Package 'anamiR'

April 14, 2017

Type Package

Title An integrated analysis package of miRNA and mRNA expression data

Version 1.0.1

Description This package is intended to identify potential
interactions of miRNA-target gene interactions from miRNA and
mRNA expression data. It contains functions for statistical
test, databases of miRNA-target gene interaction and functional
analysis.

biocViews Software, AssayDomain, GeneExpression, BiologicalQuestion,
GeneSetEnrichment, GeneTarget, Normalization, Pathways,
DifferentialExpression, GeneRegulation, ResearchField,
Genetics, Technology, Microarray, Sequencing, miRNA,
WorkflowStep

License GPL-2

URL https://github.com/AllenTiTaiWang/anamiR

BugReports https://github.com/AllenTiTaiWang/anamiR/issues

LazyData TRUE

Imports stats, DBI, limma, lumi, agricolae, RMySQL, DESeq2,
SummarizedExperiment, gplots

Depends R (>= 3.3.1), SummarizedExperiment(>= 1.1.6)

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Suggests knitr, rmarkdown

VignetteBuilder knitr

NeedsCompilation no

R topics documented:

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anamiR   *anamiR: An integrated analysis package of miRNA and mRNA expression data.*

Description

The anamiR package is used to identify miRNA-target genes interactions. The anamiR package provides a whole workflow, which contains important functions: 'normalization', 'differExp_discrete', 'negative_cor', 'miR_converter', 'database_support', 'enrichment'.

normalization

The normalization function is used to normalize the expression data with one of three methods, including normal, quantile, rank.invariant.

differExp_discrete

The differExp_discrete function is used to find the differential genes or miRNAs from given expression data with one of three statistical methods, including t.test, wilcox.test,limma and DESeq. The miRNA would remain if its p-value lower than the cutoff value.

miR_converter

The miR_annotation function is used to convert the older miRNA annotation to the miRBase 21 version.

negative_cor

The negative_cor function is used to identify the possible miRNA-target gene interactions from given miRNA and mRNA expression data by caculating the correlation coefficient between each miRNA and gene. interaction would remain if its correlation coefficient is negative and lower than cutoff value.

database_support

The database_support function would search information about miRNA-target gene interactions from an integrated database, which contains 8 algorithm predicted databases and 2 experiment validated databases. Eventually return a big table, which is in data.frame format and contains extra 10 columns for those 10 databases to count if interactions were predicted or validated by these databases.

enrichment

> The enrichment function is used to do the functional analysis from the output of 'database_support'. Not only p-value from hypergeometric test but empirical p-value from 10000 times of permutation would be provided by this function.

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database_support   *Intersect with databases for potential miRNA-target gene interactions*

Description

This function will interst potential miRNA-target gene interactions from the input matrix, which is generated by [negative_cor](#) or [miR_converter](#), with 8 predict databases and 2 validate databases about miRNA-target gene interactions. If the input caontains hundreds of interactions, it would take a few minutes to intersect all of them.

Usage

```
database_support(cor_data, org = "hsa", Sum.cutoff = 2)
```

Arguments

cor_data   matrix format generated from [negative_cor](#) or [miR_converter](#), including miRNA, gene, correlation coefficient for column names.

org   species of genes and miRNAs, only support "hsa", "mmu"

Sum.cutoff   a Threshold for total hits by predict databases. This one should not be greater than 8. Default is 2.

Value

data.frame format. Each row represent one potential interaction. The first four columns are information about interactions: miRNA, gene symbol, Ensembl ID, gene ID, as for column 5 to 12 represent the predict dataases, while column 13 to 14 are validate databases. if databases truly hit this interactions, the number in it would be 1. The column 'Sum' means total hits by 8 databases, and column 'Validate' would be TRUE if at least one validate database hit the interaction. Furthermore, 'Fold-Change' and 'P-adjust' can also be found in this output, and if the 'de novo' column contains 1 means that row is not supported by any databases. The column 'evidence' represents if the experiment for validation is strong or limited, considering <http://mirtarbase.mbc.nctu.edu.tw/>.

Examples

```
## Use the internal dataset
data("mirna", package = "anamiR", envir = environment())
data("pheno.mirna", package = "anamiR", envir = environment())
data("mrna", package = "anamiR", envir = environment())
data("pheno.mrna", package = "anamiR", envir = environment())

## SummarizedExperiment class
require(SummarizedExperiment)
mirna_se <- SummarizedExperiment(
 assays = SimpleList(counts=mirna),
 colData = pheno.mirna)
```

```
## SummarizedExperiment class
require(SummarizedExperiment)
mrna_se <- SummarizedExperiment(
 assays = SimpleList(counts=mrna),
 colData = pheno.mrna)

## Finding differential miRNA from miRNA expression data with t.test
mirna_d <- differExp_discrete(
   se = mirna_se,
   class = "ER",
   method = "t.test"
)

## Finding differential mRNA from mRNA expression data with t.test
mrna_d <- differExp_discrete(
   se = mrna_se,
   class = "ER",
   method = "t.test"
)

## Convert annotation to miRBse 21
mirna_21 <- miR_converter(data = mirna_d, original_version = 17)

## Correlation
cor <- negative_cor(mrna_data = mrna_d, mirna_data = mirna_21)

## Intersect with known databases
sup <- database_support(cor_data = cor)
```

---

differExp_continuous   *Find differential expression genes or miRNAs from given expression*
                              data

Description

This function will apply linear regression model to find differential expression genes or miRNAs
with continuous phenotype data,and then filter the genes or miRNAs (rows) which have bigger
p-value than cutoff.

Usage

```
differExp_continuous(se, class, log2 = FALSE, p_value.cutoff = 0.05)
```

Arguments

se	[SummarizedExperiment](#) for input format.
class	string. Choose one features from all rows of phenotype data.
log2	logical, if this data hasn't been log2 transformed yet, this one should be TRUE. Default is FALSE.
p_value.cutoff	an numeric value indicating a threshold of p-value for every genes or miRNAs (rows). Default is 0.05.

Value

data expression data in matrix format, with sample name in columns and gene symbol or miRNA name in rows.

See Also

[lm](#) for fitting linear models.

Examples

```
## Use the internal dataset
data("mirna", package = "anamiR", envir = environment())
data("pheno.mirna", package = "anamiR", envir = environment())

## SummarizedExperiment class
require(SummarizedExperiment)
mirna_se <- SummarizedExperiment(
 assays = SimpleList(counts=mirna),
 colData = pheno.mirna)

## Finding differential miRNA from miRNA expression data with lm
differExp_continuous(
    se = mirna_se, class = "Survival"
)
```

---

differExp_discrete   *Find differential expression genes or miRNAs from given expression data*

Description

This function will apply one of three statistical methods, including t.test, wilcox.test and limma, to find differential expression genes or miRNAs with, discrete phenotype data, and then filter the genes or miRNAs (rows) which have bigger p-value than cutoff.

Usage

```
differExp_discrete(se, class, method = c("t.test", "limma", "wilcox.test",
  "DESeq"), limma.trend = FALSE, t_test.var = FALSE, log2 = FALSE,
  p_value.cutoff = 0.05, p_adjust.method = "BH", foldchange = 0.5)
```

Arguments

se	[SummarizedExperiment](#) for input format.
class	string. Choose one features from all rows of phenotype data.
method	statistical method for finding differential genes or miRNAs, including "t.test", "wilcox.test", "limma". Default is "t.test".
limma.trend	logical, only matter when limma is chosen to be the method. From function [eBayes](#).

t_test.var	logical, only matter when limma is chosen to be the method. Whether to treat the two variances as being equal. From function `t.test`
log2	logical, if this data hasn't been log2 transformed yet, this one should be TRUE. Default is FALSE.
p_value.cutoff	an numeric value indicating a threshold of p-value for every genes or miRNAs (rows). Default is 0.05.
p_adjust.method	
	Correction method for multiple testing. (If you are using DESeq for method, this param would not affect the result) From function `p.adjust`. Default is "BH".
foldchange	an numeric value indicating a threshold of foldchange (log2) for every genes or miRNAs (rows). Default is 0.5.

Value

data expression data in matrix format, with sample name in columns and gene symbol or miRNA name in rows.

See Also

`t.test` for Student's t-Test; `wilcox.test` for Wilcoxon Rank Sum and Signed Rank Tests.

Examples

```
## Use the internal dataset
data("mirna", package = "anamiR", envir = environment())
data("pheno.mirna", package = "anamiR", envir = environment())

## SummarizedExperiment class
require(SummarizedExperiment)
mirna_se <- SummarizedExperiment(
 assays = SimpleList(counts=mirna),
 colData = pheno.mirna)

## Finding differential miRNA from miRNA expression data with t.test
mirna_d <- differExp_discrete(
   se = mirna_se,
   class = "ER",
   method = "t.test"
)
```

---

enrichment	*Enrich pathway with genes from potential miRNA-target gene interactions.*

Description

This function will do function analysis with genes from potential miRNA-target gene interactions in the input data.frame, which is generated by database_support, with total 4 kinds of pathway databases, including mouse and human two species, beseides, this function will permute 5000 times for each pathway to show an empirical p_value to avoid the bias from hypergeometric p-value, this indicating that it would take a few minutes to do functional analysis.

Usage

```
enrichment(data_support, org = "hsa", per_time = 5000)
```

Arguments

data_support	matrix format generated from [database_support](#).
org	species of genes and miRNAs, only support "hsa", "mmu"
per_time	Times of permutation about each enriched pathways, higher times, more precise empirical p-value user can obtain, meanwhile, this function would cost more time. Default is 5000.

Value

matrix format. There are 7 columns in it, including database, term, total genes of the term, targets in the term, targets in total genes of the term (p-value.

See Also

[phyper](#) for details.

Examples

```
## Use the internal dataset
data("mirna", package = "anamiR", envir = environment())
data("pheno.mirna", package = "anamiR", envir = environment())
data("mrna", package = "anamiR", envir = environment())
data("pheno.mrna", package = "anamiR", envir = environment())

## SummarizedExperiment class
require(SummarizedExperiment)
mirna_se <- SummarizedExperiment(
 assays = SimpleList(counts=mirna),
 colData = pheno.mirna)

## SummarizedExperiment class
require(SummarizedExperiment)
mrna_se <- SummarizedExperiment(
 assays = SimpleList(counts=mrna),
 colData = pheno.mrna)

## Finding differential miRNA from miRNA expression data with t.test
mirna_d <- differExp_discrete(
   se = mirna_se,
   class = "ER",
   method = "t.test"
)

## Finding differential mRNA from mRNA expression data with t.test
mrna_d <- differExp_discrete(
   se = mrna_se,
   class = "ER",
   method = "t.test"
)

## Convert annotation to miRBse 21
```

```
mirna_21 <- miR_converter(data = mirna_d, original_version = 17)

## Correlation
cor <- negative_cor(mrna_data = mrna_d, mirna_data = mirna_21)

## Intersect with known databases
sup <- database_support(cor_data = cor)

## Functional analysis
pat <- enrichment(data_support = sup, org = "hsa", per_time = 1000)
```

---

heat_vis   *Using correlation information to draw a heatmaps*

Description

This function would base on Fold-Change information from the output of negative_cor, differExp_discrete and show heatmaps to users. Note that if miRNA-gene interactions (row) from input are larger than 100, the lable in plot would be unclear.

Usage

```
heat_vis(cor_data, mrna_d, mirna_21)
```

Arguments

cor_data	matrix format generated from negative_cor.
mrna_d	differential expressed data in data.frame format, with sample name in columns and gene symbol in rows, which is generated by differExp_discrete or differExp_continuous.
mirna_21	differential expressed data in data.frame format, with sample name in columns and miRNAl in rows, which is generated by differExp_discrete or differExp_continuous, miRNA should be miRBase 21 version now.

Value

heatmap plots of miRNA and gene.

See Also

heatmap.2 for plot.

Examples

```
## Use the internal dataset
data("mirna", package = "anamiR", envir = environment())
data("pheno.mirna", package = "anamiR", envir = environment())
data("mrna", package = "anamiR", envir = environment())
data("pheno.mrna", package = "anamiR", envir = environment())

## SummarizedExperiment class
require(SummarizedExperiment)
```

```
mirna_se <- SummarizedExperiment(
 assays = SimpleList(counts=mirna),
 colData = pheno.mirna)

## SummarizedExperiment class
require(SummarizedExperiment)
mrna_se <- SummarizedExperiment(
 assays = SimpleList(counts=mrna),
 colData = pheno.mrna)

## Finding differential miRNA from miRNA expression data with t.test
mirna_d <- differExp_discrete(
   se = mirna_se,
   class = "ER",
   method = "t.test"
)

## Finding differential mRNA from mRNA expression data with t.test
mrna_d <- differExp_discrete(
   se = mrna_se,
   class = "ER",
   method = "t.test"
)

## Convert annotation to miRBse 21
mirna_21 <- miR_converter(data = mirna_d, original_version = 17)

## Correlation
cor <- negative_cor(mrna_data = mrna_d, mirna_data = mirna_21)

## Draw heatmap
heat_vis(cor, mrna_d, mirna_21)
```

---

mirna   *miRNA expression data about breast cancer*

Description

This miRNA expression dataset is originally from GSE19536. To make dataset smaller, we have selected 30 samples in columns and 489 miRNAs in rows.

Usage

```
mirna
```

Format

A data frame with 489 obs (miRNAs) and 30 variables:

BC.M.014 sample name, in column

BC.M.015 sample name, in column

BC.M.017 sample name, in column

BC.M.019 sample name, in column

BC.M.023 sample name, in column

BC.M.031 sample name, in column

BC.M.053 sample name, in column

BC.M.083 sample name, in column

BC.M.088 sample name, in column

BC.M.112 sample name, in column

BC.M.119 sample name, in column

BC.M.144 sample name, in column

BC.M.148 sample name, in column

BC.M.150 sample name, in column

BC.M.209 sample name, in column

BC.M.220 sample name, in column

BC.M.221 sample name, in column

BC.M.300 sample name, in column

BC.M.308 sample name, in column

BC.M.309 sample name, in column

BC.M.318 sample name, in column

BC.M.357 sample name, in column

BC.M.381 sample name, in column

BC.M.388 sample name, in column

BC.M.406 sample name, in column

BC.M.451 sample name, in column

BC.M.457 sample name, in column

BC.M.493 sample name, in column

BC.M.512 sample name, in column

BC.M.709 sample name, in column

Value

data.frame

Source

NCBI GEO: <http://www.ncbi.nlm.nih.gov/geo/>

---

miR_converter   *Convert miRNA annotation to the miRBase 21 version*

Description

This function will convert the miRNA names from the data frame, which is produced by differ-Exp_discrete, to the miRBase 21 version of miRNA annotation. If the input contains hundreds of miRNAs, it would take a few minutes to convert all of them.

Usage

```
miR_converter(data, remove_old = TRUE, original_version,
  latest_version = 21)
```

Arguments

data   expression data in data.frame format, with sample name in columns and miRNA name in rows.

remove_old   logical value, if the miRNA is deleted in miRBase 21, should it be removed from row? Default is TRUE.

original_version
                  the original version of miRNA in input matrix. This one is necessary.

latest_version   choose an interger under 21, and this function would convert miRNA annotation to that version. Default is 21.

Value

expression data in data.frame format, with sample name in columns and miRNA name for miRBase version 21 in rows.

Examples

```
## Use the internal dataset
data("mirna", package = "anamiR", envir = environment())
data("pheno.mirna", package = "anamiR", envir = environment())

## SummarizedExperiment class
require(SummarizedExperiment)
mirna_se <- SummarizedExperiment(
 assays = SimpleList(counts=mirna),
 colData = pheno.mirna)

## Finding differential miRNA from miRNA expression data with t.test
mirna_d <- differExp_discrete(
   se = mirna_se,
   class = "ER",
   method = "t.test"
)

## Convert annotation to miRBse 21
mirna_21 <- miR_converter(data = mirna_d, original_version = 17)
```

---

mrna   *mRNA expression data about breast cancer*

Description

This mRNA expression dataset is originally from GSE19536. To make dataset smaller, we have selected 30 samples in columns and 19210 genes in rows.

Usage

    mrna

Format

A data frame with 15000 rows (genes) and 30 variables:

BC.M.014 sample name, in column
BC.M.015 sample name, in column
BC.M.017 sample name, in column
BC.M.019 sample name, in column
BC.M.023 sample name, in column
BC.M.031 sample name, in column
BC.M.053 sample name, in column
BC.M.083 sample name, in column
BC.M.088 sample name, in column
BC.M.112 sample name, in column
BC.M.119 sample name, in column
BC.M.144 sample name, in column
BC.M.148 sample name, in column
BC.M.150 sample name, in column
BC.M.209 sample name, in column
BC.M.220 sample name, in column
BC.M.221 sample name, in column
BC.M.300 sample name, in column
BC.M.308 sample name, in column
BC.M.309 sample name, in column
BC.M.318 sample name, in column
BC.M.357 sample name, in column
BC.M.381 sample name, in column
BC.M.388 sample name, in column
BC.M.406 sample name, in column
BC.M.451 sample name, in column
BC.M.457 sample name, in column
BC.M.493 sample name, in column
BC.M.512 sample name, in column
BC.M.709 sample name, in column

Value

data.frame

Source

NCBI GEO: <http://www.ncbi.nlm.nih.gov/geo/>

---

multi_Differ	*Find differential expression groups of each genes or miRNA from expression data*

Description

This function will apply anova ,a statistical methods, for each gene or miRNA (row) to find not only whether expression data of multiple groups differential expressed or not, but also tell specifically two groups from all are differential expression.

Usage

```
multi_Differ(se, class, anova_p_value = 0.05, post_hoc = c("scheffe.test",
  "duncan.test", "HSD.test"), post_hoc_p_value = 0.05)
```

Arguments

se	[SummarizedExperiment](#) for input format.
class	string. Choose one features from all rows of phenotype data.
anova_p_value	an numeric value indicating a threshold of p-value from anova for every genes or miRNAs (rows). Default is 0.05.
post_hoc	post hoc test for anova, including "scheffe.test", "HSD.test", "duncan.test".
post_hoc_p_value	
	an numeric value indicating a threshold of p-value from post hoc test for every genes or miRNAs (rows). Default is 0.05.

Value

data.frame format with extra columns containing information about differential expressed groups among all.

See Also

[aov](#) for fit an analysis of variance model.

Examples

```
## Use the internal dataset
data("mirna", package = "anamiR", envir = environment())
data("pheno.mirna", package = "anamiR", envir = environment())

## SummarizedExperiment class
require(SummarizedExperiment)
mirna_se <- SummarizedExperiment(
```

```
  assays = SimpleList(counts=mirna),
 colData = pheno.mirna)

## Finding differential miRNA from miRNA expression data with anova
aov <- multi_Differ(se = mirna_se, class = "Subtype",
   post_hoc = "scheffe.test")
```

negative_cor   *Find the correlation coefficient between each gene and miRNA.*

Description

This function will calculate the correlation coefficient between each gene and miRNA from differential expressed data, which are produced by differExp_discrete or differExp_continuous. After filtering the positive and higher than cutoff value of correlation, this function would return a matrix with seven columns, including miRNA, gene, correlation coefficients and Fold change, P-adjust value for miRNA and gene. Each row represents one potential miRNA-target gene interaction.

Usage

```
negative_cor(mrna_data, mirna_data, method = c("pearson", "kendall",
  "spearman"), cut.off = -0.5)
```

Arguments

mrna_data	differential expressed data in data.frame format, with sample name in columns and gene symbol in rows, which is generated by differExp_discrete or differExp_continuous.
mirna_data	differential expressed data in data.frame format, with sample name in columns and miRNAl in rows, which is generated by differExp_discrete or differExp_continuous, miRNA should be miRBase 21 version now.
method	methods for calculating correlation coefficient, including "pearson", "spearman", "kendall". Default is "pearson". From function cor
cut.off	an numeric value indicating a threshold of correlation coefficient for every potential miRNA-genes interactions. Default is -0.5, however, if no interaction pass the threshold, this function would add 0.2 value in threshold until at least one interaction passed the threshold.

Value

matrix format with each row indicating one potential miRNA-target gene interaction and seven columns are miRNA, gene, correlation coefficient and Fold change, P-adjust value for miRNA and gene.

See Also

cor for calculation of correlation.

Examples

```
## Use the internal dataset
data("mirna", package = "anamiR", envir = environment())
data("pheno.mirna", package = "anamiR", envir = environment())
data("mrna", package = "anamiR", envir = environment())
data("pheno.mrna", package = "anamiR", envir = environment())

## SummarizedExperiment class
require(SummarizedExperiment)
mirna_se <- SummarizedExperiment(
 assays = SimpleList(counts=mirna),
 colData = pheno.mirna)

## SummarizedExperiment class
require(SummarizedExperiment)
mrna_se <- SummarizedExperiment(
 assays = SimpleList(counts=mrna),
 colData = pheno.mrna)

## Finding differential miRNA from miRNA expression data with t.test
mirna_d <- differExp_discrete(
   se = mirna_se,
   class = "ER",
   method = "t.test"
)

## Finding differential mRNA from mRNA expression data with t.test
mrna_d <- differExp_discrete(
   se = mrna_se,
   class = "ER",
   method = "t.test"
)

## Correlation
cor <- negative_cor(mrna_data = mrna_d, mirna_data = mirna_d,
    method = "pearson"
)
```

---

normalization   *Normalize expression data*

Description

This function will normalize the given expression data and return it in the same data format.

Usage

```
normalization(data, method = c("quantile", "normal", "rank.invariant"))
```

Arguments

data   expression data in matrix format, with sample name in columns and gene symbol
                or miRNA name in rows.

method normalization methods, including "quantile", "normal", "rank.invariant". De-
fault is "quantile". As for method "normal", we trim the extreme value and
calculate the mean in the data.

Value

[SummarizedExperiment](#) for return object.

See Also

[normalizeQuantiles](#) for quantile normalization; [rankinvariant](#) for rank invariant normalization.

Examples

```
## Use the internal dataset
data("mirna", package = "anamiR", envir = environment())

## Normalize miRNA expression data
normalization(data = mirna, method = "quantile")
```

---

pheno.mirna *phenotype data of* [mirna](#) *about breast cancer*

Description

This phenotype dataset is originally from GSE19536. It contains 3 features in row and 30 samples
in column.

Usage

```
pheno.mirna
```

Format

A data frame with 30 obs and 3 variables:

ER estrogen receptor status

Subtype breast cancer subtype

Survival disease free survival time (months)

Value

data.frame

Source

NCBI GEO: http://www.ncbi.nlm.nih.gov/geo/

pheno.mrna	*phenotype data of* mrna *about breast cancer*

Description

This phenotype dataset is originally from GSE19536. It contains 3 features in row and 30 samples in column.

Usage

```
pheno.mrna
```

Format

A data frame with 30 obs and 3 variables:

ER estrogen receptor status

Subtype breast cancer subtype

Survival disease free survival time (months)

Value

data.frame

Source

NCBI GEO: http://www.ncbi.nlm.nih.gov/geo/

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