

# Package ‘SPONGE’

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**Type** Package

**Title** Sparse Partial Correlations On Gene Expression

**Version** 1.12.0

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**Description** This package provides methods to efficiently detect competitive endogeneous 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.

**License** GPL (>=3)

**LazyData** TRUE

**RoxygenNote** 6.1.1

**Depends** R (>= 3.4)

**Suggests** testthat, knitr, rmarkdown, visNetwork, ggplot2, ggrepel, gridExtra, digest, doParallel, bigmemory

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**VignetteBuilder** knitr

**biocViews** GeneExpression, Transcription, GeneRegulation, NetworkInference, Transcriptomics, SystemsBiology, Regression

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---

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

---

### Description

ceRNA interactions

### Usage

ceRNA\_interactions

### Format

A data table of ceRNA interactions typically provided by sponge

---

`check_and_convert_expression_data`

*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.*

---

**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)
```

---

`fn_elasticnet`

*Computes an elastic net model*

---

**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\_gene\_miRNA\_F\_test    *Perform F test for gene-miRNA elastic net model*

---

**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    *Extract the model coefficients from an elastic net model*

---

**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_shared_miRNAs	<i>Identify miRNAs for which both genes have miRNA binding sites aka miRNA response elements in the competing endogenous RNA hypothesis</i>
----------------------	---

---

**Description**

Identify miRNAs for which both genes have miRNA binding sites aka miRNA response elements in the competing endogenous 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.

---

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

*Gene expression test data set*

---

### **Description**

Gene expression test data set

### **Usage**

```
gene_expr
```

### **Format**

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

---

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

---

**Description**

mircode predicted miRNA gene interactions

**Usage**

mircode\_ensg

**Format**

A matrix gene ensembl ids vs miRNA family names.  $\geq 1$  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

**Usage**

mircode\_symbol

**Format**

A matrix gene symbols vs miRNA family names.  $\geq 1$  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**

mir\_expr

**Format**

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

---

mir\_interactions      *miRNA / gene interactions*

---

**Description**

miRNA / gene interactions

**Usage**

mir\_interactions

**Format**

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

---

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

**Usage**

precomputed\_cov\_matrices

**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**

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)



---

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

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

---

**Description**

Compute competing endogenous 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 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_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
selected.genes	Operate only on a subset of genes, particularly useful for bootstrapping
gene.combinations	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 gene_expr
each.miRNA	Whether to consider individual miRNAs or pooling them.
min.cor	Consider only gene pairs with a minimum correlation specified here.
parallel.chunks	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.
random_seed	A random seed to be used for reproducible results
result_as_dt	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 logging 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 independently 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 logging 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.
mir_predicted_targets	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
elastic.net	Whether to apply elastic net regression filtering or not.
log.level	One of 'warn', 'error', 'info'
log.file	Log file to write to
var.threshold	Only consider genes and miRNA with variance > var.threshold. If this parameter is NULL no variance filtering is performed.
F.test	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.
F.test.p.adj.threshold	If F.test is TRUE, threshold to use for miRNAs to be included.

`coefficient.threshold`  
 threshold to cross for a regression coefficient to be called significant. depends on the parameter `coefficient.direction`.

`coefficient.direction`  
 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.

`select.non.targets`  
 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.

`random_seed` A random seed to be used for reproducible results

`parallel.chunks`  
 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	<i>Prepare a sponge network for plotting</i>
----------------	--

---

**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 <code>sponge_gene_miRNA_interaction_filter</code>
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	<i>Computes various node centralities</i>
--------------------------	---

---

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

---

**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      *Sponge subsampling*

---

## 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

**Usage**

targetscan\_ensg

**Format**

A matrix gene ensembl ids vs miRNA family names.  $\geq 1$  if interaction is predicted, 0 otherwise

**Source**

[http://www.targetscan.org/vert\\_71/](http://www.targetscan.org/vert_71/)

---

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

---

**Description**

targetscan predicted miRNA gene interactions

**Usage**

targetscan\_symbol

**Format**

A matrix gene symbols vs miRNA family names.  $\geq 1$  if interaction is predicted, 0 otherwise

**Source**

[http://www.targetscan.org/vert\\_71/](http://www.targetscan.org/vert_71/)

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