Package ‘MAGNAMWAR’

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phenotype variations in meta-genome with association studies. Follows
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Author  Corinne Sexton [aut],
        John Chaston [aut, cre],
        Hayden Smith [ctb]
Maintainer  John Chaston <john_chaston@byu.edu>
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after_ortho_format

**after_ortho_format**  
*Formatted output of OrthoMCL.*

**Description**

A list created by inputting the output of OrthoMCL clusters into the FormatAfterOrtho function.

**Usage**

`after_ortho_format`

**Format**

List of 2: (1) presence absence matrix, (2) protein ids:

- **pa_matrix**  matrix showing taxa presence/absence in OG
- **proteins**  matrix listing protein_id contained in each OG
after_ortho_format_grps

formatted output of OrthoMCL.

Description

A list created by inputting the output of OrthoMCL clusters into the FormatAfterOrtho function.

Usage

after_ortho_format_grps

Format

List of 2: (1) presence absence matrix, (2) protein ids:

- pa_matrix matrix showing taxa presence/absence in OG
- proteins matrix listing protein_id contained in each OG

AnalyzeOrthoMCL

Main OrthoMCL Analysis

Description

Main function for analyzing the statistical association of OG (orthologous group) presence with phenotype data.

Usage

AnalyzeOrthoMCL(mcl_data, pheno_data, model, species_name, resp = NULL, fix2 = NULL, rndm1 = NULL, rndm2 = NULL, multi = 1, time = NULL, event = NULL, time2 = NULL, startnum = 1, stopnum = "end", output_dir = NULL, sig_digits = NULL, princ_coord = 0)

Arguments

- mcl_data output of FormatAfterOrtho; a list of matrices; (1) a presence/absence matrix of taxa per OG, (2) a list of the specific protein ids within each OG
- pheno_data a data frame of phenotypic data with specific column names used to specify response variable as well as other fixed and random effects
AnalyzeOrthoMCL

model
linear model with gene presence as fixed effect (lm), linear mixed mfect models
with gene presence as fixed effect and additional variables specified as: one ran-
dom effect (lmeR1); two independent random effects (lmeR2iind); two random
effects with rndm2 nested in rndm1 (lmeR2nest); or two independent random
effects with one additional fixed effect (lmeF2); Wilcox Test with gene presence
as fixed effect (wx); Survival Tests with support for multi core design: with two
random effects (survmulti), and with two times as well as an additional fixed
variable (survmulticensor)

species_name
Column name in pheno_data containing 4-letter species designations

resp
Column name in pheno_data containing response variable

fix2
Column name in pheno_data containing second fixed effect

rndm1
Column name in pheno_data containing first random variable

rndm2
Column name in pheno_data containing second random variable

multi
(can only be used with survival tests) Number of cores

time
(can only be used with survival tests) Column name in pheno_data containing
first time

event
(can only be used with survival tests) Column name in pheno_data containing
event

time2
(can only be used with survival tests) Column name in pheno_data containing
second time

startnum
number of test to start on

stopnum
number of test to stop on

output_dir
(if using survival tests) directory where small output files will be placed before
using SurvAppendMatrix. Must specify a directory if choosing to output small
files, else only written as a matrix

sig_digits
amount of digits to display for p-values and means of data; default to NULL (no
rounding)

princ_coord
the number of principle coordinates to be included in model as fixed effects (1,
2, or 3), if a decimal is specified, as many principal coordinates as are needed to
account for that percentage of the variance will be included in the analysis

Value
A matrix with the following columns: OG, p-values, Bonferroni corrected p-values, mean
pheno-type of OG-containing taxa, mean phenotype of OG-lacking taxa, taxa included in OG, taxa not
included in OG

Examples

#Linear Model

# Not run:
mcl_mtrx <- AnalyzeOrthoMCL(after_ortho_format, pheno_data, 'lm',
'Treatment', resp='RespVar')
AnalyzeOrthoMCL

# End(Not run)

# the rest of the examples are not run for time's sake
#Linear Mixed Effect with one random effect
## Not run:
mcl_mtrx <- AnalyzeOrthoMCL(after_ortho_format, pheno_data, 'lmeR1',
'Treatment', resp='RespVar', rndm1='Experiment')

## End(Not run)

#Linear Mixed Effect with two independent random effects
## Not run:
mcl_mtrx <- AnalyzeOrthoMCL(after_ortho_format, pheno_data, 'lmeR2ind',
'Treatment', resp='RespVar', rndm1='Experiment', rndm2='Vial')

## End(Not run)

#Linear Mixed Effect with rndm2 nested in rndm1
## Not run:
mcl_mtrx <- AnalyzeOrthoMCL(after_ortho_format, pheno_data, 'lmeR2nest',
'Treatment', resp='RespVar', rndm1='Experiment', rndm2='Vial')

## End(Not run)

#Linear Mixed Effect with two independent random effects and one additional fixed effect
## Not run:
mcl_mtrx3 <- AnalyzeOrthoMCL(after_ortho_format, pheno_data, 'lmeF2',
'Treatment', resp='RespVar', fix2='Treatment', rndm1='Experiment', rndm2='Vial', princ_coord = 4)

## End(Not run)

#Wilcoxon Test
## Not run:
mcl_mtrx <- AnalyzeOrthoMCL(after_ortho_format, pheno_data, 'wx',
'Treatment', resp='RespVar')

## End(Not run)

# ~ 5 minutes
#Survival with two independent random effects, run on multiple cores
## Not run:
mcl_mtrx <- AnalyzeOrthoMCL(after_ortho_format, starv_pheno_data, 'TRT', model='survmulti',
time='t2', event='event', rndm1='EXP', rndm2='VIAL', multi=1)

## End(Not run)

# ~ 5 minutes
#Survival with two independent random effects and one additional fixed effect,
#including drops on multi cores
## Not run:
mcl_mtrx <- AnalyzeOrthoMCL(after_ortho_format, starv_pheno_data, 'TRT', model='survmulticensor',
  time='t1', time2='t2', event='event', rndm1='EXP', rndm2='VIAL', fix2='BACLO', multi=1)

## End(Not run)
#to be appended with SurvAppendMatrix

---

### CalculatePrincipalCoordinates

*Show Principal Components Breakdown*

**Description**

Function to show Principal Components statistics based on the OrthoMCL presence absence groupings.

**Usage**

```r
CalculatePrincipalCoordinates(mcl_data)
```

**Arguments**

- `mcl_data` output of `FormatAfterOrtho` – list of 2 things– 1: binary matrix indicating the presence / absence of genes in each OG and 2: vector of names of OGs

**Value**

returns a named list of principal components and accompanying proportion of variance for each

**Examples**

```r
CalculatePrincipalCoordinates(after_ortho_format)
```

---

### FormatAfterOrtho

*Format file from output of OrthoMCL algorithm before use in AnalyzeOrthoMCL*

**Description**

After running OrthoMCL and/or submitting to www.orthomcl.org, formats the output file to be used in `AnalyzeOrthoMCL`

**Usage**

```r
FormatAfterOrtho(file, format = "ortho")
```
**FormatMCLFastas**

**Arguments**

- **file**
  Path to the OrthoMCL output file

- **format**
  Specification of the method by which file was obtained: defaults to 'ortho' for output from orthomcl.org. Other option is 'groups' for output from local run of OrthoMCL software.

**Value**

a list of matrices; (1) a presence/absence matrix of taxa per OG, (2) a list of the specific protein ids within each OG

**Examples**

```r
file <- system.file('extdata', 'orthologGroups.txt', package='MAGNAMWAR')
after_ortho_format <- FormatAfterOrtho(file)

file_grps <- system.file('extdata', 'groups_example_r.txt', package='MAGNAMWAR')
after_ortho_format_grps <- FormatAfterOrtho(file_grps, format = 'groups')
```

---

**FormatMCLFastas**

Format all raw GenBank fastas to single OrthoMCL compatible fasta file

**Description**

Creates the composite fasta file for use in running OrthoMCL and/or submitting to www.orthomcl.org

**Usage**

```r
FormatMCLFastas(fa_dir, genbnk_id = 4)
```

**Arguments**

- **fa_dir**
  Path to the directory where all raw GenBank files are stored. Note, all file names must be changed to a 4-letter code representing each species and have '.fasta' file descriptor

- **genbnk_id**
  (Only necessary for the deprecated version of fasta headers) The index of the sequence ID in the GenBank pipe-separated annotation line (default: 4)

**Value**

Returns nothing, but prints the path to the final OrthoMCL compatible fasta file
**Examples**

```r
## Not run:
dir <- system.file('extdata', 'fasta_dir', package='MAGNAMWAR')
dir <- paste(dir,'/','sep='')
formatted_file <- FormatMCLFastas(dir)

## End(Not run)
```

---

**joined_mtrx**

*Final output of join_repset.*

**Description**

A data frame containing the final results of statistical analysis with protein ids, annotations, and sequences added.

**Usage**

`joined_mtrx`

**Format**

A data frame with 17 rows and 11 variables:

- **OG** taxa cluster id, as defined by OrthoMCL
- **pval1** p-value, based on presence absence
- **corrected_pval1** Bonferroni p-value, corrected by number of tests
- **mean_OGContain** mean of all taxa phenotypes in that OG
- **mean_OGLack** mean of all taxa phenotypes not in that OG
- **taxa_contain** taxa in that cluster
- **taxa_miss** taxa not in that cluster
- **rep_taxon** randomly selected representative taxa from the cluster
- **rep_id** protein id, from randomly selected representative taxa
- **rep_annot** fasta annotation, from randomly selected representative taxa
- **rep_seq** AA sequence, from randomly selected representative taxa
**joined_mtrx_grps**

*Final output of join_repset.*

**Description**

A data frame containing the final results of statistical analysis with protein ids, annotations, and sequences added.

**Usage**

`joined_mtrx_grps`

**Format**

A data frame with 10 rows and 11 variables:

- **OG** taxa cluster id, as defined by OrthoMCL
- **pval1** p-value, based on presence absence
- **corrected_pval1** Bonferroni p-value, corrected by number of tests
- **mean_OGContain** mean of all taxa phenotypes in that OG
- **mean_OGLack** mean of all taxa phenotypes not in that OG
- **taxa_contain** taxa in that cluster
- **taxa_miss** taxa not in that cluster
- **rep_taxon** randomly selected representative taxa from the cluster
- **rep_id** protein id, from randomly selected representative taxa
- **rep_annot** fasta annotation, from randomly selected representative taxa
- **rep_seq** AA sequence, from randomly selected representative taxa

**JoinRepSeq**

*Join Representative Sequences*

**Description**

Joins the OrthoMCL output matrix to representative sequences

**Usage**

`JoinRepSeq(mcl_data, fa_dir, mcl_mtrx, fastaformat = "new")`
Arguments

mcl_data  output of FormatAfterOrtho; a list of matrices; (1) a presence/absence matrix of taxa per OG, (2) a list of the specific protein ids within each OG
fa_dir Path to the directory where all raw GenBank files are stored. Note, all file names must be changed to a 4-letter code representing each species and have '.fasta' file descriptor
mcl_mtrx  OrthoMCL output matrix from AnalyzeOrthoMCL()
fastaformat options: new & old; new = no GI numbers included; defaults to new

Value

Returns the original OrthoMCL output matrix with additional columns: representative sequence taxon, representative sequence id, representative sequence annotation, representative sequence

Examples

```r
## Not run:
dir <- system.file('extdata', 'fasta_dir', package='MAGNAMWAR')
dir <- paste(dir,'/','sep=')
joined_mtrx_grps <- JoinRepSeq(after_ortho_format_grps, dir, mcl_mtrx_grps, fastaformat = 'old')
## End(Not run)
```

ManhatGrp  Manhattan Plot of All Taxa

Description

Manhattan plot that graphs all p-values for taxa.

Usage

`ManhatGrp(mcl_data, mcl_mtrx, tree = NULL)`

Arguments

mcl_data  FormatAfterOrtho output
mcl_mtrx  output of AnalyzeOrthoMCL()
tree tree file optional, used for ordering taxa along x axis

Value

a manhattan plot
**References**

Some sort of reference

**Examples**

```r
ManhatGrp(after_ortho_format, mcl_mtrx)
```

```ruby
#@param equation of line of significance, defaults to -log10((.05)/dim(pdgs)[1])
```

---

**mcl_mtrx**

*Final output of AnalyzeOrthoMCL*

**Description**

A matrix containing the final results of statistical analysis.

**Usage**

```r
mcl_mtrx
```

**Format**

A matrix with 17 rows and 7 variables:

- **OG** taxa cluster id, as defined by OrthoMCL
- **pval1** p-value, based on presence absence
- **corrected_pval1** Bonferroni p-value, corrected by number of tests
- **mean_OGContain** mean of all taxa phenotypes in that OG
- **mean_OGLack** mean of all taxa phenotypes not in that OG
- **taxa_contain** taxa in that cluster
- **taxa_miss** taxa not in that cluster

---

**mcl_mtrx_grps**

*Final output of AnalyzeOrthoMCL*

**Description**

A matrix containing the final results of statistical analysis.

**Usage**

```r
mcl_mtrx_grps
```
Format

A matrix with 10 rows and 7 variables:

- **OG**: taxa cluster id, as defined by OrthoMCL
- **pval1**: p-value, based on presence absence
- **corrected_pval1**: Bonferroni p-value, corrected by number of tests
- **mean_OGContain**: mean of all taxa phenotypes in that OG
- **mean_OGLack**: mean of all taxa phenotypes not in that OG
- **taxa_contain**: taxa in that cluster
- **taxa_miss**: taxa not in that cluster

---

**PDGPlot**  
*Plot of a PDG and Data with Standard Error Bars*

Description

Bar plot of PDG vs phenotype data with presence of taxa in PDG indicated by color

Usage

```r
PDGPlot(data, mcl_matrix, OG = "NONE", species_colname, data_colname, 
   xlab = "Taxa", ylab = "Data", ylimit = NULL, tree = NULL, 
   order = NULL, main_title = NULL)
```

Arguments

- **data**: R object of phenotype data
- **mcl_matrix**: AnalyzeOrthoMCL output
- **OG**: optional parameter, a string with the name of chosen group (OG) to be colored
- **species_colname**: name of column in phenotypic data file with taxa designations
- **data_colname**: name of column in phenotypic data file with data observations
- **xlab**: string to label barplot’s x axis
- **ylab**: string to label barplot’s y axis
- **ylimit**: optional parameter to limit y axis
- **tree**: optional parameter (defaults to NULL) Path to tree file, orders the taxa by phylogenetic distribution, else it defaults to alphabetical
- **order**: vector with order of taxa names for across the x axis (defaults to alpha ordering)
- **main_title**: string for title of the plot (defaults to OG)

Value

A barplot with taxa vs phenotypic data complete with standard error bars
**Examples**

```r
PDGPlot(pheno_data, mcl_mtrx, 'OG5_126778', 'Treatment', 'RespVar', ylimit=12)
```

<table>
<thead>
<tr>
<th>PDGvOG</th>
<th>Number of PDGs vs OGs/PDG</th>
</tr>
</thead>
</table>

**Description**

Barplot that indicates the number of PDGs vs OGs (clustered orthologous groups) in a PDG

**Usage**

```r
PDGvOG(mcl_data, num = 40, ...)
```

**Arguments**

- `mcl_data`: FormatAfterOrtho output
- `num`: an integer indicating where the x axis should end and be compiled
- `...`: args to be passed to barplot

**Value**

A barplot with a height determined by the second column and the first column abbreviated to accommodate visual spacing

**Examples**

```r
PDGvOG(after_ortho_format_grps,2)
```

<table>
<thead>
<tr>
<th>pheno_data</th>
<th>Triglyceride (TAG) content of fruit flies dataset.</th>
</tr>
</thead>
</table>

**Description**

A subset of the TAG content of fruit flies, collected in the Chaston Lab, to be used as a brief example for tests in AnalyzeOrthoMCL.

**Usage**

```r
pheno_data
```
Format

A data frame with 586 rows and 4 variables:

- **Treatment** 4-letter taxa designation of associated bacteria
- **RespVar** response variable, TAG content
- **Vial** random effect variable, vial number of flies
- **Experiment** random effect variable, experiment number of flies

Description

Presents data for each taxa including standard error bars next to a phylogenetic tree.

Usage

```r
PhyDataError(phy, data, mcl_matrix, species_colname, data_colname, 
color = NULL, OG = NULL, xlabel = "xlabel", ...)
```

Arguments

- **phy** Path to tree file
- **data** R object of phenotype data
- **mcl_matrix** AnalyzeOrthoMCL output
- **species_colname** name of column in data file with taxa designations
- **data_colname** name of column in data file with data observations
- **color** optional parameter, (defaults to NULL) assign colors to individual taxa by providing file (format: Taxa | Color)
- **OG** optional parameter, (defaults to NULL) a string with the names of chosen group to be colored
- **xlabel** string to label barplot’s x axis
- **...** argument to be passed from other methods such as parameters from barplot() function

Value

A phylogenetic tree with a barplot of the data (with standard error bars) provided matched by taxa.

References

Some sort of reference
**PrintOGSeqs**

**Examples**

```r
file <- system.file("extdata", "muscle_tree2.dnd", package="MAGNAMWAR")
PhyDataError(file, pheno_data, mcl_mtrx, species_colname = "Treatment", data_colname = "RespVar",
OG="OG5_126778", xlabel="TAG Content")
```

---

**PrintOGSeqs**

Print OG Sequences

**Description**

Print all protein sequences and annotations in a given OG

**Usage**

`PrintOGSeqs(after_ortho, OG, fasta_dir, out_dir = NULL, outfile = "none")`

**Arguments**

- `after_ortho`: output from FormatAfterOrtho
- `OG`: name of OG
- `fasta_dir`: directory to fastas
- `out_dir`: complete path to output directory
- `outfile`: name of file that will be written to

**Value**

A fasta file with all protein sequences and ids for a given OG

**Examples**

```r
## Not run:
OG <- 'OG5_126968'
file <- system.file('extdata', 'muscle_tree2.dnd', package='MAGNAMWAR')
OG <- system.file('extdata', 'fasta_dir', package='MAGNAMWAR')
dir <- paste(dir, '/', sep='')
PrintOGSeqs(after_ortho_format, OG, dir)

## End(Not run)
```
QQPlotter

Description

 Makes a qqplot of the p-values obtained through AnalyzeOrthoMCL

Usage

 QQPlotter(mcl_mtrx)

Arguments

 mcl_mtrx matrix generated by AnalyzeOrthoMCL

Value

 a qqplot of the p-values obtained through AnalyzeOrthoMCL

References

 Some sore of reference

Examples

 QQPlotter(mcl_mtrx)

RASTtoGBK

Write RAST files to Genbank formats OrthoMCL Analysis

Description

 Useful for reformating RAST files to GBK format

Usage

 RASTtoGBK(input_fasta, input_reference, out_name_path)

Arguments

 input_fasta path to input fasta file
 input_reference path to a .csv file; it should be downloaded from RAST as excel format, saved as a .csv (saved as the tab-delimited version has compatibility problems)
 out_name_path name and path of the file to write to
Examples

```r
## Not run:
lfrc_fasta <- system.file('extdata', 'RASTtoGBK/lfrc.fasta', package='MAGNAMWAR')
lfrc_reference <- system.file('extdata', 'RASTtoGBK/lfrc_lookup.csv', package='MAGNAMWAR')
lfrc_path <- system.file('extdata', 'RASTtoGBK/lfrc_out.fasta', package='MAGNAMWAR')

RASTtoGBK(lfrc_fasta, lfrc_reference, lfrc_path)

## End(Not run)
```

---

**starv_pheno_data**

*Starvation rate of fruit flies dataset.*

Description

A subset of the Starvation rate of fruit flies, collected in the Chaston Lab, to be used as a brief example for survival tests in AnalyzeOrthoMCL.

Usage

```r
starv_pheno_data
```

Format

A matrix with 543 rows and 7 variables:

- **EXP** random effect variable, experiment number of flies
- **VIAL** random effect variable, vial number of flies
- **BACLO** fixed effect variable, loss of bacteria in flies
- **TRT** 4-letter taxa designation of associated bacteria
- **t1** time 1
- **t2** time 2
- **event** event
## SurvAppendMatrix  Append Survival Test Outputs

### Description

Function used to append all .csv files that are outputted from AnalyzeOrthoMCL into one matrix.

### Usage

```r
SurvAppendMatrix(work_dir, out_name = "surv_matrix.csv", out_dir = NULL)
```

### Arguments

- **work_dir**: the directory where the output files of AnalyzeOrthoMCL are located
- **out_name**: file name of outputted matrix
- **out_dir**: the directory where the outputted matrix is placed

### Value

A csv file containing a matrix with the following columns: OG, p-values, Bonferroni corrected p-values, mean phenotype of OG-containing taxa, mean phenotype of OG-lacking taxa, taxa included in OG, taxa not included in OG

### Examples

```r
## Not run:
file <- system.file("extdata", "outputs", package="MAGNAMWAR")
directory <- paste(file, "/", sep = ")
SurvAppendMatrix(directory)

## End(Not run)
```

## WriteMCL  Print analyzed matrix

### Description

Writes a tab separated version of the analyzed OrthoMCL data with or without the joined representative sequences

### Usage

```r
WriteMCL(mtrx, filename)
```
Arguments

mtrx Matrix derived from AnalyzeOrthoMCL
filename File name to save final output

Value

The path to the written file

Examples

## Not run:
WriteMCL(mcl_mtrx, 'matrix.tsv')
#mcl_mtrx previously derived from AnalyzeOrthoMCL() or join_repset()

## End(Not run)
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