User Manual for mrMLM

1. Introduction

mrMLM, a R package, aims to provide a user-friendly interface to conduct genome-wide association study (GWAS) via a multi-locus random-SNP-effect mixed linear model (mrMLM) methodology and to visualize the results. It works on the platforms of Windows, Linux and MacOS. The GUI is based on available add-on package RGtk2, via the aid of another package gWidgetsRGtk2. The visualization of results is based on package qqman, such as Manhattan and QQ plots.

2. Installation

2.1 install GTK+

You may need to install GTK+ before installing RGtk2, because RGtk2 depends on GTK+.

For Windows user, you do as below:

Download GTK+ here (http://sourceforge.net/projects/gladewin32/files/gtk%2B-win32-runtime/2.10.11/gtk-2.10.11-win32-1.exe).

Run the resulting file (gtk-2.10.11-win32-1.exe) which is an automated installer that will help you complete the installation of Gtk2 libraries.

For Mac OS users, you do as below:

Download GTK+ here (http://sourceforge.net/projects/gtk-osx/files/latest/download).

Extract and run the resulting file (gtk-osx-docbook-1.2.tar.gz).

For Linux users, you do as below:

You may or may not upgrade the GTK libraries depending on your distribution.

There are more details on RGtk2 at RGtk2's home page (http://www.ggobi.org/rgtk2/).

2.2 install R

Download R from CRAN (https://cran.r-project.org/) and install it by running the file.

2.3 install the R packages

The following R packages are needed: RGtk2, cairoDevice, gWidgets, gWidgetsRGtk2, RGtk2Extras and qqman, which can be downloaded from CRAN (https://cran.r-project.org/). Install them in order, as some depend on others. Then install them directly within R environment using the below command:

install.packages(pkgs=c("RGtk2","cairoDevice","gWidgets","gWidgetsRGtk2","RGtk2Extras","qqman"))

2.4 Install mrMLM

The mrMLM package is freely available at the CRAN (https://cran.r-project.org/ or soyzhang@mail.hzau.edu.cn or soyzhang@hotmail.com), you can download or request this R software. Then install mrMLM software directly within R environment using the below command:
install.packages(pkgs="mrMLM")

3. Running

The procedure is described below. Launch mrMLM with in R by command:
library(pkgs="mrMLM"), after that the GUI of mrMLM turned out like the following dialog.
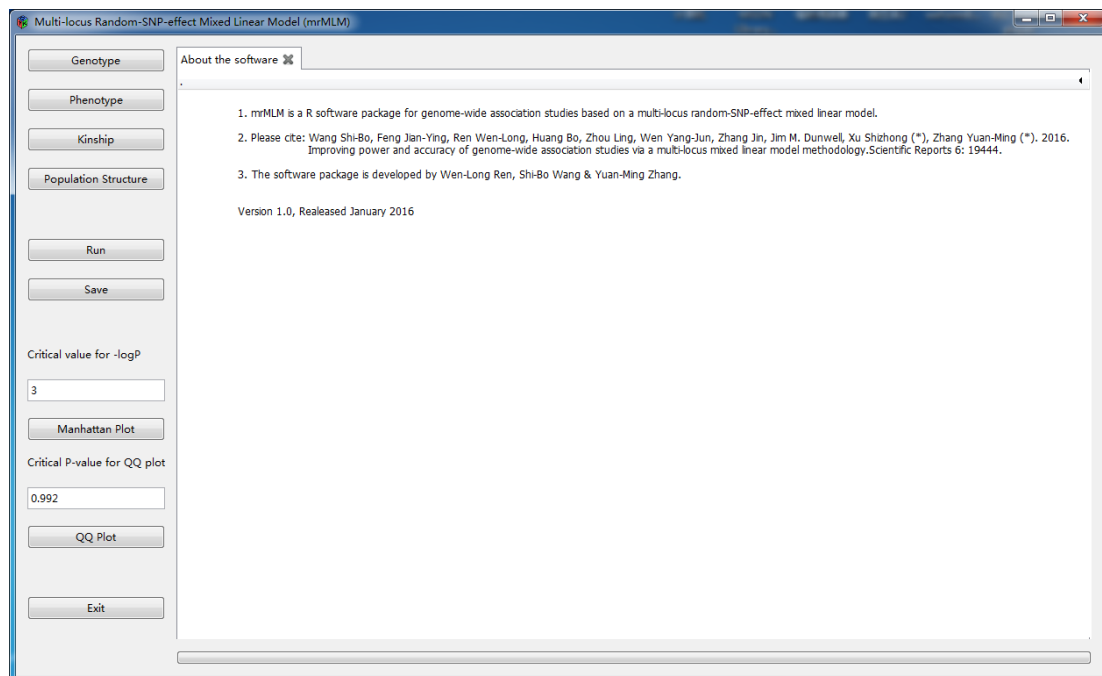

Figure 1. A snapshot of the GUI of mrMLM

To restart the GUI, the command *mrMLM()* can be issued.

3.1 Input Files

Users can use the **Genotype** button to import the genotypic data, use the **Phenotype** button to import the trait observations, use the **Kinship** button to import the kinship matrix, use the **Population Structure** button to import the population structure matrix. If one file is imported successfully, there will be one tabbed page added to the notebook. Meanwhile, the **Kinship** has two options: one is to import directly the known kinship matrix and another is to compute the kinship matrix using the mrMLM software. And the **Population Structure** has two options as well. If there is no effect of population structure on GWAS, the population structure matrix may be not included in the mixed linear model of the GWAS. If not, you should incorporate the population
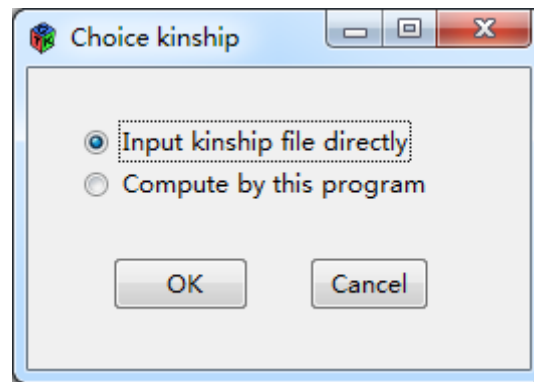
structure matrix into your GWAS.
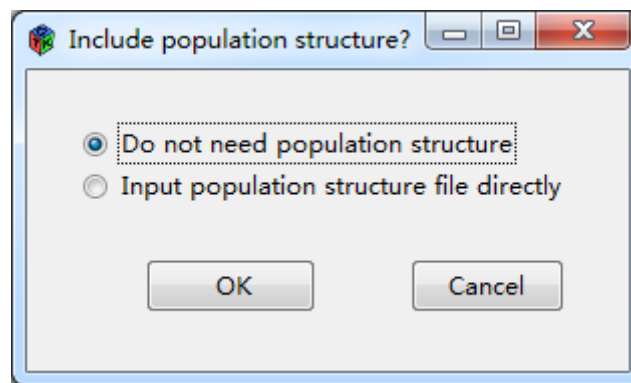

Figure 2. A snapshot of the kinship dialog

Figure3. A snapshot of the population structure dialog

3.2 Run Program

Users can use the Run button to execute the program. If the program starts, there will be a progress bar showing the running status and "Please be patient..." words in the bottom of the interface. If the work have finished, there will be "All done." instead of "Please be patient...".
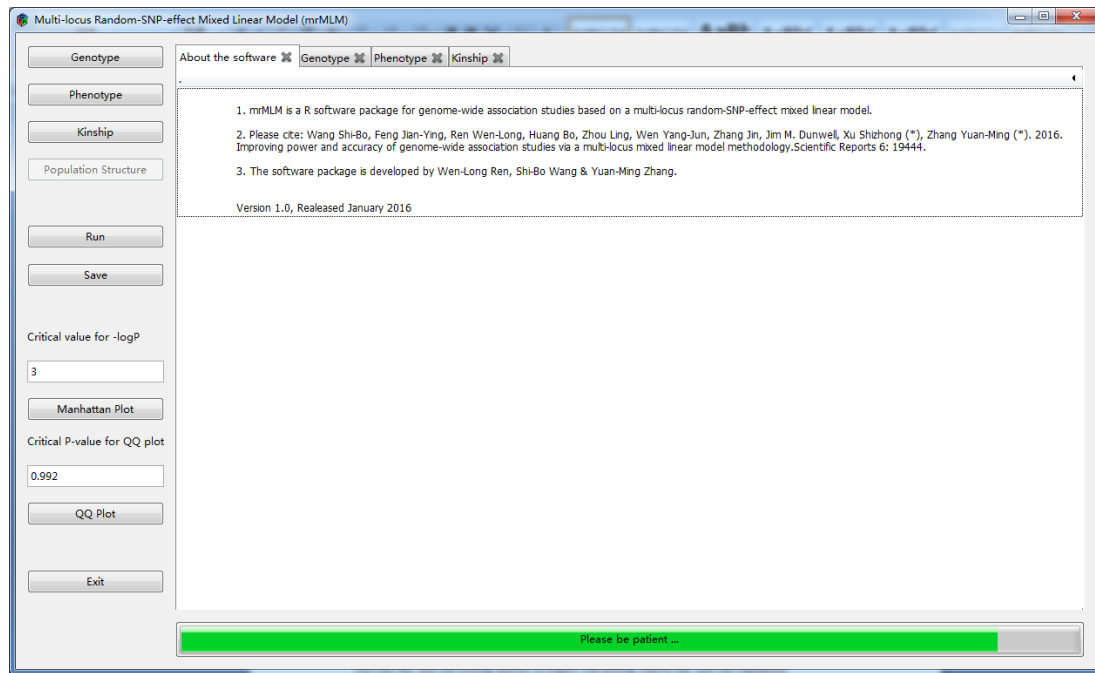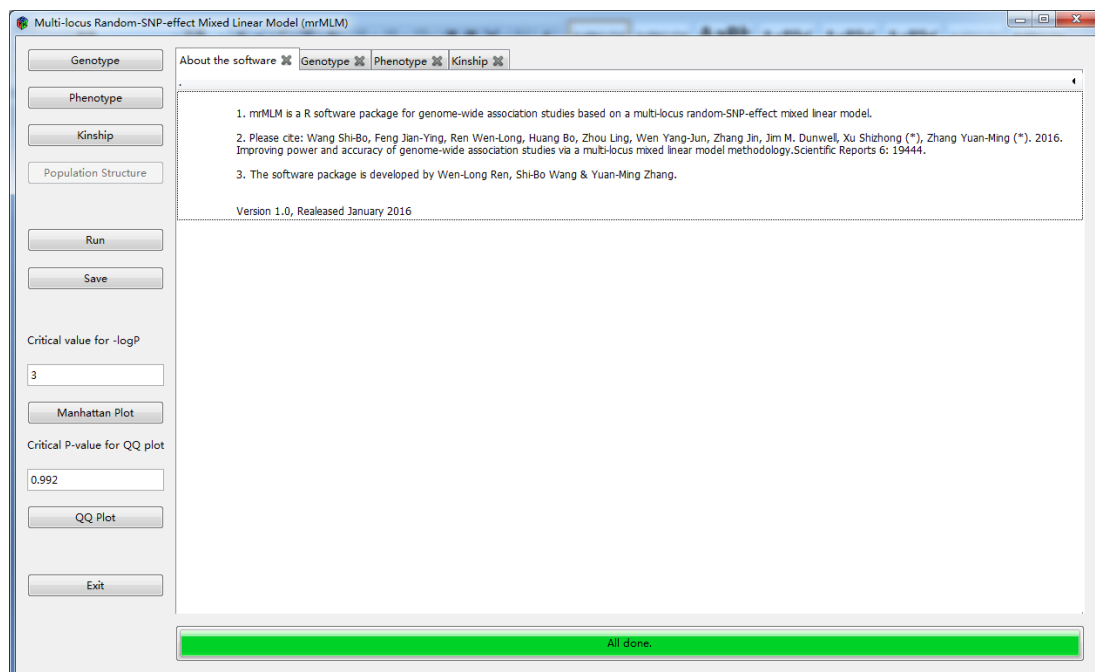
Figure 4. A snapshot of a running program

Figure 5. A snapshot of finished program

3.3 Save Results

Users can use **Save** button to save the results as ***.csv** format file. There will have a dialog to choice the pathway and the file name.
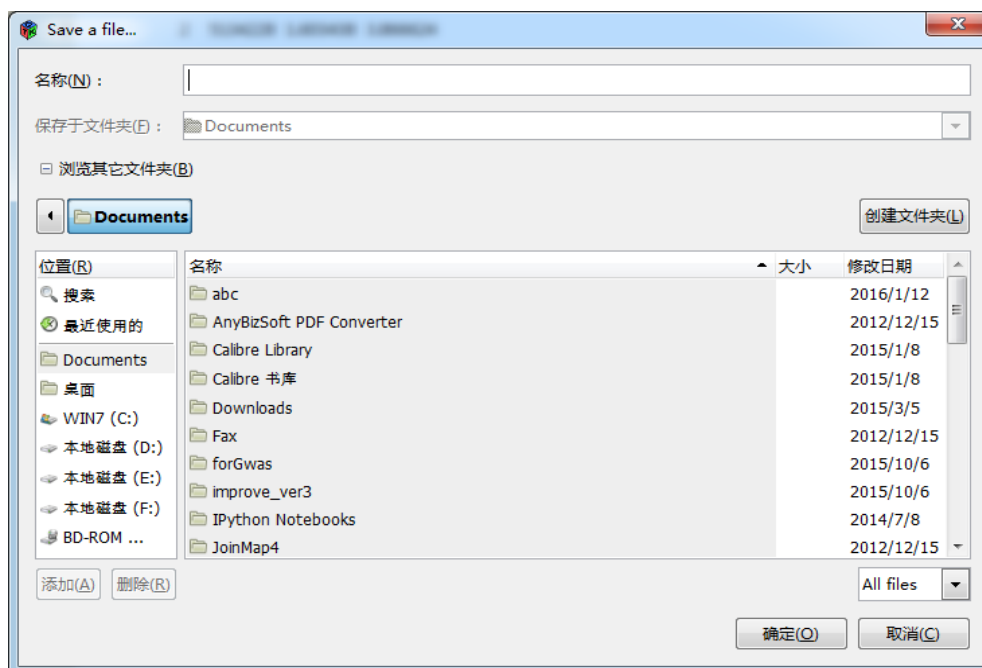
Figure6. A snapshot of **Save** dialog

Warning: It is better not to include other languages except English in the pathway and file name. Otherwise, there may be something wrong.

4. Visualization of Results

If the program have finished, you can have the visualization of the results. Before use the **Manhattan Plot** button, please set the critical value for –logP, which is defaulted the value of 3. Before use the **QQ Plot** button, please set the critical P-value for QQ plot, which is defaulted the value of 0.992.
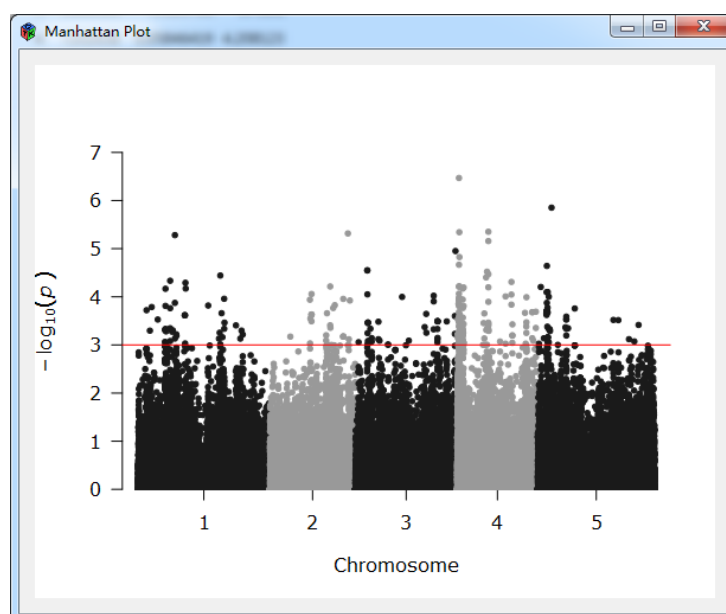

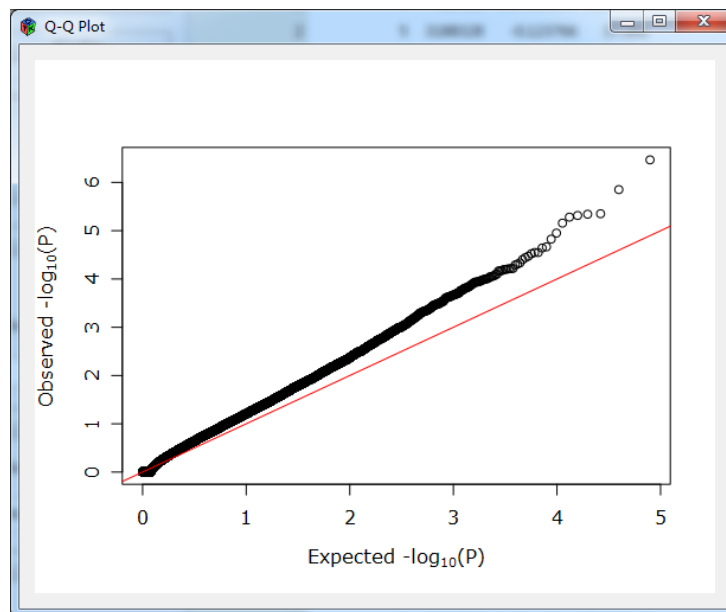
Figure 7. A snapshot of **Manhattan Plot**

Figure 8. A snapshot of **QQ Plot**