Package 'miRtest'

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Title combined miRNA- and mRNA-testing

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Type Package

LazyLoad yes

Author Stephan Artmann, Klaus Jung, Tim Beissbarth

Description combined miRNA- and mRNA-testing

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Depends corpcor, MASS

Imports globaltest, GlobalAncova, limma, RepeatedHighDim

Collate 'miRtest.R'

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R topics documented:

`A` *A*

Description

Part of expression data from Nielsen et al.

Author(s)

Stephan Artmann taken from Nielsen et al.

`contingency.table` *Contingency table.*

Description

Contingency table. Necessary for Fisher test.

Usage

```
contingency.table(gene.set, p.val, sign=0.05)
```

Arguments

`gene.set`	Vector of gene sets.
`p.val`	Vector with p-values.
`sign`	Significane threshold.

Author(s)

Stephan Artmann

fisher.combination *Fisher method of p value combination.*

Description

Fisher method of p value combination.

Usage

```
fisher.combination(p1, p2, check.range=FALSE)
```

Arguments

p1,p2	one-sided p-values that shall be combined.
check.range	If set to "TRUE" values above 1 will be set to 1.

Value

Combined p-value.

Author(s)

Stephan Artmann

generate.A *Turn a data...*

Description

Turn a data.frame indicating gene sets into the allocation matrix.

Usage

```
generate.A(df, X, Y, verbose=TRUE)
```

Arguments

df	data.frame with mRNAs in its first and miRNAs in its second column.
X	Expression matrix of miRNAs whose row names will be used to generate the list of miRNAs.
Y	Expression matrix of mRNAs whose row names will be used to generate the list of mRNAs.
verbose	Logical. Shall progress be printed?

Value

Allocation matrix A necessary for "miR.test" function.

Author(s)

Stephan Artmann

Examples

```
#######################################
### Generate random expression data ###
#######################################
# Generate random miRNA expression data of 3 miRNAs
# with 8 replicates
set.seed(1)
X = rnorm(24);
dim(X) = c(3,8);
rownames(X) = 1:3;
# Generate random mRNA expression data with 20 mRNAs
# and 10 replicates
Y = rnorm(200);
dim(Y) = c(20,10);
rownames(Y) = 1:20;
# Let's assume that we want to compare 2 miRNA groups, each of 4 replicates:
group.miRNA = factor(c(1,1,1,1,2,2,2,2));
# ... and that the corresponding mRNA experiments had 5 replicates in each group
group.mRNA = factor(c(1,1,1,1,1,2,2,2,2,2));
####################
### Perform Test ###
####################
library(miRtest)
#Let miRNA 1 attack mRNAs 1 to 9 and miRNA 2 attack mRNAs 10 to 17.
# mRNAs 18 to 20 are not attacked. miRNA 3 has no gene set.
miR = c(rep(1,9),c(rep(2,8)));
mRNAs = 1:17;
A = data.frame(mRNAs,miR); # Note that the miRNAs MUST be in the second column!
A
set.seed(1)
P = miR.test(X,Y,A,group.miRNA,group.mRNA)
P


#####################################################
### For a faster result: use other gene set tests ###
#####################################################
# Wilcoxon two-sample test is recommended for fast results
# Note that results may vary depending on how much genes correlate

P.gsWilcox = miR.test(X,Y,A,group.miRNA,group.mRNA,gene.set.tests="W")
P.gsWilcox
###########################################
### We can use an allocation matrix as A ###
```

```
##########################################
A = generate.A(A,X=X,Y=Y,verbose=FALSE);
A
# Now we can test as before
set.seed(1)
P = miR.test(X,Y,A,group.miRNA,group.mRNA,allocation.matrix=TRUE)
P


####################
### Other Designs ###
####################

# Some more complicated designs are implemented, check the vignette "miRtest" for details.
group.miRNA = 1:8
group.mRNA = 1:10
covariable.miRNA = factor(c(1,2,3,4,1,2,3,4))    ### A covariable in miRNAs.
covariable.mRNA = factor(c(1,2,3,4,5,1,2,3,4,5)) ### A covariable in mRNAs.

library(limma)
design.miRNA = model.matrix(~group.miRNA + covariable.miRNA)
design.mRNA =  model.matrix(~group.mRNA + covariable.mRNA)

P = miR.test(X,Y,A,design.miRNA=design.miRNA,design.mRNA=design.mRNA,allocation.matrix=TRUE)
P
```

`gs.test` *Internal function for gene set testing.*

Description

Internal function for gene set testing.

Usage

```
gs.test(A, X, Y, group, tests, permutation=FALSE, nrot=1000, design,
    allocation.matrix=FALSE, verbose=FALSE)
```

Arguments

`A`	Allocation matrix as in "miR.test" function.
`X`	miRNA expression matrix as in 'miR.test' function. Only necessary when allocation.matrix=TRUE.
`Y`	mRNA expression matrix as in "miR.test" function.
`group`	group as in 'miR.test' function
`tests`	Test applied, sie gene.set.tests
`permutation`	Shall permutation procedure for global tests be applied? Put 'FALSE' to use approximate results or give a number for the number of permutations.

`nrot` Number of rotations of rotation tests. Defaults to 1000 to be able to show p-values as low as 10^-3.

`design` If specified, group will be ignored. Design matrix as used in 'limma' package. Cannot be used with global tests.

`allocation.matrix`
Logical, is A an allocation matrix with mRNAs in its columns and miRNAs in its rows, or is it an allocation data.frame?

`verbose` Defaults to FALSE. If TRUE, progress is printed.

Value

List of the following, for up- and for down-regulation: Matrix with testing results for every gene set in its rows and the applied gene set test in its columns.

Author(s)

Stephan Artmann

References

Artmann, Stephan and Jung, Klaus and Bleckmann, Annalen and Beissbarth, Tim (2012). Detection of simultaneous group effects in microRNA expression and related functional gene sets. PLoS ONE 7(6):e38365, PMID: 22723856.

Brunner, E. (2009) Repeated measures under non-sphericity. Proceedings of the 6th St. Petersburg Workshop on Simulation, 605-609.

Jelle J. Goeman, Sara A. van de Geer, Floor de Kort, Hans C. van Houwelingen (2004) A global test for groups of genes: testing association with a clinical outcome. Bioinformatics 20, 93-99.

Jung, Klaus and Becker, Benjamin and Brunner, Edgar and Beissbarth, Tim (2011). Comparison of Global Tests for Functinoal Gene Sets in Two-Group Designs and Selection of Potentially Effect-causing Genes. Bioinformatics, 27: 1377-1383.

Majewski, IJ, Ritchie, ME, Phipson, B, Corbin, J, Pakusch, M, Ebert, A, Busslinger, M, Koseki, H, Hu, Y, Smyth, GK, Alexander, WS, Hilton, DJ, and Blewitt, ME (2010). Opposing roles of polycomb repressive complexes in hematopoietic stem and progenitor cells. _Blood_, published online 5 May 2010.

Mansmann, U. and Meister, R., 2005, Testing differential gene expression in functional groups, _Methods Inf Med_ 44 (3).

Smyth, G. K. (2004). Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. _Statistical Applications in Genetics and Molecular Biology_, Volume *3*, Article 3.

Wu, D, Lim, E, Francois Vaillant, F, Asselin-Labat, M-L, Visvader, JE, and Smyth, GK (2010). ROAST: rotation gene set tests for complex microarray experiments. _Bioinformatics_, published online 7 July 2010.

inverse.normal.combination *Inverse-normal method for p value combination.*

Description

Inverse-normal method for p value combination.

Usage

```
inverse.normal.combination(p1, p2)
```

Arguments

p1,p2	one-sided p-values that shall be combined.

Value

Two-sided combined p-value.

Author(s)

Stephan Artmann

limma.one.sided *Internal algorithm: Make limma test one-sided...*

Description

Internal algorithm: Make limma test one-sided

Usage

```
limma.one.sided(fit, lower=FALSE)
```

Arguments

fit	Result of "lmFit" and "eBayes" functions in "limma" package.
lower	Shall one-sided p-value indicated down-regultation?

limma.test *internal algorithm for author's convenience.*

Description

internal algorithm for author's convenience. Create a linear model with the limma package.

Usage

```
limma.test(X, group, design)
```

Arguments

X	Expression matrix.
group	Group membership of replicates.
design	Design as specified in limma (design matrix, see model.matrix).

Author(s)

Stephan Artmann

m.combine *Internal function for author's convenience and more legible code.*

Description

Internal function for author's convenience and more legible code. Applies a function to every column vector of a matrix and a vector.

Usage

```
m.combine(M, v, FUN, ...)
```

Arguments

M	The matrix for whose column vectors mapply shall be used.
v	The vector.
FUN	The function.
...	Further arguments to be given to FUN.

Author(s)

Stephan Artmann

`miR.test` *Main Function of miRtest package.*

Description

Main Function of miRtest package.

Usage

```
miR.test(X, Y, A, group.miRNA, group.mRNA, gene.set.tests="romer",
    design.miRNA, design.mRNA, adjust="none", permutation=FALSE,
    nrot=1000, allocation.matrix=FALSE, verbose=FALSE, errors=TRUE)
```

Arguments

`X`	miRNA expression matrix with genes in rows and replicates in columns
`Y`	mRNA expression matrix with genes in rows and replicates in columns
`A`	Allocation data.frame or Allocation matrix. An allocation data.frame contains the mRNAs in its first column and the miRNAs in its second column. See vignette 'miRtest' for information on Allocation matrices.
`group.miRNA`	Vector of miRNA group membership, being either numeric or a factor (**this makes a difference**). E. g. if you have four replicates in a control group and three replicates in a treated group, you may choose c(1,1,1,1,2,2,2)
`design.miRNA`	If specified, group.miRNA will be ignored. Here you can specify a design matrix as it is returned from the model.matrix 'limma' function.
`design.mRNA`	If specified, group.mRNA will be ignored. Here you can specify a design matrix as it is returned from the model.matrix 'limma' function.
`group.mRNA`	Vector of mRNA group membership, being either numeric or a factor (**this makes a difference**).E. g. if you have four replicates in a control group and three replicates in a treated group, you may choose c(1,1,1,1,2,2,2)
`gene.set.tests`	Test to be applied for gene set testing. Can be one or more of the following: 'globaltest', 'GA', 'RHD', 'KS', 'W', 'Fisher', 'roast', 'romer', or 'all' if you want to do all tests.
`adjust`	Muliple hypothesis testing adjustment. Same options as in "p.adjust" function.
`permutation`	Number of permutations for 'globaltest' or 'GlobalAncova' gene set tests. Put to "FALSE" to use the approximate p-values instead of permutation ones.
`nrot`	Number of rotations for rotation tests 'ROAST' and 'romer'
`allocation.matrix`	Logical, is A an allocation matrix with mRNAs in its columns and miRNAs in its rows, or is it an allocation data.frame?
`verbose`	Defaults to FALSE. If TRUE, output on progress is printed.
`errors`	Defaults to TRUE. If set to FALSE, some errors checking correct sizes of matrices are turned into warning messages.

Value

Matrix with testing results for every miRNA in its rows and the applied gene set test in its columns. Note that result will depend on whether multiple hypothesis testing correction was applied or not.

Author(s)

Stephan Artmann

References

Artmann, Stephan and Jung, Klaus and Bleckmann, Annalen and Beissbarth, Tim (2012). Detection of simultaneous group effects in microRNA expression and related functional gene sets. PLoS ONE 7(6):e38365, PMID: 22723856.

Brunner, E. (2009) Repeated measures under non-sphericity. Proceedings of the 6th St. Petersburg Workshop on Simulation, 605-609.

Jelle J. Goeman, Sara A. van de Geer, Floor de Kort, Hans C. van Houwelingen (2004) A global test for groups of genes: testing association with a clinical outcome. Bioinformatics 20, 93-99.

Jung, Klaus and Becker, Benjamin and Brunner, Edgar and Beissbarth, Tim (2011). Comparison of Global Tests for Functinoal Gene Sets in Two-Group Designs and Selection of Potentially Effect-causing Genes. Bioinformatics, 27: 1377-1383.

Majewski, IJ, Ritchie, ME, Phipson, B, Corbin, J, Pakusch, M, Ebert, A, Busslinger, M, Koseki, H, Hu, Y, Smyth, GK, Alexander, WS, Hilton, DJ, and Blewitt, ME (2010). Opposing roles of polycomb repressive complexes in hematopoietic stem and progenitor cells. _Blood_, published online 5 May 2010.

Mansmann, U. and Meister, R., 2005, Testing differential gene expression in functional groups, _Methods Inf Med_ 44 (3).

Smyth, G. K. (2004). Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. _Statistical Applications in Genetics and Molecular Biology_, Volume *3*, Article 3.

Wu, D, Lim, E, Francois Vaillant, F, Asselin-Labat, M-L, Visvader, JE, and Smyth, GK (2010). ROAST: rotation gene set tests for complex microarray experiments. _Bioinformatics_, published online 7 July 2010.

Examples

```
#######################################
### Generate random expression data ###
#######################################
# Generate random miRNA expression data of 3 miRNAs
# with 8 replicates
set.seed(1)
X = rnorm(24);
dim(X) = c(3,8);
rownames(X) = 1:3;
# Generate random mRNA expression data with 20 mRNAs
# and 10 replicates
Y = rnorm(200);
```

```
dim(Y) = c(20,10);
rownames(Y) = 1:20;
# Let's assume that we want to compare 2 miRNA groups, each of 4 replicates:
group.miRNA = factor(c(1,1,1,1,2,2,2,2));
# ... and that the corresponding mRNA experiments had 5 replicates in each group
group.mRNA = factor(c(1,1,1,1,1,2,2,2,2,2));
####################
### Perform Test ###
####################
library(miRtest)
#Let miRNA 1 attack mRNAs 1 to 9 and miRNA 2 attack mRNAs 10 to 17.
# mRNAs 18 to 20 are not attacked. miRNA 3 has no gene set.
miR = c(rep(1,9),c(rep(2,8)));
mRNAs = 1:17;
A = data.frame(mRNAs,miR); # Note that the miRNAs MUST be in the second column!
A
set.seed(1)
P = miR.test(X,Y,A,group.miRNA,group.mRNA)
P


#####################################################
### For a faster result: use other gene set tests ###
#####################################################
# Wilcoxon two-sample test is recommended for fast results
# Note that results may vary depending on how much genes correlate

P.gsWilcox = miR.test(X,Y,A,group.miRNA,group.mRNA,gene.set.tests="W")
P.gsWilcox
############################################
### We can use an allocation matrix as A ###
############################################
A = generate.A(A,X=X,Y=Y,verbose=FALSE);
A
# Now we can test as before
set.seed(1)
P = miR.test(X,Y,A,group.miRNA,group.mRNA,allocation.matrix=TRUE)
P


#####################
### Other Designs ###
#####################

# Some more complicated designs are implemented, check the vignette "miRtest" for details.
group.miRNA = 1:8
group.mRNA = 1:10
covariable.miRNA = factor(c(1,2,3,4,1,2,3,4))    ### A covariable in miRNAs.
covariable.mRNA = factor(c(1,2,3,4,5,1,2,3,4,5)) ### A covariable in mRNAs.

library(limma)
design.miRNA = model.matrix(~group.miRNA + covariable.miRNA)
design.mRNA =  model.matrix(~group.mRNA + covariable.mRNA)
```

```
P = miR.test(X,Y,A,design.miRNA=design.miRNA,design.mRNA=design.mRNA,allocation.matrix=TRUE)
P
```

`miRtest` *Package Description: Two-group combined miRNA- and mRNA- expression testing.*

Description

Looking for differential expression in miRNA-data can have low power. Taking their respective mRNA-gene sets on the other hand can lead to too liberal results. In Artmann et al. we proposed a method to combine both information sources and generate p-values that can detect either miRNA- and target gene set expression differences.

Details

Package:	miRtest
Type:	Package
Version:	1.7
Date:	2014-05-07
License:	GPL
LazyLoad:	yes
URL:	http://www.ncbi.nlm.nih.gov/pubmed/22723856

For a detailed help check vignette("miRtest")

You can start the test with the "miR.test" function, which needs the expression matrix X of miRNAs, the expression matrix Y of mRNAs and the allocation matrix.

Author(s)

Stephan Artmann <stephanartmann@gmx.net>, Klaus Jung, Tim Beissbarth

Maintainer: Stephan Artmann <stephanartmann@gmx.net>

References

Artmann, Stephan and Jung, Klaus and Bleckmann, Annalen and Beissbarth, Tim (2012). Detection of simultaneous group effects in microRNA expression and related functional gene sets. Plos ONE, PMID: 22723856.

Brunner, E. (2009) Repeated measures under non-sphericity. Proceedings of the 6th St. Petersburg Workshop on Simulation, 605-609.

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Jung, Klaus and Becker, Benjamin and Brunner, Edgar and Beissbarth, Tim (2011). Comparison of Global Tests for Functinoal Gene Sets in Two-Group Designs and Selection of Potentially Effect-causing Genes. Bioinformatics, 27: 1377-1383.

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Mansmann, U. and Meister, R., 2005, Testing differential gene expression in functional groups, _Methods Inf Med_ 44 (3).

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Wu, D, Lim, E, Francois Vaillant, F, Asselin-Labat, M-L, Visvader, JE, and Smyth, GK (2010). ROAST: rotation gene set tests for complex microarray experiments. _Bioinformatics_, published online 7 July 2010.

See Also

Function "generate.A" as well as main function "miR.test"

Examples

```
#######################################
### Generate random expression data ###
#######################################
# Generate random miRNA expression data of 3 miRNAs
# with 8 replicates
set.seed(1)
X = rnorm(24);
dim(X) = c(3,8);
rownames(X) = 1:3;
# Generate random mRNA expression data with 20 mRNAs
# and 10 replicates
Y = rnorm(200);
dim(Y) = c(20,10);
rownames(Y) = 1:20;
# Let's assume that we want to compare 2 miRNA groups, each of 4 replicates:
group.miRNA = factor(c(1,1,1,1,2,2,2,2));
# ... and that the corresponding mRNA experiments had 5 replicates in each group
group.mRNA = factor(c(1,1,1,1,1,2,2,2,2,2));
####################
### Perform Test ###
####################
library(miRtest)
#Let miRNA 1 attack mRNAs 1 to 9 and miRNA 2 attack mRNAs 10 to 17.
# mRNAs 18 to 20 are not attacked. miRNA 3 has no gene set.
miR = c(rep(1,9),c(rep(2,8)));
mRNAs = 1:17;
A = data.frame(mRNAs,miR); # Note that the miRNAs MUST be in the second column!
A
```

```
set.seed(1)
P = miR.test(X,Y,A,group.miRNA,group.mRNA)
P


#####################################################
### For a faster result: use other gene set tests ###
#####################################################
# Wilcoxon two-sample test is recommended for fast results
# Note that results may vary depending on how much genes correlate

P.gsWilcox = miR.test(X,Y,A,group.miRNA,group.mRNA,gene.set.tests="W")
P.gsWilcox
###########################################
### We can use an allocation matrix as A ###
###########################################
A = generate.A(A,X=X,Y=Y,verbose=FALSE);
A
# Now we can test as before
set.seed(1)
P = miR.test(X,Y,A,group.miRNA,group.mRNA,allocation.matrix=TRUE)
P


####################
### Other Designs ###
####################

# Some more complicated designs are implemented, check the vignette "miRtest" for details.
group.miRNA = 1:8
group.mRNA = 1:10
covariable.miRNA = factor(c(1,2,3,4,1,2,3,4))    ### A covariable in miRNAs.
covariable.mRNA = factor(c(1,2,3,4,5,1,2,3,4,5)) ### A covariable in mRNAs.

library(limma)
design.miRNA = model.matrix(~group.miRNA + covariable.miRNA)
design.mRNA =  model.matrix(~group.mRNA + covariable.mRNA)

P = miR.test(X,Y,A,design.miRNA=design.miRNA,design.mRNA=design.mRNA,allocation.matrix=TRUE)
P
```

X *X*

Description

Part of expression data from Nielsen et al.

Author(s)

Stephan Artmann taken from Nielsen et al.

Y *Y*

Description

Part of expression data from Nielsen et al.

Author(s)

Stephan Artmann taken from Nielsen et al.

Index