

User Manual for

GAPIT



Genomic Association and Prediction Integrated Tool

(Version 2)

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Disclaimer: While extensive testing has been performed by the Edward Buckler Lab and Zhiwu Zhang Lab at Cornell University and Washington State University respectively, results are, in general, reliable, correct or appropriate. However, results are not guaranteed for any specific set of data. We strongly recommend that users validate GAPIT results with other software packages, such as SAS, and TASSEL.

Support documents: Extensive support documents, including this user manual, source code, demonstration scripts, data, and results, are available at GAPIT website Zhiwu Zhang Laboratory: <http://zzlab.net/GAPIT>

Questions and comments: Users and developers are recommended to post questions and comments at GAPIT forum: <https://groups.google.com/forum/#!forum/gapit-forum>. Answers from other users and developers are appreciated. The GAPIT team members will periodically go through these questions and comments and address them accordingly.

Citation: Multiple statistical methods are implemented in GAPIT version 1&2. Citations of GAPIT vary depending on methods and versions used in the analysis:

Method	Method paper	GAPIT Implimentation
Compression MLM (CMLM)	Zhang et al, 2010, <i>Nature Genetics</i> ¹	Version 1 by Lipka et al, 2012, <i>Bioinformatics</i> ²
gBLUP	Zhang et al, 2007, <i>J. Anim. Science</i> ³	Version 1 by Lipka et al, 2012, <i>Bioinformatics</i> ²
Enriched CMLM	Li et al, 2014, <i>BMC Biology</i> ⁴	Version 2 by Tang et al, 2016, <i>The Plant Genome</i> ⁵
SUPER	Wang et al, 2014, <i>PLoS One</i> ⁶	Version 2 by Tang et al, 2016, <i>The Plant Genome</i> ⁵

Note: These references are listed in section of Reference.



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1 INTRODUCTION

1.1 Why GAPIT?

The gap between developing statistical methods and applying them to solve bioinformatics problems is increasing because of recent advances in genotyping technologies. It is now more affordable than ever to obtain millions of SNPs on a large number of individuals. Although software programs have been developed to implement these statistical methods, many of them cannot accommodate large data sets. Additionally, many of these computer programs require a complex user interface, and thus may not be easily accessible to researchers.

The Efficient Mixed Model Association (EMMA) R package⁷ and TASSEL⁸ are two relatively easy to use programs. The EMMA R package uses the EMMA algorithm to reduce computational time when estimating variance components in the mixed linear model (MLM)⁹. TASSEL was written in Java and can be operated through a simple graphic user interface. In addition to the EMMA algorithm, TASSEL uses the compressed MLM (CMLM)¹ and population parameters previously determined (P3D)¹ to speed up computational time and optimize statistical performance. The P3D method was independently developed by Kang *et al* (2010) and is implemented in a C program called EMMA expedited (EMMAx)¹⁰.

Identifying the genetic potential of individuals is one of the ultimate goals of genetic researchers. In plants and animals, this potential can be incorporated into selection programs. In humans, this can be used for genomic prediction of an individual's likelihood for having a disease, and medical treatments can be applied accordingly. Our recent unpublished study showed that the CMLM method developed for GWAS can be used for genomic prediction and selection, and can provide accurate predictions with a dramatic reduction in computational time.

Genomic Association and Prediction Integrated Tool (GAPIT)² is a computer package that uses EMMA, CMLM, and P3D to conduct GWAS and make genomic predictions. This package is operated in an R environment and uses a minimal amount of code. Large data sets with many SNPs can be analyzed in GAPIT by subdividing the genotypic data into multiple files. GAPIT can read in genotypic data in either HapMap format or in the numerical format required for the EMMA R package. GAPIT reports detailed results in a series of tables and graphs.

This help document and corresponding tutorials should assist with getting a new user familiar with GAPIT. Additional questions may be directed towards the authors.

1.2 Getting Started

GAPIT is a package that is run in the R software environment, which can be freely downloaded from <http://www.r-project.org> or <http://www.rstudio.com>.

GAPIT uses six R libraries: multtest, gplots, LDheatmap, genetics, EMMREML, scatterplot3d, and a modified version of the EMMA R package. Library multtest can be installed by typing these command lines:

```
source("http://www.bioconductor.org/biocLite.R")
biocLite("multtest")
```

Library `gplots`, `LDheatmap`, `genetics`, `EMMREML` and `scatterplot3d` can be installed by typing these command lines (you are required to choose a cite to download):

```
install.packages("gplots")
install.packages("LDheatmap")
install.packages("genetics")
install.packages("EMMREML")
install.packages("scatterplot3d") #The downloaded link at: http://cran.r-project.org/package=scatterplot3d
```

Once the above packages are installed, these libraries can be imported to R environment by typing these commands:

```
library(multtest)
library(gplots)
library(LDheatmap)
library(genetics)
library(EMMREML)
library(compiler) #this library is already installed in R
library("scatterplot3d")
```

The GAPIT package can be installed by typing this command line:

```
source("http://zzlab.net/GAPIT/gapit_functions.txt")
```

The EMMA library was developed by Kang *et al.* (2008). One line was added to the library to handle the grid search with “NA” likelihood. The modified library can be installed by typing this command line:

```
source("http://zzlab.net/GAPIT/emma.txt")
```

Now create a directory and set it as your working directory in R. Download the GAPIT tutorial dataset to the directory:

```
setwd("C:\\myGAPIT")
```

The easiest way of using GAPIT is to COPY/PASTE [GAPIT tutorial script](#). Here is the code from the first tutorial. After a few minutes, the GWAS and genomic prediction results will be saved in the above working directory.

```
#Step 1: Set working directory and import data
myY <- read.table("mdp_traits.txt", head = TRUE)
myG <- read.table("mdp_genotype_test.hmp.txt" , head = FALSE)

#Step 2: Run GAPIT
myGAPIT <- GAPIT(
  Y=myY,
  G=myG,
  PCA.total=3
)
```

These results are also included in the tutorial data set. All tables, including GWAS P -values and genomic breeding values are stored in comma separated value (.csv) files. All graphs, including Manhattan plots and QQ plots, are saved as portable document format (.pdf) files. These results are presented in detail in Chapter 4 (Results). Before reading next three chapters, we recommend you go directly to the tutorial chapter and run other tutorials.

1.3 How to use the GAPIT user manual?

The next three chapters (2-4) describe details on the input data, type of analysis and output of results. Chapter 5 presents scenarios to demonstrate the applications. Chapter 6 is for users to use GAPIT for prototyping. The last chapter (7) lists frequently questions and answers.

2 Data

The phenotypic data are the only data that must be directly provided by the user. Although the kinship is essential, it can be either provided by the user, or estimated automatically from the genotypic data. Genotypic data may not be needed for genomic prediction if the kinship matrix is provided by the user. Covariate variables (fixed effects), such as population structure represented by the Q matrix (subpopulation proportion) or principal components (PCs), is optional. GAPIT provides the option to calculate PCs from the genotypic data. All input files should be saved as a “Tab” delimited text file, and we recommend sorting the taxa in alphabetical order.

Notice: It is important that each taxa name is spelled, punctuated, and capitalized (R is case sensitive) the same way in each of the input data sets. If this is not done, they will be excluded from the analysis. Additionally, the taxa names must not be numeric.

2.1 Phenotypic Data

The user has the option of performing GWAS on multiple phenotypes in GAPIT. This is achieved by including all phenotypes in the text file of phenotypic data. Taxa names should be in the first column of the phenotypic data file and the remaining columns should contain the observed phenotype from each individual. Missing data should be indicated by either “NaN” or “NA”. The first ten observations in the tutorial data (mdp_traits.txt) are displayed as follows:

Taxa	EarHT	dpoll	EarDia
811	59.5	NaN	NaN
4226	65.5	59.5	32.21933
4722	81.13	71.5	32.421
33-16	64.75	64.5	NaN
38-11	92.25	68.5	37.897
A188	27.5	62	31.419
A214N	65	69	32.006
A239	47.88	61	36.064
A272	35.63	70	NaN
A441-5	53.5	67.5	35.008

The file is “Tab” delimited. The first row consists of column labels (i.e., headers). The column labels indicate the phenotype name, which is used for the remainder of the analysis.

The phenotype file can be input to R by typing command line:

```
myY <- read.table("mdp_traits.txt", head = TRUE)
```

2.2 Genotypic Data

Genotypic data are required for GWAS, but are optional for genomic prediction. In the later case, genomic prediction is performed using a kinship matrix provided by the user.

GAPIT accepts **genotypic data in either standard HapMap format** or in numeric format.

2.2.1 Hapmap Format

Hapmap is a commonly used format for storing sequence data where SNP information is stored in the rows and taxa information is stored in the columns. This format allows the SNP information (chromosome and position) and genotype of each taxa to be stored in one file.

The first 11 columns display attributes of the SNPs and the remaining columns show the nucleotides observed at each SNP for each taxa. The first row contains the header labels and each remaining row contains all the information for a single SNP. The first five individuals on the first seven SNPs from the tutorial data (mdp_genotype.hmp.txt) are presented below.

rs	alleles	chrom	pos	strand	assembly	center	protLSID	assayLSID	panel	QCcode	33-16	38-11	4226	4722	A188
PZB00859.1	A/C	1	157104	+	AGPv1	Panzea	NA	NA	maize282	NA	CC	CC	CC	CC	AA
PZA01271.1	C/G	1	1947984	+	AGPv1	Panzea	NA	NA	maize282	NA	CC	GG	CC	GG	CC
PZA03613.2	G/T	1	2914066	+	AGPv1	Panzea	NA	NA	maize282	NA	GG	GG	GG	GG	GG
PZA03613.1	A/T	1	2914171	+	AGPv1	Panzea	NA	NA	maize282	NA	TT	TT	TT	TT	TT
PZA03614.2	A/G	1	2915078	+	AGPv1	Panzea	NA	NA	maize282	NA	GG	GG	GG	GG	GG
PZA03614.1	A/T	1	2915242	+	AGPv1	Panzea	NA	NA	maize282	NA	TT	TT	TT	TT	TT
PZA00258.3	C/G	1	2973508	+	AGPv1	Panzea	NA	NA	maize282	NA	GG	CC	CC	CG	CC
PZA02962.13	A/T	1	3205252	+	AGPv1	Panzea	NA	NA	maize282	NA	TT	TT	TT	TT	TT
PZA02962.14	C/G	1	3205262	+	AGPv1	Panzea	NA	NA	maize282	NA	CC	CC	CC	CC	CC
PZA00599.25	C/T	1	3206090	+	AGPv1	Panzea	NA	NA	maize282	NA	CC	TT	CC	TT	TT

This file is read into R by typing the following command line:

```
myG <- read.table("mdp_genotype_test.hmp.txt", head = FALSE)
```

Although all of the first 11 columns are required, GAPIT uses only 3 of these: the “rs” column, which is the SNP name (e.g. “PZB00859.1”); the “chrom” column, which is the SNP’s chromosome; and the “pos”, which is the SNP’s base pair (bp) position. It is sufficient to fill in the requested information in the remaining eight columns with “NA”s. To be consistent with HapMap naming conventions, missing genotypic data are indicated by either “NN” (double bit) or “N” (single bit).

For genotypic data in HapMap format, GAPIT accepts genotypes in either double bit or in the standard IUPAC code (single bit) as following:

Genotype	AA	CC	GG	TT	AG	CT	CG	AT	GT	AC
Code	A	C	G	T	R	Y	S	W	K	M

By default, the HapMap numericalization is performed so that the sign of the allelic effect estimate (in the GAPIT output) is with respect to the nucleotide that is second in alphabetical order. For example, if the nucleotides at a SNP are “A” and “T”, then a positive allelic effect indicates that “T” is favorable. Selecting “Major.allele.zero = TRUE” in the GAPIT() function will result in the sign of the allelic effect estimate being with respect to the minor allele. In this scenario, a positive allelic effect estimate will indicate that the minor allele is favorable.

2.2.2 Numeric format

GAPIT also accepts the numeric format used by EMMA. Columns are used for SNPs and rows are used for taxa. This format is problematic in Excel because the number of SNPs used in a typical analysis exceeds the Excel column limit. Additionally, this format does not contain the chromosome and position (physical or genetic) of the SNPs. Therefore, two separate files must be provided to GAPIT. One file contains the numeric genotypic data (called the “GD” file), and the other contains the position of each SNP along the genome (called the “GM” file). *Note:* The SNPs in the “GD” and “GM” files NEED to be in the same order.

Homozygotes are denoted by “0” and “2” and heterozygotes are denoted by “1” in the “GD” file. Any numeric value between “0” and “2” can represent imputed SNP genotypes. The first row is a header file with SNP names, and the first column is the taxa name.

Example file (mdp_numeric.txt from tutorial data set):

taxa	PZB00859.1	PZA01271.1	PZA03613.2	PZA03613.1
33-16	2	0	0	2
38-11	2	2	0	2
4226	2	0	0	2
4722	2	2	0	2
A188	0	0	0	2
...				

This file is read into R by typing the following command line:

```
myGD <- read.table("mdp_numeric.txt", head = TRUE)
```

The “GM” file contains the name and location of each SNP. The first column is the SNP id, the second column is the chromosome, and the third column is the base pair position. As seen in the example, the first row is a header file.

Example file (mdp_SNP_information.txt from tutorial data set):

Name	Chromosome	Position
PZB00859.1	1	157104
PZA01271.1	1	1947984
PZA03613.2	1	2914066
PZA03613.1	1	2914171
PZA03614.2	1	2915078
...		

This file is read into R by typing the following command line:

```
myGM <- read.table("mdp_SNP_information.txt", head = TRUE)
```

2.3 Kinship

The kinship matrix file (called “KI” in GAPIT) is formatted as an n by n+1 matrix where the first column is the taxa name, and the rest is a square symmetric matrix. Unlike the other input data files, the first row of the kinship matrix file does not consist of headers.

Example (KSN.txt from tutorial data set):

33-16	2	0.228837	0.229322	0.268842	0.237145	0.0781	0.347107
38-11	0.228837	2	0.244965	0.293708	0.175211	0.079276	0.295606
4226	0.229322	0.244965	2	0.214859	0.236153	0.082693	0.283713
4722	0.268842	0.293708	0.214859	2	0.25935	0.061573	0.160104
A188	0.237145	0.175211	0.236153	0.25935	2	0.061469	0.232799
A214N	0.0781	0.079276	0.082693	0.061573	0.061469	2	0.110364
A239	0.347107	0.295606	0.283713	0.160104	0.232799	0.110364	2

This file is read into R by typing the following command line:

```
myKI <- read.table("KSN.txt", head = FALSE)
```

2.4 Covariate variables

A file containing covariates (called “CV” in GAPIT) can include information such as population structure (commonly called the “Q matrix”), which are fitted into the GWAS and GS models as fixed effects. These files are formatted similarly to the phenotypic files presented in Section 2.1. Specifically, the first column consists of taxa names, and the remaining columns contain covariate values. The first row consists of column labels. The first column can be labeled “Taxa”, and the remaining columns should be covariate names.

Example file (mdp_population_structure.txt from tutorial data set):

Taxa	Q1	Q2	Q3
33-16	0.014	0.972	0.014
38-11	0.003	0.993	0.004
4226	0.071	0.917	0.012
4722	0.035	0.854	0.111
A188	0.013	0.982	0.005
A214N	0.762	0.017	0.221
A239	0.035	0.963	0.002
A272	0.019	0.122	0.859
A441-5	0.005	0.531	0.464

This file is read into R by typing the following command line:

```
myCV <- read.table("mdp_population_structure.txt", head = TRUE)
```

2.5 Import Genotype by File Names

Genotype data can be too large that it does not fit memory requirement. It can also be saved as multiple files, such as each from a chromosome. GAPIT is capable to import genotype by their file names. The file names must be named sequentially (e.g., “mdp_genotype_chr1.hmp.txt”, “mdp_genotype_chr2.hmp.txt”, ...). For the Hapmap format, the common file name (e.g. “mdp_genotype_chr”), file name extension (e.g. “hmp.txt”) are passed to GAPIT through the “file.G”, “file.Ext.G”. The starting file and the ending file are specified by file.from and file.to parameters. When the file is not in the working directory, the file path can be passed to GAPIT through file.path parameter.

For numeric format, the common name and extension of genotype data file are passed to GAPIT through “file.GD” and “file.Ext.GD” parameters, respectively. Similarly, the common name and extension of genotype map file are passed to GAPIT through the “file.GM” and “file.Ext.GM” parameters, respectively.

2.6 Other GAPIT Input Parameters

In addition to the input parameters defined in previous sections, GAPIT has more parameters to define compression, PCA, GWAS and genomic prediction/selection (GPS; see Gallery of GAPIT Input Parameters).

Gallery of GAPIT input parameters

Parameter	Default	Options	Description
Y	NULL	User	Phenotype
KI	NULL	User	Kinship Matrix
CV	NULL	User	Covariate Variables
G	NULL	User	Genotype Data in Hapmap Format
GD	NULL	User	Genotype Data in Numeric Format
GM	NULL	User	Genotype Map for Numeric Format
file.Ext.G	NULL	User	File Extension for Genotype in Hapmap Format
file.Ext.GD	NULL	User	File Extension for Genotype Data in Numeric Format
file.Ext.GM	NULL	User	File Extension for Genotype Map for Numeric Format
file.fragment	NULL	User	The Fragment Size to Read Each Time within a File
file.G	NULL	User	The Common Name of File for Genotype in Hapmap Format
file.GD	NULL	User	The Common Name of File for Genotype Map for Numeric Format
file.GM	NULL	User	The Common Name of File for Genotype Data in Numeric Format
file.path	NULL	User	Path for Genotype Files
file.from	0	>0	The First Genotype Files Named Sequentially
file.to	0	>0	The Last Genotype Files Named Sequentially
group.by	10	>0	The Grouping Interval of Compression
group.from	1	>1	The Starting Number of Groups of Compression
group.to	10000000	>1	The Ending Number of Groups of Compression
kinship.algorithm	VanRaden	Loiselle and EMMA	Algorithm to Derive Kinship from Genotype
kinship.cluster	average	complete, ward, single, mcquitty, median, and centroid	Clustering algorithm to group individuals based on their kinship
kinship.group	Mean	Max, Min, and Median	Method to derive kinship among groups
LD.chromosome	NULL	User	Chromosome for LD analysis
LD.location	NULL	User	Location (center) of SNPs for LD analysis
LD.range	NULL	User	Range around the Central Location of SNPs for LD Analysis
PCA.total	0	>0	Total Number of PCs as Covariates
PCA.scaling	None	Scaled, Centered.and.scaled	Scale And/Or Center And Scale The SNPs Before Conducting PCA
SNP.FDR	1	>0 and <1	Threshold to Filter SNP on FDR
SNP.MAF	0	>0 and <1	Minor Allele Frequency to Filter SNPs in GWAS Reports
SNP.effect	Add	Dom	Genetic Model
SNP.P3D	TRUE	FALSE	Logic Variable to Use P3D or Not for Testing SNPs
SNP.fraction	1	>0 and <1	Fraction of SNPs Sampled to Estimate Kinship and PCs
SNP.test	TRUE	FALSE	Logic Variable to Test SNPs or Not

3 Analysis

GAPIT is designed to accurately perform GWAS and genomic prediction on large data sets. It accomplishes this through implementation of state-of-the-art statistical methods, including CMLM¹, P3D¹ or EMMAX¹⁰, and genomic prediction through CMLM. This section illustrates the options for using these methods.

3.1 Compressed Mixed Linear Model

A mixed linear model (MLM) includes both fixed and random effects. Including individuals as random effects gives an MLM the ability to incorporate information about relationships among individuals. This information about relationships is conveyed through the kinship (K) matrix, which is used in an MLM as the variance-covariance matrix between the individuals. When a genetic marker-based kinship matrix (K) is used jointly with population structure (commonly called the “Q” matrix, and can be obtained through STRUCTURE¹¹ or conducting a principal component analysis¹²), the “Q+K” approach improves statistical power compared to “Q” only⁹. An MLM can be described using Henderson’s matrix notation⁶ as follows:

$$\mathbf{Y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{e}, \quad (1)$$

where \mathbf{Y} is the vector of observed phenotypes; $\boldsymbol{\beta}$ is an unknown vector containing fixed effects, including the genetic marker, population structure (Q), and the intercept; \mathbf{u} is an unknown vector of random additive genetic effects from multiple background QTL for individuals/lines; \mathbf{X} and \mathbf{Z} are the known design matrices; and \mathbf{e} is the unobserved vector of residuals. The \mathbf{u} and \mathbf{e} vectors are assumed to be normally distributed with a null mean and a variance of:

$$\text{Var} \begin{pmatrix} \mathbf{u} \\ \mathbf{e} \end{pmatrix} = \begin{pmatrix} \mathbf{G} & \mathbf{0} \\ \mathbf{0} & \mathbf{R} \end{pmatrix} \quad (2)$$

where $\mathbf{G} = \sigma_a^2 \mathbf{K}$ with σ_a^2 as the additive genetic variance and \mathbf{K} as the kinship matrix. Homogeneous variance is assumed for the residual effect; i.e., $\mathbf{R} = \sigma_e^2 \mathbf{I}$, where σ_e^2 is the residual variance. The proportion of the total variance explained by the genetic variance is defined as heritability (h^2).

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2}, \quad (3)$$

We use cluster analysis to assign similar individuals into groups. The elements of the kinship matrix are used as similarity measures in the clustering analysis. Various linkage criteria (e.g., unweighted pair group method with arithmetic mean, UPGMA) can be used to group the lines together. The number of groups is specified by the user. Once the lines are assigned into groups, summary statistics of the kinship between and within groups are used as the elements of a reduced kinship matrix. This procedure is used to create a reduced kinship matrix for each compression level.

A series of mixed models are fitted to determine the optimal compression level. The value of the log likelihood function is obtained for each model, and the optimal compression level is defined as the one whose fitted mixed model yields the largest log likelihood function value. There are three parameters to determine the range and interval of groups for examination: `group.from`, `group.to` and `group.by`. Their defaults are 0, n and 10, where n is the total number of individuals.

3.2 Regular mixed Linear Model

Regular MLM is an extreme case of CMLM where each individual is considered as a group. It can be simply performed by setting the number of groups equal to the total number of individuals, e.g. `group.from = n` and `group.to = n`, where n is total number of individuals shared in both the genotype and phenotype files.

3.3 P3D/EMMAx

In addition to implementing compression, GAPIT can conduct EMMAx/P3D^{1,10}. If specified, the additive genetic (σ_a^2) and residual (σ_e^2) variance components will be estimated prior to conducting GWAS. These estimates are then used for each SNP where a mixed model is fitted.

3.4 Genomic Prediction

Genomic prediction is performed with the method based on genomic best linear unbiased prediction, (gBLUP)³. The method was extended to compressed best linear unbiased prediction (cBLUP) by using the CMLM approach that was proposed for GWAS¹. The genetic potential for a group, which is derived from the BLUPs of group effects in the compressed mixed model, is used as a prediction for all individuals in the group.

The groups created from compression belong to either a reference (R) or an inference (I) panel. All groups in the reference panel have at least one individual with phenotypic data, and all groups in the inference panel have no individuals with phenotypic data. Genomic prediction for groups in the inference panel is based on phenotypic ties with corresponding groups in the reference panel.

The group kinship matrix is then partitioned into R and I groups as follows:

$$\mathbf{K} = \begin{bmatrix} \mathbf{k}_{RR} & \mathbf{k}_{RI} \\ \mathbf{k}_{IR} & \mathbf{k}_{II} \end{bmatrix}, \quad (4)$$

where \mathbf{k}_{RR} is the variance-covariance matrix for all groups in the reference panel, \mathbf{k}_{RI} is the covariance matrix between the groups in the reference and inference panels, $\mathbf{k}_{IR} = (\mathbf{k}_{RI})'$ is the covariance matrix between the groups inference and reference panels, and \mathbf{k}_{II} is the variance-covariance matrix between the groups in the inference panels.

Solving of mixed linear model is performed on the reference individuals.

$$\mathbf{y}_R = \mathbf{X}_R \boldsymbol{\beta} + \mathbf{Z}_R \mathbf{u}_R + \mathbf{e}_R, \quad (5)$$

where all terms are as defined in Equation (1), and the “R” subscript denotes that only individuals in the reference panel are considered.

The genomic prediction of the inference groups is derived Henderson's formula (1984) as follows:

$$\mathbf{u}_I = \mathbf{K}_{IR} \mathbf{K}_{RR}^{-1} \mathbf{u}_R, \quad (6)$$

where \mathbf{k}_{IR} , \mathbf{k}_{RR} , and \mathbf{u}_R are as previously defined, and \mathbf{u}_I is the predicted genomic values of the individuals in the inference group.

The reliability of genomic prediction is calculated as follows:

$$\text{Reliability} = 1 - \frac{\text{PEV}}{\sigma_a^2} \quad (7)$$

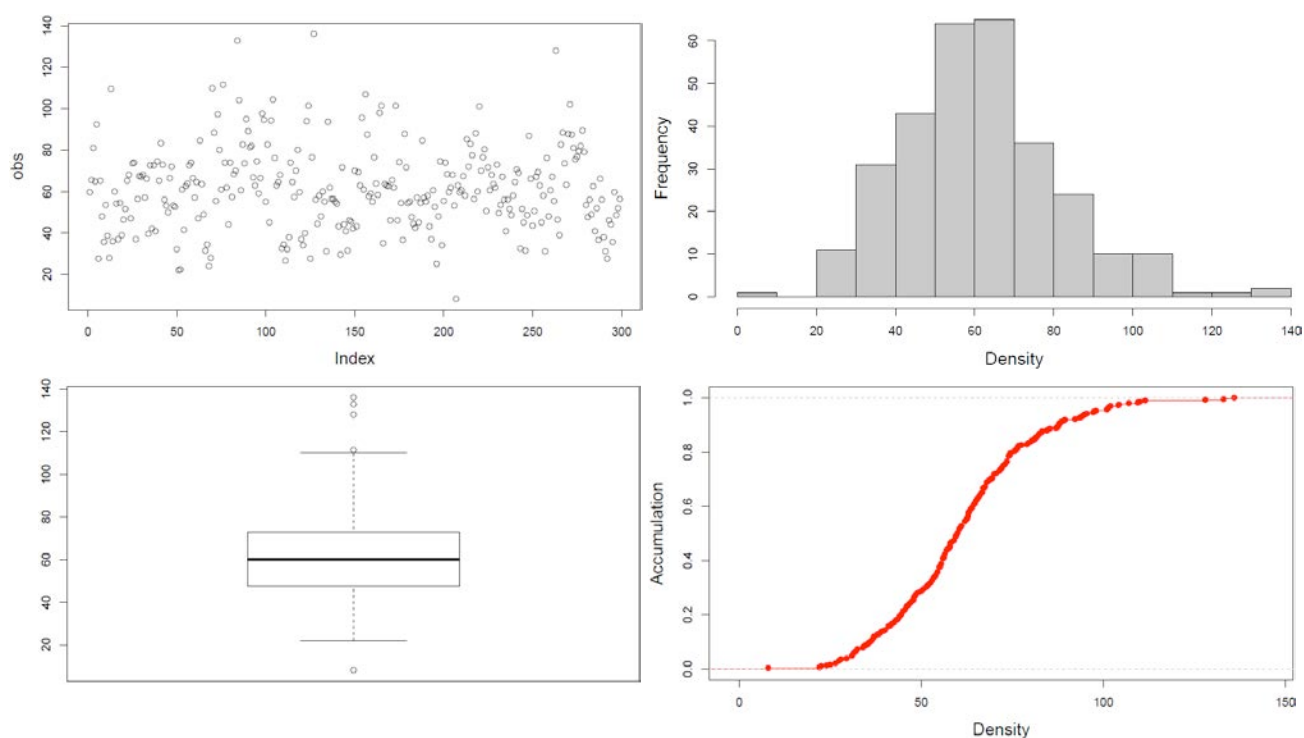
where PEV is the prediction error variance which is the diagonal element in the inverse left-hand side of the mixed model equation, and σ_a^2 is the genetic variance.

4 Results

GAPIT produces a series of output files that are saved in two formats. All tabular results are saved as comma separated value (.csv) files, and all graphs are stored as printable document format (.pdf) files. This section provides descriptions of these output files.

4.1 Phenotype diagnosis

GAPIT diagnosis phenotype in several ways, including scatter plot, histogram, box plot and accumulative distribution.



4.2 Marker density

Marker density is critical to establish Linkage Disequilibrium (LD) between markers and causal mutations. Comparison between the marker density and the LD decay over distance provides the indication if markers are dense enough to have good coverage of LD.

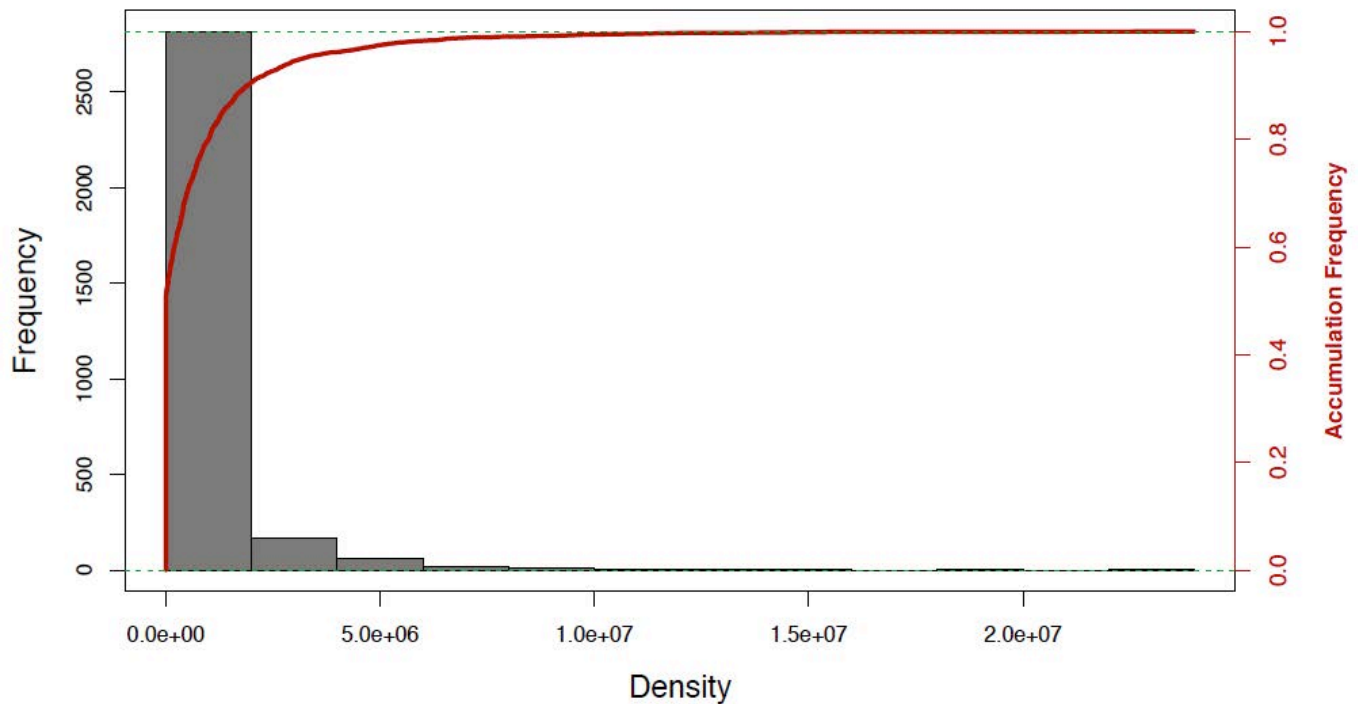


Figure 4.2.1 Frequency and accumulative frequency of marker density. Distribution of marker density is displayed as a histogram and an accumulative distribution.

4.3 Linkage Disequilibrium Decade

Linkage disequilibrium are measured as R square for pair wise markers and plotted against their distance. The moving average of adjacent markers were calculated by using a sliding windows with ten markers.

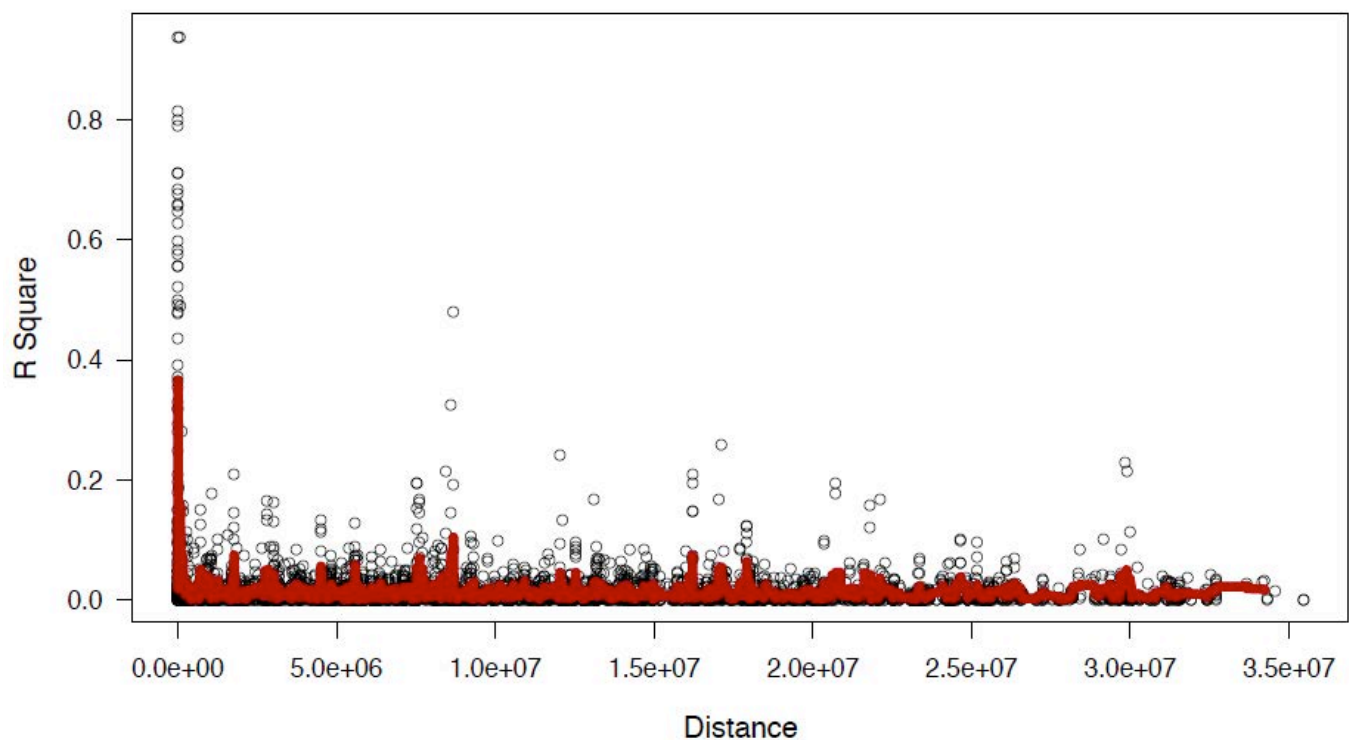
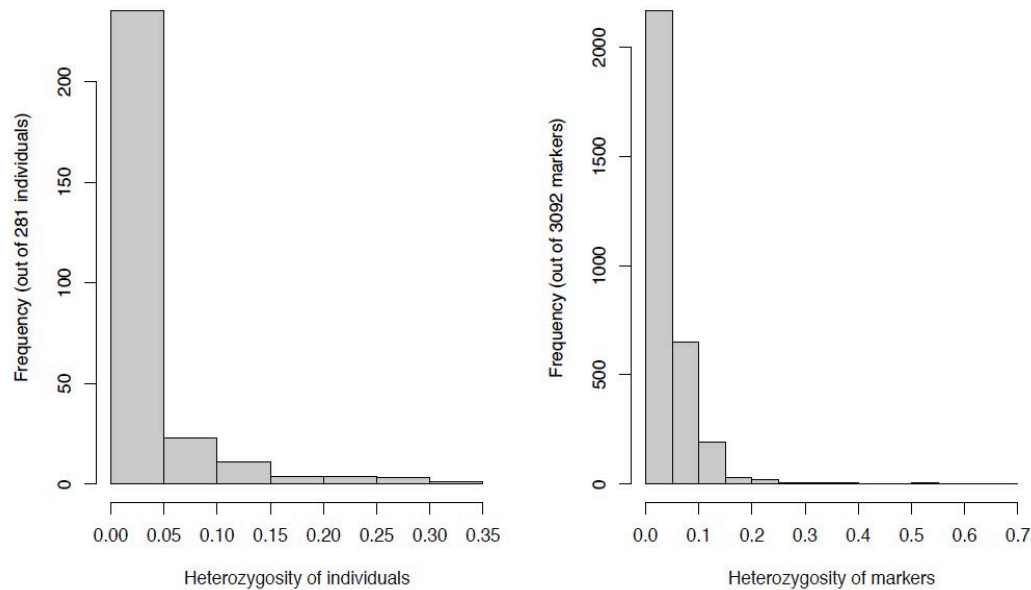


Figure 4.3.1 Linkage disequilibrium (LD) decay over distance. LDs were calculated on sliding windows with 100 adjacent genetic markers. Each dot represents a pair of distances between two markers on the window and their squared correlation coefficient. The red line is the moving average of the 10 adjacent markers.

4.4 Heterozygosity

The frequency of heterozygous were calculated for both individuals and markers. High level of heterozygosity indicated low quality. For example, over 50% of heterozygosity on inbred lines for some of markers suggested they problematic (see bottom right).



4.5 Principal Component (PC) plot

For each PC included in the GWAS and GPS models, the observed PC values are plotted.

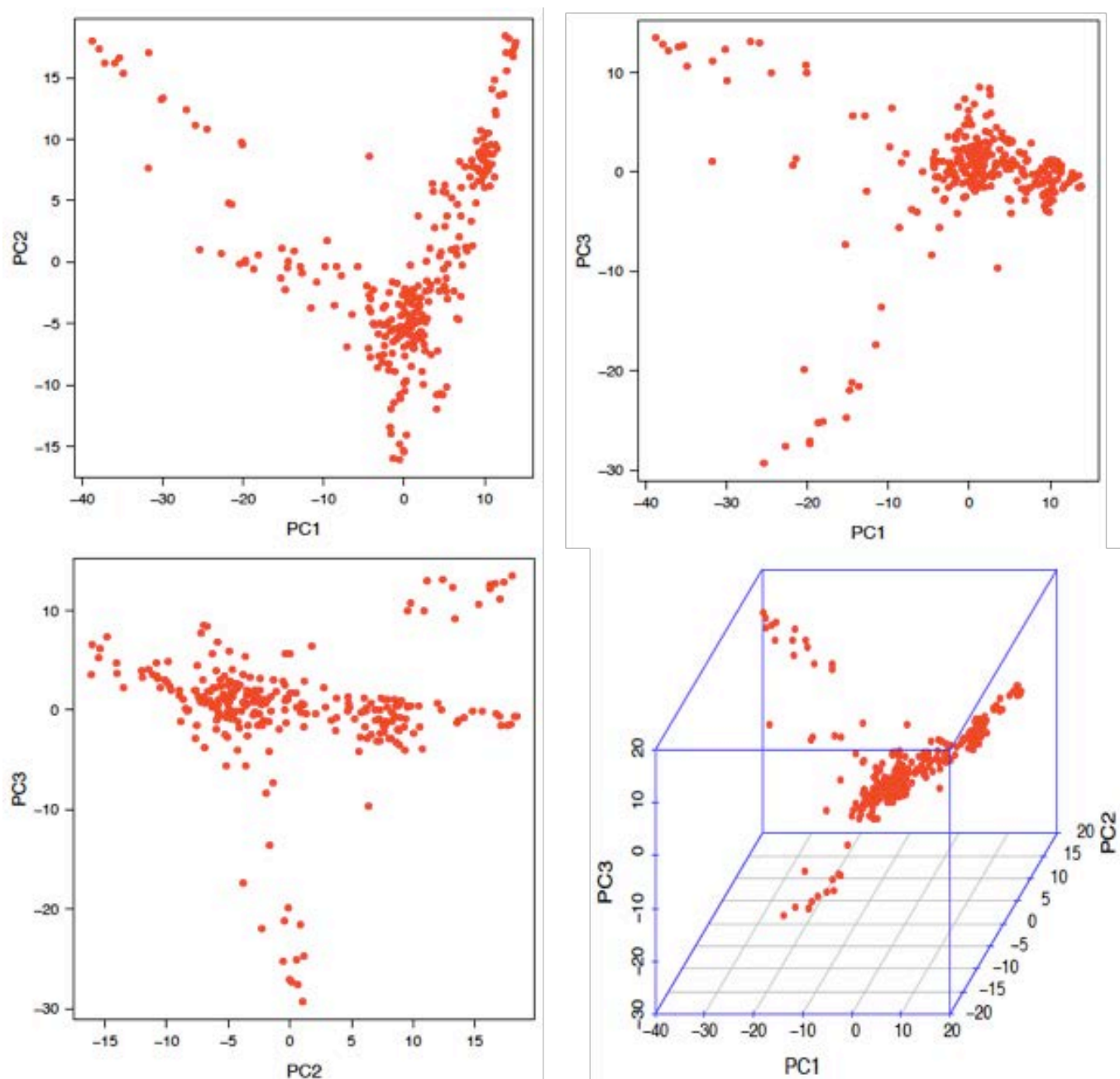


Figure 4.5.1 Pair-wise plots and 3D plots of principal component (PC).

4.6 Kinship plot

The kinship matrix used in GWAS and GPS is visualized through a heat map. To reduce computational burden, this graph is not made when the sample size exceeds 1,000.

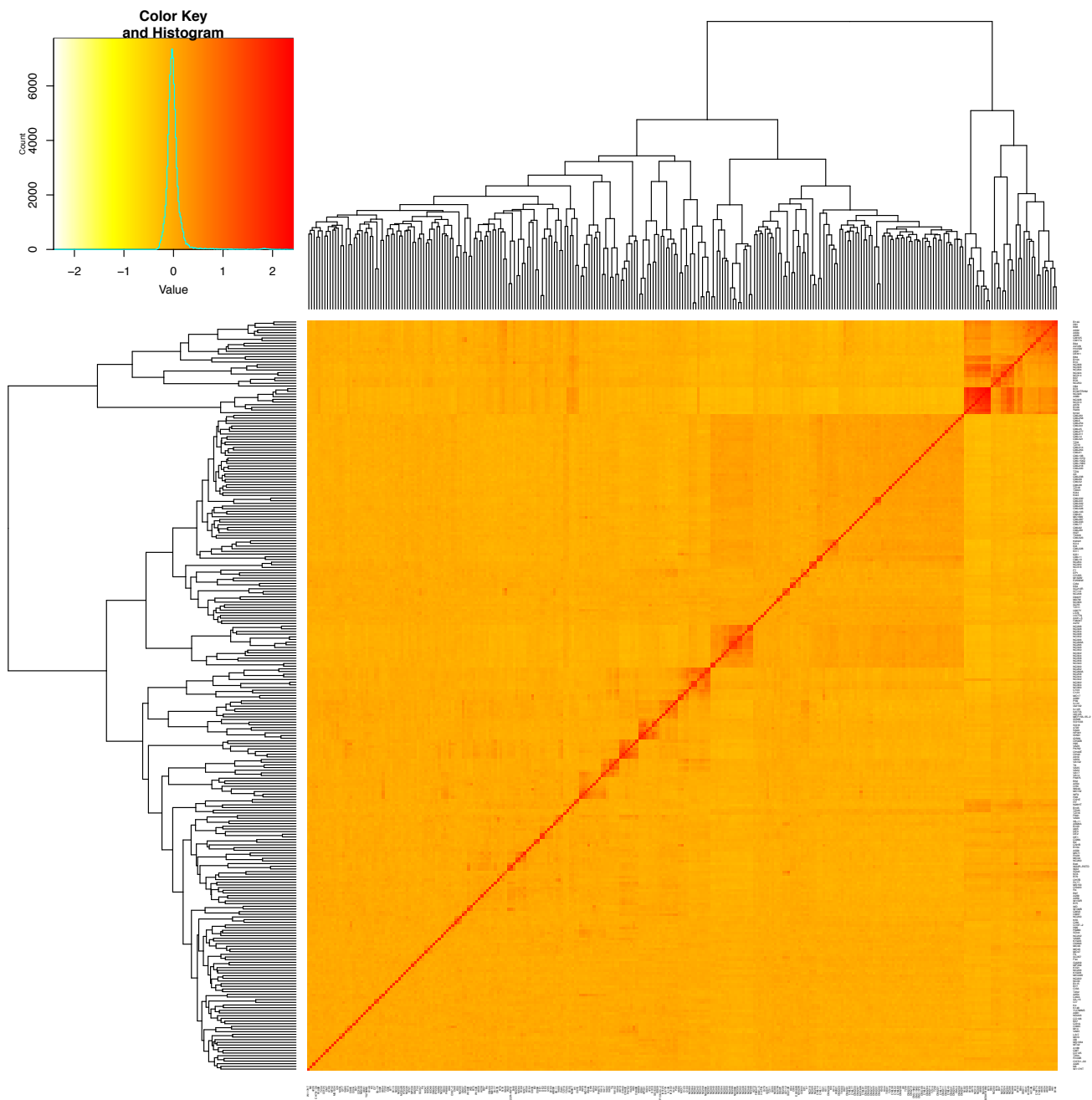


Figure 4.6.1 Kinship plot. A heat map of the values in the values in the kinship matrix is created.

4.7 QQ-plot

The quantile-quantile (QQ) –plot is a useful tool for assessing how well the model used in GWAS accounts for population structure and familial relatedness. In this plot, the negative logarithms of the P -values from the models fitted in GWAS are plotted against their expected value under the null hypothesis of no association with the trait. Because most of the SNPs tested are probably not associated with the trait, the majority of the points in the QQ-plot should lie on the diagonal line. Deviations from this line suggest the presence of spurious associations due to population structure and familial relatedness, and that the GWAS model does not sufficiently account for these spurious associations. It is expected that the SNPs on the upper right section of the graph deviate from the diagonal. These SNPs are most likely associated with the trait under study. By default, the QQ-plots in GAPIT show only a subset of the larger P -values (i.e., less significant P -values) to reduce the file size of the graph.

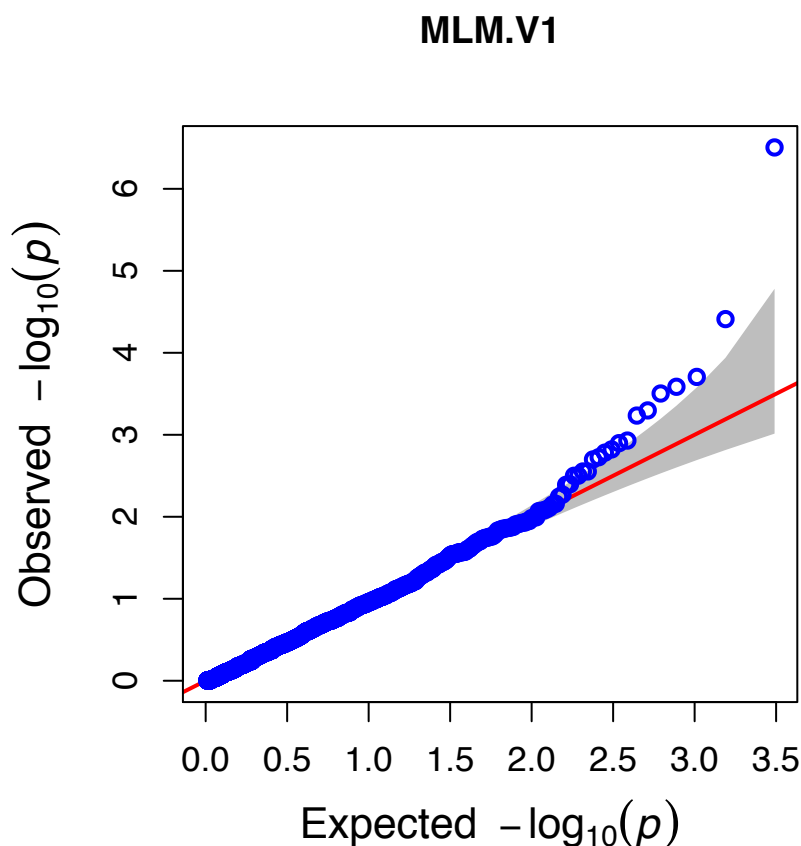


Figure 4.7.1 Quantile-quantile (QQ) –plot of P -values. The Y-axis is the observed negative base 10 logarithm of the P -values, and the X-axis is the expected observed negative base 10 logarithm of the P -values under the assumption that the P -values follow a $\text{uniform}[0,1]$ distribution. The dotted lines show the 95% confidence interval for the QQ-plot under the null hypothesis of no association between the SNP and the trait.

4.8 Manhattan Plot

The Manhattan plot is a scatter plot that summarizes GWAS results. The X-axis is the genomic position of each SNP, and the Y-axis is the negative logarithm of the P -value obtained from the GWAS model (specifically from the F -test for testing H_0 : No association between the SNP and trait). Large peaks in the Manhattan plot (i.e., “skyscrapers”) suggest that the surrounding genomic region has a strong association with the trait. GAPIT produces one Manhattan plot for the entire genome (Figure 3.4) and individual Manhattan plots for each chromosome.

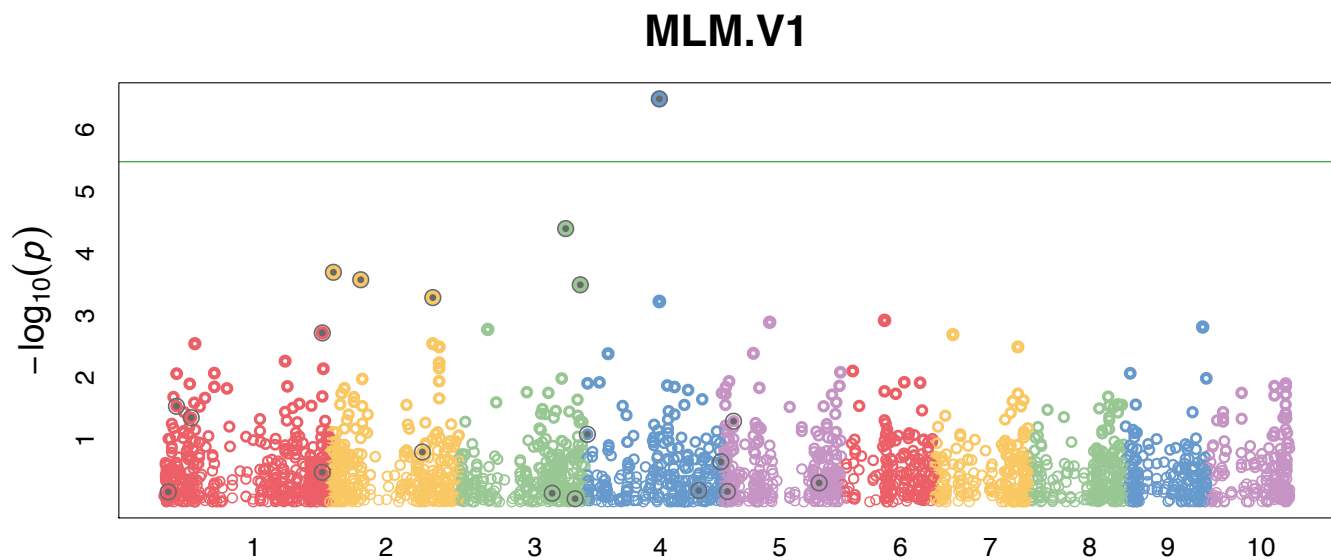


Figure 4.8.1 Manhattan plot. The X-axis is the genomic position of the SNPs in the genome, and the Y-axis is the negative log base 10 of the P -values. Each chromosome is colored differently. SNPs with stronger associations with the trait will have a larger Y-coordinate value.

4.9 Association Table

The GWAS result table provides a detailed summary of appropriate GWAS results. The rows display the results for each SNP above the user-specified minor allele frequency threshold. The SNPs sorted by their P values (from smallest to largest).

Table 4.9.1 GWAS results for all SNPs that were analyzed.

SNP	Chromosome	Position	P.value	maf	nobs	Rsquare.of.Model.without.SNP	Rsquare.of.Model.with.SNP	FDR_Adjusted_P-values
Fea2.4	4	132736424	3.13E-07	0.290035587	281	0.079004463	0.170450593	0.000966617
PZB01223.3	3	192865132	3.88E-05	0.346975089	281	0.079004463	0.137165003	0.059980668
PZA03748.1	2	7481079	0.000196769	0.195729537	281	0.079004463	0.126357611	0.193907122
PZA00394.11	2	56930271	0.00025959	0.145907473	281	0.079004463	0.124537393	0.193907122
PZA00219.6	3	219309117	0.000313461	0.379003559	281	0.079004463	0.123302809	0.193907122

This table provides the SNP id, chromosome, bp position, P -value, minor allele frequency (maf), sample size (nobs), R^2 of the model without the SNP, R^2 of the model with the SNP, and adjusted P -value following a false discovery rate (FDR)-controlling procedure¹³.

4.10 Allelic Effects Table

A separate table showing allelic effect estimates is also included in the suite of GAPIT output files. The SNPs, presented in the rows, are sorted by their position in the genome.

Table 4.10.1 Information of associated SNPs.

SNP	Chromosome	Position	DF	t Value	std Error	effect
PZB00859.1	1	157104	276	0.255638527	0.501027645	0.128081969
PZA01271.1	1	1947984	276	-0.205390736	0.451124818	-0.092656858
PZA03613.2	1	2914066	276	-1.143780776	0.4887135	-0.558981106
PZA03613.1	1	2914171	276	1.194922452	0.540019499	0.645281424
PZA03614.2	1	2915078	276	0.277681398	0.489962989	0.136053608

This table provides the SNP id, chromosome, bp position, and the allelic effect estimate of each SNP analyzed.

4.11 Compression Profile

There are seven algorithms available to cluster individuals into groups for the compressed mixed linear model. There are also four summary statistics available for calculating the group kinship matrix. When only one group number (i.e., one dimension for the group kinship matrix) is specified, a column chart is created to illustrate the compression profile for $2 \times \log$ likelihood function (the smaller the better), genetic variance, residual variance and the estimated heritability.

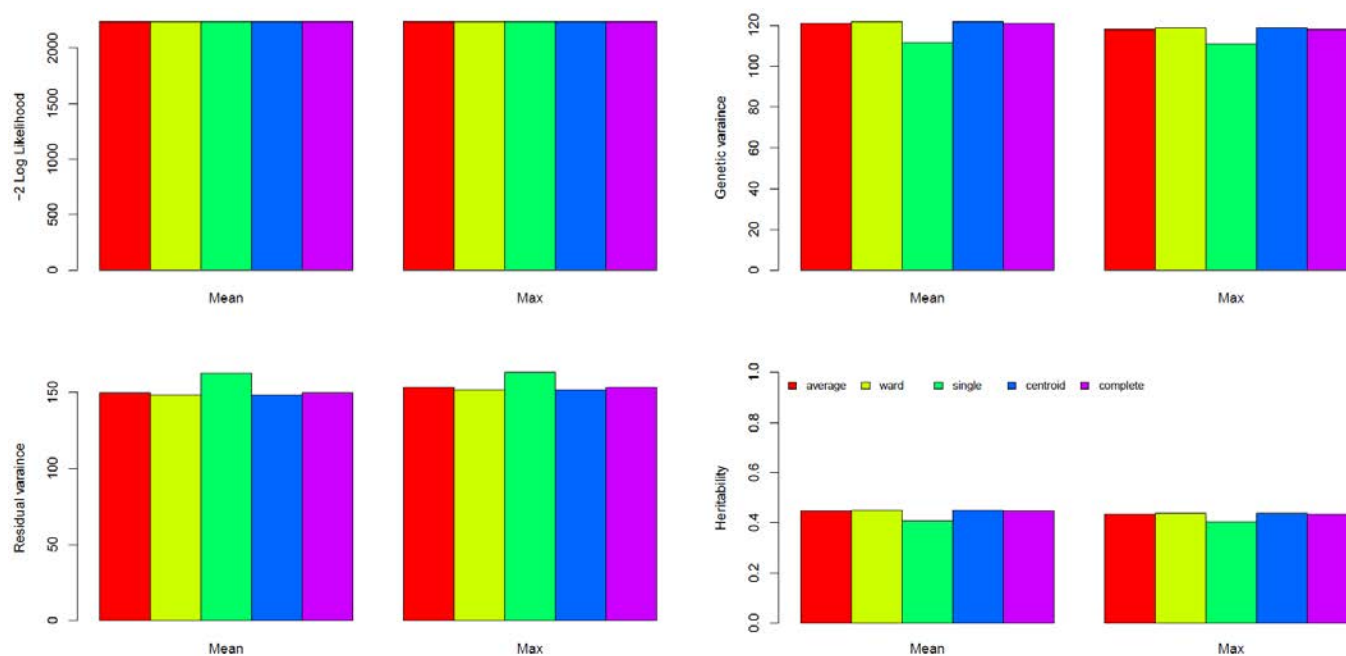


Figure 4.11.1. Compression profile with single group. The X-axis on all graphs display the summary statistic method used to obtain the group kinship matrix. The rectangles with different colors indicate the clustering algorithm used to group individuals.

Note: This graph is not created when multiple groups are specified.

When a range of groups (i.e., a range of dimensions for the group kinship matrix) is specified, a different series of graphs are created. In this situation, the X-axis displays the group number. Lines with different style and colors are used to present the combinations between clustering algorithm and the algorithm to calculate kinship among groups.

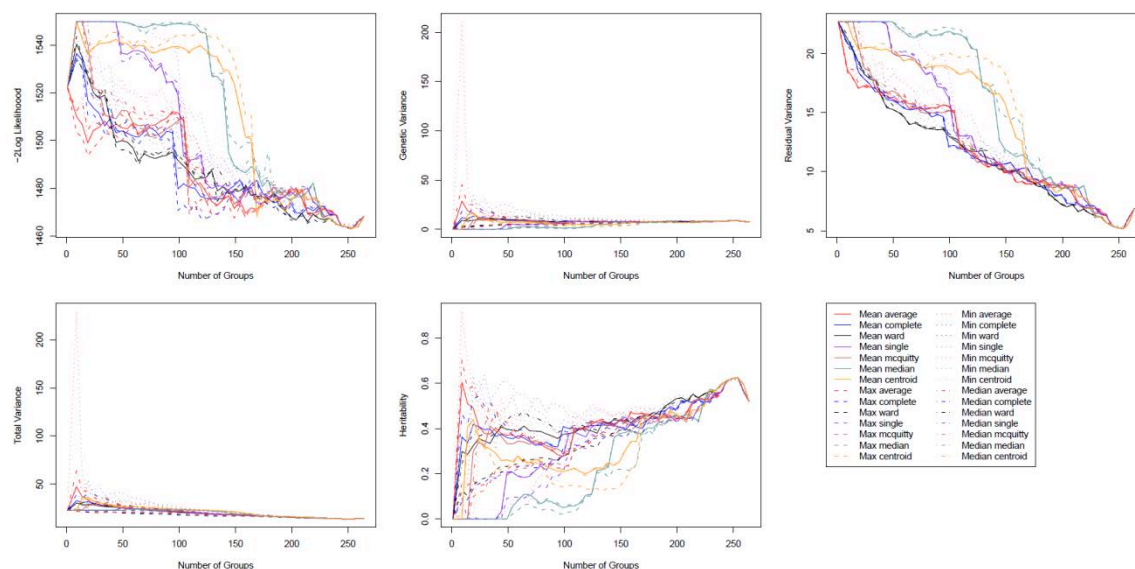


Figure 4.11.2. Compression profile over multiple groups. The X-axis on each graph is the number of groups considered, and the Y-axes on the graphs are the $-2 \times \log$ likelihood function, the estimated genetic variance components, the estimated residual variance component, the estimated total variance, and the heritability estimate. Each clustering method and group kinship type is represented as a line on each graph.

Notice: This graph is not created when only one group is specified.

4.12 The Optimum Compression

Once the optimal compression settings are determined, GAPIT produces a PDF file containing relevant detailed information. This information includes the optimal algorithm to calculate the group kinship matrix, the optimal clustering algorithm, the optimal number of groups, $2 \times \log$ likelihood function and the estimated heritability.

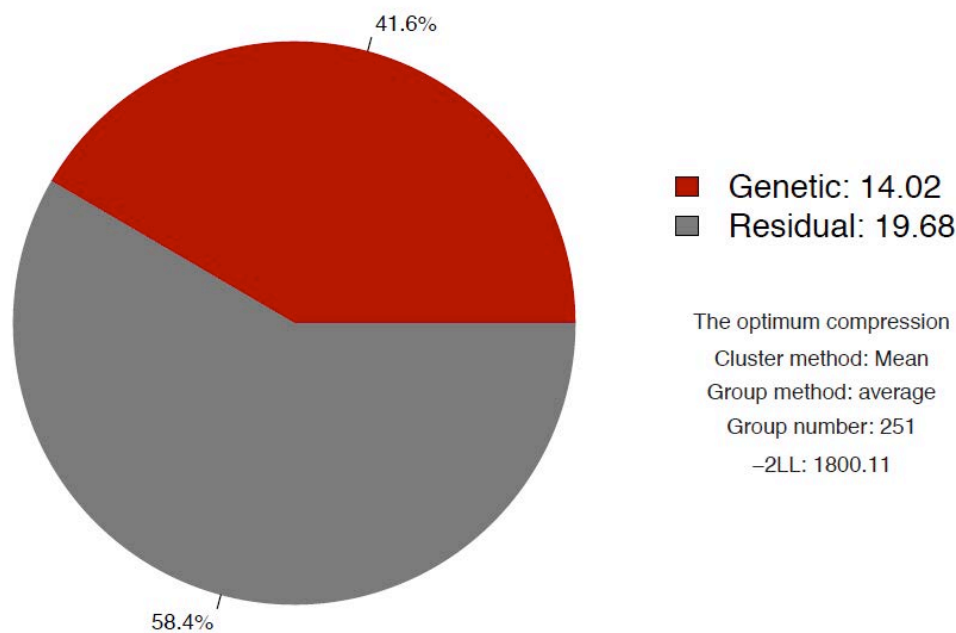


Figure 4.12.1 The profile for the optimum compression. The optimal method to calculate group kinship is “Max”, the optimal clustering method is “average”, the number of groups (ie., the dimension of the group kinship matrix) is 251, the value of $-2 \times \log$ likelihood function is 1800.11, and the heritability is 41.6%.

4.13 Model Selection Results

By selecting “Model.selection = TRUE”, forward model selection using the Bayesian information criterion (BIC) will be conducted to determine the optimal number of PCs/Covariates to include for each phenotype. The results summary (below) for model selection are stored in a .csv file called “.BIC.Model.Selection.Results”.

Table 4.13.1 Summary for Bayesian information criterion (BIC) model selection results.

Number of PCs/Covariates	BIC (larger is better) - Schwarz 1978	log Likelihood Function Value
0	-816.3884646	-807.9578633
1	-810.4406992	-799.1998974
2	-796.269878	-782.2188759
3	-798.1539656	-781.292763

The number of PCs/Covariates, the BIC value, and the log Likelihood function value are presented. In this table, the optimal number of PCs to include in the GWAS model is 2.

4.14 Genomic Prediction

The genomic prediction results are saved in a .csv file.

Table 4.14.1 Genomic Breeding values and prediction error variance.

Taxa	Group	RefInf	ID	BLUP	PEV	BLUE	Prediction
33-16	1	1	1	4.61889306	9.86652431	-9.2195121	-4.600619
38-11	2	1	2	4.81979176	10.1420079	-8.8888307	-4.069039
4226	3	1	3	-0.8609631	11.3324656	-8.8692075	-9.7301705
4722	4	1	4	5.68756264	9.29840109	-9.1363798	-3.4488171
A188	5	1	5	-4.404809	11.3810847	-9.1053928	-13.510202
A214N	6	1	6	0.15659848	17.6333752	-8.1102155	-7.9536171
A239	7	1	7	5.12523349	10.8215374	-8.9566662	-3.8314327
A272	8	1	8	-0.4981601	11.08823	-9.6011763	-10.099336
A441-5	9	1	9	-2.1785473	11.2196816	-9.7514142	-11.929961
A554	10	1	10	-4.2207138	10.4013176	-8.9041479	-13.124862
A556	11	1	11	3.39391482	11.1632936	-9.0252992	-5.6313843
A6	12	1	12	3.32945838	13.176845	-10.578872	-7.2494132
A619	13	1	13	-1.224313	8.85099809	-8.6909708	-9.9152838
A632	14	1	14	-2.4776767	21.1823568	-7.5293175	-10.006994
A634	15	1	15	-2.2882799	19.9636905	-7.6763634	-9.9646433

The individual id (taxa), group, RefInf which indicates whether the individual is in the reference group (1) or not (2), the group ID number, the BLUP, and the PEV of the BLUP.

4.15 Distribution of BLUPs and their PEV.

A graph is provided to show the joint distribution of GBV and PEV. The correlation between them is an indicator of selection among the sampled individuals¹⁴.

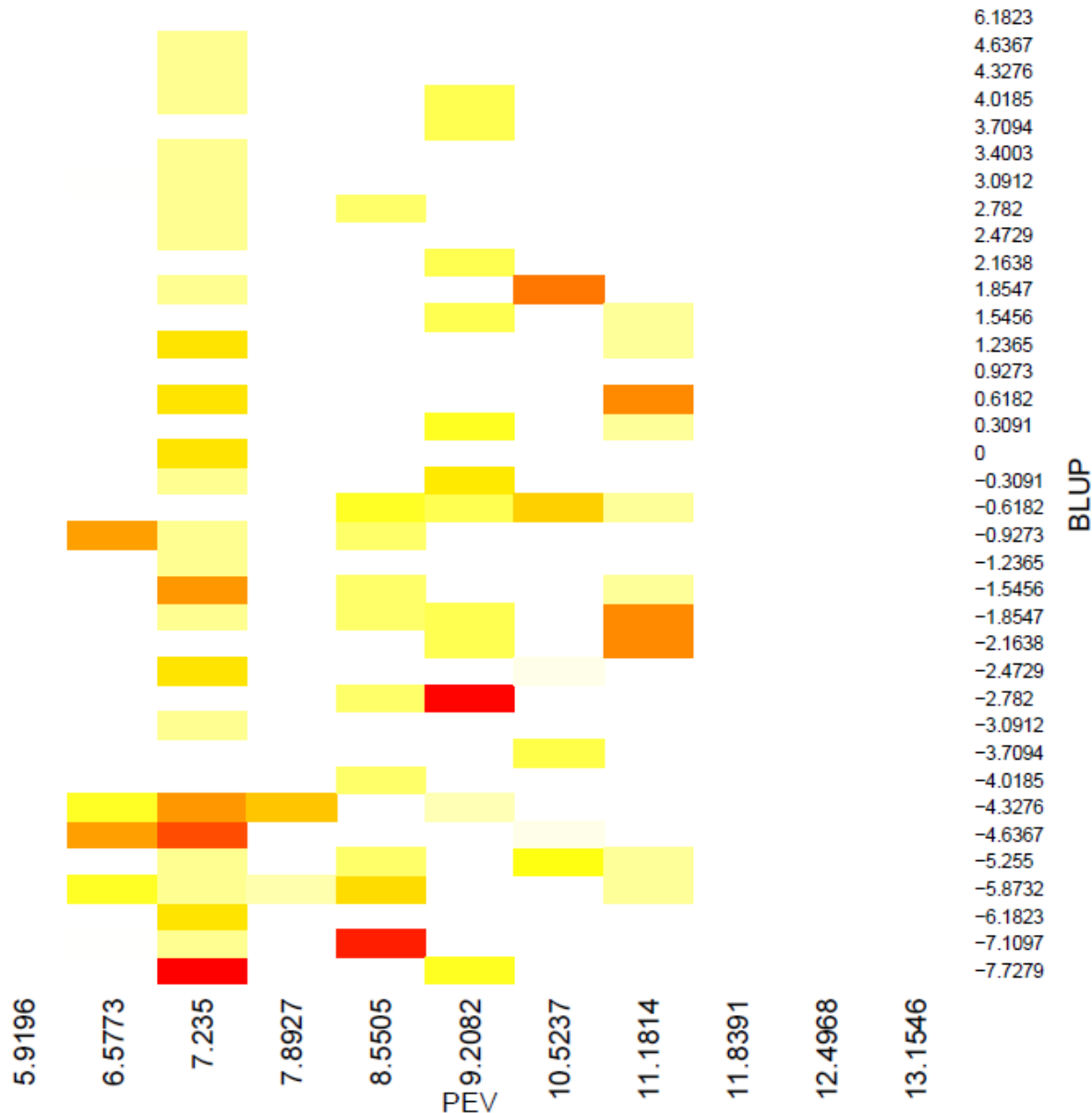


Figure 4.15.1 Joint distribution of genomic breeding value and prediction error variance.

5 Tutorials

The “Getting started” section should be reviewed before running these tutorials, and it is assumed that the GAPIT package and its required libraries have been installed. These tutorials begin with a scenario requiring minimal user input. Subsequent scenarios require a greater amount of user input. Each scenario involves two steps: reading in the data and then running the GAPIT() function. All tutorials are available on the GAPIT home page, which also contains the R source code and results for all the scenarios.

The GAPIT maize demonstration data (described at www.panzea.org) are from a maize association panel consisting of 281 diverse lines¹⁵. The genotypic data consist of 3,093 SNPs distributed across the maize genome, and are available in HapMap and numeric format. The three phenotypes included are ear height, days to pollination, and ear diameter. The kinship matrix was calculated using the method of Loiselle *et al.*¹⁶ and the fixed effects used to account for population structure were obtained from STRUCTURE¹⁷.

Notice: It is important that the correct paths to the directories are specified. Please note that two backward slashes (“\\”) are necessary when specifying these paths.

5.1 A Basic Scenario

The user needs to provide two data sets (phenotype and genotype) and one input parameter. This parameter, “PCA.total”, specifies the number of principal components (PCs) to include in the GWAS model. GAPIT will automatically calculate the kinship matrix using the VanRaden method¹⁸, perform GWAS and genomic prediction with the optimum compression level using the default clustering algorithm (average) and group kinship type (Mean). The scenario assumes that the genotype data are saved in a single file in HapMap format. If the working directory contains the tutorial data, the analysis can be performed by typing these command lines:

```
#Step 1: Set data directory and import files
myY <- read.table("mdp_traits.txt", head = TRUE)
myG <- read.table("mdp_genotype_test.hmp.txt", head = FALSE)

#Step 2: Run GAPIT
myGAPIT <- GAPIT(
  Y=myY,
  G=myG,
  PCA.total=3
)
```

5.2 Enhanced Compression

In this scenario, the user can specify additional clustering algorithms (controlled by the “kinship.cluster” parameter) and kinship summary statistic (controlled by the “kinship.group” parameter). The default is `kinship.cluster="average"`, and `kinship.group="Mean"`. Their expansion, the Enriched CMLM⁴ improves statistical power. Additionally, a specific range group numbers (i.e., dimension of the kinship matrix) can be specified. This range is controlled by the “group.from”, “group.to”, and “group.by” parameters. The analysis can be performed by typing these command lines:

```
#Step 1: Set data directory and import files
myY <- read.table("mdp_traits.txt", head = TRUE)
myG <- read.table("mdp_genotype_test.hmp.txt", head = FALSE)

#Step 2: Run GAPIT
myGAPIT <- GAPIT(
  Y=myY,
  G=myG,
  PCA.total=3,
  kinship.cluster=c("average", "complete", "ward"),
  kinship.group=c("Mean", "Max"),
  group.from=200,
  group.to=1000000,
  group.by=10
)
```

5.3 User-inputted Kinship Matrix and Covariates

This scenario assumes that the user provides a kinship matrix and covariate file. The kinship matrix or covariates (e.g., PCs) may be calculated previously or from third party software. When the PCs are input in this way, the parameter “PCA.total” should be set to 0 (default). Otherwise, PCs will be calculated within GAPIT, resulting in a singular design matrix in all model fitted for GWAS. The analysis can be performed by typing these command lines:

```
#Step 1: Set data directory and import files
myY <- read.table("mdp_traits.txt", head = TRUE)
myG <- read.table("mdp_genotype_test.hmp.txt", head = FALSE)
myKI <- read.table("KSN.txt", head = FALSE)
myCV <- read.table("Copy of Q_First_Three_Principal_Components.txt", head = TRUE)

#Step 2: Run GAPIT
myGAPIT <- GAPIT(
  Y=myY,
  G=myG,
  KI=myKI,
  CV=myCV
)
```

5.4 Genomic Prediction

Genomic prediction can be performed without running GWAS. Thus, GWAS can be turned off by using “SNP.test=FALSE” option. The analysis can be performed by typing these command lines:

```
#Step 1: Set data directory and import files
myY <- read.table("mdp_traits.txt", head = TRUE)
myKI <- read.table("KSN.txt", head = FALSE)

#Step 2: Run GAPIT
myGAPIT <- GAPIT(
  Y=myY,
  KI=myKI,
  PCA.total=3,
  SNP.test=FALSE
)
```

5.5 Multiple Genotype Files

In this scenario, the HapMap genotypic data set from Scenario 1 is subdivided into multiple genotype files, one for each chromosome. This scenario mimics the situation where the genotype file is too large to be handled in R. When this situation arises, all genotype files need to have a common name and extensions, as well as a sequential number (e.g., “mdp_genotype_chr1.hmp.txt”, “mdp_genotype_chr2.hmp.txt”, ...). The starting and ending file are indicated by the “file.from” and “file.to” parameters. The common file name (e.g., “mdp_genotype_chr”) and file name extension (e.g., “hmp.txt”) are passed to GAPIT through the “file.G”, “file.Ext.G” parameters, respectively. When “file.path” is not provided, GAPIT try to get the data from the current working directory. The analysis can be performed by typing these command lines:

```
#Step 1: Set data directory and import files
myY <- read.table("mdp_traits.txt", head = TRUE)

#Step 2: Run GAPIT
myGAPIT <- GAPIT(
  Y=myY,
  PCA.total=3,
  file.G="mdp_genotype_chr",
  file.Ext.G="hmp.txt",
  file.from=1,
  file.to=10,
  file.path="C:\\myGAPIT\\"
)
```

The three genotype file used in these scenario are from the file used in Tutorial 5.1. Their results should be identical.

5.6 Numeric Genotype Format

In this scenario, the genotype data set from Scenario 1 is formatted differently, specifically in numerical format. Two genotype files are required. One file contains the genotypic data, and the other contains the chromosome and base pair position of each SNP. These are passed to GAPIT through the “GD” and “GM” parameters, respectively. The analysis can be performed by typing these command lines:

```
#Step 1: Set data directory and import files
myY <- read.table("mdp_traits.txt", head = TRUE)
myGD <- read.table("mdp_numeric.txt", head = TRUE)
myGM <- read.table("mdp_SNP_information.txt", head = TRUE)

#Step 2: Run GAPIT
myGAPIT <- GAPIT(
  Y=myY,
  GD=myGD,
  GM=myGM,
  PCA.total=3
)
```

5.7 Numeric Genotype Format in Multiple Files

In this scenario, the numeric genotype data set from Scenario 6 is subdivided into multiple genotype files. The common name and extension of genotype data file are passed to GAPIT through “file.GD” and “file.Ext.GD” parameters, respectively. Similarly, the common name and extension of genotype map file are passed to GAPIT through the “file.GM” and “file.Ext.GM” parameters, respectively. The analysis can be performed by typing these command lines:

```
#Step 1: Set data directory and import files
myY <- read.table("mdp_traits.txt", head = TRUE)

#Step 2: Run GAPIT
myGAPIT <- GAPIT(
  Y=myY,
  PCA.total=3,
  file.GD="mdp_numeric",
  file.GM="mdp_SNP_information",
  file.Ext.GD="txt",
  file.Ext.GM="txt",
  file.from=1,
  file.to=3,
)
```

The three genotype file used in these scenario are the splits from the file used in the previous scenario. Their results should be identical.

5.8 Fractional SNPs for Kinship and PCs

The computations of kinship and PCs are extensive with large number of SNPs. Sampling a fraction of it would reduce computing time. More importantly, it would give very similar result with appropriate number of SNPs sampled. The fraction can be controlled by “Ratio” parameter in GAPIT. The sampling scheme is random. A line of “SNP.fraction=0.6” is added to the previous scenario which has 3,093 SNPs:

```
#Step 1: Set data directory and import files
myY <- read.table("mdp_traits.txt", head = TRUE)

#Step 2: Run GAPIT
myGAPIT <- GAPIT(
  Y=myY,
  PCA.total=3,
  file.GD="mdp_numeric",
  file.GM="mdp_SNP_information",
  file.Ext.GD="txt",
  file.Ext.GM="txt",
  file.from=1,
  file.to=3,
  SNP.fraction=0.6
)
```


5.9 Memory saving

With large amount of individuals, loading a entire large genotype dataset could be difficult. GAPIT load a fragment of it each time. The default of the fragment size is 512 SNPs. This number can be changed with “file.fragment” parameter in GAPIT. Here is an example of using “file.fragment = 128”.

```
#Step 1: Set data directory and import files
myY <- read.table("mdp_traits.txt", head = TRUE)

#Step 2: Run GAPIT
myGAPIT <- GAPIT(
  Y=myY,
  PCA.total=3,
  file.GD="mdp_numeric",
  file.GM="mdp_SNP_information",
  file.Ext.GD="txt",
  file.Ext.GM="txt",
  file.from=1,
  file.to=3,
  SNP.fraction=0.6,
  file.fragment = 128
)
```

This scenario is the same as previous scenario except changing “file.fragment” from default (512) to 128. As SNPs (minimune of two) are sampled withing each fragment, the final SNPs sampled would be different for different length of fragment when the SNP sample fraction is less than 100%. The results in this scenario would be different from the previous one.

5.10 Model selection

The degree of correlation with population structure varies from trait to trait. Therefore, the full set of PCs selected to account for population structure in the GWAS model are not necessary for all traits. As such, GAPIT has the capability to conduct Bayesian information criterion (BIC)-based model selection to find the optimal number of PCs for inclusion in the GWAS models. Model selection is activated by selecting “**Model.selection = TRUE**”. The results for the BIC model selection procedure are summarized in the “.BIC.Model.Selection.Results.csv” output file.

```
myY <- read.table("mdp_traits.txt", head = TRUE)
myG <- read.table("mdp_genotype_test.hmp.txt", head = FALSE)

#Step 2: Run GAPIT
myGAPIT <- GAPIT(
  Y=myY,
  G=myG,
  PCA.total=3,
  Model.selection = TRUE
)
```

5.11 SUPER

GAPIT also implements the SUPER GWAS method⁶, which extracts a small subset of SNPs and uses them in FaST-LMM. This method not only retains the computational advantage of FaST-LMM, but also increases statistical power.

```
#Step 1: Set data directory and import files
myCV <- read.table("Copy of Q_First_Three_Principal_Components.txt", head = TRUE)
myY <- read.table("mdp_traits.txt", head = TRUE)
myG <- read.table("mdp_genotype_test.hmp.txt" , head = FALSE)

#Step 2: Run GAPIT
myGAPIT_SUPER <- GAPIT(
  Y=myY[,c(1,2)],
  G=myG,
  #KI=myKI,
  CV=myCV,
  #PCA.total=3,
  sangwich.top="MLM", #options are GLM,MLM,CMLM, FaST and SUPER
  sangwich.bottom="SUPER", #options are GLM,MLM,CMLM, FaST and SUPER
  LD=0.1,
)
```

6 Prototype

The usage of GAPIT described in previous chapters barely require knowledge of R. Users can simply copy/paste the command lines from the user manual (or only tutorials) with a minimal keyboard typing such as changing file names and path. All the results are saved in the format of text files and PDF files.

This chapter describes the output of R objects from GAPIT (please see the gallery below). Using these objects require knowledge of R. This is designed for users who are interested in: 1) developing new statistical approaches or software package using the results of GAPIT as a starting point; 2) comparing GAPIT with other new or existing statistical methods or software packages; 3) studying a specific result from GAPIT.

6.1 Preparation

#Install packages (Do this section only for new installation of R)

References Download URL: <http://cran.rstudio.com/>, <http://ftp.ctex.org/mirrors/CRAN/>

#Step 0: Import library and GAPIT functions run this section each time to start R)

#####

```
library(multtest)
library("gplots")
library("LDheatmap")
library("genetics")
library(MASS)
library("compiler")
#library(RColorBrewer)
library("scatterplot3d") #Please Download and Install
                        #The downloaded link at: http://cran.r-project.org/package=scatterplot3d

source("http://www.zzlab.net/GAPIT/emma.txt")
source("http://www.zzlab.net/GAPIT/gapit_functions.txt")

#source("/Users/Zhiwu/Dropbox/Current/revolutionr/gapit/gapit_functions.txt")
```

#####

#download tutorial data and save them in myGAPIT directory under C drive and run tutorials

```
setwd("/myGAPIT")
```

6.2 Gallery of GAPIT Graphic Output

Statistical distributions of phenotype. The distributions are presented in four formats: Scatter plots, histogram , boxplot and accumulative distribution.

#Tutorial Step: Using ECMLM by Li and et. al. (BMC Biology, 2014)

#-----

```

#Step 1: Set data directory and import files
myY <- read.table("mdp_traits.txt", head = TRUE)
myG <- read.table("mdp_genotype_test.hmp.txt", head = FALSE)

#Step 2: Run GAPITc
myGAPIT <- GAPIT(
  Y=myY[,1:2],
  G=myG,
  PCA.total=3,
  kinship.cluster=c("average", "complete", "ward"),
  kinship.group=c("Mean", "Max"),
  group.from=200,
  group.to=1000000,
  group.by=10
)

```

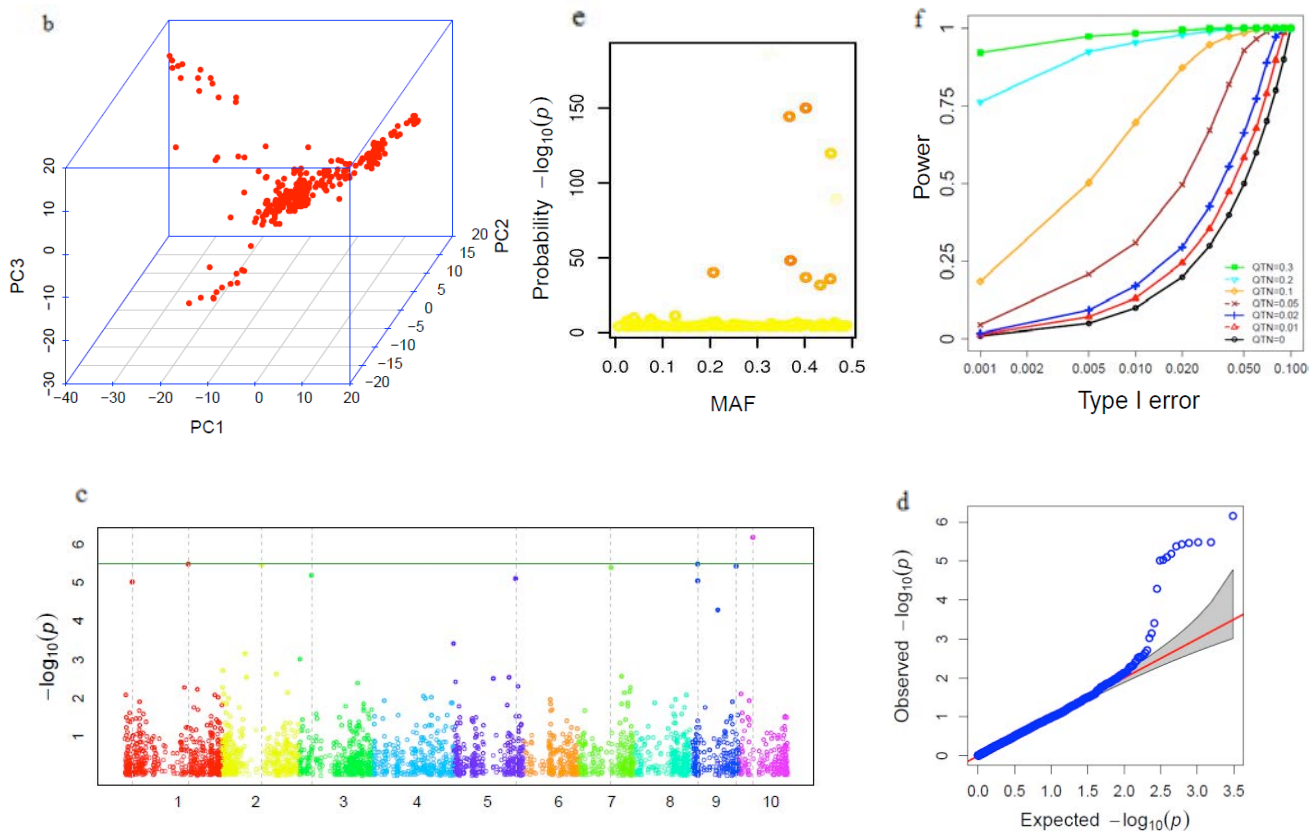
(b) Plot of the first three principal components (PC1,PC2 and PC3);

(c) Manhattan plot. -log P-values are plotted against physical map position of SNPs. Chromosomes have different colors, and simulated QTNs are marked with grey horizontal dotted lines;

(d) Quantile – quantile (QQ) plot determines how GWAS results compare to the expected results under the null hypothesis of no association;

(e) Minor allele frequency (MAF) plot. -log P-values from each marker are plotted against their MAF;

(f) Type 1 error plot, which depicts the tradeoff between type I error rate (x-axis) and power (y-axis) for quantitative trait nucleotides (QTNs) with different effect size;



#**Tutorial** Step: Basic Scenario of Compressed MLM by Zhang and et. al. (Nature Genetics, 2010)

#-----

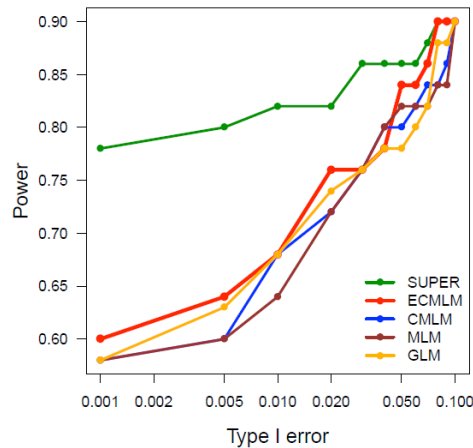
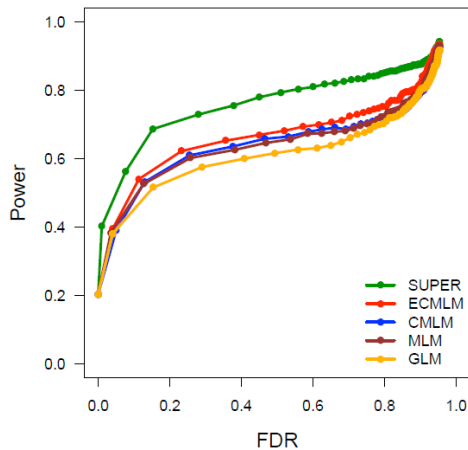
```
#Step 1: Set working directory and import data
myY <- read.table("mdp_traits.txt", head = TRUE)
myG <- read.table("mdp_genotype_test.hmp.txt", head = FALSE)

#Step 2: Run GAPIT
myGAPIT <- GAPIT(
  Y=myY,
  G=myG,
  PCA.total=3
)
```

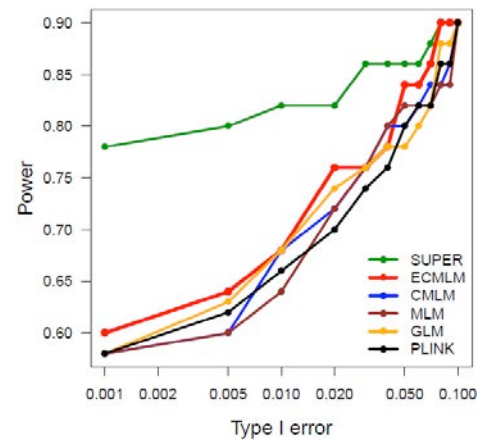
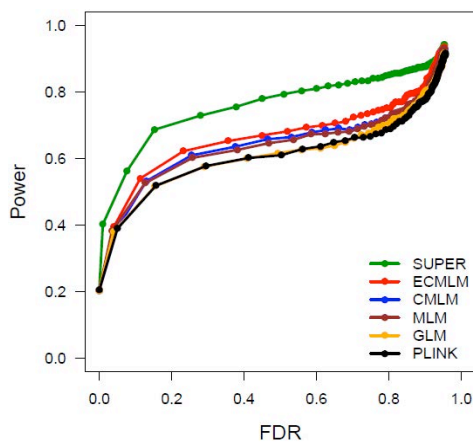
(g-1) Power-false discovery rate (FDR) plot, which displays the power (y-axis) of GLM, MLM, CMLM, ECMLM and SUPER model in different FDR (x-axis);

(g-2) Power-false discovery rate (FDR) plot, which displays the power (y-axis) of GLM, MLM, CMLM, ECMLM, SUPER and **P-LINK** model in different FDR (x-axis);

g-1



g-2



#Tutorial Step(g-1): Compare to Power against FDR for GLM,MLM,CMLM,ECMLM,SUPER
#Hint: Program running time is more than 24 hours for repetition 100 times.

```
#Step 1: Set data directory and import files
myGD <-read.table("mdp_numeric.txt", head = TRUE)
myGM <-read.table("mdp_SNP_information.txt", head = TRUE)
myKI <- read.table("KSN.txt", head = FALSE)
myG <- read.table("mdp_genotype_test.hmp.txt", head = FALSE)
```

```
#Step 2: Run GAPIT
GAPIT.Power.compare(
myG=myG,
myGD=myGD,
myGM=myGM,
myKI=myKI,
rel=100,
h2=0.9,
NQTN=5
)
```

#Object: Compare to Power-FDR with different model method for the same data

#Input:myG - genotype data

#Input:myGD - The numeric SNP file (content format: 0,1 and 2)

#Input:myGM - SNP information file (content: SNP ID, chromosome, position)

#Input:myKI - Kinship data

#input: rel - Program running time - repetition 100 times

#input: h2 - heritability

#input: NQTN - number of QTNs

```
#Output: Power-false discovery rate (FDR) plot for GLM, MLM,CMLM,ECMLM and SUPER model ;
#Output: Table of Power-FDR and TypeI for different model method
#Last update: January 1, 2015
#Note:If hava no myGD,myGM and myKI files,to generate the files by genotype data on GAPIT method:
#   myGAPIT0=GAPIT(G=myG,file.output=FALSE)
#   myGD=myGAPIT0$GD
#   myGM=myGAPIT0$GI
#   myKI=myGAPIT0$kinship
#Run Procedure
#1 Run GAPIT.Power.compare passing parameters
#2 The next simulation phenotype
#3 Operation gapit different method model to get the value of power and FDR
#4 Calculate means of repetition 100 times and comparison
#5 Output graphics and table of result
```

#Tutorial Step(g-2): Compare to Power against FDR for GLM,MLM,CMLM,ECMLM,SUPER,PLINK

#Hint: Please download P-LINK Tool for different operating system, and copy it to R work path #(set setwd("/myGAPIT") location)

References Download URL: <http://pngu.mgh.harvard.edu/~purcell/plink/download.shtml>

#P-LINK running have different forms for different operating systems, such windows: plink --ped #file1.ped --map file1.map --assoc

Program runing time is more than 24 hours for repetition 100 times.

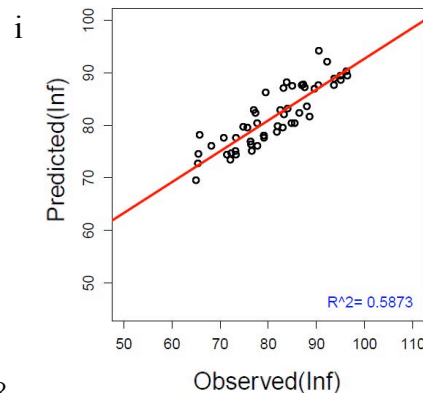
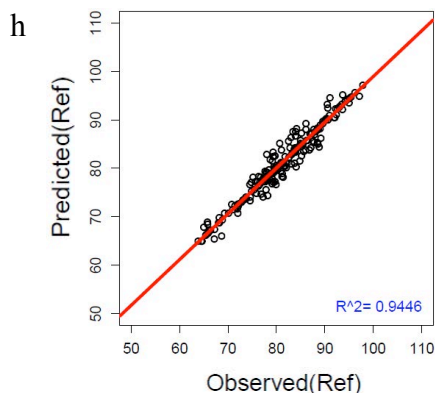
#-----

```
#Step 1: Set data directory and import files
myGD <-read.table("mdp_numeric.txt", head = TRUE)
myGM <-read.table("mdp_SNP_information.txt", head = TRUE)
myKI <- read.table("KSN.txt", head = FALSE)
myG <- read.table("mdp_genotype_test.hmp.txt", head = FALSE)
```

```
#Step 2: Run GAPIT
GAPIT.Power.compare.plink(
myG=myG,
myGD=myGD,
myGM=myGM,
myKI=myKI,
rel=100,
h2=0.9,
NQTN=5
)
```

(h) Genomic predictions vs. observed for samples of reference;

(i) Genomic predictions vs. observed for samples of inference;



#Tutorial Step: Genetic Prediction one time by cross validation

#-----

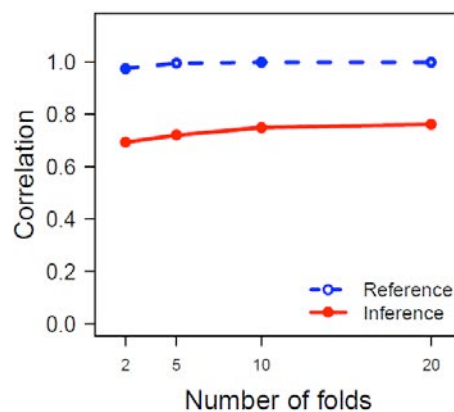
```
#Step 1: Set data directory and import files
y<-read.table("mdp_traits.txt", head = TRUE)
myK<-read.table("KSN.txt", head = FALSE)
```

```
#Step 2: Run GAPIT
OnePre<-GAPIT.Prediction(
myK=myK,
y<-y[,c(1,3)],
##y=y[,1:2],
num=5
)
```

(j) Cross validation plot, which depicts the accuracy values of genomic prediction by splitting total samples into different number of folds.

Result Demo:

j

**#Tutorial Step: Compare accuracy to different folds by replicate times**

#-----

```
#Step 1: Set data directory and import files
y<- read.table("mdp_traits.txt", head = TRUE)
myGD <-read.table("mdp_numeric.txt", head = TRUE)
```

```
#Step 2: Run GAPIT
myCross<-GAPIT.cross_validation.compare(
myGD=myGD,
y=y,
#y<-y[,c(1,3)],
rel=100,
tc<-c(2,5,10,20) ##input compare to folds num
)
```


6.3 Gallery of GAPIT File Output

Object	Dimension	Description
GWAS	mX7	GWAS results (SNPID,CHR,BP,P,MAF,DF,Effect)
GPS	nX6	GPS results (lineID,GroupID,Vategoty,BLUP,PEV)
compression	kX7	Compression result(VA,VE,REML,h2,group,cluster,method)
kinship	nXn	kinship among individuals
kinship.optimum	sXs	The optimum group kinship
PCA	NA	Principal components

6.4 Cross validation with replacement

Here we demonstrate an example of studying a specific output of GAPIT, specifically to investigate the accuracy of genome prediction through cross validation.

First we randomly set 25% of original phenotype (Y) as missing (NA) and generate a genomic prediction model by using their kinship. Then we record the correlation between the predicted phenotypic values and the original phenotype. We repeat this process for 1000 times. The average correlation is used as the criteria of genome prediction accuracy. The corresponding R code is displayed in the following box. The accuracy (correlation coefficient) over the 100 replicates (for demonstration purposes only; we suggest using 1000 replications) were 0.9203 and 0.6749 in the reference and inference (cross validation), respectively. The standard deviations were 0.078 and 0.0054 in the reference and inference, respectively.

R code for cross validation with replacement

```

#Import files
#####
myY <- read.table("mdp_traits.txt", head = TRUE)
myKI <- read.table("KSN.txt", head = FALSE)
myCV <- read.table("Copy of Q_First_Three_Principal_Components.txt", head = TRUE)

#Initial
#####
t=100 #total replicates
s=1/5 #sample of inference, e.g. set it to 1/5 for five fold cross validation
Y.raw=myY[,c(1,3)]#choos a trait
Y.raw=Y.raw[!is.na(Y.raw[,2]),] #Remove missing data
n=nrow(Y.raw)
n.missing=round(n*s)
storage.ref=matrix(NA,t,1)
storage.inf=matrix(NA,t,1)

#Loop on replicates
for(rep in 1:t){

#Set missing data
sample.missing=sample(1:n,n.missing)
if(n.missing>0){ Y0=Y.raw[-sample.missing,]
}else{Y0=Y.raw}

#Prediction
myGAPIT <- GAPIT(
Y=Y0,
KI=myKI,
CV=myCV,
group.from=254,
group.to=254,
group.by=10,
kinship.cluster=c("average"),
kinship.group=c("Mean")
)
prediction=myGAPIT$GPS

#Separate reference (with phenotype) and inference (without phenotype)
prediction.ref=prediction[prediction[,3]==1,]
prediction.inf=prediction[prediction[,3]==2,]

#Merge prediction with original Y
YP.ref <- merge(Y.raw, prediction.ref, by.x = "Taxa", by.y = "Taxa")
YP.inf <- merge(Y.raw, prediction.inf, by.x = "Taxa", by.y = "Taxa")

#Calculate correlation and store them
r.ref=cor(as.numeric(as.vector(YP.ref[,2])),as.numeric(as.vector(YP.ref[,6])) )
r.inf=cor(as.numeric(as.vector(YP.inf[,2])),as.numeric(as.vector(YP.inf[,6])) )
storage.ref[rep,1]=r.ref
storage.inf[rep,1]=r.inf
}#End of for (rep in 1:t)

storage=cbind(storage.ref,storage.inf)
colnames(storage)=c("Reference","Inference")
write.table(storage, "GAPIT.Cross.Validation.txt", quote = FALSE, sep = "\t", row.names = TRUE,col.names = NA)

```

6.5 Cross validation without replacement

Cross validation can also be performed by excluding one or a set of individuals in the reference to derive predicted phenotypic values from the genomic prediction model. Using this approach, this process can be repeated until all the individual have been excluded at least once. The correlation between the originals and the prediction (might be more than once) is used as the accuracy of prediction. The following demonstrate the process with the same data in previous section.

```
#Initial
#####
nj= 200 # number of Jack Knives, nj>0, nj!=1

Y.raw=myY[,c(1,3)]#choos a trait
Y.raw=Y.raw[!is.na(Y.raw[,2]),] #Remove missing data
n=nrow(Y.raw)

if(nj>=1){nLoop=nj}
else{
  nLoop=1/nj
}
assignment=ceiling((1:n)/(n/nLoop))
randomization=sample(1:n,n)
assignment=assignment[randomization]
nLoop=ceiling(nLoop)

#Loop on replicates
for(rep in 1:nLoop){

#Set missing data
if(nj>=1){Y0=Y.raw[assignment!=rep,]
} else{
  Y0=Y.raw[assignment==rep,]
}

#Prediction
myGAPIT <- GAPIT(
Y=Y0,
KI=myKI,
CV=myCV,
group.from=254,
group.to=254,
group.by=10,
kinship.cluster=c("average"),
kinship.group=c("Mean")
)
prediction=myGAPIT$GPS

#Separate reference (with phenotype) and inference (without phenotype)
if(rep==1){
  prediction.inf=prediction[prediction[,3]==2,]
} else{
  prediction.inf=rbind(prediction.inf,prediction[prediction[,3]==2,] )
}
} #End of for (rep in 1:t)

#Merge prediction with original Y
YP.inf <- merge(Y.raw, prediction.inf, by.x = "Taxa", by.y = "Taxa")

#Calculate correlation and store them
r.inf=cor(as.numeric(as.vector(YP.inf[,2])),as.numeric(as.vector(YP.inf[,6])) )
write.table(YP.inf, "GAPIT.Jack.Knife.txt", quote = FALSE, sep = "\t", row.names = TRUE,col.names = NA)
print(r.inf)
```

6.6 Convert HapMap format to numerical

Many software require genotype data in the numerical format. GAPIT can perform such conversion with a few lines of code as follows.

```
myG <- read.table("mdp_genotype_test.hmp.txt", head = FALSE)
myGAPIT <- GAPIT(G=myG, output.numerical=TRUE)
myGD= myGAPIT$GD
myGM= myGAPIT$GM
```

6.7 Compile SNPs from multiple GAPIT analyses into one set of results

To further expedite completion of the analysis for a large amount of SNPs, one may want to split the SNPs into multiple subgroups, and simultaneously run GAPIT on multiple clusters or R consoles. The code below reads in GWAS results and allelic effect estimates from the GAPIT analysis run on each subgroup, then combines them, recalculates the adjusted P -values using the Benjamini-Hochberg (1995) FDR-controlling procedure, and produces a combined GWAS summary table, allelic effect estimates, Manhattan plots, and QQ-plot for all SNPs.

```

#Step 0: In your working directory, create three folders, and name them "Results_1",
#"Results_2", and "Results_Combined". Put all of the GAPIT output files from one set up SNPs in
# "Results_1", and all of the GAPIT output files from the second set of SNPs in "Results_2".
# Finally, suppose that two traits named "trait1" and "trait2" are being analyzed
#####
library('MASS')
library(multtest)
library(gplots)

setwd("C:\\Folder")

#Import the source code
source("http://zzlab.net/GAPIT/emma.txt")
source("http://zzlab.net/GAPIT/gapit_functions.txt")

#Step 1: Set data directory and import files
#####
mydataPath.Results.1="C:\\Folder\\Results_1\\"
mydataPath.Results.2="C:\\Folder\\Results_2\\"

name <- c("trait1", "trait2")

#Step 2: Set the result directory to where the combined data will go
#####
setwd("C:\\Folder\\Results_Combined")

for(i in 1:length(name)){

#Read in the GWAS and Allelic effect estimates
GWAS.Results.1 <- read.csv(paste(mydataPath.Results.1,"GAPIT.",name[i],".GWAS.Results.csv",sep=""), head = TRUE)
GWAS.Results.2 <- read.csv(paste(mydataPath.Results.2,"GAPIT.",name[i],".GWAS.Results.csv",sep=""), head = TRUE)
Effect.Estimates.1 <- read.csv(paste(mydataPath.Results.1,"GAPIT.",name[i],".Allelic_Effect_Estimates.csv",sep=""), head = TRUE)
Effect.Estimates.2 <- read.csv(paste(mydataPath.Results.2,"GAPIT.",name[i],".Allelic_Effect_Estimates.csv",sep=""), head = TRUE)

#Append the the GWAS results and allelic effect estimates onto one folder
GWAS.Results <- rbind(GWAS.Results.1, GWAS.Results.2)
Effect.Estimates <- rbind(Effect.Estimates.1, Effect.Estimates.2)

#Remove the last column, which is the FDR adjusted P-values
GWAS.Results <- GWAS.Results[,-ncol(GWAS.Results)]
#Run the B-H procedure on the combined data, and append the FDR-adjusted P-values to the GWAS.Results
Conduct.FDR <- GAPIT.Perform.BH.FDR.Multiple.Correction.Procedure(PW1 = GWAS.Results,
                        FDR.Rate = 0.05, FDR.Procedure = "BH")
GWAS.Results.with.FDR <- Conduct.FDR$PWIP

#Make QQ-plots
GAPIT.QQ(P.values = Conduct.FDR$PWIP[,4], name.of.trait = name[i],DPP=50000)

#Make new Manhattan plots with the combined results
GAPIT.Manhattan(GI.MP = GWAS.Results.with.FDR[,2:4], name.of.trait = name[i],
                DPP=50000, plot.type = "Genomewise",cutOff=0.00)
GAPIT.Manhattan(GI.MP = GWAS.Results.with.FDR[,2:4], name.of.trait = name[i],
                DPP=50000, plot.type = "Chromosomewise",cutOff=0.00)

#Export the combined GWAS results
write.table(GWAS.Results.with.FDR, paste("GAPIT.", name[i], ".GWAS.Results.csv", sep = ""),
            quote = FALSE, sep = ",", row.names = FALSE,col.names = TRUE)

#Export the combined Allelic effect estimates
write.table(Effect.Estimates, paste("GAPIT.", name[i], ".Allelic_Effect_Estimates.csv", sep = ""),
            quote = FALSE, sep = ",", row.names = FALSE,col.names = TRUE)

rm(GWAS.Results.1)
rm(GWAS.Results.2)
rm(GWAS.Results)
rm(GWAS.Results.with.FDR)
rm(Effect.Estimates.1)
rm(Effect.Estimates.2)
rm(Effect.Estimates)
rm(Conduct.FDR)
} #End for(i in 1:length(name))

```

7 Appendix

7.1 Tutorial Data sets

The data set contains 26 files and can be downloaded at:

http://zzlab.net/GAPIT/gapit_tutorial_data.zip

Table 7.1. Properties of tutorial files

Name	Date	Time	Bytes
Copy of Q_First_Three_Principal_Components.txt	6/8/2011	11:00 PM	35,604
mdp_SNP_information.txt	6/14/2012	1:13 PM	73,728
mdp_SNP_information1.txt	6/14/2012	1:35 PM	23,325
mdp_SNP_information2.txt	6/14/2012	1:36 PM	23,539
mdp_SNP_information3.txt	6/14/2012	1:37 PM	26,011
KSN.txt	5/3/2011	2:56 PM	724,004
mdp_genotype_chr1.hmp.txt	9/2/2011	6:18 PM	489,479
mdp_genotype_chr2.hmp.txt	9/2/2011	6:19 PM	356,629
mdp_genotype_chr3.hmp.txt	9/2/2011	6:20 PM	322,390
mdp_genotype_chr4.hmp.txt	9/2/2011	6:20 PM	289,859
mdp_genotype_chr5.hmp.txt	9/2/2011	6:21 PM	324,154
mdp_genotype_chr6.hmp.txt	9/2/2011	6:22 PM	194,119
mdp_genotype_chr7.hmp.txt	9/2/2011	6:23 PM	223,920
mdp_genotype_chr8.hmp.txt	9/2/2011	6:23 PM	232,994
mdp_genotype_chr9.hmp.txt	9/2/2011	6:24 PM	194,047
mdp_genotype_chr10.hmp.txt	9/2/2011	6:25 PM	183,469
mdp_genotype_test.hmp.txt	4/28/2011	12:16 PM	2,796,282
mdp_genotype_test1.hmp.txt	6/2/2011	2:00 PM	904,942
mdp_genotype_test2.hmp.txt	6/2/2011	2:00 PM	905,156
mdp_genotype_test3.hmp.txt	6/2/2011	1:58 PM	989,468
mdp_genotype_test5.hmp.txt	5/3/2011	2:56 PM	724,004
mdp_traits.txt	5/20/2011	11:34 PM	6,586
mdp_numeric.txt	6/19/2012	9:40 AM	1,834,937
mdp_numeric1.txt	6/19/2012	9:41 AM	594,195
mdp_numeric2.txt	6/19/2012	9:43 AM	594,134
mdp_numeric3.txt	6/19/2012	9:44 AM	649,748

These files can be classified into following categories. We use “?” and “*” for a string with any length and one character respectively.

1. “Copy of*” is a covariate data set consisting of three PCs in maize.
mdp_SNP_information*” are genotype map files containing SNP name, chromosome, and base pair position. The ones with numeric number are the ones separated from the one does not have numeric number.

2. “KSN.txt” is a kinship matrix of a subset of the maize diversity panel calculated using the method of Loiselle *et al.* (1995).
3. “mdp_genotype_*.hmp.txt” is genotype in format of hapmap containing 3093 SNPs. The files with numeric numbers are the ones separated from “mdp_genotype_test.hmp.txt”. The files with “chr” are separated by chromosome and the ones with “test” are separated by block (1000, 1000 and 1093 SNPs).
4. “mdp_traits.txt” is phenotype containing three traits: ear height (EarHT), days to pollinating (dpoll) and ear diameter (EarDia).
5. “mdp_numeric?.txt” is numeric genotype data files paired with “mdp_SNP_information?.txt”. The one with numeric number are the ones separated from the without the numeric numbers. The first two files contain 1000 SNPs and the last one contains 1094 SNPs.

7.2 Typical ways of reading data

```
#Phenotypic Data
myY <- read.table("Phenotype_dpoll.txt", head = TRUE)

#HapMap genotype format
myG <- read.delim("mdp_genotype_test.hmp.txt", head = FALSE)

#Numerical genotype format
#-----A pair of Genotypic Data and map files-----
myGD <- read.table("mdp_numeric.txt", head = TRUE)
myGM <- read.table("mdp_SNP_information.txt", head = TRUE)

#Kinship matrix
myKI <- read.table("KSN.txt", head = FALSE)

#covariate variables (such as population structure represented by Q matrix or PC)
myCV <- read.table("Copy of Q_First_Three_Principal_Components.txt", head = TRUE)
```

7.3 Frequently Asked Questions

1. What do I do if I get frustrated?

A: The GAPIT team makes the effort to provide suggestions to any errors that users might run into. Try to find the answer from this question list. If not there, post the problem to GAPIT forum.

2. What happen if the magnitude of my trait is too small or large?

A: This will cause a problem due to rounding error. Multiple or divide by a value to make it into a reasonable range.

3. How many PCs to include?

A: The number of principal components (PCs) included in the GWAS models can be adjusted in GAPIT. To help determine the number of PCs that adequately explain population structure, a scree plot is provided in the GAPIT output (if at least one PC is selected for inclusion into the final model). Once

the ideal number of PCs is determined, GAPIT should be reran with this number PCs included in the GWAS models. We also recommend running BIC-based model selection (activated by writing `Model.selection = TRUE` in the `GAPIT()` function) because the optimal number of PCs to include may vary from trait to trait.

4. How do I report an error?

A: In order to fix the problem, please copy and paste the error message from the R environment and sent your R source code and the dataset that allow us to repeat the error.

5. What should I do with “Error in file(file, "rt") : cannot open the connection”?

A: In most cases this error is caused by incorrect file name or number of file specific is more than exist.

6. What should I do with “Error in GAPIT(... : unused argument(s) ...”?

A: In most cases this error is caused by incorrect spelling of GAPIT key word such as upper or lower case.

7. What should I do with “Error in solve.default(crossprod(X, X)) : system is computationally singular”?

A: Check covariate variables and remove the ones that are linear independent.

8. How to cite GAPIT?

A: The Bioinformatics GAPIT paper² can be cited in most of cases. As it implemented multiple statistical methods, the corresponding papers should be cited for clarity. These methods include regular MLM⁹ methods, CMLM¹, ECMLM⁴, SUPER⁶, and P3D¹.

9. Is it possible to analyze case-control studies in GAPIT?

A: GAPIT was designed to use mixed model approaches for performing GWAS and GS on quantitative phenotypes in association panels. This program may be used for case-control studies, where a set of individuals with an affliction are matched up with healthy individuals with similar demographic characteristics. Thus, case-control studies use binary phenotypes (i.e., case versus control). Because of this, the approaches used in GAPIT are not necessarily statistically appropriate for case-control studies. However, the effectiveness of the mixed model for controlling population structure is still relevant for finding biologically real associations with diseases. Because of this, we recommend augmenting GAPIT GWAS results with fitting logistic regression models at the most significant SNPs. To illustrate the analysis of case-control studies in GAPIT, we included the “for.exercise” case –control data set from the `snpStats` R program (<http://bioc.ism.ac.jp/2.8/bioc/html/snpStats.html>) plus results from running the CMLM approach in GAPIT in our tutorial data. These data consist of 500 cases and 500 controls that are simulated on human chromosome 10.

10. I am using covariates from STRUCTURE as fixed effects in my model, and GAPIT gives an error message. What should I do?

A: This error is occurring because the covariates from `STRUCTURE` are linearly dependent. In particular, for a given individual/taxa, these covariates sum to 1. To circumvent this error, remove one of the `STRUCTURE` covariates from the corresponding input file.

7.4 GAPIT Biography

Date	Version	Event
May 11, 2011	1.20	First public release with following method implemented: <ul style="list-style-type: none"> • Principal component method to encounter population structure (Price). • Unified mixed model to encounter both population structure and kinship. • EMMA method to improve the speed to estimate variance components (ratio). • Compressed mixed model to improve statistical power and speed • P3D or EMMAx to improve speed by estimating population parameters (e.g. variances and grouping) only once.
June 13, 2011	1.22	Genotype in numerical format in addition to hapmap format
August 15, 2011	1.25	Van Raden algorithm for kinship
September 2, 2011	1.31	Reading fragment within single genotype file to save memory
September 9, 2011	1.34	LD heat map
September 17, 2011	1.36	Interface change for prototyping
October 4, 2011	1.40	Genotype, kinship and PC process optimization to improve speed for multiple traits
October 24, 2011	1.41	Missing data imputation as middle, major, minor, present/absent. Heterozygous coding as the middle, left (one of the homozygous) and right (the other homozygous)
November 1, 2011	1.42	Matrix partitioning to improve speed (5-10 folds)
December 7, 2011	2.01	FaST-LMM method implemented
May 19, 2012	2.19	Splitting big genotype file
July 8, 2012	NA	GAPIT Bioinformatics paper accepted for publication
November 8, 2012	2.20	Automatic sorting taxa in kinship for regular MLM
February 14, 2013	2.25	Confidence interval on QQ plot
April 26, 2013	2.26	Labeling QTNs on Manhattan plot
July 15, 2014	2.27	SUPER implemented
August 20, 2014	2.28	ECMLM implemented
October 25, 2014	2.29	Refinement of the VanRaden kinship algorithm to accommodate centralization

Date	Version	Event
February 28, 2015	2.3.41	Report enrichment
September 05, 2015	Same as date	Uniform reports
October 12, 2015		Simulation of category phenotype
October 22, 2015		Compression BLUP
April 04, 2016		Zhang Kinship
April 04, 2016		SUPER BLUP

REFERENCES

1. Zhang, Z. *et al.* Mixed linear model approach adapted for genome-wide association studies. *Nat Genet* **42**, 355–360 (2010).
2. Lipka, A. E. *et al.* GAPIT: genome association and prediction integrated tool. *Bioinformatics* **28**, 2397–2399 (2012).
3. Zhang, Z., Todhunter, R. J., Buckler, E. S. & Van Vleck, L. D. Technical note: Use of marker-based relationships with multiple-trait derivative-free restricted maximal likelihood. *J. Anim. Sci.* **85**, 881–885 (2007).
4. Li, M. *et al.* Enrichment of statistical power for genome-wide association studies. *BMC Biol.* **12**, 73 (2014).
5. Tang, Y. *et al.* GAPIT Version 2: An Enhanced Integrated Tool for Genomic Association and Prediction. *Plant J.* **9**, (2016).
6. Wang, Q., Tian, F., Pan, Y., Buckler, E. S. & Zhang, Z. A SUPER Powerful Method for Genome Wide Association Study. *PLoS One* **9**, e107684 (2014).
7. Kang, H. M. *et al.* Efficient control of population structure in model organism association mapping. *Genetics* **178**, 1709–1723 (2008).
8. Bradbury, P. J. *et al.* TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics* **23**, 2633–2635 (2007).
9. Yu, J. M. *et al.* A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nat. Genet.* **38**, 203–208 (2006).
10. Kang, H. M. *et al.* Variance component model to account for sample structure in genome-wide association studies. *Nat. Genet.* **42**, 348–354 (2010).
11. Pritchard, J. K., Stephens, M. & Donnelly, P. Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959 (2000).
12. Zhao, K. *et al.* An Arabidopsis example of association mapping in structured samples. *PLoS Genet.* **3**, 0071–0082 (2007).
13. Benjamini, Y. & Hochberg, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J.R. Statis. Soc. B.* **57**, 289–300 (1995).
14. Guo, G. *et al.* Canine hip dysplasia is predictable by genotyping. *Osteoarthr. Cartil.* **19**, 420–429 (2011).
15. Flint-Garcia, S. A. *et al.* Maize association population: a high-resolution platform for quantitative trait locus dissection. *Plant J* **44**, 1054–1064 (2005).
16. Loiselle, B. A., Sork, V. L., Nason, J. & Graham, C. Spatial Genetic-Structure of a Tropical Understory Shrub, *Psychotria Officinalis* (Rubiaceae). *Am. J. Bot.* **82**, 1420–1425 (1995).
17. FALUSH, D., STEPHENS, M. & PRITCHARD, J. K. Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Mol. Ecol. Notes* **7**, 574–578 (2007).
18. VanRaden, P. M. Efficient methods to compute genomic predictions. *J Dairy Sci* **91**, 4414–4423 (2008).