Package ‘COTAN’

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**Type**  Package

**Title**  COexpression Tables ANalysis

**Version**  2.2.3

**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](https://github.com/seriph78/COTAN)

**BugReports**  [https://github.com/seriph78/COTAN/issues](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 \( p\text{Value} \) and the \( \text{GDI} \) (Global Differential Expression).

The COEX matrix is defined by following formula:

\[
    P_{i,j} \in \{Y, N\} \left( -1 \right)^{\#\{(i,j)\}} \frac{O_{ij} - E_{ij}}{1 \lor E_{ij}}
\]

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 \( \text{actOnCells} \) flag).

The formula can be more effectively implemented as:

\[
    \sqrt{\frac{1}{n} \sum_{i,j \in \{Y,N\}} \frac{1}{1 \lor E_{ij}} \left( O_{YY} - E_{YY} \right)}
\]

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

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

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

```r
## 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")
)
Calculating COEX

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
Calculating COEX

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

genesCoex() returns the genes' COEX values

cellsCoex() 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"

calculatingContingencyTables() 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)
genexCoex <- 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 calculate 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)
ClustersList

Description

Handle clustering <-> 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

clusters A named vector or factor that defines the clusters
clustersList A named list whose elements define the various clusters
elemNames A list of names to which associate a cluster
throwOnOverlappingClusters When TRUE, in case of overlapping clusters, the function fromClustersList and groupByClustersList will throw. This is the default. When FALSE, instead, in case of overlapping clusters, fromClustersList will return the last cluster to which each element belongs, while groupByClustersList will return a vector of positions that is longer than the given elemNames

names A list of clusters names to be merged
mergedName The name of the new merged clusters
namesList A list of lists of clusters names to be respectively merged
mergedNames The names of the new merged clusters

Details

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

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

groupByClusters() given a clustering 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)

cl1Size <- length(clustersList[["1"]])

## establish the permutation that groups clusters together
perm <- groupByClusters(clusters)
is.unsorted(head(names(clusters)[perm], cl1Size))
head(clusters[perm], cl1Size)

## 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)

---

### 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

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

- `raw dgCMatrix` - the raw UMI count matrix \( n \times m \) (gene number \( n \) \( \times \) 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

Description

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

Usage

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

```r
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
COTANObjectCreation

```r
COTANObjectCreation
```

**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**

```r
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,
```
Datasets

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

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

```r
## 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)

DEAOnClusters(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 clusterization. If not given the last available clusterization will be used, as it is probably the most significant!
clusters A clusterization to use. If given it will take precedence on the one indicated by clName
dropNoCoex When TRUE drops the names from the clusterizations with empty associated coex data.frame
keepPrefix When TRUE returns the internal name of the clusterization: the one with the CL_ prefix.
coexDF a data.frame where each column indicates the COEX for each of the clusters of the clusterization
override When TRUE silently allows overriding data for an existing clusterization name. Otherwise the default behavior will avoid potential data losses
numCells the number of overall cells in all clusters
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 cluster (this defines the height for the tree cut).

condNameList: a list of conditions’ 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 clustersSummaryData().

conditionsList: a list of conditions to use. If given they will take precedence on the ones indicated by condNameList.

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 conditions to use. If given it will take precedence on the one indicated by condName that will only indicate the relevant column name in the returned data.frame.

plotTitle: The title to use for the returned plot.

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.

n: the number of extreme COEX values to return.

markers: a list of marker genes.

pValueDF: a data.frame with In/Out p-value based on the COEX. E.G. the result of a call to pValueFromDEA()

deltaExp: a data.frame with the delta-expression in a cluster. E.G. the result of a call to clustersDeltaExpression()

method: p-value adjustment method. Defaults to "bonferroni"

clustersCoex: the COEX data.frame.

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* dendogram 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 dendogram 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 \( \frac{1}{n} \sum (1 - e^{-\theta X_i}) \), where the \( X_i \) are the positive values from DEAOnClusters() and \( \theta = \frac{-1}{\ln(0.25)} \).

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 <- DEAOnClusters(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

**Description**

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

**Usage**

funProbZero(disp, mu)

**Arguments**

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

Using \( d \) for \( \text{disp} \) and \( \mu \) for \( \text{mu} \), it returns: \((1 + d\mu)^{-\frac{1}{2}}\) when \( d > 0 \) and \( \exp((d - 1)\mu) \) otherwise. The function is continuous in \( d = 0 \), increasing in \( d \) and decreasing in \( \mu \). 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**  
**Local Differentiation Index**

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

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

genesCoexSpace() calculates genes groups based on the primary markers and uses them to prepare the genes’ COEX space data.frame.

establishGenesClusters() perform the genes’ clustering based on a pool of gene markers, using the genes’ COEX space

Value

genesCoexSpace() returns a list with:

- “SecondaryMarkers” a named list that for each secondary marker, gives the list of primary markers that selected for it
- “GCS” the COEX data.frame
- “rankGenes” a data.frame with the rank of each gene according to its p-value

establishGenesClusters() a list of:

- ”g.space” the genes’ COEX space data.frame
- ”plot.eig” the eigenvalues plot
- ”pca_clusters” the pca components data.frame
- ”tree_plot” the tree plot for the genes’ COEX space

Examples

data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)
objCOTAN <- proceedToCoex(objCOTAN, cores = 12, saveObj = FALSE)

markers <- getGenes(objCOTAN)[sample(getNumGenes(objCOTAN), 10)]
GCS <- genesCoexSpace(objCOTAN, primaryMarkers = markers,
numGenesPerMarker = 15)

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

resList <- establishGenesClusters(objCOTAN, groupMarkers = groupMarkers,
numGenesPerMarker = 11)
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 χ² 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: $- \sum c \frac{X_c = 0}{X_c = 0} \log(p_c) - \frac{X_c = 0}{X_c = 0} \log(1 - p_c)$ 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
getColorsVector() 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 ($\chi^2$).

calculatePValue() 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

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)
HandleMetaData

**Handling meta-data in COTAN objects**

**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**

```r
## 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

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)
Handling Conditions

Arguments

- **objCOTAN**: a COTAN object
- **keepPrefix**: When TRUE returns the internal name of the condition: the one with the COND_prefix.
- **condName**: the name of an existing condition.
- **name**: the name of the clusterization/condition. If not given the last available clusterization will be used, or no conditions
- **labels**: a clusterization/condition 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 clusterizations FALSE or conditions TRUE
- **conditions**: a (factors) array of condition labels
- **override**: When TRUE silently allows overriding data for an existing condition 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.
addCondition() 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

```r
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")
```

Description

These functions create heatmap COEX plots.

Usage

```r
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

- **genesLists**: A list of genes' arrays. The first array defines the genes in the columns
- **sets**: 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
genesisHeatmapPlot() 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
genesisHeatmapPlot() 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)

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

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

- **x**: a list formed by two arrays: genes with the unique gene names and values with all the values.
- **genes**: an array with all wanted genes or the string "all". When equal to "all" (the default), it recreates the entire matrix.
- **mat**: 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::dsrMatrix` 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

```r
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.
LoggingFunctions

Usage

setLoggingLevel(newLevel = 1L)

setLoggingFile(logFileName)

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

Arguments

newLevel the new default logging level. It defaults to 1
logFileName the log file.
msg the message to print
logLevel the logging level of the current message. It defaults to 2
appendLF 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: COTANLogFile

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, ",

ParametersEstimations

```r
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 (lambda) the average count for each cell (nu) 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**

```r
## 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'
estimateDispersionNuNuNlminb(
    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)

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)

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

Examples

```r
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  Raw data cleaning

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

```r
## 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 PCA 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.
genSizePlot() 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
genSizePlot() 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
genSizePlot() 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**

```r
## 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.

genes() 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.

gNumExpressedGenes() extracts the number of genes expressed for each cell. Exploits a feature of Matrix::CsparseMatrix

genesSize() extracts the genes raw library size.

gNumOfExpressingCells() 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 ($n$)

gCells() returns a character array with the cells’ names

genes() returns a character array with the genes’ names

gZeroOneProj() returns the raw count matrix projected to 0 or 1

gCellsSize() returns an array with the library sizes

gNumExpressedGenes() returns an array with the library sizes

genesSize() returns an array with the library sizes

gNumOfExpressingCells() returns an array with the library sizes
Examples

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

rawData <- getRawData(objCOTAN)
numCells <- getNumCells(objCOTAN)
numGenes <- getNumGenes(objCOTAN)
cellsNames <- getCells(objCOTAN)
genomesNames <- getGenes(objCOTAN)
zeroOne <- getZeroOneProj(objCOTAN)
cellsSize <- getCellsSize(objCOTAN)
numExpGenes <- getNumExpressedGenes(objCOTAN)
genSize <- 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.
UniformClusters

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,
  GDIIn = NULL
)

cellsUniformClustering(
  objCOTAN,
  GDIThreshold = 1.4,
  cores = 1L,
  maxIterations = 25L,
  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
gen 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
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 paced in a sub-folder.
cluster the tag of the cluster
cells the cells belonging to the cluster
clusters The clusterization to merge. If not given the last available clusterization 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

Examples

data("test.dataset")

objCOTAN <- automaticCOTANObjectCreation(raw = test.dataset,
                                          GEO = "S",
                                          sequencingMethod = "10X",
                                          sampleCondition = "Test",
                                          cores = 12L,
                                          saveObj = FALSE)
groupMarkers <- list(G1 = c("g-000010", "g-000020", "g-000030"),
G2 = c("g-000030", "g-000033"),
G3 = c("g-0000510", "g-0000530", "g-0000550",
"g-0000570", "g-0000590"))
gdiPlot <- GDIPlot(objCOTAN, genes = groupMarkers, cond = "test")
plot(gdiPlot)

## Here we override the default GDI threshold as a way to speed-up
## calculations as higher threshold implies less stringent uniformity
## It real applications it might be appropriate to change the threshold
## in cases of relatively low genes/cells number, or in cases when an
## rough clusterization is needed in the early satges of the analysis
##
splitList <- cellsUniformClustering(objCOTAN, cores = 12,
    initialResolution = 0.8,
    GDIThreshold = 1.5, saveObj = FALSE)

clusters <- splitList[["clusters"]]

firstCluster <- getCells(objCOTAN)[clusters %in% clusters[[1L]]]
checkClusterUniformity(objCOTAN,
    GDIThreshold = 1.5,
    cluster = clusters[[1L]],
    cells = firstCluster,
    cores = 12L,
    saveObj = FALSE)

objCOTAN <- addClusterization(objCOTAN,
    clName = "split",
    clusters = clusters)

objCOTAN <- addClusterizationCoex(objCOTAN,
    clName = "split",
    coexDF = splitList[["coex"]])

identical(reorderClusterization(objCOTAN)[["clusters"]], clusters)

mergedList <- mergeUniformCellsClusters(objCOTAN,
    GDIThreshold = 1.5,
    batchSize = 5L,
    clusters = clusters,
    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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