Package 'Eagle'

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```
Title Multiple Locus Association Mapping on a Genome-Wide Scale

Version 1.1.0

Maintainer Andrew George <andrew.george@csiro.au>

Author Andrew George [aut, cre],
    Joshua Bowden [ctb],
    Ryan Stephenson [ctb],
    Hyun Kang [ctb],
    Noah Zaitlen [ctb],
    Claire Wade [ctb],
    Andrew Kirby [ctb],
    David Heckerman [ctb],
    Mark Daly [ctb],
    Eleazar Eskin [ctb]
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Description An implementation of multiple-locus association mapping on a genome-wide scale. 'Eagle' can handle inbred or outbred study populations, populations of arbitrary unknown complexity, and data larger than the memory capacity of the computer. Since 'Eagle' is based on linear mixed models, it is best suited to the analysis of data on continuous traits. However, it can tolerate non-normal data. 'Eagle' reports, as its findings, the best set of snp in strongest association with a trait. To perform an analysis, run 'OpenGUI()'. This opens a web browser to the menudriven user interface for the input of data, and for performing genome-wide analysis.

```
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Depends R (>= 3.4)

Imports matrixcalc, shiny, shinythemes, shinyBS, shinyjs, stats, utils, parallel, data.table, tcltk

LinkingTo RcppEigen, Rcpp

Roxygen list(wrap=FALSE)

LazyData true

ByteCompile TRUE

NeedsCompilation yes

URL http://eagle.r-forge.r-project.org

Contact eaglehelp@csiro.au
```

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Eagle-package

A short title line describing what the package does

Description

A more detailed description of what the package does. A length of about one to five lines is recommended.

Details

This section should provide a more detailed overview of how to use the package, including the most important functions.

Author(s)

Your Name, email optional.

Maintainer: Your Name <your@email.com>

References

This optional section can contain literature or other references for background information.

See Also

Optional links to other man pages

```
## Not run:
    ## Optional simple examples of the most important functions
    ## These can be in \dontrun{} and \donttest{} blocks.

## End(Not run)
```

AM	multiple-locus association mapping	

Description

AM performs association mapping within a multiple-locus linear mixed model framework. AM finds the best set of marker loci in strongest association with a trait while simultaneously accounting for any fixed effects and the genetic background.

Usage

```
AM(trait = NULL, fformula = NULL, availmemGb = 8, geno = NULL,
pheno = NULL, map = NULL, Zmat = NULL, ncpu = detectCores(),
ngpu = 0, quiet = TRUE, maxit = 20)
```

Arguments

trait	the name of the column in the phenotype data file that contains the trait data. The name is case sensitive and must match exactly the column name in the phenotype data file.
fformula	the right hand side formula for the fixed effects. See below for details. If not specified, only an overall mean will be fitted.
availmemGb	a numeric value. It specifies the amount of available memory (in Gigabytes). This should be set to the maximum practical value of available memory for the analysis.
geno	the R object obtained from running ReadMarker. This must be specified.
pheno	the R object obtained from running ReadPheno. This must be specified.
map	the R object obtained from running ReadMap. If not specified, a generic map will be assumed.
Zmat	the R object obtained from running ReadZmat. If not specified, an identity matrix will be assumed.
ncpu	a integer value for the number of CPU that are available for distributed computing. The default is to determine the number of CPU automatically.
ngpu	a integer value for the number of gpu available for computation. The default is to assume there are no gpu available. This option has not yet been implemented.
quiet	a logical value. If set to TRUE, additional runtime output is printed. This is useful for error checking and monitoring the progress of a large analysis.
maxit	an integer value for the maximum number of forward steps to be performed. This will rarely need adjusting.

Details

How to perform a basic AM analysis:

Suppose,

• the snp data are contained in the file geno.txt which is a plain space separated text file with no column headings. The file is located in the current working directory. It contains numeric genotype values 0, 1, and 2 for snp genotypes AA, AB, and BB, respectively. It also contains the numeric value X for a missing genotype.

- the phenotype data is contained in the file pheno.txt which is a plain space separated text file containing a single column with the trait data. The first row of the file has the column heading 'y'. The file is located in the current working directory.
- there is no map data.

To analyse these data, we would use the following three functions:

```
geno_obj <- ReadMarker(filename='geno.txt', AA=0, AB=1, BB=2, type="text", missing='X')
pheno_obj <- ReadPheno(filename='pheno.txt')
res <- AM(trait='y', geno=geno_obj, pheno=pheno_obj)</pre>
```

A table of results is printed to the screen and saved in the R object res.

How to perform a more complicated AM analysis:

Suppose,

- the snp data are contained in the file geno.ped which is a 'PLINK' ped file. See ReadMarker for details. The file is located in /my/dir. Let's assume the file is large, say 50 gigabytes, and our computer only has 32 gigabytes of RAM.
- the phenotype data is contained in the file pheno.txt which is a plain space separated text file with six columns. The first row of the file contains the column headings. The first column is a trait and is labeled y1. The second column is another trait and is labeled y2. The third and fourth columns are nuisance variables and are labeled cov1 and cov2. The fifth and sixth columns are the first two principal components to account for population substructure and are labeled pc1 and pc2. The file contains missing data that are coded as 99. The file is located in /my/dir.
- the map data is contained in the file map.txt, is also located in /my/dir, and the first row has the column headings.
- An 'AM' analysis is performed where the trait of interest is y2, the fixed effects part of the model is cov1 + cov2 + pc1 + pc2, and the available memory is set to 32 gigabytes.

To analyse these data, we would run the following:

A table of results is printed to the screen and saved in the R object res.

How to perform an analysis where individuals have multiple observations: Suppose,

- the snp data are contained in the file geno.ped which is a 'PLINK' ped file. See ReadMarker for details. The file is located in /my/dir. Let's assume the file is large, say 50 gigabytes, and our computer only has 32 gigabytes of RAM.
- the phenotype data is contained in the file pheno.txt which is a plain space separated text file with six columns. The first row of the file contains the column headings. The first column is a trait and is labeled y1. The second column is another trait and is labeled y2. The third and fourth columns are nuisance variables and are labeled cov1 and cov2. The fifth and sixth columns are the first two principal components to account for population substructure and are

labeled pc1 and pc2. The file contains missing data that are coded as 99. The file is located in /my/dir.

- the Z matrix data are contained in the file Zmatrix.txt. The file is located in /my/dir.This file is a design matrix that only contains zeros and ones where each row must contain only a single one in the column that matches the individual's trait value to their corresponding genotype.
- the map data is contained in the file map.txt, is also located in /my/dir, and the first row has the column headings.
- An 'AM' analysis is performed where the trait of interest is y2, the fixed effects part of the model is cov1 + cov2 + pc1 + pc2, and the available memory is set to 32 gigabytes.

To analyse these data, we would run the following:

A table of results is printed to the screen and saved in the R object res.

Dealing with missing marker data:

AM can tolerate some missing marker data. However, ideally, a specialized genotype imputation program such as 'BEAGLE', 'MACH', 'fastPHASE', or 'PHASE2', should be used to impute the missing marker data before being read into 'Eagle'.

Dealing with missing trait data:

AM deals automatically with individuals with missing trait data. These individuals are removed from the analysis and a warning message is generated.

Dealing with missing explanatory variable values:

AM deals automatically with individuals with missing explanatory variable values. These individuals are removed from the analysis and a warning message is generated

Error Checking:

Most errors occur when reading in the data. However, as an extra precaution, if quiet=TRUE, then additional output is printed during the running of AM. If AM is failing, then this output can be useful for diagnosing the problem.

Value

A list with the following components:

trait column name of the trait being used by 'AM'.

fformula Right hand size formula of the fixed effects part of the linear mixed model.

indxNA a vector containing the row indexes of those individuals, whose trait and fixed effects data contain missing values and have been removed from the analysis.

Mrk a vector with the names of the snp in strongest and significant association with the trait.If no loci are found to be significant, then this component is NA.

Chr the chromosomes on which the identified snp lie.

Pos the map positions for the identified snp.

Indx the column indexes in the marker file of the identified snp.

ncpu number of cpu used for the calculations.

availmemGb amount of RAM in gigabytes that has been set by the user.

quiet boolean value of the parameter.

extBIC numeric vector with the extended BIC values for the loci found to be in significant association with the trait.

See Also

ReadMarker, ReadPheno, ReadZmat, and ReadMap

```
## Not run:
# Since the following code takes longer than 5 seconds to run, it has been tagged as dontrun.
 # However, the code can be run by the user.
 # Example
 #-----
 # read the map
 # File is a plain space separated text file with the first row
 # the column headings
 complete.name <- system.file('extdata', 'map.txt',</pre>
                                  package='Eagle')
 map_obj <- ReadMap(filename=complete.name)</pre>
 # read marker data
 # Reading in a PLINK ped file
# and setting the available memory on the machine for the reading of the data to 8 gigabytes
 complete.name <- system.file('extdata', 'geno.ped',</pre>
                                    package='Eagle')
 geno_obj <- ReadMarker(filename=complete.name, type='PLINK', availmemGb=8)</pre>
 # read phenotype data
 #~~~~~~~
 # Read in a plain text file with data on a single trait and two covariates
 # The first row of the text file contains the column names y, cov1, and cov2.
 complete.name <- system.file('extdata', 'pheno.txt', package='Eagle')</pre>
 pheno_obj <- ReadPheno(filename=complete.name)</pre>
# Performing multiple-locus genome-wide association mapping with a model
  with no fixed effects except for an intercept.
```

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OpenGUI

Browser-based Graphical User Interface

Description

Opens a web browser to act as a user-friendly user interface to 'Eagle'

Usage

OpenGUI()

Details

OpenGUI is an easy to use web-based user interface for 'Eagle'. By clicking on the navigation tabs at the top of a page, data can be read and analysed. By using this user interface, a user can avoid having to write R code.

Note that even though a web browser is being used as the user interface, everything remains local to the computer.

Examples

```
## Not run:
# opens a web browser
OpenGUI()
## End(Not run)
```

ReadMap

Read map file

Description

Read in the marker map data.

Usage

```
ReadMap(filename = NULL, csv = FALSE, header = TRUE)
```

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Arguments

filename contains the name of the map file. The file name needs to be in quotes.

csv a logical value. When TRUE, a csv file format is assumed. When FALSE, a space

separated format is assumed.

header a logical value. When TRUE, the first row of the file contains the column head-

ings.

Details

Association mapping, unlike classical linkage mapping, does not require a map to find marker-trait associations. So, reading in a map file is optional. If a map file is supplied, then the marker names from this file are used when reporting the findings from AM. If a map file is not supplied, then generic names M1, M2, ..., are assigned to the marker loci where the number refers to the column number in the marker file.

A space separated text file with column headings is assumed as the default input. The map file can have three or four columns. If the map file has three columns, then it is assumed that the three columns are the marker locus names, the chromosome number, and the map position (in any units). If the map file has four columns as with a 'PLINK map file, then the columns are assumed to be the marker locus names, the chromosome number, the map position in centimorgans, and the map position in base pairs.

Missing values are allowed but not in the first column of the file (i.e. the marker labels are not allowed to be missing).

The order of the marker loci in this file is assumed to be the same order as the loci in the marker data file.

The first column of the map file is assumed to contain the marker names.

Value

a data frame is returned of the map data.

See Also

ReadMarker and ReadPheno.

```
# Read in example map data from ./extdata/
# find the full location of the map data
complete.name <- system.file('extdata', 'map.txt', package='Eagle')
# read in map data
map_obj <- ReadMap(filename=complete.name)
# look at first few rows of the map file
head(map_obj)</pre>
```

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|--|

Description

A function for reading in marker data. Two types of data can be read.

Usage

```
ReadMarker(filename = NULL, type = "text", missing = NULL, AA = NULL,
   AB = NULL, BB = NULL, availmemGb = 16, quiet = TRUE)
```

Arguments

filename	contains the name of the marker file. The file name needs to be in quotes.
type	specify the type of file. Choices are 'text' (the default) and PLINK.
missing	the number or character for a missing genotype in the text file. There is no need to specify this for a PLINK ped file. Missing allele values in a PLINK file must be coded as '0' or '-'.
AA	the character or number corresponding to the 'AA' snp genotype in the marker genotype file. This need only be specified if the file type is 'text'. If a character then it must be in quotes.
AB	the character or number corresponding to the 'AB' snp genotype in the marker genotype file. This need only be specified if the file type is 'text'. This can be left unspecified if there are no heterozygous genotypes (i.e. the individuals are inbred). Only a single heterozygous genotype is allowed ('Eagle' does not distinguish between 'AB' and 'BA'). If specified and a character, it must be in quotes.
BB	the character or number corresponding to the 'BB' snp genotype in the marker genotype file. This need only be specified if the file type is 'text'. If a character, then it must be in quotes.
availmemGb	a numeric value. It specifies the amount of available memory (in Gigabytes). This should be set to be as large as possible for best performance.
quiet	a logical value. If set to TRUE, additional runtime output is printed.

Details

ReadMarker can handle two different types of marker data; namely, genotype data in a plain text file, and PLINK ped files.

Reading in a plain text file containing the marker genotypes: To load a text file that contains snp genotypes, run ReadMarker with filename set to the name of the file, and AA, AB, BB set to the corresponding genotype values. The genotype values in the text file can be numeric, character, or a mix of both.

We make the following assumptions

- The text file does not contain row or column headings
- The file is allowed to contain missing genotypes that have been coded according to missing
- · Individuals are diploid

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• The rows of the text file are the individuals and the columns are the marker loci

- The file is space separated
- The mapping of the observed genotypes in the marker file to AA, AB, and BB, remains the same for all loci
- Individuals are outbred when AA, AB, and BB are specified and inbred when only AA, and BB are specified
- For a text file, the same alphanumeric value is used for all missing marker genotypes. For a PLINK ped file, the missing allele is allowed to be '0' or '-'.

For example, suppose we have a space separated text file with marker genotype data collected from five snp loci on three individuals where the snp genotype AA has been coded 0, the snp genotype AB has been coded 1, the snp genotype BB has been coded 2, and missing genotypes are coded as 99

```
0 1 2 0 2
1 1 0 2 0
2 2 1 1 99
```

The file is called geno.txt and is located in the directory /my/dir/.

To load these data, we would use the command

```
geno_obj <- ReadMarker(filename='/my/dir/geno.txt', AA=0, AB=1, BB=2, type='text', missing=99)</pre>
```

where the results from running the function are placed in geno_obj.

As another example, suppose we have a space separated text file with marker genotype data collected from five snp loci on three individuals where the snp genotype AA has been coded a/a, the snp genotype AB has been coded a/b, and the snp genotype BB has been coded b/b

```
a/a a/b b/b a/a b/b
a/b a/b a/a b/b a/a
b/b b/b a/b a/b NA
```

The file is called geno.txt and is located in the same directory from which R is being run (i.e. the working directory).

To load these data, we would use the command

where the results from running the function are placed in geno_obj.

Reading in a PLINK ped file: PLINK is a well known toolkit for the analysis of genome-wide association data. See https://www.cog-genomics.org/plink2 for details.

Full details of PLINK ped files can be found https://www.cog-genomics.org/plink/1.9/ formats#ped. Briefly, the PED file is a space delimited file (tabs are not allowed): the first six columns are mandatory:

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

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Here, these columns can be any values since ReadMarker ignores these columns.

Genotypes (column 7 onwards) 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 must have two alleles specified. Missing alleles (i.e 0 or -) are allowed. No column headings should be given.

As an example, suppose we have data on three individuals genotyped for four snp loci

FAM001	101	0	0	1	0	Α	G	C	C	C	G	Α	Α
FAM001	201	0	0	2	0	A	A	C	T	G	G	T	A
FAM001	300	101	201	2	0	G	A	T	T	C	G	A	T

Then to load these data, we would use the command

```
geno_obj <- ReadMarker(filename='PLINK.ped', type='PLINK')</pre>
```

where geno_obj is used by AM, and the file PLINK.ped is located in the working directory (i.e. the directory from which R is being run).

Reading in other formats: Having first installed the stand-alone PLINK software, it is possible to convert other file formats into PLINK ped files. See https://www.cog-genomics.org/plink/1.9/formats for details.

For example, to convert vcf file into a PLINK ped file, at the unix prompt, use the PLINK command

```
PLINK --vcf filename.vcf --recode --out newfilename
and to convert a binary ped file (bed) into a ped file, use the PLINK command
PLINK --bfile filename --recode --tab --out newfilename
```

Value

To allow AM to handle data larger than the memory capacity of a machine, ReadMarker doesn't load the marker data into memory. Instead, it creates a reformatted file of the marker data and its transpose. The object returned by ReadMarker is a list object with the elements asciifileM, asciifileMt, and dim_of_ascii_M which is the full file name (name and path) of the reformatted file for the marker data, the full file name of the reformatted file for the transpose of the marker data, and a 2 element vector with the first element the number of individuals and the second element the number of marker loci.

```
#------
# Example 1
#------
# Read in the genotype data contained in the text file geno.txt
#
# The function system.file() gives the full file name (name + full path).
complete.name <- system.file('extdata', 'geno.txt', package='Eagle')
#
# The full path and name of the file is
print(complete.name)
# Here, 0 values are being treated as genotype AA,</pre>
```

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```
# 1 values are being treated as genotype AB,
# and 2 values are being treated as genotype BB.
# 4 gigabytes of memory has been specified.
# The file is space separated with the rows the individuals
# and the columns the snp loci.
geno_obj <- ReadMarker(filename=complete.name, type='text', AA=0, AB=1, BB=2, availmemGb=4)</pre>
# view list contents of geno_obj
print(geno_obj)
#-----
# Example 2
#-----
# Read in the allelic data contained in the PLINK ped file geno.ped
# The function system.file() gives the full file name (name + full path).
complete.name <- system.file('extdata', 'geno.ped', package='Eagle')</pre>
# The full path and name of the file is
print(complete.name)
# Here, the first 6 columns are being ignored and the allelic
# information in columns 7 - 10002 is being converted into a reformatted file.
# 4 gigabytes of memory has been specified.
# The file is space separated with the rows the individuals
# and the columns the snp loci.
geno_obj <- ReadMarker(filename=complete.name, type='PLINK', availmemGb=4)</pre>
# view list contents of geno_obj
print(geno_obj)
```

ReadPheno

Read phenotype file

Description

Read in the phenotype data.

Usage

```
ReadPheno(filename = NULL, header = TRUE, csv = FALSE, missing = NULL)
```

Arguments

filename	contains the name of the phenotype file. The file name needs to be in quotes.
header	a logical value. When TRUE, the first row of the file contains the names of the columns. Default is TRUE.
CSV	a logical value. When TRUE, a csv file format is assumed. When FALSE, a space separated format is assumed. Default is FALSE.
missing	the number or character for a missing phenotype value.

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Details

ReadPheno reads in the phenotype data which are data measured on traits and any fixed effects (or predictors/features/explanatory variables). A space separated plain text file is assumed. Each row in this file corresponds to an individual. The number of rows in the phenotype file must be the same as the number of rows in the marker data file. Also, the ordering of the individuals must be the same in the two files. A space separated file with column headings is the default but can be changed with the header and csv options.

The phenotype file may contain multiple traits and fixed effects variables.

Missing values are allowed. Eagle is told which value should be treated as missing by setting the missing parameter to the value.

For example, suppose we have three individuals for which we have collected data on two quantitative traits (y1 and y2), and four explanatory variables (age, weight, height, and sex). The data looks like

y1	y2	age	weight	height	sex
112.02	-3.123	26	75	168.5	M
156.44	1.2	45	102	NA	NA
10.3	NA	28	98	189.4	F

where the first row has the column headings and the next three rows contain the observed data on three individuals.

To load these data, we would use the command

```
pheno_obj <- ReadPheno(filename='pheno.dat', missing='NA')</pre>
```

where pheno.dat is the name of the phenotype file, and pheno_obj is the R object that contains the results from reading in the phenotype data. The file is located in the working directory so there is no need to specify the full path, just the file name is suffice.

Dealing with missing trait data:

AM deals automatically with individuals with missing trait data. These individuals are removed from the analysis and a warning message is generated.

Dealing with missing fixed effects values:

AM deals automatically with individuals with missing fixed effects values. These individuals are removed from the analysis and a warning message is generated

Value

a data frame is returned of the phenotype data. If header is true, the names of the columns will be as specified by the first row of the phenotype file. If header is FALSE, generic names are supplied by R in the form of V1, V2, etc. If no column headings are given, these generic names will need to be used in the trait and fformula parameters in AM. You can print out the column names of the data frame by using

```
names(pheno_obj)
```

The column names are also printed along with other summary information when ReadPheno is run.

See Also

ReadMarker for reading in marker data, AM for performing association mapping.

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Examples

```
# Read in phenotype data from ./extdata/
# find the full location of the phenotype data
complete.name <- system.file('extdata', 'pheno.txt', package='Eagle')
pheno_obj <- ReadPheno(filename=complete.name)
## print a couple of lines of the data file
head(pheno_obj)</pre>
```

ReadZmat

Read Z matrix

Description

Read in the Z matrix that assigns groups/strains/lines to their trait measurements.

Usage

```
ReadZmat(filename = NULL)
```

Arguments

filename

contains the name of the Z matrix file. The file name needs to be in quotes.

Details

The underlying linear mixed model is of the form

$$Y = X\beta + Zu_q + e$$

where Z is a (n x n_g) matrix that contains ones and zeros, n is the number of trait measurements, and n_g is the number of groups/strains/lines. If n and n_g are the same, then there is no need to specify Z.

A space separated text file is assumed. Each row of the matrix contains multiple zeroes but only a single one. The file cannot contain column or row headings.

Value

a data matrix is returned of the Z matrix.

See Also

ReadMarker and ReadPheno.

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Examples

```
# Read in example Z matrix from ./extdata/
# find the full location of the Z matrix data
complete.name <- system.file('extdata', 'Z.txt', package='Eagle')
# read in Z matrix data
Z_obj <- ReadZmat(filename=complete.name)
# look at first few rows of the Z matrix file
head(Z_obj)</pre>
```

SummaryAM

Summary of multiple locus association mapping results

Description

A summary function that provides additional information on the significant marker-trait associations found by ${\sf AM}$

Usage

```
SummaryAM(AMobj = NULL, pheno = NULL, geno = NULL, map = NULL)
```

Arguments

AMobj	the (list) object obtained from running AM. Must be specified.
pheno	the (data frame) object obtained from running ReadPheno. Must be specified.
geno	the (list) object obtained from running ReadMarker. Must be specified.
map	the (data frame) object obtained from running ReadMap. The default is to assume a map object has not been supplied. Optional.

Details

SummaryAM produces two tables of results. First, a table of results is produced with the additive effect size and p-value for each fixed effect in the final model. Second, a table of results is produced with the proportion of phenotypes variance explained by the different multiple-locus models. Each row in this table is the proportion of phenotype variance after the marker locus has been added to the multiple locus model. Our calculations of variance explained are based on Sun et al. (2010).

References

Sun G., Zhu C., Kramer MH., Yang S-S., et al. 2010. Variation explained in mixed model association mapping. Heredity 105, 330-340.

See Also

AM

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```
# Since the following code takes longer than 5 seconds to run, it has been tagged as dontrun.
 # However, the code can be run by the user.
 #-----
 # read the map
 # File is a plain space separated text file with the first row
 # the column headings
 complete.name <- system.file('extdata', 'map.txt',</pre>
                                package='Eagle')
 map_obj <- ReadMap(filename=complete.name)</pre>
 # to look at the first few rows of the map file
head(map_obj)
 # read marker data
 #-----
 # Reading in a PLINK ped file
 # and setting the available memory on the machine for the reading of the data to 8 gigabytes
 complete.name <- system.file('extdata', 'geno.ped',</pre>
                                  package='Eagle')
 geno_obj <- ReadMarker(filename=complete.name, type='PLINK', availmemGb=8)</pre>
 #-----
 # read phenotype data
 # Read in a plain text file with data on a single trait and two fixed effects
 # The first row of the text file contains the column names y, cov1, and cov2.
 complete.name <- system.file('extdata', 'pheno.txt', package='Eagle')</pre>
 pheno_obj <- ReadPheno(filename=complete.name)</pre>
 #-----
 # Perform multiple-locus genome-wide association mapping
 res <- AM(trait = 'y',
                        fformula=c("cov1 + cov2"),
                        map = map_obj,
                        pheno = pheno_obj,
                        geno = geno_obj, availmemGb=8)
 #-----
 # Produce additional summary information
 #-----
 SummaryAM(AMobj=res, pheno=pheno_obj, geno=geno_obj, map=map_obj)
## End(Not run)
```

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