

Package ‘SPONGE’

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Type Package

Title Sparse Partial Correlations On Gene Expression

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Description This package provides methods to efficiently detect competitive endogenous RNA interactions between two genes. Such interactions are mediated by one or several miRNAs such that both gene and miRNA expression data for a larger number of samples is needed as input. The SPONGE package now also includes spongEffects: ceRNA modules offer patient-specific insights into the miRNA regulatory landscape.

License GPL (>=3)

LazyData TRUE

LazyDataCompression xz

Depends R (>= 3.6)

Suggests testthat, knitr, rmarkdown, visNetwork, ggrepel, gridExtra, digest, doParallel, bigmemory, GSVA

Imports methods, Biobase, stats, ppcor, logger, foreach, doRNG, data.table, MASS, expm, gRbase, glmnet, igraph, iterators, caret, dplyr, biomaRt, randomForest, ggridges, cvms, ComplexHeatmap, ggplot2, MetBrewer, rlang, tnet, ggpubr, stringr, tidyr, tibble

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SPONGE-package	<i>Package shared imports</i>
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Description

Centralized imports for functions used across multiple files. These roxygen directives generate ‘NAMESPACE’ entries via roxygen2.

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build_classifier_central_genes
build classifiers for central genes

Description

build classifiers for central genes

Usage

```
build_classifier_central_genes(
  train_gene_expr,
  test_gene_expr,
  train_enrichment_modules,
  test_enrichment_modules,
  train_meta_data,
  test_meta_data,
  train_meta_data_type = "TCGA",
  test_meta_data_type = "TCGA",
  metric = "Exact_match",
  tunegrid_c = c(1:100),
  n.folds = 10,
  repetitions = 3
)
```

Arguments

```
train_gene_expr      expression data of train dataset, genenames must be in rownames
test_gene_expr       expression data of test dataset, genenames must be in rownames
train_enrichment_modules
                     return of enrichment_modules()
test_enrichment_modules
                     return of enrichment_modules()
train_meta_data      meta data of train dataset
test_meta_data       meta data of test dataset
train_meta_data_type TCGA or METABRIC
```

test_meta_data_type	TCGA or METABRIC
metric	metric (Exact_match, Accuracy) (default: Exact_match)
tunegrid_c	defines the grid for the hyperparameter optimization during cross validation (caret package) (default: 1:100)
n_folds	number of folds to be calculated
repetitions	number of k-fold cv iterations (default: 3)

Value

model for central genes

calibrate_model	<i>tests and trains a model for a disease using a training and test data set (e.g., TCGA-BRCA and METABRIC)</i>
-----------------	---

Description

tests and trains a model for a disease using a training and test data set (e.g., TCGA-BRCA and METABRIC)

Usage

```
calibrate_model(
  Input,
  modules_metadata,
  label,
  sampleIDs,
  Metric = "Exact_match",
  tunegrid_c = c(1:100),
  n_folds = 10,
  repetitions = 3
)
```

Arguments

Input	Features to use for model calibration.
modules_metadata	metadata table containing information about samples/patients
label	Column of metadata to use as label in classification model
sampleIDs	Column of metadata containing sample/patient IDs to be matched with column names of spongEffects scores
Metric	metric (Exact_match, Accuracy) (default: Exact_match)
tunegrid_c	defines the grid for the hyperparameter optimization during cross validation (caret package) (default: 1:100)
n_folds	number of folds (default: 10)
repetitions	number of k-fold cv iterations (default: 3)

Value

returns a list with the trained model and the prediction results Calibrate classification RF classification model
returns a list with the trained model and the prediction results

ceRNA_interactions	<i>ceRNA interactions</i>
--------------------	---------------------------

Description

ceRNA interactions

Usage

data(ceRNA_interactions)

Format

A data table of ceRNA interactions typically provided by sponge

check_and_convert_expression_data	<i>Checks if expression data is in matrix or ExpressionSet format and converts the latter to a standard matrix. Alternatively, a big.matrix descriptor object can be supplied to make use of shared memory between parallelized workers through the bigmemory package.</i>
-----------------------------------	--

Description

Checks if expression data is in matrix or ExpressionSet format and converts the latter to a standard matrix. Alternatively, a big.matrix descriptor object can be supplied to make use of shared memory between parallelized workers through the bigmemory package.

Usage

check_and_convert_expression_data(expr_data)

Arguments

expr_data expr_data as matrix or ExpressionSet

Value

expr_data as matrix

Examples

Not run: check_and_convert_expression_data(gene_expr)

define_modules	<i>Functions to define Sponge modules, created as all the first neighbors of the most central genes</i>
----------------	---

Description

Functions to define Sponge modules, created as all the first neighbors of the most central genes

Usage

```
define_modules(  
  network,  
  central.modules = F,  
  remove.central = T,  
  set.parallel = T  
)
```

Arguments

- network Network as dataframe and list of central nodes. First two columns of the dataframe should contain the information of the nodes connected by edges.
- central.modules consider central gene as part of the module (default: False)
- remove.central Possibility of keeping or removing (default) central genes in the modules (default: T)
- set.parallel paralleling calculation of define_modules() (default: F)

Value

List of modules. Module names are the corresponding central genes.

enrichment_modules	<i>Calculate enrichment scores</i>
--------------------	------------------------------------

Description

Calculate enrichment scores

Usage

```
enrichment_modules(  
  Expr.matrix,  
  modules,  
  bin.size = 100,  
  min.size = 10,  
  max.size = 200,  
  min.expr = 10,  
  method = "OE",  
  cores = 1  
)
```

Arguments

Expr.matrix	ceRNA expression matrix
modules	Result of define_modules()
bin.size	bin size (default: 100)
min.size	minimum module size (default: 10)
max.size	maximum module size (default: 200)
min.expr	minimum expression (default: 10)
method	Enrichment to be used (Overall Enrichment: OE or Gene Set Variation Analysis: GSVA) (default: OE)
cores	number of cores to be used to calculate enrichment scores with gsva or ssgea methods. Default 1

Value

matrix containing module enrichment scores (module x samples)

ensembl.df	<i>example potential central nodes</i>
------------	--

Description

example potential central nodes

Usage

```
data(ensembl.df)
```

Format

(downloaded via biomaRt)

filter_ceRNA_network	<i>prepare ceRNA network and network centralities from SPONGE / SPONGEdb for spongEffects</i>
----------------------	---

Description

prepare ceRNA network and network centralities from SPONGE / SPONGEdb for spongEffects

Usage

```
filter_ceRNA_network(
  sponge_effects,
  Node_Centrality = NA,
  add_weighted_centrality = T,
  mscor.threshold = NA,
  padj.threshold = NA
)
```

Arguments

sponge_effects the ceRNA network downloaded as R object from SPONGEdb (Hoffmann et al., 2021) or created by SPONGE (List et al., 2019) (ends with `_sponge_results` in the SPONGE vignette)

Node_Centrality the network analysis downloaded as R object from SPONGEdb (Hoffmann et al., 2021) or created by SPONGE and containing centrality measures. (List et al., 2019) (ends with `_networkAnalysis` in the SPONGE vignette, you can also use your own network centrality measurements) if `network_analysis` is NA then the function only filters the ceRNA network, otherwise it will filter the given network centralities, but will not recalculate them based on the filtered ceRNA network.

add_weighted_centrality calculate and add weighted centrality measures to previously available centralities. Default = T

mscor.threshold mscor threshold to be filtered (default: NA)

padj.threshold adjusted p-value to be filtered (default: NA)

Value

list of filtered ceRNA network and network centralities. You can access it with `list$objectname` for further spongEffects steps

fn_combined_centrality

Function to calculate centrality scores Calculation of combined centrality scores as proposed by Del Rio et al. (2009)

Description

Function to calculate centrality scores Calculation of combined centrality scores as proposed by Del Rio et al. (2009)

Usage

```
fn_combined_centrality(CentralityMeasures)
```

Arguments

CentralityMeasures
dataframe with centrality score measures as columns and samples as rows

Value

Vector containing combined centrality scores

fn_discretize_spongeeffects

discretize #' (functions taken from: Jerby-Arnon et al. 2018)

Description

discretize #' (functions taken from: Jerby-Arnon et al. 2018)

Usage

```
fn_discretize_spongeeffects(v, n.cat)
```

Arguments

v gene distance (defined by mother function OE module function)
n.cat size of the bins (defined by mother function OE module function)

Value

discretized

fn_elasticnet	<i>Computes an elastic net model</i>
---------------	--------------------------------------

Description

Computes an elastic net model

Usage

```
fn_elasticnet(x, y, alpha.step = 0.1)
```

Arguments

x	miRNA expression matrix
y	gene expression vector
alpha.step	Step size for alpha, the tuning parameter for elastic net.

Value

The best model, i.e. the one for which the selected alpha yielded the smallest residual sum of squares error

fn_exact_match_summary	<i>Calibrate classification method</i>
------------------------	--

Description

Calibrate classification method

Usage

```
fn_exact_match_summary(data, lev = NULL, model = NULL)
```

Arguments

data	Dataframe with module scores/covariates (modules x samples) AND outcome variable
lev	(default: NULL)
model	(default: NULL)

Value

Model and confusion matrix in a list

fn_filter_network	<i>Preprocessing ceRNA network</i>
-------------------	------------------------------------

Description

Preprocessing ceRNA network

Usage

```
fn_filter_network(network, mscor.threshold = 0.1, padj.threshold = 0.01)
```

Arguments

network	ceRNA network as data (typically present in the outputs of sponge)
mscor.threshold	mscor threshold (default 0.1)
padj.threshold	adjusted p-value threshold (default 0.01)

Value

filtered ceRNA network

fn_gene_miRNA_F_test	<i>Perform F test for gene-miRNA elastic net model</i>
----------------------	--

Description

Perform F test for gene-miRNA elastic net model

Usage

```
fn_gene_miRNA_F_test(g_expr, m_expr, model, p.adj.threshold = NULL)
```

Arguments

g_expr	A gene expression matrix with samples in rows and genes in columns
m_expr	A miRNA expression matrix with samples in rows and genes in columns. Sample number and order has to agree with above gene expression matrix
model	A nested elastic net model to be tested
p.adj.threshold	Threshold for FDR corrected p-value

Value

return data frame with miRNA, fstat and adjusted p.value (BH).

fn_get_model_coef	<i>Extract the model coefficients from an elastic net model</i>
-------------------	---

Description

Extract the model coefficients from an elastic net model

Usage

```
fn_get_model_coef(model)
```

Arguments

model	An elastic net model
-------	----------------------

Value

A data frame with miRNAs and coefficients

fn_get_rss	<i>Compute the residual sum of squares error for an elastic net model</i>
------------	---

Description

Compute the residual sum of squares error for an elastic net model

Usage

```
fn_get_rss(model, x, y)
```

Arguments

model	The elastic net model
x	The miRNA expression
y	The gene expression

Value

the RSS

fn_get_semi_random_OE *Function to calculate semi random enrichment scores of modules OE (functions taken from: Jerby-Arnon et al. 2018)*

Description

Function to calculate semi random enrichment scores of modules OE (functions taken from: Jerby-Arnon et al. 2018)

Usage

```
fn_get_semi_random_OE(r, genes.dist.q, b.sign, num.rounds = 1000)
```

Arguments

r	expression matrix
genes.dist.q	values of the genes after binning (result of binning)
b.sign	does the signature contain less than 2 genes? (controll parameter) (is set by mother function (OE module function))
num.rounds	number of rounds (default: 1000)

Value

random signature scores

fn_get_shared_miRNAs *Identify miRNAs for which both genes have miRNA binding sites aka miRNA response elements in the competing endogeneous RNA hypothesis*

Description

Identify miRNAs for which both genes have miRNA binding sites aka miRNA response elements in the competing endogeneous RNA hypothesis

Usage

```
fn_get_shared_miRNAs(geneA, geneB, mir_interactions)
```

Arguments

geneA	The first gene
geneB	The second gene
mir_interactions	A named list of genes, where for each gene all miRNA interacting partners are listed

Value

A vector with shared RNAs of the two genes.

fn_OE_module	<i>Function to calculate enrichment scores of modules OE (functions taken from: Jerby-Arnon et al. 2018)</i>
--------------	--

Description

Function to calculate enrichment scores of modules OE (functions taken from: Jerby-Arnon et al. 2018)

Usage

```
fn_OE_module(  
  NormCount,  
  gene.sign,  
  bin.size = 100,  
  num.rounds = 1000,  
  set_seed = 42  
)
```

Arguments

NormCount	normalized counts
gene.sign	significant genes
bin.size	bin size (default: 100)
num.rounds	number of rounds (default: 1000)
set_seed	seed size (default: 42)

Value

Signature scores

fn_RF_classifier	<i>RF classification model</i>
------------------	--------------------------------

Description

RF classification model

Usage

```
fn_RF_classifier(
  Input.object,
  K,
  rep,
  metric = "Exact_match",
  tunegrid,
  set_seed = 42
)
```

Arguments

Input.object	data.frame made by predictors and dependent variable
K	number of folds (k-fold)
rep	number of times repeating the cross validation
metric	metric (Exact_match, Accuracy) (default: Exact_match)
tunegrid	defines the grid for the hyperparameter optimization during cross validation (caret package)
set_seed	set seed (default: 42)

Value

Model and confusion matrix in a list

fn_weighted_degree	<i>Function to calculate centrality scores Calculation of weighted degree scores based on Opsahl et al. (2010) Hyperparameter to tune: Alpha = 0 -> degree centrality as defined in Freeman, 1978 (number of edges).</i>
--------------------	---

Description

Function to calculate centrality scores Calculation of weighted degree scores based on Opsahl et al. (2010) Hyperparameter to tune: Alpha = 0 -> degree centrality as defined in Freeman, 1978 (number of edges).

Usage

```
fn_weighted_degree(network, undirected = T, Alpha = 1)
```

Arguments

network	Network formatted as a dataframe with three columns containing respectively node1, node2 and weights
undirected	directionality of the network (default: T)
Alpha	degree centrality as defined in Barrat et al., 2004 (default: 1)

Value

Dataframe containing information about nodes and their weighted centrality measure

genes_pairwise_combinations

Compute all pairwise interactions for a number of genes as indices

Description

Compute all pairwise interactions for a number of genes as indices

Usage

```
genes_pairwise_combinations(number.of.genes)
```

Arguments

number.of.genes	Number of genes for which all pairwise interactions are needed
-----------------	--

Value

data frame with one row per unique pairwise combination. To be used as input for the sponge method.

gene_expr	<i>Gene expression test data set</i>
-----------	--------------------------------------

Description

Gene expression test data set

Usage

data(gene_expr)

Format

A data frame of expression values with samples in columns and genes in rows

get_central_modules	<i>prepare ceRNA network and network centralities from SPONGE / SPONGEdb</i>
---------------------	--

Description

prepare ceRNA network and network centralities from SPONGE / SPONGEdb

Usage

```
get_central_modules(  
  central_nodes,  
  node_centrality,  
  ceRNA_class = c("lncRNA", "circRNA", "protein_coding"),  
  centrality_measure = "Weighted_Degree",  
  cutoff = 1000  
)
```

Arguments

- | | |
|--------------------|---|
| central_nodes | Vector containing Ensemble IDs of the chosen RNAs to use as central nodes for the modules. |
| node_centrality | output from filter_ceRNA_network() or own measurement, if own measurement taken, please provide node_centrality_column |
| ceRNA_class | default c("lncRNA","circRNA","protein_coding") (see http://www.ensembl.org/info/genome/genebuild/b) |
| centrality_measure | Type of centrality measure to use. (Default: "Weighted_Degree", calculated in filter_ceRNA_network()) |
| cutoff | the top cutoff modules will be returned (default: 1000) |

Value

top cutoff modules, with selected RNAs as central genes

mircode_ensg	<i>mircode predicted miRNA gene interactions</i>
--------------	--

Description

mircode predicted miRNA gene interactions

Format

A matrix gene ensembl ids vs miRNA family names. >=1 if interaction is predicted, 0 otherwise

Source

<http://www.mircode.org/download.php>

mircode_symbol	<i>mircode predicted miRNA gene interactions</i>
----------------	--

Description

mircode predicted miRNA gene interactions

Format

A matrix gene symbols vs miRNA family names. >=1 if interaction is predicted, 0 otherwise

Source

<http://www.mircode.org/download.php>

mir_expr	<i>miRNA expression test data set</i>
----------	---------------------------------------

Description

miRNA expression test data set

Usage

```
data(mir_expr)
```

Format

A data frame of expression values with samples in columns and miRNA in rows

mir_interactions	<i>miRNA / gene interactions</i>
------------------	----------------------------------

Description

miRNA / gene interactions

Usage

```
data(mir_interactions)
```

Format

A data frame of regression coefficients typically provided by `sponge_gene_miRNA_interaction_filter`

plot_accuracy_sensitivity_specificity	<i>list of plots for (1) accuracy and (2) sensitivity + specificity (see Boniolo and Hoffmann 2022 et al. Fig. 3a and Fig. 3b)</i>
---------------------------------------	--

Description

list of plots for (1) accuracy and (2) sensitivity + specificity (see Boniolo and Hoffmann 2022 et al. Fig. 3a and Fig. 3b)

Usage

```
plot_accuracy_sensitivity_specificity(
  trained_model,
  central_genes_model = NA,
  all_expression_model = NA,
  random_model,
  training_dataset_name = "TCGA",
  testing_dataset_name = "TCGA",
  subtypes
)
```

Arguments

trained_model	returned from <code>train_and_test_model</code>
central_genes_model	returned from <code>build_classifier_central_genes()</code>
all_expression_model	training and testing like <code>central_genes_model</code> but on ALL common expression data

`random_model` returned from `train_and_test_model` using the randomization
`training_dataset_name`
 name of training (e.g., TCGA)
`testing_dataset_name`
 name of testing set (e.g., METABRIC)
`subtypes` array of subtypes (e.g., `c("Normal", "LumA", "LumB", "Her2", "Basal")`)

Value

list of plots for (1) accuracy and (2) sensitivity + specificity

`plot_confusion_matrices`

plots the confusion matrix from `spongEffects train_and_test()` (see Boniolo and Hoffmann 2022 et al. Fig. 3a and Fig. 3b)

Description

plots the confusion matrix from `spongEffects train_and_test()` (see Boniolo and Hoffmann 2022 et al. Fig. 3a and Fig. 3b)

Usage

`plot_confusion_matrices(trained_model, subtypes.testing.factors)`

Arguments

`trained_model` returned from `train_and_test_model`
`subtypes.testing.factors`
 subtypes of testing samples as factors

Value

plot of the confusion matrix
 returns confusion matrix plots of the trained model

plot_density_scores	<i>plots the density of the model scores for subtypes (see Boniolo and Hoffmann 2022 et al. Fig. 2)</i>
---------------------	---

Description

plots the density of the model scores for subtypes (see Boniolo and Hoffmann 2022 et al. Fig. 2)

Usage

```
plot_density_scores(trained_model, spongEffects, meta_data, label, sampleIDs)
```

Arguments

trained_model	returned from train_and_test_model
spongEffects	output of enrichment_modules()
meta_data	metadata of samples (retrieved from prepare_tcga_for_spongEffects() or from prepare_metabric_for_spongEffects())
label	Column of metadata to use as label in classification model
sampleIDs	Column of metadata containing sample/patient IDs to be matched with column names of spongEffects scores

Value

plots density scores for subtypes

plot_heatmaps	<i>plots the heatmaps from training_and_test_model (see Boniolo and Hoffmann 2022 et al. Fig. 6)</i>
---------------	--

Description

plots the heatmaps from training_and_test_model (see Boniolo and Hoffmann 2022 et al. Fig. 6)

Usage

```
plot_heatmaps(
  trained_model,
  spongEffects,
  meta_data,
  label,
  sampleIDs,
  Modules_to_Plot = 2,
  show.rownames = F,
  show.colnames = F
)
```

Arguments

trained_model	returned from train_and_test_model
spongEffects	output of enrichment_modules()
meta_data	metadata of samples (retrieved from prepare_tcga_for_spongEffects() or from prepare_metabric_for_spongEffects())
label	Column of metadata to use as label in classification model
sampleIDs	Column of metadata containing sample/patient IDs to be matched with column names of spongEffects scores
Modules_to_Plot	Number of modules to plot in the heatmap. Default = 2
show.rownames	Add row names (i.e. module names) to the heatmap. Default = F
show.colnames	Add column names (i.e. sample names) to the heatmap. Default = F

Value

ComplexHeatmap object NOT FUNCTIONAL

plot_involved_miRNAs_to_modules

plots the heatmap of miRNAs invovled in the interactions of the modules (see Boniolo and Hoffmann 2022 et al. Fig. 7a)

Description

plots the heatmap of miRNAs invovled in the interactions of the modules (see Boniolo and Hoffmann 2022 et al. Fig. 7a)

Usage

```
plot_involved_miRNAs_to_modules(
  sponge_modules,
  trained_model,
  gene_mirna_candidates,
  k_modules = 25,
  filter_miRNAs = 3,
  bioMart_gene_symbol_columns = "hgnc_symbol",
  bioMart_gene_ensembl = "hsapiens_gene_ensembl",
  width = 5,
  length = 5,
  show_row_names = T,
  show_column_names = T,
  show_annotation_column = F,
  title = "Frequency",
  legend_height = 1.5,
```

```

labels_gp_fontsize = 8,
title_gp_fontsize = 8,
legend_width = 3,
column_title = "Module",
row_title = "miRNA",
row_title_gp_fontsize = 10,
column_title_gp_fontsize = 10,
row_names_gp_fontsize = 7,
column_names_gp_fontsize = 7,
column_names_rot = 45,
unit = "cm"
)

```

Arguments

```

sponge_modules  result of define_modules()
trained_model   returned from train_and_test_model
gene_mirna_candidates
                output of SPONGE or SPONGEdb (miRNAs_significance)
k_modules       top k modules to be shown (default: 25)
filter_miRNAs   min rowsum to be reach of miRNAs (default: 3.0)
bioMart_gene_symbol_columns
                bioMart dataset column for gene symbols (e.g. human: hgnc_symbol, mouse:
                mgi_symbol)
bioMart_gene_ensembl
                bioMart gene ensemble name (e.g., hsapiens_gene_ensembl).
width           the width of the heatmap (default: 5)
length         the length of the heatmap (default: 5)
show_row_names  show row names (default: T)
show_column_names
                show column names (default: T)
show_annotation_column
                add annotation column to columns (default: F)
title           the title of the plot (default: "Frequency")
legend_height   the height of the legend (default: 1.5)
labels_gp_fontsize
                the font size of the labels (default: 8)
title_gp_fontsize
                the font size of the title (default: 8)
legend_width    the width of the legend (default: 3)
column_title    the column title (default: "Module")
row_title       the title of the rows (default: "miRNA")
row_title_gp_fontsize
                the font size of the row title (default: 10)

```

column_title_gp_fontsize
 the font size of the column title (default: 10)
 row_names_gp_fontsize
 the font size of the row names (default: 7)
 column_names_gp_fontsize
 the font size of the column names (default: 7)
 column_names_rot
 the rotation angel of the column names (default: 45)
 unit
 either cm or inch (see ComplexHeatmap parameter)

Value

plot object

plot_top_modules	<i>plots the top x gini index modules (see Boniolo and Hoffmann 2022 et al. Figure 5)</i>
------------------	---

Description

plots the top x gini index modules (see Boniolo and Hoffmann 2022 et al. Figure 5)

Usage

```
plot_top_modules(
  trained_model,
  k_modules = 25,
  k_modules_red = 10,
  text_size = 16
)
```

Arguments

trained_model returned from train_and_test_model
 k_modules top k modules to be shown (default: 25)
 k_modules_red top k modules shown in red - NOTE: must be smaller than k_modules (default: 10)
 text_size text size (default 16)

Value

plot object for lollipop plot

```
precomputed_cov_matrices
```

covariance matrices under the null hypothesis that sensitivity correlation is zero

Description

covariance matrices under the null hypothesis that sensitivity correlation is zero

Format

A list (different gene-gene correlations k) of lists (different number of miRNAs m) of covariance matrices

```
precomputed_null_model
```

A null model for testing purposes

Description

A null model for testing purposes

Usage

```
data(precomputed_null_model)
```

Format

A list (different gene-gene correlations k) of lists (different number of miRNAs m) of sampled mscor values (100 each, computed from 100 samples)

```
prepare_metabric_for_spongEffects
```

prepare METABRIC formats for spongEffects

Description

prepare METABRIC formats for spongEffects

Usage

```
prepare_metabric_for_spongEffects(
  metabric_expression,
  metabric_metadata,
  subtypes_of_interest,
  bioMart_gene_ensembl = "hsapiens_gene_ensembl",
  bioMart_gene_symbol_columns = "hgnc_symbol"
)
```

Arguments

metabric_expression
 filepath to expression data in metabric format

metabric_metadata
 filepath to metabric metadata in metabric format

subtypes_of_interest
 array e.g., c("LumA", "LumB", "Her2", "Basal", "Normal")

bioMart_gene_ensembl
 bioMart gene ensemble name (e.g., hsapiens_gene_ensembl). (See <https://www.bioconductor.org/packages>)
 (default: hsapiens_gene_ensembl)

bioMart_gene_symbol_columns
 bioMart dataset column for gene symbols (e.g. human: hgnc_symbol, mouse: mgi_symbol) (default: hgnc_symbol)

Value

list with metabric expression and metadata. You can access it with list\$objectname for further spongEffects steps

```
prepare_tcga_for_spongEffects
```

prepare TCGA formats for spongEffects

Description

prepare TCGA formats for spongEffects

Usage

```
prepare_tcga_for_spongEffects(
  tcga_cancer_symbol,
  normal_cerNA_expression_data,
  tumor_cerNA_expression_data,
  normal_metadata,
  tumor_metadata,
  clinical_data,
```

```
tumor_stages_of_interest,
subtypes_of_interest
)
```

Arguments

```
tcga_cancer_symbol
    e.g., BRCA for breast cancer
normal_ceRNA_expression_data
    normal ceRNA expression data (same structure as input for SPONGE)
tumor_ceRNA_expression_data
    tumor ceRNA expression data (same structure as input for SPONGE)
normal_metadata
    metadata for normal samples (TCGA format style, needs to include column:
    sampleID, PATIENT_ID)
tumor_metadata
    metadata for tumor samples (TCGA format style, needs to include column: sam-
    pleID, PATIENT_ID)
clinical_data
    clinical data for all patients (TCGA format style, needs to include column: PA-
    TIENT_ID, AJCC_PATHOLOGIC_TUMOR_STAGE)
tumor_stages_of_interest
    array e.g., c(STAGE I', 'STAGE IA', 'STAGE IB', 'STAGE II', 'STAGE IIA')
subtypes_of_interest
    array e.g., c("LumA", "LumB", "Her2", "Basal", "Normal")
```

Value

list of prepared data. You can access it with `list$objectname` for further `spongEffects` steps

Random_spongEffects	<i>Define random modules</i>
---------------------	------------------------------

Description

Define random modules

Usage

```
Random_spongEffects(
  sponge_modules,
  gene_expr,
  min.size = 10,
  bin.size = 100,
  max.size = 200,
  min.expression = 10,
  replace = F,
  method = "OE",
  cores = 1
)
```

Arguments

sponge_modules	result of define_modules()
gene_expr	Input expression matrix
min.size	minimum module size (default: 10)
bin.size	bin size (default: 100)
max.size	maximum module size (default: 200)
min.expression	minimum expression (default: 10)
replace	Possibility of keeping or removing (default) central genes in the modules (default: F)
method	Enrichment to be used (Overall Enrichment: OE or Gene Set Variation Analysis: GSVA) (default: OE)
cores	number of cores to be used to calculate enrichment scores with gsva or ssgsea methods. Default 1

Value

A list with randomly defined modules and related enrichment scores

sample_zero_mscor_cov *Sampling zero multiple miRNA sensitivity covariance matrices*

Description

Sampling zero multiple miRNA sensitivity covariance matrices

Usage

```
sample_zero_mscor_cov(
  m,
  number_of_solutions,
  number_of_attempts = 1000,
  gene_gene_correlation = NULL,
  random_seed = NULL,
  log.level = "ERROR"
)
```

Arguments

m	number of miRNAs, i.e. number of columns of the matrix
number_of_solutions	stop after this many instances have been samples
number_of_attempts	give up after that many attempts

gene_gene_correlation optional, define the correlation of the first two elements, i.e. the genes.

random_seed A random seed to be used for reproducible results

log.level the log level, typically set to INFO, set to DEBUG for verbose logging

Value

a list of covariance matrices with zero sensitivity correlation

Examples

```
sample_zero_mscor_cov(m = 1,
  number_of_solutions = 1,
  gene_gene_correlation = 0.5)
```

sample_zero_mscor_data

Sample mscor coefficients from pre-computed covariance matrices

Description

Sample mscor coefficients from pre-computed covariance matrices

Usage

```
sample_zero_mscor_data(
  cov_matrices,
  number_of_samples = 100,
  number_of_datasets = 100
)
```

Arguments

cov_matrices a list of pre-computed covariance matrices

number_of_samples the number of samples available in the expression data

number_of_datasets the number of mscor coefficients to be sampled from each covariance matrix

Value

a vector of mscor coefficients

See Also

sample_zero_mscor_cov

Examples

```
#we select from the pre-computed covariance matrices in SPONGE
#100 for m = 5 miRNAs and gene-gene correlation 0.6
cov_matrices_selected <- precomputed_cov_matrices[["5"]][["0.6"]]
sample_zero_mscor_data(cov_matrices = cov_matrices_selected,
  number_of_samples = 200, number_of_datasets = 10)
```

sponge	<i>Compute competing endogeneous RNA interactions using Sparse Partial correlations ON Gene Expression (SPONGE)</i>
--------	---

Description

Compute competing endogeneous RNA interactions using Sparse Partial correlations ON Gene Expression (SPONGE)

Usage

```
sponge(
  gene_expr,
  mir_expr,
  mir_interactions = NULL,
  log.level = "ERROR",
  log.every.n = 1e+05,
  log.file = NULL,
  selected.genes = NULL,
  gene.combinations = NULL,
  each.miRNA = FALSE,
  min.cor = 0.1,
  parallel.chunks = 1000,
  random_seed = NULL,
  result_as_dt = FALSE
)
```

Arguments

gene_expr	A gene expression matrix with samples in rows and featurns in columns. Alternatively an object of class ExpressionSet.
mir_expr	A miRNA expression matrix with samples in rows and features in columns. Alternatively an object of class ExpressionSet.
mir_interactions	A named list of genes, where for each gene we list all miRNA interaction partners that should be considered.
log.level	The log level, can be one of "info", "debug", "error"
log.every.n	write to the log after every n steps
log.file	write log to a file, particularly useful for parallelization

<code>selected.genes</code>	Operate only on a subset of genes, particularly useful for bootstrapping
<code>gene.combinations</code>	A data frame of combinations of genes to be tested. Gene names are taken from the first two columns and have to match the names used for <code>gene_expr</code>
<code>each.miRNA</code>	Whether to consider individual miRNAs or pooling them.
<code>min.cor</code>	Consider only gene pairs with a minimum correlation specified here.
<code>parallel.chunks</code>	Split into this number of tasks if parallel processing is set up. The number should be high enough to guarantee equal distribution of the work load in parallel execution. However, if the number is too large, e.g. in the worst case one chunk per computation, the overhead causes more computing time than can be saved by parallel execution. Register a parallel backend that is compatible with <code>foreach</code> to use this feature. More information can be found in the documentation of the <code>foreach</code> / <code>doParallel</code> packages.
<code>random_seed</code>	A random seed to be used for reproducible results
<code>result_as_dt</code>	whether to return results as data table or data frame

Value

A data frame with significant gene-gene competitive endogenous RNA or 'sponge' interactions

Examples

```
#First, extract miRNA candidates for each of the genes
#using sponge_gene_miRNA_interaction_filter. Here we use a prepared
#dataset mir_interactions.

#Second we compute ceRNA interactions for all pairwise combinations of genes
#using all miRNAs remaining after filtering through elasticnet.
ceRNA_interactions <- sponge(
  gene_expr = gene_expr,
  mir_expr = mir_expr,
  mir_interactions = mir_interactions)
```

```
sponge_build_null_model
```

Build null model for p-value computation

Description

Build null model for p-value computation

Usage

```
sponge_build_null_model(
  number_of_datasets = 1e+05,
  number_of_samples,
  cov_matrices = precomputed_cov_matrices,
  ks = seq(0.2, 0.9, 0.1),
  m_max = 8,
  log.level = "ERROR"
)
```

Arguments

number_of_datasets	the number of datasets defining the precision of the p-value
number_of_samples	the number of samples in the expression data
cov_matrices	pre-computed covariance matrices
ks	a sequence of gene-gene correlation values for which null models are computed
m_max	null models are build for each elt in ks for 1 to m_max miRNAs
log.level	The log level of the logger package

Value

a list (for various values of m) of lists (for various values of k) of lists of simulated data sets, drawn from a set of precomputed covariance matrices

Examples

```
sponge_build_null_model(100, 100,
  cov_matrices = precomputed_cov_matrices[1:3], m_max = 3)
```

sponge_compute_p_values

Compute p-values for SPONGE interactions

Description

This method uses pre-computed covariance matrices that were created for various gene-gene correlations (0.2 to 0.9 in steps of 0.1) and number of miRNAs (between 1 and 8) under the null hypothesis that the sensitivity correlation is zero. Datasets are sampled from this null model and allow for an empirical p-value to be computed that is only significant if the sensitivity correlation is higher than can be expected by chance given the number of samples, correlation and number of miRNAs. p-values are adjusted indepdently for each parameter combination using Benjamini-Hochberg FDR correction.

Usage

```
sponge_compute_p_values(sponge_result, null_model, log.level = "ERROR")
```

Arguments

`sponge_result` A data frame from a sponge call
`null_model` optional, pre-computed simulated data
`log.level` The log level of the logger package

Value

A data frame with sponge results, now including p-values and adjusted p-value

See Also

`sponge_build_null_model`

Examples

```
sponge_compute_p_values(ceRNA_interactions,
  null_model = precomputed_null_model)
```

```
sponge_edge_centralities
```

Computes edge centralities

Description

Computes edge betweenness centrality for the ceRNA interaction network induced by the results of the SPONGE method.

Usage

```
sponge_edge_centralities(sponge_result)
```

Arguments

`sponge_result` The output generated by the sponge method.

Value

data table or data frame with gene, degree, eigenvector and betweenness

See Also

`sponge`

Examples

```
sponge_edge_centralities(ceRNA_interactions)
```

```
sponge_gene_miRNA_interaction_filter
```

Determine miRNA-gene interactions to be considered in SPONGE

Description

The purpose of this method is to limit the number of miRNA-gene interactions we need to consider in SPONGE. There are 3 filtering steps: 1. variance filter (optional). Only consider genes and miRNAs with variance > var.threshold. 2. miRNA target database filter (optional). Use a miRNA target database provided by the user to filter for those miRNA gene interactions for which evidence exists. This can either be predicted target interactions or experimentally validated ones. 3. For each remaining interaction of a gene and its regulating miRNAs use elastic net regression to achieve a) Feature selection: We only retain miRNAs that influence gene expression b) Effect strength: The sign of the coefficients allows us to filter for miRNAs that down-regulate gene expression. Moreover, we can use the coefficients to rank the miRNAs by their relative effect strength. We strongly recommend setting up a parallel backend compatible with the foreach package. See example and the documentation of the foreach and doParallel packages.

Usage

```
sponge_gene_miRNA_interaction_filter(
  gene_expr,
  mir_expr,
  mir_predicted_targets,
  elastic.net = TRUE,
  log.level = "ERROR",
  log.file = NULL,
  var.threshold = NULL,
  F.test = FALSE,
  F.test.p.adj.threshold = 0.05,
  coefficient.threshold = -0.05,
  coefficient.direction = "<",
  select.non.targets = FALSE,
  random_seed = NULL,
  parallel.chunks = 100
)
```

Arguments

gene_expr	A gene expression matrix with samples in rows and features in columns. Alternatively an object of class ExpressionSet.
mir_expr	A miRNA expression matrix with samples in rows and features in columns. Alternatively an object of class ExpressionSet.

<code>mir_predicted_targets</code>	A data frame with miRNA in cols and genes in rows. A 0 indicates the miRNA is not predicted to target the gene, >0 otherwise. If this parameter is NULL all miRNA-gene interactions are tested
<code>elastic.net</code>	Whether to apply elastic net regression filtering or not.
<code>log.level</code>	One of 'warn', 'error', 'info'
<code>log.file</code>	Log file to write to
<code>var.threshold</code>	Only consider genes and miRNA with variance > var.threshold. If this parameter is NULL no variance filtering is performed.
<code>F.test</code>	If true, an F-test is performed on each model parameter to assess its importance for the model based on the RSS of the full model vs the RSS of the nested model without the miRNA in question. This is time consuming and has the potential disadvantage that correlated miRNAs are removed even though they might play a role in ceRNA interactions. Use at your own risk.
<code>F.test.p.adj.threshold</code>	If F.test is TRUE, threshold to use for miRNAs to be included.
<code>coefficient.threshold</code>	threshold to cross for a regression coefficient to be called significant. depends on the parameter coefficient.direction.
<code>coefficient.direction</code>	If "<", coefficient has to be lower than coefficient.threshold, if ">", coefficient has to be larger than threshold. If NULL, the absolute value of the coefficient has to be larger than the threshold.
<code>select.non.targets</code>	For testing effect of miRNA target information. If TRUE, the method determines as usual which miRNAs are potentially targeting a gene. However, these are then replaced by a random sample of non-targeting miRNAs (without seeds) of the same size. Useful for testing if observed effects are caused by miRNA regulation.
<code>random_seed</code>	A random seed to be used for reproducible results
<code>parallel.chunks</code>	Split into this number of tasks if parallel processing is set up. The number should be high enough to guarantee equal distribution of the work load in parallel execution. However, if the number is too large, e.g. in the worst case one chunk per computation, the overhead causes more computing time than can be saved by parallel execution. Register a parallel backend that is compatible with foreach to use this feature. More information can be found in the documentation of the foreach / doParallel packages.

Value

A list of genes, where for each gene, the regulating miRNA are included as a data frame. For `F.test = TRUE` this is a data frame with fstat and p-value for each miRNA. Else it is a data frame with the model coefficients.

See Also

sponge

Examples

```
#library(doParallel)
#cl <- makePSOCKcluster(2)
#registerDoParallel(cl)
genes_miRNA_candidates <- sponge_gene_miRNA_interaction_filter(
  gene_expr = gene_expr,
  mir_expr = mir_expr,
  mir_predicted_targets = targetscan_symbol)
#stopCluster(cl)

#If we also perform an F-test, only few of the above miRNAs remain
genes_miRNA_candidates <- sponge_gene_miRNA_interaction_filter(
  gene_expr = gene_expr,
  mir_expr = mir_expr,
  mir_predicted_targets = targetscan_symbol,
  F.test = TRUE,
  F.test.p.adj.threshold = 0.05)
```

sponge_network

*Prepare a sponge network for plotting***Description**

Prepare a sponge network for plotting

Usage

```
sponge_network(
  sponge_result,
  mir_data,
  target.genes = NULL,
  show.sponge.interaction = TRUE,
  show.mirnas = "none",
  min.interactions = 3
)
```

Arguments

sponge_result	ceRNA interactions as produced by the sponge method.
mir_data	miRNA interactions as produced by sponge_gene_miRNA_interaction_filter
target.genes	a character vector to select a subset of genes
show.sponge.interaction	whether to connect ceRNAs
show.mirnas	one of none, shared, all
min.interactions	minimum degree of a gene to be shown

Value

a list of nodes and edges

Examples

```
sponge_network(ceRNA_interactions, mir_interactions)
```

```
sponge_node_centralities
```

Computes various node centralities

Description

Computes degree, eigenvector centrality and betweenness centrality for the ceRNA interaction network induced by the results of the SPONGE method

Usage

```
sponge_node_centralities(sponge_result, directed = FALSE)
```

Arguments

`sponge_result` output of the sponge method

`directed` Whether to consider the input network as directed or not.

Value

data table or data frame with gene, degree, eigenvector and betweenness

See Also

sponge

Examples

```
sponge_node_centralities(ceRNA_interactions)
```

sponge_plot_network *Plot a sponge network*

Description

Plot a sponge network

Usage

```
sponge_plot_network(
  sponge_result,
  mir_data,
  layout = "layout.fruchterman.reingold",
  force.directed = FALSE,
  ...
)
```

Arguments

`sponge_result` ceRNA interactions as produced by the sponge method.
`mir_data` miRNA interactions as produced by `sponge_gene_miRNA_interaction_filter`
`layout` one of the layout methods supported in the `visNetwork` package
`force.directed` whether to produce a force directed network, gets slow for large networks
`...` further params for `sponge_network`

Value

shows a plot

Examples

```
sponge_plot_network(ceRNA_interactions, mir_interactions)
```

sponge_plot_network_centralities
plot node network centralities

Description

plot node network centralities

Usage

```
sponge_plot_network_centralities(
  network_centralities,
  measure = "all",
  x = "degree",
  top = 5,
  base_size = 18
)
```

Arguments

network_centralities	a result from sponge_node_centralities()
measure	one of 'all', 'degree', 'ev' or 'btw'
x	plot against another column in the data table, defaults to degree
top	label the top x samples in the plot
base_size	size of the text in the plot

Value

a plot

Examples

```
## Not run:
network_centralities <- sponge_node_centralities(ceRNA_interactions)
sponge_plot_network_centralities(network_centralities)
## End(Not run)
```

sponge_plot_simulation_results

Plot simulation results for different null models

Description

Plot simulation results for different null models

Usage

```
sponge_plot_simulation_results(null_model_data)
```

Arguments

null_model_data	the output of sponge_build_null_model
-----------------	---------------------------------------

Value

a ggplot2 object

Examples

```
sponge_plot_simulation_results(precomputed_null_model)
```

sponge_run_benchmark	<i>run sponge benchmark where various settings, i.e. with or without regression, single or pooled miRNAs, are compared.</i>
----------------------	---

Description

run sponge benchmark where various settings, i.e. with or without regression, single or pooled miRNAs, are compared.

Usage

```
sponge_run_benchmark(  
  gene_expr,  
  mir_expr,  
  mir_predicted_targets,  
  number_of_samples = 100,  
  number_of_datasets = 100,  
  number_of_genes_to_test = c(25),  
  compute_significance = FALSE,  
  folder = NULL  
)
```

Arguments

gene_expr	A gene expression matrix with samples in rows and features in columns. Alternatively an object of class ExpressionSet.
mir_expr	A miRNA expression matrix with samples in rows and features in columns. Alternatively an object of class ExpressionSet.
mir_predicted_targets	(a list of) mir interaction sources such as targetscan, etc.
number_of_samples	number of samples in the null model
number_of_datasets	number of datasets to sample from the null model
number_of_genes_to_test	a vector of numbers of genes to be tested, e.g. c(250,500)
compute_significance	whether to compute p-values
folder	where the results should be saved, if NULL no output to disk

Value

a list (regression, no regression) of lists (single miRNA, pooled miRNAs) of benchmark results

Examples

```
sponge_run_benchmark(gene_expr = gene_expr, mir_expr = mir_expr,
  mir_predicted_targets = targetscan_symbol,
  number_of_genes_to_test = c(10), folder = NULL)
```

sponge_subsampling	<i>Sponge subsampling</i>
--------------------	---------------------------

Description

Sponge subsampling

Usage

```
sponge_subsampling(
  subsample.n = 100,
  subsample.repeats = 10,
  subsample.with.replacement = FALSE,
  subsample.plot = FALSE,
  gene_expr,
  mir_expr,
  ...
)
```

Arguments

subsample.n	the number of samples to be drawn in each round
subsample.repeats	how often should the subsampling be done?
subsample.with.replacement	logical, should we allow samples to be used repeatedly
subsample.plot	logical, should the results be plotted as box plots
gene_expr	A gene expression matrix with samples in rows and features in columns. Alternatively an object of class ExpressionSet.
mir_expr	A miRNA expression matrix with samples in rows and features in columns. Alternatively an object of class ExpressionSet.
...	parameters passed on to the sponge function

Value

a summary of the results with mean and standard deviations of the correlation and sensitive correlation.

References

sponge

Examples

```
sponge_subsampling(gene_expr = gene_expr,  
mir_expr = mir_expr, mir_interactions = mir_interactions,  
subsample.n = 10, subsample.repeats = 1)
```

targetscan_ensg	<i>targetscan predicted miRNA gene interactions</i>
-----------------	---

Description

targetscan predicted miRNA gene interactions

Format

A matrix gene ensembl ids vs miRNA family names. >=1 if interaction is predicted, 0 otherwise

Source

http://www.targetscan.org/vert_71/

targetscan_symbol	<i>targetscan predicted miRNA gene interactions</i>
-------------------	---

Description

targetscan predicted miRNA gene interactions

Format

A matrix gene symbols vs miRNA family names. >=1 if interaction is predicted, 0 otherwise

Source

http://www.targetscan.org/vert_71/

test_cancer_gene_expr *example test expression data for spongEffects*

Description

example test expression data for spongEffects

Usage

```
data(test_cancer_gene_expr)
```

Format

a matrix with gene expression data

test_cancer_metadata *example test sample meta data for spongEffects*

Description

example test sample meta data for spongEffects

Usage

```
data(test_cancer_metadata)
```

Format

a data frame with sample meta data, SUBTYPE must be inside your dataframe

test_cancer_mir_expr *example test miRNA data for spongEffects*

Description

example test miRNA data for spongEffects

Usage

```
data(test_cancer_mir_expr)
```

Format

a matrix with miRNA expression data

`train_cancer_gene_expr`*example training expression data for spongEffects*

Description

example training expression data for spongEffects

Usage

```
data(train_cancer_gene_expr)
```

Format

a matrix with gene expression data

`train_cancer_metadata` *example training sample meta data for spongEffects*

Description

example training sample meta data for spongEffects

Usage

```
data(train_cancer_metadata)
```

Format

a data frame with sample meta data, SUBTYPE must be inside your dataframe

`train_cancer_mir_expr` *example training miRNA data for spongEffects*

Description

example training miRNA data for spongEffects

Usage

```
data(train_cancer_mir_expr)
```

Format

a matrix with miRNA expression data

```
train_ceRNA_interactions
```

example train ceRNA interactions for spongEffects

Description

example train ceRNA interactions for spongEffects

Usage

```
data(train_ceRNA_interactions)
```

Format

(obtained by SPONGE method)

```
train_genes_miRNA_candidates
```

example candidate genes for miRNA filtering

Description

example candidate genes for miRNA filtering

Usage

```
data(train_genes_miRNA_candidates)
```

Format

A data frame with candidate genes and associated miRNA scores

```
train_network_centralities
```

example train network centralities for spongEffects

Description

example train network centralities for spongEffects

Usage

```
data(train_network_centralities)
```

Format

(obtained by SPONGE method)

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