column is by default `ADD` meaning the additive effects of allele dosage. Adding the option

```
--genotypic
```

will generate file which will have two extra tests per SNP, corresponding to two extra rows: `DOMDEV` and `GENO_2DF` which represent a separate test of the dominance component or a 2 df joint test of both additive and dominance (i.e. corresponding the the general, genotypic model in the `--model` command). Unlike the dominance model is the `--model`, `DOMDEV` refers to a variable coded 0,1,0 for the three genotypes `AA,Aa,aa`, i.e. representing the *dominance deviation* from additivity, rather specifying that a particular allele is dominant or recessive. That is, the `DOMDEV` term is fitted jointly with the `ADD` term in a single model.

**NOTE!** The coding PLINK uses with the 2 df `--genotypic` model involves two variables representing an additive effect and a dominance deviation;

|  | A | D |
|---|---|---|
| AA | 0 | 0 |
| AB | 1 | 1 |
| BB | 2 | 0 |

Although the 2df test will be identical, you would **not** expect to see similar p-values, etc for the two individual terms if instead you used a different version of ”genotypic” coding, e.g. in another analysis package, such as using dummy variables to represent genotypes:

|  | G1 | G2 |
|---|---|---|
| AA | 0 | 0 |
| AB | 1 | 0 |
| BB | 0 | 1 |

That is, although fundamentally the same, in terms of the 2df test, the interpretation of the two individual terms is different in these two cases. To achieve this coding in PLINK (v1.02 onwards), add the `--hethom` flag as well as `--genotypic`.

In a related note, you would not always expect the `ADD` p-value to be the same when entering in the dominance term as it is without it; if in doubt, you are advised to stick to just interpreting the 2 df test if using the `--genotypic` option.

To specify a model assuming full dominance (or recessive) for the minor allele (i.e. rather than the 2 df model mentioned above), you can specify with either

```
--dominant
```

or

```
--recessive
```

### 9.10.2 Covariates and interactions

If a covariate file is also specified, then **all** covariates in that file will be included in the regression model, labelled `COV1`, `COV2`, etc. This is different to other commands which take only a single covariate (possibly working in conjunction with the `--mcovar` option).

**NOTE** The `--covar-name` or `--covar-number` commands can be used to select a subset of all covariates in the file, described here.

For example, if the covariate file is made as described here and contains 2 covariates then the command

```
plink --bfile mydata --linear --genotypic --covar mycov.txt
```

will add two extra tests per SNP, `COV1` and `COV2`. The p-value for the SNP term or terms in the model will be adjusted for the covariates; that is, a single model is fit to the data (that also includes a dominance term, as the `--genotypic` flag was also set):

```
Y = b0 + b1.ADD + b2.DOMDEV + b3.COV1 + b4.COV2 + e
```

(Note, using this notation, the genotypic test is of `b1=b2=0`.)

The output per each SNP might look something like:

```
CHR        SNP      BP  A1       TEST   NMISS       OR      STAT         P
  5   rs000001   10001   A        ADD     664   0.7806    -1.942   0.05216
  5   rs000001   10001   A     DOMDEV     664   0.9395   -0.3562    0.7217
  5   rs000001   10001   A       COV1     664   0.9723   -0.7894    0.4299
  5   rs000001   10001   A       COV2     664    1.159    0.5132    0.6078
  5   rs000001   10001   A   GENO_2DF     664       NA     5.059    0.0797
```

That is, this represent coefficients from four terms in a multiple regression of disease on `ADD`, `DOMDEV`, `COV1` and `COV2` jointly. The final test is a 2df test that tests the coefficients for `ADD` and `DOMDEV` together. Importantly, the p-values for each line reflect the effect of the entity under the `TEST` column, not of the SNP whilst controlling for that particular covariate. (That is, p=0.0797 is the 2df test of the SNP whilst controlling for `COV1` and `COV2`.)

**HINT** To suppress the multiple lines of output for each covariate (which often are not of interest in themselves) add the flag `--hide-covar`, i.e. the above would just read as follows for this SNP:

```
CHR        SNP      BP  A1       TEST   NMISS       OR      STAT         P
  5   rs000001   10001   A        ADD     664   0.7806    -1.942   0.05216
  5   rs000001   10001   A   GENO_2DF     664       NA     5.059    0.0797
```

**HINT** To condition analysis on a specific SNP when using `--linear` or `--logistic`, use the `--condition` option, e.g.

```
plink --bfile mydata --linear --condition rs123456
```

will test all SNPs but adding the allelic dosage for `rs123456` as a covariate. This command can be used in conjunction with `--covar` and the other options listed here. To condition on multiple SNPs, use, for example,

```
plink --bfile mydata --linear --condition-list snps.txt
```

where `snps.txt` is a plain text file contain a list of SNPs which are to be included as covariates. The output will now include terms that correspond to the SNPs listed in the file `snps.txt`.

The conditioning SNPs are entered into the model simply as covariates, using a simple 0, 1, 2 allele dosage coding. That is, for two conditioning SNPs, `rs1001` and `rs1002` say, and also a standard covariate, the model would be

```
Y = b0 + b1.ADD + b2.rs1001 + b3.rs1002 + b4.COV1 + e
```

If the `b1` coefficient for the test SNP is still significant after entering these covariates, this would suggest that it does indeeed have an effect independent of `rs1001`, `rs1002` and the other covariate. (The other coefficients may still be highly significant, but these reflect the effects of the conditioning SNPs and covariates, not the test SNP.)

If the `--sex` flag is added, then sex will be entered as a covariate in the model (coded 1 for male, 0 for female), e.g

```
plink --bfile mydata --logistic --sex
```

If the option `--interaction` is added, then terms will be entered which correspond to SNP x covariate interactions (with `DOMDEV` as well as `ADD` if `--genotypic` is specified). In the case of two covariates, without `--genotypic`, for example, the command

```
plink --bfile mydata --linear --covar tmp.cov --interaction
```

results in the model

```
Y = b0 + b1.ADD + b2.COV1 + b3.COV2 + b4.ADDxCOV1 + b5.ADDxCOV2 + e
```

**NOTE** Please remember that when interaction terms are included in the model, the significance of the main effects can not necessarily be interpreted straightforwardly (i.e. they will depend on the arbitrary coding of the variables). In otherwords, when including the `--interaction` flag, you should probably only interpret the interaction p-value. Please refer to any standard text of regression models if you are unclear on this.

Finally, a `--test-all` option drops all the terms in the model in a multiple degree of freedom test.

### 9.10.3 Flexibly specifying the model

Use command such as `--covar` and `--interaction` will automatically enter all covariates and possible SNP x covariate interactions. If one does not want to test all of these, then use the `--parameters` flag to extract only the ones of interest.

For example, to take the example above:

```
Y = b0 + b1.ADD + b2.COV1 + b3.COV2 + b4.ADDxCOV1 + b5.ADDxCOV2 + e
```

If one only wanted `ADD`, the two covariates and the `ADDxCOV2` but **not** the `ADDxCOV1` interaction, then, from the above example, you could use

```
plink --bfile mydata --linear --covar tmp.cov --interaction --parameters 1,2,3,5
```

That is, `--parameters` takes a comman-separated list of integers, starting from 1, that represent the terms in the model (in the order in which they would appear if the command were run without the `--parameters` flag). In this case:

```
ADD          [1]
COV1         [2]
COV2         [3]
ADD x COV1   [4]  <-- excluded
ADD x COV2   [5]
```

### 9.10.4 Flexibly specifying joint tests

To perform a user-defined joint test of more than one parameter, use the `--tests` option. This takes a comma-delimited set of parameter numbers, for example: if the model is

```
ADD         [1]
COV1        [2]
COV2        [3]
ADDxCOV1    [4]
ADDxCOV2    [5]
```

then

```
plink --bfile mydate --linear --covar file.cov --interaction --tests 1,4,5
```

represeents a 3 degree of freedom test of ADD and the two interactions.

Note, if this is used in conjunction with the `--parameters` option, then the coding here refers to the reduced model – for example, the command

```
plink --bfile mydate --linear --covar file.cov --interaction --parameters 1,2,3,5
--tests 1,4
```

performs a joint test of ADD and ADDxCOV2 (2df test) whilst controlling for main effects of COV1 and COV2, i.e. we *do not* use `--tests 1,5`, as there are now only 4 terms in the model:

| | | --parameters 1,2,3,5 | --tests 1,4 |
|---|---|---|---|
| ADD | [1] | [1] | TEST |
| COV1 | [2] | [2] | |
| COV2 | [3] | [3] | |
| ADDxCOV1 | [4] | n/a | |
| ADDxCOV2 | [5] | [4] | TEST |

In other words, we fit the model

```
Y = b0 + b1.ADD + b2.COV1 + b3.COV2 + b4.ADDxCOV2 + e
```

and jointly test the hypothesis

```
H0: b1 = b4 = 0
```

As mentioned above, use `--test-all` to drop all terms in the model in a single joint test.

### 9.10.5 Multicollinearity

A common problem with multiple regression is that of multi-collinearity: when the predictor variables are too strongly correlated to each other, the parameter estimates will become unstable. PLINK tries to detect this, and will display NA for the test statistic and p-value for all terms in the model if there is evidence of multi-collinearity. One common instance where this would occur would be if one includes the `--genotypic` option but a SNP only has two of the three possible genotype classes: in this case, ADD and DOM will be perfectly correlated and PLINK will display NA for both tests; this is basically telling you that you should re-run without the `--genotypic` option for that particular SNP. Similar principles apply to including covariates and interactions terms: the more terms you include, the more likely you are to have problems.

The `--vif` option can be used to specify the variance inflation factor (VIF) used in the initial test for multicollinearity. The default value is 10 – smaller values represent more stringent tests.

**HINT** If you have a quantitative trait, only want an additive model and have only a single binary covariate, use the `--gxe` option (described above) instead of `--linear`: it will run much faster (being based on a more simple test of the difference of two regression slopes; it will not necessarily give numerically identical results to the multiple regression approach, but asymptotically both tests should be similar).

## 9.11 Set-based tests

These set-based tests are particularly suited to large-scale candidate gene studies as opposed to whole genome association studies, as they use permutaiton.

**NOTE** The basis of the set-based test has been changed in version 1.04 onwards.

This analysis works as follows:

- For each set, for each SNP determine which other SNPs are in LD, above a certain threshold $R$
- Perform standard single SNP analysis (which might be basic case/control association, family-based TDT or quantitative trait analysis).

- For each set, select up to $N$ "independent" SNPs (as defined in step 1) with p-values below $P$. The best SNP is selected first; subsequent SNPs are selected in order of descreasing statistical significance, after removing SNPs in LD with previously selected SNPs.
- From these subsets of SNPs, the statistic for each set is calculated as the mean of these single SNP statistics
- Permute the dataset a large number of times, keeping LD between SNPs constant (i.e. permute phenotype labels)
- For each permuted dataset, repeat steps 2 to 4 above.
- Empirical p-value for set (`EMP1`) is the number of times the permuted set-statistic exceeds the original one for that set.

Note that the empirical p-values are corrected for the multiple SNPs within a set (taking account of the LD between these SNPs). They are not corrected for multiple testing if there is more than one set, however (i.e. there is no equivalent of `EMP2` (see the page on permutation).

The critical parameters described above, $R$, $N$ and $P$ can all be altered by the user, as described below.

To perform a set-based test the critical keywords are

```
--set-test
--set my.set
--mperm 10000
```

which state that we are performing a set-based test, which set-file to use and how many permutations to perform (this last command is necessary). As mentioned above, the `--assoc` command could be replaced by `--tdt`, or `--logistic`, etc.

The set file `my.set` is in form

```
SET1
rs1234
rs28384
rs29334
END
SET2
rs4774
rs662662
rs77262
END
...
```

For example,

```
plink --file mydata --set-test --set my.set --mperm 10000 --assoc
```

would display in the LOG file the following critical parameters with their default values

```
Performed LD-based set test, with parameters:
     r-squared  (--set-r2)   = 0.5
     p-value    (--set-p)    = 0.05
     max # SNPs (--set-max)  = 5
```

The output is written to a file with a `.set.mperm` extension, for example

```
plink.assoc.set.mperm
```

with the fields

```
SET     Set name
```

```
     NSNP    Number of SNPs in set
     NSIG    Total number of SNPs below p-value threshold
     ISIG    Number of significant SNPs also passing LD-criterion
     STAT    Average test statistic based on ISIG SNPs
     EMP1    Empirical set-based p-value
     SNPS    List of SNPs in the set
```

For example, here is output from a case/control dataset with SNPs for five related genes (lines truncated)

```
         SET   NSNP   NSIG   ISIG         STAT         EMP1 SNPS
      GABRB2     45      0      0            0            1 NA
      GABRA6      6      4      3        5.199      0.09489 rs3811991|rs2197414|...
      GABRA1     22     11      5        5.951      0.09459 rs4254937|rs4260711|...
      GABRG2     24      0      0            0            1 NA
       GABRP     17      2      1         7.64       0.0269 rs7736504
```

Here the first gene, *GABRB2* has 45 SNPs, but none of these are significant at p=0.05, and so the empirival p-value is necessarily 1.00. The next gene has 6 SNPs, 4 of which are significant, but only 3 of which are independently significant based on an r-squared threshold of 0.5. The `STAT` of 5.199 is the average chi-squared statistic across these three SNPs. It should not be interpreted in itself – rather, you should consider the `EMP1` significance value based on it. In this case, P=0.095. The final gene, *GABRP* is nominally significant here, P=0.027, but this does not correct for the 5 genes tested of course.

Naturally, different thresholds will produce different results. Depending on the unknown genetic architecture, these may vary substantially and meaningfully so. In general, if the set represents a very large pathway (dozens of genes) you might want to increase `--set-max`. There are probably no hard and fast rules with regard to how to set `--set-p` and `--set-r2`, except to say that running under a large number of settings and selecting the most significant is not a good idea.

Running with a "stricter" set of values

```
     --set-r2 0.1
     --set-p 0.01
     --set-max 2
```

we see a broadly similar pattern of results; naturally, the thresholding on p-value means that *GABRA6* goes from showing some signal to asbolutely no signal.

```
         SET   NSNP   NSIG   ISIG         STAT         EMP1 SNPS
      GABRB2     45      0      0            0            1 NA
      GABRA6      6      0      0            0            1 NA
      GABRA1     22      2      2        7.464      0.05949 rs4254937|rs4260711
      GABRG2     24      0      0            0            1 NA
       GABRP     17      1      1         7.64      0.06309 rs7736504
```

Alternatively, a more inclusive setting might be something like

```
     --set-r2 0.8
     --set-p 1
     --set-max 10
```

which, in this particular case, happens to yield slightly stronger signals for *GABRA6* and *GABRA1* but weaker for *GABRp* (lines truncated)

```
         SET   NSNP   NSIG   ISIG         STAT         EMP1 SNPS
      GABRB2     45     12     10        1.749       0.7162 hCV26311691|...
      GABRA6      6      6      6        3.998       0.0184 rs3811991|...
      GABRA1     22     13     10        5.277       0.0182 rs4254937|...
      GABRG2     24     11     10       0.6976       0.9099 hCV3167705|...
       GABRP     17     10     10        2.753       0.1225 rs7736504|...
```

**HINT** Two extremes are to perform a test based on a) the best single SNP result per set:

```
--set-max 1
--set-p 1
```

or to use all SNPs in a set:

```
--set-max 99999
--set-p 1
--set-r2 1
```

## 9.12 Adjustment for multiple testing: Bonferroni, Sidak, FDR, etc

To generate a file of adjusted significance values that correct for all tests performed and other metrics, use the option:

```
plink --file mydata --assoc --adjust
```

which generates the file

```
plink.adjust
```

which contains the fields

| | |
|---|---|
| CHR | Chromosome number |
| SNP | SNP identifer |
| UNADJ | Unadjusted p-value |
| GC | Genomic-control corrected p-values |
| BONF | Bonferroni single-step adjusted p-values |
| HOLM | Holm (1979) step-down adjusted p-values |
| SIDAK_SS | Sidak single-step adjusted p-values |
| SIDAK_SD | Sidak step-down adjusted p-values |
| FDR_BH | Benjamini & Hochberg (1995) step-up FDR control |
| FDR_BY | Benjamini & Yekutieli (2001) step-up FDR control |

This file is sorted by significance value rather than genomic location, the most significant results being at the top.

**WARNING** Currently, these procedures are only implemented for asymptotic significance values for the standard TDT and association (disease trait and quantitative trait, `--assoc`, `--linear`, `--logistic`) tests and the 2x2xK Cochran-Mantel-Haenszel test. Future versions will allow these results for empirical significance values and for other tests (e.g. epistasis, etc).

# Chapter 10

# Family-based association analysis

The main focus of PLINK is for population-based samples. There is some support for family-based analyses however, described in this section, for disease traits and quantitative traits.

## 10.1 Family-based association (TDT)

`PLINK` supports basic family-based association testing for disease traits, using the TDT and a variant of this test that also incorporates parental phenotype information, the *parenTDT*.

To run a basic TDT analysis for family data:

```
plink --file mydata --tdt
```

which generates the file

```
plink.tdt
```

If permutation has been requested, then either

```
plink.tdt.perm
```

or

```
plink.tdt.mperm
```

will be generated also. The main output file, `plink.tdt`, contains the following fields:

| | |
|---|---|
| CHR | Chromosome number |
| SNP | SNP identifier |
| A1 | Minor allele code |
| A2 | Major allele code |
| T | Transmitted minor allele count |
| U | Untransmitted allele count |
| OR | TDT odds ratio |
| CHISQ | TDT chi-square statistic |
| P | TDT asymptotic p-value |
| A:U_PAR | Parental discordance counts |
| CHISQ_PAR | Parental discordance statistic |
| P_PAR | Parental discordance asymptotic p-value |
| CHISQ_COM | Combined test statistic |
| P_COM | Combined test asymptotic p-value |

If the `--ci` option has been requested, then two additional fields will appear after `TDT_OR`:

| | |
|---|---|
| L95 | Lower 95% confidence interval for TDT odds ratio |
| U95 | Upper 95% confidence interval for TDT odds ratio |

(naturally, if a value other than 0.95 was used as the argument for the `--ci` option, it will appear here instead.)

The TDT statistic is calculated simply as

```
(b-c)^2 / (b+c)
```

where `b` and `c` are the number of transmitted and untransmitted alleles as shown in `plink.tdt`; under the null, it is distributed as a 1df chi-squared.

The parental discordance test is based on counting the number of alleles in affected versus unaffected parents, treating each nuclear family parental pair as a matched pair. These counts can be combined with the `T` and `U` counts of the basic TDT to give a combined test statistic, also shown in the output. The parenTDT assumes homogeneity within families rather than between families, in terms of population stratification. If parents are measured on the phenotype, then this test can add considerable power to family-based association analysis, whilst providing a strong degree (but not complete) protection against population stratification. The increase in power will depend on the proportion of parents that are discordant for the disease. This approach is described in Purcell *et al* AJHG (2005). `PLINK` uses a more simple approach to calculate the `PAR` and `COM` statistics, however: if

```
                Unaffeced parent
                A/A   A/B   B/B
Affected A/A     -     p     r
 parent  A/B     x     -     q
         B/B     z     y     -
```

i.e. such that the `A:U_PAR` fields represents `p+q+2r :  x+y+2z`, then

```
PAR = ( (p+q+2r) - (x+y+2z) )^2
       / ( p+q+x+y+4(r+z) )
```

and

```
COM =  ( ( b+p+q+2r ) - ( c+x+y+2z ) )^2
        / ( b+p+q+c+x+y+4(r+z) )
```

Both statistics follow a 1 df chi-squared distribution under the null.

When running the `--tdt` option, `PLINK` will first perform a check for Mendel errors and make missing the offending genotypes.

Using the `--tdt` option, if permutation is requested (using either `--perm` or `--mperm`) a file entitled either

```
plink.tdt.perm
```

or

```
plink.tdt.mperm
```

will be generated: the empirical p-value will be based on the standard TDT test. The permutation procedure will flip transmitted/untransmitted status constantly for all SNPs for a given family, thereby preserving the LD and linkage information between markers and siblings.

## 10.2 parenTDT

The parenTDT, described in the paragraph above, is automatically included when using the `--tdt` option. These alternate commands generate the same output as for the `--tdt` command, described above, except the permutation is based not on the standard TDT, but either the parenTDT if using the option

```
plink --file mydata --parentdt1
```

or, the combined test (TDT and parenTDT) if using the option

```
plink --file mydata --parentdt2
```

## 10.3 Parent of origin analysis

When performing family-based TDT analysis, it is possible to separately consider transmissions from heterozygous fathers versus heterozygous mothers to affected offspring. This is performed by adding the `--poo` to request parent-of-origin analysis:

```
plink --file mydata --tdt --poo
```

which generates the file `plink.tdt.poo`. If permutation is also requested, this also generates the file `plink.tdt.poo.perm` or `plink.tdt.poo.mperm`, depending which permutation procedure is used. The main output file has the following format:

| | |
|---|---|
| CHR | Chromosome number |
| SNP | SNP identifier |
| A1:A2 | Allele 1 : allele 2 codes |
| T:U_PAT | Paternal transmitted : untransmitted counts |
| OR_PAT | Paternal odds ratio |
| CHISQ_PAT | Paternal chi-squared test |
| T:U_MAT | Maternal, as above |
| OR_MAT | Matneral, as above |
| CHISQ_MAT | Maternal, as above |
| Z_POO | Z score for difference in paternal versus maternal odds ratios |
| P_POO | Asymptotic p-value for parent-of-origin test |

If permutation is requested, the default test statistic is the absolute value of the Z score for the parent-of-origin test (i.e. making a two-sided test). The flags `--pat` and `--mat` indicate that the permutation statistic should be the paternal TDT chi-squared statistics, or the maternal statistic, instead.

**NOTE** When both parents are heterozygous, these ambiguous transmissions are counted as 0.5 for both mother and father – this is why the T:U counts will often not be whole numbers.

## 10.4 DFAM: family-based association for disease traits

The DFAM procedure in PLINK implements the sib-TDT and also allows for unrelated individuals to be included (via a clustered-analysis using the Cochran-Mantel-Haesnzel). To perform this test:

```
plink --bfile mydata --dfam
```

which generates the file

```
plink.dfam
```

which contains the fields

| | |
|---|---|
| CHR | Chromosome code |
| SNP | SNP identifier |
| A1:A2 | Minor and major allele codes |
| OBS | Number of observed minor alleles |
| EXP | Number of expected minor alleles |
| CHISQ | Chi-squared test statistic |
| P | Asymptotic p-value |

This test can therefore be used to combine discordant sibship data, parent-offspring trio data and unrelated case/control data in a single analysis.

**NOTE** If you are analysing a sibling-only sample (i.e. no parents) then also add the `--nonfounders` option; otherwise, all SNPs will be pruned out at the filtering stage, as PLINK will by default only consider founder alleles when calculating allele frequency, Hardy-Weinberg, etc.

## 10.5 QFAM: family-based association tests for quantitative traits

PLINK offers a somewhat ad-hoc procedure to perform family-based tests of association with quantitative phenotypes: the QFAM procedure, which uses permutation to account for the dependence between related individuals. It adopts the between/within model as used by Fulker et al (1999, AJHG) and Abecasis et al (2000, AJHG) as implemented in the QTDT package. However, rather than fitting a maximum likelihood variance components model, as QTDT does, PLINK performs a simple linear regression of phenotype on genotype, but then uses a special permutation procedure to correct for family structure.

There are several ways to run QFAM: a total association test (between and within components)

```
plink --bfile mydata --qfam-total --mperm 100000
```

or a within-family test

```
plink --bfile mydata --qfam --mperm 100000
```

or a test including parental phenotypes

```
plink --bfile mydata --qfam-parents --mperm 100000
```

(Also, `--qfam-between` will look only at the between-family component of association).

**NOTE** In all cases above, we have used `--mperm` to specify permutation; adaptive permutation can also be used with QFAM (`--perm`). Permutation is necessary for the QFAM test.

The columns in the QFAM permutation result files are:

| | |
|---|---|
| CHR | Chromosome code |
| SNP | SNP identifier |
| STAT | Test statistic (ignore) |
| EMP1 | Pointwise empirical p-value |
| NP | Number of permutations performed |

The columns in the non-permutation file (e.g. `plink.qfam.total`, if `plink.qfam.total.mperm` contains the permuted results) are as follows:

| | |
|---|---|
| CHR | Chromosome code |
| SNP | SNP identifier |
| A1 | Minor allele (corresponds to beta given below; absent in earlier PLINK releases) |
| TEST | Type of test, TOT, WITH and BET |
| NMISS | Number of non-missing individuals in analysis |
| BETA | Regression coefficient |
| STAT | Test statistic (ignore; not corrected for family-structure) |
| P | Asymptotic p-value (ignore; use empirical p-value) |

These results are from a standard `--linear` type analysis, i.e. which ignores family structure. They are displayed so that the direction of effect may be determined (from the `BETA`) – but otherwise, only the empirical p-value from the permuted results file should be looked at.

The B and W components are calcalated using parental genotypes if they are available for both parents, otherwise siblings are used. Singletons can be included in this analysis (i.e. B=G and W=0 for them): for example, the scores are shown below for a few configurations, when parents are available:

| Genotype | G |
|---|---|
| AA | 1 |
| Aa | 0 |
| aa | -1 |

```
B = ( Pat + Mat ) / 2
W = G - B
```

| Pat | Mat | Offspring | G | B | W |
|---|---|---|---|---|---|
| AA | AA | AA | 1 | 1 | 0 |

```
Aa      AA      AA              1      0.5   0.5
Aa      AA      Aa              0      0.5   -0.5
aa      AA      Aa              0      0     0
etc
```

The QFAM permutation procedure breaks down the genotypes into between (B) and within (W) components, permutes them independently (i.e. at the family level, either swapping the B component for one family with another family, or flipping the sign of all W's in a family with 50:50 chance) and then (for the total association test) reconstructs the individual level "genotypes" as the sum of the new B's and W's i.e:

```
1) G -> B + W (individual-level)
2a) Permute B (family-level) -> B'
2b) Permute W (family-level) -> W'
3) B' + W' -> G' (individual-level)
```

The logic is that we know how to permute both B and W separately whilst maintaining the familial structural component, and they are orthogonal components, so we should permute them separately, but then recombine them as a single individual-level genotypic score.

**NOTE** The total `--qfam-total` test is designed to extract all association information from a family-based sample, controlling for relatedness: it is not robust to stratification. Use the `--qfam` for a strictly within family test.

In many circumstances, the standard QTDT as implemented in Goncalo Abecasis' QTDT `http://www.sph.umich.edu/csg/abecasis/QTDT/` program will perhaps be more appropriate. The disadvantages of the QFAM procedure are

- that it uses permutation and so is slower
- appears to be slightly less powerful when there is a higher residual correlation

On the plus side, the advantages of the QFAM procedure are

- that is uses permutation and so is appropriate for non-normal phenotypes; it could also be used for disease phenotypes, although it will not be appropriate for affected-only TDT style designs
- that it can be applied to genome-wide data easily (albeit not necessarily quickly)

**Technical note** As a technical point: when permuting genotype between families in this way, one has to be careful with missing genotype data, particularly in the instance in which a family is completely missing. Because a missing B component cannot be recombined with a non-missing W component, and vice versa, this process would tend to increase the amount of missingness in permutations versus the original data.

One could exclude individuals with missing genotypes first and permute separately for each SNP, but this would no longer maintain the correlation between SNPs (and require more computation). Instead, we use the following scheme. We permute once per replicate (e.g. a table of F (original family) and F' (permuted family), true and permuted families). e.g. but let's say that 2 is missing their B component (denoted 2*) For example:

```
F  F'
0  5
1  2*  <- remove ?
2* 4   <- remove ?
3  1
4  0
5  3
```

This would knock out families 1 and 2 from the permutation. We therefore permute once to create a single table for permutation for all SNPs, but then resursively edit the table on a SNP-by-SNP basis, to regroup the missing families, by swapping missing F' families: in this case, swap 2* with 4 (the other partner of 2*), e.g.

```
F  F'
0  5
1  4
2* 2* <- remove
3  1
4  0
5  3
```

So now we have a permuted sample but the total level of missingness is the same. This procedure still generates valid, completely random permutations of the non-missing genotype data and trys to maintain as much of the correlation between SNPs as possible (i.e. as typically only a small % of genotypes are missing and so we do not need to edit the table much).

# Chapter 11

# Permutation procedures

Permutation procedures provide a computationally intensive approach to generating significance levels empirically. Such values have desirable properties: for example, relaxing assumptions about normality of continuous phenotypes and Hardy-Weinberg equilibrium, dealing with rare alleles and small sample sizes, providing a framework for correction for multiple testing, and controlling for identified substructure or familial relationships by permuting only within cluster.

### 11.0.1 Conceptual overview of permutation procedures

Permutation procedures are available for a variety of tests, as described below. For some tests, however, these procedures are not available (e.g. SNP x SNP epistasis tests). For other tests, permutation is necessary to obtain any significance values at all (e.g. set-based tests).

The permutation tests described below come can be categorized in two ways:

- Label-swapping versus gene-dropping
- Adaptive versus max(T)

### 11.0.2 Label-swapping and gene-dropping

In samples of unrelated individuals, one simply swaps labels (assuming that individuals are interchangeable under the null) to provide a new dataset sampled under the null hypothesis. Note that only the phenotype-genotype relationship is destroyed by permutation: the patterns of LD between SNPs will remain the same under the observed and permuted samples. For family data, it might be better (or in the case of affected-only designs such as the TDT, necessary) to perform gene-dropping permutation instead. In it's most simple form, this just involves flipping which allele is transmitted from parent to offspring with 50:50 probability. This approach can extend to general pedigrees also, dropping genes from founders down the generations.

For quantitative traits, or samples in which both affected and unaffected non-founders are present, one can then perform a basic test of association (with disease, or with a quantitative trait) treating the pedigree data as if they were all unrelated (i.e. just using the `--assoc` option) but creating permuted datasets by gene-dropping will both control for stratification and the non-independence of related individuals (i.e. as these will also be properties of every permuted dataset). It is possible to maintain LD between SNPs by applying the same series of 50:50 flip/no-flip decisions to all SNPs in the same permuted replicate for a given transmission. In addition, it is possible to control for linkage by applying the same series of flip/no-flip decisions to all siblings in the same nuclear family. Both these features are automatically applied in `PLINK`.

### 11.0.3 Adaptive and max(T) permutation

Using either label-swapping or gene-dropping, there are two basic approaches to performing the permutations. The default mode is to use an ***adaptive*** permutation approach, in which we give up permuting SNPs that are clearly going to be non-significant more quickly than SNPs that look interesting. In otherwords, if after only 10 permutations we see that for 9 of these the permuted test statistic for a given SNP is larger than the observed test statistic, there is little point in carrying on, as this SNP is incredibly unlikely to ever achieve a highly significant result. This greatly speeds up the permutation procedure, as most SNPs (that are not highly significant) will drop out quite quickly, making it possible to properly evaluate significance for the handful of SNPs that require millions of permutations. Naturally, the precision with which one has estimated the significance p-value (i.e. relating from the number of permutations performed) will be correlated the significance value itself – but for most purposes, this is precisely what one wants, as it is of little interest whether a clearly un-associated SNP really has a p-value of 0.78 or 0.87.

In contrast, ***max(T)*** permutation does not drop SNPs along the way. If 1000 permutations are specified, then all 1000 will be performed, for all SNPs. The benefit of doing this is that two sets of empirical significance values can then be calculated – pointwise estimates of an individual SNPs significance, but also a value that controls for that fact that thousands of other SNPs were tested. This is achieved by comparing each observed test statistic against the maximum of all permuted statistics (i.e. over all SNPs) for each single replicate. In otherwords, the p-value now controls the familywise error rate, as the p-value reflects the chance of seeing a test statistic this large, given you've performed as many tests as you have. Because the permutation schemes preserve the correlational structure between SNPs, this provides a less stringent correction for multiple testing in comparison to the Bonferroni, which assumes all tests are independent. Because it is now the corrected p-value that is of interest, it is typically sufficient to perform a much smaller number of tests – i.e. it is probably not necessary to demonstrate that something is genome-wide significant beyond 0.05 or 0.01.

### 11.0.4 Computational issues

`PLINK` performs the basic tests of association reasonably quickly – for small datasets both permutation procedures will be feasible. For example, for a dataset comprising 100,000 SNPs measured on 350 individuals, each permutation (for all 100K SNPs) takes approximately 2 seconds on a modern Linux workstation. At this speed, it will take just over 1 day to perform 50,000 permutations using the max(T) mode and label-swapping. With the same dataset, using adaptive mode, the entire analysis is finished much quicker (although the empirical p-values are, of course, not corrected for multiple testing). For larger datasets (e.g. 1000s of individuals measured on ¿500K SNPs) things will slow down, although this will be linear with the number of genotypes – if one has access to a cluster, however, the max(T) approach lends itself to easy parrallelization (i.e. if one can set many jobs running analysing the same data, it is easy to combine the empirical p-values afterwards).

By default, `PLINK` will select a random seed for the permutations, based on the system clock. To specify a fixed seed instead add the command

```
--seed 6377474
```

where the parameter a (large) integer seed.

## 11.1 Basic (adaptive) permutation procedure

The default method for permutation is the adaptive method. To obtain a max(T) permutation p-value, see the section below. For either either case/control or quantitative trait association analysis, use the option:

```
plink --file mydata --assoc --perm
```

to initiate adaptive permutation testing. As well as the `plink.assoc` or `plink.qassoc` output file, adding the `--perm` option will generate a file named:

```
plink.assoc.perm
```

which contains the fields:

| | |
|---|---|
| CHR | Chromosome |
| SNP | SNP ID |
| STAT | Test statistic |
| EMP1 | Empirical p-value (adaptive) |
| NP | Number of permutations performed for this SNP |

An alternate scheme is also available, that may under some circumstances be useful. Specifically, this approach fixes the observed marginal counts for the 2-by-3 tables that is case/control status by the two alleles *and the missing allele count*. After permuting case/control label, only two cells in the table, e.g. missing and A2 alleles for controls, are counted, the rest of the table is filled in on the basis of the fixed marginal values. This speeds up the permutation procedure a little, and also implicitly downweights association results where there is a lot of missing genotype data that is non-random with respect to genotype and case/control status. Naturally, this approach can not provide total protection against the problem of non-random missing genotype data. Also, for SNPs with lots of missing data, this test will be conservative, whether the missingness is non-random or not. For these reasons, this is not the default option, although this approach might be one worth exploring further. To use this alternate permutation scheme, use the `--p2` flag:

```
plink --file mydata --assoc --perm --p2
```

or

```
plink --file mydata --assoc --mperm 1000 --p2
```

## 11.2 Adaptive permutation parameters

Although the `--perm` option invokes adaptive permutation by default, there are various parameters that alter the behavior of the adaptive process that can be tweaked using the `--aperm` option, followed by six parameters: for example,

```
plink --file mydata --assoc --aperm 10 1000000 0.0001 0.01 5 0.001
```

The six arguments (along with the default values) are:

| | |
|---|---|
| Minimum number of permutations per SNP | 5 |
| Maximum number of permutations per SNP | 1000000 |
| Alpha level threshold (alpha) | 0 |
| Confidence interval on empirical p-value (beta) | 0.0001 |
| Interval to prune test list (intercept) | 1 |
| Interval to prune test list (slope) | 0.001 |

These are interpreted as follows: for every SNP, at least 5 permutations will be performed, but no more than 1000000. After 5 permutations, the p-values will be evaluated to see which SNPs we can prune. The first interval value means to perform this pruning every 5 replicates; the second pruning parameter (0.001) means that the rate of pruning slows down with increasing number of replicates (i.e. pruning is, in this case, performed every 5+0.001R replicates where R is the current number of replicates). At each pruning stage, a `100*(1 - beta / 2T)%` confidence interval is calculated for each empirical p-value, where `beta` is, in this case 0.01, and `T` is the number of SNPs. Using the normal approximation to the binomial, we prune any SNP for which the lower confidence bound is greater than `alpha` or the upper confidence bound is less than `alpha`.

## 11.3 max(T) permutation

To perform the max(T) permutation procedure, use the `--mperm` option, which takes a single paramter, the number of permutations to be performed: e.g. to use with the TDT test:

```
plink --file mydata --tdt --mperm 5000
```

which will generate (along with the `plink.tdt` file) an file named

```
plink.tdt.mperm
```

which contains the fields:

```
CHR     Chromosome
SNP     SNP ID
STAT    Test statistic
EMP1    Empirical p-value (pointwise)
EMP2    Corrected empirical p-valie (max(T) / familywise)
```

**Hint** If multiple runs of `PLINK` are performed on the same dataset in parallel, using a computer cluster to speed up the max(T) permutations, then the resulting estimates of empirical significance can be combined across runs as follows. Empirical p-values are calculated as `(R+1)/(N+1)` where `R` is the number of times the permuted test is greater than the observed test; `N` is the number of permutations. Therefore, therefore, given `p_i`, the empirical p-value for the ith run, this implies that `p_i*(N_i+1)-1` replicates passed the observed value. The overall empirical p-value should then be:

```
( SUM_i  p_i * ( N_i + 1 ) - 1  + 1 ) / ( SUM_i  N_i  + 1 )
```

To produce output files that contain either the best statistic per replicate, or all statistics per replicate, use either option

```
--mperm-save
```

or

```
--mperm-save-all
```

along with the usual `--mperm` command. The first command generates a file

```
plink.mperm.dummp.best
```

which contains two columns. The first is the replicate number (0 represents the original data, the remaining rows 1 to $R$ where $R$ is the number of permutations specified). The second column is the maximum test statistic over all SNPs for that replicate. The second command, `--mperm-save-all` produces a file

```
plink.mperm.dump.all
```

that could be a very large file: the test statistic for all SNPs for all replicates. As before, the first row is the original data; the first column represents the replicate number; all other columns represent the test statistic values for each SNP (`NA` if this cannot be calculated). These two files might be of use if, for example, you wish to create your own wrapper around PLINK to perform higher-order corrections for multiple testing, e.g. if more than one phenotype is tested per SNP. In most cases, for this purpose, the first form should suffice.

## 11.4 Gene-dropping permutation

To perform gene-dropping permutation, use the `--genedrop` option, combined with the standard `--assoc` option. Either adaptive: e.g.

```
plink --file mydata --assoc --genedrop
```

or max(T) permutation: e.g.

```
plink --file mydata --assoc --genedrop --mperm 10000
```

can be specified.

This analysis option is equally applicable to disease and quantitative traits, although at least some non-founder individuals should be unaffected. Currently, an individual must have both parents genotyped for genedropping. For founders and for individuals without two genotyped parents, their genotypes are unchanged throughout all genedropping permutations.

It is possible to combine label-swapping with gene-dropping, however, to handle different family/sample configurations. That is, the basic gene-dropping procedure will leave untouched all individuals without two parents, making them uninformative for the test of association. One can think of at least three classes of groups of people without two parents in the dataset: founders/parents, siblings and unrelated singletons. Label-swapping within these groups can provide additional sources information for association that control different levels of the between/within family components of association.

There are three options, which can be used together, are:

```
--swap-sibs               within family
--swap-parents            partial within-family
--swap-unrel              between family
```

which label-swap between sibs without genotyped parents (swapping only within families), between parents only (swapping only within families), or between all singletons (unrelated individuals) (swapping between familes).

### 11.4.1 Basic within family QTDT

This test only considers information from individuals with two genotyped parents:

```
plink --file mydata --assoc --genedrop
```

### 11.4.2 Discordant sibling test

Although gene-dropping only considers individuals with two parents to be informative, valid family-based tests can include information from full-siblings – by label-swapping only within each full sibship that does not otherwise have parents, it is possible to augment the power of the gene-dropping approach:

```
plink --file mydata --assoc --genedrop --swap-sibs
```

### 11.4.3 parenTDT/parenQTDT

This test additionally incorporates information from phenotypically discordant parents (for either quantitative or disease triats). This provides more information for association, but provides a weaker level of protection against stratification (i.e. it assumes that mother and father pairs are well matched in terms of subpopulation stratum).

```
plink --file mydata --assoc --genedrop --swap-parents
```

### 11.4.4 Standard association for singleton, unrelated individuals

If a sample is a mixture of families and unrelated individuals (e.g. case/control and offspring/parent trios combined) then adding this option as well as the `--gene-drop` option will perform label-swapping permutation for all unrelated individuals.

```
plink --file mydata --assoc --genedrop --swap-unrel
```

One or more of these options can be included with the `--genedrop` option. These features allow between and within family components of association to be included in analysis. Below are the results of some simple, proof-of-principle simulations, to illustrate parental discordance test:

Here is a subset of the results: in all cases, we have an unselected quantitative trait measured in parent/offspring nuclear families. The four models:

- no stratification, no QTL

- no stratification, QTL
- stratification between families (i.e. mother and father from same subpopulation), no QTL
- stratification within families (i.e. mother and father might not be from same subpopulation), no QTL

The three analytic procedures:

- standard QTL test (i.e. ignore family structure, which we know is incorrect)
- gene-dropping permutation (i.e. within family QTDT)
- gene-dropping + parental label-swapping (i.e. parenQTDT)

From simulation, the empirically estimated power/type I error rates (for a nominal value of 0.05) are:

```
500 trios (QT)
  I     II    III
A 0.121 0.045 0.053
B 0.841 0.239 0.563
C 0.461 0.056 0.056
D 0.505 0.055 0.501
500 tetrads (QT)
  I     II    III
A 0.173 0.043 0.050
B 0.900 0.363 0.653
C 0.439 0.042 0.045
D 0.390 0.044 0.421
```

That is,

- method I is, as expected, liberal (e.g. for tetrads, we see type I error rate of 17.3% instead of 5%). Subsequent values for this test should therefore be ignored in the table
- the parenQTDT (III) (as implemented by gene-dropping) is considerably more powerful than the standard within-family test that ignores parental phenotypes (II) – i.e. 65% versus 36% for tetrads, in this particular instance.
- the parenQTDT is robust to stratification so long as it is between-family (condition C) – i.e. it only assumes that mum and dad are matched on strata, not the whole sample. When this does not hold (condition D), then we get spurious association, as expected.

**HINT** For disease traits, the parenTDT test is automatically performed by the `--tdt` option (as long as there are at least 10 phenotypically discordant parental pairs in the sample). See the section of standard association testing for more details.

## 11.5 Within-cluster permutation

To perform label-swapping permutaion only *within clusters*, you must supply either a cluster file with the `--within` option, or indicate that family ID is to be used as the cluster variable, with the `--family` option. Then any label-swapping permutation procedure will only swap phenotype labels between individuals within the same cluster. For example,

```
plink --file mydata --assoc --within mydata.clst --perm
```

if the file `mydata.clst` were (for a PED file containing only 6 individuals, the file format is family ID, individual ID, cluster):

```
F1  1  1
F2  1  1
F3  1  1
F4  1  2
F5  1  2
F6  1  3
```

this would imply that only sets 1,2,3 and 4,5 could be permuted. That is, 1 and 3 could swap phenotypes, but not 1 and 5, for example. In this way, any between-cluster effects are preserved in each permuted dataset, which thereby controls for them.

To permute with family ID as the cluster variable for label-swapping, use the

```
plink --file mydata --assoc --family --perm
```

Note that label-swapping within families is different from gene-dropping. This approach would be appropriate for sibship data, for example, when no parents are available. The assumption is that individuals within family unit are interchangeable under the null – as such, you should not include mixtures of full siblings and half siblings, or siblings and parents, for example, in the same cluster using this approach.

**Note** Other options for stratified analyses are described on the previous page

## 11.6 Generating permuted phenotype filesets

To generate a phenotype file with $N$ permuted phenotypes in it, use the function

```
plink --bfile mydata --make-perm-pheno 10
```

which will make a file

```
plink.pphe
```

with 10 phenotypes (listed after the FID and IID as the first two columns). This can then be used in any further analysis with PLINK or any other program. This command can be combined with `--within`, to generate permuted phenotype files in which individuals' phenotypes are only swapped within each level of the stratifying cluster variable, e.g.

```
plink --bfile mydata --make-perm-pheno 10 --within strata1.dat
```

# Chapter 12

# Multimarker haplotype tests

All tests described above are based on single SNP tests. It is also possible to impute haplotypes based on multimarker predictors using the standard E-M algorithm and to perform simple tests based on the distribution of probabilistically-inferred set of haplotypes for each individual.

As well as the autosomes, X and haploid chromosomes should be appropriately handled. Phasing can either be based on a sample of unrelated individuals, or certain kinds of family data. First, all founders are phased using the E-M algorithm; then all descendents of these founders are phased given the set of possible parental phases and assuming random-mating. Currently it is not possible to phase sibships without parents. The current implementation of the phasing and haplotype testing algorithm is designed focus on relatively small regions of the genome, rather than to phase whole chromosomes at once.

**HINT!** Another approach to haplotype-testing can be found under the page describing proxy association. This set of methods essentially just provide a different interface to the exact same E-M phasing and haplotype-testing algorithms, one that is centered around a specific reference SNP.

## 12.1 Specification of haplotypes to be estimated

Haplotype testing in PLINK requires that the user supplies a file listing the haplotypes to be tested (Some precomputed lists are given below which might be useful in some circumstances.) The formats of these files are described below. An alternative is to specify a simple, sliding window of fixed haplotype size (also described below).

The command

```
plink --file mydata --hap myfile.hlist
```

will read the file `myfile.hlist`, each row of which is expected to have one of the three following formats:
***1) Particular allele specified***
The first format specifies a particular haplotype at a given locus. Two example rows of this format are:

```
rs1001 5 0 201  1 2   TC    snp1 snp2
rs1002 5 0 202  A C   TTA   snp1 snp3 snp4
...
```

The columns represent:

```
Col 1  : Imputed SNP name
Col 2  : Imputed SNP chromosome
Col 3  : Imputed SNP genetic distance (default: Morgan coding)
Col 4  : Imputed SNP physical position (bp units)
Col 5  : Imputed SNP allele 1 name
Col 6  : Imputed SNP allele 2 name
Col 7  : Tag SNP allele/haplotype that equals imputed SNP allele 1
Col 8+ : Tag SNP(s) [in same order as haplotype in Col 7]
```

Here we have explicitly specified the `TC` and `TTA` haplotypes. For example, in the first case, SNPs `snp1` and `snp2` may have all four common haplotypes seen in the sample, `TT`, `CT` and `CC` as well as `TC`; this command would select only the `TC` haplotype to be imputed, or as the focus of haplotype analysis. The imputed SNP, `rs1001` therefore has the following alleles:

```
TC/TC    1/1
TC/*     1/2
*/*      2/2
```

and will be positioned on chromosome 5, and base-positon 201. Haplotypes other than `TC` will be coded 2.

The imputed SNP details (alleles, etc) will only be used if the `--hap-impute` option has been requested. For `--hap-assoc` and `--hap-tdt` options (which consider all possible phases rather than just imputing the most likely) these are not considered (but they are still required in this input file).

***2) 'Wildcard' specification***

Alternatively, all haplotypes at a given locus above the `--maf` threshold can be automatically estimated by entering a line in `myfile.hlist` as follows:

```
* snp1 snp2 snp3
* snp1 snp2
```

i.e. where the first character is an asterisk `*`, which would, taking just the first line for example, create all 3-SNP haplotypes for the SNPs labelled in the MAP file as `snp1`, `snp2` and `snp3`, above the minor allele frequency threshold. If the haplotypes were, for example, `AAC`, `AGG` and `TGG`, then the following names would be automatically assigned:

```
H1_AAC_
H1_AGG_
H1_TGG_
```

Haplotypes based on subsequent lines in the file would be labelled `H2_*_`, `H3_*_`, etc. In this case, all two-SNP haplotypes for `snp1` and `snp2` would start `H2_`. The chromosome and position flags for the new haplotypes are set to equal the first SNP of the set.

***3) 'Named wildcard' specification***

Finally, this format is identical to the previous wildcard specification, except a name can be given to the haplotype. This uses `**` instead of `*` to start a row; the second entry is then interpreted as the name of the haplotype locus rather than the first SNP. For example:

```
** BLOCK1 snp1 snp2 snp3
** BLOCK2 snp6 snp7
```

The only difference is that `BLOCK1` and `BLOCK2` names will be used in the output instead of `H1` and `H2` being assigned automatically.

***4) Sliding window specification***

Finally, instead of specifying a haplotype file with the `--hap` option, you can use the `--hap-window` option to specifty all haplotypes in sliding windows of a fixed number of SNPs (shifting 1 SNP at a time).

```
plink --bfile mydata --hap-window 3 --hap-assoc
```

to form all 3-SNP haplotypes across the entire dataset (respecting chromosome boundaries, however). In this case the windows will be automatically named `WIN1`, `WIN2`, etc. This command can take a comma-delimited list of values, e.g.

```
--hap-window 1,2,3
```

to perform all single SNP tests (1-SNP haplotypes) as well as sliding windows of all 2-SNP and 3-SNP haplotypes.

## 12.2 Precomputed lists of multimarker tests

Below are links to some PLINK-formatted lists of multimarker tests selected for Affymetrix 500K and Illumina whole genome products, based on consideration of the CEU Phase 2 HapMap (at r-squared=0.8 threshold). One should download the appropriate file and run with the `--hap` option (after ensuring that any strand issues have been resolved). These files were generated by Itsik Pe'er and others, as described in this manuscript:

```
Pe'er I, de Bakker PI, Maller J, Yelensky R, Altshuler D
& Daly MJ (2006) Evaluating and improving power in whole-genome
association studies using fixed marker sets. Nat Genet, 38(6): 605-6.
```

- Affymetrix.GeneChip.500k.both.CEU.0.8.tests.zip `http://pngu.mgh.harvard.edu/~purcell/dist/mmtests/Affymetrix.GeneChip.500k.both.CEU.0.8.tests.zip`
- Illumina.HumanHap.300k.CEU.0.8.tests.zip `http://pngu.mgh.harvard.edu/~purcell/dist/mmtests/Illumina.HumanHap.300k.CEU.0.8.tests.zip`
- Illumina.HumanHap.550k.CEU.0.8.tests.zip `http://pngu.mgh.harvard.edu/~purcell/dist/mmtests/Illumina.HumanHap.550k.CEU.0.8.tests.zip`
- Illumina.HumanHap.650k.CEU.0.8.tests.zip `http://pngu.mgh.harvard.edu/~purcell/dist/mmtests/Illumina.HumanHap.650k.CEU.0.8.tests.zip`

These tables list all tags for every common HapMap SNP, at the given r-squared threshold. The same haplotype may therefore appear multiple times (i.e. if it tags more than 1 SNP). The haplotypes are specified in terms of the + (positive) strand relative to the HapMap. You might need to reformat your data prior to using these files (using the `--flip` command, for instance) before you can use them.

**Note** These tables obviously assume that all tags on present in the final, post-quality-control dataset: i.e. if certain SNPs have been removed, it will be better to reselect the predictors – that is, these lists should really only be used as a first pass, for convenience.

## 12.3 Estimating haplotype frequencies

To obtain the haplotype frequencies for all haplotypes in each window, use the option:

```
plink --file mydata --hap myfile.hlist --hap-freq
```

which will generate the file

```
plink.freq.hap
```

which contains the fields (no header)

```
LOCUS        Haplotype locus / window name
HAPLOTYPE    Haplotype identifer
F            Frequency in sample (founders)
```

## 12.4 Testing for haplotype-based case/control and quantitative trait association

In a population-based sample of unrelated individuals, case/control and quantitative traits can be analysed for haplotype associations, using the option, for example,

```
plink --file mydata --hap myfile.hlist --hap-assoc
```

which will generate haplotype-specific tests (1df) for both disease and quantitative traits; for disease traits only, an omnibus association statistic will also be computed. This option generates the file

```
plink.assoc.hap
```

which contains the following fields:

```
LOCUS        Haplotype locus / window name
HAPLOTYPE    Haplotype identifer / "OMNIBUS"
F_A          Frequency in cases
F_U          Frequency in controls
CHISQ        Test for association
DF           Degrees of freedom
P            Asymptotic p-value
SNPS         SNPs forming the haplotype
```

or

```
plink.qassoc.hap
```

which contains the following fields:

```
LOCUS        Haplotype locus / window name
HAPLOTYPE    Haplotype identifer
NANAL        Number of individuals in analysis
BETA         Regression coefficient
RSQ          Proportion variance explained
STAT         Test statistic (T)
P            Asymptotic p-value
SNPS         SNPs forming the haplotype
```

In all cases, the tests are based on the expected number of haplotypes each individual has (which might be fractional). The case/control omnibus test is a H-1 degree of freedom test, if there are H haplotypes.

## 12.5 Haplotype-based association tests with GLMs

The following options use linear and logistic regression to perform haplotye-based association analysis. The two main commands, `--hap-linear` and `--hap-logistic` are analogous to `--linear` and `--logistic`, described here.

The main advantages of these commands over the above approaches, are that they can include one or more covariates and allow for permutation. The disadvantage is that they will run a little more slowly.

The basic command is

```
plink --file mydata --hap myfile.hlist --hap-logistic
```

(alternatively, for a quantitative outcome, use `--hap-linear`; aside from minor differences in the output, the discussion below applies equally to both forms of these commands).

**NOTE** Here the haplotypes to be tested are specified in a file with the `--hap` command, but one could alternatively use a sliding window analysis, e.g. to cover all 2, 3 and 4-SNP windows, e.g. `--hap-window 2,3,4`

The output is in the file

```
plink.assoc.hap.logistic
```

(or `plink.assoc.hap.linear`) which has the fields:

```
NSNP    Number of SNPs in this haplotype
NHAP    Number of common haplotypes (threshold determined by --mhf, 0.01 default)
 CHR    Chromosome code
 BP1    Physical position of left-most (5') SNP (base-pair)
```

|  |  |
|---|---|
| BP2 | Physical position of right-most (3') SNP (base-pair) |
| SNP1 | SNP ID of left-most (5') SNP |
| SNP2 | SNP ID of left-most (3') SNP |
| HAPLOTYPE | Haplotype |
| F | Frequency in sample |
| OR | Estimated odds ratio |
| STAT | Test statistic (T from Wald test) |
| P | Asymptotic p-value |

for example: (*spaces between rows added for clarity*)

```
     NSNP NHAP  CHR        BP1        BP2       SNP1       SNP2  HAPLOTYPE        F       OR     STAT
P
        2    2   22   15462210   15462259 rs11089263 rs11089264         AA    0.345     1.31    0.693
0.405
        2    2   22   15462210   15462259 rs11089263 rs11089264         CG    0.655    0.762    0.693
0.405
        3    3   22   15688352   15690057   rs165650   rs165757        GTG    0.117    0.544     1.46
0.227
        3    3   22   15688352   15690057   rs165650   rs165757        CTG   0.0167    0.406    0.525
0.469
        3    3   22   15688352   15690057   rs165650   rs165757        CGA    0.867      1.7     1.56
0.212
        5    5   22   15691787   15699058   rs175152   rs165914      ACACT    0.129    0.515     2.13
0.144
        5    5   22   15691787   15699058   rs175152   rs165914      CCACT    0.236    0.917   0.0566
0.812
        5    5   22   15691787   15699058   rs175152   rs165914      CCACG   0.0169     1.74    0.198
0.656
        5    5   22   15691787   15699058   rs175152   rs165914      CTGTG    0.085    0.565     1.11
0.292
        5    5   22   15691787   15699058   rs175152   rs165914      CTATG    0.533     1.88     3.36
0.0666
        5    4   22   15902049   15939567  rs2845389  rs4819958      GTAAA   0.0857    0.719    0.388
0.533
        5    4   22   15902049   15939567  rs2845389  rs4819958      GTGAA     0.32     1.04   0.0185
0.892
        5    4   22   15902049   15939567  rs2845389  rs4819958      CCGGG    0.303    0.548     2.97
0.0847
        5    4   22   15902049   15939567  rs2845389  rs4819958      GCGGG    0.292     1.82     3.28
0.0701
```

which illustrates results for the first four haplotype window positions (e.g. the second window position contains 3 SNPs, and there are 3 common haplotypes, `GTG`, `CTG` and `CGA`).

The additional command

```
--hap-omnibus
```

instructs PLINK to perform instead of H-1 haplotype-specific tests for H haplotypes (of each versus all others), a single H-1 df omnibus test (jointly estimating a testing all haplotype effects at that position). This will result in a single row per window, with the following slightly different format. Now the first four window positions have only a single line of output, and a single p-value (the degree of freedom will be `NHAP-1`). Also, there is no haplotype-specific output (e.g. haplotype names, frequencies or odds ratios):

```
NSNP NHAP  CHR          BP1          BP2       SNP1       SNP2     STAT        P
   2    2   22     15462210     15462259 rs11089263 rs11089264    0.693    0.405
   3    3   22     15688352     15690057   rs165650   rs165757     1.57    0.457
   5    5   22     15691787     15699058   rs175152   rs165914     5.08    0.279
   5    4   22     15902049     15939567  rs2845389  rs4819958      4.4    0.222
```

As mentioned above, covariates can be incorporated with the

```
--covar myfile.txt
```

command. Note that the coefficients and p-values for the covariates are not listed in these output files (unlike the default for the `--logistic` command).

Permutation procedures can be used, with the command

```
--mperm 10000
```

to specify, for example, ten thousand permutations. The empirical p-values from this analysis are listed in the file

```
plink.assoc.hap.logistic.mperm
```

Note that there will be no SNP name listed in the permutation output file: rather, it will be in the form:

```
 TEST         EMP1         EMP2
   T0       0.4158            1
   T1       0.1782            1
   T2       0.2475            1
   T3       0.1683            1
 ...
```

The number of rows, and the order of the output, will be the same as for the asymptotic results file, so they can be easily aligned. e.g. here `T0` would correspond to either the first omnibus test, or the first haplotype-specific test, `T1` the second, etc.

## 12.6 Haplotype-based TDT association test

If the case/control data are being analysed, use the option

```
plink --file mydata --hap myfile.hlist --hap-tdt
```

to test for TDT haplotype-specific association. This option generates the file

```
plink.tdt.hap
```

which contains the following fields:

```
LOCUS        Haplotype locus / window name
HAPLOTYPE    Haplotype identifer / "OMNIBUS"
T            Number of transmitted haplotypes
U            Number of untransmitted haplotypes
CHISQ        Test for association
P            Asymptotic p-value
```

## 12.7 Imputing multimarker haplotypes

If the `--hap-impute` option is also given, this will create two new files:

```
plink --file mydata --hap myfile.hlist --hap-impute
```

will generate the file:

```
plink.impute.ped
plink.impute.map
```

based on the most likely E-M phase reconstructed haplotypes. One could then simply treat the most likely haplotype assignments as SNPs and use all the standard analytic options of `PLINK`, e.g. `--assoc`.

**Warning** ***This represents a quick and dirty approach to haplotype testing***. Depending on how accurately the haplotypes have been imputed (i.e. the range of maximum posterior probabilities per individual) some bias will be introduced into subsequent tests based on these 'SNPs'. Typically, as long as cases and controls are phased together, as they are here, this bias is likely to be quite small and so should not substantively impact results (unpublished simulation results, SMP). Furthermore, exact methods can be used to refine the association for the putative hits discovered by this approach.

**NOTE** Future versions will allow for a binary PED file to be created from the `--hap-impute` command. You do **not** need to specify `--recode` when using `--hap-impute`.

## 12.8 Tabulating individuals' haplotype phases

To obtain a summary of all possible haplotype phases and the corresponding posterior probabilities (i.e. given genotype data), use the command:

```
plink --file mydata --hap myfile.hlist --hap-phase
```

which will generate the file

```
plink.phase-*
```

where `*` is the name of the 'window' (i.e. the row of the haplotype list file). That is, if the haplotype list contains multiple rows, then multiple phase files will be generated. These files contain the fields, where each row is one possible haplotype phase for one individual:

```
FID       Family ID
IID       Individual ID
PH        Phase number for that individual (0-based)
HAP1      First haplotype, H1
HAP2      Second haplotype, H2
POSTPROB  P(H1,H2 | G )
BEST      1 if most likely phase for that individual
```

# Chapter 13

# LD calculations

PLINK includes a set of options to calculate pairwise linkage disequilibrium between SNPs, and to present or process this information in various ways. Also see the functions on haplotype analyisis.

## 13.1 Pairwise LD measures for a single pair of SNPs

The command `--ld` followed by two SNP identifiers prints the following LD statistics to the LOG file, for a single pair of SNPs: r-squared, D', the estimated haplotype frequencies and those expected under linkage equilibrium, and indicates which haplotypes are in phase (i.e. occuring more often than expected by chance). For example:

```
plink --bfile mydata --ld rs2840528 rs7545940
```

gives the following output

```
LD information for SNP pair [ rs2840528 rs7545940 ]
   R-sq = 0.592     D' = 0.936
   Haplotype     Frequency    Expectation under LE
   ---------     ---------    --------------------
       GC          0.013           0.199
       AC          0.435           0.245
       GT          0.441           0.250
       AT          0.111           0.307
   In phase alleles are GT/AC
```

The LD statistics presented here are based on haplotype frequencies estimated via the EM algorithm. Only founders are used in these calculations.

## 13.2 Pairwise LD measures for multiple SNPs (genome-wide)

Correlations based on genotype allele counts (i.e. w/out phasing, and for founders only) can be obtained with the commands

```
plink --file mydata --r
```

or

```
plink --file mydata --r2
```

That is, this calculates for each SNP the correlation between two variables, coded 0, 1 or 2 to represent the number of non-reference alleles at each. The squared correlation based on genotypic allele counts is therefore not identical to the r-sq as estimated from haplotype frequencies (see above), although it will typically be very similar. Because it is faster to calculate, it provides a good way to screen for strong LD. The estimated value for the example in the section above (rs2840528,rs7545940) is 0.5748 (versus 0.592).

Both commands create a file called

```
plink.ld
```

with a list of R or R-squared values in it.

### 13.2.1 Filtering the output

By default, several filters on imposed on which pairwise calculations are calculated and reported. To only analyse SNPs that are not more than 10 SNPs apart, for example, use the option (default is 10 SNPs)

```
--ld-window 10
```

to specify a kb window in addition (default 1Mb)

```
--ld-window-kb 1000
```

and to report only values above a particular value (this only applies when the `--r2` and not the `--r` command is used) (default is 0.2)

```
--ld-window-r2 0.2
```

The default for `--ld-window-r2` is set at 0.2 to reduce the size of output files when many comparisons are made: to get all pairs reported, set `--ld-window-r2` to 0.

### 13.2.2 Obtaining LD values for a specific SNP versus all others

To obtain all LD values for a set of SNPs versus one specific SNP, use the `--ld-snp` command in conjunction with `--r2`. For example, to get a list of all values for every SNP within 1Mb of `rs12345`, use the command

```
plink --file mydata
      --r2
      --ld-snp rs12345
      --ld-window-kb 1000
      --ld-window 99999
      --ld-window-r2 0
```

The `--ld-window` and `--ld-window-r2` commands effectively means that output will be shown for *all* other SNPs within 1Mb of `rs12345`.

Similar to the `--ld-snp` command, but for multiple seed SNPs: to obtain all LD values from a group of SNPs with other SNPs, use the command

```
--ld-snp-list mysnps.txt
```

where `mysnps.txt` is a list of SNPs.

### 13.2.3 Obtaining a matrix of LD values

Alternatively, it is possible to add the `--matrix` option, which creates a matrix of LD values rather than a list: in this case, all SNP pairs are calculated and reported, even for SNPs on different chromosomes.

**Note** To force all SNP-by-SNP cross-chromosome comparisons with the standard output format (e.g. without `--matrix`) add the flag

```
--inter-chr
```

instead. This can be combined with `--ld-window-r2`, for example to list all inter-chromosomal SNPs pairs with very high R-squared values. **Warning**: this command could take an excessively long time to run if applied to large datasets with many SNPs.

## 13.3 Functions to select tag SNPs for specified SNP sets

The command

```
plink --bfile mydata --show-tags mysnps.txt
```

where `mysnps.txt` is just a list of SNP IDs, generates a file

```
plink.tags
```

that lists all the SNPs in the dataset that tag the SNPs in `mysnps.txt` (including the SNPs in the original file). A message is also written to the LOG file that indicates how many new SNPs were added

```
Reading SNPs to tag from [ mysnps.txt ]
Read 10 SNPs to tag, of which 10 are unique and present
In total, added 2 tag SNPs
Writing tag list to [ plink.tags ]
```

meaning that `plink.tags` will contain 12 SNPs. This command could be useful, for example, if one wants to generate a list of SNPs that tag all known coding SNPs, or a list of known disease-associated SNPs.

If the option

```
--list-all
```

is also added, then an additional file is generated that gives some more details for each target SNP (i.e. each SNP listed in `mysnps.txt`, in the above example) regarding how many and which tags were set for it. The file is named

```
plink.tags.list
```

and has the following fields

| | |
|---|---|
| SNP | Target SNP ID |
| CHR | Chromosome code |
| BP | Physical position (base-pair) |
| NTAG | Number of other SNPs that tag this SNP |
| LEFT | Physical position of left-most (5') tagging SNP (bp) |
| RIGHT | Physical position of right-most (3') tagging SNP (bp) |
| KBSPAN | Kilobase size of region implied by LEFT-RIGHT |
| TAGS | List of SNPs that tag target |

For example:

```
<small>
          SNP  CHR         BP NTAG       LEFT      RIGHT   KBSPAN TAGS
    rs2542334   22   16694612    2   16693517   16695440    1.923 rs415170|rs2587108
    rs2587108   22   16695440    2   16693517   16695440    1.923 rs415170|rs2542334
     rs873387   22   16713566    0   16713566   16713566        0 NONE
      rs11917   22   16717565    2   16717565   16742194   24.629 rs1057721|rs2075444
    rs1057721   22   16718397    2   16717565   16742194   24.629 rs11917|rs2075444
    rs9605422   22   16737494    0   16737494   16737494        0 NONE
    rs2075444   22   16742194    2   16717565   16742194   24.629 rs11917|rs1057721
    rs4819644   22   16744470    0   16744470   16744470        0 NONE
    rs2083882   22   16769795    0   16769795   16769795        0 NONE
    rs5992907   22   16796453    5   16796453   16830384   33.931 rs400509|rs396012|rs415651|rs384
     rs400509   22   16800853    3   16796453   16813039   16.586 rs5992907|rs396012|rs384215
     rs396012   22   16806587    3   16796453   16813039   16.586 rs5992907|rs400509|rs384215
    rs7293187   22   16807274    0   16807274   16807274        0 NONE
</small>
```

The settings for declaring that a SNP tags another SNP can be varied with the commands

```
--tag-r2 0.5
```

to specify a minimum r-squared (based on the genotypic correlation, see above); in this case it is set to a value of 0.5 as being necessary to declare that one SNP tags another (the default is 0.8). Also,

```
--tag-kb 1000
```

will constrain the search for tags to be within a megabase (the default is 250kb).

**HINT** If you specify the filename for the `--show-tags` command to be the keyword `all`, then PLINK will only generate the `plink.tags.list` file, but for all SNPs in the dataset. (This means that you cannot have a file actually called `all` used as the input for the `--show-tags` command of course).

**NOTE** You can add the `--tag-mode2` command to specify an alternative input and output format. In this case, we assume the input file contains two columns, with the second field being either 0 or 1 to indicate whether or not this is a target SNP:

```
rs00001  0
rs00002  0
rs00003  1
rs00004  0
rs00005  1
rs00006  0
```

The output is in a similar form, except that tagging SNPs will now have a `1` in the second field:

```
rs00001  0
rs00002  0
rs00003  1
rs00004  1
rs00005  1
rs00006  1
```

i.e. this above example would be equivalent to the original input file

```
rs00003
rs00005
```

and output file

```
rs00003
rs00004
rs00005
rs00006
```

indicating that SNPs `rs00004` and `rs00006` have been added as tags.

**NOTE** This function does not pick the minimal set of SNPs required to tag all common variation in a region, in the way tagging algorithms typically work (e.g. such as Tagger `http://www.broad.mit.edu/mpg/tagger/`). Rather, this utility function is designed merely to indicate which other SNPs tag a one or more of a pre-specified list of SNPs.

## 13.4 Haplotyp block estimation

The command

```
plink --bfile mydata --blocks
```

generates two files

```
plink.blocks
```

and

```
plink.blocks.det
```

Haplotype blocks are estimated following the default procedure in Haploview `http://www.broad.mit.edu/mpg/haploview/`.

The first file lists each block (2 or more SNPs) on a row, starting with an asterisk symbol (*), for example:

```
* rs7527871 rs2840528 rs7545940
* rs2296442 rs2246732
* rs10752728 rs897635
* rs10489588 rs9661525 rs2993510
```

This format can be used with the `--hap` command, for example to test each haplotype in each block for assocaition, or to estimate the haplotype frequencies: for example,

```
plink --bfile mydata --hap plink.blocks --hap-freq
```

The second file, `plink.blocks.det` is similar to the first, but contains some addition information:

| | |
|---|---|
| CHR | Chromosome identifier |
| BP1 | The start position (base-pair units) of this block |
| BP2 | The end position (base-pair units) of this block |
| KB | The kilobase distanced spanned by this block |
| NSNPS | The number of SNPs in this block |
| SNPS | List of SNPs in this block |

for example

```
CHR         BP1         BP2          KB  NSNPS SNPS
  1     2313888     2331789      17.902      3 rs7527871|rs2840528|rs7545940
  1     2462779     2482556      19.778      2 rs2296442|rs2246732
  1     2867411     2869431       2.021      2 rs10752728|rs897635
  1     2974991     2979823       4.833      3 rs10489588|rs9661525|rs2993510
  ....
```

Chapter 14

# Conditional haplotype-based association testing

This page describes `PLINK` functions that are aimed at dissecting a haplotypic association. These functions largely include and extend the functionality offered in the older WHAP `http://pngu.mgh.harvard.edu/~purcell/whap/` software package, which is no longer supported.

For reference, the main ways of specifying conditional haplotype tests, that modify the behaviour of main `--chap` command, are given here; they are also described in more detail below. Each row here is mutually exclusive, e.g. you would not want to, or be able to, specify `--control` and `--alt-snp` at the same time:

- Test whether SNPs have independent haplotyic effects (`--independent-effect SNP,SNP,SNP`)
- Test whether a set of SNPs explain an omnibus association (`--control SNP,SNP,...`)
- Test whether a specific set of haplotypes explain an omnibus association (`--control HAPLOTYPE,HAPLOTYPE,...`)
- Test specific haplotypes for association (`--specific-haplotype HAPLOTYPE`)
- Specify alternative and null haplotypic models in terms of sets of SNPs (`--alt-snp SNP,SNP-SNP,...` and/or `--null-snp SNP,SNP-SNP,...`)
- Specify alternative and null haplotypic models in terms of sets of haplotypes (`--alt-group HAPLOTYPE,HAPLOTYPE,...` and/or `--null-group HAPLOTYPE,HAPLOTYPE,...` )
- Test a one or more simple SNP effects, potentially controlling for haplotype effects (`--test-snp SNP,SNP-SNP,...`)

It is also possible to include one or more continuous or binary covariates, which can include other SNPs outside of the phased region.

This page contains the following sections:

- Basic usage
- Specifying the type of test
- General specification of haplotype groupings
- Including covariates and other SNPs

The value of using `--chap` over `--hap-assoc` is that covariates can be included, and more complex conditional tests can be specified. The value of using `--hap-assoc` (described here) over `--chap` is that it is designed to iterate over very many SNPs in a single go, whereas the `--chap` test is more designed to focus on one specific set of SNPs. The `--hap-logistic` and `--hap-linear` commands, described here, are also designed for large numbers of tests; they do allow for covariates and permutation, but not the conditional tests described below.

## 14.1 Basic usage for conditional haplotype-based testing

The `--chap` command is used in conjunction with the `--hap-snps` command to specify a set of SNPs to phase, form haplotypes and test for association (in samples of unreated individuals only):

```
plink --bfile mydata --hap-snps rs1001-rs1005 --chap
```

which generates a file

```
plink.chap
```

The `--hap-snps` command can take a comma-delimited list of SNPs, including ranges, e.g. if the MAP file specifies the following SNPs and physical positions:

```
1 rs1001 0 101200
1 rs1002 0 102030
1 rs1003 0 107394
1 rs1004 0 107499
1 rs1005 0 113990
```

then the command

```
--hap-snps rs1001-rs1003,rs1005
```

includes all SNPs except `rs1004`, for example. The hyphen/minus symbol specifies all SNPs within a range (based on sorted physical position).

**NOTE** No spaces are allowed in this kind of comma-delimited list. Also, note that currently this will not work if SNP names have hypen characters in them. In this case, to use a different delimter for any ranges specified on the command line, add the "–d" flag (which can be any non-whitespace character except a comma (although be cautious if using characters with special meanings on command lines)

```
--d + --hap-snps SNP-A10001+SNP-A10020
```

to obtain a range between `SNP-A10001` and `SNP-A10020`.

The default test is an *omnibus* haplotype test: that is, if there are *H* haplotypes, then `--chap` performs an *H-1* df test comparing the alternate (each haplotype having a unqiue effect) versus the null (no haplotypes having any different effect). In each case, one haplotype is arbitrarily chosen to be the reference haplotype. The coefficients must be interpreted with respect to that haplotype, but otherwise the coding makes no difference.

For binary disease traits, the test is based on a likelihood ratio test. For continuous traits, the test is based on an F-test comparing the alternate and null models. For continuous traits, the `--chap` command also displays the proportion of variance in the outcome explained by the regression model (R-squared) as well as an adjusted R-squared (that takes model complexity into account).

For example, here is a `plink.chap` output file representing a basic omnibus test:

```
+++ PLINK conditional haplotype test results +++
5 SNPs, and 6 common haplotypes ( MHF >= 0.01 ) from 32 possible
 CHR          BP          SNP   A1   A2          F
   1      101200       rs1001    C    A       0.45
   1      102030       rs1002    A    C     0.2362
   1      107394       rs1003    A    C     0.4325
   1      107499       rs1004    T    G     0.2362
   1      113990       rs1005    A    C     0.4487
Haplogrouping: each set allowed a unique effect
Alternate model
    AAATA    AACTA    CCCGA    ACAGC    CCCGC    ACCGC
Null model
    AAATA, AACTA, CCCGA, ACAGC, CCCGC, ACCGC
     HAPLO         FREQ        OR(A)        OR(N)
```

```
      -------       ------      -------      -------
        AAATA        0.169      (-ref-)      (-ref-)
        AACTA       0.0673        2.619         |
        CCCGA        0.212       0.8942         |
        ACAGC        0.264       0.6839         |
        CCCGC        0.237        1.025         |
        ACCGC       0.0502        1.038         |
      -------       ------      -------      -------
   Model comparison test statistics:
                             Alternate       Null
                  -2LL :         535.4      554.5
     Likelihood ratio test: chi-square = 19.11
                           df = 5
                           p = 0.001836
```

There are several points to note:

- At the top of the output, `PLINK` lists the SNPs (`SNP`) involved in the test, their chromosomal (`CHR`) and base-pair (`BP`) positions, their alleles (`A1` and `A2`) and the minor allele frequency (`F`).
- It is reported that there are 5 common haplotypes: this filter (default value of 0.01) can be changed by adding, for example, the `--mhf 0.05` command (minimum haplotype frequency).
- The next section presents the *haplogrouping* under the null and alternate models. If two haplotypes are in the same `set` , it means they are treated as identical in terms of their effect on phenotype (i.e. a single regression coefficient is used for that group). For the basic omnibus test the haplogrouping will always take this simple form: under the alternate all haplotypes in their own set, whilst under the null all haplotypes are in one set. This output is more useful in interpreting some of the other conditional haplotype tests that are introduced below.
- The next section contains the estimated regression coefficients for each haplotype under the alternate and null models, as well as the frequency (`FREQ`) of each haplotype. For continuous traits, the coefficients are labelled `BETA`; for disease traits they are labelled `OR` and are in fact transformed to be odds ratios, i.e. exp(beta). The `(-ref-)` indicates which haplotype has been selected to be the baseline, reference category. If a haplotype has instead a pipe (vertical bar) `|` symbol, it implies that this haplotype is grouped with the one above it (and so it will not have a regression coefficient of its own). In the case of this simple null model as shown here, this implies that all haplotypes are equated with `AAATA`, the reference haplotype (i.e. there is no effect of any haplotype).
- When the null model is not so straightforward (as in the examples below), the rows are separated into the null-model haplogroups for clarity. In this case, certain *sub-null* model comparisons are also presented, to the right of the table of coefficients: these are shown and described below.
- The final section presents the overall model statistics: for a linear trait these are the R-squared (sometimes called the coefficient of determination) and adjusted R-squared, as well as the F-test. For disease traits, as in this case, only the sample log-likelihood under each model (-2LL) and the likelihood ratio test are presented. In both cases, the degrees of freedom is the number of parameters in the alternate model minus the number in the null model.

The interpretation of this particular analysis would be that overall variation at this locus appears to influence the trait, with p = 0.001836. Using the commands introduced below, we can perform various conditional tests to explore this *omnibus* result.

**HINT** To obtain confidence intervals on the estimated odds ratios or regression coefficients, add the flag

```
--ci 0.95
```

for example; the output will now be as follows:

```
    HAPLO        FREQ                  OR(A)                   OR(N)
  -------      ------    ----------------------    ----------------------
    AAATA       0.169                  (-ref-)                 (-ref-)
    AACTA      0.0673        2.619 (1.24; 5.54 )                     |
    CCCGA       0.212        0.8942 (0.57; 1.4 )                     |
    ACAGC       0.264      0.6839 (0.438; 1.07 )                     |
    CCCGC       0.237        1.025 (0.657; 1.6 )                     |
    ACCGC      0.0502       1.038 (0.507; 2.12 )                     |
  -------      ------    ----------------------    ----------------------
```

## 14.2 Specifying the type of test

If no other commands are given, the `--chap` test will perform an omnibus haplotypic association test. Various other options can be used to refine the type of test. In this section we introduce three commonly used tests; in the section below we introduce a more general way in which any two (nested) models can be compared.

### 14.2.1 Testing a specific haplotype

It is possible to specify a particular haplotype to be tested against all others: for example, `CCCGA`

```
./plink --file mydata --hap-snps rs10001-rs10005 --chap --specific-haplotype CCCGA
```

This creates the following two haplogroupings:

```
Alternate model
    AAATA, AACTA, ACAGC, CCCGC, ACCGC    CCCGA
Null model
    AAATA, AACTA, CCCGA, ACAGC, CCCGC, ACCGC
```

which hopefully begins to indicate how these groupings should be interpreted in relation to the tests they imply.

The main body of the output is:

```
    HAPLO        FREQ      OR(A)      OR(N)
  -------      ------    -------    -------
    AAATA       0.169    (-ref-)    (-ref-)
    AACTA     0.06728       |          |
    ACAGC      0.2635       |          |
    CCCGC      0.2375       |          |
    ACCGC     0.05022       |          |
    CCCGA      0.2125     0.9153       |
  -------      ------    -------    -------
```

which shows that now under the alternate all haplotypes are grouped together except for `CCCGA`; versus all other haplotypes, this has an estimated odds ratio of 0.9153.

**NOTE** Of course, the estimated odds ratio for `CCCGA` was different in the first example given above (when it was 0.8942) because the reference category was different (it was then only `AAATA` as opposed to all other SNPs). In other words, remember that the odds ratios are only interpretable in relation to some specific baseline, reference category.

Finally, we see the model compariston test is non-significant

```
Likelihood ratio test: chi-square = 0.2653
                       df = 1
                       p = 0.6065
```

The option `--each-vs-others` will add an extra column to the output, if there is more than one haplotype-grouping under the alternate model, which provides p-values for haplotype-specific tests of that haplotye (or haplotype group) versus all others. For example,

```
./plink --file mydata --hap-snps rs10001-rs10005 --chap --each-vs-others
```

which produces output with the new `SPEC(A)` field

```
    HAPLO         FREQ        OR(A)      SPEC(A)        OR(N)
  -------       ------      -------    ---------      -------
    AAATA        0.169      (-ref-)        0.537      (-ref-)
    AACTA      0.06728        2.619    0.0001791         |
    CCCGA       0.2125       0.8942       0.6065         |
    ACAGC       0.2635       0.6839     0.003466         |
    CCCGC       0.2375        1.025       0.5132         |
    ACCGC      0.05022        1.038        0.787         |
  -------       ------      -------      -------    ---------
```

which contains p-values for all haplotype-specific tests (i.e. as above, the haplotype `CCCGA` has the p-value of 0.6065 as above, i.e. that haplotype versus all others). The benefit of the `--specific-haplotype` command versus `--each-vs-others` is that it also produces the odds ratio for that haplotype.

These *haplotype specific* tests are of course similar to the basic test given by the `--hap-assoc` command, e.g.

```
./plink --file mydata --hap-snps rs10001-rs10005 --hap-assoc
```

which generates the output file

```
plink.assoc.hap
```

which contains the line

```
 LOCUS  HAPLOTYPE    F_A    F_U   CHISQ  DF       P  SNPS
  WIN1      CCCGA  0.205   0.22  0.2689   1  0.6041  rs1001|rs1002|rs1003|rs1004|rs1005
```

This command frames the test in a slightly different way and presents different statistics (i.e. it does not use logistic regression, case and control frequencies are presented instead of odds ratios, etc) but the p-value is, as expected, very similar (p=0.6041 from `--hap-assoc` versus p=0.6065 from the `--chap` test). Note that they are not expected to be numerically identical however.

### 14.2.2 Testing whether SNPs have independent effects

It is possible to ask whether one or more SNPs have an effect that is independent of the other SNPs in the model, framing the question in terms of haplotypes. This conditional test essentially stratifies by the haplotyic background: for the SNP(s) under scruntiny, we only compare the alleles/haplotypes that have a similar haplotypic background.

Before proceeding to the conditional haplotype tests, let's first consider the simple, single SNP effects for the example dataset:

```
./plink --file mydata --assoc
```

which generates the file `plink.assoc` which is as follows:

```
 CHR     SNP       BP   A1      F_A      F_U   A2     CHISQ         P        OR
   1  rs1001   101200    C   0.4525   0.4475    A    0.0202     0.887      1.02
   1  rs1002   102030    A   0.2775    0.195    C     7.544   0.00602     1.586
   1  rs1003   107394    A    0.395     0.47    C     4.584   0.03228    0.7362
   1  rs1004   107499    T   0.2775    0.195    G     7.544   0.00602     1.586
   1  rs1005   113990    A   0.4825    0.415    C     3.644   0.05495     1.314
```

Here we see that SNPs `rs1002` and `rs1004` have the strongest associations, although `rs1003` and `rs1005` show marginal trends.

Next, to obtain a quick view of the LD in this small region, we can generate the matrix of r-squared (LD) values (i.e. note: this is using r-squared as a measure of LD, which is distinct from the coefficient of determination which descibes the fitted regression models).

```
./plink --file mydata --r2 --ld-window-r2 0
```

This command, by default, only outputs values for SNPs that have an r-squared greater than 0.2, are within 1 Mb and 10 SNPs of each other; these can be changed with the options `--ld-window-r2`, `ld-window-kb` and `--ld-window` respectively; in this case, we requested all SNPs to be reported with `--ld-window-r2`. The file

```
plink.ld
```

contains the fields

```
CHR_A    SNP_A  CHR_B    SNP_B          R2
    1   rs1001      1   rs1002     0.260769
    1   rs1001      1   rs1003     0.628703
    1   rs1001      1   rs1004     0.260769
    1   rs1001      1   rs1005  0.000357147
    1   rs1002      1   rs1003    0.0964906
    1   rs1002      1   rs1004            1
    1   rs1002      1   rs1005     0.398912
    1   rs1003      1   rs1004    0.0964906
    1   rs1003      1   rs1005   0.00919232
    1   rs1004      1   rs1005     0.398912
```

Here we see that `rs1002` and `rs1004` are in complete LD, but that there is also moderate (r-squared above 0.2) LD between many other pairs of SNPs.

Moving then to the conditional tests: using the dataset above, to test for an independent effect of `rs1003`, for example (independent of the haplotypic effects formed by the remaining SNPs), one would issue the command:

```
./plink --file mydata --hap-snps rs1001-rs1005 --chap --independent-effect rs1003
```

The haplogroupings implied by this command are

```
Alternate model
    AAATA    AACTA    CCCGA    ACAGC    CCCGC    ACCGC
Null model
    AAATA, AACTA    CCCGA    ACAGC, ACCGC    CCCGC
```

The test SNP, `rs1003`, is the middle SNP in the 5-SNP haplotype (an `A/C` SNP). In comparison to the alternate model, we now see that the null is formed by grouping two pairs of haplotypes; each pair is identical except for `rs1003`: i.e.

`AA`**A**`TA, AA`**C**`TA`

and

`AC`**A**`GC, AC`**C**`GC`

In each case here, the comparison between alternate and null models is to equate the effects of these haplotypes (i.e. implicitly providing a test for whether `rs1003` has any effect). A haplotype such as `CCCGA` is effectively left out of the analysis: although it contains a `C` allele for `rs1003`, we never see the corresponding `CCAGA` haplotype to perform a stratified analysis.

The main output for this test is shown below:

```
     HAPLO         FREQ        OR(A)         OR(N)    SUBNULL P
   -------       ------      -------       -------  -----------
```

```
          AAATA        0.169      (-ref-)      (-ref-)     0.008016
          AACTA      0.06728        2.619         |
          CCCGA       0.2125       0.8942       0.6907          n/a
          ACAGC       0.2635       0.6839       0.5628       0.2643
          ACCGC      0.05022        1.038         |
          CCCGC       0.2375        1.025       0.7897          n/a
        -------       ------      -------      -------  -----------
    Model comparison test statistics:
                                Alternate       Null
                       -2LL :       535.4      544.4
      Likelihood ratio test: chi-square = 8.982
                             df = 2
                             p = 0.01121
```

There are two new features to note: first, the null model is no longer a simple unitary group; the rows are separated out into the groups defined by the null model. That is, *null* does **not** mean *no effect of any haplotype*; rather, it is used in the statistical sense of the default, more simple model compared to the alternate: the model which we want to try to *nullify*.

Under the null, haplotypes `AAATA` and `AACTA` have a single parameter (both are the reference category); haplotypes `ACAGC` and `ACCGC` have an estimated odds ratio of 0.5628 (versus the reference group).

The second new addition is of the *sub-null* test p-values in the right-most column. These will only appear when the null model contains more than one group for which there was more than one group in the alternate model (i.e. groups in which haplotype effects have been equated within group). Whereas the likelihood ratio test at the bottom is a joint 2df test (for whether the two sets of haplotypes can be equated; equivalently, for whether `rs1003` has an independent effect), the sub-model p-values represent a test of just that part of the model, i.e. a 1 df likelihood ratio test for whether `AAATA` and `AACTA` do indeed have similar odds ratios has the p-value of 0.008016.

One way of interpreting these results would be that `rs1003` has an effect on the `AA-TA` haplotype background, but not the `AC-GC` background. However, drawing such a conclusion in this simple manner is not advised – p-values should not be interpreted in this direct manner, and also the power of the test will vary by the frequency of the haplotype background. ( A feature will be added that enables one to ask specifically whether or not the effect of `rs1003` varies between these two haplotype backgrounds: this involves the specification of linear constraints between parameters.)

Note that it is not always possible to perform a test of independent effects: for example, consider `rs1002`: given the set of common haplotypes under study, we see it is perfectly correlated with `rs1004` (i.e. we only ever see the `AT` and `CG` haplotypes for these two SNPs. We therefore never see both alleles of `rs1002` on the same haplotypic background. As such, the null model is the same as the alternate: PLINK therefore reports

```
      Likelihood ratio test:  ( not a valid comparison: identical models, df = 0 )
```

It is also possible to see whether more than one SNP has an independent effect: this is still a haplotypic test (of haplotypes formed by the two or more SNPs), but the test is stratified by the haplotypic background formed by the remaining SNPs. For example:

```
./plink --file mydata --hap-snps rs1001-rs1005 --chap --independent-effect rs1003,rs1004
```

leads to the haplogrouping

```
    Alternate model
        AAATA    AACTA    CCCGA    ACAGC    CCCGC    ACCGC
    Null model
        AAATA, AACTA    CCCGA    ACAGC, ACCGC    CCCGC
```

and the main test statistics

```
          HAPLO         FREQ        OR(A)        OR(N)    SUBNULL P
        -------       ------      -------      -------  -----------
```

```
      AAATA        0.169      (-ref-)      (-ref-)     0.008016
      AACTA      0.06728        2.619         |
      CCCGA       0.2125       0.8942       0.6907          n/a
      ACAGC       0.2635       0.6839       0.5628       0.2643
      ACCGC      0.05022        1.038         |
      CCCGC       0.2375        1.025       0.7897          n/a
    -------       ------      -------      -------  -----------
  Model comparison test statistics:
                               Alternate       Null
                      -2LL :       535.4      544.4
    Likelihood ratio test: chi-square = 8.982
                           df = 2
                           p = 0.01121
```

In this particular case, this test of independent effects of `rs1003` and `rs1004` happens to give exactly the same results as the test of `rs1003` by itself, which will be made clear from examining the haplogroupings. Note that, in both cases, the test is a two degree of freedom test.

### 14.2.3 Omnibus test controlling for X

To perform an omnibus test but controlling for a particular haplotype of set of haplotypes, you can use the `--control` command. The haplotypes can either be directly specified, or implied through the list of SNPs specified. This test is a complement to the `--independent-effect` test.

Typically, one would use this test in the case of a significant omnibus assocation result. For example, we could ask whether we still see the association even if we control for haplotypes of SNPs `rs1002` and `rs1004` (the two most highly associated SNPs, that are in complete LD with each other):

```
./plink --file mydata --hap-snps rs1001-rs1005 --chap --control rs1002,rs1004
```

which gives implied haplogroupings:

```
Alternate model
    AAATA    AACTA    CCCGA    ACAGC    CCCGC    ACCGC
Null model
    AAATA, AACTA   CCCGA, ACAGC, CCCGC, ACCGC
```

In this case, rather than make the null model a single set, the `--control` command separates the haplotypes out into distinct groups based on the sub-haplotypes at SNPs `rs1002` and `rs1004`, i.e.

A**A**A**T**A, A**A**C**T**A    C**C**C**G**A, A**C**A**G**C, C**C**C**G**C, A**C**C**G**C

The regression coefficient table is:

```
  HAPLO         FREQ        OR(A)        OR(N)    SUBNULL P
-------       ------      -------      -------  -----------
  AAATA        0.169      (-ref-)      (-ref-)     0.008016
  AACTA      0.06728        2.619         |
  CCCGA       0.2125       0.8942       0.6603       0.2087
  ACAGC       0.2635       0.6839         |
  CCCGC       0.2375        1.025         |
  ACCGC      0.05022        1.038         |
-------       ------      -------      -------  -----------
```

and model comparison statistics are:

```
                           Alternate       Null
                  -2LL :       535.4      547.7
Likelihood ratio test: chi-square = 12.32
                       df = 4
```

```
                                   p = 0.01515
```

This is a 4 df test because 4 haplotypes are grouped with another haplotype (i.e. the 4 | symbols in the output).

One would conclude from this analysis that there is still a significant effect at this locus even controlling from the haplotypic effects of `rs1002` and `rs1004`. In otherwords, the command

```
--control rs1002,rs1004
```

is identical to

```
--indepedent-effect rs1001,rs1003,rs1005
```

in this instance. Unlike the `--independent-effect`, the `--control` command does allow for hapltoype(s) to be specified, instead of SNPs: for example, we might ask whether the omnibus test is significant controlling for `ACAGC`:

```
./plink --file mydata --hap-snps rs1001-rs1005 --chap --control ACAGC
```

which gives the following haplogrouping

```
Alternate model
    AAATA    AACTA    CCCGA    ACAGC    CCCGC    ACCGC
Null model
    AAATA, AACTA, CCCGA, CCCGC, ACCGC    ACAGC
```

i.e., effectively leaving `ACAGC` out of the test, and this table of coefficients

```
     HAPLO          FREQ         OR(A)         OR(N)
   -------        ------       -------       -------
     AAATA         0.169       (-ref-)       (-ref-)
     AACTA       0.06728         2.619          |
     CCCGA        0.2125        0.8942          |
     CCCGC        0.2375         1.025          |
     ACCGC       0.05022         1.038          |
     ACAGC        0.2635        0.6839         0.624
   -------        ------       -------       -------
Model comparison test statistics:
                             Alternate       Null
                  -2LL :        535.4        546
  Likelihood ratio test: chi-square = 10.56
                         df = 4
                         p = 0.03194
```

In otherwords, there is still a marginal omnibus assocation (p=0.032) after controlling for `ACAGC`. Repeating this test for each haplotype:

```
 HAPLOTYPE (--control)      P-VALUE (omnibus association)
     AAATA                  0.0008895
     AACTA                  0.2803
     CCCGA                  0.0008441
     CCCGC                  0.0009084
     ACCGC                  0.0007738
     ACAGC                  0.03194
```

which would suggest that there is no significant signal after controlling for `AACTA`, at the p=0.05 level at least. This is consistent with the true model: these data are in fact simulated, and `AACTA` was in fact the disease haplotype.

Finally, it is possible to specify multiple, comma-delimited haplotypes for the `--control` command.

## 14.3 General specification of haplotype groupings

Rather than use any of the above *convenience* functions for specifying tests, one can directly specify the haplogrouping, in one of two ways: by manually specifying the haplotypes, or the SNPs, to include under both alternate and null models.

### 14.3.1 Manually specifying haplotypes

With the `--alt-group` and `--null-group` commands, it is possible to directly specify the haplogrouping. These commands take a comma-delimited list of *sets*, where the equals symbol is used to specify equality of haplotypes. For example, the command

```
--independent-effect rs1003
```

which gives rise to the following haplogroups

```
Alternate model
    AAATA    AACTA    CCCGA    ACAGC    CCCGC    ACCGC
Null model
    AAATA, AACTA    CCCGA    ACAGC, ACCGC    CCCGC
```

which could instead have been directly specified

```
--alt-group AAATA,AACTA,CCCGA,ACAGC,CCCGC,ACCGC
--null-group AAATA=AACTA,CCCGA,ACAGC=ACCGC,CCCGC
```

Note how the `=` symbol is used to define sets. When using these commands, the default for the alternate is as specified above, so this command could have been excluded. Also, it is not necessary to specify all haplotypes: if a haplotype is not specified, it will revert to its default grouping (i.e. depending on whether this is for the alternate or null). In other words, the same effect could have been achieved just with the single command

```
--null-group AAATA=AACTA,ACAGC=ACCGC
```

Finally, there are two *wild-cards*, one of which can be used in these two commands:

```
*    Group all haplotypes not otherwise explicitly mentioned
%    Separate all haplotypes not otherwise explicitly mentioned
```

In other words, implicitly there is always a base-line of

```
--alt-group %
--null-group *
```

To just equate two haplotypes, for instance, but keeping everything else the same, one might use

```
--null-group AAATA=AACTA,%
```

i.e. which means "under the null, allow each haplotype to have a unique effect (`%`), with the exception of `AACTA` and `AACTA`, which should be grouped with each other".

### 14.3.2 Manually specifying SNPs

With the `--alt-snp` and `--null-snp` commands, it is possible to specify which SNPs should be used to form haplotypes. By default, all SNPs are included in the alternate, no SNPs are included in the null: this leads to the default haplogrouping of the omnibus test.

To illustrate this command, by reference to the `--independent-effect` specification, for example: the command

```
--independent-effect rs1003
```

is equivalent to

```
--alt-snp rs1001-rs1005 --null-snp rs1003
```

## 14.4 Covariates and additional SNPs

Covariates can be included with the `--covar` option, the same as for `--linear` and `--logistic` models. By default, all covariates in that file with be used. Covariates always feature under both the alternate and null models.

```
./plink --file mydata --hap-snps rs1001-rs1005 --chap --covar myfile.cov
```

which generates an additional set of entries in the `plink.chap` output file, representing the coefficients (no other statistical tests are performed for the covariates, i.e. no p-values, etc):

```
COVAR                      OR(A)       OR(N)
-----                    -------     -------
 COV1                     0.7834      0.8499
```

In a similar manner, additional SNPs can be included, which can be SNPs other than those included in the `--hap-snps` command. These SNPs are not considered in any way during the phasing process: the alleles are simply entered in an allelic dosage manner. The command `--condition` and a list of SNPs, or `--condition-list` followed by a filename with a list of SNP names, includes these.

```
./plink --file mydata --hap-snps rs1001-rs1005 --chap --condition rs1006
```

which adds the following lines in the output file

```
 SNPS                      OR(A)       OR(N)
-----                    -------     -------
rs1006                     1.038       2.899
```

Unlike for standard covariates, it is also possible to request that a SNP effect be dropped under the null model, which allows, for example, for a test of a SNP controlling for a set of haplotypes at a different locus: here, one would want to include all haplotype effects under the null, and use the `--test-snp` command to drop one or more of the conditioning SNPs:

```
./plink --file mydata --hap-snps rs1001-rs1005 --chap --null-group % --condition
rs1006 --test-snp rs1006
```

which would instead show

```
 SNPS                      OR(A)       OR(N)
-----                    -------     -------
rs1006                     1.038   (dropped)
```

and an extra degree of freedom would be added to the model comparison test. As the `--null-group %` command was used to effectively control for all haplotypic effects whilst testing this particular SNP, `rs1006`, the test will be a 1 df test,

```
Likelihood ratio test: chi-square = 0.0007377
                       df = 1
                       p = 0.9783
```

It is also possible to specify more than one conditioning SNP (and to drop none, some or all of these under the null): for example,

```
./plink --file mydata --hap-snps rs1001-rs1005 --chap --null-group % --condition
rs1006,rs1007 --test-snp rs1006
```

## 14.5 General setting of linear constraints

*to be completed*

# Chapter 15

# Proxy association

This page describes a convenience function designed to provide a quick representation of a single SNP association, in terms of the surrounding haplotypic background. Specifically, given a particular (reference) SNP this approach involves a) finding flanking markers and haplotypes (proxies) that are in strong linkage disequilibrium with the reference SNP and, b) testing these proxies for association with disease, within a haplotype-based framework.

There are three main applications of this utility, which are described in more detail and with examples in the main text below:

- technical validation of single SNP results ( by looking for flanking haplotypes involving different markers that also show the same result )
- refining a single SNP association signal ( is there a stronger association with a local haplotype? )
- more robust single SNP tests ( by framing single SNP tests within a haplotypic framework, some degree of control against non-random genotyping failure can be achieved )

The proxy approach also forms the basis of the imputation methods in PLINK, described separately. The methods are identical in fact, the only difference in imputation mode is the presence of a reference set of individuals that is handled specially.

The proxy methods use the same basic EM algorithm used by the other haplotyping methods in PLINK. The only difference is that the proxy methods put a wrapper around the basic haplotyping procedure that a) provides some methods to automatically select proxies to phase given a designated reference SNP, and b) frames the subsequent tests and summaries in terms of groups of haplotypes that track the reference SNP.

## 15.1 Proxy association: basic usage

The basic proxy association method for a particular SNP is invoked with the `--proxy-assoc` option:

```
plink --file mydata --proxy-assoc rs6703905
```

which generates a file

```
plink.proxy.report
```

This file contains three main sections, describing the local flanking SNPs, haplotypes and "proxies" for the reference SNP, and will be described below in turn. The full output file is shown here:

```
*** Proxy haplotype association report for rs13232128 ***
       SNP       MAF      GENO       KB       RSQ       OR    CHISQ        P
 rs1389273     0.286  0.00173     -99.2    0.0932    0.916     2.61    0.106
rs10236783     0.253   0.0236     -66.9     0.214    0.875      5.7    0.017
```

```
rs17556689    0.328  0.00259    -66.7    0.282      1.1     3.09    0.079
rs17135491    0.153  0.00317    -2.59    0.153    0.934    0.955    0.328
rs13232128    0.494   0.0179        0        *    0.828     14.9 0.000112
 rs1826529    0.487  0.00461     9.72    0.674    0.883     6.58   0.0103
   ....*.       FREQ         OR      CHISQ          P
   GTTGAG     0.0171       1.02     0.0221      0.882
   AGTGAG     0.0166      0.876      0.712      0.399
   GGTGAG      0.103       0.91       1.56      0.212
   GGCAAG     0.0226       0.97     0.0475      0.827
   ATTAAG      0.111      0.853       4.96      0.026
   GTTAAG     0.0615      0.877       2.09      0.149
   AGTAAG     0.0557      0.881       1.73      0.188
   GGTAAG     0.0513      0.949      0.306       0.58
   GGCAGG     0.0365       1.39       7.56    0.00596
   ATTAAT     0.0233      0.825       1.78      0.183
   GGCAGT      0.249       1.05      0.893      0.345
   ATTAGT     0.0201       1.31       3.26     0.0711
   AGTAGT      0.049       1.08      0.741      0.389
   GGTAGT       0.13       1.16       5.17      0.023
Haplotype frequency estimation based on 6938 of 6938 founder chromosomes
Omnibus haplotype test statistic: 23.3, df = 13, p = 0.0377
Of 125 subhaplotypes considered, 8 met proxy criteria
      HAP       FREQ        RSQ         OR      CHISQ          P
   ..T. G      0.422       0.72      0.843       12.3   0.000449
   .G.. T      0.453      0.705       1.15       8.62    0.00333
   .G.A T      0.445      0.693       1.14       7.22     0.0072
   GG.. T      0.399      0.561       1.14       7.15     0.0075
   .... G      0.487      0.674      0.883       6.58     0.0103
   ...A T      0.505      0.661       1.12       5.56     0.0184
   G... T      0.415      0.542       1.11       4.99     0.0255
   G..A T      0.408      0.535        1.1       4.19     0.0408
```

The first section lists the reference SNP (rs13232128) and 5 flanking SNPs that have been automatically selected as proxies. For each SNP, the minor allele frequency (MAF), genotyping failure rate (GENO) and distance to the reference SNP (KB) is given. A measure of single SNP association is also given for each SNP: odds ratio (OR), chi-squared statistic (CHISQ) and asymptotic p-value (P).

***Importantly***, however, these single SNP tests are not quite the same as from the basic `--assoc` command, as they are formed within the haplotypic context of the flanking SNPs. That is, for example, a single SNP test of the 5th SNP is formed by grouping the haplotypes as shown below, and testing for a difference in the frequency of the first group (containing `A` at the 5th position) versus the second group (all containing `G`).

GTTG-**A**-G
AGTG-**A**-G
GGTG-**A**-G
GGCA-**A**-G
ATTA-**A**-G
GTTA-**A**-G
AGTA-**A**-G
GGTA-**A**-G
ATTA-**A**-T
 *versus*
GGCA-**G**-G
GGCA-**G**-T

```
ATTA-G-T
AGTA-G-T
GGTA-G-T
```

Because the test is conducted in the context of a haplotypic test, it has some slightly different properties to the standard association test, which can sometimes be used to advantage. In particular, when there is strong LD in the region, the haplotype information will often help to fill in missing genotype data for single SNPs. Therefore, rather than throwing away individuals with missing genotype data, it is possible to try to reconstruct it from the surrounding region: this can lessen the impact of non-random genotyping failure causing spurious associations, as described below.

In this example, note that no other surrounding SNPs appear to show strong association with disease, compared to the reference SNP: when looking at the pattern of LD (RSQ column) we see that there are no SNPs with very high LD (e.g. over 0.8) to the reference SNP, so this is not necessarily surprising. Other *haplotypes* might be, however: this is what the rest of the report considers. The second section lists the haplotypes formed in that region given all flanking proxy SNPs (including the reference SNP) and the frequency and association with disease of each of these haplotypes.

Finally, the third part of the report contains that information on single proxies SNPs or haplotypes of two of more proxies (subhaplotypes) but excluding the reference SNP, that are in LD with the reference SNP; this list is sorted by strength of association with disease and filtered by other criteria, described below. For example, the first line in the above example is:

```
      HAP       FREQ        RSQ         OR      CHISQ          P
    ..T. G     0.422       0.72      0.843       12.3   0.000449
```

This suggest although no single SNP shows a similar association to the reference SNP in this region, a haplotype does show association results of a similar magnitude and is correlated with the reference SNP (in this case, the `TG` haplotype formed by the third and last proxy SNPs).

So, in this particular example, this might be taken as additional support for the association: it is of course still possible that the association is just due to chance, or due to population stratification, etc, but this would suggest that it is unlikely to be due to some technical genotyping artefact that was specific to the reference SNP, as we are also seeing the same signal from other SNPs (or, as in this case, a haplotype formed from two other SNPs).

Naturally, if one considers enough proxy haplotypes, some are bound to show stronger association with disease than the reference SNP merely due to chance. One should therefore be careful in how these tests are interpreted, i.e. not to forget the multiple testing that is implicit here.

This kind of analysis represents the typical use case for proxy association: we may have a single SNP association result, but the SNP might be rare or have a higher genotyping failure rate than we would like. Rather than exclude that SNP altogether, one option is to include the SNP in analysis, assess evidence for assocation, and then also ask whether other SNPs show the same signal. The assumption is that although the true alleles at the proxy SNPs are (hopefully) not independent of the reference SNP (i.e. there is LD) any technical genotyping artefact that influenced the reference SNP is unlikely to also be impacting the proxy SNPs (i.e. the implicit model of genotyping failure is that most SNPs are okay, but a few SNPs might fail: as such, we can use the surrounding genotype data to fill-in failed genotypes, even if these SNPs failed in a very biased way, e.g. if only `TT` homozygotes tended to fail and only in cases).

### 15.1.1 Heuristic for selection of proxy SNPs

The main parameters for SNP selection are:

- LD thresholds between the index and proxies, and between the proxies themselves
- Maximum number of SNPs and kb range to search for proxies
- Maximum number of proxies to include

There are four main commands to influence the search strategy for proxies:

```
--proxy-r2        A  B  C
--proxy-window    # SNPs to search
--proxy-kb        kb distance
--proxy-maxsnp    # SNPs to include in final set
```

Proxies are chosen based on LD with the reference SNP as follows. Proxies are examined one at a time in order of strongest to weakest LD with the reference. A proxy must be above a certain minimum r-squared threshold with the reference (criterion *A*), although if we already have two proxies selected, a different threshold is used (criterion *B*). In both cases, for a proxy to be added, it must not have an r-squared greater than criterion *C* with any proxy already selected. For common SNPs, the default values for A, B and C are:

```
Is a proxy?
      A) r-sq > 0.00 with reference          if < 2 proxies selected
      B) r-sq > 0.25 with reference          if 2 or more proxies selected
      C) r-sq < 0.50 with any other proxy
```

Setting *A* lower than *B*, and to 0 by default, ensures that we always allow a chance of finding a 2-SNP haplotype that might tag the reference SNP, even if no single SNP does.

By default, proxy association selects up to 5 (`--proxy-maxsnp`) SNPs flanking the reference SNP, from a search of 15 SNPs (`--proxy-window`) either side of the reference, at most 250 kb away (`--proxy-kb`).

The defaults vary depending on the frequency of the index SNP: for rarer SNPs (MAF less than 0.1), a slightly larger search space will be used. This threshold can be changed with the command

```
--proxy-b-threshold 0.05
```

In contrast to common SNPs, for which the defaults are:

```
--proxy-r2        0.00  0.25  0.50
--proxy-window    15
--proxy-kb        250
--proxy-maxsnp    5
```

these values for rarer SNPs (as defined by `--proxy-b-threshold`) and the commands that can be used to change them:

```
--proxy-b-r2        0.00  0.01  0.50
--proxy-b-window    30
--proxy-b-kb        500
--proxy-b-maxsnp    510
```

In other words, the search space is increased for rarer SNPs, to increase the chance that a good haplotypic proxy is found even if there is no other single SNP that well captures the variation at the index site.

In addition, proxies must by default be above 0.01 MAF and below 0.05 genotyping failure rate. To explicitly select only more common proxies with very high genotyping rate (e.g. to verify association at a reference SNP with lower genotyping rate and a very rare allele), then set values for

```
--proxy-maf
```

and

```
--proxy-geno
```

¡/tt¿ appropriately (these mirror the basic `--maf` and `--geno` commands).

Finally, there are some parameters that determine the behavior of the haplotypic proxy search (the 3rd section of the verbose output). Haplotypes formed by proxies must have a frequency of at least 0.01; these haplotypes must show an r-squared of at least 0.5 with the reference; when considering all possible subhaplotypes, only permutations of up to 3 SNP-haplotypes are considered.

Overall, it is possible to change the behaviour of the basic proxy selection heuristic with the following commands:

- to select a different number of flanking SNPs (`--proxy-window`)

- to filter proxy SNPs on distance to reference (`--proxy-kb`)
- to specify the maximum number of proxies (`--proxy-maxsnp`)
- to filter proxy SNPs on LD with reference (`--proxy-r2`)
- to not filter proxy SNPs on LD (`--proxy-no-r2-filter`, i.e. same as `--proxy-r2 0 0 1`)
- to filter proxy SNPs on MAF (`--proxy-maf`)
- to filter proxy SNPs on genotyping rate (`--proxy-geno`)
- to select a specifc set of flanking SNPs (`--proxy-flanking`)
- to filter haplotypes based on frequency (`--proxy-mhf`)
- to filter haplotypes based on LD with reference (`--proxy-sub-r2`)
- to select different levels of subhaplotype search (`--proxy-sub-maxsnp`)

For example, to select up to 6 SNPs, that are above 0.10 MAF and 0.01 genotyping failure rate, that are within 100 kb and 10 SNPs of the reference SNP and that have an r-squared of at least 0.1 with the refernce but no greater than 0.5 with an already-selected proxy SNP; and then to look at all haplotype proxies that are above 0.005 minor haplotype frequency and have an r-squared of at least 0.8 with the reference SNP, use the command (line breaks added for clarity):

```
plink --file mydata --proxy-assoc rs6703905
                    --proxy-maxsnp 6
                    --proxy-r2 0.1 0.1 0.5
                    --proxy-window 10
                    --proxy-kb 100
                    --proxy-maf 0.1
                    --proxy-geno 0.01
                    --proxy-sub-r2 0.8
                    --proxy-mhf 0.005
```

As mentioned, rather than use the heuristic above, you can specify a particular set of SNPs with the command

```
plink --file mydata --proxy-assoc rs6703905 --proxy-flanking my.proxy.list
```

where `my.proxy.list` is a file listing the SNPs you wish to use as proxies for `rs6703905`, for example.

**Warning** There will possibly be a very, very large number of possible combinations to consider if you make both `--proxy-maxsnp` and `--proxy-sub-maxsnp` too large, meaning that the analysis will take a very long time to run. You should probably keep `--proxy-maxsnp` less than 10 and `--proxy-sub-maxsnp` less than 6.

**HINT** To speed up the proxy report, you need only load in the relevant chromosomal region: that is, use the `--snp` and `--window` options:

```
plink --bfile mydata --proxy-assoc rs12345 --snp rs12345 --window 300
```

### 15.1.2 Specifying the type of association test

By default, the `--proxy-assoc` command only applies to population-based samples of unrelated individuals. It is suitable for either disease (case/control) or quantitative trait outcomes: the appropriate test will automatically be selected depending on the phenotype.

The basic command cannot include covariates: however, if the flag `--proxy-glm` is added, then the routines that correspond to `--linear` and `--logistic` are used instead to test the proxy association, meaning that covariates can be included (this is slightly slower than the default analysis), e.g.

```
plink --bfile mydata --proxy-assoc rs12345 --proxy-glm --covar mycov.txt
```

**BETA** There is preliminary support for the TDT in this context with the `--proxy-tdt` option; this has not yet been fully tested however, and we do not yet suggest you use it generally.

## 15.2 Refining a single SNP association

The proxy association report is primarily designed simply to provide a convenient way to automatically scan for evidence of the same association signal coming from different sets of markers (that are assumed to be independent in terms of technical artefact but not LD). Of course, it is entirely possible that a 'proxy' may show a markedly stronger association than the original reference SNP. In this way, one might think of using the `--proxy-assoc` method as a way to refine an association signal, or fine-map a region. In a whole genome context, there is clearly nothing special about the particular SNP genotyped that shows association: it may be representing just the tip of an iceberg in association space, and certain haplotypes might have a stronger association. One strategy and way of using haplotype inflormation in a whole genome context, therefore, might be to scan all single SNPs for modest levels of association, and then exhaustively search the haplotype space surrounding those SNPs, *but constraining the search to only haplotypes that are in LD with the original SNP* (in this way, keeping the multiple testing burden somewhat under control, as although many more tests are added, they will all be quite highly correlated).

As implied in the section above, remember that taking just the best proxy association result (i.e. the top listed in the 3rd section of the report) will capitalize on chance and so these best values will not follow asymptotic null test statistic distributions. These p-values are perhaps best interpreted either against a set genome-wide significance threshold, or corrected for the number of subhaplotypes tested for a given reference SNP.

## 15.3 Automating for multiple references SNPs

To faciliate looking at more than one reference SNP at a time, you can use the command

```
plink --bfile mydata --proxy-assoc all
```

or

```
plink --bfile mydata --proxy-assoc all --proxy-list hits.list
```

That is, instead of a SNP name after `--proxy-assoc`, put the keyword `all`. PLINK will then treat as the reference, one at a time, either all SNPs in the dataset (first usage) or in the subset listed in the file `hits.list` (second usage).

By default, only a restricted degree of output is given, and no "subhaplotype" tests are performed when more than one SNP is specified as the reference (i.e. these correspond to the third section in the above example output). To get the full report for every SNP (all listed in a single file) add the option

```
--proxy-verbose
```

In non-verbose mode, the output is as follows, in a file

```
plink.assoc.proxy
```

with fields

| | |
|---|---|
| CHR | Chromosome code |
| SNP | Reference SNP |
| BP | Physical position |
| A1 | Name of first allele |
| A2 | Name of second allele |
| GENO | Genotyping for the reference SNP |
| NPRX | Number of proxy SNPs used to tag reference SNP |
| INFO | Information metric for each reference SNP |
| F_A | Reference SNP allele frequency in cases (disease traits) |
| F_U | Reference SNP allele frequency in controls (disease traits) |
| OR | Odds ratio (for disease traits) |
| P | Asymptotic p-value for test of association |
| PROXIES | (Optional, given --proxy-show-proxies) Displays actual proxy SNPs used |

For, example, here are some lines from such an output file, in this case with the

```
--proxy-show-proxies
```

flag added, which appends the final `PROXIES` field to the output (lines truncated)

```
CHR        SNP       BP A1 A2    GENO NPRX  INFO    F_A    F_U   OR     P PROXIES
 17   rs731971 29529017  T  A 0.00605    3  1.01  0.103  0.104 1.02 0.849 rs4794990|rs11652429|...
 17  rs4794990 29529302  T  C 0.00346    3  1.01  0.102  0.104 1.02 0.838 rs731971|rs11652429|...
 17 rs12938546 29530562  C  A 0.00115    3 0.996 0.0322 0.0381 1.19 0.186 rs7359592|rs887071|...
 17 rs11652429 29531710  G  C 0.00115    5     1   0.32   0.32    1 0.984 rs11080256|rs1024613|...
```

The p-values reported here take account of the fact that the SNP has been probabilistically reconstructed. For example, the first line indicates that for `rs731971` three proxy SNPs were selected, `rs4794990`, `rs11652429` and `rs887071`.

The `GENO` and `INFO` have more meaning in the context of *imputation*, as described here, which involves running proxy association/imputation with a reference panel, such as the HapMap.

## 15.4 Providing some degree of robustness to non-random genotyping failure

When performing tests in a haplotype-context, the E-M algorithm is used to estimate haplotpe frequencies and each individual's posterior haplotype phase probabilities. The association test is then based on these fractional counts (i.e. allowing for ambiguity in inferred haplotypes). As such, missing genotypes are quite naturally accommodated in this framework: if for example an individual has genotypes for these 3 SNPs, then two haplotype phases are considered:

```
Observed           Possible
genotypes    -->   haplotypes
A/C A/C G/G  -->   AAG / CCG
                   ACG / CAG
```

whereas if the third SNP has a missing genotype (and if the other allele is `T`, for example) then the standard approach is just to consider a larger, consistent set (which are of course weighted by the current estimate of the population haploytpe frequencies):

```
Observed           Possible             Possible
genotypes    -->   genotypes     -->    haplotypes
A/C A/C 0/0  -->   A/C A/C G/G   -->    AAG / CCG
                                        ACG / CAG
```

```
-->   A/C A/C T/T   -->   AAT / CCT
                          ACT / CAT
-->   A/C A/C G/T   -->   AAG / CCT
                          ACG / CAT
                          AAT / CCG
                          ACT / CAG
```

In this way, ***if*** there is strong LD between SNPs, we can use the genotypes at flanking SNPs to effectively 'fill-in' missing genotype data. One advantage of this is that, if the genotypes are not missing at random for any given SNP, then it can give a less biased test to fill in the true values using LD information, rather than just to treat those genotypes as missing. This motivates a reframing of the basic single SNP association statistic in terms of groups of haplotypes rather than just as single SNPs (as shown above in the first example). Consider this example, involving simulated data, where the following haplotypes were simulated with these frequencies (in both cases and controls, so we would not expect any association with disease; 500 cases and 500 controls were generated).

| Haplotype | Population frequency |
|---|---|
| AABAB | 0.4 |
| AABBA | 0.2 |
| ABBBA | 0.2 |
| BBBBB | 0.1 |
| AAABB | 0.1 |

We will label the five SNPs, `snp1`, `snp2`, etc. Some non-random genotyping failure was simulated: in cases only, the `BB` genotype of `snp3` only has a genotyping rate of 0.5 (i.e. half were set to missing). Such as pattern of genotyping failure, which is non-random with respect to both phenotype and genotype, can tend to produce spurious association results. For example, here are the basic single SNP results:

```
plink --file sim1 --assoc
```

which gives the output

```
CHR  SNP   A1    F_A     F_U   A2      CHISQ          P          OR
  1 snp1    B   0.102   0.106   A    0.08585     0.7695       0.958
  1 snp2    B   0.297    0.31   A     0.3997     0.5272      0.9403
  1 snp3    A  0.1812   0.118   B      12.02  0.0005271       1.654
  1 snp4    A   0.406   0.383   B      1.107     0.2927       1.101
  1 snp5    A   0.388   0.393   B    0.05252     0.8187      0.9792
```

Note how `snp3` shows a strong association (this is solely due to the non-random drop-out of genotypes for this SNP). However, the proxy association will, in this case, correct this:

```
plink --file sim1 --proxy-assoc snp3 --mind 1 --geno 1
```

Note that we use `--mind` and `--geno` to ensure that PLINK does not discard any individuals, in this particular case (i.e. we will use the flanking SNPs to fill in the missing data). This analysis gives the following output

```
      *** Proxy haplotype association report for snp3 ***
 SNP      MAF     GENO       KB      RSQ       OR    CHISQ        P
snp1    0.104        0   -0.002   0.0145    0.958   0.0859     0.77
snp2    0.303        0   -0.001   0.0544     0.94      0.4    0.527
snp3    0.141    0.213        0        *    0.868    0.993    0.319
snp4    0.394        0    0.001   0.0813      1.1     1.11    0.293
snp5     0.39        0    0.002     0.08    0.979   0.0525    0.819
         ..*..       FREQ         OR      CHISQ          P
         ABBBA      0.199      0.945      0.254      0.615
         AABBA      0.191       1.03     0.0518       0.82
```

```
          AABAB      0.394        1.1        1.11      0.293
          AAABB      0.111      0.868       0.993      0.319
          BBBBB      0.104      0.958      0.0859       0.77
Haplotype frequency estimation based on 2000 of 2000 founder chromosomes
Omnibus haplotype test statistic: 1.88, df = 4, p = 0.759
            HAP       FREQ        RSQ          OR      CHISQ          P
          .A BB      0.111          1       0.868      0.993      0.319
          A. BB      0.111          1       0.868      0.993      0.319
```

In otherwords, instead of removing individuals who are missing for `snp3` (which is implicitly what a single SNP association statistic would do) we use the flanking data to fill in the unobserved genotypes. Even if these are misssing not-at-random, if there is strong LD then we will often be able to do a good job at guessing the true genotype. Note that the other SNPs (that have no missing genotype data) have identical association p-values under basic association test as under this constrained haplotype test, as would be expected (i.e. under most normal conditions, there is no loss of power in using a proxy-association approach).

**IMPORTANT** It is very important to remember that this test is not a panacea for the problem of missing data: many times there will not be sufficient LD to accurately reconstruct the missing genotype within the E-M. Future versions of PLINK aim to add diagnostics to indicate when this is the case; also, one might select the SNPs that define the flanking region more intelligently (e.g. making use of known patterns of LD, etc).

As such, thie results of this test should most probably be interpreted as follows: if a highly significant basic single SNP association result is not significant by this method, one would worry about biased missingness for that SNP; if a highly significant basic single SNP result remains highly significant, this is only meaningful when there is strong LD.

Of course, it is possible that other biases that are specific to haplotype analysis (the ability to estimate rare haplotype frequencies, etc) will impact these proxy tests, the effects of stratification may be more pronounced, etc. As such, these tests should be interpreted only as complementary pieces of information along with the basic SNP result, rather than as water-tight proof of an unbiased association *per se*.

However, if one knew up front that non-random genotyping drop-out might be an issue (for example, cases and controls from from different labs, different genotyping procedures used, etc) then it might seem prudent to take this approach.

**Note** Normally individuals are removed from the haplotype analysis if they are missing more than 50% of their genotypes for a given haplotype: in this case, we try to not remove individuals, but rather let the E-M fill in the missing data, so the rate is changed to 0.9 by default; this can be altered with the `--hap-miss` option.

# Chapter 16

# SNP imputation and association testing

This page describes PLINK functions to impute SNPs that are not directly genotyped but are present on a reference panel such as the HapMap. As well as imputing genotypes (either making the most likely call, or outputting the posterior probabilities of each genotype, or the dosage) some simple association tests can be framed in this context. These methods do not necessarily need whole-genome data to work however: with dense SNP genotyping in a particular region, these methods could still straightforwardly be applied.

These methods utilise the proxy association set of commands. The approach is a simple one, essentially based around the concept of multi-marker tagging. This approach is designed to provide a straightforward albeit *quick and dirty* approach to imputation for common variants: it is unlikely to be optimal, particularly for rarer alleles, when compared to other imputation methods available.

In the text below, an *observed* SNP refers to one that was genotyped in both the reference and the WGAS sample. An *imputed* SNP refers to one that only appears in the reference panel.

**IMPORTANT** These features are still in *beta* meaning that they are still actively being updated, optimised, changed and fixed. As such, you are advised only to use these routines in an exploratory manner, if at all. More details regarding the specific procedures, and interpretation of results, will be posted presently. That is, rather than e-mail for more details, please wait until they are posted here.

## 16.1 Basic steps for using PLINK imputation functions

The first step is to create a single fileset with the reference panel merged in with your dataset. We assume that the HapMap CEU founders will be used in this example.

**HINT** A PLINK binary fileset of the Phase 2 HapMap data can be downloaded from here. For studies of individuals of European ancestry, the CEU founder fileset will be the one to download from that link.

Given the HapMap data, `hapmap-ceu.*` or `hapmap-ceu-all.*`, for example, you merge in your WGAS data as follows,

```
./plink --bfile hapmap-ceu --bmerge mydata.bed mydata.bim mydata.fam --make-bed --out
merged
```

In imputation mode, the reference panel is denoted by making those individuals have a ***missing value for the phenotype***. You will therefore need to edit the `.fam` files to make the 6th column (phenotype) `0` for all HapMap individuals and `1` (control) or `2` (case) for the individuals in your sample. If you have trio data, make sure that no observed individuals have missing phenotypes (i.e. set parents to controls in a TDT context, rather than have a missing phenotype code).

### 16.1.1 Strand issues

The HapMap SNPs are all given on the +ve strand, and so it is your responsibility to ensure that your data are aligned also, for the merge to work. The `--flip` command can help changing strand. If there are strand problems, PLINK will report a list of SNPs that did not match in terms of strand. Naturally, if there are SNPs `A/T` or `C/G` SNPs in your dataset, these will potentially go unflagged. As such, it is always a good idea to check allele frequencies between the HapMap and the WGAS sample to identify grossly deviant SNPs and/or undetected strand issues (i.e. create an alternate phenotype file with the HapMap individuals coded as controls and the rest of WGAS data as cases, and run a basic association command). The `--flip-scan` command can also help to detect some incorrectly aligned variants.

**NOTE** This will create a **very large** dataset and take some time; particularly if you have a parallel computing environment available, you might want to split the files and the merge procedures up by chromosomes, e.g. first download the archive with the HapMap CEU founder fileset split by chromosome, then merge each chromosome separately:

```
./plink --bfile mydata --chr 1 --make-bed --out data-1

./plink --bfile mydata --chr 2 --make-bed --out data-2
```

etc, followed by

```
./plink --bfile hapmap-ceu-chr1 --bmerge data-1.bed data-1.bim data-1.fam --make-bed
--out merged-1

./plink --bfile hapmap-ceu-chr2 --bmerge data-2.bed data-2.bim data-2.fam --make-bed
--out merged-2
```

This will create 22 separate filesets (`merged-1`, `merged-2`, etc) and all the following routines can then be run separately on each.

## 16.2 Combined imputation and association analysis of case/control data

Given the merged fileset, containing both the reference panel and the (more sparse) WGAS samples, PLINK will attempt to perform case/control association for every SNP (both observed and imputed) with the following command:

```
./plink --bfile merged-1 --proxy-assoc all
```

which will generate an output file

```
plink.assoc.proxy
```

with the fields

| | |
|---|---|
| CHR | Chromosome code |
| SNP | SNP identifier |
| BP | Physical position (base-pairs) |
| A1 | First allele code (not necessarily minor allele) |
| A2 | Second allele code (not necessarily major allele) |
| GENO | Genotyping rate in entire sample *and* reference panel |
| NPRX | Number of proxy SNPs selected |
| INFO | Information content metric |
| F_A | Allele 1 frequency in cases |
| F_U | Allele 1 frequency in controls |

|  |  |
|---|---|
| OR | Odds ratio |
| P | Significance value of case/control association test |

The fields INFO and NPRX refer to how well PLINK managed, if at all, to impute the SNP. If NPRX is zero, then it could not be even poorly imputed. If INFO ranges from between 0 and 1, although it can be greater than 1 occasionally. A higher value general means a better imputed SNP; roughly speaking, only looking at imputed SNPs with a INFO value greater than 0.8 or so is probably good practice. More specific details on these metrics will be posted soon.

## 16.3 Modifying options for basic imputation/association testing

One of the most important modofying options for the --proxy-assoc test is --proxy-drop, which means that the *observed* SNPs are dropped, one at a time, from the WGAS sample when they are tested as the reference SNP (i.e. they will be re-imputed given the surrounding SNPs). That is, the command,

```
./plink --bfile merged-1 --proxy-assoc all --proxy-drop
```

would mean that every single SNP test statistic in plink.assoc.proxy would not involve a single observed genotype for that particular SNP; as such, running this association test with the --proxy-drop command is a good idea as it will provide both a means to assess the performance of the imputation (by comparing the results against the results of the observed genotypes) but also of an extra level of QC (if you still see a significant result, it cannot be due to technical artifacts specific to that SNP, as no observed genotypes were used in the test for that SNP).

The value of not using --proxy-drop always with --proxy-assoc (given that the basic --assoc command more straightforwardly calculates association for observed SNPs) is if there is a reasonable amount of missing genotype data for an observed SNP and you want to use imputation to recover it. (Although, in this case, there is perhaps less need to use a separate reference panel in any case, and so the standard proxy association approach, without any reference panel, can be used.)

### 16.3.1 Parameters modifying selection of proxies

Imputation in this context works simply by selecting a set of proxy SNPs (using the reference panel information) and then phasing these SNPs in both reference panel and WGAS sample jointly. By grouping haplotypes, the corresponding single SNP tests of *imputed* SNPs can then be straightforwardly performed.

There are a number of parameters that impact the choice of proxy SNPs. Fine tuning of these parameters is still in progress. These parameters will be described in more detail shortly. For now, the default parameters should be sufficient in most cases. See the proxy association page for a description of the parameters, the defaults, and how they can be changed.

## 16.4 Imputing discrete genotype calls

The association test described above performs imputation on-the-fly and does not save the imputed genotype calls or probabilities. To do so, and to generate other metrics of imputation performance, use the --proxy-impute command.

To generate summary statistics for the imputation performance of each SNP, use the command

```
./plink --bfile merged-1 --proxy-impute all
```

which produces a file

plink.proxy.impute

which has the fields

|  |  |
|---|---|
| CHR | Chromosome |

| | |
|---|---|
| SNP | SNP ID |
| NPRX | Number of proxy SNPs |
| INFO | Information metric |
| TOTAL_N | Total number of WGAS sample genotypes (exc. reference panel) |
| OBSERVD | Proportion of these w/ observerd genotypes |
| IMPUTED | Proportion of these imputed |
| OVERLAP | Proportion of SNPs with both an imputed and overlapping |
| CONCORD | Concordance rate in the overlapping set |

Here are some example lines:

```
 CHR            SNP NPRX     INFO  TOTAL_N  OBSERVD  IMPUTED  OVERLAP  CONCORD
  18      rs7233673    5    0.993     3469        0    0.991        0       NA
  18      rs7233597    5    0.998     3469    0.999    0.993    0.992    0.986
  18      rs7505507    4    0.632     3469    0.999    0.332    0.332    0.891
```

e.g. the first line represents an unobserved SNP, for which 99% of individuals were imputed; the second line was an observed SNP, but if we drop it and try to re-impute, we get 99.3%; the concordance rate between imputed and genotyped is 98.6% for this SNP. The final line represents a SNP that did not perform as well: we only impute a third of genotypes and these are less than 90% concordant (this was an observed SNP also). In this case, we see the `INFO` score is lower (below 0.8) for this third SNP than for the other two: at the standard 0.8 threshold this SNP would have been ignored in any case.

The required confidence threshold for making a call can be changed with, for example,

```
--proxy-impute-threshold 0.8
```

(it is set to 0.95 by default currently).

To give genotype-specific concordances, use the additional option:

```
--proxy-genotypic-concordance
```

then a set of extra fields are append to the `plink.proxy.impute` output

| | |
|---|---|
| F_AA | Frequency of true 'AA' genotype |
| I_AA | Proportion imputed for true AA genotype |
| C_AA | Concordance rate for true AA genotype |
| F_AB | As above, for 'AB' genotype |
| ... | ... |

That is, for a very rare SNP, overall concordance would be high just by chance, even if none of the rare genotypes were correctly called. This option is therefore useful to get a better picture of imputation performance (when the observed genotype is also available).

In additon, if

```
--proxy-show-proxies
```

is also specified, an extra `PROXIES` field will appear in `plink.proxy.impute` showing the specific SNPs selected.

To perform imputation and save the dosages (fractional count of 0 to 2 alleles for each genotype), add the `--proxy-dosage` option;

```
./plink --bfile merged-1 --proxy-impute all --proxy-dosage
```

which produces a file

```
plink.proxy.impute.dosage
```

in which each imputed SNP is represented as a row; the fields (which does not have any header row)

```
SNP Identifier
Allele 1 code
Allele 2 code
Information content score for SNP
```

```
Allele dosage for first individual in sample
Allele dosage for second individual in sample
...
Allele dosage for final individual in sample
```

This file can then be analysed outside of PLINK.

To perform imputation and save the called (most likely) genotypes in a new fileset, add the `--make-bed` option;

```
./plink --bfile merged-1 --proxy-impute all --make-bed --out imputed-1
```

By default, PLINK will only replace genotypes that were missing in the original WGAS sample; to make PLINK re-impute all genotypes (whether they were actually observed or not), add the `--proxy-replace` flag,

```
./plink --bfile merged-1 --proxy-impute all --proxy-replace --make-bed --out imputed-1
```

**Note** Future versions will do obvious things, like let you generate proxy-impute and proxy-assoc output files in the same run (you can't now).

**Important** Making discrete calls for the most likely genotype will necessarily introduce error and bias in the all but perfectly imputed SNPs. As such, one should take care in the analysis and interpretation of imputed datasets – they should not be treated as if they were directly observed with certainty. In particular, one should be particularly cautious when combining multiple imputed files, particularly if different platforms were used and/or if the files also differ by disease state. Indeed, such an analysis is currently not recommended.

## 16.5 Verbose output options

To get a verbose output for a single SNP in the association mode, use instead of the `all` keyword the specific SNP name:

```
--proxy-assoc rs123235
```

See the web-page on proxy association methods to interpret this output.

You can also specify verbose imputation for one or more SNPs, e.g.

```
--proxy-impute rs8096534  --proxy-verbose
```

which will add extra lines to the file `plink.proxy.impute` representing the actual calls per person:

```
rs8096534       78-03C15376 TBI-78-03C15376-1   01 01 0 1 0
rs8096534       78-03C15377 TBI-78-03C15377-1   00 00 1 0 0
rs8096534       78-03C15378 TBI-78-03C15378-1   01 01 0 1 0
rs8096534       78-03C15398 TBI-78-03C15398-1   00 00 1 0 0
rs8096534       78-03C15448 TBI-78-03C15448-1   01 01 0 1 0
rs8096534       78-03C20292 TBI-78-03C20292-1   11 11 0 0 1
rs8096534       78-03C20300 TBI-78-03C20300-1   11 10 0 0.08199 0.918
rs8096534       78-03C20317 TBI-78-03C20317-1   01 01 0 1 0
rs8096534       78-03C20335 TBI-78-03C20335-1   01 01 0 1 0
...
```

where the fields are (note: currently there is no header for these fields)

```
SNP     SNP identifier
FID     Family ID
IID     Individual ID
OBS     Observed genotype (coded 00,01,11 = AA,AB,BB,  10 = missing)
IMP     Imputed genotype (as above)
PAA     Probability of 'AA' genotype
```

```
PAB     Probability of 'AB' genotype
PBB     Probability of 'BB' genotype (i.e. these last 3 numbers sum to 1.00)
```

In addition, after these lines you will see a table of counts which summarises the actual calls versus the true values (if known). Ideally, you would observe high numbers down the diagonal therefore (the columns are the same as the rows):

```
Imputation matrix (rows observed, columns imputed)
A/A     292     2       0       1
A/G     0       1389    8       55
G/G     0       5       1585    130
0/0     1       1       0       0
```

and this is then followed by the normal, single-line non-verbose report for that SNP

```
 CHR            SNP NPRX     INFO  TOTAL_N  OBSERVD  IMPUTED  OVERLAP  CONCORD
  18      rs8096534    5    0.961     3469    0.999    0.946    0.946    0.995
```

Although you are able to specify `--proxy-impute all` and `--proxy-verbose` together, be warned that this will typically result in a very large output file for real data. It is better used for single SNPs in its current format.

# Chapter 17

# LD-based result clumping procedure

This page describes PLINK's ability to group SNP-based results across one or more datasets or analyses, based on empirical estimates of linkage disequilibrium between SNPs. The basic procedure was inspired by a script written by Ben Voight.

There are probably two main applications for this method:

- To report the top $X$ single SNP results from a genome-wide scan in terms of a smaller number of *clumps* of correlated SNPs (i.e. to assess how many independent loci are associated, for example)
- To provide a quick way to combine sets of results from two or more studies, when the studies might also be genotyped on different marker sets

## 17.1 Basic usage for LD-based clumping

The `--clump` command is used to specify one or more result files (i.e. precomputed analyses of some kind). By default, PLINK scans these files and extracts fields with the headers SNP and P. For example:

```
plink --file mydata --clump mytest1.assoc
```

which generates a file

```
plink.clumped
```

The actual genotype dataset specified here (i.e. the `mydata.*` fileset) may or may not be the same dataset that was used to generate the results in `mytest1.assoc`. The `mydata` fileset is only used to calculate linkage disequilibrium between the SNPs that feature in `mytest1.assoc` (i.e. the analyses are not re-run).

There are four main parameters that determine the level of clumping, listed here in terms of the command flag used to change them and their default values:

```
--clump-p1 0.0001          Significance threshold for index SNPs
--clump-p2 0.01            Secondary significance threshold for clumped SNPs
--clump-r2 0.50            LD threshold for clumping
--clump-kb 250             Physical distance threshold for clumping
```

The clumping procedure takes all SNPs that are significant at threshold *p1* that have not already been clumped (denoting these as *index SNPs*) and forms clumps of all other SNPs that are within a certain kb distance from the index SNP (default 250kb) and that are in linkage disequilibrium with the index SNP, based on an r-squared threshold (default 0.50). These SNPs are then subsetted based on the result for that SNP, as illustrated below. This is a greedy algorithm and so each SNP will only appear in a single clump, if at all.

In the default, non-verbose mode, the default output lists all index SNPs and a summary of the other SNPs that are clumped with this SNP: (note, SNP IDs and positions are made-up in the example below):

```
    CHR  F         SNP         BP          P  TOTAL   NSIG    S05    S01   S001  S0001    SP2
      8  1   rs1234564   15716326  5.01e-07      0      0      0      0      0      0   NONE
     14  1   rs1205236   69831825  1.46e-06      0      0      0      0      0      0   NONE
      2  1  rs16331058  114547107  2.33e-06      3      0      0      0      0      3   rs2366902(1)
      2  1    rs759966   54902416  9.28e-06      4      0      0      0      3      1   rs12538389(1)
     11  1   rs8031586   44633498  9.75e-06      1      0      0      0      0      1   rs802328(1)
     12  1  rs12431413   30028246  9.89e-06      0      0      0      0      0      0   NONE
      6  1  rs14966070   62091121  1.07e-05      0      0      0      0      0      0   NONE
```

where the fields are as follows

| | |
|---|---|
| CHR | Chromosome code |
| F | Results fileset code (1,2,...) |
| SNP | SNP identifier |
| BP | Physical position of SNP (base-pairs) |
| TOTAL | Total number of other SNPs in clump (i.e. passing --clump-kb and --clump-r2 thresholds) |
| NSIG | Number of clumped SNPs that are not significant ( p > 0.05 ) |
| S05 | Number of clumped SNPs 0.01 < p < 0.05 |
| S01 | Number of clumped SNPs 0.001 < p < 0.01 |
| S001 | Number of clumped SNPs 0.0001 < p < 0.001 |
| S0001 | Number of clumped SNPs p < 0.0001 |
| SP2 | List of SNPs names (and fileset code) clumped and significant at --clump-p2 |

That is, the `TOTAL` field lists all SNPs that are clumped with the index SNP, irrespective of the p-value for those SNPs. This number is then split into those clumped SNPs that are not significant (p¿0.05) and various other groups defined by significance thresholds. For SNPs that are significant at the *p2* threshold, they are listed explicitly. The `(1)` after each SNP name refers to the results file they came from (in this case, there is only a single result file specified, so all values are 1).

To specify more than a single result file, use a comma-delimited list after `--clump` (without any spaces between file names), for example:

```
plink --bfile mydata --clump mytest1.assoc,mytest2.assoc
```

To specify a field labelled other than `P`, use the command

```
plink --bfile mydata --clump mytest1.assoc --clump-field P_CMH
```

for example.

**NOTE** The same fields are extracted from all results files (e.g. `SNP` and `P`) – i.e. it is not possible to specify different fields from different files.

**NOTE** All results are interpreted as p-values – i.e. it is not possible to specify a Z-statistic, as significance is always defined as less than the threshold. Finally, by default a SNP is not allowed to appear in more than one clump, either as an index or non-index SNP. If you add the command, then a SNP that has appeared as a non-index SNP in one clump can appear as a non-index SNP in other clumps:

```
--clump-allow-overlap
```

## 17.2 Verbose report

For a more detailed report of the SNPs in each clump, add the flag `--clump-verbose`

```
plink --bfile mydata --clump mytest1.assoc --clump-verbose
```

which produces a report as follows:

```
 CHR    F          SNP         BP          P    TOTAL   NSIG    S05    S01   S001  S0001
   8    1    rs1234564   15716326  5.019e-07        0      0      0      0      0      0
------------------------------------------------------------------
 CHR    F          SNP         BP          P    TOTAL   NSIG    S05    S01   S001  S0001
  14    1    rs1205236   69831825  1.469e-06        0      0      0      0      0      0
------------------------------------------------------------------
 CHR    F          SNP         BP          P    TOTAL   NSIG    S05    S01   S001  S0001

   2    1   rs16331058  114547107  2.337e-06        3      0      0      0      0      3
                               KB      RSQ  ALLELES    F           P
  (INDEX)   rs16331058        0.0    1.000        A    1    2.34e-06
             rs2366902      -75.4    0.611    AT/GC    1    4.42e-05
             rs1274528      -47.4    0.555    AC/GT    1    1.28e-05
             rs3200591      -22.3    0.964    AT/GC    1    2.68e-05
------------------------------------------------------------------
  etc
```

For example, for the third SNP, `rs16331058` we see there are 3 other SNPs that fulfil the specified criteria (kb distance less than 250kb, r-squared greater then 0.5 and p-value of less than *p2* threshold of 0.01), and they are listed explicitly in verbose mode. As well as the kb and r-squared for each SNP (relative to `rs16331058`) we see listed the fileset which the result comes from (`F` – in this case, all are listed 1, as there was only one result file specified) and p-value. Also, the alleles column indicates for the index SNP what the minor allele is (`A`); for the other SNPs, the two haplotypes that are more common than expected are listed (e.g. for SNPs `A/B` and `1/2`, then if `P(A1) > P(A)P(1)` it will list `A1/B2`, otherwise `A2/B1`.

### 17.2.1 Annotation by SNP details and genomic co-ordinates

Another useful verbose-mode option is `--clump-anotate` which takes as a parameter a comma-delimited list of header names, e.g.

```
        --clump-annotate A1,OR
```

and will then list these items in the verbose report mode (e.g. minor allele and odds ratio, in this case, if the results file were a `plink.assoc` file). The output would then appear as, for example,

```
 CHR    F          SNP         BP          P    TOTAL   NSIG    S05    S01   S001  S0001

   2    1   rs16331058  114547107  2.337e-06        3      0      0      0      0      3
                               KB      RSQ  ALLELES    F           P       ANNOT
  (INDEX)   rs16331058        0.0    1.000        A    1    2.34e-06     A, 1.23
             rs2366902      -75.4    0.611    AT/GC    1    4.42e-05     T, 1.17
             rs1274528      -47.4    0.555    AC/GT    1    1.28e-05     C, 1.22
             rs3200591      -22.3    0.964    AT/GC    1    2.68e-05     T, 1.19
```

i.e. here we can see that for `rs2366902` the minor allele `T` had an odds ratio of 1.17; this is consistent with the index SNP, as the haplotype `AT` is more common than expected (i.e. indicating the direction of the LD).

**NOTE** The allele coding in the `ALLELES` field is taken directly from the specified genotype data, i.e. `mydata.*` in this case, whereas the allele coding in the `ANNOT` field is taken (if available and `--clump-annotate` selects an allele field) from the results file. It is up to the user to ensure that these match to be interpretable (i.e. in terms of number versus letter coding, but more importantly in terms of strand, etc, which might be an issue if the genotype data is a file different from that which the results were calculated on, e.g. see below for an example).

A further option is `--clump-range`, which takes a gene-list or region-list file as a parameter. For example, this might be a list of all RefSeq genes, as available here. The command

```
  plink --bfile mydata --clump myresults.assoc --clump-range glist-hg18
```

would, for example, generate the additional file

```
plink.clumped.ranges
```

which has the fields

| | |
|---|---|
| CHR | Chromosome code |
| SNP | Index SNP per clump |
| P | p-value |
| N | Number of clumped SNPs |
| POS | Genomic co-ordinates |
| KB | kb span of clumped SNPs |
| RANGES | List of ranges/genes that intersect the clumped region |

For example, the first four rows of a simulated, random study are:

```
 CHR         SNP          P      N                           POS         KB RANGES
  17   rs9944528  1.927e-05      2     chr17:77894039..77933018     38.979 [UTS2R,SKIP,FLJ35767]
   9  rs17534370  1.958e-05      1      chr9:70297172..70297172          0 [PGM5]
  11  rs12418173  1.965e-05      7   chr11:112102294..112133479     31.185 []
```

which indicates that rs9944528 has one other SNP that clumps with it (N=2), which is just under 40kb away, spanning three genes; the next SNP doesn't have any clumped partners and falls in the *PGM5* gene; the third SNP has 6 other clumped SNPs, spanning just over 30kb, but no genes are in that interval.

If the `--clump-range` flag is added in `--clump-verbose` mode, the output looks slightly different. In this case, the special `plink.clumped.ranges` file is not produced: now all the output is in the `plink.clumped` file:

```
 CHR    F          SNP         BP          P    TOTAL   NSIG    S05    S01   S001  S0001
  17    1    rs9944528   77894039   1.93e-05        1      0      0      0      0      1
                               KB      RSQ  ALLELES    F            P
  (INDEX)    rs9944528          0    1.000        C    1     1.93e-05
             rs7207095         39    0.648    CG/GA    1     2.83e-05
          RANGE: chr17:77894039..77933018
           SPAN: 38kb
    GENES w/SNPs: SKIP
           GENES: UTS2R,SKIP,FLJ35767
-------------------------------------------------------------------
 CHR    F          SNP         BP          P    TOTAL   NSIG    S05    S01   S001  S0001
   9    1   rs17534370   70297172   1.96e-05        0      0      0      0      0      0
            GENES: PGM5
-------------------------------------------------------------------
 CHR    F          SNP         BP          P    TOTAL   NSIG    S05    S01   S001  S0001
  11    1   rs12418173  112133479   1.96e-05        6      0      0      0      2      4
                               KB      RSQ  ALLELES    F            P
  (INDEX)   rs12418173          0    1.000        G    1     1.96e-05
            rs12800322      -31.2    0.902    GG/AC    1     0.000133
             rs1870496      -30.7    0.853    GC/AT    1     0.000267
             rs2199197      -20.1        1    GG/AA    1     9.76e-05
             rs7931135      -16.7        1    GG/AA    1     1.96e-05
            rs12418739      -10.8        1    GA/AC    1      3.5e-05
              rs898311      -4.98        1    GT/AC    1     1.96e-05
          RANGE: chr11:112102294..112133479
           SPAN: 31kb
    GENES w/SNPs:
           GENES:
```

```
------------------------------------------------------------------
```

Note, if there is more than 1 SNP in a clump, we distinguish here between whether or not one of the clumped SNPs is actually within the a specified region or gene (`GENES w/SNPs`) versus whether that gene or region is just within the general clumped range (`GENES`).

Naturally, any file can be used with `--clump-range` – the regions do not have to correspond to actual genes, but they could be regions of interest identified by other means.

Finally, the command

```
--clump-range-border 20
```

adds a 20kb border to the start and stop of each gene or region.

## 17.3 Combining multiple result files (potentially from different SNP panels)

When more than one output file is specified, e.g. as

```
plink --bfile mydata --clump mytest1.assoc,mytest2.assoc,mytest3.assoc
```

there are two other options that can modify the behaviour of `--clump`. First,

```
--clump-index-first
```

indicates that index SNPs should only taken from the first result file listed (`mytest1.assoc` in the example above). In other words, this allows for an asymmetric comparison, in which we ask only whether or not a result in a particular file has any other SNPs (in that same, or in different files) that could be clumped.

Second, the additional option

```
--clump-replicate
```

means that only clumps containing clumped SNPs with *p2*-significant results in *more than one* result file are shown. This could be used in the following context: imagine one had data for two different whole-genome scans, for the same phenotype but performed on different platforms, e.g. Affymetrix and Illumina. A quick way to compare these sets of results would be to use the HapMap as a common dataset (i.e. containing all SNPs on both platforms, or the majority of these in any case) as follows:

```
plink --bfile hapmap --clump affymetrix.assoc,illumina.assoc --clump-verbose --clump-replicate
```

This assumes that you have made the fileset `hapmap.*` to contain all SNPs for one of the analysis panels, e.g. CEU. In this context, we are only interested in hits (e.g. p-values less than 1e-3) that are seen across the studies, by using the `--clump-replicate` flag (i.e. only clumps where `F` is seen to have values of both 1 and 2 for *p2*-significant SNPs). In this case, it also probably makes sense to equate the *p1* and *p2* thresholds, by adding, for example,

```
--clump-p1 1e-3 --clump-p2 1e-3
```

Finally, by also adding the

```
--clump-annotate A1,OR
```

flag, you can see whether or not there appears to be a consistent direction of effect also (by putting together the direction of odds ratios with the over-represented haplotype to tie together the two or three SNPs).

## 17.4 Selecting the single best proxy

The command

```
--clump-best
```

produces an additional file

```
plink.clumped.best
```

which contains the fields

```
INDEX     Index SNP identifier
PSNP      Best proxy SNP
RSQ       LD (r-squared) between index and proxy
KB        Physical distance between index and proxy
P         p-value for proxy SNP
ALLELES   The associated haplotypes for the index and proxy SNP
F         Which file (from --clump) this result came from
```

For example, if we use the command

```
plink --bfile mydata --clump myresults-a.assoc,myresults-b.assoc --clump-best
```

based on dummy simulated data result files `myresults-a.assoc` and `myresults-b.assoc`, the first few lines of `plink.clumped` are as follows:

```
CHR    F         SNP         BP          P  TOTAL  NSIG  S05  S01 S001 S0001  SP2
 11    1   rs2513514   75922141   2.27e-07      3     0    0    0    1     2  rs2508756(1),...
 20    1   rs6110115   13911728   8.24e-07      9     0    2    3    2     2  rs6079243(1),...
 11    1   rs2508756   75921549   1.07e-06      0     0    0    0    0     0  NONE
 15    1  rs16976702   54120691   1.15e-06      1     0    0    0    1     0  rs16976702(2)
```

The corresponding `plink.clumped.best` file shows the single best proxy SNP for each index SNP. This information could have been extracted manually after using the `--clump-verbose`, but the `--clump-best` option simply makes this easier.

```
     INDEX        PSNP    RSQ        KB         P  ALLELES        F
 rs2513514   rs2513514      1         0  8.04e-05    AA/GG        2
 rs6110115   rs6110115      1         0   0.00145    CC/AA        2
 rs2508756          NA     NA        NA        NA       NA       NA
rs16976702  rs16976702      1         0    0.0009    GG/CC        2
```

For example, the best SNP, rs2513514 (which had the lowest p-value in this case for `F` 1, i.e. `myresults-a.assoc`) has a single best proxy of rs2513514, the same SNP, but in `F` 2, i.e. `myresults-b.assoc`. The third SNP here, rs2508756, does not have any proxy SNP that meets the criteria for clumping (`--clump-r2`, `--clump-p2`, etc).

**Warning** If the same SNP existed in both `myresults-a.assoc` and `myresults-b.assoc` then the `P` value and `ALLELES` would always, arbitrarily be selected from the first file. See the note below also.

One might often want to add the three options

```
--clump-index-first
--clump-replicate
--clump-allow-overlap
```

along with `--clump-best`. This would pose the question: what is the best proxy in `myresults-b.assoc` (i.e. `--clump-replicate` forces a cross-file proxy) for the top results in `myresults-a.assoc` (e.g. `--clump-index-first` forces the first-listed file to contain index SNPs only). The `--clump-allow-overlap` will mean that a proxy SNP can be selected for more than one index SNP, if it is the best. These may sometimes be the same SNP, if it is present in both result sets, otherwise it will rely on all SNPs being present in the `mydata` fileset, and will use LD information to select the best proxy.

**NOTE** By *best* proxy, we mean the SNP with the strongest LD to the index, rather than the best p-value. Which SNP has the greatest LD will be based on the genotype data and will therefore be the same for all result files. As such, this command should be used in such a way that only one result file is being queried for the best proxy at a time. That is, used without `--clump-replicate`, only a single result file should be specified with `--clump`. If used with `--clump-replicate` then a) `--clump-index-first` should always be used and no more than two result files should be specified with `--clump`. That is, in this second usage, this command will try to find the best proxy in the second result file for each index SNP selected from the first file. Otherwise, if the same SNP is present in more than one result file, only details for the first encountered will be reported.

Overall, the most command usage of this will be to select the best SNP proxy in file `B` for the hits in `A`, i.e. in the form:

```
./plink --bfile mydata
        --clump myresults-a.assoc,myresults-b.assoc
        --clump-best
        --clump-replicate
        --clump-index-first
        --clump-allow-overlap
        --clump-p1 1e-4
        --clump-p2 1
        --clump-kb 250
        --clump-r2 0.2
```

That is: this will select the SNP from `B` that is in highest LD with each SNP in `A` that has a p-value less than 1e-4 in `A`. The same SNP in `B` is allowed to be the best proxy for more than one SNP in `A` (`--clump-allow-overlap`). The best proxy will be reported no matter what p-value it has in `B` (`--clump-p2 1`) although it must satisfy the criteria of being at least above r-sq of 0.2 and within 250kb.

# Chapter 18

# Epistasis

For disease-trait population-based samples, it is possible to test for epistasis. The epistasis test can either be case-only or case-control. All pairwise combinations of SNPs can be tested: although this may or may not be desirable in statistical terms, it is computationally feasible for moderate datasets using PLINK, e.g. the 4.5 billion two-locus tests generated from a 100K data set took just over 24 hours to run, for approximately 500 individuals (with the `--fast-epistasis` command).

Alternatively, sets can be specified (e.g. to test only the most significant 100 SNPs against all other SNPs, or against themselves, etc). The output consists only pairwise epistatic results above a certain significance value; also, for each SNP, a summary of all the pairwise epistatic tests is given (e.g. maximum test, proportion of tests significant at a certain threshold, etc).

To test for gene-by-environment interaction, see either the section on stratified analyses for disease traits, or the section on QTL GxE for quantitative traits.

**IMPORTANT!** These tests for epistasis are currently only applicable for population-based samples, not family-based.

## 18.1 SNP x SNP epistasis

To test SNP x SNP epistasis for case/control population-based samplse, use the command

```
plink --file mydaya --epistasis
```

which will send output to the files

```
plink.epi.cc
plink.epi.cc.summary
```

where `cc` = case-control; for quantitative traits, `cc` will be replaced by `qt`.

The default test uses either linear or logistic regression, depending on whether the phenoype is a quantitative or binary trait. PLINK makes a model based on allele dosage for each SNP, `A` and `B`, and fits the model in the form of

```
Y ~ b0 + b1.A + b2.B + b3.AB + e
```

The test for interaction is based on the coefficient `b3`. This test therefore only considers allelic by allelic epistasis. Currently, covariates can not be included when using this command. Similarly, permutation, and use of modifier commands such as `--genotypic`, `--within` or `--sex`, etc, are not currently available.

**Important** The `--epistasis` command is set up for testing a potentially very large number of SNP by SNP comparisons, most of which would not be significant or of interest. Because the output may contains millions or billions of line, the default is to only output tests with p-values less than 1e-4, as specified by the `--epi1` option (see below). If your dataset is much smaller and you definitely want to see all the output, add `--epi1 1` . If you do not, odds are you'll see a blank output file except for the header (i.e. immediately telling you that none of the tests were significant at 1e-4).

**Specifying which SNPs to test**

There are different modes for specifying which SNPs are tested: `ALL x ALL`

```
  plink --file mydata --epistasis
```

`SET1 x SET1` *where epi.set contains only 1 set*

```
  plink --file mydata --epistasis --set-test --set epi.set
```

`SET1 x ALL` *where epi.set contains only 1 set*

```
  plink --file mydata --epistasis --set-test --set epi.set --set-by-all
```

`SET1 x SET2` *where epi.set contains 2 sets*

```
  plink --file mydata --epistasis --set-test --set epi.set
```

For the 'symmetrical' cases (ALLxALL and SET1xSET1) then only unique pairs are analysed.

For the other two cases (SET1xALL, SET1xSET2) then all pairs are analysed (e.g. will perform SNPA x SNPB as well as SNPB x SNPA, if A and B are in both SET1 and SET2). It will not try to analysis SNPA x SNPA however.

**The output**

The output can be controlled via

```
  plink --file mydata --epistasis --epi1 0.0001
```

which means only record results that are significant p¡=0.0001. (This prevents too much output from being generated). The output is in the form

| | |
|---|---|
| CHR1 | Chromosome of first SNP |
| SNP1 | Identifier for first SNP |
| CHR2 | Chromosome of second SNP |
| SNP2 | Identifier for second SNP |
| OR_INT | Odds ratio for interaction |
| STAT | Chi-square statistic, 1df |
| P | Asymptotic p-value |

The odds ratio for interaction is interpreted in the standard manner: a value of 1.0 indicates no effect. To better visualise the manner of an interaction, use the `--twolocus` command to produce a report. For example:

```
  plink --bfile mydata --twolocus rs9442385 rs4486391
```

generates the file

```
plink.twolocus
```

which contains counts and frequencies of the two locus genotypes, e.g. (there is no interaction evident in this case):

```
All individuals
===============
                   rs4486391
                   1/1    1/4    4/4    0/0    */*
  rs9442385  4/4     4      5      7      1     17
             4/3     7     15     14      0     36
```

```
          3/3      6     20     10      0     36
          0/0      0      1      0      0      1
          */*     17     41     31      1     90
                rs4486391
                 1/1    1/4    4/4    0/0    */*
rs9442385 4/4  0.044  0.056  0.078  0.011  0.189
          4/3  0.078  0.167  0.156  0.000  0.400
          3/3  0.067  0.222  0.111  0.000  0.400
          0/0  0.000  0.011  0.000  0.000  0.011
          */*  0.189  0.456  0.344  0.011  1.000
```

For case/control data, two similar sets of tables are included which stratify the two-locus genotype counts by cases and controls

A second part of the output: for each SNP in SET1, or in ALL if no sets were specified, is information about the number of significant epistatic tests that SNP featured in (i.e. either with ALL other SNPs, with SET1, or with SET2). The threshold `--epi2` determines this:

```
plink --file mydata --epistasis --epi1 0.0001 --epi2 0.05
```

The output in the `plink.epi.cc.summary` file containts the following fields:

```
CHR        Chromosome
SNP        SNP identifier
N_SIG      # significant epistatic tests (p <= "--epi2" threshold)
N_TOT      # of valid tests (i.e. non-zero allele counts, etc)
PROP       Proportion significant of valid tests
BEST_CHISQ Highest statistic for this SNP
BEST_CHR   Chromosome of best SNP
BEST_SNP   SNP identifier of best SNP
```

This file should be interpreted as giving only a very rough idea about the extent of epistasis and which SNPs seem to be interacting (although, of course, this is a naive statistic as we do not take LD into account – i.e. `PROP` does not represent the number of *independent* epistatic results).

### 18.1.1 A faster epistasis option

For disease traits only, an approximate but faster method can be used to screen for epistasis: use the `--fast-epistasis` command instead of `--epistasis`. This test is based on a Z-score for difference in SNP1-SNP2 assocation (odds ratio) between cases and controls (or in cases only, in a case-only analysis). For more details, see this page.

## 18.2 Case-only epistasis

For case-only epistatic analysis,

```
plink --file mydata --fast-epistasis --case-only
```

sends output to (`co` = case-only)

```
plink.epi.co
plink.epi.co.summary
```

All other options are as described above.

Currently, in case-only analysis, only SNPs that are more than 1 Mb apart, or on different chromosomes, are included in case-only tests. This behavior can be changed with the `--gap` option, with the distance specified kb: for example, to specify a gap of 5 Mb,

```
plink --file mydata --fast-epistasis --case-only --gap 5000
```

This option is important, as the case-only test for epistasis assumes that the two SNPs are in linkage equilibrium in the general population.

## 18.3 Gene-based tests of epistasis

**WARNING** This test is still under heavy development and not ready for use.

# Chapter 19

# R plugin functions

This page describes PLINK's limited support for R-based 'plug-in' functions. In this manner, users can extend the basic functionality of PLINK to better meet their own needs.

R `http://www.r-project.org/` is a powerful, freely-available package for statistical computing. PLINK uses the Rserve `http://www.rforge.net/Rserve/` package to communicate with R. There are some notes on installing and running the Rserve package below.

The idea is that some analyses, such as survival analysis for example, are already implemented in R but not available in PLINK. Having a simple interface for accessing such R functionality, allows one to benefit from both the data-handling features of PLINK (i.e. it being designed specifically to handle large SNP datasets efficiently, in a way that the basic R package is not) as well as the ever-increasing library of statistical tools in R. Also, this should provide an easy way to prototype new methods, etc.

Currently there is only support for SNP-based analyses. As of version 1.05, multiple values can be returned for each SNP, as defined by the user. Potentially (if there is interest/specific suggestions) these features will be expanded to allow other units of analysis and broader communcation with R.

**Note** Currently, there is only support for R-plugins for Linux-based and Mac OS PLINK distributions.

**Note** Version 1.04 onwards of PLINK has updated the client code to support the latest version of Rserve. You should re-install Rserve (see notes below) to make sure you have the latest version.

## 19.1 Basic usage for R plug-ins

Assuming Rserve has been installed and is running locally (see below) and that the file `myscript.R` contains the R code conforming to the standard for a PLINK plug-in (see here), then the command is simply

```
plink --file mydata --R myscript.R
```

which generates a file

```
plink.auto.R
```

This file contains the raw output for each SNP, which is whatever vector of numeric values the user returned from their script, and some details about the SNP. There is no header row; each row has the following fields.

```
Chromosome position
SNP id
Physical position (base-pair)
Minor allele (A1)
First return value from R-plugin
Second return value from R-plugin
...
```

Depending on how you set up the R script, each row may or may not have the same number of columns. Currently it is not possible to return strings of other R objects.

If `Rserve` is running on anything other than the default port, you can specify an alternate port number by adding

```
--R-port 8221
```

for example.

## 19.2 Defining the R plug-in function

PLINK expects a function in the exact form

```
Rplink <- function(PHENO,GENO,CLUSTER,COVAR)
```

to be defined in the supplied file. This function is expected to return a numeric vector, with as many elements are there are SNPs. Internally, PLINK will call the `Rplink` function – it must be written exactly as shown here. The objects refer to:

```
PHENO     vector of phenotypes (n)
GENO      matrix of genotypes (n x l)
CLUSTER   vector of cluster membership codes (n)
COVAR     matrix of covariates (n x c)
```

where `n` is the number of individuals (after pruning, filtering, etc) and `c` is the number of covariates (if any). PLINK generates these objects internally, so the user can assume these exist for when the `Rplink()` function is called. (In practice, the number of SNPs, `l` will probably be smaller than the total number of SNPs in the file, as PLINK passes the genotype data into R in batches rather than all in one go).

Genotypes are coded 0, 1 or 2 copies of the minor allele, and NA, as per the `--recodeA` option.

For each SNP, PLINK expects the function to return a numeric vector of values. This need not have the same number of values for each SNP (although this will make subsequently parsing of the output file harder, potentionally). If the desired return vector is `r`, then the actual return vector must be

```
c( length(r) , r )
```

That is, PLINK expects back a long string of values, where it reads how many values to read for the first SNP, reads them, then reads how many values to read for the second SNP, reads them, etc. By also using the abve formulation to specify the return vector, PLINK will be able to parse the output.

An example R plug-in is shown here – this is probably the most straightforward template for an R-plugin, in which the `apply()` function is used to iteratively call the nested function (`f1()`), once per SNP, in this case. For example, the file `myscript.R` might contain the following plug-in:

```
Rplink <- function(PHENO,GENO,CLUSTER,COVAR)
 f1 <- function(x)

    r <- mean(x, na.rm=T) / 2
    c( length(r) , r )

 as.numeric( apply(GENO, 2 , f1) )
```

If you are not familiar with the R language, there are a number of excellent resources available from the main R webpage `http://www.r-project.org/`.

Within the body of the main `Rplink()` function, there are no constraints on what you can do, as long as the return value is in the proper format, as described above. In this example, within the main body of the `Rplink()` function we first define a function that will be applied to each SNP, called `f1()`. Unlike the `Rplink()` function, you can call this whatever you want, or have as many functions as you want. The function `f1()` calculates the allele frequency for each SNP (as the genotypes are coded as the count of the minor allele, 0,1,2). The second line applies this function to each column of the genotype data, using the `apply(` *`data , row/col , function`* `)` command.

Another, perhaps more useful, example is implementing survival analysis within PLINK: here we define a function, `f1()` to return the p-value for the first coefficient; we assume here that a censoring variable was loaded into PLINK as the first covariate (i.e. the R `Surv` function takes two parameters, the survival time and censoring status). (This is probably not the optimal way to implement this analysis, but is intended purely as an example of what can be done.)

```
library(survival)
Rplink <- function(PHENO,GENO,CLUSTER,COVAR)

 f1 <- function(s)

   m <- summary( coxph( Surv( PHENO , COVAR[,1] ) ~ s ) )
   r <- c( m$coef , m$loglik, m$n )
   c( length(r) , r )

apply( GENO , 2 , f1 )
```

In other words, the general format is

```
 load any libraries or auxiliary data from a file first
<b>Rplink <- function(PHENO,GENO,CLUSTER,COVAR)
</b>
 f1 <- function(x)

   do something to generate per-SNP return vector r
  c( length(r) , r )

apply( GENO , 2 , f1 )
```

## 19.3 Example of debugging an R plug-in

To generate a text file that contains the R commands PLINK would have run (rather than actually trying to run them – this is useful for debugging purposes), add the following flag

```
plink --file mydata --R myscript.R --R-debug
```

To illustrate the debug function, consider this example, in which we try to implement a logistic regression. The file

```
mylog.R
```

which contains the function

```
Rplink <- function(PHENO,GENO,CLUSTER,COVAR)

  f1 <- function(s)

   m <- glm( PHENO ~ s , family="binomial" )
   r <- summary(m)$coef[8]
   c( length(r) , r )

 apply( GENO , 2 , f1 )
```

and we have a dataset with three SNPs; the internal PLINK logistic regression command

```
plink --file mydata --logistic
```

yields

```
CHR  SNP         BP   A1       TEST    NMISS       ODDS         STAT            P
  1 snp0      10000    A        ADD      200      1.256         1.15       0.2501
  1 snp1      10001    B        ADD      200     0.9028      -0.5112       0.6092
  1 snp2      10002    B        ADD      200     0.6085       -2.242      0.02499
```

Trying to run the R implementation:

```
plink --file mydata --R mylog.R
```

we obtain a set of invalid p-values in plink.auto.R

```
1 snp0 10000  A  NA
1 snp1 10001  B  NA
1 snp2 10002  B  NA
```

To find out what is happening, we will run the same command with the debug option

```
plink --file mydata --R mylog.R --R-debug
```

This writes to the file `plink.auto.R` the actual commands that would be passed to R, including the data and the function:

```
n <- 200
PHENO <- c( 2, 1, 1, 2, 2, 2, 1, 2, 1, 1, 1, 1, 1, 1, 1, 1, 2, 1, 2,
1, 2, 1, 2, 1, 2, 2, 1, 1, 1, 2, 1, 1, 2, 2, 2, 2, 1, 2, 1, 1, 1, 2,
2, 2, 1, 1, 1, 1, 1, 2, 2, 1, 1, 2, 2, 2, 2, 1, 1, 1, 2, 1, 1, 2, 1,
2, 1, 1, 2, 2, 2, 1, 2, 2, 1, 1, 1, 1, 2, 2, 1, 1, 1, 1, 2, 1, 2, 1,
2, 2, 1, 2, 1, 1, 2, 2, 2, 1, 2, 2, 1, 1, 1, 1, 2, 2, 2, 2, 2, 1, 1,
1, 1, 1, 2, 2, 1, 2, 2, 2, 2, 1, 2, 2, 1, 1, 2, 2, 2, 2, 2, 2, 1, 1,
1, 2, 1, 2, 2, 2, 1, 1, 1, 1, 1, 2, 2, 1, 1, 1, 2, 1, 2, 1, 1, 2, 1,
1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 1, 2, 2, 2, 1, 1, 2, 1, 1, 2, 2, 1, 1,
2, 2, 2, 1, 2, 1, 2, 1, 1, 2, 1, 2, 1, 1, 1, 1, 1, 2, 1, 1 )
COVAR <- matrix( NA , nrow = n , ncol = 0 , byrow = T)
CLUSTER <- c( 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 )
l <- 3
g <- c( 1, 2, 1, 2, 2, 1, 1, 1, 1, 2, 2, 0, 0, 1, 1, 0, 1, 2, 1, 1, 1,
2, 1, 1, 2, 1, 1, 0, 1, 1, 1, 0, 1, 2, 1, 1, 1, 0, 0, 0, 0, 1, 1, 1,
1, 1, 1, 1, 0, 1, 2, 1, 1, 1, 1, 0, 1, 1, 0, 2, 2, 0, 1, 1, 2, 0, 1,
1, 1, 2, 1, 1, 1, 1, 1, 0, 2, 2, 0, 0, 2, 1, 1, 1, 2, 1, 1, 0, 1, 1,
1, 1, 2, 2, 2, 1, 0, 2, 0, 1, 1, 1, 0, 0, 1, 0, 1, 1, 0, 1, 1, 0, 1,
0, 2, 2, 1, 0, 0, 0, 1, 0, 1, 2, 2, 2, 1, 0, 0, 0, 2, 1, 2, 2, 1, 1,
1, 1, 0, 0, 1, 1, 1, 1, 1, 2, 1, 1, 0, 1, 2, 2, 1, 2, 2, 1, 2, 0, 1,
1, 1, 1, 2, 1, 1, 0, 1, 0, 1, 1, 2, 1, 2, 1, 1, 1, 1, 1, 0, 1, 1, 1,
2, 2, 1, 2, 1, 1, 2, 2, 0, 0, 1, 2, 1, 0, 0, 1, 1, 2, 1, 2, 2, 2, 0,
1, 1, 0, 2, 1, 1, 2, 1, 0, 1, 1, 0, 1, 1, 1, 1, 2, 1, 1, 0, 1, 1, 0,
0, 1, 1, 2, 1, 0, 1, 2, 0, 2, 1, 1, 1, 0, 0, 2, 1, 1, 1, 2, 0, 1, 1,
1, 1, 1, 2, 1, 2, 0, 1, 1, 0, 1, 0, 2, 1, 0, 2, 1, 2, 2, 0, 0, 0, 1,
1, 2, 1, 1, 1, 0, 2, 1, 0, 2, 2, 1, 1, 2, 1, 1, 1, 2, 0, 1, 1, 0, 1,
2, 2, 2, 0, 1, 1, 1, 1, 1, 2, 1, 1, 2, 1, 2, 0, 0, 2, 0, 2, 1, 1, 1,
1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 2, 1, 0, 0, 1, 1, 0, 2, 1, 1, 1, 2,
2, 2, 1, 1, 0, 0, 2, 2, 1, 2, 2, 0, 2, 2, 2, 2, 0, 1, 2, 2, 2, 2, 0,
0, 0, 1, 1, 1, 2, 1, 1, 2, 1, 1, 1, 2, 2, 2, 0, 1, 2, 0, 0, 1, 1, 1,
0, 1, 1, 1, 0, 0, 1, 1, 2, 1, 0, 1, 0, 2, 2, 1, 2, 1, 1, 1, 0, 1, 1,
1, 1, 1, 1, 1, 0, 1, 0, 1, 1, 2, 1, 1, 0, 0, 0, 0, 1, 0, 1, 0, 2, 2,
2, 2, 1, 2, 1, 1, 1, 2, 1, 2, 0, 0, 1, 0, 1, 2, 1, 0, 2, 0, 1, 1, 0,
1, 0, 1, 1, 0, 2, 0, 1, 2, 1, 1, 2, 2, 1, 2, 0, 2, 0, 2, 0, 0, 1, 1,
1, 1, 2, 1, 0, 2, 0, 1, 1, 0, 1, 2, 2, 2, 1, 0, 1, 2, 1, 2, 1, 2, 0,
0, 1, 0, 1, 1, 2, 0, 1, 1, 2, 1, 0, 1, 2, 1, 0, 2, 2, 2, 2, 2, 2, 1,
0, 2, 1, 2, 1, 1, 1, 1, 2, 0, 1, 1, 1, 2, 2, 1, 0, 1, 1, 2, 1, 1, 0,
1, 1, 2, 1, 1, 1, 2, 1, 1, 1, 1, 1, 2, 0, 2, 0, 2, 2, 1, 0, 1, 2, 1,
0, 2, 0, 0, 1, 0, 2, 1, 0, 2, 1, 1, 1, 0, 1, 1, 1, 1, 1, 1, 0, 2, 2,
0, 1, 2, 1 )
GENO <- matrix( g , nrow = n ,byrow=T)
GENO[GENO == -1 ] <- NA
Rplink <- function(PHENO,GENO,CLUSTER,COVAR)

f1 <- function(s)
   m <- glm( PHENO-1 ~ s , family="binomial" )
   r <- summary(m)$coef[8]
   c( length(r), r)

apply( GENO , 2 , f1 )
```

In R, load this function

```
source("plink.auto.R")
```

and then try to run the Rplink function

```
Rplink(PHENO,GENO,CLUSTER,COVAR)
```

and you will see the error message

```
Error in eval(expr, envir, enclos) : y values must be 0 <= y <= 1
```

which indicates that R is expecting a 0/1 coding for this particular function, not the default 1/2 coding used by PLINK for the phenotype/dependent variable. You might therefore want to change the relevant line of the function from

```
m <- glm( PHENO ~ s , family="binomial" )
```

to

```
m <- glm( PHENO==2 ~ s , family="binomial" )
```

for example. Then, repeating the above debug procedure, you would see in R

```
Rplink(PHENO,GENO,CLUSTER,COVAR)
```

gives

```
[1] 0.25013412 0.60921037 0.02499268
```

which are the correct p-values. So, now the function is fixed running

```
plink --file mydata --R mylog.R
```

would generate the same set of p-values as the PLINK logistic command, in `plink.auto.R`

```
     1 snp0 10000  A  0.250134
     1 snp1 10001  B  0.60921
     1 snp2 10002  B  0.0249927
```

This basic function could then be extended to return the coefficients also, or to use different analytic approaches available in R.

## 19.4 Setting up the Rserve package

First, you must ensure that you have `Rserve` installed on your system. Normally, this will involve just typing, ***at the R command prompt (not the system shell prompt)***

```
install.packages("Rserve")
```

**HINT** For this to work, R must have been configured with `--enable-R-shlib`.

When using any R-based PLINK plug-in, Rserve must be running in the background before invoking the PLINK command. To start Rserve, just type at the shell prompt

```
R CMD Rserve
```

(note, you may need to change `Rserve` to the full path of where Rserve was installed), or, within R, type at the R prompt

```
library(Rserve)
Rserve()
```

Please see the Rserve documentation `http://www.rforge.net/Rserve/doc.html` for further support.

# Chapter 20

# SNP annotation database lookup

This page describes PLINK's ability to output basic annotation information on SNPs on common WGAS genotyping platforms, via a web-based lookup function.

The SNP annotation data were compiled by Patrick Sullivan's lab `http://genetics.unc.edu/faculty/sullivan.htm`; the original data files are available here `https://slep.unc.edu/evidence/`.

**NOTE** All gene names must be HUGO standard gene names . For example, the serotonin transporter is SLC6A4 (not HTT or SERT).

If you use these annotations in a publication, include the following sentence and corresponding references:

```
Using the PLINK retrieval interface, SNP annotations were created using
the TAMAL database (1) based chiefly on UCSC genome browser files (2),
HapMap (3), and dbSNP (4).
```

- Hemminger BM, Saelim B, Sullivan PF. TAMAL: An integrated approach to choosing SNPs for genetic studies of human complex traits. Bioinformatics 2006;22:626-7.
- Hinrichs AS, Karolchik D, Baertsch R, Barber GP, Bejerano G, Clawson H, Diekhans M, Furey TS, Harte RA, Hsu F, Hillman-Jackson J, Kuhn RM, Pedersen JS, Pohl A, Raney BJ, Rosenbloom KR, Siepel A, Smith KE, Sugnet CW, Sultan-Qurraie A, Thomas DJ, Trumbower H, Weber RJ, Weirauch M, Zweig AS, Haussler D, Kent WJ. The UCSC Genome Browser Database: update 2006. Nucleic Acids Res 2006;34:D590-8.
- Altshuler D, Brooks LD, Chakravarti A, Collins FS, Daly MJ, Donnelly P. A haplotype map of the human genome. Nature 2005;437:1299-320.
- Wheeler DL, Barrett T, Benson DA, Bryant SH, Canese K, Chetvernin V, Church DM, DiCuccio M, Edgar R, Federhen S, Geer LY, Helmberg W, Kapustin Y, Kenton DL, Khovayko O, Lipman DJ, Madden TL, Maglott DR, Ostell J, Pruitt KD, Schuler GD, Schriml LM, Sequeira E, Sherry ST, Sirotkin K, Souvorov A, Starchenko G, Suzek TO, Tatusov R, Tatusova TA, Wagner L, Yaschenko E. Database resources of the National Center for Biotechnology Information. Nucleic Acids Res 2006;34:D173-80.

## 20.1 Basic usage for SNP lookup function

The basic command is, for example,

```
plink --lookup rs1475515
```

which outputs to the LOG file the following information

```
PLINK-SNP (WGAS SNP annotation courtesy of Patrick Sullivan)
```

```
Connecting to web...
SNP ID                          : rs1475515
Affy ID                         :
Affy 5.0                        : no
Affy 6.0                        : no
Perlegen ID                     :
Perlgen 600                     : no
Illumina 650                    : yes
Illumina 550                    : no
Non-syn SNP                     : no
SNP Error                       : no
SNP Pos Duplication             : no
Chromosome                      : 1
Strand                          : -
HG17 Position (bp)              : 228459232
HG18 Position (bp)              : 230219120
Pseudo-autosomal region?        : N/A
NCBI reference allele           : T
UCSC reference allele           : A
Observed alleles                : C/T
Human alleles                   : C/T
Predominant human allele        : A
Chimp allele                    : T
Macaque allele                  : T
dbSNP MAF                       : 0.038
HapMap CEU MAF                  : 0
HapMap ASI MAF                  : 0
HapMap YRI MAF                  : 0.15
HapMap CEU Strand               : -
HapMap CEU Allele               : C
HapMap ASI Allele               : C
HapMap YRI Allele               : C
In gene transcript              :
In gene coding region           :
Nearby Genes(KB distance)       :
Segmental duplication?          : no
Copy Number Variant?            : no
Conservation >95% pctile?       : no
Conservation >99% pctile?       : no
Disease-causing region?         : no
miRNA target? (TargetScan)      : no
miRNA target? (PicTAR)          : no
Regulatory potential?           : yes
Promotor region? (Stanford)     : no
Promotor region? (firstEF)      : no
Transfactor binding site        : no
Enhancer?                       : no
Exon?                           : no
Consensus splice site?          : no
5' UTR?                         : no
3' UTR?                         : no
------------------------------------------------
```

To perform a lookup query on a batch of SNPs rather than 1 at a time, use the command

```
plink --lookup-list hits.list
```

where `hits.list` is just a list of SNP IDs (RS numbers); this will generate a file

```
plink.snp.annot
```

containing multiple reports of the above kind. There is a limit to the number of SNPs that can be submitted at one time (currently 200).

## 20.2 Gene-based SNP lookup

It is possible to dump all SNPs in a gene with the command

```
plink --lookup-gene DISC1
```

which does two things: writes some gene-centric informationto the LOG file, and lists all the SNPs that feature on common WGAS platforms to the file

```
plink.snp.list
```

By default, SNPs within 20kb upstream and downstream of the gene are recorded. To change this, add the command

```
--lookup-gene-kb 0
```

or

```
--lookup-gene-kb 100
```

for example.

In the information written to the LOG file, there is a strong bias towards neuropsychiatrically-relevant information, reflecting the research interests of the creator. For example, the output for `DISC1` is: (note: there are a few relatively redundant or uninformative fields currently that will be removed in future releases)

```
Looking up gene information (and SNPs +/- 20 kb)
Connecting to web... Writing SNP details to [ plink.snp.list ]
Gene Name                              : DISC1
Product                                : disrupted in schizophrenia 1 isoform Es
Entry                                  : 1
CCDS Name                              : CCDS31056.1
KG ID                                  : uc001hux.1
SwissProt ID                           : Q9NRI5-4
Hugo ID                                : 2888
Hugo alias                             :
Hugo old gene names                    :
Has gene name?                         : no
HG18 strand                            : +
HG18 chrom                             : 1
HG18 TX Start                          : 229829236
HG18 TX End                            : 229924970
HG18 CDS Start                         : 229829236
HG18 CDS End                           : 229924970
HG18 TX Length                         : 95734
HG18 TX Length Percentile              : 96
HG17 strand                            : -
HG17 chrom                             : 0
HG17 TX Start                          : 0
HG17 TX End                            : 0
```

```
     HG17 CDS Start                         : 0
     HG17 CDS End                           : 0
     HG17 TX Length                         : 0
     Has HG17 pos                           : no
     mRNA accession numbers                 : NM_001012958.1 ENST00000317586 OTTHUMT00000092355
     Protein accession numbers              : NP_001012976.1 ENSP00000320784 OTTHUMP00000035959
     Pseudoautosomal HG18                   : no
     Pseudoautosomal HG17                   : no
     Brain expressed 50th percentile        : yes
     Brain expressed 75th percentile        : yes
     Correlated cortex expression           : NA
     Correlated lymphoblastoid expression   : yes
     Number association studies from SZGene : 20
     Annotation from SLEP database          : ? Schizophrenia [PMID=16033310]/Schizoaffective
                                            : disorder, susceptibility to, 181500 (3) [OMIM=605210]
                                            : /Schizophrenia, susceptibility to, 604906 (3)
[OMIM=605210]
     Association studies from GAD database  : psych (16)
     ----------------------------------------------------------
```

It is possible to supply a list of genes to lookup, with the command

```
plink --lookup-gene-list mygenes.txt
```

that will dump the SNPs from multple genes in a SET file format, e.g. where the file

```
mygenes.txt
```

is something like

```
COMT
DISC1
CACNA1C
...
```

These could then be subsequently extracted with the command

```
--extract plink.snp.list
```

as the `END` comments and gene names will just be ignored if these are not SNP IDs in the MAP file.

## 20.3 Description of the annotation information

For a detailed description of the annotation fields and how they were compiled, please see Patrick Sullivan's PDF `https://slep.unc.edu/evidence/files/README\_annotations.pdf`

# Chapter 21

# SNP simulation routine

PLINK provides an interface to a very simplistic SNP simulation routine, designed to generate large SNP datasets for population-based, case/control studies. This function is largely intended as a convenience function for generating data to prototype new methods, comparing the power of different approaches, etc, rather than producing *realistic* whole genome data. Critically, all SNPs simulated are ***unlinked and in linkage equilibrium***.

## 21.1 Basic usage

The basic command to simulate a SNP data file is the `--simulate` option,

```
./plink --simulate wgas.sim --make-bed --out sim1
```

which takes as a parameter the name of a file (here `wgas.sim`) that describes the to-be-simulated data. The simulation file `wgas.sim` is as follows:

```
100000  null      0.00 1.00  1.00 1.00
100     disease   0.00 1.00  2.00 mult
```

These files can have 1 or more rows, where each row has exactly five fields, as follows

```
Number of SNPs in this set
Label of this set of SNPs
Lower allele frequency range
Upper allele frequency range
Odds ratio for disease, heterozygote
Odds ratio for disease, homozyygote (or "mult")
```

Specifying `mult` implies a multiplicative risk for the homozygote, e.g. 2*2=4 in the above example.

Given this file, PLINK would generate 100,000 SNPs with no association with disease. Each SNP would have its own population allele frequency, generated as a uniform number between, in this case, 0.00 and 1.00. In addition, 100 extra SNPs will be simulated that are associated with disease (population odds ratio of 2.00).

The names of each SNP would follow from the label (which must be unqiue), with a number appended, e.g.

```
null_0
null_1
null_2
...
disease_99
```

An exception is that if a set only contains a single SNP, nothing is appended to the label. This is useful in generating multiple samples from the same population, as described below.

Obviously, a uniform allele frequency range is not realistic: one could instead specify a series of bins to enrich for rarer SNPs, if so desired, to build a more realistic spectrum of allele frequencies (not that the example below is meant to be more realistic).

```
20000  nullA     0.00 0.05  1.00 1.00
10000  nullB     0.05 0.10  1.00 1.00
 5000  nullC     0.10 0.20  1.00 1.00
10000  nullD     0.20 0.99  1.00 1.00
  ...
```

As well as generating the actual data, the `--simulate` outputs to the LOG file the following:

```
Reading simulation parameters from [ wgas.sim ]
Writing SNP population frequencies to [ plink.simfreq ]
Read 2 sets of SNPs, specifying 100100 SNPs in total
Simulating 100 cases and 100 controls
Assuming a disease prevalence of 0.01
```

The `plink.simfreq` file is described below. By default, 100 cases and 100 controls are generated. This can be changed with the command-line options

```
--simulate-ncases 5000
```

and

```
--simulate-ncontrols 5000
```

for example. Likewise, the default disease prevalence is assumed to be 0.01. This can be changed with

```
--simulate-prevalence 0.05
```

for example.

In the example above, the simulated data were directly saved to a binary fileset: this need not be the case. For example, any other analysis command could instead have been applied, e.g. `--simulate` acts just like `--file` or `--bfile`:

```
./plink --simulate wgas.sim --assoc
```

although the actual simulated data would be subsequently lost of course.

**Hint** This tool only generates individuals drawn from a homogeneous population, but you can easily imagine using several `--simulate` runs then using `PLINK` commands to merge the resulting files to specify more complex scenarios, e.g. representing population stratification, allelic heterogeneity, etc.

## 21.2 Specification of LD between marker and causal variant

It is also possible to simulate data in which the observed marker SNP is only in incomplete LD with the actual causal allele. This is achieved with either the `--simulate-tags` or `--simulate-haps` options, e.g.

```
plink --simulate ld.sim --simulate-tags
```

PLINK now expects 9 fields instead of 6 in the simulation file: namely,

```
Number of SNPs in this set
Label of this set of SNPs
Lower allele frequency range, causal variant
Upper allele frequency range, causal variant
Lower allele frequency range, marker
Upper allele frequency range, marker
Marker / causal variant LD (D-prime)
```

```
Odds ratio for disease, heterozygote causal variant
Odds ratio for disease, homozyygote causval variant, (or "mult")
```

For example,

```
5    snp    0.05 0.05      0.5 0.5    0.5   2 mult
```

Implies 5 CVs, each of 5% MAF and 2-fold multiplicative effect size (each in linkage equilibrium still) but with 10 additional markers, each of 50% MAF, each in complete LD with D'=0.5 with their respective CV (i.e. pairs of markers are simulated). The command

```
plink --simulate ld.sim --simulate-tags --assoc
```

will therefore generate only 10 SNPs, that are the markers that tag the CVs, i.e. note the frequency of these variants is around 50%

```
CHR      SNP           BP   A1       F_A       F_U   A2        CHISQ           P          OR
  1    snp_0            1    D     0.474    0.5045    d        3.723     0.05368      0.8851
  1    snp_1            2    D     0.484    0.4985    d       0.8413       0.359      0.9436
  1    snp_2            3    D     0.493    0.4775    d       0.9618      0.3267       1.064
  1    snp_3            4    D     0.486     0.494    d       0.2561      0.6128      0.9685
  1    snp_4            5    D    0.4925    0.5005    d        0.256      0.6129      0.9685
```

In contrast, the related command

```
plink --simulate ld.sim --simulate-haps --assoc
```

will output both the causal variant and the marker, with _M appended to the marker name:

```
CHR      SNP           BP   A1       F_A       F_U   A2        CHISQ           P          OR
  1    snp_0            1    D    0.0995     0.044    d        46.25   1.042e-11       2.401
  1  snp_0_M            2    B    0.4885     0.494    A        0.121      0.7279      0.9782
  1    snp_1            3    D    0.0875     0.044    d         30.8   2.853e-08       2.083
  1  snp_1_M            4    B    0.4815    0.5055    A        2.304       0.129      0.9084
  1    snp_2            5    D     0.088    0.0575    d        13.79   0.0002044       1.582
  1  snp_2_M            6    B     0.459    0.5115    A        11.03   0.0008943      0.8103
  1    snp_3            7    D    0.0965     0.047    d        36.79   1.316e-09       2.166
  1  snp_3_M            8    B    0.5005     0.491    A        0.361      0.5479       1.039
  1    snp_4            9    D     0.093    0.0535    d        22.98   1.634e-06       1.814
  1  snp_4_M           10    B    0.4895     0.496    A        0.169       0.681      0.9743
```

**WARNING** Again, please note that this procedure does not produce anything like realistic patterns of LD as one would expect to observe in whole genome datasets: rather, this simply simulates pairs of markers, for which there is LD within, but not between, pairs.

## 21.3 Resimulating a sample from the same population

The `--simulate` command also generates the file `plink.simfreq`. This records, for each SNP of the two sets, `null` and `disease` from the `wgas.sim` example, the *actual* allele frequency chosen for that particular SNP when simulating the data. For example,

```
1 null_0   0.1885 0.1885       1
1 null_1   0.424675 0.424675   1
1 null_2   0.12797 0.12797     1
1 null_3   0.544394 0.544394   1
1 null_4   0.938641 0.938641   1
....
```

Conveniently, this information is output in the same format as the original simulation file: note how the upper and lower allele frequency range is converged to specify a particular value, i.e. the first row shows a range of 0.1885 to 0.1885, i.e. effectively forcing the allele frequency for the first SNP to be 0.1885. This can be useful, as to generate a new independent dataset *from the same population as the first*, you would simply use the `plink.simfreq` output file, as input for a new `--simulate` command, see below.

Putting this together, one might imagine setting up a simple screen/replicate simulation design: first we generate the original WGAS screening data

```
./plink --simulate wgas.sim --make-bed --out screen
```

run our association test

```
./plink --bfile screen --assoc
```

and extract a list of significant SNPs (here using the Unix `gawk` command, to filter on the p-value column, 9)

```
gawk ' NR>1 && $9 < 1e-3  print $2  ' plink.assoc > positives
```

and then generate and test these same SNPs in an independent sample

```
./plink --simulate screen.simfreq --extract positives --assoc --out replication
```

etc. By labeling true disease SNPs and null SNPs sensibly as above, you can tell how many true positives and false positives appear at the screening and the replication stages, e.g. using Unix `bash` shell scripting to summarise results:

```
t=1e-3
s0=‘fgrep null plink.assoc | gawk ' $9 < t ' t=$t | wc -l‘
s1=‘fgrep disease plink.assoc | gawk ' $9 < t ' t=$t | wc -l‘
echo "Detected $s1 true positives and $s0 false positives in screening"
t=1e-2
s0=‘fgrep null replication.assoc | gawk ' $9 < t ' t=$t | wc -l‘
s1=‘fgrep disease replication.assoc | gawk ' $9 < t ' t=$t | wc -l‘
echo "Of these, $s1 true positives and $s0 false positives replicate"
```

# Chapter 22

# SNP scoring routine

PLINK provides a simple means to generate *scores* or *profiles* for individuals based on a simple allelic scoring system involving one or more SNPs. One potential use of such would be to assign a single quantitative index of genetic load, perhaps to build simple multi-SNP prediction models, or just as a quick way to identify a list of individuals containing one or more of a set of variants of interest.

## 22.1 Basic usage

The basic command to generate a score is the `--score` option, e.g.

```
./plink --bfile mydata --score myprofile.raw
```

which takes as a parameter the name of a file (here `myprofile.raw`) that describes the scoring system. This file has the format of one or more lines, each with exactly three fields

```
SNP ID
Reference allele
Score (numeric)
```

for example

```
SNPA   A    1.95
SNPB   C    2.04
SNPC   C   -0.98
SNPD   C   -0.24
```

These scores can be based on whatever you want. One choice might be the log of the odds ratio for significantly associated SNPs, for example. Then, running the command above would generate a file

```
plink.profile
```

with one individual per row and the fields:

```
FID     Family ID
IID     Individual ID
PHENO   Phenotype for that
CNT     Number of non-missing SNPs used for scoring
CNT2    The number of named alleles
SCORE   Total score for that individual
```

The score is simply a sum across SNPs of the number of reference alleles (0,1 or 2) at that SNP multiplied by the score for that SNP. For, example,

```
Variant(1/2)          A/T         C/G         A/C        C/G
Freq. of allele 1     0.20        0.43        0.02       0.38
```

```
Ind 1 genotype        A/A         G/G         A/C        0/0
# ref alleles          2           0           1         2*0.38 (=expectation)
Score           (  2*1.95    +   0*2.04  +  1*(-0.98) +  2*0.38*(-0.24) ) / 4
               =    2.74 / 4   =  0.68
```

The score 2.74/4 (the average score per non-missing SNP) could then be used, e.g. as a covariate, or a predictor of disease if it is scored in a sample that is independent from the one used to generate the original scoring weights. Obviously, a score profile based on some effect size measure from a large number of SNPs will necessarily be highly correlated with the phenotype in the original sample: i.e. this in no (straightforward) way provides additional statistical evidence for associations *in that sample*.

## 22.2 Multiple scores from SNP subsets

To calculate multiple scores from subsets of SNPs in a single `--score` file, it is possible to use the two commands, each followed by a filename, e.g.

```
--q-score-file snpval.dat
--q-score-range q.ranges
```

in addition to `--score`, where `snpval.dat` is a file that contains for each SNP a number (e.g. that might be the p-value from some test)

```
rs00001  0.234
rs00002  0.046
rs00003  0.887
...
```

and `q.ranges` is a file in which each row corresponds to a different score, containing a label, then a lower and upper bound for the values as given in the other file, e.g.

```
S1  0.00 0.01
S2  0.00 0.20
S3  0.10 0.50
```

would create three score files,

```
plink.S1.profile
plink.S2.profile
plink.S3.profile
```

in which the first only uses SNPs that have a value in `snpval.txt` between 0.0 and 0.01; the second uses only SNPs which have a value between 0.00 and 0.20, etc.

## 22.3 Misc. options

By default, if a genotype in the score is missing for a particular individual, then the expected value is imputed, i.e. based on the sample allele frequency. To change this behavior, add the flag

```
--score-no-mean-imputation
```

which means the above example would be calculated as

```
Score           (  2*1.95    +   0*2.04  +  1*(-0.98)  ) / 3
               =    2.92 / 3   =  0.97
```

# Chapter 23

# Rare copy number variant (CNV) data

This page describes some basic file formats, convenience functions and analysis options for rare copy number variant (CNV) data. Support for common copy number polymorphisms (CNPs) is described here.

Copy number variants are represented as *segments*. These segments are essentially represented and analysed in a similar manner to how PLINK handles runs of homozygosity (defined by a start and stop site on a given chromosome). Allelic (i.e. basic SNP) information is not considered here: `PLINK` skips the usual procedure of reading in SNP genotype data.

Here we assume that some other software package such as the Birdsuite `http://www.broad.mit.edu/mpg/birdsuite/` package has previously been used to make calls for either specific copy-number variable genotypes or to identify particular genomic regions in individuals that are deletions or duplications, based on the raw data. That is, PLINK only offers functions for downstream analysis of CNV data, not for identifying CNVs in the first place, i.e. similar to the distinction between SNP genotype calling versus the subsequent analysis of those calls.

In this section, we describe the basic format for rare CNV data; the steps involved in making a MAP file and loading the data. We consider ways to filter the CNV lists by type, genomic location or frequency. We describe options for relating CNVs to phenotype, either at the level of genome-wide burden or looking for specific associations. Finally, we detail the tools for producing reports of any genes intersected by CNVs and for displaying groups of overlapping CNVs.

## 23.1 Basic support for segmental CNV data

The basic command for reading a list of segmental CN variants is

```
plink --cnv-list mydata.cnv
      --fam mydata.fam
      --map mydata.cnv.map
```

which can be abbreviated

```
plink --cfile mydata
```

(note that the map file must have the `.cnv.map` map extension). The CNV list file `mydata.cnv` has the format

```
FID     Family ID
IID     Individual ID
```

| | |
|---|---|
| CHR | Chromosome |
| BP1 | Start position (base-pair) |
| BP2 | End position (base-pair) |
| TYPE | Type of variant, e.g. 0,1 or 3,4 copies |
| SCORE | Confidence score associated with variant |
| SITES | Number of probes in the variant |

Having a header row is optional; if the first line starts with `FID` it will be ignored.

**Note** The `SCORE` and `SITES` values are not used in any direct way, except potentially as variates to filter segments on, as described below. That is, the values of these do not fundamentally impact the way analysis is performed by `PLINK` itself (they might alter the meaning of the results of course, e.g. if including low-confidence calls into the analysis!). In other words, if whatever software was used to generate the CNV calls does not supply some conceptually similar values, it is okay to simply put dummy codes (e.g. all 0) in these two fields.

The first few lines of a small example file is shown here:

```
FID    IID   CHR        BP1        BP2  TYPE   SCORE  SITE
P1     P1      4   71338469   71459318     1      27     0
P1     P1      5   31250352   32213542     1    34.2     0
P1     P1      7   53205351   53481230     3    18.2     0
P2     P2     11   86736484   87074601     1      22     0
P2     P2     14   47817280   47930190     4    55.1     0
...
```

The FAM file format is the first 6 fields of a PED file, described here; this file lists the sex, phenotype and founder status of each individual. The MAP file format is described here, although the next section how this can be automatically created using the `--cnv-make-map` command.

## 23.2 Creating MAP files for CNV data

Prior to any analysis, a dummy MAP first needs to be created (this step only needs to be performed once per CNV file). This PLINK-generated MAP file has dummy entries that correspond to the start and stop sites of all segments. This facilitates subsequent parsing and analysis of CNV data by `PLINK`. The `--cnv-make-map` command is used as follows:

```
plink --cnv-list mydata.cnv --cnv-make-map
```

which creates a file

```
plink.cnv.map
```

which will look just like a standard MAP file but with dummy markers:

```
1       p1-51593   0    51593
1       p1-51598   0    51598
1       p1-51666   0    51666
1       p1-52282   0    52282
1       p1-69061   0    69061
...
```

where the marker names start with the `p` prefix and contain chromosome and base-position information.

As an (unrealistic) example to illustrate how the mapping works, consider the following, with 3 segments, spanning ”positions” 1 to 8, 4 to 12 and 16 to 23. In this case, 6 unqiue map positions would be created, the three start positions and the three stop positions.

```
Base                 1111111111222222
Position   1234567890123456789012345
Marker #   1..2...3...4...5......6..
```

```
           |  |   |   |   |      |
                      |   |      |
Segments   *------*       |      |
              *-------*
                          *------*
```

The new MAP file would then be

```
1 p1-1   0  1
1 p1-4   0  4
1 p1-8   0  8
1 p1-12  0  12
1 p1-16  0  16
1 p1-23  0  23
```

Given such a MAP file, these three segments would then be perfectly mapped to the corresponding markers (`p1-1` to `p1-8`, `p1-4` to `p1-12` and `p1-16` to `p1-23`). The created MAP file is then specified in subsequent segmental CNV analyses (using `--cnv-list`) with the standard `--map` command (or `--cfile` command).

## 23.3 Loading CNV data files

Once a suitable MAP file has been created, i.e. with dummy markers that correspond to the position of every start and stop site of all segments, use the `--cnv-list` command again to load in the CNV segment data. As mentioned above, in addition to the basic CNV file, a MAP (previously generated) and FAM file (continaing ID and phenotype information) also need to be specified. For example.

```
plink --map plink.cnv.map --fam mydata.fam --cnv-list mydata.cnv
```

Alternatively, if the MAP, FAM and CNV list files all have the same root, the command

```
plink --cfile study1
```

is equivalent, i.e. it implies the following files exist

```
study1.cnv
study1.cnv.map
study1.fam
```

By default either command will simply load in the CNV data and produce a report in the LOG file, enumerating the number of CN states in the total dataset and any filtering processes applied. For example,

```
Reading segment list (CNVs) from [ cnv1.list ]
714 of 2203 mapped as valid segments
1872 mapped to a person, of which 714 passed filters
   CopyN  Count
   0      46
   1      339
   3      200
   4      129
Writing segment summary to [ plink.cnv.indiv ]
```

This indicates that of 2203 total segments (i.e. should correspond to number of lines in the `cnv1.list` file, allowing for any header) 1872 are mapped to a person in the dataset. In other words, some of the segments in `cnv1.list` are for individuals not in `cnv1.fam`. These are simply ignored; for example, these individuals might have been filtered out of the study for other reasons, e.g. QC based on standard SNP genotypes. Of these, 714 passed the further set of filters, as described below. As described below, segments can be filtered based on genomic location, frequency, size, quality score/number of sites and type (duplication or deletion).

It will also be reported in the LOG file if some of the segments do not map to a marker in the MAP file: if this is because you've used `--chr` or similar commands to restrict the portion of the data examined, you can safely ignore this line; otherwise, it might mean that the appropriate MAP file wasn't created (e.g. using `--cnv-make-map`) for that CNV file.

By default, `PLINK` will create a file that summarises per individual events (after any filtering has been applied), in a file named

```
plink.cnv.indiv
```

which has the fields, one row per person, in the same order as the original FAM file:

```
FID     Family ID
IID     Individual ID
PHE     Phenotype
NSEG    Number of segments that individual has
KB      Total kilobase distance spanned by segments
KBAVG   Average segment size
```

PLINK will also create a file

```
plink.cnv.summary
```

that represents a count of CNVs, in cases (`AFF`) and controls (`UNAFF`) that overlap each map position.

## 23.4 Checking for overlapping CNV calls (within the same individual)

As a sanity check of a CNV file: to check whether segments are overlapping for the same person (e.g. if a deletion and a duplication event had been specified for the same person in the same region, or if the same event is listed twice), use the option

```
plink --cfile mydata --cnv-check-no-overlap
```

If there is overlap, this writes a warning to the LOG, with the number of implicated events:

```
Within-individual CNV overlap detected, involving 2 CNVs
```

and creates a file

```
plink.cnv.overlap
```

that lists these offending segments, with the format:

```
FID   Family ID
IID   Individual ID
CHR   Chromosome code
BP1   Segment start (bp)
BP2   Segment end (bp)
```

## 23.5 Filtering of CNV data based on CNV type

The segments read in can be filtered in a number of ways. First, one can specify to read in only either deletions (`TYPE` is less than 2) or duplications (`TYPE` is greater than 2), with the options,

```
--cnv-del
```

and

```
--cnv-dup
```

Segments can also be filtered based on a minimum size (kb), score or number of sites contributing with the following commands:

```
--cnv-kb 50
--cnv-score 3
--cnv-sites 5
```

The default minimum segment size is 20kb; none of the other filters have a default setting that would exclude anything. Also, corresponding maximum thresholds can be set:

```
--cnv-max-kb 2000
--cnv-max-score 10
--cnv-max-sites 10
```

As mentioned above, the `SCORE` and `SITES` fields are not used for any other purpose in analysis, and so if you do not have this information, can can safely enter dummy information (e.g. a value of 1 for every CNV).

The set of individuals for whom segment data are based on can be modified with the standard `--keep` and `--remove` options, to exclude people from the analysis.

## 23.6 Filtering of CNV data based on genomic location

It is possible to extract a specific set of segments that overlap with one or more regions as specified in a file, e.g. that might contain the genomic co-ordinates for genes or segmental duplications, etc. Use the command

```
--cnv-intersect regions.list
```

The file `regions.list` should be in the following format: one range per line, whitespace-separated:

```
CHR     Chromosome code (1-22, X, Y, XY, MT, 0)
BP1     Start of range, physical position in base units
BP2     End of range, as above
MISC    Any other fields after 3rd ignored
```

For example, if `regions.list` were

```
2 30000000 35000000  REGION1
2 60000000 62000000
X 10000000 20000000  Linkage hotspot
```

then

```
plink --cfile mydata --cnv-intersect regions.list
```

would extract all segments in `mydata.cnv` that at least partially span these three regions (5Mb and 2Mb on chromosome 2 and 10Mb on chromosome X), ignoring the comments or gene names. A typical type of file used with `--cnv-intersect` will often be a list of genes (such as available in the resources page).

Alternatively, you can use

```
--cnv-exclude regions.list
```

to filter out a specific set of segments, i.e. to remove any CNVs that overlap with one or more regions specified in the file `regions.list`.

Assuming the region file has consistent, unique names in the fourth field, the command

```
--cnv-subset mylist.txt
```

takes a list of region names and extracts just these from the main `--cnv-intersect`, `--cnv-exclude` (or `--cnv-count`, as described below) list. e.g. if `mylist.txt` contained

```
REGION1
REGION2
```

and `region.list` where

```
2 30000000 35000000  REGION1
2 60000000 62000000  GENE22
X 10000000 20000000  LinkageHotspot
```

then only the first region (chromosome 3, 30Mb to 35Mb, labelled `REGION1`) would be extracted, as `REGION2` does not exist. The `--cnv-subset` command requires that the `regions.list` file has exactly four fields (i.e. always a unique region/gene name in the fourth field).

### 23.6.1 Defining overlap for partially overlapping CNVs and regions

The basic intersection or exclusion commands will select all segments that are at least partially in the specified region. Alternatively, one can select only segments that have at least *X* percent of them in the specified region, for example

```
--cnv-overlap 0.50
```

would only include (`--cnv-intersect`), or exclude (`--cnv-exclude`), events that have at least 50% of their length spanned by the region.

There are two other variant forms of the overlap command, which change the denominator in calculating the proportion overlap:

```
--cnv-union-overlap 0.50
```

which defines overlap as the ratio of the intersection and the union, also

```
--cnv-region-overlap 0.50
```

which defines overlap as the ratio of the intersection and the length of the region (rather than the CNV). For example,

```
------|-----|---------------------   Region/gene
----------+++++++++++++++---------   CNV (duplication, +)
----------XXX---------------------   Intersection
----------XXXXXXXXXXXXXXX---------   Denominator for basic overlap
------XXXXXXXXXXXXXXXXXXX---------   Denominator for union overlap
------XXXXXXX---------------------   Denominator for region overlap
```

In this example, if we take each character to represent a standard length

```
Default overlap = 3 / 15
Union overlap = 3 / 19
Region overlap = 3 / 7
```

This next example illustrates how the overlap statistics can then subsequently be used to include or exclude specific CNVs: if overlap threshold were set to 0.5, then only the first of therse two CNVs would be selected by `--cnv-intersect`

```
------|----------|----------------
--------OOOOOOOOOOXXX-------------   Selected
------------OOOOOOXXXXXXXXXXXXXX--   Not selected
```

The default setting is equivalent to setting `--cnv-overlap 0` (i.e. *more than* 0% must overlap).

Finally, the command

```
--cnv-disrupt
```

will select only CNVs that start or stop *within* a region specified in the region list (i.e. resulting in a partially deleted or duplicated gene or region). The normal overlap commands cannot be used in conjunction with the `--cnv-disrupt` defintion of whether or not a CNV overlaps a gene.

### 23.6.2 Filtering by chromosomal co-ordinates

In addition, the standard commands for filtering chromosomal positions are still applicable, for example

```
--chr 5
```

or

```
--chr 2 --from-mb 20 --to-mb 25
```

Note that for a CNV to be included when using these filters, both the start and stop site must fall *within* the prespecified range (i.e. a CNV spanning from 19 to 24Mb on chromosome 2 would not be included in the above example).

## 23.7 Filtering of CNV data based on frequency

It is also possible to exclude based on the frequency of CNVs at a particular position. There are two main approaches to this: by assigning frequencies for *regions* and then applying the same routines as for the range-intersection command described above, or alternatively by assigning each CNV a single, specific count.

These commands, and the differences between them, are described more fully on this page. As well as the two basic approaches described above, one can specify different degrees of overlap when calculating frequencies, which can alter the result of frequency filtering.

The key commands and some examples are given here. To remove segments that map to regions with more than 10 segments

```
--cnv-freq-exclude-above 10
```

To remove any segments that only have at most 4 copies

```
--cnv-freq-exclude-below 5
```

To remove any segments not in regions with exactly 5 copies

```
--cnv-freq-exclude-exact 5
```

and correspondingly to include only segments in regions with exactly 5 copies

```
--cnv-freq-include-exact 5
```

As with the earlier range intersection commands, the definition of *intersection* can be *soft*, specified with the `--cnv-overlap` option. In most cases here, one would probably want to allow for a soft filtering, e.g. with `--cnv-overlap 0.5` for example.

For example, given the following segments, and counts below

```
      Segments   *------*
                    *------------*
                                *------*
        Counts 001112222211111112211111100000
Common regions      XXXXX       XX
```

then `--cnv-freq-exclude-above 1` would remove all three segments if `--cnv-overlap 0` (the default) were set. This is because each CNV has at least some part of it that intersects with a region that contains more than 1 CNV. However, if `--cnv-overlap` were instead set to 0.5, for example, then only the top segment would be removed (as the other two segments have more than 50% of their length outside of a region with more than 1 segment). If the overlap were set higher still, then in this example no CNVs would be removed by the command `--cnv-freq-exclude-above 1`.

**NOTE** Because multiple CNVs at the same region will not all exactly overlap, and may be spanned by distinct larger events, or contain smaller events, in other individuals, then requesting that you include only CNVs with exactly five copies for example (`--cnv-freq-include-exact 5`) does **not** mean that at all positions in the genome you will always see either 0 or 5 copies. Rather, the selection process works exactly as specified above. Please see this page for further details.

### 23.7.1 Alternative frequency filtering specification

The alternate approach is invoked with the command

```
--cnv-freq-method2 0.5
```

where the value following it represents an overlap parameter (there is no need to specify the `--cnv-overlap` command directly when using `--cnv-freq-method2`). Based on this overlap, PLINK will assign a specific count to each CNV that represents the number of CNVs that overlap it (including itself) based on a union intersection overlap definition with the specified proportion parameter, between that CNV and all CNVs.

This approach is illustrated in the page, that gives more details on the frequency filtering commands including a comparison to the region-based approach to filtering, described above.

If the `--cnv-freq-method2` command is used, then the other frequency filtering commands will use the CNV-based counts to include of exclude CNVs, for example

```
plink --cfile mydata
      --cnv-freq-method2 0.5
      --cnv-freq-exclude-above 10
```

If `--cnv-write` (see below) is specified with `--cnv-freq-method2`, then the additional command

```
--cnv-write-freq
```

will add a field `FREQ` to the `plink.cnv` file generated that shows the frequency for each CNV. Also, the `--cnv-seglist` command (see below) can be modified with `--cnv-write-freq` (to report the frequency as a number at the start and stop of each CNV instead of the usual codes).

### 23.7.2 Miscellaneous commands frequency filtering commands

To keep only segments that are unique to either cases or to controls

```
--cnv-unique
```

This can be used in conjunction with other frequency filter commands. To drop individuals from the file who do not have at least one segment after filtering, add the flag

```
--cnv-drop-no-segment
```

This can make the `plink.cnv.indiv` summary files easy to browse, for example.

## 23.8 Association analysis of segmental CNV data

To perform a set of global test of CNV burden in cases versus controls, add the

```
--cnv-indiv-perm
```

option as well as

```
--mperm 10000
```

for example (i.e. permutation is required). By default, this reports on four tests, which use these metrics to calculate burden in both cases and controls

| | |
|---|---|
| RATE | Number of segments |
| PROP | Proportion of sample with one or more segment |
| TOTKB | Total kb length spanned |
| AVGKB | Average segment size |

Tests are based (1-sided) on comparing these metrics in cases versus controls, evaluated by permutation.

If a list of regions is supplied in a file, e.g. `gene.list` and the command

```
--cnv-count gene.list
```

then an extra test is added

| | |
|---|---|
| GRATE | Number of regions/genes spanned by CNVs |
| GPROP | Number of CNVs with at least one gene |
| GRICH | Number of regions/genes per total CNV kb |

These tests respect all the normal filtering commands, with the exception that `--cnv-intersect` and `--cnv-exclude` cannot be used if `--cnv-count` is also being used.

The mean metrics in cases and controls are reported in the file

```
plink.cnv.grp.summary
```

when the `--cnv-indiv-perm` command is used. For example: this gives the number of events (`N`) in cases and controls, the rate per person, the proportion of cases/controls to have at least one event, the total distance spanned per person and the average event size per person.

```
 TEST     GRP      AFF    UNAFF
    N     ALL      528      362
 RATE     ALL   0.1557   0.1138
 PROP     ALL   0.1309   0.1041
TOTKB     ALL    290.8    265.4
AVGKB     ALL    249.8    243.3
```

As usual, if the `--within` command is added and a cluster file specified, then any permutations are performed within cluster. In this case, the statistics displayed in the `plink.cnv.grp.summary` file are also split out by the strata as well as presented in total (as indicated by the `GRP` field).

## 23.9 Association mapping with segmental CNV data

To perform a simple permutation-based test of association of segmental CNV data for case/control phenotypes, add the option

```
--mperm 50000
```

to perform, for example, 50,000 null permutations to generate empirical p-values. T he results are saved in the file

```
plink.cnv.summary.mperm
```

This is a standard empirical p-value file: `EMP1` and `EMP2` represent pointwise and genome-wide corrected p-values, respectively. Both tests are 1-sided by default.

You can consult the corresponding

```
plink.cnv.summary
```

that is also generated for details of the association: this file has the fields

```
CHR    Chromosome code
SNP    SNP identifier (dummy SNP, see below)
BP     Base-pair position
AFF    Number of affected individuals with a segment at this position
UNAFF  Number of unaffected individuals
```

To instead perform a 2-sided test (i.e. allowing that events might be more common in controls) add the flag

```
--cnv-test-2sided
```

To perform an analysis in which the total number of events within a sliding window is compared between cases and controls (rather than the number overlapping a single position) add the flag

```
--cnv-test-window 50
```

where the parameter is the kb window either side of the test position. As before, the association results are reported per marker, but now the counts indicate the total number of segments that overlap any of the 100kb window surrounding the test position (+/- 50kb), rather than just the test position itself. Significance is evaluated by permutation as before.

## 23.10 Association mapping with segmental CNV data: regional tests

To perform a test of association for CNVs in particular regions, use the command

```
./plink --cfile mydata --cnv-intersect glist-hg18 --cnv-test-region --mperm 10000
```

where `glist-hg18` contains a list of genes (as available from the resources page. The output is written to

```
plink.cnv.regional.summary
```

which has the fields

```
CHR       Chromosome code
REGION    Name of region
BP1       Start position of region
BP2       End position of region
AFF       Number of case CNVs spanning region
UNAFF     Number of control CNVs spanning region
```

and the permutation results are written to

```
plink.cnv.regional.summary.mperm
```

which has the fields

```
CHR     Chromosome code
REGION  Name of region
STAT    Statistic
EMP1    Empirical p-value, per region
EMP2    Empirical p-value, corrected for all tests
```

For example, the line

```
CHR           REGION          BP1          BP2      AFF    UNAFF
  1           TTLL10      1079148      1143176        2        3
  ...
```

implies 2 case CNVs (note, PLINK does not distinguish whether these CNVs belong to the same individual or not) and 3 control CNVs span the gene `TTLL10`. The standard commands for regions in CNV analysis such as `--cnv-border` and `--cnv-overlap` can be used in this context.

## 23.11 Association mapping with segmental CNV data: quantitative traits

To test for association between rare CNVs and a quantitative trait, use the same commands as for disease traits. PLINK will automatically detect that the phenotype is continuous. For example, if the file `pheno.qt` contains a quantitative trait, the command

```
./plink --cfile mydata --pheno qt.dat --mperm 10000
```

will generate a file

```
plink.cnv.qt.summary
```

which contains the fields

```
CHR    Chromosome code
SNP    Dummy label for map position
BP     Physical position (base-pairs)
NCNV   Number of individiuals with a CNV here
```

```
M1     QT mean in individuls with a CNV here
M0     QT mean in individuals without a CNV here
```

and the file

```
plink.cnv.qt.summary.mperm
```

that contains the empirical p-values, `EMP1` and `EMP2`, as for disease traits. The only difference is that the quantitative trait test is, by default, two-sided. To perform a 1-sided CNV test, add the command

```
--cnv-test-1sided
```

**NOTE** Currently, genome-wide burden (`--cnv-indiv-perm`), window-based (`--cnv-test-window`) and region-based (`--cnv-test-region`) CNV association tests are not available for quantitative traits.

## 23.12 Writing new CNV lists

Given a set of filters applied, you can output as a new CNV file the filtered subset, with the command

```
--cnv-write
```

For example, to make a new file using only deletions over 200kb but not more than 1000kb, with a quality score of 10 or more, use the command

```
plink --cfile cnv1
      --cnv-del
      --cnv-kb 200
      --cnv-max-kb 1000
      --cnv-score 10
      --cnv-write
      --out hiqual-large-deletions
```

which will generate two new files

```
hiqual-large-deletions.cnv
hiqual-large-deletions.fam
```

To obtan a corresponding MAP file, so that you can subsequently use

```
--cfile hiqual-large-deletions
```

give the command

```
plink --cnv-list hiqual-large-deletions.cnv --cnv-make-map --out hiqual-large-deletions
```

(although note that this will overwrite the LOG file generated by the `--cnv-write` command).

### 23.12.1 Creating UCSC browser CNV tracks

As opposed to listing CNVs in PLINK format with `--cnv-write`, the command `--cnv-track` will generate a UCSC-friendly BED file (note: this is distinct from a PLINK binary PED file) that can be uploaded to their browser for convenient viewing.

```
plink --cfile mydata --cnv-track --out mycnvs
```

which generates a file

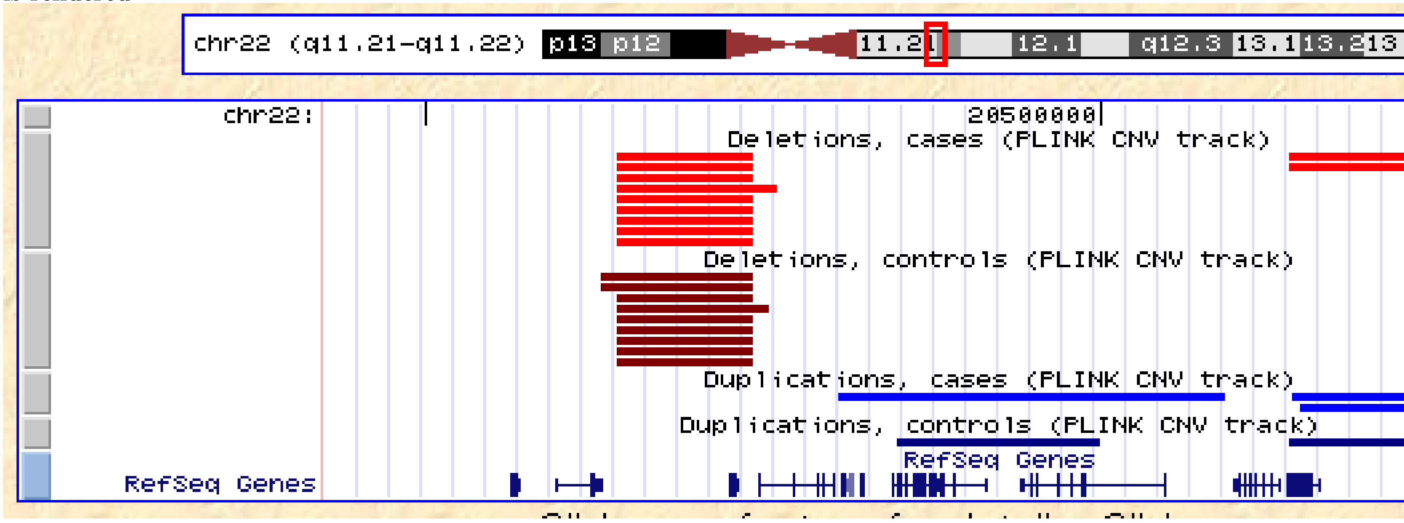
```
plink.cnv.bed
```

The filtering commands described above can be combined with this option.

By using the *Manage custom tracks* option on the UCSC genome browser `http://genome.ucsc.edu/cgi-bin/hgGateway`, one can easily visualise the CNV data, along side other genomic features. For example, the file (IID and `SCORE`, `SITES` information is omitted for clarity)

```
FID IID  CHR      BP1        BP2   TYPE    SCORE    SITES
... ...   22   20140420   20241877    1      ...      ...
... ...   22   20140420   20241877    1      ...      ...
... ...   22   20129453   20241877    1      ...      ...
... ...   22   20140609   20241877    1      ...      ...
... ...   22   20140420   20241877    1      ...      ...
... ...   22   20140420   20241877    1      ...      ...
... ...   22   20639721   20793965    1      ...      ...
... ...   22   20639721   20765489    1      ...      ...
... ...   22   20305076   20591362    3      ...      ...
... ...   22   20646213   20756780    3      ...      ...
... ...   22   20140420   20259122    1      ...      ...
... ...   22   20639866   20787533    3      ...      ...
... ...   22   20140420   20241877    1      ...      ...
... ...   22   20140420   20241877    1      ...      ...
... ...   22   20140420   20241877    1      ...      ...
... ...   22   20140420   20241877    1      ...      ...
... ...   22   20348901   20498220    3      ...      ...
... ...   22   20140420   20241877    1      ...      ...
... ...   22   20140420   20241877    1      ...      ...
... ...   22   20639643   20793173    3      ...      ...
... ...   22   20140420   20241877    1      ...      ...
... ...   22   20141114   20241877    1      ...      ...
... ...   22   20140420   20254215    1      ...      ...
... ...   22   20140420   20241877    1      ...      ...
... ...   22   20129130   20241877    1      ...      ...
```

is rendered


chr22 (q11.21-q11.22)
p13
p12
11.21
12.1
q12.3
13.1
13.2
chr22:
20500000
Deletions, cases (PLINK CNV track)
Deletions, controls (PLINK CNV track)
Duplications, cases (PLINK CNV track)
Duplications, controls (PLINK CNV track)
RefSeq Genes
RefSeq Genes

Note that the CNVs are split by deletion versus duplication (red versus blue) and case versus control (light versus dark).

Additionally, a poor-man's version of this plot can be obtained with the command

```
--cnv-seglist
```

which produces a file

```
plink.cnv.seglist
```

which, for the CNV list above, can be seen here. Deletions and duplications are represented by `+` and `-` symbols at the start of each CNV; case and control status is represented as `A` and `U`.

Finally, it is also possible to report CNVs annotated by the regions or genes they span (see `--cnv-verbose-report-regions` described below.

## 23.13 Listing intersected genes and regions

With the `--cnv-intersect` (or `--cnv-exclude`) command, you can add the flag

```
--cnv-report-regions
```

which will create a file

```
plink.reg
```

listing only the regions that intersect (or do not intersect) with any of the CNVs (given the filtering and overlap commands that might also be specified). For example, to obtain a list of genes that are intersected by a rare case singleton deletions over 500kb (i.e. event seen only once)

```
plink --cfile mydata
      --filter-cases
      --cnv-freq-exclude-above 1
      --cnv-del
      --cnv-kb 500
      --cnv-report-regions
      --cnv-intersect glist-hg18
```

Alternatively, the command

```
--cnv-verbose-report-regions
```

produces a verbose form of `plink.reg`, which does not just list the regions or genes intersected but lists the specific segmental CNVs also. This can be used in conjunction with, for example,

```
--cnv-subset genes.txt
```

in order to produce reports on specific genes of interest. For example if `genes.txt` contained

```
HES4
ISG15
```

then

```
plink --cfile mydata
      --cnv-verbose-report-regions
      --cnv-intersect glist-hg18
      --cnv-border 20
      --cnv-subset genes.txt
```

would produce a file

```
plink.reg
```

that, for each gene/region, contains the following fields

```
FID        Family ID
IID        Individual ID
```

```
     PHE        Phenotype
     CHR        Chromosome code
     BP1        Start position (base-pair)
     BP2        Stop position (base-pair)
     TYPE       DELetion or DUPlication
     KB         Kilobase length of CNV
     OLAP       Overlap (extent of CNV covered by gene)
     OLAP_U     Union overlap (ration of intersection to union)
     OLAP_R     Region overlap (extent of gene covered by CNV)
```

that might contain something like the following report

```
 RANGE (+/- 20kb )  [ 1 924206 925333 HES4 ]
      FID   IID   PHE  CHR       BP1      BP2  TYPE      KB     OLAP   OLAP_U   OLAP_R
     P001     1     2    1    789258  1232396   DUP   443.1  0.09281  0.09281        1
     P002     1     1    1    826576  1304312   DEL   477.7  0.08609  0.08609        1
     P003     1     2    1    864765  1913364   DUP    1049  0.03922  0.03922        1
     P004     1     1    1    890974  1258710   DUP   367.7   0.1118   0.1118        1
 RANGE (+/- 20kb )  [ 1 938709 939782 ISG15 ]
      FID   IID   PHE  CHR       BP1      BP2  TYPE      KB     OLAP   OLAP_U   OLAP_R
     P001     1     2    1    789258  1232396   DUP   443.1  0.09269  0.09269        1
     P002     1     1    1    826576  1304312   DEL   477.7  0.08598  0.08598        1
     P003     1     2    1    864765  1913364   DUP    1049  0.03917  0.03917        1
     P004     1     1    1    890974  1258710   DUP   367.7   0.1117   0.1117        1
```

That is, this is a list of any CNV that at least partially overlaps these two genes. The exact behavior can be modified with flags such as `--cnv-del`, `--cnv-kb`, `--cnv-disrupt`, `--cnv-overlap`, `--filter-cases`, etc.

## 23.14 Reporting sets of overlapping segmental CNVs

Finally, there are two option to group or report sets of segments that span a particular position. In the first case, use the option

```
--segment-group
```

which takes all segments in a given region (whole genome unless otherwise specified) and forms ”pools” of overlapping segments. Several pools of overlapping segments will be created; these will be listed in order of decreasing size (number of segments); note that the same segment can appear in multiple pools (e.g. if A overlaps with C, and B overlaps with C, but A and B do not overlap). The pools give information as described below.

The more restricted form of this command forms a single pool of all segments that overlap a particular position, which takes a single parameter of a marker name; typically these will be the dummy `pos*` markers created by the `--cnv-make-map` command.

```
--segment-spanning pos119
```

In this case, for some made-up data, we see from the `plink.cnv.summary` file that there are 8 cases and 6 controls with a segment spanning a particular position, `pos586`

```
     CHR         SNP           BP      AFF    UNAFF
     ...         ...          ...      ...      ...
       1      pos586     16631570        8        6
     ...         ...          ...      ...      ...
```

In this case, there is unsurprisingly no association between segmental CNVs and disease: for example, the corresponding position in the `plink.cnv.summary.mperm` file shows an empirical p-value of 0.35, but of p=1 if adjusted for multiple testing (`EMP2`)

```
CHR      SNP        STAT        EMP1        EMP2
...      ...         ...         ...         ...
  1   pos586    0.419408    0.351324           1
...      ...         ...         ...         ...
```

Naturally, one would usually be more interested in following up significantly associated regions of course... Nonetheless, if so desired we can see which segments (given any of the filtering specified) are spanning this position, with `--segment-spanning`, which gives the following:

```
POOL       FID       IID   PHE  CHR       BP1       BP2       KB  TYPE  SCORE
  S1   PT-2378   PT-2378     2   12  16631570  16751087  119.517   DEL  10.23
  S1   PT-268D   PT-268D     2   12  16631494  16732162  100.668   DEL    9.3
  S1   PT-2M8O   PT-2M8O     1   12  16631441  16751082  119.641   DEL  31.23
  S1   PT-2FZ9   PT-2FZ9     2   12  16631436  16751045  119.609   DEL   15.2
  S1   PT-287D   PT-287D     1   12  16616579  17183201  566.622   DUP  200.3
  S1   PT-2C91   PT-2C91     2   12  16616579  16751045  134.466   DEL   14.3
  S1   PT-28A8   PT-28A8     1   12  16616579  16751045  134.466   DEL    8.3
  S1   PT-2FPB   PT-2FPB     1   12  16616579  16714372   97.793   DEL   11.1
  S1   PT-28IG   PT-28IG     2   12  16616579  16708856   92.277   DEL   10.3
  S1   PT-2E5N   PT-2E5N     2   12  16614664  16715703  101.039   DEL   9.87
  S1   PT-2FVL   PT-2FVL     1   12  16614664  16751045  136.381   DEL  10.67
  S1   PT-2DYE   PT-2DYE     2   12  16614664  16715489  100.825   DEL  11.82
  S1   PT-264I   PT-264I     2   12  16614664  16751045  136.381   DEL   14.2
  S1   PT-25WZ   PT-25WZ     1   12  16591338  16715767  124.429   DEL   14.7
  S1       CON        14   8:6   12  16631570  16708856   77.286    NA     NA
  S1     UNION        14   8:6   12  16591338  17183201  591.863    NA     NA
```

For CNV data (in contrast to shared segments based on homozygosity or IBD sharing) the extra fields of `TYPE` (deletion or duplication) and `SCORE` (some metric of quality/confidence of CNV call) are also presented.

Here we see the 14 segments listed, 8 cases and 6 controls. The `CON` and `UNION` lines at the end of the pool give the consensus region (i.e. shared by all segments) and the total distance spanned by all. The `PHE` field gives the phenotype for each individual.

Note that the way in which the dummy markers are selected will effectively mean that every possibly unique position, in terms of counts of segments, is evaluated. The actual base pair regions of any dummy marker is itself probably not of interest: given a sigificant (set of) SNPs, the strategy would be to select any one and generate the corresponding pool to see what and where the association maps to.

# Chapter 24

# Resources available for download

This page contains links to several freely-available resources, mostly generated by other individuals. All these resources are provided ”as is”, without any guarantees regarding their correctness or utility.

## 24.1 The Phase 2 HapMap as a PLINK fileset

The HapMap `http://www.hapmap.org` genotype data (the latest is release 23) are available here as PLINK binary filesets. The SNPs are currently coded according NCBI build 36 coordinates on the forward strand. Several versions are available here: the entire dataset (a single, very large fileset: you will need a computer with at least 2Gb of RAM to load this file).

The *filtered* SNP set refers to a list of SNPs that have MAF greater than 0.01 and genotyping rate greater than 0.95 in the 60 CEU founders. This fileset is probably a good starting place for imputation in samples of European descent. Filtered versions of the other HapMap panels will be made available shortly.

| **Description** | **File size** | **File name** |
|---|---|---|
| Entire HapMap (release 23, 270 individuals, 3.96 million SNPs) | 120M | hapmap_r23a.zip `http://pngu.mgh.harvard.edu/~purcell/dist/hapmap\_r23a.zip` |
| CEU (release 23, 90 individuals, 3.96 million SNPs) | 59M | hapmap_CEU_r23a.zip `http://pngu.mgh.harvard.edu/~purcell/dist/hapmap\_CEU\_r23a.zip` |
| YRI (release 23, 90 individuals, 3.88 million SNPs) | 65M | hapmap_YRI_r23a.zip `http://pngu.mgh.harvard.edu/~purcell/dist/hapmap\_YRI\_r23a.zip` |
| JPT+CHB (release 23, 90 individuals, 3.99 million SNPs) | 58M | hapmap_JPT_CHB_r23a.zip `http://pngu.mgh.harvard.edu/~purcell/dist/hapmap\_JPT\_CHB\_r23a.z` |
| CEU founders (release 23, 60 individuals, filtered 2.3 million SNPs) | 31M | hapmap_CEU_r23a_filtered.zip `http://pngu.mgh.harvard.edu/~purcell/dist/hapmap\_CEU\_r23a\_fi` |
| YRI founders (release 23, 60 individuals, filtered 2.6 million SNPs) | 38M | hapmap_YRI_r23a_filtered.zip `http://pngu.mgh.harvard.edu/~purcell/dist/hapmap\_YRI\_r23a\_fil` |
| JPT+CHB founders (release 23, 90 individuals, filtered 2.2 million SNPs) | 33M | hapmap_JPT_CHB_r23a_filtered.zip `http://pngu.mgh.harvard.edu/~purcell/dist/hapmap\_JPT\_CH` |

| **Description** | **File size** | **File name** |
|---|---|---|
| Entire HapMap (release 22, 270 individuals, 3.96 million SNPs) | 110M | hapmap_r22.zip `http://pngu.mgh.harvard.edu/~purcell/dist/hapmap\_r22.zip` |
| CEU founders (release 22, 60 individuals, 3.96 million SNPs) | 49M | hapmap-ceu-all.zip `http://pngu.mgh.harvard.edu/~purcell/dist/hapmap-ceu-all.zip` |
| CEU founders (release 22, 60 individuals, filtered 2.2 million SNPs) | 29M | hapmap-ceu.zip `http://pngu.mgh.harvard.edu/~purcell/dist/hapmap-ceu.zip` |
| CEU founders (release 22, as above, files split by chromosome, 1-22 and X) | 29M | hapmap-ceu-by-chr.zip `http://pngu.mgh.harvard.edu/~purcell/dist/hapmap-ceu-by-chr.zip` |

| **Description** | **File name** |
|---|---|
| Hapmap individuals with population information ( FID, IID, POP ) | hapmap.pop `http://pngu.mgh.harvard.edu/~purcell/dist/hapmap.pop` |

## 24.2 Teaching materials and example dataset

A tutorial can be downloaded from here; the material is similar to the online tutorial but slightly more involved. As it currently stands, it is designed to first use *gPLINK* to perform a set of basic tests and QC procedures and then move to standard *PLINK* for more in-depth analysis.

It is designed to work on a standard modern laptop computer or equivalent desktop. It was written for vesion 1.02 of PLINK, but should remain compatible with future releases.

| **Description** | **File size** | **File name** |
|---|---|---|
| ZIP archive containing data | 15M | example.zip `http://pngu.mgh.harvard.edu/~purcell/dist/example.zip` |
| ZIP archive containing teaching materials | 1.3M | teaching.zip `http://pngu.mgh.harvard.edu/~purcell/dist/teaching.zip` |

You are feel free to use, modify or distribute these files in any way you wish, although giving me appropriate credit for the materials would be appreciated.

The `example.zip` archive contains

| | |
|---|---|
| `wgas1.ped` | `Whole-genome SNP data example PED file` |
| `wgas1.map` | `Corresponding MAP file` |
| `extra.ped` | `Follow-up genotyping for a particular region` |
| `extra.map` | `Corresponding MAP file` |

```
pop.cov               Population membership variable
command-list.txt      List of all commands for 2nd part of practical
```

The `teaching.zip` archive contains a PowerPoint and a Word file:

```
practical-1-slides.ppt
practical-2-notes.doc
```

These two files cover the first and second half of the tutorial respectively. The second document assumes the first half has already been completed (but also contains some introductory remarks concerning the data). I will probably update the Word document to also include the early commands covered in the PowerPoint/gPLINK part (i.e. so that the entire practical can be performed from the command line rather than using gPLINK). The list of commands (`command-list.txt`) is included so that people can cut-and-paste commands in, rather than type. If using DOS, it is a good idea to first increase the window width (right click on header on DOS window, Properties, Layout and increase buffer and window width to around 120 characters).

Everything should be fairly self-explantory after looking through the PowerPoint file and Word document.

## 24.3 Multimarker test lists

These files, generated by Itsik Pe'er and others, facilitate the 'multi-marker predictor' approach to association testing, as described in the manusctipt:

```
Pe'er I, de Bakker PI, Maller J, Yelensky R, Altshuler D
& Daly MJ (2006) Evaluating and improving power in whole-genome
association studies using fixed marker sets. Nat Genet, 38(6): 605-6.
```

They are PLINK-formatted lists of multimarker tests selected for Affymetrix 500K and Illumina whole genome products, based on consideration of the CEU Phase 2 HapMap (at r-squared=0.8 threshold). One should download the appropriate file and run with the `--hap` option (after ensuring that any strand issues have been resolved).

- Affymetrix.GeneChip.500k.both.CEU.0.8.tests.zip `http://pngu.mgh.harvard.edu/~purcell/dist/mmtests/Affymetrix.GeneChip.500k.both.CEU.0.8.tests.zip`
- Illumina.HumanHap.300k.CEU.0.8.tests.zip `http://pngu.mgh.harvard.edu/~purcell/dist/mmtests/Illumina.HumanHap.300k.CEU.0.8.tests.zip`
- Illumina.HumanHap.550k.CEU.0.8.tests.zip `http://pngu.mgh.harvard.edu/~purcell/dist/mmtests/Illumina.HumanHap.550k.CEU.0.8.tests.zip`
- Illumina.HumanHap.650k.CEU.0.8.tests.zip `http://pngu.mgh.harvard.edu/~purcell/dist/mmtests/Illumina.HumanHap.650k.CEU.0.8.tests.zip`

**Note** These haplotypes are specified in terms of the +ve (positive) strand relative to the HapMap. You might need to reformat your data prior to using these files (using the `--flip` command, for instance) before you can use them.

**Note** These tables list all tags for every common HapMap SNP, at the given r-squared threshold. The same haplotype may therefore appear multiple times (i.e. if it tags more than 1 SNP).

**Note** These tables obviously assume that all tags on present in the final, post-quality-control dataset: i.e. if certain SNPs have been removed, it will be better to reselect the predictors – that is, these lists should really only be used as a first pass, for convenience.

In general, however, quite possibily an easier and better strategy is instead to analyse the data within an imputation context, e.g. utilising the proxy association procedures rather than using these fixed lists.

## 24.4 Gene sets

**NOTE** The gene range lists below have replaced this old gene SET file: you are advised to use the lists below rather than this file.

Here is a `PLINK`-format SET file, containing a genome-wide set of genes (N=18272). The co-ordinates are based on NCBI B36 assembly, dbSNP 126; a gene is arbitrarily defined as including 50kb upstream and downstream.

`Download (ZIP archive):` gene-list.zip `http://pngu.mgh.harvard.edu/~purcell/dist/gene-list.zip`

## 24.5 Gene range lists

These are gene lists: files containing lists of genes, based on either hg17 or hg18 co-ordinates. The format is one gene per row,

```
Chromosome
Start position (bp)
Stop position (bp)
Gene name
```

These lists can be used with PLINK commands such as `--make-set`, `--range`, `--gene-list`, `--cnv-intersect`, `--clump-range`, etc. These gene lists were downloaded from UCSC table browser for all RefSeq genes on July 24th 2008. Overlapping isoforms of the same gene were combined to form a single full length version of the gene. Isoforms that didn't overlap were left as duplicates of that gene.

Rather than using the gene sets (described above), we suggest using these gene lists to make gene sets on the fly (using `--make-set-border` if so desired, to add a fixed kb border on the fly).

`Gene list (hg18):` glist-hg18
`Gene list (hg17):` glist-hg17 `http://pngu.mgh.harvard.edu/~purcell/dist/glist-hg17`

# Chapter 25

# ID helper

PLINK includes a set of utitily options designed to help manage ID-related project data. In large projects, ID schemes can be difficult to manage. This set of options is aimed at scenarios in which individuals have been assigned multiple IDs, meaning that multiple lookup tables are needed to translate between schemes, although more basic tasks (e.g. joining multiple files based on a single shared ID) are supported. In particular, these options will:

- Combine multiple (partially overlapping) ID schemes
- Spot inconsistencies
- Track other (non-unique) attributes along with identifier information
- Filter subsets of this database, for quick look-ups
- Allow ID aliases
- Allow individuals to be uniquely specified by two or more IDs, such as family ID and individual ID
- Automatically collate and update ID schemes in external files
- Merge multiple files based on multiple ID schemes

These functions are generic, in that they are not tied to any particular format or scheme of IDs used by PLINK. In fact, the "individuals" need not be samples, but could be anything, e.g. SNPs with RS numbers and vendor-specific codes. These options are specifically aimed for cases where ID data, along with limited amounts of secondary attributes (e.g. sex, age, etc; or chromosome, map position in the case of SNPs, etc) are stored in flat, rectangular text files.

Obviously there are many other ways to perform such tasks, for example, using any standard relational database, a perl script or Excel. Depending on your needs, you may or may not find the options implemented here quicker, easier or more reliable than some these alternatives.

## 25.1 Example of usage

As an example: consider the following case, in which ID information is spread across four files: family and individual IDs from two sites, `collab12.txt` (site ID, family ID and individual ID)

```
1    F00001  1
1    F00001  2
1    F00001  3
1    F00002  1
1    F00002  2
```

```
1    F00002  3
2    C101    P1
2    C101    M2
2    C101    C2
```

Similar information from a third sample, but with some additional information appended:

```
3 1 F00001_1    3/12/09         F
3 1 F00001_2    NA              NA
3 1 F00001_3    3/17/09         M
```

Then we have a report back from the genotyping lab, on some of the samples (and which also includes some other samples)

```
SITE FID       IID    GENO   PASS
1    F00001      1    S001      Y
1    F00001      2    S002      Y
1    F00001      3    S003      Y
1    F00002      2    S004      N
1    F00002      3    S005      Y
2    C101       P1    S006      Y
2    C101       M2    S007      N
2    C101       C2    S008      Y
2    X1         X1    S009      Y
```

Finally, we also have information on yet a further set of IDs assigned in a follow-up stage of the project, that are tied to the IDs assigned by the genotyping lab, rather than the original collaborator IDs:

```
S001    fu_01_a
S002    fu_01_b
S003    fu_01_c
S005    fu_01_d
S006    fu_01_e
S008    fu_01_f
S009    fu_01_g
```

As described, below, the following *dictionary* file (proj1.dict) is specified to track this information:

```
collab12.txt  SITE FID IID           : joint=SITE,FID,IID
collab3.txt   SITE FID IID DATE SEX  : attrib=DATE,SEX missing=NA
geno.txt      SITE FID IID GENO PASS : attrib=PASS header
followup.txt  GENO FUID
```

and the command

```
plink --id-dict proj1.dict
```

will collate all the files (after checking for inconsistencies, etc) into a single table, with missing values inserted where appropriate:

```
DATE        FID        FUID     GENO          IID  PASS   SEX  SITE
    .     F00001     fu_01_a     S001            1     Y     .     1
    .     F00001     fu_01_b     S002            2     Y     .     1
    .     F00001     fu_01_c     S003            3     Y     .     1
    .     F00002           .        .            1     .     .     1
    .     F00002           .     S004            2     N     .     1
    .     F00002     fu_01_d     S005            3     Y     .     1
    .       C101     fu_01_e     S006           P1     Y     .     2
    .       C101           .     S007           M2     N     .     2
    .       C101     fu_01_f     S008           C2     Y     .     2
```

```
3/12/09      1         .       .    F00001_1    .    F    3
      .      1         .       .    F00001_2    .    .    3
3/17/09      1         .       .    F00001_3    .    M    3
      .     X1    fu_01_g    S009         X1    Y    .    2
```

There are then numerous commands that can search this database, and update or match external files based on any of the ID schemes. There is also a command for joining two or more files based on a single ID scheme, which does not require a dictionary/database to be specified. This could be of use, for example, to quickly line up partially overlapping output from PLINK, based on SNP RS numbers, for example.

## 25.2 Overview

The idea is that all data are kept in simple plain text files, and that the complete "master file" is then generated on-the-fly. This makes it easier to add and edit individual components of the ID database (i.e. the individual files).

**Note** In contrast to a full database, there is no support for hierarchical, relational data structures. That is, all observations in all tables must be of the same fundamental unit (e.g. a single individual).

Consider we have three sets of IDs, labelled A, B and C, on up to four individuals. These are described across two files, `id1.txt`, which lists the A and B schemes (coded here for clarity to simply be `a1`, `a2`, etc)

```
a1 b1
a2 b2
a3 b3
a4 b4
```

and `id2.txt`, which contains the B and C codes for 3 individuals:

```
b2 c2
b1 c1
b3 c3
```

For example, the individual labelled `a1` under the A scheme is called `b1` under the B scheme. Note that in `id2.txt` the individuals are in a different order and one individual (`a4/b4`) does not appear in the second file.

Importantly, all ID values and files should conform to the following:

- values are delimited by 1 or more whitespace characters (tab or space)
- one observation/individual per row/line; each line must have same number of fields
- values cannot contain spaces, tabs, commas (,) or plus (+) characters
- missing values must be explicitly indicated (by "." or another specified code, see below)

A *dicitonary* file describing these ID tables would be as follows, e.g. in the file `example.dict`

```
id1.txt A B
id2.txt B C
```

The dictionary file lists each file in the database, followed by the field names in each. This dictionary thereby specifies that the second field in `id1.txt` should correspond with the first field in `id2.txt` as they both represent the B ID scheme. The dictionary file can also contain other commands, described below. Dictionaries can include full paths (i.e. database files can reside in different directories).

The basic command

```
plink --id-dict example.dict
```

will load all the ID data, check for consistency and generate the following in the LOG file

```
ID helper, with dictionary [ example.dict ]
Read 3 unique fields
Reading [ id1.txt ] with fields : A, B
Reading [ id2.txt ] with fields : B, C
Writing output to [ plink.id ]
4 unique records retrieved
```

The default behavior is to generate a file

```
plink.id
```

that contains all the fields, with a header row included:

```
A       B       C
a1      b1      c1
a2      b2      c2
a3      b3      c3
a4      b4      .
```

Because the last individual wasn't listed for the C field, a missing character (period/full stop ".") is entered.

## 25.3 Consistency checks

Imagine that one of the IDs had been entered incorrectly, for example if `id2.txt` has `c2` repeated:

```
b2 c2
b1 c1
b3 c2
```

PLINK would report this probelm when loading the file, pointing out the inconsistency:

```
*** Problems were detected in the ID lists:
Two unique entries [ B = b2 and b3 ] that match elsewhere
 a) A=a2 B=b2 C=c2
 b) B=b3 C=c2
```

That is, PLINK has spotted that two entries are matched for the C field, but have different values for the B field. As these values are assumed to be unique identifiers, this is an inconsistency that must be fixed by the user. Inconsistencies across files or involving more than 2 ID fields can also be spotted.

## 25.4 Attributes

In the example above, consider that `id2.txt` has been fixed, but that we now have a third file, `id3.txt`:

```
a1 c1 M Wave1
a2 c2 M Wave2
a3 c3 F Wave2
a4 c4 F Wave1
```

The third and fourth fields have non-unique values (e.g. M, for male, is repeated). In this example, this is because they contain information (attributes) that we want to track along with the sample IDs, but which is not an ID itself, i.e. the sex and source of the sample. It is possible to indicate the certain fields are to be treated not as *identifiers* (that, by definition, should be unique for each individual) but instead as *attributes*, as follows: the dictionary now reads:

```
id1.txt A B
id2.txt B C
id3.txt A C Sex Source : attrib=Sex,Source
```

using the `attrib=` keyword after a colon `:` character to specify that the fields `Sex` and `Source` are attributes, not idenitifiers. This effectively means that duplicates are allowed, and that these values will not be considered when attempting to reconcile individuals across files.

**Note** All dictionary commands follow the filename and field headings; a colon character must come before any keyword; all items must be on the same line.

The LOG file now reads

```
ID helper, with dictionary [ e.dict ]
Read 5 unique fields
   Attribute fields: Sex Source
Reading [ id1.txt ] with fields : A, B
Reading [ id2.txt ] with fields : B, C
Reading [ id3.txt ] with fields : A, C, Sex, Source
```

noting that `Sex` and `Source` are attributes. The output file `plink.id` now reads

```
 A       B       C      Sex    Source
a1      b1      c1        M     Wave1
a2      b2      c2        M     Wave2
a3      b3      c3        F     Wave2
a4      b4      c4        F     Wave1
```

Note that the columns are sorted in alphabetical order. Also note that we now see the fourth individual's value for the `C` field in this third file (`c4`) and so it is no longer missing.

## 25.5 Aliases

PLINK supports the use of aliases, where variant forms of an ID value are understood to map to the same individual. For example, an individual sample might have been sent for genotyping twice and received two distinct IDs, that we really want to treat as refering to the same person.

Aliases can be specified in two ways: either by listing the same ID field twice (or more) in a file, or by entering a comma-delimited list of terms as a single value. For example, if the dictionary line is

```
a.txt C C
```

and the file `a.txt` is

```
c1 .
c2 .
c3 c3_w2
```

For the first two individuals, there are no aliases specified (as there is a missing value for the second field). For the third individual, this indicates that any instance of `c3_w2` for the C field should be treated as an alias for `c3`.

Equivalently, the original `id2.txt` could simply be modified as follows:

```
b2 c2
b1 c1
b3 c3,c3_w2
```

i.e. a comma-delimited list of two or more values indicates the additional values are aliases for the original value. Note that aliases must always be unique. The first value encountered is always the preferred value, to which aliases are converted.

For example, if the file `id3.txt` was in fact,

```
a1 c1    10 Wave1
a2 c2    10 Wave2
a3 c3_w2 12 Wave2
a4 c4    23 Wave1
```

but the appropriate alias for `c3` had been specified in one of the two ways mentioned above, PLINK should run correctly, automatically converting `c3_w2` to `c3` and producing the output file `plink.id`

```
A       B       C       Sex     Source
a1      b1      c1      M       Wave1
a2      b2      c2      M       Wave2
a3      b3      c3      F       Wave2
a4      b4      c4      F       Wave1
```

Finally, the command `--id-alias` generates a file `plink.id.eq` that lists all aliases and the preferred value that are found in the database: e.g. (other aliases listed here just for illustration)

```
FIELD     PREF      EQUIV
    C       c3      c3_w2
    C       c3         C3
    A       a1      ID-a1
```

## 25.6 Joint ID specification

An individual can be uniquely specified by a combination of two or more IDs instead of a single ID, for example, by a family ID and individual ID, or a project ID and an individual ID. This is represented in the dictionary as follows:

```
id1.list  PROJ  FID  IID  : joint=FID,IID
```

Note, if a joint ID is specified, then all joint IDs must appear in subsequent files, e.g. a dictionary file that read as follows:

```
id1.list  PROJ     FID   IID  : joint=FID,IID
id2.list  CLIN_ID  IID
```

would give an error

```
ERROR: Need to specify all joint fields in dictionary, [id2.list ]
```

A correct dictionary would read: (note, the order of the fields within the file is not important)

```
id1.list  PROJ     FID   IID  : joint=FID,IID
id2.list  CLIN_ID  IID   FID
```

This means that a different individuals can share the same `FID`, for example:

```
FID     IID
F0001   1
F0001   2
F0002   1
F0002   2
```

now denote four unique individuals.

**NOTE** You can create joint IDs containing more than two fields, e.g. `joint=X,Y,Z`. The order of the joint fields does not need to be the same in all files. Also, you only need to specify the "joint=X,Y,.." command once in the dictionary. Finally, you can also have multiple joint fields:

```
id1.list  SITE   PROJ   FID          IID  : joint=FID,IID joint=SITE,PROJ
id2.list  FID    IID    CLIN_ID
id3.list  SITE   PROJ   RECRUIT_ID
```

**HINT** The `set:field=value` command, described below, can be used to create joint IDs. This can be useful to ensure no accidental overlap of ID schemes between files from different sources. See below for an example.

## 25.7 Filtering / lookup options

It is possible to restrict the output to certain rows or columns of the total database. For example, to only output fields `C` and `Sex`, add the command

```
--id-table C,Sex
```

To lookup all fields on a particular individual, e.g. with a given ID value for the B ID scheme, use the command

```
--id-lookup B=b2
```

This prints a message to the LOG indicating that a lookup is being performed

```
Lookup up items matching:
  B = b2  (id)
```

and the output file now only contains a single row

```
 A      B      C   Sex    Source
a2     b2     c2    M      Wave2
```

It is possible to lookup an individual based on an alias, e.g. in the example above,

```
--id-lookup C=c3_w2
```

produces the output in the LOG

```
Lookup up items matching:
  C = c3  (id)
```

indicating that the query term alias has been replaced with the preferred value, and the output is

```
 A      B      C   Sex    Source
a3     b3     c3    F      Wave2
```

Lookups can also be based on attributes and involve multiple fields, in which case the row must match all the specified field values:

```
   --id-lookup Sex=M,Source=Wave2
```

for example

```
Looking up items matching:
  Sex = M  (attribute)
  Source = Wave2  (attribute)
Writing output to [ plink.id ]
1 unique records retrieved
```

and the output in `plink.id` is

```
 A      B      C   Sex    Source
a2     b2     c2    M      Wave2
```

**NOTE** It is **not** currently possible to specify ranges of numerical values (e.g. less than 10) or wildcards, (e.g. Wave*) when performing `--id-lookup`.

## 25.8 Replace ID schemes in external files

The command

```
--id-replace [options] file old ID new ID
```

will use the information specified in the dictionary to read in an external file (i.e. not specified in the dictionary) and replace or update the IDs as requested. Consider the data file `mydata.dat`:

```
 A   v1 v2 v3 v4   v5
a1    0  0  1  1 0.23
```

```
  a3    1  1  0  1 0.35
  a5    0  0  0  1 0.54
```

Then the command

```
plink --id-dict ex.dict --id-replace [header] mydata.dat A C
```

will lookup up the value for A in mydata.dat, using the fact that this file has a header row, and replace it, if possible, with the value for C for that person. This prints the following in the LOG:

```
Replacing A with C from [ mydata.dat ]
Writing new file to [ plink.rep ]
Set to keep original value for unmatched observations
Could not find matches for 1 lines
```

The file plink.rep contains the updated file:

```
C  v1  v2  v3  v4  v5
c1  0   0   1   1  0.23
c3  1   1   0   1  0.35
a5  0   0   0   1  0.54
```

The last line did not match any entry in the database (a5) and so, by default, it is left as is. Otherwise, the appropriate C ID schemes have been swapped in for the other two indiviauls, and the header has been changed.

To change to default behavior when a non-matching individual is encountered, use one of the following options: warn, skip, miss or list. For example,

```
plink --id-dict ex.dict --id-replace [header,warn] mydata.dat A C
```

will produce an error in the LOG file

```
 ERROR: Could not find replacement for a5
```

and not proceed any further. The option

```
plink --id-dict ex.dict --id-replace [header,skip] mydata.dat A C
```

will simply ignore that line, not printing it in plink.rep which will now read

```
C  v1  v2  v3  v4  v5
c1  0   0   1   1  0.23
c3  1   1   0   1  0.35
```

The option

```
plink --id-dict ex.dict --id-replace [header,miss] mydata.dat A C
```

will replace the non-matching ID with the missing code NA,

```
C  v1  v2  v3  v4  v5
c1  0   0   1   1  0.23
c3  1   1   0   1  0.35
NA  0   0   0   1  0.54
```

Finally, the option

```
plink --id-dict ex.dict --id-replace [header,list] mydata.dat A C
```

will list in plink.rep any individual that *did not match*: in this case, it will just list

```
 a5
```

It is possible to combine both aliases (in the target file) and joint IDs (as both the target and replacement ID) with the --id-replace function. This is specified by use of the plus "+" symbol, e.g.

```
plink --id-dict ex.dict --id-replace [header] mydata2.dat GENOID FID+IID
```

will replace the single entry of GENOID with the two values for FID and IID.

Finally, if the file does not contain a header row, use the field option:

```
plink --id-dict ex.dict --id-replace [field=1] mydata.dat A C
```

which tells PLINK that column 1 of mydata.dat contains the A file. If the target ID is a joint ID, the same notation can be used in this case:

```
plink --id-dict ex.dict --id-replace [field=2+5] mydata2.dat FID+IID GENOID
```

for example, to indicate that FID is in column 2 and IID is in column 3. In this case, column 5 will be printed as blank, and so effectively skipped. When the replacing ID is a joint ID, all joint values replace the first matched field, i.e. in this case would have been inserted as columns 2, 3, etc, if the replacement field was in fact a joint ID rather than just GENOID.

## 25.9 Match multiple files based on IDs

This option takes an index file and one or more other files and sorts these files to match the order of the index file (inserting blank rows if needed, or dropping rows if they are not present in the index file, as specified), using IDs as defined in the dictionary, in the format

```
<em>
    --id-match [options] file ID  file ID  file ID ...
</em>
```

For example,

```
plink --id-dict ex.dict --id-match dat1.dat A,1 dat2.txt C dat3.txt C
```

would generate a new file

```
plink.match
```

that lines up the the rows in dat2.txt and dat3.txt to match dat1.dat, using the ID database specified by ex.dict. The IDs are specified as follows:

```
A       Field A, assume header exists and contains A
A,2     Field A, 2nd column of file, assume no header
A+B     Joint ID A and B, assume header exists
A+B,2+3 Joint ID A and B, in 2nd and 3rd columns, no header
```

Therefore, the above implies that dat1.dat does not contain a header row, but the other two files do. That is, by specifying a number following a comma, we implicitly tell PLINK both that no header exists, and which column to look in. Otherwise we assume the header should contain the named field (an error will be reported otherwise). In all cases the files to be matched must be rectangular, i.e. having the same number of whitespace-delimited fields.

To print only the rows that are present in all files, add the option [complete] as follows:

```
--id-match [complete] f1.txt ID f2.txt ID
```

Otherwise by default, missing values are printed when the data are not present in one of the files.

**NOTE** For any individuals not found in the database, they are listed in a file named plink.noid and a message is printed in the LOG file.

## 25.10 Quick match multiple files based on IDs, without a dictionary

If the `--id-match` command is used without specifying a data dictionary, i.e. there is no `--id-dict`, then we assume a simple correspondence of ID schemes between files. This can provide a quick way to join up rectangular text files based on a common key, e.g.

```
./plink --id-match f1.txt ID f2.txt ID,2 f3.txt IID
```

Note: when a field position is specified, it does not matter what the field is named (as there is no database to look it up in, in any case). Similarly, the ID field may have a different name in some files, e.g. `IID` not `ID` in `f3.txt`. Importantly, however, we assume the specific entries in these files all come from the same ID scheme, i.e. otherwise a dictionary should be specified to map between schemes.

## 25.11 Miscellaneous

The dictionary file can specify whether the file has a header row by adding the keyword `header` in the dictionary. The `missing=` keyword can also be used to specify one or more missing value codes, that are specific to that file.

```
id1.list A B : header
id2.list B C
id3.list C D : attrib=D header missing=NA,-9
```

### 25.11.1 The set command

For an attribute, or part of a joint ID, it is possible to use the `set` command to specify that all individuals in that file have a particular ID value inserted. This can be useful, for example, if samples from several sources are being grouped, and one wants to ensure no accidental overlap between samples: e.g. if one site sends a file `site1.txt` with individuals

```
ID
 1
 2
 3
 4
```

and another site sends a similar file, `site2.txt`, that refers to three different individuals

```
1
2
3
```

the dictionary `ex2.dict` could read

```
site1.txt ID : set:SITE=1 joint=ID,SITE header
site2.txt ID : set:SITE=2
```

then

```
plink --id-dict ex2.dict
```

will produce a file `plink.id` that reads

```
ID  SITE
 1     1
 2     1
 3     1
```

```
    4     1
    1     2
    2     2
    3     2
```

Note the specific format, with a colon and equals sign but no spaces:

```
set:field=value
```

### 25.11.2 List all instances of an ID across files

To get a list of all instances of an ID value across multiple files, use the command

```
plink --id-dict ex.dict --id-dump A=a1
```

will list to the LOG file

```
Reporting rows that match [ A=a1 ]
id1.txt : A = a1
id1.txt : B = b1
id3.txt : A = a1
id3.txt : C = c1
id3.txt : Sex = M
id3.txt : Source = Wave1
```

This can be useful in tracking down where incorrect IDs are located across multiple files, for example, in order to manually resolve inconsistencies, etc.

# Chapter 26

# Miscellaneous

This page details a collection of options and commands that did not get proper mention elsewhere.

## 26.1 Output modifiers

One convenient filter is

```
--pfilter 1e-3
```

which will, for example, only report statistics with p-values less than 1e-3.

**NOTE** This is operation for the basic association tests, but do not expect this to work for all methods that return a p-value.

To obtain -log10(p) values instead of p-values in the `*adjusted` file, add the flag (this does not change the output of p-values in other files)

```
--log10
```

To fix the value of lambda used for the genomic control in the `*adjusted` file, instead of estimating it from the data, use the option, for example

```
--lambda 1.2
```

To obtain an extra set of columns that facilitates making a Q-Q plot in the `*.adjusted` file, add the option

```
--qq-plot
```

This will work with either basic p-values, or with `--log10` p-values.

## 26.2 Analyses with different species

PLINK differentiates between species only in terms of the number of chromosomes and which are sex-linked or haploid. Several non-human species are supported, by adding one of the following flags

```
--dog
--horse
--cow
--sheep
--rice
--mouse
```

**NOTE** This flag needs to be added to every analysis. If you work primarily with one of these non-human species, you might want to make a link or wrapper to make, e.g. `myplink` always add the flag, e.g.

```
./plink --dog
```

or compile PLINK with the option fixed (in options.cpp, edit the appropriate line, by setting one of these to `true`:

```
bool par::species_dog = false;
bool par::species_cow = false;
bool par::species_horse = false;
bool par::species_sheep = false;
bool par::species_rice = false;
```

## 26.3 Known issues

Development of `PLINK` is ongoing: as such, there is always likely to be a list of features, listed here, that are only partialy implemented, or have known problems not yet fixed. A list of known issues can be found on the warnings page:

```
http://pngu.mgh.harvard.edu/purcell/plink/warnings.shtml
```

# Chapter 27

# FAQ and Hints

This section contains a small but expanding set of answers to questions and hints.

- Can I convert my binary PED fileset back into a standard PED/MAP fileset?
- Can I speed up loading large files?
- Why are no individuals included in my analysis?
- Why are my results different from an analysis using program `X`?
- How large a file can PLINK handle?
- Why does my linear/logistic regression output have all `NA`'s?
- What kind of computer do I need to run PLINK?
- Can I analyse multiple phenotypes in a single run (e.g. for gene expression datasets)?
- How does PLINK handle the X chromosome in association tests?
- Can/why can't gPLINK perform a particular PLINK command?
- When I include covariates with `--linear` or `--logistic`, what do the p-values mean?

## 27.1 Can I convert my binary PED fileset back into a standard PED/MAP fileset?

Yes. Use the `--recode` option, for example:

```
plink --bfile mydata --recode --out mynewdata
```

You might also want to use the variant `--recode12` and `--recodeAD` forms, described here.

## 27.2 To speed up input of a large fileset

As well as using the binary fileformat, which greatly increases speed of loading relative to the PED/MAP format, if you know that you have already excluded all the individuals you want (with the per-individual genotyping threshold option), then setting

```
--mind 1
```

will skip the step where per-individual genotyping rates are calculated, which can reduce the time taken to load the file. Note, the command `--all` is equivalent to specifying `--mind 1 --geno 1 --maf 0` (i.e. do not apply any filters).

## 27.3 Why are no indidividuals included in the analysis?

A common cause for this is either that all individuals are non-founders (e.g. a sibling pair dataset) and PLINK, by default, only uses founders to calculate allele frequencies. The

```
--non-founders
```

option can force these individuals in.

An alternative is that none of the individuals have a valid sex code – in this case, they are all set to missing status, unless the

```
--allow-no-sex
```

option is given. You are strongly recommended to enter the correct sex codes for all individuals however, so they can be appropriate treated in any subsequent analyses involving the sex chromosomes.

## 27.4 Why are my results different from an analysis using program `X`?

This is obviously a difficult question to answer without specific details. Therefore, if you send me a question along these lines and want to get an answer, please make it as specific as possible, to put it bluntly! Ideally, include example data that replicates the problem / illustrates the difference.

There is always the possibility that the difference could be due to a bug in `PLINK`, which is obviously something I would want to track down and fix. Similarly, it could be due to a bug in the other software. Perhaps more likely, the difference might arise from one of two general sources

- The analytic routines themselves are slightly different. Are the results dramatically different? Do not expect exact numerically similarity between similar analyses (i.e. even for a simple case, `--assoc`, `--fisher` and `--logistic` will give slightly different p-values for a simple single SNP test, but this is to be expected). So, is the difference really meaningful? Perhaps more importantly, are you sure the other routine really is implementing a similar test, with similar assumptions, etc?
- A common reason for *apparent* differences between PLINK and other analysis packages is that `PLINK` implements some default filtering of the data, i.e. first removing individuals or SNPs with below threshold genotyping rate. Look at the LOG file to check that *exactly* the same set of individuals were actually included in both analyses. In other words: be sure to check how missing data were handled in each case.

## 27.5 How large a file can PLINK handle?

There are no fixed limits to the size of the data file; it uses currently 1 byte for 4 SNP genotypes and some overhead per SNP and per individual. This means that you should be able to get datasets of, say, 1 million SNPs and up to 5000 individuals, in a machine with 2GB RAM without causing too much stress/swapping, etc. That is,

```
5000 * 1e6 / 4 = 1.25e9 bytes = ~1GB.
```

Things scale more or less linearly after that. So for a very large file 4 times the size (20K individuals for example), an 8GB or 16GB machine would be required to load the data in a single run).

For datasets with very many SNPs, even the list of SNP names and storage information can take a reasonable amount of space, even if the number of individuals is small (i.e. for the Phase 2 HapMap data, most of the space is taken up with the SNP name and position information, rather than the genotypes themselves).

You can test the capacity of `PLINK` and your machine by entering the commands

```
plink --dummy 15000 500000 --make-bed --out test1
```

to generate a dummy file of, in this instance, 15,000 individuals genotyped on 600,000 SNPs. If you do not get an `Out of memory error`, then it has worked. Note that dealing with files this size will take a while. Of course, in many cases it would be easy to split up the data and do per-chromosome analyses if need be, which would help on smaller machines.

## 27.6 Why does my linear/logistic regression output have all `NA`'s?

PLINK will set the output to be all `NA`s if it was unable to fit the regression model. Common causes for this are:

- There is no variation in the phenotype or one or more of the predictor variables: are you sure the right variables were selected, and that no filters were applied meaning that the individuals left are all cases, for example? Is the SNP monomorhpic?

- The second reason is that the correlation between predictor variables is too strong. PLINK uses the variance inflation factor criterion (VIF) to check for multi-collinearity. If two or more variables perfectly predict each other, PLINK will (correctly) print all `NA`s to the output, indicating that the model can not be fit. Sometimes, PLINK may be overly-conservative in calling such problems however, which is particularly likely to occur if you add more covariates and allow for interactions between terms (as the interaction terms will correlate with the main effect variables). The default VIF is 10; try setting this value higher with the `--vif` option, to say 100. The VIF is 1/(1-R) where R is the multiple correlation coefficient between one predictor variable and all others. A value of 100 implies R=0.99. If one variable or more variables fail the VIF test, then the entire model is not run and `NA`s appear in the output.

## 27.7 What kind of computer do I need to run PLINK?

There are no special requirements: PLINK should be able to be compiled for any machine for which a recent C/C++ compiler is available. Pre-compiled binary versions are distributed from this website for Linux, MS-DOS and Mac machines.

In terms of speed, memory and diskspace, obviously more is usually better. The suggestions below are really minimum values to make life easy for a "normal" sized study (i.e. many analyses could easily be run on much smaller machines; some analyses will require more resources, etc).

The FAQ above about dataset limits gives some indication of the amount of RAM needed for large studies. Basically, for any whole genome scale studies you would want at least 2Gb of RAM; 4 or 8Gb would be desirable.

In terms of disk space: the main storage requirements will result from the raw data (e.g. CEL files, etc) rather than genotype files or most PLINK results files. However, certain PLINK files can be large: e.g. `.genome` files for large samples, dosage output for whole-genome imputation of all HapMap SNPs, etc. Therefore, a large hard drive is desirable: not including storage for CEL files, a drive of at least 200Gb would be good.

PLINK does not specifically take advantage of multi-core processors. For large datasets, a fast processor is desirable (e.g. at least 3GHz). The majority of analyses described in these pages can be performed on a single processor. For certain analyses (e.g. epistasis, using permutation procedures on very large datasets, IBS calculation on very large datasets, etc) then access to a parallel computing cluster, if possible, is very desirable and sometimes necessary.

In terms of operating systems, there should not be major differences in performance: using a Linux/Unix environment probably has some advantages in terms of the existing text file processing utilities typically available, and the more powerful shell scripting options, but probably personal preference and institutional support is a bigger consideration. There is a definite advantage to ensuring a C/C++ compiler exists on the system so that the source code version of PLINK can be compiled for your particular system however – this may give some performance advantages and allows access to the development source code (i.e. to receive a patched version that fixes a particular problem or adds a new feature before the next release in generally available).

## 27.8 Can I analyse multiple phenotypes in a single run (e.g. for gene expression datasets)?

For most association commands, you can specify the `--all-pheno` option to automatically loop over all phenotypes in an alternate phenotype file:

```
plink --bfile mydata --pheno phenos.raw --all-pheno --linear --covar covar.dat
```

If there are *N* phenotypes, this will generate *N* separate output files. If a header row was supplied in the alternate phenotype file, then each file will have the phenotype name appended (it is up to the user therefore to ensure that the phenotype names are unique). If not, the output files are simply numbered, `P1`, `P2`, etc, (e.g. `plink.P1.assoc`, etc).

This works for most basic association commands that consider all SNPs (e.g. `--assoc`, `--logistic`, `--fisher`, `--cmh`, etc) but currently not for any haplotype analysis or epistasis options.

## 27.9 How does PLINK handle the X chromosome in association tests?

By default, in the linear and logistic (`--linear`, `--logistic`) models, for alleles `A` and `B`, males are coded

```
A   ->   0
B   ->   1
```

and females are coded

```
AA  ->   0
AB  ->   1
BB  ->   2
```

and additionally sex (0=male,1=female) is also automatically included as a covariate. It is therefore important not to include sex as a separate covariate in a covariate file ever, but rather to use the special `--sex` command that tells PLINK to add sex as coded in the PED/FAM file as the covariate (in this way, it is not double entered for X chromosome markers). If the sample is all female or all male, PLINK will know not to add sex as an additional covariate for X chromosome markers.

The basic association tests that are allelic (`--assoc`, `--mh`, etc) do not need any special changes for X chromosome markers: the above only applies to the linear and logistic models where the individual, not the allele, is the unit of analysis. Similarly, the TDT remains unchanged. For the `--model` test and Hardy-Weinberg calculations, male X chromosome genotypes are excluded.

Not all analyses currently handle X chromosomes markers (for example, LD pruning, epistasis, IBS calculations) but support will be added in future.

## 27.10 Can/why can't gPLINK perform a particular PLINK command?

`gPLINK` is intended only as a lightweight interface to some of the basic PLINK commands. It is designed to provide an easy way to become familiar with PLINK and to perform certain very basic operations for users who are not yet familiar with command line interfaces. It is not the recommended mode for using PLINK for anything beyond the most basic analyses and there are no immediate plans to extend gPLINK any further to incorporate new commands that are added to PLINK.

## 27.11 When I include covariates with `--linear` or `--logistic`, what do the p-values mean?

If one or more covariates are included (by `--covar`) when using `--linear` or `--logistic`, PLINK performs a multiple regression analysis and reports the coefficients and p-values for each term (i.e. SNP, covariates, any interaction terms). The only term omitted from the report is the intercept.

The p-values for the covariates **do not** represent the test for the SNP-phenotype association after controlling for the covariate. That is the first row (`ADD`). Rather, the covariate term is the test associated with the covariate-phenotype association. These p-values might be extremely significant (e.g. if one covaries for smoking in an analysis of heart disease, etc) but this does not mean that the SNP has a highly significant effect necessarily. For example:

```
CHR        SNP      BP  A1   TEST  NMISS      BETA     STAT           P
  1  rs1234567  742429   G    ADD   1495  -0.03335  -0.1732      0.8625
  1  rs1234567  742429   G   COV1   1495    0.1143    9.748  8.321e-022
```

suggests that the covariate is highly correlated with the outcome (which will often be already known, presumably), but there is no evidence that the SNP is in any way correlated with phenotype. These correspond to the partial regression coefficient terms of a muliple regression

```
Y ~ m + b1.ADD + b2.COV1 + e
```

where p=0.8625 is the Wald test for `b1`, p=8e-22 is the Wald test for `b2`, the covariate-phenotype relationship. To repeat: it does not mean that the SNP-phenotype test has a p=8e-22 after controlling for `COV1`.

# Appendix A

# Reference Tables

## A.1 Options

| Option | Parameter/default | Description |
|---|---|---|
| **Basic input/output** | | |
| `--file` | plink | Specify .ped and .map files |
| `--ped` | plink.ped | Specify .ped file |
| `--map` | plink.map | Specify .map file |
| | | |
| `--no-sex` | | PED file does not contain column 5 (sex) |
| `--no-parents` | | PED file does not contain columns 3,4 (parents) |
| `--no-fid` | | PED file does not contain column 1 (family ID) |
| `--no-pheno` | | PED file does not contain column 6 (phenotype) |
| `--liability` | | PED file does contain liability (column 7) |
| `--map3` | | Specify 3-column MAP file format |
| | | |
| `--tfile` | plink | Specify .tped and .tfam files |
| `--tped` | plink.tped | Specify .tped file |
| `--tfam` | plink.tfam | Specify .tfam file |
| | | |
| `--lfile` | plink | Specify long-format: LGEN, FAM and MAP |
| | | |
| `--bfile` | plink | Specify .bed, .bim and .fam |
| `--bed` | plink.bed | Specify .bed file |
| `--bim` | plink.bim | Specify .bim file |
| `--fam` | plink.fam | Specify .fam file |
| | | |
| `--out` | plink | Specify output root filename |
| `--silent` | | Suppress output to console |
| | | |
| `--pheno` | phenofile | Specify alternate phenotype |
| `--make-pheno` | file value | Specify binary phenotype, with cases have value |
| `--make-pheno` | file * | Specify binary phenotype, with cases are present |
| `--mpheno` | var # | Specify which, if >1 phenotype column |
| `--pheno-name` | var name | Instead of `--mpheno`, if a header row exists |
| `--all-pheno` | | Perform association for all phenotypes in file |
| `--loop-assoc` | clusterfile | Perform association for each level of cluster versis all others |
| | | |
| `--covar` | covarfile | Specify covariate |
| `--mcovar` | var # | Specify which, if >1 covariate column (for use with `--gxe`) |
| `--covar-name` | list | Specify 1 or more covariates by name |
| `--covar-number` | list | Specify 1 or more covariates by number |
| | | |
| `--within` | filename | Specify clustering scheme |
| `--mwithin` | var # | Specify which, if >1 cluster column |
| | | |
| `--script` | filename | Include command-line options from file |
| | | |
| **Selection of SNPs and individuals** | | |
| `--chr` | N | Select a particular chromosome N |
| `--gene` | name | Select a particular gene, given a SET file (`--set`) |
| | | |
| `--from` | SNP | Select range from this SNP ... |
| `--to` | SNP | ... to this SNP (must be on same chromosome) |
| | | |
| `--snps` | SNP list | Select comma-delimited list of SNPs, allowing for ranges, e.g. snp1,snp2,snp6-snp12 |
| | | |
| `--snp` | SNP | Select this SNP ... |
| `--window` | kb | ... and (optionally) all SNPs in the surrounding kb window |
| | | |
| `--from-bp` | bp | Select SNPs within this window... |
| `--to-bp` | bp | ... specified in base-pair position |
| | | |
| `--from-kb` | kb | Select SNPs within this window... |
| `--to-kb` | kb | ... specified in kilobases |
| | | |
| `--from-mb` | mb | Select SNPs within this window... |
| `--to-mb` | mb | ... specified in megabases |
| | | |
| `--extract` | snplist | Extract list of SNPs |
| `--exclude` | snplist | Exclude list of SNPs |
| | | |
| `--keep` | indlist | Keep only these individuals |
| `--remove` | indlist | Remove these individuals |
| | | |
| `--keep-before-remove` | | Perform keep before remove (default opposite) |
| `--exclude-before-extract` | | Perform exclude before extract (default opposite) |
| | | |
| `--filter` | filename value | Filter individuals matching value |
| `--mfilter` | var # | Specify filter value, if >1 filter column |
| | | |
| `--filter-cases` | | Include only cases |
| `--filter-controls` | | Include only controls |
| `--filter-males` | | Include only males |
| `--filter-females` | | Include only females |
| `--filter-founders` | | Include only founders |
| `--filter-nonfounders` | | Include only nonfounders |

| | | |
|---|---|---|
| --prune | | Remove individuals with missing phenotypes |

**Other data management options**

| | | |
|---|---|---|
| --make-bed | | Make .bed, .fam and .bim |
| --recode | | Output new .ped and .map files |
| --recode12 | | As above, with 1/2 allele coding |
| --recodeHV | | As above, with Haploview .info file |
| --recode-fastphase | | Ouput fastphase format file |
| --recode-bimbam | | Ouput bimbam format file |
| --recode-structure | | Ouput structure format file |
| --recodeA | | Raw data file with additive coding |
| --recodeAD | | Raw data file with additive/dominance coding |
| --tab | | Delimit --recode and --recode12 with tabs |
| --list | | Output one genotype per line, list of FIDs and IIDs |
| --plist | FID1 IID1 FID2 IID2 | Pairwise listing of genotypes for two individuals |
| --write-snplist | | List only the (filtered) SNPs in the dataset |
| --update-map | filename | Update physical positions in a map file |
| --update-cm | | Update genetic distances in a map file |
| --update-name | | Update SNP names in a map file |
| --update-chr | | Update chromosome codes in a map file |
| --update-ids | file | Update FIDs and IIDs in a file |
| --update-sex | file | Update sex information in a file |
| --update-parents | file | Update parent codes in a file |
| --write-covar | | Output ordered, filtered covariate file |
| --with-phenotype | | Include PED/phenotype information in new covariate file |
| --dummy-coding | | Downcode categorical covariates to binary dummy variables |
| --merge | pedfile, mapfile | Merge in a PED/MAP fileset |
| --bmerge | bedfile, bimfile, famfile | Merge in a binary fileset |
| --merge-list | list file | Merge multiple standard and/or binary filesets |
| --merge-mode | 1 | Specify merge mode (1-7) |
| --zero-cluster | filename | Zero-out specific SNPs for specific clusters |
| --oblig-missing | filename | SNPs/clusters that are obligatory missing |
| --oblig-cluster | filename | Individuals/clusters defining obligatory missingness |
| --flip | snplist | Flip strand of SNPs in list |
| --flip-subset | individual-list | Flip strand of SNPs only for these individuals in list |
| --flip-scan | | LD-based heuristic to look for SNPs flipped between cases and controls |
| --1 | | 0/1 unaffected/affected coding |
| --compound-genotypes | | Use AA, AG, 00 coding (no spaces between alleles in PED file) |
| --missing-phenotype | -9 | Missing phenotype code |
| --missing-genotype | 0 | Missing genotype code |
| --output-missing-phenotype | -9 | Missing phenotype code for output |
| --output-missing-genotype | 0 | Missing genotype code for output |
| --allele1234 | | Convert (A,C,G,T) to (1,2,3,4) |
| --alleleACGT | | Convert (1,2,3,4) to (A,C,G,T) |
| --update-alleles | file | Update allele codes in a file |
| --reference-allele | file | Force a particular reference (A1) allele |
| --keep-allele-order | | Do not flip A1 to be the minor allele |
| --allow-no-sex | | Do not set ambiguously-sexed individuals missing |
| --must-have-sex | | When making a new dataset, do set ambiguously-sexed individuals missing |
| --set-hh-missing | | Making new fileset, set heterozygous haploids missing |
| --set-me-missing | | Making new fileset, set Mendel errors missing |
| --make-founders | | Set non-founders without two parents to founders |
| --pedigree | | When performing TDT, dump parsed family structure |
| --tucc | | Make pseudo case/control pairs form trio data |

**Reporting summary statistics**

| | | |
|---|---|---|
| --freq | | Allele frequencies |
| --counts | | Modifies --freq to report actual allele counts |
| --nonfounders | | Include all individuals in MAF/HWE calculations |
| --missing | | Missing rates (per individual, per SNP) |
| --test-missing | | Test of missingness differing by case/control status |
| --test-mishap | | Haplotype-based test for non-random missingness |
| --cluster-missing | | IBM clustering |
| --hardy | | Report Hardy-Weinberg disequilibrium tests (exact) |
| --hardy2 | | Report Hardy-Weinberg disequilibrium tests (asymptotic) |
| --mendel | | Report Mendel error checks |
| --check-sex | | Use X chromosome data to check an individual's assigned sex |
| --impute-sex | | Use X chromosome data to impute an individual's assigned sex |
| --within | cluster file | Stratify frequencies and missing rates by clusters |

**Inclusion thresholds**

| | | |
|---|---|---|
| --maf | 0.01 | Minor allele frequency |
| --max-maf | 1 | Maximum minor allele frequency |

| | | |
|---|---|---|
| `--geno` | 0.1 | Maximum per-SNP missing |
| `--mind` | 0.1 | Maximum per-person missing |
| `--hwe` | 0.001 | Hardy-Weinberg disequilibrium p-value (exact) |
| `--hwe2` | 0.001 | Hardy-Weinberg disequilibrium p-value (asymptotic) |
| `--hwe-all` | | HW filtering based on all founder individuals for binary trait (instead of just unaffecteds) |
| `--me` | 0.1 0.1 | Mendel error rate thresholds (per SNP, per family) |
| `--cell` | 5 | Minimum genotype cell count for `--model` |
| `--min` | 0 | Minimum pi-hat for `--genome` output |
| `--max` | 1 | Maximum pi-hat for `--genome` output |

**Quality scores**

| | | |
|---|---|---|
| `--qual-scores` | file | SNP based quality scores filter |
| `--qual-threshold` | 0.8 | SNP quality score threshold |
| `--qual-max-threshold` | 1 | SNP maximum quality scores threshold |
| `--qual-geno-scores` | file | Genotype-based quality scores filter |
| `--qual-geno-threshold` | 0.8 | Genotype quality score threshold |
| `--qual-geno-max-threshold` | 1 | Genotype maximum quality scores threshold |

**IBS stratification / clustering**

| | | |
|---|---|---|
| `--genome` | | Calculate IBS distances between all individuals |
| `--cluster` | | Perform clustering |
| `--matrix` | | Output IBS (similarity) matrix |
| `--distance-matrix` | | Output 1-IBS (distance) matrix |
| `--mc` | 0 | Maximum cluster size |
| `--cc` | | Cluster by phenotype |
| `--mcc` | 0 0 | Maximum number of cases/controls per cluster |
| `--ibm` | 0.01 | Constrain IBS matching on IBM matching |
| `--ppc` | 0.01 | IBS test p-value threshold (was `--pmerge`) |
| `--ppc-gap` | 500kb | Skip SNPs within this for PPC test |
| `--match` | match-file | Specify external categorical matching criteria |
| `--match-type` | match-type-file | Specify external categorical matching direction (+/- match) |
| `--qmatch` | match-file | Specify external quantitative matching criteria |
| `--qt` | threshold-file | Specify quantitative matching thresholds |
| `--neighbour` | N M | Outlier statistics (for nearest neighbours N to M) |

**Whole genome summary statistics**

| | | |
|---|---|---|
| `--genome` | | Output genome-wide IBS/IBD |
| `--rel-check` | | Only calculate IBS/IBD for members of same family (FID) |
| `--read-genome` | genome-file | Read previously-computed genome values |
| `--nudge` | | Adjusted estimated IBD values |
| `--impossible` | | Indicate 'impossible' estimated IBD values |
| `--het` | | Individual inbreeding F / heterozygosity |
| `--homozyg-kb` | kb | Identify runs of homozygosity (kb) |
| `--homozyg-snp` | N SNPs | Identify runs of homozygosity (# SNPs) |
| `--homozyg-het` | N hets | Allow for N hets in run of homozygosity |
| `--homozyg-group` | | Group pools of overlapping segments |
| `--homozyg-match` | 0.95 | Identity threshold for allelic matching overlapping segments |
| `--homozyg-verbose` | | Display actual genotypes for each pool |

**Association analysis procedures**

| | | |
|---|---|---|
| `--assoc` | | Case/control or QTL association |
| `--fisher` | | Fisher's exact (allelic) test |
| `--model` | | Cochran-Armitage and full-model C/C association |
| `--model --fisher` | | Exact full-model tests |
| `--T2` | | Hotelling's T(2) multilocus test |
| `--mh` | | Cochran-Mantel-Haenszel SNPxDISEASE—STRATA |
| `--mh2` | | Cochran-Mantel-Haenszel SNPxSTRATA—DISEASE |
| `--bd` | | Breslow-Day homogeneity of odds ratios test |
| `--homog` | | Partitioning chi-square homogeneity of odds ratios test |
| `--gxe` | | QTL interaction test (dichotomous covariate only) |
| `--linear` | | Test for quantitative traits and multiple covariates |
| `--logistic` | | Test for disease traits and multiple covariates |
| `--genotypic` | | Include dominance term in model, and 2df model |
| `--dominant` | | Fit dominant model for minor allele |
| `--recessive` | | Fit recessive model for minor allele |
| `--condition` | SNP | Include additive effect of SNP in model |
| `--condition-list` | filename | Include additive effects of these SNPs in model |
| `--sex` | | Include sex effect in model |
| `--interaction` | | Include SNP x covariate interactions |
| `--test-all` | | Joint test of all terms in model |
| `--parameters` | 1,2,... | Fit only a subset of model terms |
| `--tests` | 1,2,... | Joint test of user-specified set of parameters |
| `--beta` | | Make `--logistic` return coefficients, not odds ratios |
| `--tdt` | | Family-based TDT and parenTDT (permute TDT) |
| `--parentdt1` | | As above, except permuted statistic is parental test |
| `--parentdt2` | | As above, except permuted statistic is combined test |
| `--poo` | | Parent-of-origin analysis in TDT |
| `--dfam` | | Disease family-test (families and unrelateds) |
| `--ci` | 0.95 | Confidence interval for CMH odds ratios |
| `--set-test` | | Set-based association (requires `--mperm`) |

| | | |
|---|---|---|
| `--set-p` | p-value | p-value threshold for set-based test |
| `--set-r2` | r$\hat{2}$ | R-squared threshold for set-based test |
| `--set-max` | N SNPs | Maximum number of SNPs in set |

**Permutation procedure options**

| | | |
|---|---|---|
| `--perm` | | Run permutations (adaptive-mode) |
| `--mperm` | 1000 | # of permutations in max-perm mode |
| `--aperm` | ... | Parameters (six) for adaptive permutation mode |
| `--rank` | | Modifies `--mperm` for rank-based permutation |
| | | |
| `--model-trend` | | Use CA-trend test from `--model` |
| `--model-gen` | | Use genotypic test from `--model` |
| `--model-dom` | | Use dominant test from `--model` |
| `--model-rec` | | Use recessive test from `--model` |
| | | |
| `--genedrop` | | Permutation by gene-dropping simulation (family-data) |
| `--swap-parents` | | Labal-swap permutation for parents when gene-dropping |
| `--swap-sibs` | | Labal-swap permutation for siblings when gene-dropping |
| `--swap-unrel` | | Labal-swap permutation for unrelateds when gene-dropping |
| | | |
| `--family` | | Make Family ID the cluster |
| | | |
| `--p2` | | Alternate permutation scheme (C/C only) |

**Epistasis analysis**

| | | |
|---|---|---|
| `--epistasis` | | Perform SNP x SNP epistatic analysis |
| `--fast-epistasis` | | Quick SNP x SNP screening for C/C data |
| `--twolocus` | SNP SNP | Display contingency table for two SNPs |
| `--case-only` | | Case-only epistatic analysis |
| `--gap` | 1000 | Gap (kb) for SNP x SNP case-only epistasis tests |
| `--epi1` | 0.0001 | Output p-value threshold: pairs |
| `--epi2` | 0.01 | Output p-value threshold: summary |
| –> `--set-by-all` | | Test set 1 SNPs paired with all others |
| `--nop` | | Do not calculate p-values (fast screening) |
| | | |
| `--genepi` | | Gene-based test for epistasis |

**Haplotype inference and linkage disequilibrium**

| | | |
|---|---|---|
| `--hap-snps` | snplist | Specify a list of SNPs to phase |
| `--hap-window` | N | Specify haplotype sliding window |
| `--hap` | tagfilename | Multimarker predictor / haplotype list |
| `--whap` | tagfilename | Weighted haplotype test list |
| | | |
| `--hap-assoc` | | Perform haplotype-based case/control association |
| `--hap-tdt` | | Perform haplotype-based TDT |
| `--hap-freq` | | Output haplotype frequencies for entire sample |
| `--hap-phase` | | Output individual haplotype phases |
| `--hap-phase-wide` | | Output individual haplotype phases, wide-format |
| | | |
| `--hap-impute` | | Create fileset with imputed haplotypes as SNPs |
| `--hap-pp` | 0.8 | Posterior probability threshold |
| `--hap-miss` | 0.5 | Proportion of missing genotypes allowed |
| `--hap-min-phase-prob` | 0.01 | Minimum reported phase probability |
| `--hap-max-phase` | N | Maximum number of phases considered per person |
| `--mhf` | 0.01 | Minor haplotype frequency threshold |

**Proxy association and imputation methods**

| | | |
|---|---|---|
| `--proxy-assoc` | SNP/all | Proxy association methods |
| `--proxy-glm` | | Use linear models in proxy association |
| `--proxy-drop` | | Drop then re-impute observed genotypes |
| `--proxy-tdt` | SNP/all | Proxy TDT association methods |
| | | |
| `--proxy-impute` | SNP/all | Proxy imputation methods |
| `--proxy-replace` | | Replace observed genotypes |
| `--proxy-dosage` | | Also output dosage file |
| `--proxy-impute-threshold` | 0.95 | Per-genotype threshold to impute for an individual |
| | | |
| `--proxy-list` | file | Specify SNPs to impute/test |
| `--proxy-flanking` | file | Specify proxies for single reference SNP |
| | | |
| `--proxy-r2` | 0 0.05 0.5 | Proxy selection LD parameters |
| `--proxy-maxsnp` | 5 | Maximum number of proxies tto select |
| `--proxy-window` | 15 | Proxy SNP search space (SNPs) |
| `--proxy-kb` | 250 | Proxy SNP search space (kb) |
| | | |
| `--proxy-b-threshold` | 0.1 | MAF threshold for *rare* alleles (plan B) |
| `--proxy-b-r2` | 0 0.05 0.5 | Alternate proxy selection LD parameters |
| `--proxy-b-maxsnp` | 0.1 | Alternate maximum number of proxies to use |
| `--proxy-b-window` | 0.1 | Alternate proxy SNP search space (SNPs) |
| `--proxy-b-kb` | 250 | Alternate proxy SNP search space (kb) |
| | | |
| `--proxy-maf` | 0.01 | Proxy SNP MAF threshold |
| `--proxy-geno` | 0.05 | Proxy SNP missingness threshold |
| `--proxy-r2-no-filter` | | No LD-based proxy selection |
| | | |
| `--proxy-mhf` | 0.05 | Proxy haplotype frequency threshold |
| `--proxy-sub-r2` | 0.8 | Minimum r-squared with reference for haplotypic proxies (verbose mode) |

| | | |
|---|---|---|
| `--proxy-sub-maxsnp` | 3 | Maximum number of SNPs per haplotypic proxy (verbose mode) |
| `--proxy-verbose` | | Verbose mode |
| `--proxy-show-proxies` | | List actual proxies in non-verbose mode |
| `--proxy-genotypic-concordance` | | In imputation, show genotypic-specific concordance |

**Conditional haplotype association tests**

| | | |
|---|---|---|
| `--chap` | | Main conditional-haplotype test command |
| `--specific-haplotype` | haplotype(s) | Test for specific haplogroup effect |
| `--independent-effect` | snps | Test for independent effect |
| `--control` | snp(s)/haplotype(s) | Control for certain effects |
| `--alt-snp` | | Specify SNP groupings under alternate |
| `--null-snp` | | Specify SNP groupings under null |
| `--alt-group` | | Specify haplogroupings under alternate |
| `--null-group` | | Specify haplogroupings under null |
| `--test-snp` | | Drop 1 or more conditioning SNPs |
| `--each-versus-others` | | Each all haplogroup-specific p-values |
| `--each-vs-others` | | As above |

**LD-based result clumping**

| | | |
|---|---|---|
| `--clump` | file(s) | Comma-delimited result files |
| `--clump-p1` | 1e-4 | p-value threshold for index SNPs |
| `--clump-p2` | 1e-2 | p-value threshold for clumped SNPs |
| `--clump-r2` | 0.2 | r$\hat{2}$ (LD) threshold for clumping |
| `--clump-kb` | 250 | kb-threshold for clumping |
| `--clump-replicate` | | Only report multi-file clumps |
| `--clump-best` | | For each SNP in the first file, find the best proxy from the other files |
| `--clump-verbose` | | Specifty verbose output |
| `--clump-range` | filename | Add gene/region range information to clumped output |
| `--clump-range-border` | kb | Use a kb border around each gene/region |
| `--clump-annotate` | field(s) | Include these fields in verbose mode |
| `--clump-field` | field | Specifty p-value field other than `P` |
| `--clump-index-first` | | Only index based on first results file |
| `--clump-allow-overlap` | | Specify that a SNP can appear in more than one clump |

**Gene annotation of SNP results**

| | | |
|---|---|---|
| `--gene-report` | filename | Results file to perform gene-report on |
| `--gene-list` | filename | List of genes/regions for reporting |
| `--gene-list-border` | kb | Add a kb border aroud each gene/region |
| `--gene-subset` | filename | Only report on a subset of genes, listed here |
| `--gene-report-empty` | | Report genes without any informative SNPs |

**LD pruning and pairwise LD**

| | | |
|---|---|---|
| `--indep` | N M VIF | VIF pruning (N-SNP window, shifted at M-SNP intervals) |
| `--indep-pairwise` | N M r$\hat{2}$ | r$\hat{2}$ pruning (as above) |
| `--r` | | Pairwise SNPxSNP LD (r) |
| `--r2` | | Pairwise SNPxSNP LD (r$\hat{2}$) |
| `--ld-window` | N | Limit pairwise SNPxSNP to within a *N* SNP window |

**Definition of SETs**

| | | |
|---|---|---|
| `--set` | setfilename | SET definitions |
| `--subset` | filename | Only read of subset of SETs from `--set` |
| `--set-table` | | Output a SNP by SET matrix |

**Copy number variants (CNV) analysis**

| | | |
|---|---|---|
| `--gfile` | fileset | Load generic variant file |
| `--cfile` | fileset | Load segmental CNV fileset (CNV, FAM, MAP) |
| `--cnv-list` | filename | Load segmental CNV list |
| `--cnv-del` | | Filter only deletions |
| `--cnv-dup` | | Filter only duplications |
| `--cnv-intersect` | filename | Include segments intersecting with regions |
| `--cnv-exclude` | filename | Exclude segments intersecting with regions |
| `--cnv-disrupt` | | Include/Exclude segments that start or stop within a gene/region |
| `--cnv-count` | filename | Count number of regions intersected by CNVs |
| `--cnv-border` | kb | Add a kb border around each region |
| `--cnv-freq-excldue-above` | N | Exclude CNVs overlapping regions with more than N CNVs |
| `--cnv-freq-excldue-below` | N | Exclude CNVs overlapping regions with fewer than N CNVs |
| `--cnv-freq-excldue-exact` | N | Exclude CNVs overlapping regions with exactly N CNVs |
| `--cnv-freq-incldue-exact` | N | Include CNVs overlapping regions with exactly N CNVs |
| `--cnv-freq-method2` | | Use alternative method for determining CNV frequency |
| `--cnv-overlap` | N | Define overlap of CNV and region by CNV length |
| `--cnv-union-overlap` | N | Define overlap of CNV and region by union |
| `--cnv-region-overlap` | N | Define overlap of CNV and region by region length |
| `--cnv-write` | | Create a new CNV and FAM file |
| `--cnv-write-freq` | | Include frequency counts if `--cnv-freq-method2` specified |
| `--cnv-make-map` | | Create a new MAP file from a CNV and FAM file |
| `--cnv-report-regions` | | List regions that are intersected by CNVs |
| `--cnv-verbose-report-regions` | | Verbose listing of regions that are intersected by CNVs |
| `--cnv-subset` | filename | Define overlap of CNV and region by region length |

| | | |
|---|---|---|
| --cnv-track | kb | Create a UCSC-compatible BED track for viewing CNVs |
| --cnv-blue | kb | Make this CNV track blue |
| --cnv-red | kb | Make this CNV track red |
| --cnv-green | kb | Make this CNV track green |
| --cnv-brown | kb | Make this CNV track brown |
| --cnv-kb | N | Exclude segments below N kb |
| --cnv-max-kb | N | Exclude segments above N kb |
| --cnv-score | N | Exclude segments below N score |
| --cnv-max-score | N | Exclude segments above N score |
| --cnv-drop-no-segment | | Remove individuals with no segments |
| --cnv-unique | | Exclude CNVs seen in both cases and controls |
| --cnv-seglist | kb | Create a printout of CNVs |
| --cnv-indiv-perm | | Permutation test for genome-wide CNV burden |
| --cnv-test-2sided | | Use 2-sided approach for empirical p-values |
| --cnv-test-window | kb | Extend test to a region extending kb distance on either side of position |
| --cnv-test-region | kb | Test regions for CNV case/control differences |
| **Data simulation options** | | |
| --simulate | filename | Simulate SNP population-based data |
| --simulate-ncases | 100 | Number of cases to simulate |
| --simulate-ncontrols | 100 | Number of controls to simulate |
| --simulate-prevalence | 0.01 | Disease prevalence in population |
| --dummy | N M | Generate dataset of N individuals on M SNPs |
| **Misc analysis output options** | | |
| --adjust | | Output adjusted p-values and calculate genomic control |
| --lambda | X | Set lambda to X instead of estimating from data |
| --qq-plot | | Generate entries to faciliate a Q-Q plot in adjusted output |
| **Misc.** | | |
| --help | | Display list of options |
| --dog | | Set chromosome codes for dog |
| --mouse | | Set chromosome codes for mouse |
| --horse | | Set chromosome codes for horse |
| --cow | | Set chromosome codes for cow |
| --sheep | | Set chromosome codes for sheep |
| --lookup | SNP rs# | Lookup WGAS SNP annotation information |
| --lookup-gene | gene name | List all SNPs in gene |
| --lookup-list | snplist filename | SNP annotation for multiple SNPs |

## A.2 Output files (alphabetical listing)

| Filename | Main associated command(s) | Description |
|---|---|---|
| plink.adjust | --adjust | Adjusted significance values (multiple testing) |
| plink.assoc | --assoc | Association results |
| plink.assoc.hap | --hap-assoc | Haplotype-based association results |
| plink.assoc.linear | --linear | Linear regression model |
| plink.assoc.logistic | --logistic | Logistic regression model |
| plink.assoc.mperm | --assoc --mperm | maxT permutation empirical p-values |
| plink.assoc.perm | --assoc --perm | Adaptive permutation empirical p-values |
| plink.assoc.proxy | --proxy-assoc | Proxy association results |
| plink.assoc.set | --assoc --set | Set-based association results |
| plink.bed | --make-bed | Binary PED file |
| plink.bim | --make-bed | Binary MAP file |
| plink.chap | --chap | Conditional haplotype tests |
| plink.cov | --write-covar | Ordered, filtered covariate file |
| plink.clumped | --clump | LD-based results clumping |
| plink.clumped.best | --clump-best | Single best LD-based clumping |
| plink.clumped.ranges | --clump-range | Gene/region report for clumps |
| plink.cluster0 | --cluster | Progress of IBS clustering |
| plink.cluster1 | --cluster | IBS cluster solution, format 1 |
| plink.cluster2 | --cluster | IBS cluster solution, format 2 |
| plink.cluster3 | --cluster | IBS cluster solution, format 3 |
| plink.cluster3.missing | --cluster-missing | IBM cluster solution, format 3 |
| plink.cmh | --mh | Cochran-Mantel-Haenszel test 1 |
| plink.cmh2 | --mh2 | Cochran-Mantel-Haenszel test 2 |
| plink.cnv.indiv | --cnv-list | Copy number variant per individual summary |
| plink.cnv.overlap | --cnv-list | Copy number variant overlap |
| plink.cnv.summary | --cnv-list | Copy number variant summary |
| plink.cnv.summary.mperm | --cnv-list | Copy number variant test |
| plink.diff | --merge-mode 6/7 | Difference file |
| plink.epi-cc1 | --epistasis | Epistasis: case/control pairwise results |
| plink.epi-cc2 | --epistasis | Epistasis: case/control summary results |
| plink.epi-co1 | --epistasis --case-only | Epistasis: case-only pairwise results |
| plink.epi-co2 | --epistasis --case-only | Epistasis: case-only summary results |
| plink.fam | --make-bed | Binary FAM file |
| plink.fmendel | --mendel | Mendel errors, per family |
| plink.frq | --freq | Allele frequency table |
| plink.frq.count | --freq --counts | Allele counts table |
| plink.frq.hap | --hap-freq | Allele frequency table |
| plink.genepi.dat | --genepi | Gene-based epistasis R dataset |
| plink.genepi.R | --genepi | Gene-based epistasis R script |
| plink.genome | --genome | Genome-wide IBD/IBS pairwise measures |
| plink.het | --het | Individual inbreeding coefficients |
| plink.hh | | List of heterozygous haploid genotypes (SNPs/individuals) |

| | | |
|---|---|---|
| plink.hom | --homozyg-snp --homozyg-kb | Runs of homozygosity |
| plink.hom.overlap | --homozyg-group | Pools of overlapping runs of homozygosity |
| plink.homog | --homog | Between strata homogeneity test |
| plink.hwe | --hardy | Hardy-Weinberg test statistics |
| plink.imendel | --mendel | Mendel errors, per individual |
| plink.imiss | --missing | Missing rates, per individual |
| plink.info | --recodeHV | Info file for Haploview filesets |
| plink.irem | --mind | List of individuals removed for low genotyping |
| plink.imputed.map | --hap-impute | Imputed from multi-marker predictors |
| plink.impute.ped | --hap-impute | Imputed from multi-marker predictors |
| plink.list | --list | Recoded LIST file |
| plink.lmendel | --mendel | Mendel errors, per locus |
| plink.lmiss | --missing | Missing rates, per locus |
| plink.log | | Log file (always generated) |
| plink.map | --recode | Recoded MAP file |
| plink.mdist | --cluster --matrix | IBS distance matrix |
| plink.mdist.missing | --cluster-missing | IBM distance matrix |
| plink.mendel | --mendel | Mendel errors, per error |
| plink.mishap | --hap | List of SNPs that show problem phasing (could not be found or on wrong chromosome) |
| plink.missing | --test-missing | Test of differences in C/C missing rates |
| plink.missing.hap | --test-mishap | Haplotype-based test of non-random genotyping failure |
| plink.missnp | --merge | List of SNPs that show strand problems when merging files (more than 2 alleles) |
| plink.model | --model | Full-model association results |
| plink.model.best.mperm | --model --mperm | Best full-model association max(T) permutation results |
| plink.model.best.perm | --model --perm | Best full-model association adaptive permutation results |
| plink.model.gen.mperm | --model --mperm --model-gen | Genotypic association max(T) permutation results |
| plink.model.gen.perm | --model --perm --model-gen | Genotypic association adaptive permutation results |
| plink.model.dom.mperm | --model --mperm --model-dom | Dominant association max(T) permutation results |
| plink.model.dom.perm | --model --perm --model-dom | Dominant association adaptive permutation results |
| plink.model.trend.mperm | --model --mperm --model-trend | Trend test association max(T) permutation results |
| plink.model.trend.perm | --model --perm --model-trend | Trend test association adaptive permutation results |
| plink.model.rec.mperm | --model --mperm --model-rec | Recessive association max(T) permutation results |
| plink.model.rec.perm | --model --perm --model-rec | Recessive association adaptive permutation results |
| plink.nof | | List of SNPs with no observed founders |
| plink.nosex | | List of individuals with ambiguous sex code |
| plink.nearest | --cluster --neighbour | Nearest neighbour (IBS) statistics |
| plink.pdump | --pedigree | Information on pedigree structure |
| plink.ped | --recode | Recoded PED file |
| plink.phase-* | --hap --phase | Haplotype phases (one file per locus) |
| plink.plist | --plist | Pairwise list of two people's genotypes |
| plink.proxy.impute | --proxy-impute | Proxy imputation output |
| plink.proxy.impute.dosage | --proxy-impute --proxy-dosage | Proxy imputation dosage output |
| plink.proxy.report | --proxy-assoc | Verbose proxy association output |
| plink.prune.in | --indep --indep-pairwise | List of remaining SNPs (i.e. not pruned) |
| plink.prune.out | --indep --indep-pairwise | List of pruned-out SNPs |
| plink.qassoc | --assoc | Quantitative trait association results |
| plink.qassoc.gxe | --gxe | Quantitative trait interaction results |
| plink.range.report | --cnv-verbose-report-regions | Listing of CNVs by genes/regions |
| plink.raw | --recodeAD | Recoded additive/dominance format file |
| plink.snplist | --write-snplist | List of SNPs in the dataset |
| plink.T2 | --T2 | Hotelling's T(2) test results |
| plink.tdt | --tdt | TDT/parenTDT asymptotic results |
| plink.tdt.hap | --tdt | TDT/parenTDT permutaion results |
| plink.tdt.mperm | --tdt | TDT/parenTDT max(T) permutation results |
| plink.tdt.perm | --tdt | TDT/parenTDT adaptive permutation results |
| plink.tdt.poo | --tdt --poo | TDT parent-of-origin results |
| plink.tdt.poo.mperm | --tdt --poo --mperm | TDT parent-of-origin max(T) permutation results |
| plink.tdt.poo.perm | --tdt --poo --perm | TDT parent-of-origin adaptive permutation results |
| plink.tdt.poo.set | --tdt --poo --set --mperm | TDT parent-of-origin set-based results |
| plink.tdt.set | --tdt --set --mperm | TDT/parenTDT set-based results |
| plink.tfam | --transpose / --tfile | FAM for for transposed fileset |
| plink.tped | --transpose / --tfile | PED file for transposed fileset |
| plink.twolocus | --twolocus | SNP x SNP contingency table |

# Appendix B

# Order of major operations in PLINK

This section contains a rough flow-chart of some of the main operations in PLINK. In particular, it is designed to indicate the order in which certain operations are performed (i.e. whether SNPs are excluded before or after merging files, etc), and also when PLINK halts operation, e.g. after certain commands, meaning that certain combinations are not feasible.

Most of these steps are optional (i.e. will only occur if a specific command has been issued on the command line).

- Parse command line
- Check version, unused options, warnings
- Define chromosome set (human, or `--mouse`, `--rice`, etc)
- Run ID-helper utility (`--id-dict` and `--id-match`), then **QUIT**
- Run SNP-annotation (`--lookup` and `--lookup-gene`), then **QUIT**
- Read input, either:
  - Dummy dataset(`--dummy`), or
  - Simulated dataset (`--simulate`), or
  - Maps for CNVs (`--cfile`, `--cnv-list`), or
  - Binary filset (`--bfile`), or
  - PED fileset (`--file`), or
  - LGEN fileset (`--lfile`), or
  - Transposed fileset (`--tfile`), or
  - Maps for generic variants (`--gfile`)
- At this stage, the following filters apply directly when loading (Note: some other filters not mentioned below are done later, e.g. `--snps`, `--extract`, `--remove`, `--filter-males`):
  - `--chr`
  - `--snp`, `--window`
  - `--from`, `--to`
  - `--from-kb`, `--to-kb`, etc
- Check for duplicate individual or SNP names

- Merge one or more filesets (`--merge`, `--bmerge`, `--merge-list`)
- Swap in alternate phenotype file (`--pheno`), or make a new phenotype (`--make-pheno`)
- Remove individuals with missing phenotypes (`--prune`)
- Update SNP information (`--update-map`)
- Update FAM information (`--update-ids`, `--update-sex`, ...)
- Update allele information (`--update-alleles`)
- Flip strand (`--flip`)
- Recode alleles 1234/ACGT (`--alleleACGT`, `--allele1234` )
- Either, if (`--exclude-before-extract`), then
  - extract any SNPs (`--extract`)
  - then exclude any SNPs (`--exclude`)
- otherwise
  - exclude any SNPs (`--exclude`)
  - then extract any SNPs (`--extract`)
- Either, if (`--keep-before-remove`), then
  - keep any individuals (`--keep`)
  - then remove any individuals (`--remove`)
- otherwise
  - remove any individuals (`--remove`)
  - then keep any individuals (`--keep`)
- Filter SNPs based on attributes (`--attrib`)
- Filter individuals based on attributes (`--attrib-indiv`)
- Filter SNPs based on quality scores (`--qual-scores`)
- Filter genotypes based on quality scores (`--qual-geno-scores`)
- Random thinning of SNPs (`--thin`)
- Read `--genome-lists`
- Read list of obligatory missing genotypes (`--oblig-missing`)
- Filter based on a variable (`--filter`)
- Filter based on sex, phenotype, etc (`--filter-males`, `--filter-cases`, ...)
- Read covariate file (`--covar`)
- Read cluster file (`--within`)
- Zero-out specific genotypes (`--zero-cluster`)
- Process rare CNV data

  - Read CNV list, map to genomic positions
  - Filter on genes, sizes, types, etc (`--cnv-intersect`, `--cnv-del`, `--cnv-kb`, etc)
  - Write back any genes, regions intersected (`--cnv-report-regions`)
  - Filter CNVs based on frequency (`--cnv-freq-exclude-above`, etc)
  - Report basic count of CNVs in LOG file
  - Write a new CNV list, map file (`--cnv-write`, `--cnv-make-map`)
  - Calculate per-individual CNV summary statistics
  - Calculate per-position CNV summaries
  - Make summary displays(`--cnv-track`, `--cnv-seglist`)
  - Find overlapping CNVs as pools (`--segment-group`)
  - Perform association / genome-wide burden test (`--mperm`, `--cnv-indiv-perm`)
  - **QUIT**

- Process generic variant data (`--gfile`)
  - Read GVAR data (might be on top of existing, standard file)
  - Calculate frequency statistics for each allele, CNP state
  - Perform linear/logistic regression of phenotype on CNP states
  - **QUIT**

- Main SNP filters
  - Count founders and nonfounders
  - Calculate per-individual genotyping rate, remove individuals below threshold (`--missing`, `--mind`)
  - Calculate (or read from file (`--read-freq`) allele frequencies
  - Determine per SNP missing genotype rate, **after removing individuals**, exclude below threshold (`--geno`)
  - Determine minor (reference) allele
  - List of heterozygous hets found, by default set to missing
  - List SNPs with no founder genotypes observed
  - Write allele frequencies to file (`--freq`)
  - Calculate HWE statistics per SNP (`--hardy`, `--hwe`); after `--hardy`, then **QUIT**
  - Report genotyping rate per SNP and per individual as calculated above (`--missing`)
  - Remove SNPs below the MAF filter (`--maf`)

- Re-report basic case/control counts to LOG

- Re-specify reference alleles (`--reference-allele` )

- Make family units, if needed; perform Mendel checks (`--mendel`, `--me`, `--tdt`, etc)

- Reset pat and mat codes of non-founders if parents not present (`--make-founders`)

- Perform sex-check (`--check-sex`)

- Create pseudo case/control units from trio data (`--tucc`)

- Write permuted phenotype file (`--make-perm-pheno`), **QUIT**

- Write table of SNPs/set scoring (`--set-table`), **QUIT**
- Write covariate file (`--write-covar`), then **QUIT**
- Write cluster file (`--write-cluster`), then **QUIT**
- Write snplist file (`--write-snplist`), then **QUIT**
- Write binary fileset file (`--make-bed`), then **QUIT**
- Write other file formats for genotype data (`--recode`, `--recodeA`, `--list`, `--two-locus`, etc), then **QUIT**
- Create and output a SET file given ranges (`--make-set`), then **QUIT**
- LD-based clumping of association results, (`--clump`), then **QUIT**
- Generate lists of SNPs tagging other SNPs (`--show-tags`), then **QUIT**
- Generate haplotype blocks (`--blocks`), then **QUIT**
- Determine if conditioning SNPs used (`--condition`)
- Perform IBS, cluster analysis and MDS analysis (`--cluster`, `--mds-plot`, `--neighbour`), then **QUIT**
- Test for differences in IBS between groups (`--ibs-test`), then **QUIT**
- Calculate genome-wide IBS and IBD (`--genome`), then **QUIT**
- Calculate F inbreeding statistic (`--het`)
- Calculate runs of homozygosity (`--homozyg`), then **QUIT**
- Perform LD-based pruning of SNP (`--indep`, `--indep-pairwise`), then **QUIT**
- Perform LD-based scan for strand flips (`--flipscan`), then **QUIT**
- Calculate and display pairwise LD (`--r2`, `--ld`), then **QUIT**
- General haplotype estimation, (association, phase reports, frequencies) `--hap`)
  - Phasing
  - Report haplotype frequencies
  - Report hapotype phases
  - Perform mis-hap test for non-missing randomness
  - Proxy association and imputation
  - **QUIT**
- SNP-by-SNP epistasis tests (`--epistasis`), then **QUIT**
- Score per-individual risk profiles (`--score`), then **QUIT**
- Run R-plugin on dataset (`--R`), then **QUIT**
- For main association tests, loop over all phenotypes, (`--all-pheno`)
  - Perform assocaition test (`--mh`, `--model`, `--assoc`, `--fisher`, `--linear`, `--logistic`, `--homog`, `--qfam`, `--tdt`, `--poo`, `--dfam`, `--gxe`, etc)
  - Perform haplotype association test (`--hap-assoc`, `--hap-tdt`)

  - Perform conditional haplotype test (`--chap`), then **QUIT**
  - Perform `--test-missing`
  - If specified, repeat the above tests with permuted datasets
  - Go to next phenotype
- Perform PLINK segmental sharing test
- Definitely **QUIT**