

Package ‘COTAN’

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Type Package

Title COexpression Tables ANalysis

Version 1.2.0

Description Statistical and computational method to analyze the co-expression of gene pairs at single cell level. It provides the foundation for single-cell gene interactome analysis. The basic idea is studying the zero UMI counts' distribution instead of focusing on positive counts; this is done with a generalized contingency tables framework. COTAN can effectively assess the correlated or anti-correlated expression of gene pairs. It provides a numerical index related to the correlation and an approximate p-value for the associated independence test. COTAN can also evaluate whether single genes are differentially expressed, scoring them with a newly defined global differentiation index. Moreover, this approach provides ways to plot and cluster genes according to their co-expression pattern with other genes, effectively helping the study of gene interactions and becoming a new tool to identify cell-identity marker genes.

URL <https://github.com/seriph78/COTAN>

BugReports <https://github.com/seriph78/COTAN/issues>

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R topics documented:

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| | |
|------------------------|------------------------|
| <i>add.row.to.meta</i> | <i>add.row.to.meta</i> |
|------------------------|------------------------|

Description

This function is used to add a line of information to the information data frame (metadata).

Usage

```
add.row.to.meta(object, text.line)

## S4 method for signature 'scCOTAN'
add.row.to.meta(object, text.line)
```

Arguments

object a COTAN object
text.line an array containing the information

Value

the updated COTAN object

Examples

```
data("ERCC.cotan")
text <- c("Test", "This is a test")
ERCC.cotan <- add.row.to.meta(ERCC.cotan, text)
get.metadata(ERCC.cotan)
```

```
automatic.COTAN.object.creation  
    automatic.COTAN.object.creation
```

Description

automatic.COTAN.object.creation

Usage

```
automatic.COTAN.object.creation(  
  df,  
  out_dir,  
  GEO,  
  sc.method,  
  cond,  
  mt = FALSE,  
  mt_prefix = "^mt",  
  cores = 1  
)  
  
## S4 method for signature 'data.frame'  
automatic.COTAN.object.creation(  
  df,  
  out_dir,  
  GEO,  
  sc.method,  
  cond,  
  mt = FALSE,  
  mt_prefix = "^mt",  
  cores = 1  
)
```

Arguments

| | |
|-----------|---|
| df | dataframe with the row counts |
| out_dir | directory for the output |
| GEO | GEO or other code that identify the dataset |
| sc.method | Type of single cell RNA-seq method used |
| cond | A string that will identify the sample or condition. It will be part of the final file name. |
| mt | A boolean (default F). If T mitochondrial genes will be kept in the analysis, otherwise they will be removed. |
| mt_prefix | is the prefix that identify the mitochondrial genes (default is the mouse prefix: "^mt") |
| cores | number of cores to be used |

Value

It return the COTAN object. It will also store it directly in the output directory

Examples

```
data("raw.dataset")
obj <- automatic.COTAN.object.creation(df= raw.dataset,
  out_dir = tempdir(),
  GEO = "test_GEO",
  sc.method = "test_method",
  cond = "test")
```

clean

clean

Description

Main function that can be used to check and clean the dataset. It also produce (using the function `fun_linear`) and store the estimators for `nu` and `lambda`. It also fill the `raw.norm` (`raw / nu`) and `n_cell` (the initial number of cells in the dataset)

Usage

```
clean(object)

## S4 method for signature 'scCOTAN'
clean(object)
```

Arguments

`object` COTAN object

Value

a list of objects containing: "cl1" is the first cell cluster, "cl2" is the second cell cluster, "pca.cell.2" is a `ggplot2` cell pca plot, "object" is the COTAN object with saved the estimated `lambda` and `mu`, "mu_estimator", "D" "pca_cells" pca numeric data.

Examples

```
data("ERCC.cotan")
ttm <- clean(ERCC.cotan)
```

cotan_analysis *cotan_analysis*

Description

This is the main function that estimates the a vector to store all the negative binomial dispersion factors. It need to be run after [clean](#)

Usage

```
cotan_analysis(object, cores = 1)

## S4 method for signature 'scCOTAN'
cotan_analysis(object, cores = 1)
```

Arguments

| | |
|--------|--|
| object | A COTAN object |
| cores | number of cores to use. Default is 11. |

Value

a COTAN object

Examples

```
data("ERCC.cotan")
ERCC.cotan <- cotan_analysis(ERCC.cotan)
```

drop.genes.cells *drop.genes.cells*

Description

This finction remove an array of genes and/or cells from the original object raw matrix.

Usage

```
drop.genes.cells(object, genes = c(), cells = c())

## S4 method for signature 'scCOTAN'
drop.genes.cells(object, genes = c(), cells = c())
```

Arguments

object a COTAN object
 genes an array of gene names
 cells an array of cell names

Value

the original object but with the raw matrix without the indicated cells and/or genes.

Examples

```
data("ERCC.cotan")
genes.to.rem = names(get.genes(ERCC.cotan)[1:10])
cells.to.rem = names(get.cell.size(ERCC.cotan)[1:10])
ERCC.cotan = drop.genes.cells(ERCC.cotan,genes.to.rem,cells.to.rem )
```

| | |
|------------|---------------------|
| ERCC.cotan | <i>COTAN object</i> |
|------------|---------------------|

Description

The COTAN object for the ERCC dataset.

Usage

```
ERCC.cotan
```

Format

A structure with:

raw the raw dataset: 88 fake genes for 1015 fake cells

raw.norm raw divided for nu

coex

nu UDE

lambda average gene expression

a

hk genes expressed in all cells

n_cells final number of cells

meta meta data

Source

<https://support.10xgenomics.com/single-cell-gene-expression/datasets/1.1.0/ercc?>

| | |
|---------|-------------------------|
| ERCCraw | <i>Raw ERCC dataset</i> |
|---------|-------------------------|

Description

ERCC dataset

Usage

ERCCraw

Format

A data frame

Source

ERCC

| | |
|--------------------|---------------------------|
| est.min.parameters | <i>est.min.parameters</i> |
|--------------------|---------------------------|

Description

This function estimates the nu and a parameters to minimize the...

Usage

```
est.min.parameters(object, par)
```

```
## S4 method for signature 'scCOTAN'  
est.min.parameters(object, par)
```

Arguments

| | |
|--------|-----------------------------------|
| object | A COTAN object |
| par | A vector of inicial a and log(nu) |

Value

vector

Examples

```
data("ERCC.cotan")
```

| | |
|--------------|---------------------|
| extract.coex | <i>extract.coex</i> |
|--------------|---------------------|

Description

This function extract a complete or a prtial coex matrix from the COTAN object.

Usage

```
extract.coex(object, genes = "all")

## S4 method for signature 'scCOTAN'
extract.coex(object, genes = "all")
```

Arguments

| | |
|--------|--|
| object | A COTAN object |
| genes | A vector of gene names. These will be on the columns of the final data frame. By defaults the function will use all genes. |

Value

the coex values data frame

Examples

```
data("ERCC.cotan")
coex <- extract.coex(ERCC.cotan)
```

| | |
|------------|-------------------|
| fun_linear | <i>fun_linear</i> |
|------------|-------------------|

Description

Internal function to estimate the cell efficiency

Usage

```
fun_linear(object)
```

Arguments

| | |
|--------|--------------|
| object | COTAN object |
|--------|--------------|

Value

a list of object (dist_cells, to_clust, pca_cells, t_to_clust, mu_estimator, object)

`get.a`*get.a*

Description

This function extract the a array.

Usage

```
get.a(object)
```

```
## S4 method for signature 'scCOTAN'  
get.a(object)
```

Arguments

object A COTAN object

Value

the a array.

Examples

```
data("ERCC.cotan")  
get.a(ERCC.cotan)[1:10]
```

`get.cell.number`*get.cell.number*

Description

This function extract number of analysed cells.

Usage

```
get.cell.number(object)
```

```
## S4 method for signature 'scCOTAN'  
get.cell.number(object)
```

Arguments

object A COTAN object

Value

the cell number value.

Examples

```
data("ERCC.cotan")  
get.cell.number(ERCC.cotan)
```

`get.cell.size` *get.cell.size*

Description

This function extract the cell raw library size.

Usage

```
get.cell.size(object)  
  
## S4 method for signature 'scCOTAN'  
get.cell.size(object)
```

Arguments

object A COTAN object

Value

an array with the library sizes

Examples

```
data("ERCC.cotan")  
get.cell.size(ERCC.cotan)[1:10]
```

```
get.coex          get.coex
```

Description

This function estimates and stores the coex matrix in the coex field. It need to be run after [cotan_analysis](#)

Usage

```
get.coex(object)

## S4 method for signature 'scCOTAN'
get.coex(object)
```

Arguments

object A COTAN object

Value

It returns a COTAN object

Examples

```
data("ERCC.cotan")
obj <- get.coex(ERCC.cotan)
```

```
get.constitutive.genes
get.constitutive.genes This function return the genes expressed in all
cells in the dataset.
```

Description

`get.constitutive.genes` This function return the genes expressed in all cells in the dataset.

Usage

```
get.constitutive.genes(object)

## S4 method for signature 'scCOTAN'
get.constitutive.genes(object)
```

Arguments

object A COTAN object

Value

an array containing all genes expressed in all cells

Examples

```
data("ERCC.cotan")
get.constitutive.genes(ERCC.cotan)
```

```
get.expected.ct      get.expected.ct
```

Description

This function is used to get the expected contingency table for a given pair of genes.

Usage

```
get.expected.ct(object, g1, g2)

## S4 method for signature 'scCOTAN'
get.expected.ct(object, g1, g2)
```

Arguments

| | |
|--------|------------------|
| object | The cotan object |
| g1 | A gene |
| g2 | The other gene |

Value

A contingency table as dataframe

Examples

```
data("ERCC.cotan")
g1 <- get.genes(ERCC.cotan)[sample(length(get.genes(ERCC.cotan)), 1)]
g2 <- get.genes(ERCC.cotan)[sample(length(get.genes(ERCC.cotan)), 1)]
while (g1 %in% get.constitutive.genes(ERCC.cotan)) {
  g1 <- get.genes(ERCC.cotan)[sample(length(get.genes(ERCC.cotan)), 1)]
}

while (g2 %in% get.constitutive.genes(ERCC.cotan)) {
  g2 <- get.genes(ERCC.cotan)[sample(length(get.genes(ERCC.cotan)), 1)]
}
get.expected.ct(object = ERCC.cotan, g1 = g1, g2 = g2)
```

| | |
|----------------------|----------------|
| <code>get.GDI</code> | <i>get.GDI</i> |
|----------------------|----------------|

Description

This function produce a dataframe with the GDI for each genes.

Usage

```
get.GDI(object, type = "S")

## S4 method for signature 'scCOTAN'
get.GDI(object, type = "S")
```

Arguments

| | |
|---------------------|---|
| <code>object</code> | A COTAN object |
| <code>type</code> | Type of statistic to be used. Default is "S": Pearson's chi-squared test statistics. "G" is G-test statistics |

Value

A dataframe

Examples

```
data("ERCC.cotan")
quant.p <- get.GDI(ERCC.cotan)
```

| | |
|--|------------------------------------|
| <code>get.gene.coexpression.space</code> | <i>get.gene.coexpression.space</i> |
|--|------------------------------------|

Description

To make the GDI more specific, it may be desirable to restrict the set of genes against which GDI is computed to a selected subset V with the recommendation to include a consistent fraction of cell-identity genes, and possibly focusing on markers specific for the biological question of interest (for instance neural cortex layering markers). In this case we denote it as local differentiation index (LDI) relative to V .

Usage

```
get.gene.coexpression.space(object, n.genes.for.marker = 25, primary.markers)

## S4 method for signature 'scCOTAN'
get.gene.coexpression.space(object, n.genes.for.marker = 25, primary.markers)
```

Arguments

object The COTAN object.

n.genes.for.marker The number of genes correlated with the primary markers that we want to consider. By default this is 25.

primary.markers A vector of primary marker names.

Value

A dataframe

Examples

```
data("ERCC.cotan")
df <- get.gene.coexpression.space(ERCC.cotan, n.genes.for.marker = 10,
primary.markers=get.genes(ERCC.cotan)[sample(length(get.genes(ERCC.cotan)),5)])
```

| | |
|-----------|------------------|
| get.genes | <i>get.genes</i> |
|-----------|------------------|

Description

This function extract all genes in the dataset.

Usage

```
get.genes(object)

## S4 method for signature 'scCOTAN'
get.genes(object)
```

Arguments

object A COTAN object

Value

a gene array

Examples

```
data("ERCC.cotan")
get.genes(ERCC.cotan)[1:10]
```

| | |
|-------------------------|-------------------|
| <code>get.lambda</code> | <i>get.lambda</i> |
|-------------------------|-------------------|

Description

This function extract the lambda array (mean expression for each gene).

Usage

```
get.lambda(object)

## S4 method for signature 'scCOTAN'
get.lambda(object)
```

Arguments

object A COTAN object

Value

the lambda array.

Examples

```
data("ERCC.cotan")
get.lambda(ERCC.cotan)[1:10]
```

| | |
|---------------------------|---------------------|
| <code>get.metadata</code> | <i>get.metadata</i> |
|---------------------------|---------------------|

Description

This function extract the meta date stored for the dataset.

Usage

```
get.metadata(object)

## S4 method for signature 'scCOTAN'
get.metadata(object)
```

Arguments

object A COTAN object

Value

the metatdata dataframe

Examples

```
data("ERCC.cotan")  
get.metadata(ERCC.cotan)
```

| | |
|---------------------------|---------------------|
| <code>get.normdata</code> | <i>get.normdata</i> |
|---------------------------|---------------------|

Description

This function extract the normalized count table.

Usage

```
get.normdata(object)  
  
## S4 method for signature 'scCOTAN'  
get.normdata(object)
```

Arguments

object A COTAN object

Value

the normalized count dataframe (divided by nu).

Examples

```
data("ERCC.cotan")  
get.normdata(ERCC.cotan)[1:10,1:10]
```

| | |
|---------------------|---------------|
| <code>get.nu</code> | <i>get.nu</i> |
|---------------------|---------------|

Description

This function extract the nu array.

Usage

```
get.nu(object)

## S4 method for signature 'scCOTAN'
get.nu(object)
```

Arguments

| | |
|---------------------|----------------|
| <code>object</code> | A COTAN object |
|---------------------|----------------|

Value

the nu array.

Examples

```
data("ERCC.cotan")
get.nu(ERCC.cotan)[1:10]
```

| | |
|------------------------------|------------------------|
| <code>get.observed.ct</code> | <i>get.observed.ct</i> |
|------------------------------|------------------------|

Description

This function is used to get the observed contingency table for a given pair of genes.

Usage

```
get.observed.ct(object, g1, g2)

## S4 method for signature 'scCOTAN'
get.observed.ct(object, g1, g2)
```

Arguments

| | |
|---------------------|------------------|
| <code>object</code> | The cotan object |
| <code>g1</code> | A gene |
| <code>g2</code> | The other gene |

Value

A contingency table as dataframe

Examples

```
data("ERCC.cotan")
g1 <- get.genes(ERCC.cotan)[sample(length(get.genes(ERCC.cotan)), 1)]
g2 <- get.genes(ERCC.cotan)[sample(length(get.genes(ERCC.cotan)), 1)]
get.observed.ct(object = ERCC.cotan, g1 = g1, g2 = g2)
```

| | |
|----------|-----------------|
| get.pval | <i>get.pval</i> |
|----------|-----------------|

Description

This function computes the p-values for genes in the COTAN object. It can be used genome-wide or setting some specific genes of interest. By default it computes the p-values using the S statistics (χ^2)

Usage

```
get.pval(object, gene.set.col = c(), gene.set.row = c(), type_stat = "S")

## S4 method for signature 'scCOTAN'
get.pval(object, gene.set.col = c(), gene.set.row = c(), type_stat = "S")
```

Arguments

| | |
|--------------|--|
| object | a COTAN object |
| gene.set.col | an array of genes. It will be put in columns. If left empty the function will do it genome-wide. |
| gene.set.row | an array of genes. It will be put in rows. If left empty the function will do it genome-wide. |
| type_stat | By default it computes the S (χ^2) |

Value

a p-value matrix

Examples

```
data("ERCC.cotan")
ERCC.cotan <- get.pval(ERCC.cotan, type_stat="S")
```

| | |
|--------------------------|--------------------|
| <code>get.rawdata</code> | <i>get.rawdata</i> |
|--------------------------|--------------------|

Description

This function extract the raw count table.

Usage

```
get.rawdata(object)

## S4 method for signature 'scCOTAN'
get.rawdata(object)
```

Arguments

`object` A COTAN object

Value

the raw count dataframe

Examples

```
data("ERCC.cotan")
get.rawdata(ERCC.cotan)[1:10,1:10]
```

| | |
|-------------------------|-------------------|
| <code>get.subset</code> | <i>get.subset</i> |
|-------------------------|-------------------|

Description

This function extract the row data for a subset of clusters.

Usage

```
get.subset(object, cluster.names)

## S4 method for signature 'scCOTAN'
get.subset(object, cluster.names)
```

Arguments

`object` A COTAN object
`cluster.names` A cluster names array

Value

the subset raw count dataframe

Examples

```
data("ERCC.cotan")
```

initRaw

initRaw

Description

It starts to fill some fields of cotan object.

Usage

```
initRaw(object, GEO, sc.method = "10X", cond)

## S4 method for signature 'scCOTAN'
initRaw(object, GEO, sc.method = "10X", cond)
```

Arguments

| | |
|------------------------|---|
| <code>object</code> | the dataframe containing the raw data: it should be a <code>data.frame</code> , not a matrix. |
| <code>GEO</code> | a code reporting the GEO identification or other specific dataset code |
| <code>sc.method</code> | a string reporting the method used for the sequencing |
| <code>cond</code> | a string reporting the specific sample condition or time point |

Value

A COTAN object to store all information

Examples

```
data("raw.dataset")
obj <- new("scCOTAN", raw = raw.dataset)
obj <- initRaw(obj, GEO="code" , sc.method="10X", cond = "mouse dataset")
```

| | |
|---------------|----------------------|
| mat2vec_rfast | <i>mat2vec_rfast</i> |
|---------------|----------------------|

Description

mat2vec_rfast

Usage

```
mat2vec_rfast(mat)

## S4 method for signature 'matrix'
mat2vec_rfast(mat)
```

Arguments

mat a symmetric matrix with all genes as row and column names

Value

a list formed by two arrays: "genes" with the gene names and "values" with all unique values.

Examples

```
mat <- matrix(0,nrow = 10, ncol = 10)
mat <- Rfast::lower_tri.assign(mat,c(1:55),diag = TRUE)
mat <- Rfast::upper_tri.assign(mat,v = Rfast::upper_tri(Rfast::transpose(mat)))
v <- mat2vec_rfast(mat)
```

| | |
|----------|-----------------|
| plot_GDI | <i>plot_GDI</i> |
|----------|-----------------|

Description

This function directly evaluate and plot the GDI for a sample.

Usage

```
plot_GDI(object, cond, type = "S")

## S4 method for signature 'scCOTAN'
plot_GDI(object, cond, type = "S")
```

Arguments

| | |
|--------|--|
| object | A COTAN object |
| cond | A string corresponding to the condition/sample (it is used only for the title) |
| type | Type of statistic to be used. Default is "S": Pearson's chi-squared test statistics. "G" is G-test statistics |

Value

A ggplot2 object

Examples

```
data("ERCC.cotan")
plot_GDI(ERCC.cotan, cond = "ERCC")
```

plot_general.heatmap *plot_general.heatmap*

Description

This function is used to plot an heatmap made using only some genes, as markers, and collecting all other genes correlated with these markers with a p-value smaller than the set threshold. Than all relations are plotted. Primary markers will be plotted as groups of rows. Markers list will be plotted as columns.

Usage

```
plot_general.heatmap(
  prim.markers = c("Satb2", "Bcl11b", "Cux1", "Fezf2", "Tbr1"),
  markers.list = c(),
  dir,
  condition,
  p_value = 0.001,
  symmetric = TRUE
)

## S4 method for signature 'ANY'
plot_general.heatmap(
  prim.markers = c("Satb2", "Bcl11b", "Cux1", "Fezf2", "Tbr1"),
  markers.list = c(),
  dir,
  condition,
  p_value = 0.001,
  symmetric = TRUE
)
```

Arguments

| | |
|--------------|--|
| prim.markers | A set of genes plotted as rows. |
| markers.list | A set of genes plotted as columns. |
| dir | The directory where the COTAN object is stored. |
| condition | The prefix for the COTAN object file. |
| p_value | The p-value threshold |
| symmetric | A boolean: default F. If T the union of prim.markers and marker.list is sets as both rows and column genes |

Value

A ggplot2 object

Examples

```
data("ERCC.cotan")
data_dir <- tempdir()
saveRDS(ERCC.cotan, file = file.path(data_dir,"ERCC.cotan.RDS"))
# some genes
primary.markers <- c("ERCC-00154","ERCC-00156","ERCC-00164")
#a example of named list of different gene set
gene.sets.list <- list("primary.markers"=primary.markers,
                      "2.R" = c("ERCC-00170","ERCC-00158"),
                      "3.S"=c("ERCC-00160","ERCC-00162"))
plot_general.heatmap(prim.markers = primary.markers, p_value = 0.05, markers.list =gene.sets.list ,
condition ="ERCC" ,dir = paste0(data_dir,"/"))
```

plot_heatmap

plot_heatmap

Description

This is the function that create the heatmap of one or more COTAN object.

Usage

```
plot_heatmap(p_val.tr = 0.05, df_genes, sets, conditions, dir)

## S4 method for signature 'ANY'
plot_heatmap(p_val.tr = 0.05, df_genes, sets, conditions, dir)
```

Arguments

| | |
|------------|--|
| p_val.tr | p-value threshold. Default is 0.05 |
| df_genes | this is a list of gene array. The first array will define genes in the columns. |
| sets | This is a numeric array indicating from which fields of the previous list will be considered |
| conditions | An array of prefixes indicating the different files. |
| dir | The directory in which are all COTAN files (corresponding to the previous prefixes) |

Value

a ggplot object

Examples

```
data("ERCC.cotan")
data_dir <- tempdir()
saveRDS(ERCC.cotan, file = file.path(data_dir, "ERCC.cotan.RDS"))
# some genes
primary.markers <- c("ERCC-00154", "ERCC-00156", "ERCC-00164")
#a example of named list of different gene set
gene.sets.list <- list("primary.markers"=primary.markers,
                      "2.R" = c("ERCC-00170", "ERCC-00158"),
                      "3.S"=c("ERCC-00160", "ERCC-00162"))
plot_heatmap(p_v = 0.05, df_genes =gene.sets.list ,
             sets =c(2,3) ,conditions =c("ERCC") ,dir = paste0(data_dir, "/"))
```

raw.dataset

Raw sample dataset

Description

A subsample of a real sc-RNAseq dataset

Usage

```
raw.dataset
```

Format

A data frame with 2000 genes and 815 cells:

Source

GEO GSM2861514

| | |
|---------------|---------------|
| scCOTAN-class | scCOTAN-class |
|---------------|---------------|

Description

Define my COTAN structure

Value

the object class

Slots

raw ANY. To store the raw data matrix

raw.norm ANY. To store the raw data matrix divided for the cell efficiency estimated (nu)

coex ANY. The coex matrix (sparse)

nu vector.

lambda vector.

a vector.

hk vector.

n_cells numeric.

meta data.frame.

yes_yes ANY.

clusters vector.

cluster_data data.frame.

Examples

```
data("ERCCraw")  
obj <- new("scCOTAN",raw = data)
```

| | |
|---------------|----------------------|
| vec2mat_rfast | <i>vec2mat_rfast</i> |
|---------------|----------------------|

Description

vec2mat_rfast

Usage

```
vec2mat_rfast(x, genes = "all")  
  
## S4 method for signature 'list'  
vec2mat_rfast(x, genes = "all")
```

Arguments

| | |
|-------|--|
| x | a list formed by two arrays: "genes" with the gene names and "values" with all unique values. |
| genes | a vector with all wanted genes or "all". By default is equal to "all" in this way it recreate the entire coex dataframe. |

Value

a dataframe

Examples

```
v <- list("genes" = paste0("gene.",c(1:10)), "values"=c(1:55))  
genes <- c("gene.3", "gene.4", "gene.7")  
df <- vec2mat_rfast(v, genes)  
df2 <- vec2mat_rfast(v)
```

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