

Package ‘COTAN’

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Type Package

Title COexpression Tables ANalysis

Version 2.2.4

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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Description

These are the functions and methods used to calculate the **COEX** matrices according to the COTAN model. From there it is possible to calculate the associated *pValue* and the *GDI (Global Differential Expression)*

The **COEX** matrix is defined by following formula:

$$\frac{\sum_{i,j \in \{Y, N\}} (-1)^{\#\{i,j\}} \frac{O_{ij} - E_{ij}}{1 \vee E_{ij}}}{\sqrt{n \sum_{i,j \in \{Y, N\}} \frac{1}{1 \vee E_{ij}}}}$$

where O and E are the observed and expected contingency tables and n is the relevant numerosity (the number of genes/cells depending on given actOnCells flag).

The formula can be more effectively implemented as:

$$\sqrt{\frac{1}{n} \sum_{i,j \in \{Y, N\}} \frac{1}{1 \vee E_{ij}}} (O_{YY} - E_{YY})$$

once one notices that $O_{ij} - E_{ij} = (-1)^{\#\{i,j\}} r$ for some constant r for all $i, j \in \{Y, N\}$.

The latter follows from the fact that the relevant marginal sums of the the expected contingency tables were enforced to match the marginal sums of the observed ones.

Usage

```
## S4 method for signature 'COTAN'
getGenesCoex(
  objCOTAN,
  genes = vector(mode = "character"),
  zeroDiagonal = TRUE,
  ignoreSync = FALSE
)

## S4 method for signature 'COTAN'
getCellsCoex(
  objCOTAN,
  cells = vector(mode = "character"),
  zeroDiagonal = TRUE,
  ignoreSync = FALSE
)

## S4 method for signature 'COTAN'
```

```
dropGenesCoex(objCOTAN)

## S4 method for signature 'COTAN'
dropCellsCoex(objCOTAN)

## S4 method for signature 'COTAN'
calculateMu(objCOTAN)

observedContingencyTablesYY(
  objCOTAN,
  actOnCells = FALSE,
  asDspMatrices = FALSE
)

observedContingencyTables(objCOTAN, actOnCells = FALSE, asDspMatrices = FALSE)

expectedContingencyTablesNN(
  objCOTAN,
  actOnCells = FALSE,
  asDspMatrices = FALSE,
  optimizeForSpeed = TRUE
)

expectedContingencyTables(
  objCOTAN,
  actOnCells = FALSE,
  asDspMatrices = FALSE,
  optimizeForSpeed = TRUE
)

contingencyTables(objCOTAN, g1, g2)

## S4 method for signature 'COTAN'
calculateCoex(objCOTAN, actOnCells = FALSE, optimizeForSpeed = TRUE)

calculateS(
  objCOTAN,
  geneSubsetCol = vector(mode = "character"),
  geneSubsetRow = vector(mode = "character")
)

calculateG(
  objCOTAN,
  geneSubsetCol = vector(mode = "character"),
  geneSubsetRow = vector(mode = "character")
)
```

Arguments

objCOTAN	a COTAN object
genes	A vector of gene names. It will exclude any gene not on the list. By defaults the function will keep all genes.
zeroDiagonal	When TRUE sets the diagonal to zero.
ignoreSync	When TRUE ignores whether the lambda/nu/dispersion have been updated since the COEX matrix was calculated.
cells	A vector of cell names. It will exclude any cell not on the list. By defaults the function will keep all cells.
actOnCells	Boolean; when TRUE the function works for the cells, otherwise for the genes
asDspMatrices	Boolean; when TRUE the function will return only packed dense symmetric matrices
optimizeForSpeed	Boolean; when TRUE the function will use Rfast parallel algorithms that on the flip side use more memory
g1	a gene
g2	another gene
geneSubsetCol	an array of genes. It will be put in columns. If left empty the function will do it genome-wide.
geneSubsetRow	an array of genes. It will be put in rows. If left empty the function will do it genome-wide.

Details

getGenesCoex() extracts a complete (or a partial after genes dropping) genes' COEX matrix from the COTAN object.

getCellsCoex() extracts a complete (or a partial after cells dropping) cells' COEX matrix from the COTAN object.

dropGenesCoex() drops the genesCoex member from the given COTAN object

dropCellsCoex() drops the cellsCoex member from the given COTAN object

calculateMu() calculates the vector $\mu = \lambda \times \nu^T$

observedContingencyTablesYY() calculates observed *Yes/Yes* field of the contingency table

observedContingencyTables() calculates the observed contingency tables. When the parameter asDspMatrices == TRUE, the method will effectively throw away the lower half from the returned observedYN and observedNY matrices, but, since they are transpose one of another, the full information is still available.

expectedContingencyTablesNN() calculates the expected No/No field of the contingency table

expectedContingencyTables() calculates the expected values of contingency tables. When the parameter asDspMatrices == TRUE, the method will effectively throw away the lower half from the returned expectedYN and expectedNY matrices, but, since they are transpose one of another, the full information is still available.

contingencyTables() returns the observed and expected contingency tables for a given pair of genes. The implementation runs the same algorithms used to calculate the full observed/expected

contingency tables, but restricted to only the relevant genes and thus much faster and less memory intensive

calculateCoex() estimates and stores the COEX matrix in the cellCoex or genesCoex field depending on given actOnCells flag. It also calculates the percentage of *problematic* genes/cells pairs. A pair is *problematic* when one or more of the expected counts were significantly smaller than 1 (< 0.5). These small expected values signal that scant information is present for such a pair.

calculateS() calculates the statistics **S** for genes contingency tables. It always has the diagonal set to zero.

calculateG() calculates the statistics *G-test* for genes contingency tables. It always has the diagonal set to zero. It is proportional to the genes' presence mutual information.

Value

getGenesCoex() returns the genes' COEX values

getCellsCoex() returns the cells' COEX values

dropGenesCoex() returns the updated COTAN object

dropCellsCoex() returns the updated COTAN object

calculateMu() returns the mu matrix

observedContingencyTablesYY() returns a list with the *Yes/Yes* observed contingency table as matrix and the *Yes* observed vector

observedContingencyTables() returns the observed contingency tables as named list with elements: "observedNN", "observedNY", "observedYN", "observedYY"

expectedContingencyTablesNN() returns a list with the *No/No* expected contingency table as matrix and the *No* expected vector

expectedContingencyTables() returns the expected contingency tables as named list with elements: "expectedNN", "expectedNY", "expectedYN", "expectedYY"

contingencyTables() returns a list containing the observed and expected contingency tables

calculateCoex() returns the updated COTAN object

calculateS() returns the S matrix

calculateG() returns the G matrix

Note

The sum of the matrices returned by the function observedContingencyTables() and expectedContingencyTables() will have the same value on all elements. This value is the number of genes/cells depending on the parameter actOnCells being TRUE/FALSE.

See Also

[ParametersEstimations](#) for more details.

Examples

```

data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)
objCOTAN <- initializeMetaDataset(objCOTAN, GEO = "test_GEO",
                                sequencingMethod = "distribution_sampling",
                                sampleCondition = "reconstructed_dataset")

objCOTAN <- clean(objCOTAN)

objCOTAN <- estimateDispersionBisection(objCOTAN, cores = 12)

## Now the `COTAN` object is ready to calculate the genes' `COEX`

## mu <- calculateMu(objCOTAN)
## observedY <- observedContingencyTablesYY(objCOTAN, asDspMatrices = TRUE)
obs <- observedContingencyTables(objCOTAN, asDspMatrices = TRUE)

## expectedN <- expectedContingencyTablesNN(objCOTAN, asDspMatrices = TRUE)
exp <- expectedContingencyTables(objCOTAN, asDspMatrices = TRUE)

objCOTAN <- calculateCoex(objCOTAN, actOnCells = FALSE)
genesCoex <- getGenesCoex(objCOTAN)

## S <- calculateS(objCOTAN)
## G <- calculateG(objCOTAN)
## pValue <- calculatePValue(objCOTAN)
GDI <- calculateGDI(objCOTAN)

## Touching any of the lambda/nu/dispersino parameters invalidates the `COEX`
## matrix and derivatives, so it can be dropped it from the `COTAN` object
objCOTAN <- dropGenesCoex(objCOTAN)

objCOTAN <- estimateDispersionNuBisection(objCOTAN, cores = 12)

## Now the `COTAN` object is ready to calculate the cells' `COEX`
## In case one need to caclualte both it is more sensible to run the above
## before any `COEX` evaluation

g1 <- getGenes(objCOTAN)[sample(getNumGenes(objCOTAN), 1)]
g2 <- getGenes(objCOTAN)[sample(getNumGenes(objCOTAN), 1)]
tables <- contingencyTables(objCOTAN, g1 = g1, g2 = g2)
tables

objCOTAN <- calculateCoex(objCOTAN, actOnCells = TRUE)
cellsCoex <- getCellsCoex(objCOTAN)

objCOTAN <- dropCellsCoex(objCOTAN)

```

Description

Handle *clusterization* <-> *clusters* list conversions, *clusters* grouping and merge

Usage

```
toClustersList(clusters)

fromClustersList(
  clustersList,
  elemNames = vector(mode = "character"),
  throwOnOverlappingClusters = TRUE
)

groupByClustersList(elemNames, clustersList, throwOnOverlappingClusters = TRUE)

groupByClusters(clusters)

mergeClusters(clusters, names, mergedName = "")

multiMergeClusters(clusters, namesList, mergedNames = NULL)
```

Arguments

<code>clusters</code>	A named vector or factor that defines the <i>clusters</i>
<code>clustersList</code>	A named list whose elements define the various clusters
<code>elemNames</code>	A list of names to which associate a cluster
<code>throwOnOverlappingClusters</code>	When TRUE, in case of overlapping clusters, the function <code>fromClustersList</code> and <code>groupByClustersList</code> will throw. This is the default. When FALSE, instead, in case of overlapping clusters, <code>fromClustersList</code> will return the last cluster to which each element belongs, while <code>groupByClustersList</code> will return a vector of positions that is longer than the given <code>elemNames</code>
<code>names</code>	A list of <i>clusters</i> names to be merged
<code>mergedName</code>	The name of the new merged clusters
<code>namesList</code>	A list of lists of <i>clusters</i> names to be respectively merged
<code>mergedNames</code>	The names of the new merged <i>clusters</i>

Details

`toClustersList()` given a *clusterization*, creates a list of *clusters* (i.e. for each *cluster*, which elements compose the *cluster*)

`fromClustersList()` given a list of *clusters* returns a *clusterization* (i.e. a named vector that for each element indicates to which cluster it belongs)

`groupByClusters()` given a *clusterization* returns a permutation, such that using the permutation on the input the *clusters* are grouped together

groupByClustersList() given the elements' names and a list of *clusters* returns a permutation, such that using the permutation on the given names the *clusters* are grouped together.

mergeClusters() given a *clusterization*, creates a new one where the given *clusters* are merged.

multiMergeClusters() given a *clusterization*, creates a new one where the given sets of *clusters* are merged.

Value

toClustersList() returns a list of clusters

fromClustersList() returns a clusterization. If the given elemNames contain values not present in the clustersList, those will be marked as "-1"

groupByClusters() and groupByClustersList() return a permutation that groups the clusters together. For each cluster the positions are guaranteed to be in increasing order. In case, all elements not corresponding to any cluster are grouped together as the last group

mergeClusters() returns a new *clusterization* with the wanted *clusters* being merged. If less than 2 *cluster* names were passed the function will emit a warning and return the initial *clusterization*

multiMergeClusters() returns a new *clusterization* with the wanted *clusters* being merged by consecutive iterations of [mergeClusters\(\)](#) on the given namesList

Examples

```
## create a clusterization
clusters <- paste0("",sample(7, 100, replace = TRUE))
names(clusters) <- paste0("E_",formatC(1:100, width = 3, flag = "0"))

## create a clusters list from a clusterization
clustersList <- toClustersList(clusters)
head(clustersList, 1)

## recreate the clusterization from the cluster list
clusters2 <- fromClustersList(clustersList, names(clusters))
all.equal(factor(clusters), clusters2)

c11Size <- length(clustersList[["1"]])

## establish the permutation that groups clusters together
perm <- groupByClusters(clusters)
!is.unsorted(head(names(clusters)[perm],c11Size))
head(clusters[perm], c11Size)

## it is possible to have the list of the element names different
## from the names in the clusters list
selectedNames <- paste0("E_",formatC(11:110, width = 3, flag = "0"))
perm2 <- groupByClustersList(selectedNames, toClustersList(clusters))
all.equal(perm2[91:100], c(91:100))

## is is possible to merge a few clusters together
clustersMerged <- mergeClusters(clusters, names = c("7", "2"),
                               mergedName = "7_2")
```

```

sum(table(clusters)[c(2, 7)]) == table(clustersMerged)[["7__2"]]

## it is also possible to do multiple merges at once!
## Note the default new clusters' names
clustersMerged2 <-
  multiMergeClusters(clusters2, namesList = list(c("2", "7"),
                                                c("1", "3", "5")))
table(clustersMerged2)

```

COTAN

COTAN

Description

Constructor of the class COTAN

Usage

```
COTAN(raw = "ANY")
```

Arguments

`raw` any object that can be converted to a matrix, but with row (genes) and column (cells) names

Value

a COTAN object

Examples

```

data("test.dataset")
obj <- COTAN(raw = test.dataset)

```

COTAN-class

Definition of the COTAN class

Description

Definition of the COTAN class

Slots

`raw dgMatrix` - the raw UMI count matrix $n \times m$ (gene number \times cell number)
`genesCoex dspMatrix` - the correlation of COTAN between genes, $n \times n$
`cellsCoex dspMatrix` - the correlation of COTAN between cells, $m \times m$
`metaDataset` `data.frame`
`metaCells` `data.frame`
`clustersCoex` a list of COEX `data.frames` for each clustering in the `metaCells`

COTANObjectCreation *Automatic COTAN shortcuts*

Description

These functions take (or create) a COTAN object and run all the necessary steps until the genes' COEX matrix is calculated.

takes a newly created COTAN object (or the result of a call to `dropGenesCells()`) and applies all steps until the genes' COEX matrix is stored in the object

Usage

```

## S4 method for signature 'COTAN'
proceedToCoex(
  objCOTAN,
  calcCoex = TRUE,
  cores = 1L,
  saveObj = TRUE,
  outDir = "."
)

automaticCOTANObjectCreation(
  raw,
  GEO,
  sequencingMethod,
  sampleCondition,
  calcCoex = TRUE,
  cores = 1L,
  saveObj = TRUE,
  outDir = "."
)

```

Arguments

`objCOTAN` a newly created COTAN object
`calcCoex` a Boolean to determine whether to calculate the genes' COEX or stop just before at the `estimateDispersionBisection()` step

cores	number of cores to be used
saveObj	Boolean flag; when TRUE saves intermediate analyses and plots to file
outDir	an existing directory for the analysis output.
raw	a matrix or dataframe with the raw counts
GEO	a code reporting the GEO identification or other specific dataset code
sequencingMethod	a string reporting the method used for the sequencing
sampleCondition	a string reporting the specific sample condition or time point.

Details

`proceedToCoex()` takes a newly created COTAN object (or the result of a call to `dropGenesCells()`) and runs `calculateCoex()`

`automaticCOTANObjectCreation()` takes a raw dataset, creates and initializes a COTAN objects and runs `proceedToCoex()`

Value

`proceedToCoex()` returns the updated COTAN object with genes' COEX calculated. If asked to, it will also store the object, along all relevant clean-plots, in the output directory.

`automaticCOTANObjectCreation()` returns the new COTAN object with genes' COEX calculated. When asked, it will also store the object, along all relevant clean-plots, in the output directory.

Examples

```
data("test.dataset")

## In case one needs to run more steps to clean the dataset the following
## might apply
##
## objCOTAN <- COTAN(raw = test.dataset)
## objCOTAN <- initializeMetaDataset(objCOTAN,
##                                GEO = "test",
##                                sequencingMethod = "artificial",
##                                sampleCondition = "test dataset")
## # in case the genes' `COEX` is not needed it can be skipped
## # (e.g. for [cellsUniformClustering()])
## objCOTAN <- proceedToCoex(objCOTAN, calcCoex = FALSE,
##                            cores = 12, saveObj = FALSE)

## Otherwise it is possible to run all at once.
objCOTAN <- automaticCOTANObjectCreation(
  raw = test.dataset,
  GEO = "code",
  sequencingMethod = "10X",
  sampleCondition = "mouse_dataset",
  calcCoex = TRUE,
```

```
saveObj = FALSE,  
outDir = tempdir(),  
cores = 12)
```

Datasets

Data-sets

Description

Simple data-sets included in the package

Usage

```
data(raw.dataset)  
  
data(ERCCraw)  
  
data(test.dataset)  
  
data(test.dataset.clusters1)  
  
data(test.dataset.clusters2)
```

Format

`raw.dataset` is a data frame with 2000 genes and 815 cells
`ERCCraw` is a data.frame
`test.dataset` is a data.frame with 600 genes and 1200 cells
`test.dataset.clusters1` is a character array
`test.dataset.clusters2` is a character array

Details

`raw.dataset` is a sub-sample of a real *scRNA-seq* data-set
`ERCCraw` dataset
`test.dataset` is an artificial data set obtained by sampling target negative binomial distributions on a set of 600 genes on 2 two cells *clusters* of 600 cells each. Each *clusters* has its own set of parameters for the distributions even, but a fraction of the genes has the same expression in both *clusters*.
`test.dataset.clusters1` is the *clusterization* obtained running `cellsUniformClustering()` on the `test.dataset`
`test.dataset.clusters2` is the *clusterization* obtained running `mergeUniformCellsClusters()` on the `test.dataset` using the previous *clusterization*

Source

GEO GSM2861514
ERCC

estimateNuLinearByCluster,COTAN-method
Handling cells' clusterization and related functions

Description

These functions manage the *clusterizations* and their associated *cluster* COEX data .frames.

A *clusterization* is any partition of the cells where to each cell it is assigned a **label**; a group of cells with the same label is called *cluster*.

For each *cluster* is also possible to define a COEX value for each gene, indicating its increased or decreased expression in the *cluster* compared to the whole background. A data.frame with these values listed in a column for each *cluster* is stored separately for each *clusterization* in the clustersCoex member.

The formulae for this *In/Out* COEX are similar to those used in the `calculateCoex()` method, with the **role** of the second gene taken by the *In/Out* status of the cells with respect to each *cluster*.

Usage

```
## S4 method for signature 'COTAN'
estimateNuLinearByCluster(objCOTAN, clName = "", clusters = NULL)

## S4 method for signature 'COTAN'
getClusterizations(objCOTAN, dropNoCoex = FALSE, keepPrefix = FALSE)

## S4 method for signature 'COTAN'
getClusterizationName(objCOTAN, clName = "", keepPrefix = FALSE)

## S4 method for signature 'COTAN'
getClusterizationData(objCOTAN, clName = "")

getClusters(objCOTAN, clName = "")

## S4 method for signature 'COTAN'
getClustersCoex(objCOTAN)

## S4 method for signature 'COTAN'
addClusterization(
  objCOTAN,
  clName,
  clusters,
  coexDF = data.frame(),
```

```
    override = FALSE
  )

  ## S4 method for signature 'COTAN'
  addClusterizationCoex(objCOTAN, clName, coexDF)

  ## S4 method for signature 'COTAN'
  dropClusterization(objCOTAN, clName)

  DEAOncClusters(objCOTAN, clName = "", clusters = NULL)

  pValueFromDEA(coexDF, numCells)

  UMAPPlot(df, clusters = NULL, elements = NULL, title = "")

  clustersDeltaExpression(objCOTAN, clName = "", clusters = NULL)

  clustersMarkersHeatmapPlot(
    objCOTAN,
    groupMarkers,
    clName = "",
    clusters = NULL,
    kCuts = 3L,
    condNameList = NULL,
    conditionsList = NULL
  )

  clustersSummaryData(
    objCOTAN,
    clName = "",
    clusters = NULL,
    condName = "",
    conditions = NULL
  )

  clustersSummaryPlot(
    objCOTAN,
    clName = "",
    clusters = NULL,
    condName = "",
    conditions = NULL,
    plotTitle = ""
  )

  clustersTreePlot(
    objCOTAN,
    kCuts,
    clName = "",
```

```

    distance = "cosine",
    hclustMethod = "ward.D2"
  )

  findClustersMarkers(
    objCOTAN,
    n = 10L,
    markers = NULL,
    clName = "",
    clusters = NULL,
    coexDF = NULL,
    pValueDF = NULL,
    deltaExp = NULL,
    method = "bonferroni"
  )

  geneSetEnrichment(clustersCoex, groupMarkers)

  reorderClusterization(
    objCOTAN,
    clName = "",
    clusters = NULL,
    coexDF = NULL,
    reverse = FALSE,
    keepMinusOne = TRUE,
    distance = "cosine",
    hclustMethod = "ward.D2"
  )

```

Arguments

objCOTAN	a COTAN object
clName	The name of the <i>clusterization</i> . If not given the last available <i>clusterization</i> will be used, as it is probably the most significant!
clusters	A <i>clusterization</i> to use. If given it will take precedence on the one indicated by clName
dropNoCoex	When TRUE drops the names from the <i>clusterizations</i> with empty associated coex data.frame
keepPrefix	When TRUE returns the internal name of the <i>clusterization</i> : the one with the CL_ prefix.
coexDF	a data.frame where each column indicates the COEX for each of the <i>clusters</i> of the <i>clusterization</i>
override	When TRUE silently allows overriding data for an existing <i>clusterization</i> name. Otherwise the default behavior will avoid potential data losses
numCells	the number of overall cells in all <i>clusters</i>
df	the data.frame to plot. It must have a row names containing the given elements

elements	a named list of elements to label. Each array in the list will have different color
title	a string giving the plot title. Will default to UMAP Plot if not specified
groupMarkers	a named list with an element for each group comprised of one or more marker genes
kCuts	the number of estimated <i>cluster</i> (this defines the height for the tree cut)
condNameList	a list of <i>conditions</i> ' names to be used for additional columns in the final plot. When none are given no new columns will be added using data extracted via the function <code>clustersSummaryData()</code>
conditionsList	a list of <i>conditions</i> to use. If given they will take precedence on the ones indicated by <code>condNameList</code>
condName	The name of a condition in the COTAN object to further separate the cells in more sub-groups. When no condition is given it is assumed to be the same for all cells (no further sub-divisions)
conditions	The <i>conditions</i> to use. If given it will take precedence on the one indicated by <code>condName</code> that will only indicate the relevant column name in the returned <code>data.frame</code>
plotTitle	The title to use for the returned plot
distance	type of distance to use (default is "cosine", "euclidean" and the others from <code>parallelDist::parDist()</code> are also available)
hclustMethod	It defaults is "ward.D2" but can be any of the methods defined by the <code>stats::hclust()</code> function.
n	the number of extreme COEX values to return
markers	a list of marker genes
pValueDF	a <code>data.frame</code> with <i>In/Out p-value</i> based on the COEX. E.G. the result of a call to <code>pValueFromDEA()</code>
deltaExp	a <code>data.frame</code> with the <i>delta-expression</i> in a <i>cluster</i> . E.G. the result of a call to <code>clustersDeltaExpression()</code>
method	<i>p-value</i> adjustment method. Defaults to "bonferroni"
clustersCoex	the COEX <code>data.frame</code>
reverse	a flag to the output order
keepMinusOne	a flag to decide whether to keep the cluster "-1" (representing the non-clustered cells) untouched

Details

`estimateNuLinearByCluster()` does a linear estimation of *nu*: cells' counts averages normalized *cluster* by *cluster*

`getClusterizations()` extracts the list of the *clusterizations* defined in the COTAN object.

`getClusterizationName()` normalizes the given *clusterization* name or, if none were given, returns the name of last available *clusterization* in the COTAN object. It can return the *clusterization internal name* if needed

`getClusterizationData()` extracts the asked *clusterization* and its associated COEX data.frame from the COTAN object

`getClusters()` extracts the asked *clusterization* from the COTAN object

`getClustersCoex()` extracts the full clusterCoex member list

`addClusterization()` adds a *clusterization* to the current COTAN object, by adding a new column in the metaCells data.frame and adding a new element in the clustersCoex list using the passed in COEX data.frame or an empty data.frame if none were passed in.

`addClusterizationCoex()` adds a *clusterization* COEX data.frame to the current COTAN object. It requires the named *clusterization* to be already present.

`dropClusterization()` drops a *clusterization* from the current COTAN object, by removing the corresponding column in the metaCells data.frame and the corresponding COEX data.frame from the clustersCoex list.

`DEAOnClusters()` is used to run the Differential Expression analysis using the COTAN contingency tables on each *cluster* in the given *clusterization*

`pValueFromDEA()` is used to convert to *p-value* the Differential Expression analysis using the COTAN contingency tables on each *cluster* in the given *clusterization*

`UMAPPlot()` plots the given data.frame containing genes information related to clusters after applying the UMAP transformation.

`clustersDeltaExpression()` estimates the change in genes' expression inside the *cluster* compared to the average situation in the data set.

`clustersMarkersHeatmapPlot()` returns the heatmap plot of a summary score for each *cluster* and each gene marker list in the given *clusterization*. It also returns the numerosity and percentage of each *cluster* on the right and a gene *clusterization* dendrogram on the left (as returned by the function [geneSetEnrichment\(\)](#)) that allows to estimate which markers groups are more or less expressed in each *cluster* so it is easier to derive the *clusters'* cell types.

`clustersSummaryData()` calculates various statistics about each cluster (with an optional further condition to separate the cells).

`clustersSummaryPlot()` calculates various statistics about each cluster via [clustersSummaryData\(\)](#) and puts them together into a plot.

`clustersTreePlot()` returns the dendrogram plot where the given *clusters* are placed on the base of their relative distance. Also if needed calculates and stores the DEA of the relevant *clusterization*.

`findClustersMarkers()` takes in a COTAN object and a *clusterization* and produces a data.frame with the n most positively enriched and the n most negatively enriched genes for each *cluster*. The function also provides whether and the found genes are in the given markers list or not. It also returns the *p-value* and the *adjusted p-value* using the [stats::p.adjust\(\)](#)

`geneSetEnrichment()` returns a cumulative score of enrichment in a *cluster* over a gene set. In formulae it calculates $\frac{1}{n} \sum_i (1 - e^{-\theta X_i})$, where the X_i are the positive values from [DEAOnClusters\(\)](#) and $\theta = -\frac{1}{0.1} \ln(0.25)$

`reorderClusterization()` takes in a *clusterizations* and reorder its labels so that in the new order near labels indicate near clusters according to a DEA based distance

Value

estimateNuLinearByCluster() returns the updated COTAN object

getClusterizations() returns a vector of *clusterization* names, usually without the CL_ prefix

getClusterizationName() returns the normalized *clusterization* name or NULL if no *clusterizations* are present

getClusterizationData() returns a list with 2 elements:

- "clusters" the named cluster labels array
- "coex" the associated COEX data.frame. This will be an **empty** data.frame when not specified for the relevant *clusterization*

getClusters() returns the named cluster labels array

getClustersCoex() returns the list with a COEX data.frame for each *clusterization*. When not empty, each data.frame contains a COEX column for each *cluster*.

addClusterization() returns the updated COTAN object

addClusterizationCoex() returns the updated COTAN object

dropClusterization() returns the updated COTAN object

DEAOnClusters() returns the co-expression data.frame for the genes in each *cluster*

pValueFromDEA() returns a data.frame with the *p-values* corresponding to the given *coex*

UMAPPlot() returns a ggplot2 object

clustersDeltaExpression() returns a data.frame with the weighted discrepancy of the expression of each gene within the *cluster* against model expectations

clustersMarkersHeatmapPlot() returns a list with:

- "heatmapPlot" the complete heatmap plot
- "dataScore" the data.frame with the score values

clustersSummaryData() returns a data.frame with the following statistics: The calculated statistics are:

- "clName" the *cluster labels*
- "condName" the relevant condition (that sub-divides the *clusters*)
- "CellNumber" the number of cells in the group
- "MeanUDE" the average "UDE" in the group of cells
- "MedianUDE" the median "UDE" in the group of cells
- "ExpGenes25" the number of genes expressed in at the least 25% of the cells in the group
- "ExpGenes" the number of genes expressed at the least once in any of the cells in the group
- "CellPercentage" fraction of the cells with respect to the total cells

clustersSummaryPlot() returns a list with a data.frame and a ggplot objects

- "data" contains the data,
- "plot" is the returned plot

clustersTreePlot() returns a list with 2 objects:

- "dend" a ggplot2 object representing the dendrogram plot
- "objCOTAN" the updated COTAN object

findClustersMarkers() returns a data.frame containing n top/bottom COEX scores for each *cluster*

geneSetEnrichment() returns a data.frame with the cumulative score

reorderClusterization() returns a list with 2 elements:

- "clusters" the newly reordered cluster labels array
- "coex" the associated COEX data.frame

Examples

```
data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)
objCOTAN <- clean(objCOTAN)
objCOTAN <- estimateDispersionBisection(objCOTAN, cores = 12)

data("test.dataset.clusters1")
clusters <- test.dataset.clusters1

coexDF <- DEAOncusters(objCOTAN, clusters = clusters)

groupMarkers <- list(G1 = c("g-000010", "g-000020", "g-000030"),
                    G2 = c("g-000300", "g-000330"),
                    G3 = c("g-000510", "g-000530", "g-000550",
                          "g-000570", "g-000590"))

umapPlot <- UMAPPlot(coexDF, clusters = NULL, elements = groupMarkers)
plot(umapPlot)

objCOTAN <- addClusterization(objCOTAN, clName = "first_clusterization",
                             clusters = clusters, coexDF = coexDF)

objCOTAN <- estimateNuLinearByCluster(objCOTAN, clusters = clusters)

clSummaryPlotAndData <-
  clustersSummaryPlot(objCOTAN, clName = "first_clusterization",
                    plotTitle = "first clusterization")
##plot(clSummaryPlotAndData[["plot"]])

##objCOTAN <- dropClusterization(objCOTAN, "first_clusterization")

clusterizations <- getClusterizations(objCOTAN, dropNoCoex = TRUE)

enrichment <- geneSetEnrichment(clustersCoex = coexDF,
                                groupMarkers = groupMarkers)

clHeatmapPlotAndData <- clustersMarkersHeatmapPlot(objCOTAN, groupMarkers)
##plot(clHeatmapPlotAndData[["heatmapPlot"]])
```

```
conditions <- as.integer(substring(getCells(objCOTAN), 3L))
conditions <- factor(ifelse(conditions <= 600, "L", "H"))
names(conditions) <- getCells(objCOTAN)

clHeatmapPlotAndData2 <-
  clustersMarkersHeatmapPlot(objCOTAN, groupMarkers, kCuts = 2,
                             condNameList = list("High/Low"),
                             conditionsList = list(conditions))
##plot(clHeatmapPlotAndData2[["heatmapPlot"]])

clName <- getClusterizationName(objCOTAN)

clusterDataList <- getClusterizationData(objCOTAN, clName = clName)

clusters <- getClusters(objCOTAN, clName = clName)

allClustersCoexDF <- getClustersCoex(objCOTAN)

deltaExpression <- clustersDeltaExpression(objCOTAN, clusters = clusters)

summaryData <- clustersSummaryData(objCOTAN)

treePlotAndObj <- clustersTreePlot(objCOTAN, 2)
objCOTAN <- treePlotAndObj[["objCOTAN"]]
plot(treePlotAndObj[["dend"]])

clMarkers <- findClustersMarkers(objCOTAN, clusters = clusters)
```

funProbZero

funProbZero

Description

Private function that gives the probability of a sample gene count being zero given the given the dispersion and mu

Usage

```
funProbZero(dis, mu)
```

Arguments

disp the estimated dispersion (can be a n -sized vector)
mu the lambda times nu value (can be a $n \times m$ matrix)

Details

Using d for `disp` and μ for `mu`, it returns: $(1 + d\mu)^{-\frac{1}{d}}$ when $d > 0$ and $\exp((d - 1)\mu)$ otherwise. The function is continuous in $d = 0$, increasing in d and decreasing in μ . It returns 0 when $d = -\infty$ or $\mu = \infty$. It returns 1 when $\mu = 0$.

Value

the probability (matrix) that a count is identically zero

GenesCoexSpace	<i>Local Differentiation Index</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, 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 the selected subset.

Usage

```
genesCoexSpace(objCOTAN, primaryMarkers, numGenesPerMarker = 25L)
```

```
establishGenesClusters(
  objCOTAN,
  groupMarkers,
  numGenesPerMarker = 25L,
  kCuts = 6L,
  distance = "cosine",
  hclustMethod = "ward.D2"
)
```

Arguments

<code>objCOTAN</code>	a COTAN object
<code>primaryMarkers</code>	A vector of primary marker names.
<code>numGenesPerMarker</code>	the number of correlated genes to keep as other markers (default 25)
<code>groupMarkers</code>	a named list with an element for each group comprised of one or more marker genes
<code>kCuts</code>	the number of estimated <i>cluster</i> (this defines the height for the tree cut)
<code>distance</code>	type of distance to use (default is "cosine", "euclidean" and the others from parallelDist::parDist() are also available)
<code>hclustMethod</code>	default is "ward.D2" but can be any method defined by stats::hclust() function

GenesStatistics *Calculations of genes statistics*

Description

A collection of functions returning various statistics associated to the genes. In particular the *discrepancy* between the expected probabilities of zero and their actual occurrences, both at single gene level or looking at genes' pairs

Usage

```
calculateGenesCE(objCOTAN)

calculateGDI(objCOTAN, statType = "S")

calculatePValue(
  objCOTAN,
  statType = "S",
  geneSubsetCol = vector(mode = "character"),
  geneSubsetRow = vector(mode = "character")
)

calculatePDI(
  objCOTAN,
  statType = "S",
  geneSubsetCol = vector(mode = "character"),
  geneSubsetRow = vector(mode = "character")
)
```

Arguments

objCOTAN	a COTAN object
statType	Which statistics to use to compute the p-values. By default it will use the "S" (Pearson's χ^2 test) otherwise the "G" (G-test)
geneSubsetCol	an array of genes. It will be put in columns. If left empty the function will do it genome-wide.
geneSubsetRow	an array of genes. It will be put in rows. If left empty the function will do it genome-wide.

Details

calculateGenesCE() is used to calculate the discrepancy between the expected probability of zero and the observed zeros across all cells for each gene as *cross-entropy*: $-\sum_c \mathbb{1}_{X_c=0} \log(p_c) - \mathbb{1}_{X_c \neq 0} \log(1 - p_c)$ where X_c is the observed count and p_c the probability of zero

calculateGDI() produces a data.frame with the *GDI* for each gene based on the COEX matrix

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

calculatePDI() computes the p-values for genes in the COTAN object using `calculatePValue()` and takes their $\log(-\log(\cdot))$ to calculate the genes' *Pair Differential Index*

Value

calculateGenesCE() returns a named array with the *cross-entropy* of each gene

calculateGDI() returns a `data.frame` with the *GDI* data

calculatePValue() returns a *p-value* matrix as `dspMatrix`

calculatePDI() returns a *Pair Differential Index* matrix as `dspMatrix`

getColorsVector	<i>getColorsVector</i>
-----------------	------------------------

Description

This function returns a list of colors based on the `brewer.pal()` function

Usage

```
getColorsVector(numNeededColors = 0L)
```

Arguments

numNeededColors

The number of returned colors. If omitted it returns all available colors

Details

The colors are taken from the `brewer.pal.info()` sets with Set1, Set2, Set3 placed first.

Value

an array of RGB colors of the wanted size

Examples

```
colorsVector <- getColorsVector(17)
```

Description

Much of the information stored in the COTAN object is compacted into three data.frames:

- "metaDataset" - contains all general information about the data-set
- "metaGenes" - contains genes' related information along the lambda and dispersion vectors and the fully-expressed flag
- "metaCells" - contains cells' related information along the nu vector, the fully-expressing flag, the *clusterizations* and the *conditions*

Usage

```
## S4 method for signature 'COTAN'
getMetadataDataset(objCOTAN)

## S4 method for signature 'COTAN'
getMetadataElement(objCOTAN, tag)

## S4 method for signature 'COTAN'
getMetadataGenes(objCOTAN)

## S4 method for signature 'COTAN'
getMetadataCells(objCOTAN)

## S4 method for signature 'COTAN'
getDims(objCOTAN)

datasetTags()

## S4 method for signature 'COTAN'
initializeMetaDataset(objCOTAN, GEO, sequencingMethod, sampleCondition)

## S4 method for signature 'COTAN'
addElementToMetaDataset(objCOTAN, tag, value)

setColumnInDF(df, colToSet, colName, rowNames = vector(mode = "character"))
```

Arguments

objCOTAN	a COTAN object
tag	the new information tag
GEO	a code reporting the GEO identification or other specific data-set code

sequencingMethod	a string reporting the method used for the sequencing
sampleCondition	a string reporting the specific sample condition or time point
value	a value (or an array) containing the information
df	the data.frame
colToSet	the the column to add
colName	the name of the new or existing column in the data.frame
rowNames	when not empty, if the input data.frame has no real row names, the new row names of the resulting data.frame

Details

getMetadataDataset() extracts the meta-data stored for the current data-set.

getMetadataElement() extracts the value associated with the given tag if present or an empty string otherwise.

getMetadataGenes() extracts the meta-data stored for the genes

getMetadataCells() extracts the meta-data stored for the cells

getDims() extracts the sizes of all slots of the COTAN object

datasetTags() defines a list of short names associated to an enumeration. It also defines the relative long names as they appear in the meta-data

initializeMetaDataset() initializes meta-data data-set

addElementToMetaDataset() is used to add a line of information to the meta-data data.frame. If the tag was already used it will update the associated value(s) instead

setColumnInDF() is a function to append, if missing, or resets, if present, a column into a data.frame, whether the data.frame is empty or not. The given rowNames are used only in the case the data.frame has only the default row numbers, so this function cannot be used to override row names

Value

getMetadataDataset() returns the meta-data data.frame

getMetadataElement() returns a string with the relevant value

getMetadataGenes() returns the genes' meta-data data.frame

getMetadataCells() returns the cells' meta-data data.frame

getDims() returns a named list with the sizes of the slots

datasetTags() a named character array with the standard labels used in the metaDataset of the COTAN objects

initializeMetaDataset() returns the given COTAN object with the updated metaDataset

addElementToMetaDataset() returns the updated COTAN object

setColumnInDF() returns the updated, or the newly created, data.frame

Examples

```

data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)

objCOTAN <- initializeMetaDataset(objCOTAN, GEO = "test_GEO",
                                sequencingMethod = "distribution_sampling",
                                sampleCondition = "reconstructed_dataset")

objCOTAN <- addElementToMetaDataset(objCOTAN, "Test",
                                    c("These are ", "some values"))

dataSetInfo <- getMetadataDataset(objCOTAN)

numInitialCells <- getMetadataElement(objCOTAN, "cells")

metaGenes <- getMetadataGenes(objCOTAN)

metaCells <- getMetadataCells(objCOTAN)

allSizes <- getDims(objCOTAN)

```

HandlingConditions *Handling cells' conditions and related functions*

Description

These functions manage the *conditions*.

A *condition* is a set of **labels** that can be assigned to cells: one **label** per cell. This is especially useful in cases when the data-set is the result of merging multiple experiments' raw data

Usage

```

## S4 method for signature 'COTAN'
getAllConditions(objCOTAN, keepPrefix = FALSE)

## S4 method for signature 'COTAN'
getConditionName(objCOTAN, condName = "", keepPrefix = FALSE)

## S4 method for signature 'COTAN'
getCondition(objCOTAN, condName = "")

normalizeNameAndLabels(objCOTAN, name = "", labels = NULL, isCond = FALSE)

## S4 method for signature 'COTAN'
addCondition(objCOTAN, condName, conditions, override = FALSE)

## S4 method for signature 'COTAN'
dropCondition(objCOTAN, condName)

```

Arguments

objCOTAN	a COTAN object
keepPrefix	When TRUE returns the internal name of the <i>condition</i> : the one with the COND_ prefix.
condName	the name of an existing <i>condition</i> .
name	the name of the <i>clusterization/condition</i> . If not given the last available <i>clusterization</i> will be used, or no <i>conditions</i>
labels	a <i>clusterization/condition</i> to use. If given it will take precedence on the one indicated by name
isCond	a Boolean to indicate whether the function is dealing with <i>clusterizations</i> FALSE or <i>conditions</i> TRUE
conditions	a (factors) array of <i>condition labels</i>
override	When TRUE silently allows overriding data for an existing <i>condition</i> name. Otherwise the default behavior will avoid potential data losses

Details

getAllConditions() extracts the list of the *conditions* defined in the COTAN object.

getConditionName() normalizes the given *condition* name or, if none were given, returns the name of last available *condition* in the COTAN object. It can return the *condition internal name* if needed

getCondition() extracts the asked *condition* from the COTAN object

normalizeNameAndLabels() takes a pair of name/labels and normalize them based on the available information in the COTAN object

addCcondition() adds a *condition* to the current COTAN object, by adding a new column in the metaCells data.frame

dropCondition() drops a *condition* from the current COTAN object, by removing the corresponding column in the metaCells data.frame

Value

getAllConditions() returns a vector of *conditions* names, usually without the COND_ prefix

getConditionName() returns the normalized *condition* name or NULL if no *conditions* are present

getCondition() returns a named factor with the *condition*

normalizeNameAndLabels() returns a list with:

- "name" the relevant name
- "labels" the relevant *clusterization/condition*

addCondition() returns the updated COTAN object

dropCondition() returns the updated COTAN object

Examples

```

data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)

genre <- rep(c("F", "M"), getNumCells(objCOTAN) / 2)
objCOTAN <- addCondition(objCOTAN, condName = "Genre", conditions = genre)

##objCOTAN <- dropCondition(objCOTAN, "Genre")

conditionsNames <- getAllConditions(objCOTAN)

condName <- getConditionName(objCOTAN)

condition <- getCondition(objCOTAN, condName = condName)
isa(condition, "factor")

nameAndCond <- normalizeNameAndLabels(objCOTAN, name = condName,
                                     isCond = TRUE)
isa(nameAndCond[["labels"]], "factor")

```

HeatmapPlots

Heatmap Plots

Description

These functions create heatmap COEX plots.

Usage

```

heatmapPlot(genesLists, sets, conditions, dir, pValueThreshold = 0.01)

genesHeatmapPlot(
  objCOTAN,
  primaryMarkers,
  secondaryMarkers = vector(mode = "character"),
  pValueThreshold = 0.01,
  symmetric = TRUE
)

cellsHeatmapPlot(objCOTAN, cells = NULL, clusters = NULL)

plotTheme(plotKind = "common", textSize = 14L)

```

Arguments

<code>genesLists</code>	A list of genes' arrays. The first array defines the genes in the columns
<code>sets</code>	A numeric array indicating which fields in the previous list should be used

conditions	An array of prefixes indicating the different files
dir	The directory in which are all COTAN files (corresponding to the previous prefixes)
pValueThreshold	The p-value threshold. Default is 0.01
objCOTAN	a COTAN object
primaryMarkers	A set of genes plotted as rows
secondaryMarkers	A set of genes plotted as columns
symmetric	A Boolean: default TRUE. When TRUE the union of primaryMarkers and secondaryMarkers is used for both rows and column genes
cells	Which cells to plot (all if no argument is given)
clusters	Use this clusterization to select/reorder the cells to plot
plotKind	a string indicating the plot kind
textSize	axes and strip text size (default=14)

Details

heatmapPlot() creates the heatmap of one or more COTAN objects

genesHeatmapPlot() 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.

cellsHeatmapPlot() creates the heatmap plot of the cells' COEX matrix

plotTheme() returns the appropriate theme for the selected plot kind. Supported kinds are: "common", "pca", "genes", "UDE", "heatmap", "GDI", "UMAP", "size-plot"

Value

heatmapPlot() returns a ggplot2 object

genesHeatmapPlot() returns a ggplot2 object

cellsHeatmapPlot() returns the cells' COEX *heatmap* plot

plotTheme() returns a ggplot2::theme object

See Also

[ggplot2::theme\(\)](#) and [ggplot2::ggplot\(\)](#)

Examples

```
data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)
objCOTAN <- clean(objCOTAN)
objCOTAN <- estimateDispersionNuBisection(objCOTAN, cores = 12)
objCOTAN <- calculateCoex(objCOTAN, actOnCells = FALSE)
```

```

objCOTAN <- calculateCoex(objCOTAN, actOnCells = TRUE)

## Save the `COTAN` object to file
data_dir <- tempdir()
saveRDS(objCOTAN, file = file.path(data_dir, "test.dataset.cotan.RDS"))

## some genes
primaryMarkers <- c("g-000010", "g-000020", "g-000030")

## an example of named list of different gene set
groupMarkers <- list(G1 = primaryMarkers,
                    G2 = c("g-000300", "g-000330"),
                    G3 = c("g-000510", "g-000530", "g-000550",
                          "g-000570", "g-000590"))

hPlot <- heatmapPlot(genesLists = groupMarkers, sets = c(2, 3),
                    pValueThreshold = 0.05, conditions = c("test.dataset"),
                    dir = paste0(data_dir, "/"))
plot(hPlot)

ghPlot <- genesHeatmapPlot(objCOTAN, primaryMarkers = primaryMarkers,
                          secondaryMarkers = groupMarkers,
                          pValueThreshold = 0.05, symmetric = FALSE)
plot(ghPlot)

clusters <- c(rep_len("1", getNumCells(objCOTAN)/2),
             rep_len("2", getNumCells(objCOTAN)/2))
names(clusters) <- getCells(objCOTAN)

chPlot <- cellsHeatmapPlot(objCOTAN, clusters = clusters)
plot(chPlot)

theme <- plotTheme("pca")

```

LegacyFastSymmMatrix *Handle symmetric matrix <-> vector conversions*

Description

Converts a symmetric matrix into a compacted symmetric matrix and vice-versa.

Usage

```

vec2mat_rfast(x, genes = "all")

mat2vec_rfast(mat)

```


Arguments

<code>x</code>	a list formed by two arrays: genes with the unique gene names and values with all the values.
<code>genes</code>	an array with all wanted genes or the string "all". When equal to "all" (the default), it recreates the entire matrix.
<code>mat</code>	a square (possibly symmetric) matrix with all genes as row and column names.

Details

This is a legacy function related to old scCOTAN objects. Use the more appropriate `Matrix::dspMatrix` type for similar functionality.

`mat2vec_rfast` will forcibly make its argument symmetric.

Value

`vec2mat_rfast` returns the reconstructed symmetric matrix

`mat2vec_rfast` a list formed by two arrays:

- genes with the unique gene names,
- values with all the values.

Examples

```
v <- list("genes" = paste0("gene_", c(1:9)), "values" = c(1:45))

M <- vec2mat_rfast(v)
all.equal(rownames(M), v[["genes"]])
all.equal(colnames(M), v[["genes"]])

genes <- paste0("gene_", sample.int(ncol(M), 3))

m <- vec2mat_rfast(v, genes)
all.equal(rownames(m), v[["genes"]])
all.equal(colnames(m), genes)

v2 <- mat2vec_rfast(M)
all.equal(v, v2)
```

Description

Logging is currently supported for all COTAN functions. It is possible to see the output on the terminal and/or on a log file. The level of output on terminal is controlled by the `COTAN.LogLevel` option while the logging on file is always at its maximum verbosity

Usage

```

setLoggingLevel(newLevel = 1L)

setLoggingFile(logFileName)

logThis(msg, logLevel = 2L, appendLF = TRUE)

```

Arguments

<code>newLevel</code>	the new default logging level. It defaults to 1
<code>logFileName</code>	the log file.
<code>msg</code>	the message to print
<code>logLevel</code>	the logging level of the current message. It defaults to 2
<code>appendLF</code>	whether to add a new-line character at the end of the message

Details

`setLoggingLevel()` sets the COTAN logging level. It set the `COTAN.LogLevel` options to one of the following values:

- 0 - Always on log messages
- 1 - Major log messages
- 2 - Minor log messages
- 3 - All log messages

`setLoggingFile()` sets the log file for all COTAN output logs. By default no logging happens on a file (only on the console). Using this function COTAN will use the indicated file to dump the logs produced by all `logThis()` commands, independently from the log level. It stores the connection created by the call to `bzfile()` in the option: `COTAN.LogFile`

`logThis()` prints the given message string if the current log level is greater or equal to the given log level (it always prints its message on file if active). It uses `message()` to actually print the messages on the `stderr()` connection, so it is subject to `suppressMessages()`

Value

`setLoggingLevel()` returns the old logging level or default level if not set yet.
`logThis()` returns TRUE if the message has been printed on the terminal

Examples

```

setLoggingLevel(3) # for debugging purposes only

setLoggingFile("./COTAN_Test1.log") # for debugging purposes only
logThis("Some log message")
setLoggingFile("") # closes the log file

logThis("LogLevel 0 messages will always show, ",

```

```

    logLevel = 0, appendLF = FALSE)
  suppressMessages(logThis("unless all messages are suppressed",
    logLevel = 0))

```

ParametersEstimations *Estimation of the COTAN model's parameters*

Description

These functions are used to estimate the COTAN model's parameters. That is the average count for each gene (λ) the average count for each cell (ν) and the dispersion parameter for each gene to match the probability of zero.

The estimator methods are named *Linear* if they can be calculated as a linear statistic of the raw data or *Bisection* if they are found via a parallel bisection solver.

Usage

```

## S4 method for signature 'COTAN'
estimateLambdaLinear(objCOTAN)

```

```

## S4 method for signature 'COTAN'
estimateNuLinear(objCOTAN)

```

```

## S4 method for signature 'COTAN'
estimateDispersionBisection(
  objCOTAN,
  threshold = 0.001,
  cores = 1L,
  maxIterations = 100L,
  chunkSize = 1024L
)

```

```

## S4 method for signature 'COTAN'
estimateNuBisection(
  objCOTAN,
  threshold = 0.001,
  cores = 1L,
  maxIterations = 100L,
  chunkSize = 1024L
)

```

```

## S4 method for signature 'COTAN'
estimateDispersionNuBisection(
  objCOTAN,
  threshold = 0.001,
  cores = 1L,

```

```

    maxIterations = 100L,
    chunkSize = 1024L,
    enforceNuAverageToOne = TRUE
)

## S4 method for signature 'COTAN'
estimateDispersionNuNlminb(
  objCOTAN,
  threshold = 0.001,
  maxIterations = 50L,
  chunkSize = 1024L,
  enforceNuAverageToOne = TRUE
)

## S4 method for signature 'COTAN'
getNormalizedData(objCOTAN)

## S4 method for signature 'COTAN'
getNu(objCOTAN)

## S4 method for signature 'COTAN'
getLambda(objCOTAN)

## S4 method for signature 'COTAN'
getDispersion(objCOTAN)

estimatorsAreReady(objCOTAN)

```

Arguments

objCOTAN	a COTAN object
threshold	minimal solution precision
cores	number of cores to use. Default is 1.
maxIterations	max number of iterations (avoids infinite loops)
chunkSize	number of genes to solve in batch in a single core. Default is 1024.
enforceNuAverageToOne	a Boolean on whether to keep the average nu equal to 1

Details

estimateLambdaLinear() does a linear estimation of lambda (genes' counts averages)

estimateNuLinear() does a linear estimation of nu (normalized cells' counts averages)

estimateDispersionBisection() estimates the negative binomial dispersion factor for each gene (a). Determines the dispersion such that, for each gene, the probability of zero count matches the number of observed zeros. It assumes [estimateNuLinear\(\)](#) being already run.

`estimateNuBisection()` estimates the nu vector of a COTAN object by bisection. It determines the nu parameters such that, for each cell, the probability of zero counts matches the number of observed zeros. It assumes `estimateDispersionBisection()` being already run. Since this breaks the assumption that the average nu is 1, it is recommended not to run this in isolation but use `estimateDispersionNuBisection()` instead.

`estimateDispersionNuBisection()` estimates the dispersion and nu field of a COTAN object by running sequentially a bisection for each parameter.

`estimateDispersionNuNlminb()` estimates the nu and dispersion parameters to minimize the discrepancy between the observed and expected probability of zero. It uses the `stats::nlminb()` solver, but since the joint parameters have too high dimensionality, it converges too slowly to be actually useful in real cases.

`getNormalizedData()` extracts the *normalized* count table (i.e. divided by nu)

`getNu()` extracts the nu array (normalized cells' counts averages)

`getLambda()` extracts the lambda array (mean expression for each gene)

`getDispersion()` extracts the dispersion array (a)

`estimatorsAreReady()` checks whether the estimators arrays lambda, nu, dispersion are available

Value

`estimateLambdaLinear()` returns the updated COTAN object

`estimateNuLinear()` returns the updated COTAN object

`estimateDispersionBisection()` returns the updated COTAN object

`estimateNuBisection()` returns the updated COTAN object

`estimateDispersionNuBisection()` returns the updated COTAN object

`estimateDispersionNuNlminb()` returns the updated COTAN object

`getNormalizedData()` returns the normalized count data.frame

`getNu()` returns the nu array

`getLambda()` returns the lambda array

`getDispersion()` returns the dispersion array

`estimatorsAreReady()` returns a boolean specifying whether all three arrays are non-empty

Examples

```
data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)

objCOTAN <- estimateLambdaLinear(objCOTAN)
lambda <- getLambda(objCOTAN)

objCOTAN <- estimateNuLinear(objCOTAN)
nu <- getNu(objCOTAN)

objCOTAN <- estimateDispersionBisection(objCOTAN, cores = 12)
dispersion <- getDispersion(objCOTAN)
```

```

objCOTAN <- estimateDispersionNuBisection(objCOTAN, cores = 12,
                                         enforceNuAverageToOne = TRUE)
nu <- getNu(objCOTAN)
dispersion <- getDispersion(objCOTAN)

rawNorm <- getNormalizedData(objCOTAN)

```

RawDataCleaning	<i>Raw data cleaning</i>
-----------------	--------------------------

Description

These methods are to be used to clean the raw data. That is drop any number of genes/cells that are too sparse or too present to allow proper calibration of the COTAN model.

We call genes that are expressed in all cells *Fully-Expressed* while cells that express all genes in the data are called *Fully-Expressing*. In case it has been made quite easy to exclude the flagged genes/cells in the user calculations.

Usage

```

## S4 method for signature 'COTAN'
flagNotFullyExpressedGenes(objCOTAN)

## S4 method for signature 'COTAN'
flagNotFullyExpressingCells(objCOTAN)

## S4 method for signature 'COTAN'
getFullyExpressedGenes(objCOTAN)

## S4 method for signature 'COTAN'
getFullyExpressingCells(objCOTAN)

## S4 method for signature 'COTAN'
findFullyExpressedGenes(objCOTAN, cellsThreshold = 0.99)

## S4 method for signature 'COTAN'
findFullyExpressingCells(objCOTAN, genesThreshold = 0.99)

## S4 method for signature 'COTAN'
dropGenesCells(
  objCOTAN,
  genes = vector(mode = "character"),
  cells = vector(mode = "character")
)

```

```

ECDPlot(objCOTAN, yCut)

## S4 method for signature 'COTAN'
clean(
  objCOTAN,
  cellsCutoff = 0.003,
  genesCutoff = 0.002,
  cellsThreshold = 0.99,
  genesThreshold = 0.99
)

cleanPlots(objCOTAN, includePCA = TRUE)

cellSizePlot(objCOTAN, splitPattern = " ", numCol = 2L)

genesSizePlot(objCOTAN, splitPattern = " ", numCol = 2L)

mitochondrialPercentagePlot(
  objCOTAN,
  splitPattern = " ",
  numCol = 2L,
  genePrefix = "^MT-"
)

scatterPlot(objCOTAN, splitPattern = " ", numCol = 2L, splitSamples = FALSE)

```

Arguments

objCOTAN	a COTAN object
cellsThreshold	any gene that is expressed in more cells than threshold times the total number of cells will be marked as fully-expressed . Default threshold is 0.99 (99.0%)
genesThreshold	any cell that is expressing more genes than threshold times the total number of genes will be marked as fully-expressing . Default threshold is 0.99 (99.0%)
genes	an array of gene names
cells	an array of cell names
yCut	y threshold of library size to drop
cellsCutoff	clean() will delete from the raw data any gene that is expressed in less cells than threshold times the total number of cells. Default cutoff is 0.003 (0.3%)
genesCutoff	clean() will delete from the raw data any cell that is expressing less genes than threshold times the total number of genes. Default cutoff is 0.002 (0.2%)
includePCA	a Boolean flag to determine whether to calculate the <i>PCA</i> associated with the normalized matrix. When TRUE the first four elements of the returned list will be NULL
splitPattern	Pattern used to extract, from the column names, the sample field (default " ")
numCol	Once the column names are split by splitPattern, the column number with the sample name (default 2)

genePrefix Prefix for the mitochondrial genes (default "^MT-" for Human, mouse "^mt-")
 splitSamples Boolean. Whether to plot each sample in a different panel (default FALSE)

Details

flagNotFullyExpressedGenes() returns a Boolean array with TRUE for those genes that are not fully-expressed.

flagNotFullyExpressingCells() returns a Boolean vector with TRUE for those cells that are not expressing all genes

getFullyExpressedGenes() returns the genes expressed in all cells of the dataset

getFullyExpressingCells() returns the cells that did express all genes of the dataset

findFullyExpressedGenes() determines the fully-expressed genes inside the raw data

findFullyExpressingCells() determines the cells that are expressing all genes in the dataset

dropGenesCells() removes an array of genes and/or cells from the current COTAN object.

ECDPlot() plots the empirical distribution function of library sizes (UMI number). It helps to define where to drop "cells" that are simple background signal.

clean() is the main method that can be used to check and clean the dataset. It will discard any genes that has less than 3 non-zero counts per thousand cells and all cells expressing less than 2 per thousand genes. It also produces and stores the estimators for nu and lambda

cleanPlots() creates the plots associated to the output of the `clean()` method.

cellSizePlot() plots the raw library size for each cell and sample.

genesSizePlot() plots the raw gene number (reads > 0) for each cell and sample

mitochondrialPercentagePlot() plots the raw library size for each cell and sample.

scatterPlot() creates a plot that check the relation between the library size and the number of genes detected.

Value

flagNotFullyExpressedGenes() returns a Booleans array with TRUE for genes that are not fully-expressed

flagNotFullyExpressingCells() returns an array of Booleans with TRUE for cells that are not expressing all genes

getFullyExpressedGenes() returns an array containing all genes that are expressed in all cells

getFullyExpressingCells() returns an array containing all cells that express all genes

findFullyExpressedGenes() returns the given COTAN object with updated **fully-expressed** genes' information

findFullyExpressingCells() returns the given COTAN object with updated **fully-expressing** cells' information

dropGenesCells() returns a completely new COTAN object with the new raw data obtained after the indicated genes/cells were expunged. All remaining data is dropped too as no more relevant with the restricted matrix. Exceptions are:

- the meta-data for the data-set that gets kept unchanged

- the meta-data of genes/cells that gets restricted to the remaining elements. The columns calculated via estimate and find methods are dropped too

`ECDPlot()` returns an ECD plot

`clean()` returns the updated COTAN object

`cleanPlots()` returns a list of ggplot2 plots:

- "pcaCells" is for pca cells
- "pcaCellsData" is the data of the pca cells (can be plotted)
- "genes" is for B group cells' genes
- "UDE" is for cells' UDE against their pca
- "nu" is for cell *nu*
- "zoomedNu" is the same but zoomed on the left and with an estimate for the low *nu* threshold that defines problematic cells

`cellSizePlot()` returns the violin-boxplot plot

`genesSizePlot()` returns the violin-boxplot plot

`mitochondrialPercentagePlot()` returns a list with:

- "plot" a violin-boxplot object
- "sizes" a sizes data.frame

`scatterPlot()` returns the scatter plot

Examples

```
library(zeallot)

data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)

genes.to.rem <- getGenes(objCOTAN)[grep('^MT', getGenes(objCOTAN))]
cells.to.rem <- getCells(objCOTAN)[which(getCellsSize(objCOTAN) == 0)]
objCOTAN <- dropGenesCells(objCOTAN, genes.to.rem, cells.to.rem)

objCOTAN <- clean(objCOTAN)

objCOTAN <- findFullyExpressedGenes(objCOTAN)
goodPos <- flagNotFullyExpressedGenes(objCOTAN)

objCOTAN <- findFullyExpressingCells(objCOTAN)
goodPos <- flagNotFullyExpressingCells(objCOTAN)

feGenes <- getFullyExpressedGenes(objCOTAN)

feCells <- getFullyExpressingCells(objCOTAN)

## These plots might help to identify genes/cells that need to be dropped
ecdPlot <- ECDPlot(objCOTAN, yCut = 100)
```

```

plot(ecdPlot)

# This creates many informative plots useful to determine whether
# there is still something to drop...
# Here we use the tuple-like assignment feature of the `zeallot` package
c(pcaCellsPlot, ., genesPlot, UDEPlot, ., zNuPlot) %<-% cleanPlots(objCOTAN)
plot(pcaCellsPlot)
plot(UDEPlot)
plot(zNuPlot)

lsPlot <- cellSizePlot(objCOTAN)
plot(lsPlot)

gsPlot <- genesSizePlot(objCOTAN)
plot(gsPlot)

mitPercPlot <-
  mitochondrialPercentagePlot(objCOTAN, genePrefix = "g-0000")[[ "plot" ]]
plot(mitPercPlot)

scPlot <- scatterPlot(objCOTAN)
plot(scPlot)

```

RawDataGetters

Raw data COTAN accessors

Description

These methods extract information out of a just created COTAN object. The accessors have **read-only** access to the object.

Usage

```

## S4 method for signature 'COTAN'
getRawData(objCOTAN)

## S4 method for signature 'COTAN'
getNumCells(objCOTAN)

## S4 method for signature 'COTAN'
getNumGenes(objCOTAN)

## S4 method for signature 'COTAN'
getCells(objCOTAN)

## S4 method for signature 'COTAN'
getGenes(objCOTAN)

```

```
## S4 method for signature 'COTAN'  
getZeroOneProj(objCOTAN)  
  
## S4 method for signature 'COTAN'  
getCellsSize(objCOTAN)  
  
## S4 method for signature 'COTAN'  
getNumExpressedGenes(objCOTAN)  
  
## S4 method for signature 'COTAN'  
getGenesSize(objCOTAN)  
  
## S4 method for signature 'COTAN'  
getNumOfExpressingCells(objCOTAN)
```

Arguments

objCOTAN a COTAN object

Details

getRawData() extracts the raw count table.
getNumCells() extracts the number of cells in the sample (m)
getNumGenes() extracts the number of genes in the sample (n)
getCells() extract all cells in the dataset.
getGenes() extract all genes in the dataset.
getZeroOneProj() extracts the raw count table where any positive number has been replaced with 1
getCellsSize() extracts the cell raw library size.
getNumExpressedGenes() extracts the number of genes expressed for each cell. Exploits a feature of [Matrix::CsparseMatrix](#)
getGenesSize() extracts the genes raw library size.
getNumOfExpressingCells() extracts, for each gene, the number of cells that are expressing it. Exploits a feature of [Matrix::CsparseMatrix](#)

Value

getRawData() returns the raw count sparse matrix
getNumCells() returns the number of cells in the sample (m)
getNumGenes() returns the number of genes in the sample (n)
getCells() returns a character array with the cells' names
getGenes() returns a character array with the genes' names
getZeroOneProj() returns the raw count matrix projected to 0 or 1
getCellsSize() returns an array with the library sizes

`getNumExpressedGenes()` returns an array with the library sizes
`getGenesSize()` returns an array with the library sizes
`getNumOfExpressingCells()` returns an array with the library sizes

Examples

```

data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)

rowData <- getRawData(objCOTAN)

numCells <- getNumCells(objCOTAN)

numGenes <- getNumGenes(objCOTAN)

cellsNames <- getCells(objCOTAN)

genesNames <- getGenes(objCOTAN)

zeroOne <- getZeroOneProj(objCOTAN)

cellsSize <- getCellsSize(objCOTAN)

numExpGenes <- getNumExpressedGenes(objCOTAN)

genesSize <- getGenesSize(objCOTAN)

numExpCells <- getNumOfExpressingCells(objCOTAN)
  
```

scCOTAN-class

scCOTAN-class (for legacy usage)

Description

Define scCOTAN structure

Value

a scCOTAN object

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

`nu` vector.

lambda vector.
a vector.
hk vector.
n_cells numeric.
meta data.frame.
yes_yes ANY. Unused and deprecated. Kept for backward compatibility only
clusters vector.
cluster_data data.frame.

UniformClusters	<i>Uniform Clusters</i>
-----------------	-------------------------

Description

This group of functions takes in input a COTAN object and handle the task of dividing the dataset into **Uniform Clusters**, that is *clusters* that have an homogeneous genes' expression. This condition is checked by calculating the GDI of the *cluster* and verifying that no more than a small fraction of the genes have their GDI level above the given GDIThreshold

Usage

```
GDIPlot(  
  objCOTAN,  
  genes,  
  condition = "",  
  statType = "S",  
  GDIThreshold = 1.4,  
  GDIIIn = NULL  
)  
  
cellsUniformClustering(  
  objCOTAN,  
  GDIThreshold = 1.4,  
  cores = 1L,  
  maxIterations = 25L,  
  initialClusters = NULL,  
  initialResolution = 0.8,  
  distance = "cosine",  
  hclustMethod = "ward.D2",  
  saveObj = TRUE,  
  outDir = "."  
)  
  
checkClusterUniformity(  
  objCOTAN,
```

```

    cluster,
    cells,
    GDIThreshold = 1.4,
    cores = 1L,
    saveObj = TRUE,
    outDir = "."
)

mergeUniformCellsClusters(
  objCOTAN,
  clusters = NULL,
  GDIThreshold = 1.4,
  batchSize = 10L,
  cores = 1L,
  distance = "cosine",
  hclustMethod = "ward.D2",
  saveObj = TRUE,
  outDir = "."
)

```

Arguments

objCOTAN	a COTAN object
genes	a named list of genes to label. Each array will have different color.
condition	a string corresponding to the condition/sample (it is used only for the title).
statType	type of statistic to be used. Default is "S": Pearson's chi-squared test statistics. "G" is G-test statistics
GDIThreshold	the threshold level that discriminates uniform clusters. It defaults to 1.4
GDIIn	when the GDI data frame was already calculated, it can be put here to speed up the process (default is NULL)
cores	number cores used
maxIterations	max number of re-clustering iterations. It defaults to 25
initialClusters	an existing <i>clusterization</i> to use as starting point: the <i>clusters</i> deemed uniform will be kept and the rest processed as normal
initialResolution	a number indicating how refined are the clusters before checking for uniformity . It defaults to 0.8, the same as Seurat::FindClusters()
distance	type of distance to use (default is "cosine", "euclidean" and the others from parallelDist::parDist() are also available)
hclustMethod	It defaults is "ward.D2" but can be any of the methods defined by the stats::hclust() function.
saveObj	Boolean flag; when TRUE saves intermediate analyses and plots to file
outDir	an existing directory for the analysis output. The effective output will be placed in a sub-folder.

cluster	the tag of the <i>cluster</i>
cells	the cells belonging to the <i>cluster</i>
clusters	The <i>clusterization</i> to merge. If not given the last available <i>clusterization</i> will be used, as it is probably the most significant!
batchSize	Number pairs to test in a single round. If none of them succeeds the merge stops

Details

GDIPlot() directly evaluates and plots the GDI for a sample.

cellsUniformClustering() finds a **Uniform** *clusterizations* by means of the GDI. Once a preliminary *clusterization* is obtained from the Seurat-package methods, each *cluster* is checked for **uniformity** via the function `checkClusterUniformity()`. Once all *clusters* are checked, all cells from the **non-uniform** clusters are pooled together for another iteration of the entire process, until all *clusters* are deemed **uniform**. In the case only a few cells are left out (≤ 50), those are flagged as "-1" and the process is stopped.

checkClusterUniformity() takes a COTAN object and a cells' *cluster* and checks whether the latter is **uniform** by GDI. The function runs COTAN to check whether the GDI is lower than the given GDIThreshold for the 99% of the genes. If the GDI results to be too high for too many genes, the *cluster* is deemed **non-uniform**.

mergeUniformCellsClusters() takes in a **uniform** *clusterization* and iteratively checks whether merging two *near clusters* would form a **uniform** *cluster* still. This function uses the *cosine distance* to establish the *nearest clusters pairs*. It will use the `checkClusterUniformity()` function to check whether the merged *clusters* are **uniform**. The function will stop once no *near pairs* of clusters are mergeable in a single batch

Value

GDIPlot() returns a ggplot2 object

cellsUniformClustering() returns a list with 2 elements:

- "clusters" the newly found cluster labels array
- "coex" the associated COEX data.frame

checkClusterUniformity returns a list with:

- "isUniform": a flag indicating whether the *cluster* is **uniform**
- "fractionAbove": the percentage of genes with GDI above the threshold
- "1stPercentile": the quantile associated to the highest percentile

a list with:

- "clusters" the merged cluster labels array
- "coex" the associated COEX data.frame


```
cores = 12L,  
distance = "cosine",  
hclustMethod = "ward.D2",  
saveObj = FALSE)  
  
objCOTAN <- addClusterization(objCOTAN,  
  clName = "merged",  
  clusters = mergedList[["clusters"]],  
  coexDF = mergedList[["coex"]])  
  
identical(reorderClusterization(objCOTAN), mergedList)
```

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