# Package 'MultiRNAflow'

June 23, 2025

**Title** An R package for integrated analysis of temporal RNA-seq data with multiple biological conditions

Version 1.6.0

**Description** Our R package MultiRNAflow provides an easy to use unified framework allowing to automatically make both unsupervised and supervised (DE) analysis for datasets with an arbitrary number of biological conditions and time points. In particular, our code makes a deep downstream analysis of DE information, e.g. identifying temporal patterns across biological conditions and DE genes which are specific to a biological condition for each time.

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URL https://github.com/loubator/MultiRNAflow

## BugReports https://github.com/loubator/MultiRNAflow/issues

## **Depends** Mfuzz (>= 2.58.0), R (>= 4.3)

Imports Biobase (>= 2.54.0), ComplexHeatmap (>= 2.14.0), DESeq2 (>= 1.38.1), factoextra (>= 1.0.7), FactoMineR (>= 2.6), ggalluvial (>= 0.12.3), ggplot2 (>= 3.4.0), ggplotify (>= 0.1.2), ggrepel (>= 0.9.2), gprofiler2 (>= 0.2.1), graphics (>= 4.2.2), grDevices (>= 4.2.2), grid (>= 4.2.2), plot3D (>= 1.4), plot3Drgl (>= 1.0.3), reshape2 (>= 1.4.4), S4Vectors (>= 0.36.2), stats (>= 4.2.2), SummarizedExperiment (>= 1.28.0), UpSetR (>= 1.4.0), utils (>= 4.2.2)

Suggests BiocGenerics (>= 0.40.0), BiocStyle, e1071 (>= 1.7.12), knitr, rmarkdown, testthat (>= 3.0.0)

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```

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MultiRNAflow-package MultiRNAflow: An R package for integrated analysis of temporal RNAseq data with multiple biological conditions

## Description

Our R package MultiRNAflow provides an easy to use unified framework allowing to automatically make both unsupervised and supervised (DE) analysis for datasets with an arbitrary number of biological conditions and time points. In particular, our code makes a deep downstream analysis of DE information, e.g. identifying temporal patterns across biological conditions and DE genes which are specific to a biological condition for each time.

## Details

The main functions are:

- DATAnormalization to normalize raw count data
- PCAanalysis to perform PCA analysis with FactoMineR::PCA()
- HCPCanalysis to perform hierarchical clustering with FactoMineR::HCPC()
- MFUZZanalysis to perform temporal clustering with Mfuzz::mfuzz.plot2()
- DEanalysisGlobal to perform differential analysis with DESeq2::DESeq()
- GSEAQuickAnalysis to perform enrichment analysis with gprofiler2::gost()
- GSEApreprocessing to return preprocessing file for official software and online tools performing enrichment analysis

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## See Also

Useful links:

- https://github.com/loubator/MultiRNAflow
- Report bugs at https://github.com/loubator/MultiRNAflow/issues

CharacterNumbers	Transformation of a vector of integers into a vector of class "charac-
	ter".

#### Description

Transformation of a vector of integers into a vector of class "character" so that lexicographic order of characters corresponds to the numerical order of time measurements.

# Usage

CharacterNumbers(Vect.number)

## Arguments

Vect.number Vector of integers.

## Details

An appropriate number of character "0" is added in front of the numerical characters corresponding to the decimal writing of each integer in Vect.number so that the order of elements of the vector is preserved. For example, "9">"11", but "09"<"11".

## Value

A vector where each integer is transformed in class "character".

## **ColnamesToFactors**

## See Also

The function is called by ColnamesToFactors().

# Examples

```
CharacterNumbers(Vect.number=c(0,1,9,11,90,99,100,101))
CharacterNumbers(Vect.number=0:11)
CharacterNumbers(Vect.number=1:8)
```

ColnamesToFactors	Extraction of factors information and suitable column names creation
	from the column names of a dataset.

#### Description

This function generates new reduced column names according to the presence of biological conditions and/or time points, and extract the different factors (individual's names, time measurements, biological conditions) from the column names of the dataset (see Details).

#### Usage

```
ColnamesToFactors(
   ExprData,
   Column.gene,
   Group.position,
   Time.position,
   Individual.position
)
```

## Arguments

ExprData	Data.frame with $N_g$ rows and $(N_{s+k})$ columns, where $N_g$ is the number of genes, $N_s$ is the number of samples and $k = 1$ if a column is used to specify gene names, or $k = 0$ otherwise. If $k = 1$ , the position of the column containing gene names is given by Column.gene. The data.frame contains numeric values giving gene expressions of each gene in each sample. Gene expressions can be raw counts or normalized raw counts. Column names of the data.frame must describe each sample's information (individual, biological condition and time)
	and have the structure described in the section Details.
Column.gene	Integer indicating the column where gene names are given. Set Column.gene=NULI if there is no such column.
Group.position	Integer indicating the position of group information in the string of characters in each sample names (see Details). Set Group.position=NULL if there is only one or no biological information in the string of character in each sample name.

Time.position Integer indicating the position of time measurement information in the string of characters in each sample names (see Details). Set Time.position=NULL if there is only one or no time measurement information in the string of character in each sample name.

#### Individual.position

Integer indicating the position of the name of the individual (e.g patient, replicate, mouse, yeasts culture ...) in the string of characters in each sample names (see Details). The names of different individuals must be all different. Furthermore, if individual names are just numbers, they will be transform in a vector of class "character" by CharacterNumbers() and a "r" will be added to each individual name ("r" for replicate).

## Details

The column names of ExprData must be a vector of strings of characters containing

- a string of characters (if k = 1) which is the label of the column containing gene names.
- $N_s$  sample names which must be strings of characters containing at least : the name of the individual (e.g patient, mouse, yeasts culture), its biological condition (if there is at least two) and the time where data have been collected if there is at least two; (must be either 't0', 'T0' or '0' for time 0, 't1', 'T1' or '1' for time 1, ...).

All these sample information must be separated by underscores in the sample name. For instance 'CLL\_P\_t0\_r1', corresponds to the patient 'r1' belonging to the biological condition 'P' and where data were collected at time 't0'. I this example, 'CLL' describe the type of cells (here chronic lymphocytic leukemia) and is not used in our analysis.

In the string of characters 'CLL\_P\_t0\_r1', 'r1' is localized after the third underscore, so Individual.position=4, 'P' is localized after the first underscore, so Group.position=2 and 't0' is localized after the second underscore, so Time.position=3.

#### Value

The function returns new column names of the dataset, a vector indicating the name of the individual for each sample, a vector indicating the time for each sample and/or a vector indicating the biological condition for each sample.

#### See Also

The ColnamesToFactors() function is used by the following functions of our package: DATAprepSE(), PCApreprocessing(), MFUZZclustersNumber() and MFUZZanalysis().

#### Examples

Time.position=2,
Individual.position=3)

print(res.test.colnames)

DATAnormalization Normalization of raw counts (Main Function).

## Description

From raw counts, this function realizes one of the three methods of normalization of the package DESeq2:

- Relative Log Expression (rle) transformation (see BiocGenerics::estimateSizeFactors())
- Regularized Log (rlog) transformation (see DESeq2::rlog())
- Variance Stabilizing Transformation (vst) transformation (see DESeq2::vst())

## Usage

```
DATAnormalization(
   SEres,
   Normalization = "vst",
   Blind.rlog.vst = FALSE,
   Plot.Boxplot = TRUE,
   Colored.By.Factors = FALSE,
   Color.Group = NULL,
   Plot.genes = FALSE,
   path.result = NULL,
   Name.folder.norm = NULL
)
```

#### Arguments

SEres	Results of the function DATAprepSE().
Normalization	"rle", "vst", "rlog" and "rpkm". "rle", "vst" and "rlog" correspond to a method of normalization proposed by DESeq2 (see BiocGenerics::estimateSizeFactors() for "rle", DESeq2::rlog() for "rlog" and DESeq2::vst() for "vst"). "rpkm" corresponds to a RPKM normalization after a "rle" normalization.
Blind.rlog.vst	TRUE or FALSE. FALSE by default. See input 'blind' in DESeq2::rlog(). It is recommended to set Blind.rlog.vst=FALSE for downstream analysis.
Plot.Boxplot	TRUE or FALSE. TRUE by default. If Plot.Boxplot=TRUE, the function DATAplotBoxplotSamples() will be called and boxplots will be plotted. Otherwise, no boxplots will be plotted.
Colored.By.Fact	ors
	TRUE or FALSE. FALSE by default. If TRUE, boxplots will be colored with different colors for different time measurements (if data were collected at different time points). Otherwise, boxplots will be colored with different colors for different biological conditions.

Color.Group	NULL or a data.frame with $N_{bc}$ rows and two columns where $N_{bc}$ is the number of biological conditions. NULL by default. If Color.Group is a data.frame, the first column must contain the name of each biological condition and the sec- ond column must contain the colors associated to each biological condition. If Color.Group=NULL, the function will automatically attribute a color for each bi- ological condition. If samples belong to different time points only, Color.Group will not be used.
Plot.genes	TRUE or FALSE. FALSE by default. If TRUE, points representing gene expressions (normalized or raw counts) will be plotted for each sample. Otherwise, only boxplots will be plotted.
path.result	Character or NULL. NULL by default. Path to save all results. If path.result contains a sub folder entitled "1_Normalization_Name.folder.norm" all results will be saved in the sub folder "1_Normalization_Name.folder.norm". Other- wise, a sub folder entitled "1_Normalization_Name.folder.norm" will be cre- ated in path.result and all results will be saved in "1_Normalization_Name.folder.norm". If NULL, the results will not be saved in a folder.NULL as default.
Name.folder.nom	rm
	Character or NULL. NULL by default. If Name.folder.norm is a character, the folder name which will contain the results will be "1_Normalization_Name.folder.norm". Otherwise, the folder name will be "1_Normalization".

# Details

All results are built from the results of the function DATAprepSE().

#### Value

The function returns a SummarizedExperiment object (SEresNorm) identical as SEres but

- with the normalized count data saved in assays(SEresNORM)[[2]]
- with the boxplot of normalized count saved in the metadata Results[[1]][[1]] of SEresNorm.

The boxplot is plotted if Plot.Boxplot=TRUE.

## See Also

```
The DATAnormalization() function calls our R function DATAprepSE(), and the R functions BiocGenerics::estimateSizeFactors(), DESeq2::rlog() and DESeq2::vst() in order to realized the normalization.
```

## Examples

## DATAplotBoxplotSamples

Visualization of the distribution of all gene expressions using a boxplot for each sample.

# Description

From the results of either our R function DATAprepSE() or our R function DATAnormalization() (raw counts or normalized raw counts), the function plots the distribution of all gene expressions using a boxplot for each sample.

## Usage

```
DATAplotBoxplotSamples(
   SEres,
   Log2.transformation = TRUE,
   Colored.By.Factors = FALSE,
   Color.Group = NULL,
   Plot.genes = FALSE,
   y.label = NULL
)
```

#### Arguments

SEres	Results of the function DATAprepSE() or DATAnormalization().
Log2.transforma	tion
	TRUE or FALSE. TRUE by default. If TRUE, each numeric value $x$ in ExprData will
	become $log_2(x+1)$ (see Details).
Colored.By.Fact	ors
	TRUE or FALSE. FALSE by default. If TRUE, boxplots will be colored with different colors for different time measurements (if data were collected at different time points). Otherwise, boxplots will be colored with different colors for different biological conditions.
Color.Group	NULL or a data.frame with $N_{bc}$ rows and two columns where $N_{bc}$ is the number of biological conditions. NULL by default. If Color.Group is a data.frame, the first column must contain the name of each biological condition and the second column must contain the colors associated to each biological condition. If Color.Group=NULL, the function will automatically attribute a color for each biological condition. If samples belong to different time points only, Color.Group will not be used.
Plot.genes	TRUE or FALSE. FALSE by default. If TRUE, points representing gene expression (normalized or raw counts) will be added for each sample.

#### **DATAplotBoxplotSamples**

y.label	NULL or a character. NULL by default. If y.label is a character, it will be the y
	label of the graph. If y.label=NULL, the label will be either "log2(Gene expres-
	sion + 1)" (if Log2.transformation=TRUE) or "Gene expression" (if Log2.transformation=FALSE).

#### Details

The boxplot allows to visualize six summary statistics (see ggplot2::geom\_boxplot()):

- the median
- two hinges: first and third quartiles denoted Q1 and Q3.
- two whiskers: W1 := Q1 1.5 \* IQR and W3 := Q3 + 1.5 \* IQR with IQR = Q3 Q1, the interquartile range.
- outliers: data beyond the end of the whiskers are called "outlying" points and are plotted in black.

For better visualization of the six summary statistics described above, raw counts must be transformed using the function  $log_2(x + 1)$ . This transformation is automatically performed by other functions of the package, such as DATAnormalization(). Log2.transformation will be set as TRUE in DATAnormalization() if Normalization ="rle", otherwise Log2.transformation=FALSE.

## Value

The function returns a graph which plots the distribution of all gene expressions using a boxplot for each sample (see ggplot2::geom\_boxplot()).

## See Also

The DATAplotBoxplotSamples() function

- is used by the following function of our package: DATAnormalization().
- calls the R functions ggplot2::geom\_boxplot and ggplot2::geom\_jitter in order to print the boxplot.

#### Examples

DATAplotExpression1Gene

Plot expression of one gene.

## Description

The function allows to plot the gene expression profile of one gene only according to time and/or biological conditions.

#### Usage

```
DATAplotExpression1Gene(
   SEres,
   row.gene = 1,
   Color.Group = NULL,
   ylabel = "Expression"
)
```

#### Arguments

SEres	$Results \ of \ either \ our \ R \ function \ DATAprepSE(), or \ our \ R \ function \ DATAnormalization().$
row.gene	Non negative integer indicating the row of the gene to be plotted.
Color.Group	NULL or a data.frame with $N_{bc}$ rows and two columns where $N_{bc}$ is the number
	of biological conditions. If Color. Group is a data.frame, the first column must
	contain the name of each biological condition and the second column must con-
	tain the colors associated to each biological condition. If Color.Group=NULL,
	the function will automatically attribute a color for each biological condition. If
	samples belong to different time points only, Color. Group will not be used.
ylabel	Character corresponding to the label of the y-axis. By default, ylab="Expression".

## Details

All results are built from either the results of our R function DATAprepSE() or the results of our R function DATAnormalization().

# Value

The function plots for the gene selected with the input row.gene

- In the case where samples belong to different time points only : the evolution of the expression of each replicate across time and the evolution of the mean and the standard deviation of the expression across time.
- In the case where samples belong to different biological conditions only: a violin plot (see ggplot2::geom\_violin()), and error bars (standard deviation) (see ggplot2::geom\_errorbar()) for each biological condition.
- In the case where samples belong to different time points and different biological conditions : the evolution of the expression of each replicate across time and the evolution of the mean and the standard deviation of the expression across time for each biological condition.

### See Also

The DATAplotExpression1Gene() function is used by the following function of our package: DATAplotExpressionGenes().

## Examples

DATAplotExpressionGenes

Plot expression of a subset of genes.

#### Description

The function allows to plot gene expression profiles according to time and/or biological conditions.

#### Usage

```
DATAplotExpressionGenes(
   SEresNorm,
   Vector.row.gene,
   DATAnorm = TRUE,
   Color.Group = NULL,
   Plot.Expression = TRUE,
   path.result = NULL,
   Name.folder.profile = NULL
)
```

#### Arguments

SEresNorm Results of the function DATAnormalization(). Vector.row.gene

Vector of non negative integers indicating the rows of the genes to be plotted.

DATAnorm TRUE or FALSE. TRUE by default. TRUE means the function plots gene normalized expression profiles. FALSE means the function plots gene raw expression profiles.

Color.Group NULL or a data.frame with  $N_{bc}$  rows and two columns where  $N_{bc}$  is the number of biological conditions. If Color.Group is a data.frame, the first column must contain the name of each biological condition and the second column must contain the colors associated to each biological condition. If Color.Group=NULL, the function will automatically attribute a color for each biological condition. If samples belong to different time points only, Color.Group will not be used. NULL by default.

#### Plot.Expression

TRUE or FALSE. TRUE by default. If TRUE, the graph will be plotted. Otherwise no graph will be plotted.

path.result Character or NULL.NULL by default. Path to save all results. If path.result contains a sub folder entitled "1\_UnsupervisedAnalysis\_Name.folder.profile" and a sub sub folder, "1-5\_ProfileExpression\_Name.folder.profile" all results will be saved in the sub folder "1\_UnsupervisedAnalysis\_Name.folder.profile/ 1-5\_ProfileExpression\_Name.folder.profile". Otherwise, a sub folder entitled "1\_UnsupervisedAnalysis\_Name.folder.profile" and/or a sub sub folder "1-5\_ProfileExpression\_Name.folder.profile" will be created in path.result and all results will be saved in "1\_UnsupervisedAnalysis\_Name.folder.profile/ 1-5\_ProfileExpression\_Name.folder.profile". If NULL, the results will not be saved in a folder. NULL as default.

Name.folder.profile

Character or NULL. NULL by default. If Name.folder.profile is a character, the folder and sub folder names which will contain the PCA graphs will respectively be "1\_UnsupervisedAnalysis\_Name.folder.profile" and "1-5\_ProfileExpression\_Name.folder.profile" Otherwise, the folder and sub folder names will respectively be "1\_Unsuper-visedAnalysis" and "1-5\_ProfileExpression".

## Details

All results are built from the results of our function DATAnormalization().

#### Value

The function returns the same SummarizedExperiment class object SEresNorm with the a graph for each gene (depending on the experimental design) selected with the input Vector.row.gene (saved in the metadata Results[[1]][[5]] of SEresNorm)

- In the case where samples belong to different time points only : the evolution of the expression of each replicate across time and the evolution of the mean and the standard deviation of the expression across time.
- In the case where samples belong to different biological conditions only: a violin plot (see ggplot2::geom\_violin()), and error bars (standard deviation) (see ggplot2::geom\_errorbar()) for each biological condition.
- In the case where samples belong to different time points and different biological conditions : the evolution of the expression of each replicate across time and the evolution of the mean and the standard deviation of the expression across time for each biological condition.

The function plots the different graph if Plot.Expression=TRUE.

## See Also

The function calls our R function DATAnormalization() fisrt, then DATAplotExpression1Gene() for each selected genes with Vector.row.gene.

#### Examples

```
## Simulation raw counts
 resSIMcount <- RawCountsSimulation(Nb.Group=2, Nb.Time=3, Nb.per.GT=4,</pre>
                                  Nb.Gene=10)
 ## Preprocessing step
 resDATAprepSE <- DATAprepSE(RawCounts=resSIMcount$Sim.dat,</pre>
                            Column.gene=1,
                            Group.position=1,
                            Time.position=2,
                            Individual.position=3)
 ## Normalization
 resNorm <- DATAnormalization(SEres=resDATAprepSE,</pre>
                             Normalization="rle",
                             Plot.Boxplot=FALSE,
                             Colored.By.Factors=FALSE)
 resEVOgenes <- DATAplotExpressionGenes(SEresNorm=resNorm,</pre>
                                      Vector.row.gene=c(1,3),
                                      DATAnorm=TRUE,
                                      Color.Group=NULL,
                                      Plot.Expression=TRUE,
                                      path.result=NULL,
                                      Name.folder.profile=NULL)
DATAprepSE
                       Data preparation for exploratory and statistical analysis (Main Func-
                        tion)
```

## Description

This function creates automatically a SummarizedExperiment (SE) object from raw counts data to store

- information for exploratory (unsupervised) analysis using the R function SummarizedExperiment::SummarizedExper
- a DESeq2 object from raw counts data in order to store all information for statistical (supervised) analysis using the R function DESeq2::DESeqDataSetFromMatrix().

# DATAprepSE

# Usage

```
DATAprepSE(
   RawCounts,
   Column.gene,
   Group.position,
   Time.position,
   Individual.position,
   colData = NULL,
   VARfilter = 0,
   SUMfilter = 0,
   RNAlength = NULL
)
```

# Arguments

RawCounts	Data.frame with $N_g$ rows and $(N_s + k)$ columns, where $N_g$ is the number of genes, $N_s$ is the number of samples and $k = 1$ if a column is used to specify gene names, or $k = 0$ otherwise. If $k = 1$ , the position of the column containing gene names is given by Column.gene. The data.frame contains non negative integers giving gene expressions of each gene in each sample. Column names of the data.frame must describe each sample's information (individual, biological condition and time) and have the structure described in the section Details.
Column.gene	Integer indicating the column where gene names are given. Set Column.gene=NULL if there is no such column.
Group.position	Integer indicating the position of group information in the string of characters in each sample names (see Details). Set Group.position=NULL if there is only one or no biological information in the string of character in each sample name.
Time.position	Integer indicating the position of time measurement information in the string of characters in each sample names (see Details). Set Time.position=NULL if there is only one or no time measurement information in the string of character in each sample name.
Individual.posi	tion
	Integer indicating the position of the name of the individual (e.g patient, repli- cate, mouse, yeasts culture) in the string of characters in each sample names (see Details). The names of different individuals must be all different. Further- more, if individual names are just numbers, they will be transform in a vector of class "character" by CharacterNumbers() and a "r" will be added to each individual name ("r" for replicate).
colData	NULL or data.frame with $N_s$ rows and two or three columns describing the samples. NULL as default. Optional input (see Details). If Group.position, Time.position and Individual.position are filled, set colData=NULL.
	<ul> <li>If samples belong to different times point and different biological condition         <ul> <li>the first column must contain the biological condition for each sample. The column name must be "Group".</li> <li>the second column must contain the time measurement for each sample.</li> </ul> </li> </ul>

	<ul> <li>The third column must contain the individual name for each sample. The column name must be "ID".</li> </ul>
	• If samples belong to different times point or different biological condition
	<ul> <li>the first column must contain, either the biological condition for each sample, or the time measurement for each sample. The column name must be either "Group", or "Time".</li> </ul>
	<ul> <li>The second column must contain the individual name for each sample. The column name must be "ID".</li> </ul>
VARfilter	Positive numeric value, 0 as default. All rows of RawCounts which the variance of counts is strictly under the threshold VARfilter are deleted
SUMfilter	Positive numeric value, 0 as default. All rows of RawCounts which the sum of counts is strictly under the threshold SUMfilter are deleted.
RNAlength	NULL or "hsapiens" or data.frame with two columns. NULL as default.
	• if RNAlength is a data.frame
	- the first column must contain gene names (similar to those of RawCounts)
	<ul> <li>the second columns must contain the median of the transcript length of each gene of the first column and all rows of RawCounts whose genes are not included in the first column of RNAlength will be deleted.</li> </ul>
	• if RNAlength=NULL, no rows will be deleted.
	If RNAlength is either "hsapiens" or a data.frame, Column.gene can not be NULL.

#### Details

The column names of RawCounts must be a vector of strings of characters containing

- a string of characters (if k = 1) which is the label of the column containing gene names.
- $N_s$  sample names which must be strings of characters containing at least : the name of the individual (e.g patient, mouse, yeasts culture), its biological condition (if there is at least two) and the time where data have been collected if there is at least two; (must be either 't0', 'T0' or '0' for time 0, 't1', 'T1' or '1' for time 1, ...).

All these sample information must be separated by underscores in the sample name. For instance 'CLL\_P\_t0\_r1', corresponds to the patient 'r1' belonging to the biological condition 'P' and where data were collected at time 't0'. I this example, 'CLL' describe the type of cells (here chronic lymphocytic leukemia) and is not used in our analysis.

In the string of characters 'CLL\_P\_t0\_r1', 'r1' is localized after the third underscore, so Individual.position=4, 'P' is localized after the first underscore, so Group.position=2 and 't0' is localized after the second underscore, so Time.position=3.

If the user does not have all these sample information separated by underscores in the sample name, the user can build the data.frame colData describing the samples.

#### Value

The function returns a SummarizedExperiment object containing all information for exploratory (unsupervised) analysis and DE statistical analysis.

#### **DEanalysisGlobal**

## See Also

The DATAprepSE() function

- is used by the following functions of our package : DATAnormalization(), DEanalysisGlobal().
- calls the R function DESeq2::DESeqDataSetFromMatrix() in order to create the DESeq2
   object and SummarizedExperiment::SummarizedExperiment() in order to create the SummarizedExperiment object

## Examples

```
BgCdEx <- rep(c("P", "NP"), each=27)</pre>
TimeEx <- rep(paste0("t", seq_len(9) - 1), times=6)</pre>
IndvEx <- rep(paste0("pcl", seq_len(6)), each=9)</pre>
SampleNAMEex <- paste(BgCdEx, IndvEx, TimeEx, sep="_")</pre>
RawCountEx <- data.frame(Gene.name=paste0("Name", seq_len(10)),</pre>
                        matrix(sample(seq_len(100),
                                       length(SampleNAMEex)*10,
                                       replace=TRUE),
                                ncol=length(SampleNAMEex), nrow=10))
colnames(RawCountEx) <- c("Gene.name", SampleNAMEex)</pre>
##-----
                                             -----##
resDATAprepSE <- DATAprepSE(RawCounts=RawCountEx,</pre>
                            Column.gene=1,
                            Group.position=1,
                            Time.position=3,
                            Individual.position=2)
##
## colDataEx <- data.frame(Group=BgCdEx, Time=TimeEx, ID=IndvEx)</pre>
```

DEanalysisGlobal Realization of the DE analysis (Main Function).

#### Description

The function realizes the DE analysis in three cases: either samples belonging to different time measurements, or samples belonging to different biological conditions, or samples belonging to different time measurements and different biological conditions.

#### Usage

```
DEanalysisGlobal(
   SEres,
   pval.min = 0.05,
   pval.vect.t = NULL,
   log.FC.min = 1,
   LRT.supp.info = FALSE,
   Plot.DE.graph = TRUE,
```

```
path.result = NULL,
Name.folder.DE = NULL
)
```

## Arguments

SEres	Results of either our R function DATAprepSE(), or our R function DATAnormalization()
pval.min	Numeric value between 0 and 1. A gene will be considered as differentially expressed (DE) between two biological conditions if its Benjamini-Hochberg adjusted p-value (see stats::p.adjust()) is below the threshold pval.min. Default value is 0.05.
pval.vect.t	NULL or vector of dimension $T - 1$ filled with numeric values between 0 and 1, with $T$ the number of time measurements. A gene will be considered as differentially expressed (DE) between the time ti and the reference time t0 if its Benjamini-Hochberg adjusted p-value (see stats::p.adjust()) is below the i-th threshold of pval.vect.t. If NULL, pval.vect.t will be vector of dimension $T - 1$ filled with pval.min.
log.FC.min	Non negative numeric value. If the $log_2$ fold change between biological con- ditions or times has an absolute value below the threshold log.FC.min, then the gene is not selected even if is considered as DE. Default value is 1. If log.FC.min=0, all DE genes will be kept.
LRT.supp.info	TRUE or FALSE. If TRUE, the algorithm realizes another statistical test in order to detect if, among all biological conditions and/or times, at least one has a different behavior than the others (see the input test in DESeq2::DESeq()).
Plot.DE.graph	TRUE or FALSE. TRUE as default. If TRUE, all graphs will be plotted. Otherwise no graph will be plotted.
path.result	Character or NULL. Path to save all results. If path.result contains a sub folder entitled "DEanalysis_Name.folder.DE" all results will be saved in the sub folder "DEanalysis_Name.folder.DE". Otherwise, a sub folder entitled "DEanalysis_Name.folder.DE" will be created in path.result and all results will be saved in "DEanalysis_Name.folder.DE". If NULL, the results will not be saved in a folder.NULL as default.
Name.folder.DE	Character or NULL. If Name.folder.DE is a character, the folder names which will contain all results will be "DEanalysis_Name.folder.DE". Otherwise, the folder name will be "DEanalysis".

## Details

All results are built from the results of either our R function DATAprepSE(), or our R function DATAprepSE().

# Value

The function returns the same SummarizedExperiment class object SEres with the rle normalized count data (cf DATAnormalization()) automatically realized by DESeq2::DESeq() and saved in assays(SEresNORM)\$rle, and with the following results saved in the metadata Results[[2]][[2]] of SEres, depending on the experimental design.

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#### DEanalysisGlobal

- If samples belong to different biological conditions only (see DEanalysisGroup()), the function returns
  - a data.frame (output rowData(SEres)) which contains
    - \* pvalues, log2 fold change and DE genes between each pairs of biological conditions.
    - \* a binary column (1 and 0) where 1 means the gene is DE between at least one pair of biological conditions.
    - \*  $N_{bc}$  binary columns, where  $N_{bc}$  is the number of biological conditions, which gives the specific genes for each biological condition. A '1' in one of these columns means the gene is specific to the biological condition associated to the given column. 0 otherwise. A gene is called specific to a given biological condition BC1, if the gene is DE between BC1 and any other biological conditions, but not DE between any pair of other biological conditions.
    - \*  $N_{bc}$  columns filled with -1, 0 and 1, one per biological condition. A '1' in one of these columns means the gene is up-regulated (or over-expressed) for the biological condition associated to the given column. A gene is called up-regulated for a given biological condition BC1 if the gene is specific to the biological conditions. A '-1' in one of these columns means the gene is down-regulated (or under-expressed) for the biological condition associated to the given column. A gene is called down-regulated for a given biological condition BC1 if the gene is specific to the biological condition BC1 and expressions in BC1 are lower than in the other biological conditions. A '0' in one of these columns means the gene is not specific to the biological condition associated to the given column.
  - an UpSet plot (Venn diagram displayed as a barplot) which gives the number of genes for each possible intersection (see DEplotVennBarplotGroup()). We consider that a set of pairs of biological conditions forms an intersection if there is at least one gene which is DE for each of these pairs of biological conditions, but not for the others.
  - a barplot which gives the number of genes categorized as "Upregulated" and "Down-Rugulated", per biological condition (see DEplotBarplot()).
  - a barplot which gives the number of genes categorized as "Upregulated", "DownRugulated" and "Other", per biological condition (see DEplotBarplot()). A gene is categorized as 'Other', for a given biological condition, if the gene is not specific to the given biological condition. So this barplot, only plotted when there are strictly more than two biological conditions, is similar to the previous barplot but with the category "Other".
  - a list (output List.Glossary) containing the glossary of the column names of DE.results.
  - a list (output Summary.Inputs) containing a summary of sample information and inputs of DEanalysisGlobal().
- If data belong to different time points only (see DEanalysisTime()), the function returns
  - a data.frame (output rowData(SEres)) which contains
    - \* gene names
    - \* pvalues, log2 fold change and DE genes between each time ti versus the reference time t0.
    - \* a binary column (1 and 0) where 1 means the gene is DE at at least between one time ti versus the reference time t0.
    - \* a column where each element is succession of 0 and 1. The positions of '1' indicate the set of times ti such that the gene is DE between ti and the reference time t0.

- an alluvial graph of differentially expressed (DE) genes (see DEplotAlluvial())
- a graph showing the number of DE genes as a function of time for each temporal group (see DEplotAlluvial()). By temporal group, we mean the sets of genes which are first DE at the same time.
- a barplot which gives the number of DE genes per time (see DEplotBarplotTime())
- an UpSet plot which gives the number of genes per temporal pattern (see DEplotVennBarplotTime()).
   By temporal pattern, we mean the set of times ti such that the gene is DE between ti and the reference time t0.
- a similar UpSet plot where each bar is split in different colors corresponding to all possible numbers of DE times where genes are over expressed in a given temporal pattern.
- a list (output List.Glossary) containing the glossary of the column names of DE.results.
- a list (output Summary. Inputs) containing a summary of sample information and inputs of DEanalysisGlobal().
- If data belong to different time points and different biological conditions (see DEanalysisTimeAndGroup()), the function returns
  - a data.frame (output rowData(SEres)) which contains
    - \* gene names
    - \* Results from the temporal statistical analysis
      - pvalues, log2 fold change and DE genes between each pairs of biological conditions for each fixed time.
      - $N_{bc}$  binary columns (0 and 1), one per biological condition (with  $N_{bc}$  the number of biological conditions). A 1 in one of these two columns means the gene is DE at least between one time ti versus the reference time t0, for the biological condition associated to the given column.
      - $N_{bc}$  columns, one per biological condition, where each element is succession of 0 and 1. The positions of 1 in one of these two columns, indicate the set of times ti such that the gene is DE between ti and the reference time t0, for the biological condition associated to the given column.
    - \* Results from the statistical analysis by biological condition
      - pvalues, log2 fold change and DE genes between each time ti and the reference time t0 for each biological condition.
      - $\cdot$  T binary columns (0 and 1), one per time (with T the number of time measurements). A 1 in one of these columns, means the gene is DE between at least one pair of biological conditions, for the fixed time associated to the given column.
      - $T \times N_{bc}$  binary columns, which give the genes specific for each biological condition at each time ti. A 1 in one of these columns means the gene is specific to the biological condition at a fixed time associated to the given column. 0 otherwise. A gene is called specific to a given biological condition BC1 at a time ti, if the gene is DE between BC1 and any other biological conditions at time ti, but not DE between any pair of other biological conditions at time ti.
      - $T \times N_{bc}$  columns filled with -1, 0 and 1. A 1 in one of these columns means the gene is up-regulated (or over-expressed) for the biological condition at a fixed time associated to the given column. A gene is called up-regulated for a given biological condition BC1 at time ti if the gene is specific to the biological condition BC1 at time ti and expressions in BC1 at time ti are higher than in the other biological conditions at time ti. A -1 in one of these columns means the gene is down-regulated

(or under-expressed) for the biological condition at a fixed time associated to the given column. A gene is called down-regulated for a given biological condition at a time ti BC1 if the gene is specific to the biological condition BC1 at time ti and expressions in BC1 at time ti are lower than in the other biological conditions at time ti. A 0 in one of these columns means the gene is not specific to the biological condition at a fixed time associated to the given column.

- $N_{bc}$  binary columns (0 and 1). A 1 in one of these columns means the gene is specific at at least one time ti, for the biological condition associated to the given column. 0 otherwise.
- \* Results from the combination of temporal and biological statistical analysis
  - $T \times N_{bc}$  binary columns, which give the signatures genes for each biological condition at each time ti. A 1 in one of these columns means the gene is signature gene to the biological condition at a fixed time associated to the given column. 0 otherwise. A gene is called signature of a biological condition BC1 at a given time ti, if the gene is specific to the biological condition BC1 at time ti and DE between ti versus the reference time t0 for the biological condition BC1.
  - $N_{bc}$  binary columns (0 and 1). A 1 in one of these columns means the gene is signature at at least one time ti, for the biological condition associated to the given column. 0 otherwise.
- the following plots from the temporal statistical analysis
  - \* a barplot which gives the number of DE genes between ti and the reference time t0, for each time ti (except the reference time t0) and biological condition (see DEplotBarplotFacetGrid()).
  - \*  $N_{bc}$  alluvial graphs of DE genes (see DEplotAlluvial()), one per biological condition.
  - \*  $N_{bc}$  graphs showing the number of DE genes as a function of time for each temporal group, one per biological condition. By temporal group, we mean the sets of genes which are first DE at the same time.
  - \*  $2 \times N_{bc}$  UpSet plot showing the number of DE genes belonging to each DE temporal pattern, for each biological condition. By temporal pattern, we mean the set of times ti such that the gene is DE between ti and the reference time t0 (see DEplotVennBarplotTime()).
  - \* an alluvial graph for DE genes which are DE at least one time for each group.
- the following plots from the statistical analysis by biological condition
  - \* a barplot which gives the number of specific DE genes for each biological condition and time (see DEplotBarplotFacetGrid()).
  - \*  $N_{bc}(N_{bc}-1)/2$  UpSet plot which give the number of genes for each possible intersection (set of pairs of biological conditions), one per time (see DEplotVennBarplotGroup()).
  - \* an alluvial graph of genes which are specific at least one time (see DEplotAlluvial()).
- the following plots from the combination of temporal and biological statistical analysis
  - \* a barplot which gives the number of signature genes for each biological condition and time (see DEplotBarplotFacetGrid()).
  - \* a barplot showing the number of genes which are DE at at least one time, specific at at least one time and signature at at least one time, for each biological condition.
  - \* an alluvial graph of genes which are signature at least one time (see DEplotAlluvial()).

## Examples

```
data(RawCounts_Antoszewski2022_MOUSEsub500)
## No time points. We take only two groups for the speed of the example
RawCounts_T1Wt <- RawCounts_Antoszewski2022_MOUSEsub500[seq_len(200),</pre>
                                               seq_len(7)]
##------##
## Preprocessing
resDATAprepSE <- DATAprepSE(RawCounts=RawCounts_T1Wt,</pre>
                       Column.gene=1,
                       Group.position=1,
                       Time.position=NULL,
                       Individual.position=2)
##------##
## DE analysis
resDE <- DEanalysisGlobal(SEres=resDATAprepSE,</pre>
                     pval.min=0.05,
                     pval.vect.t=NULL,
                     log.FC.min=1,
                     LRT.supp.info=FALSE,
                      Plot.DE.graph=TRUE,
                      path.result=NULL,
                      Name.folder.DE=NULL)
```

DEanalysisGroup DE Analysis when samples belong to different biological conditions.

#### Description

The function realizes from the DESeq2::DESeq() output the analysis of DE genes between all pairs of biological conditions.

## Usage

```
DEanalysisGroup(
   DESeq.result,
   pval.min = 0.05,
   log.FC.min = 1,
   LRT.supp.info = TRUE,
   Plot.DE.graph = TRUE,
   path.result = NULL,
   SubFile.name = NULL
```

)

## Arguments

DESeq.result Output from the function DESeq2::DESeq().

pval.min	Numeric value between 0 and 1. A gene will be considered as differentially expressed (DE) between two biological conditions if its Benjamini-Hochberg adjusted p-value (see stats::p.adjust()) is below the threshold pval.min. Default value is 0.05.
log.FC.min	Non negative numeric value. If the log2 fold change between biological con- ditions or times has an absolute value below the threshold log.FC.min, then the gene is not selected even if is considered as DE. Default value is 1. If log.FC.min=0, all DE genes will be kept.
LRT.supp.info	TRUE or FALSE. If TRUE, the algorithm realizes another statistical test in order to detect if, among all biological conditions and/or times, at least one has a different behavior than the others (see the input 'test' in DESeq2::DESeq()).
Plot.DE.graph	TRUE or FALSE. TRUE as default. If TRUE, all graphs will be plotted. Otherwise no graph will be plotted.
path.result	Character or NULL. If path.result is a character, it must be a path to a folder, all graphs will be saved in path.result. If NULL, the results will not be saved in a folder. NULL as default.
SubFile.name	Character or NULL. If SubFile.name is a character, each saved file names will contain the strings of characters "_SubFile.name". If NULL, no suffix will be added.

#### Value

The function returns the same DESeqDataSet class object DESeq.result with the following results, saved in the metadata DEresultsGroup of DESeq.result:

- a data.frame (output DEsummary of DEresultsGroup) which contains
  - gene names
  - pvalues, log2 fold change and DE genes between each pairs of biological conditions.
  - a binary column (1 and 0) where 1 means the gene is DE between at least one pair of biological conditions.
  - $N_{bc}$  binary columns, where  $N_{bc}$  is the number of biological conditions, which gives the specific genes for each biological condition. A '1' in one of these columns means the gene is specific to the biological condition associated to the given column. 0 otherwise. A gene is called specific to a given biological condition BC1, if the gene is DE between BC1 and any other biological conditions, but not DE between any pair of other biological conditions.
  - $N_{bc}$  columns filled with -1, 0 and 1, one per biological condition. A '1' in one of these columns means the gene is up-regulated (or over-expressed) for the biological condition associated to the given column. A gene is called up-regulated for a given biological condition BC1 if the gene is specific to the biological condition BC1 and expressions in BC1 are higher than in the other biological conditions. A '-1' in one of these columns means the gene is down-regulated (or under-expressed) for the biological condition associated to the given column. A gene is called down-regulated for a given biological condition BC1 if the gene is called down-regulated for a given biological condition BC1 if the gene is specific to the biological condition BC1 and expressions in BC1 if the gene is specific to the biological condition BC1 and expressions in BC1 are lower than in the other biological conditions. A '0' in one of these columns means the gene is not specific to the biological condition associated to the given column.

- A contingency matrix (output Summary.DEanalysis of DEresultsGroup) which gives for each biological condition the number of genes categorized as "Upregulated", "DownRugulated" and "Other". A gene is categorized as 'Other', for a given biological condition, if the gene is not specific to the given biological condition. The category 'Other' does not exist when there are only two biological conditions.
- an UpSet plot (Venn diagram displayed as a barplot) which gives the number of genes for each possible intersection (see DEplotVennBarplotGroup()). We consider that a set of pairs of biological conditions forms an intersection if there is at least one gene which is DE for each of these pairs of biological conditions, but not for the others.
- a barplot which gives the number of genes categorized as "Upregulated" and "DownRugulated", per biological condition (see DEplotBarplot()).
- a barplot which gives the number of genes categorized as "Upregulated", "DownRugulated" and "Other", per biological condition (see DEplotBarplot()). So this barplot, only plotted when there are strictly more than two biological conditions, is similar to the previous barplot but with the category "Other".

#### See Also

The outputs of the function are used by the main function DEanalysisGlobal().

#### Examples

```
## Data
data(RawCounts_Antoszewski2022_MOUSEsub500)
## No time points. We take only two groups for the speed of the example
RawCounts_T1Wt<-RawCounts_Antoszewski2022_MOUSEsub500[seq_len(200),</pre>
                                                   seq_len(7)]
## Preprocessing step
resDATAprepSEmus1<- DATAprepSE(RawCounts=RawCounts_T1Wt,
                             Column.gene=1,
                             Group.position=1,
                             Time.position=NULL,
                             Individual.position=2)
DESeq2preprocess <- S4Vectors::metadata(resDATAprepSEmus1)$DESeq2obj</pre>
DESeq2obj <- DESeq2preprocess$DESeq2preproceesing</pre>
dds.DE.G <- DESeq2::DESeq(DESeq2obj, quiet=TRUE, betaPrior=FALSE)</pre>
res.sum.group <- DEanalysisGroup(DESeq.result=dds.DE.G,
                               pval.min=0.01,
                               log.FC.min=1,
                               LRT.supp.info=FALSE,
                               Plot.DE.graph=TRUE,
                               path.result=NULL,
                               SubFile.name=NULL)
```

#### Description

From the results from our function DEanalysisGlobal(), the function extracts from the SummarizedExperiment class outputs of the subset of genes selected with the inputs Set.Operation and ColumnsCriteria, and saves them in a SummarizeExperiment object.

#### Usage

```
DEanalysisSubData(
   SEresDE,
   ColumnsCriteria = 1,
   Set.Operation = "union",
   Save.SubData = FALSE
)
```

#### Arguments

SEresDE	A SummarizedExperiment class object. Output from DEanalysisGlobal() (see Examples).
ColumnsCriteria	
	A vector of integers where each integer indicates a column of SummarizedExperiment::rowData(SErest These columns should either contain only binary values, or may contain other numerical value, in which case extracted outputs from SEresDE will be those with >0 values (see Details).
Set.Operation	A character. The user must choose between "union" (default), "intersect", "set- diff" (see Details).
Save.SubData	TRUE or FALSE or a Character. FALSE as default. If TRUE, two csv files (see Value) will be saved in the folder "2_SupervisedAnalysis_Name.folder.DE" (see DEanalysisGlobal()).

## Details

We have the following three cases:

- If Set.Operation="union" then the rows extracted from the different datasets included in SEresDE are those such that the sum of the selected columns of SummarizedExperiment::rowData(SEresDE) by ColumnsCriteria is >0. For example, the selected genes can be those DE at least at t1 or t2 (versus the reference time t0).
- If Set.Operation="intersect" then the rows extracted from the different datasets included in SEresDE are those such that the product of the selected columns of SummarizedExperiment::rowData(SEresDE) by ColumnsCriteria is >0. For example, the selected genes can be those DE at times t1 and t2 (versus the reference time t0).

#### DEanalysisTime

• If Set.Operation="setdiff" then the rows extracted from the different datasets included in SEresDE are those such that only one element of the selected columns of SummarizedExperiment::rowData(SEresDE) by ColumnsCriteria is >0. For example, the selected genes can be those DE at times t1 only and at times t2 only (versus the reference time t0).

#### Value

The function returns a SummarizeExperiment class object containing

- sub data.frames of the different dataset included in SEresDE containing only the rows specified by ColumnsCriteria and Set.Operation.
- the DE results saved in SEresDE of genes selected by ColumnsCriteria and Set.Operation.
- The genes specified by ColumnsCriteria and Set.Operation.

## Examples

```
## Simulation raw counts
resSIMcount <- RawCountsSimulation(Nb.Group=2, Nb.Time=1, Nb.per.GT=4,
                               Nb.Gene=5)
## Preprocessing step
resDATAprepSE <- DATAprepSE(RawCounts=resSIMcount$Sim.dat,</pre>
                         Column.gene=1,
                         Group.position=1,
                         Time.position=NULL,
                         Individual.position=2)
## Transformation of resDATAprepSE into results of DEanalysisGlobal
resultsExamples <- data.frame(Gene=paste0("Gene", seq_len(5)),</pre>
                           DE1=c(0, 1, 0, 0, 1),
                           DE2=c(0, 1, 0, 1, 0))
listPATHnameEx <- list(Path.result=NULL, Folder.result=NULL)</pre>
SummarizedExperiment::rowData(resDATAprepSE) <- resultsExamples</pre>
S4Vectors::metadata(resDATAprepSE)$DESeq2obj$pathNAME <- listPATHnameEx
S4Vectors::metadata(resDATAprepSE)$DESeq2obj$SEidentification<-"SEresultsDE"
                          -----##
##-----
## results of DEanalysisSubData
resDEsub <- DEanalysisSubData(SEresDE=resDATAprepSE,</pre>
                           ColumnsCriteria=c(2, 3),
                           Set.Operation="union",
                           Save.SubData=FALSE)
```

DEanalysisTime

DE analysis when samples belong to different time points only.

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# DEanalysisTime

# Description

The function realizes from the DESeq2::DESeq() output the analysis of DE genes between each time versus the reference time t0.

# Usage

```
DEanalysisTime(
   DESeq.result,
   pval.min = 0.05,
   pval.vect.t = NULL,
   log.FC.min = 1,
   LRT.supp.info = FALSE,
   Plot.DE.graph = TRUE,
   path.result = NULL,
   SubFile.name = NULL
)
```

## Arguments

DESeq.result	Output from the function DESeq2::DESeq().
pval.min	Numeric value between 0 and 1. A gene will be considered as differentially expressed (DE) between two biological conditions if its Benjamini-Hochberg adjusted p-value (see <pre>stats::p.adjust()</pre> ) is below the threshold pval.min. Default value is 0.05
pval.vect.t	NULL or vector of dimension $T-1$ filled with numeric values between 0 and 1, with $T$ the number of time measurements. A gene will be considered as differentially expressed (DE) between the time ti and the reference time t0 if its Benjamini-Hochberg adjusted p-value (see stats::p.adjust()) is below the i-th threshold of pval.vect.t. If NULL, pval.vect.t will be vector of dimension $T-1$ filled with pval.min.
log.FC.min	Non negative numeric value. If the $log_2$ fold change between biological conditions or times has an absolute value below the threshold log.FC.min, then the gene is not selected even if is considered as DE. Default value is 1. If log.FC.min=0, all DE genes will be kept.
LRT.supp.info	TRUE or FALSE. If TRUE, the algorithm realizes another statistical test in order to detect if, among all biological conditions and/or times, at least one has a different behavior than the others (see the input 'test' in DESeq2::DESeq()).
Plot.DE.graph	TRUE or FALSE. TRUE as default. If TRUE, all graphs will be plotted. Otherwise no graph will be plotted.
path.result	Character or NULL. If path.result is a character, it must be a path to a folder, all graphs will be saved in path.result. If NULL, the results will not be saved in a folder. NULL as default.
SubFile.name	Character or NULL If SubFile.name is a character, each saved file names will contain the strings of characters "_SubFile.name". If NULL, no suffix will be added.

#### Value

The function returns the same DESeqDataSet class object DESeq.result with the following results, saved in the metadata DEresultsTime of DESeq.result:

- a data.frame (output DEsummary of DEresultsTime) which contains
  - gene names
  - pvalues, log2 fold change and DE genes between each time ti versus the reference time t0.
  - a binary column (1 and 0) where 1 means the gene is DE at least at between one time ti versus the reference time t0.
  - a column where each element is succession of 0 and 1. The positions of '1' indicate the set of times ti such that the gene is DE between ti and the reference time t0.
- an alluvial graph of differentially expressed (DE) genes (see DEplotAlluvial())
- a graph showing the number of DE genes as a function of time for each temporal group (see DEplotAlluvial()). By temporal group, we mean the sets of genes which are first DE at the same time.
- a barplot which gives the number of DE genes per time (see DEplotBarplotTime())
- an UpSet plot (Venn diagram displayed as a barplot) which gives the number of genes per temporal pattern (see DEplotVennBarplotTime()). By temporal pattern, we mean the set of times ti such that the gene is DE between ti and the reference time t0.
- a similar UpSet plot where each bar is split in different colors corresponding to all possible numbers of DE times where genes are over expressed in a given temporal pattern.

#### See Also

The outputs of the function will be used by the main function DEanalysisGlobal().

#### Examples

```
##------##
dds.DE.T <- DESeq2::DESeq(DESeq2obj, quiet=TRUE, betaPrior=FALSE)
##
res.T <- DEanalysisTime(DESeq.result=dds.DE.T,</pre>
```

pval.min=0.05, pval.vect.t=c(0.01,0.05,0.05), log.FC.min=1, LRT.supp.info=FALSE, Plot.DE.graph=TRUE, path.result=NULL, SubFile.name=NULL)

```
DEanalysisTimeAndGroup
```

*DE* analysis when samples belong to different biological condition and time points.

## Description

The function realizes from the DESeq2::DESeq() output the analysis of :

- DE genes between all pairs of biological conditions for each fixed time.
- DE genes between all times ti and the reference time t0for each biological condition.

## Usage

```
DEanalysisTimeAndGroup(
   DESeq.result,
   LRT.supp.info = TRUE,
   log.FC.min,
   pval.min,
   pval.vect.t,
   Plot.DE.graph = TRUE,
   path.result,
   SubFile.name
)
```

## Arguments

DESeq.result	Output from the function DESeq2::DESeq().
LRT.supp.info	TRUE or FALSE. If TRUE, the algorithm realizes another statistical test in order to detect if, among all biological conditions and/or times, at least one has a different behavior than the others (see the input test in DESeq2::DESeq()).
log.FC.min	Non negative numeric value. If the log2 fold change between biological con- ditions or times has an absolute value below the threshold log.FC.min, then the gene is not selected even if is considered as DE. Default value is 1. If log.FC.min=0, all DE genes will be kept.
pval.min	Numeric value between 0 and 1. A gene will be considered as differentially expressed (DE) between two biological conditions if its Benjamini-Hochberg adjusted p-value (see stats::p.adjust()) is below the threshold pval.min. Default value is 0.05

pval.vect.t	NULL or vector of dimension $T-1$ filled with numeric values between 0 and 1, with $T$ the number of time measurements. A gene will be considered as differentially expressed (DE) between the time ti and the reference time t0 if its Benjamini-Hochberg adjusted p-value (see stats::p.adjust()) is below the i-th threshold of pval.vect.t. If NULL, pval.vect.t will be vector of dimension $T-1$ filled with pval.min.
Plot.DE.graph	TRUE or FALSE. TRUE as default. If TRUE, all graphs will be plotted. Otherwise no graph will be plotted.
path.result	Character or NULL. If path.result is a character, it must be a path to a folder, all graphs will be saved in different sub-folders in path.result. If NULL, the results will not be saved. NULL as default.
SubFile.name	Character or NULL. If SubFile.name is a character, each saved file names and created folders names will contain the strings of characters "_SubFile.name". If NULL, no suffix will be added.

## Value

The function returns the same DESeqDataSet class object DESeq.result with the following results, saved in the metadata DEresultsTimeGroup of DESeq.result:

- a data.frame (output DEsummary of DEresultsTimeGroup) which contains
  - gene names
  - Results from the temporal statistical analysis
    - \* pvalues, log2 fold change and DE genes between each pairs of biological conditions for each fixed time.
    - \*  $N_{bc}$  binary columns (0 and 1), one per biological condition (with  $N_{bc}$  the number of biological conditions). A 1 in one of these two columns means the gene is DE at least between one time ti versus the reference time t0, for the biological condition associated to the given column.
    - \*  $N_{bc}$  columns, one per biological condition, where each element is succession of 0 and 1. The positions of 1 in one of these two columns, indicate the set of times ti such that the gene is DE between ti and the reference time t0, for the biological condition associated to the given column.
  - Results from the statistical analysis by biological condition
    - \* pvalues, log2 fold change and DE genes between each time ti and the reference time t0 for each biological condition.
    - \* T binary columns (0 and 1), one per time (with T the number of time measurements).
       A 1 in one of these columns, means the gene is DE between at least one pair of biological conditions, for the fixed time associated to the given column.
    - \*  $T \times N_{bc}$  binary columns, which give the genes specific for each biological condition at each time ti. A 1 in one of these columns means the gene is specific to the biological condition at a fixed time associated to the given column. 0 otherwise. A gene is called specific to a given biological condition BC1 at a time ti, if the gene is DE between BC1 and any other biological conditions at time ti, but not DE between any pair of other biological conditions at time ti.

- \*  $T \times N_{bc}$  columns filled with -1, 0 and 1. A 1 in one of these columns means the gene is up-regulated (or over-expressed) for the biological condition at a fixed time associated to the given column. A gene is called up-regulated for a given biological condition BC1 at time ti if the gene is specific to the biological condition BC1 at time ti and expressions in BC1 at time ti are higher than in the other biological conditions at time ti. A -1 in one of these columns means the gene is down-regulated (or under-expressed) for the biological condition at a fixed time associated to the given column. A gene is called down-regulated for a given biological condition at a time ti BC1 if the gene is specific to the biological condition BC1 at time ti are lower than in the other biological conditions at time ti are lower than in the other biological conditions at time ti are lower than in the other biological conditions at time ti. A 0 in one of these columns means the gene is not specific to the biological condition at a fixed time associated to the given column.
- \*  $N_{bc}$  binary columns (0 and 1). A 1 in one of these columns means the gene is specific at least at one time ti, for the biological condition associated to the given column. 0 otherwise.
- Results from the combination of temporal and biological statistical analysis
  - \*  $T \times N_{bc}$  binary columns, which give the signatures genes for each biological condition at each time ti. A 1 in one of these columns means the gene is signature gene to the biological condition at a fixed time associated to the given column. 0 otherwise. A gene is called signature of a biological condition BC1 at a given time ti, if the gene is specific to the biological condition BC1 at time ti and DE between ti versus the reference time t0 for the biological condition BC1.
  - \*  $N_{bc}$  binary columns (0 and 1). A 1 in one of these columns means the gene is signature at least at one time ti, for the biological condition associated to the given column. 0 otherwise.
- the following plots from the temporal statistical analysis
  - a barplot which gives the number of DE genes between ti and the reference time t0, for each time ti (except the reference time t0) and biological condition (see DEplotBarplotFacetGrid()).
  - $N_{bc}$  alluvial graphs of DE genes (see DEplotAlluvial()), one per biological condition.
  - $N_{bc}$  graphs showing the number of DE genes as a function of time for each temporal group, one per biological condition. By temporal group, we mean the sets of genes which are first DE at the same time.
  - $2 \times N_{bc}$  UpSet plot showing the number of DE genes belonging to each DE temporal pattern, for each biological condition. By temporal pattern, we mean the set of times ti such that the gene is DE between ti and the reference time t0 (see DEplotVennBarplotTime()).
  - an alluvial graph for DE genes which are DE at least one time for each group.
- the following plots from the statistical analysis by biological condition
  - a barplot which gives the number of specific DE genes for each biological condition and time (see DEplotBarplotFacetGrid()).
  - $N_{bc}(N_{bc}-1)/2$  UpSet plot which give the number of genes for each possible intersection (set of pairs of biological conditions), one per time (see DEplotVennBarplotGroup()).
  - an alluvial graph of genes which are specific at least one time (see DEplotAlluvial()).
- the following plots from the combination of temporal and biological statistical analysis
  - a barplot which gives the number of signature genes for each biological condition and time (see DEplotBarplotFacetGrid()).

- a barplot showing the number of genes which are DE at least at one time, specific at least at one time and signature at least at one time, for each biological condition.
- an alluvial graph of genes which are signature at least one time (see DEplotAlluvial()).

# See Also

The outputs of the function will be used by the main function DEanalysisGlobal().

#### Examples

```
data(RawCounts_Schleiss2021_CLLsub500)
## We take only the first three times (both group) for the speed of
## the example
Index3t<-c(2:4,11:13,20:22, 29:31,38:40,47:49)
RawCounts_3t<-RawCounts_Schleiss2021_CLLsub500[seq_len(200), c(1,Index3t)]</pre>
## Preprocessing step
resDATAprepSEleuk <- DATAprepSE(RawCounts=RawCounts_3t,</pre>
                               Column.gene=1,
                               Group.position=2,
                               Time.position=4,
                               Individual.position=3)
DESeq2preprocess <- S4Vectors::metadata(resDATAprepSEleuk)$DESeq2obj</pre>
DESeq2obj <- DESeq2preprocess$DESeq2preproceesing</pre>
##------##
dds.DE <- DESeq2::DESeq(DESeq2obj)</pre>
##
res.G.T <- DEanalysisTimeAndGroup(DESeq.result=dds.DE,</pre>
                                 LRT.supp.info=FALSE,
                                 pval.min=0.05,
                                 pval.vect.t=NULL,
                                 log.FC.min=0.1,
                                 Plot.DE.graph=TRUE,
                                 path.result=NULL,
                                 SubFile.name=NULL)
```

DEplotAlluvial

Alluvial graphs of differentially expressed (DE) genes

#### Description

The function takes as input a binary table with  $N_q$  lines corresponding to genes and

- if Temporal.Group=TRUE : T 1 columns corresponding to times (with T the number of time points). A '1' in the n-th row and t-th column means that the n-th gene is differentially expressed (DE) at time t, compared with the reference time t0.
- if Temporal.Group=FALSE : G columns corresponding to the number of group. A '1' in the *n*-th row and g-th column means that the n-th gene is

- DE at least one time ti, compared with the reference time t0, for the group g.
- specific at least one time ti, compared with the reference time t0, for the group g (see DEanalysisTimeAndGroup() for the notion 'specific').
- a signature gene at least one time ti, compared with the reference time t0, for the group g (see DEanalysisTimeAndGroup() for the notion 'signature').

#### The function plots

- if Temporal.Group=TRUE, two graphs: an alluvial graph and a plot showing the time evolution of the number of DE genes within each temporal group. By temporal group, we mean the sets of genes which are first DE at the same time.
- if Temporal.Group=FALSE : an alluvial graph.

#### Usage

```
DEplotAlluvial(
  table.DE.time,
  Temporal.Group = TRUE,
  title.alluvial = NULL,
  title.evolution = NULL
)
```

#### Arguments

table.DE.time	Binary matrix (table filled with 0 and 1) with $N_g$ rows and $T - 1$ columns with $N_g$ the number of genes and $T - 1$ the number of time points.
Temporal.Group	TRUE or FALSE, FALSE as default (see Description).
title.alluvial	String of characters or NULL, NULL as default. The input title.allluvial corresponds to the title of the alluvial graph. If title is a string of characters, title will be the title of the alluvial graph. If title=NULL, the title of the alluvial graph will be 'Alluvial graph'.
title.evolution	1
	String of characters or NULL, NULL as default. Only applied if Temporal.Group=TRUE. The input title.evolution corresponds to the title of the second graph (see Description). If title is a string of characters, it will be to the title of the second graph. If title=NULL, the title of the second graph will be 'Time evolution of the number of DE genes within each temporal group'.

## Details

The names of the columns of the table will be the axis labels in the plots. If the table has no column names, the function will automatically create column names (t1,t2,...).

## Value

The function returns, as described in description

• if Temporal.Group=TRUE, two graphs: an alluvial graph and a plot showing the time evolution of the number of DE genes within each temporal group. By temporal group, we mean the sets of genes which are first DE at the same time.

• if Temporal.Group=FALSE : an alluvial graph.

#### See Also

The DEplotAlluvial() function

- is used by the following functions of our package: DEanalysisTime() and DEanalysisTimeAndGroup().
- calls the R package ggplot2 in order to plot the two graphs.

#### Examples

DEplotBarplot Barplot of DE genes from a contingency table.

## Description

From a contingency table between two variables, the function plots a barplot of the frequency distribution of one variable against the other (see Details).

#### Usage

```
DEplotBarplot(ContingencyTable, dodge = TRUE)
```

#### Arguments

Contingency	「able
-------------	-------

A numeric data.frame, corresponding to a contingency table, of dimension N1\*N2, with N1 and N2, respectively the number of levels in the first and second variable (see examples and details).

dodge TRUE or FALSE. FALSE means multiple bars in the barplot (one per level of the first variable) one for each fixed level of the other variable. TRUE means multiple bars will be dodged side-to-side (see ggplot2::geom\_bar()).

#### **DEplotBarplot**

#### Details

A contingency table (or cross-tabulation) is a table that displays the frequency distribution of two variables (each containing several levels), i.e. the number of observation recorded per pair of levels. The function plots a single barplot from ContingencyTable.

This function is called by DEanalysisGroup() and DEanalysisTimeAndGroup(). These two functions produce several contingency tables, giving information about specific and particular DE genes, as described below.

First, we look for all genes that are DE between at least two biological conditions. A gene will be called specific to a given biological condition BC1, if the gene is DE between BC1 and any other biological conditions, but not DE between any pair of other biological conditions. Then each DE gene will be categorized as follow:

- If a gene is not specific, the gene will be categorized as 'Other'. The category 'Other' does not exist when there are only two biological conditions.
- If a gene is specific to a given biological condition BC1 and expressions in BC1 are higher than in the other biological conditions, the gene will be categorized as 'Upregulated'.
- If a gene is specific to a given biological condition BC1 and expressions in BC1 are lower than in the other biological conditions, the gene will be categorized as 'Downregulated'.

The functions DEanalysisGroup() and DEanalysisTimeAndGroup() produce two contingency table that allow to plot both

- the number of genes categorized as 'Other', 'Upregulated' and 'Downregulated' (only when there are strictly more than two biological conditions).
- the number of genes categorized 'Upregulated' and 'Downregulated'.

Second, we look for all genes that are DE between at least one time point (except t0) and t0 for each biological condition. A gene will be categorized as 'particular' to a given biological condition BC1 for a given time point ti (except t0), if the gene is DE between ti and t0 for the biological condition BC1, but not DE between ti and t0 for the other biological conditions. A gene will be categorized as 'common' to all biological conditions, if the gene is DE between ti and t0 for all biological conditions. Otherwise, a gene will categorized as 'Other'.

The function DEanalysisTimeAndGroup() produces a contingency table that allow to plot the number of 'specific', 'common' and 'other' genes for each ti (except t0).

# Value

A barplot using ggplot2 (see details).

#### See Also

The DEplotBarplot() function

- is used by the following functions of our package: DEanalysisGroup() and DEanalysisTimeAndGroup().
- calls the R package ggplot2 in order to plot the barplot.

# Examples

DEplotBarplotFacetGrid

Faceted barplot of specific DE genes

## Description

The function creates a faceted barplot from a data.frame containing two or three qualitative variables and one quantitative variable.

#### Usage

```
DEplotBarplotFacetGrid(
  Data,
  Abs.col,
  Legend.col,
  Facet.col,
  Value.col,
  Color.Legend = NULL,
  LabsPlot = c("", "")
)
```

#### Arguments

Data	Data.frame containing three or four columns. One must contain quantitative variable and the other qualitative variables.
Abs.col	Integer indicating the column of Data which will be used for the x-axis. The selected column must be one of the qualitative variables and must be identical to Legend.col if there are only two qualitative variables. Otherwise, Abs.col and Legend.col must be different.
Legend.col	Integer indicating the column of Data which is used for the color of the barplots. The selected column must be one of the qualitative variables and must be identical to Abs.col if there are only two qualitative variables. Otherwise, Abs.col and Legend.col must be different.

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Facet.col	Integer indicating the column of Data which is used for separating barplots in different panels, one per level of the qualitative variable. The selected column must be one of the qualitative variables.
Value.col	Integer indicating the column of Data which contains numeric values.
Color.Legend	Data.frame or NULL. If Color.Legend is a data.frame, the data.frame must have two columns and $N_{bc}$ rows where $N_{bc}$ is the number of biological conditions. The first column must contain the name of the $N_{bc}$ different biological conditions and the second column must the color associated to each biological condition. If Color.Legend=NULL, the function will automatically attribute a color for each biological condition.
LabsPlot	Vector of two characters indicating the x-axis label and the y-axis label of the facet grid barplot. By default, LabsPlot=c("", "").

### Value

The function will plot a facet grid barplot. The function is called by our function DEanalysisTimeAndGroup() in order to plot the number of specific (up- or down-regulated) DE genes per biological condition for each time points.

# See Also

The function

- is called by the function DEanalysisTimeAndGroup()
- calls the R functions ggplot2::facet\_grid() and ggplot2::geom\_bar().

```
Group.ex <- c('G1', 'G2', 'G3')
Time.ex <- c('t1', 't2', 't3', 't4')
Spe.sign.ex <- c("Pos", "Neg")</pre>
GtimesT <- length(Group.ex)*length(Time.ex)</pre>
Nb.Spe <- sample(3:60, GtimesT, replace=FALSE)</pre>
Nb.Spe.sign <- sample(3:60, 2*GtimesT, replace=FALSE)</pre>
Melt.Dat.1 <- data.frame(Group=rep(Group.ex, times=length(Time.ex)),</pre>
                      Time=rep(Time.ex, each=length(Group.ex)),
                      Nb.Spe.DE=Nb.Spe)
DEplotBarplotFacetGrid(Data=Melt.Dat.1, Abs.col=2, Legend.col=2,
                    Facet.col=1, Value.col=3, Color.Legend=NULL)
DEplotBarplotFacetGrid(Data=Melt.Dat.1, Abs.col=1, Legend.col=1,
                   Facet.col=2, Value.col=3, Color.Legend=NULL)
##------##
Melt.Dat.2 <- data.frame(Group=rep(Group.ex, times=length(Time.ex)*2),</pre>
                      Time=rep(Time.ex, each=length(Group.ex)*2),
                      Spe.sign=rep(Spe.sign.ex, times=2*GtimesT),
                      Nb.Spe.DE=Nb.Spe.sign)
```

DEplotBarplotTime Barplot of DE genes per time

### Description

The function takes as input two tables

- a binary table with  $N_g$  rows corresponding to genes and T-1 columns corresponding to times (with T the number of time points). A '1' in the n-th row and i-th column means that the n-th gene is differentially expressed (DE) at time ti, compared with the reference time t0.
- a numeric matrix with positive and negative values with  $N_g$  rows corresponding to genes and T-1 columns corresponding to times. The element in n-th row and i-th column corresponds to the log2 fold change between the time ti and the reference time t0 for the n-th gene. If the gene is DE and the sign is positive, then the gene n will be considered as over-expressed (up-regulated) at the time ti. If the gene is DE and the sign is negative, then the gene n will be considered as under-expressed (down-regulated) at the time ti.

The function plots two graphs: a barplot showing the number of DE genes per time and a barplot showing the number of under- and over-expressed genes per times.

### Usage

DEplotBarplotTime(table.DE.time, Log2.FC.matrix)

# Arguments

table.DE.time	Binary matrix (table filled with 0 and 1) with $N_q$ rows and $T-1$ columns with
	$N_g$ the number of genes and T the number of time points.
Log2.FC.matrix	Numeric matrix with positive and negative with $N_a$ rows and $T-1$ columns.

#### Value

The function plots two graphs: a barplot showing the number of DE genes per time and a barplot showing the number of under and over expressed genes per times.

# **DEplotHeatmaps**

### Examples

DEplotHeatmaps Heatmaps of DE genes

### Description

The function returns two heatmaps: one heatmap of gene expressions between samples and selected genes and a correlation heatmap between samples from the output of DEanalysisGlobal().

### Usage

```
DEplotHeatmaps(
   SEresDE,
   ColumnsCriteria = 2,
   Set.Operation = "union",
   NbGene.analysis = 20,
   Color.Group = NULL,
   SizeLabelRows = 5,
   SizeLabelCols = 5,
   Display.plots = TRUE,
   Save.plots = FALSE
)
```

### Arguments

SEresDE	A SummarizedExperiment class object. Output from DEanalysisGlobal() (see Examples).
ColumnsCriteria	3
	A vector of integers where each integer indicates a column of SummarizedExperiment::rowData(SErest These columns should either contain only binary values, or may contain other numerical value, in which case extracted outputs from SEresDE will be those with >0 values (see Details).
Set.Operation	A character. The user must choose between "union" (default), "intersect", "set- diff" (see Details).

NbGene.analysis	
	An integer or NULL. If it is an integer, the heatmaps will be plotted with the NbGene.analysis genes which have the highest sum of absolute log2 fold change, among the DE genes selected using ColumnsCriteria and Set.Operation. If NULL, all the DE selected genes will be used for both heatmaps.
Color.Group	NULL or a data.frame with $N_{bc}$ rows and two columns where $N_{bc}$ is the number of biological conditions. If Color.Group is a data.frame, the first column must contain the name of each biological condition and the second column must con- tain the colors associated to each biological condition. If Color.Group=NULL, the function will automatically attribute a color for each biological condition. If samples belong to different time points only, Color.Group will not be used.
SizeLabelRows	Numeric >0. Size of the labels for the genes in the heatmaps.
SizeLabelCols	Numeric >0. Size of the labels for the samples in the heatmaps.
Display.plots	TRUE or FALSE. TRUE as default. If TRUE, all graphs will be plotted. Otherwise no graph will be plotted.
Save.plots	TRUE or FALSE or a Character. If Save.plots=FALSE, the different files will not be saved. If Save.plots=TRUE and the path.result of DEanalysisGlobal() is not NULL, all files will be saved in "2_SupervisedAnalysis_Name.folder.DE/ 2-4_Supplementary_Plots_Name.folder.DE/Plots_Heatmaps". If Save.plots is a character, it must be a path and all files will be saved in the sub-folder "Plots_Heatmaps".

# Details

We have the following three cases:

- If Set.Operation="union" then the rows extracted from the different datasets (raw counts, normalized data and SummarizedExperiment::rowData(SEresDE)) included in the SummarizedExperiment class object SEresDE are those such that the sum of the selected columns of SummarizedExperiment::rowData(SEresDE) given in ColumnsCriteria is >0. This means that the selected genes are those having at least one '1' in one of the selected columns.
- If Set.Operation="intersect" then the rows extracted from the different datasets (raw counts, normalized data and SummarizedExperiment::rowData(SEresDE)) included in the SummarizedExperiment class object SEresDE are those such that the product of the selected columns of SummarizedExperiment::rowData(SEresDE) given in ColumnsCriteria is >0. This means that the selected genes are those having '1' in all of the selected columns.
- If Set.Operation="setdiff" then the rows extracted from the different datasets (raw counts, normalized data and SummarizedExperiment::rowData(SEresDE)) included in the SummarizedExperiment class object SEresDE are those such that only one element of the selected columns of SummarizedExperiment::rowData(SEresDE) given in ColumnsCriteria is >0. This means that the selected genes are those having '1' in only one of the selected columns.

# Value

The function returns the same SummarizedExperiment class object SEresDE with two heatmaps saved in the metadata Results[[2]][[4]] of SEresDE

• a correlation heatmap between samples (correlation heatmap)

### DEplotVennBarplotGroup

• a heatmap across samples and genes called Zscore heatmap, for a subset of genes that can be selected by the user.

The two heatmaps are plotted if Display.plots=TRUE. The second heatmap is build from the normalized count data after being both centered and scaled (Zscore).

# See Also

The function calls the function ComplexHeatmap::Heatmap() in order to plot the Heatmaps.

#### Examples

```
## data importation
data("RawCounts_Antoszewski2022_MOUSEsub500")
## No time points. We take only two groups for the speed of the example
dataT1wt <- RawCounts_Antoszewski2022_MOUSEsub500[seq_len(200), seq_len(7)]</pre>
## Preprocessing with Results of DEanalysisGlobal()
resDATAprepSE <- DATAprepSE(RawCounts=dataT1wt,</pre>
                        Column.gene=1,
                        Group.position=1,
                        Time.position=NULL,
                        Individual.position=2)
## DE analysis
resDET1wt <- DEanalysisGlobal(SEres=resDATAprepSE,</pre>
                          pval.min=0.05,
                          pval.vect.t=NULL,
                          log.FC.min=1,
                          LRT.supp.info=FALSE,
                          Plot.DE.graph=FALSE,
                          path.result=NULL,
                          Name.folder.DE=NULL)
##------##
resHeatmap <- DEplotHeatmaps(SEresDE=resDET1wt,</pre>
                         ColumnsCriteria=3, ## Specific genes N1haT1ko
                         Set.Operation="union",
                         NbGene.analysis=20,
                         Color.Group=NULL,
                         SizeLabelRows=5,
                         SizeLabelCols=5,
                         Display.plots=TRUE,
                         Save.plots=FALSE)
```

DEplotVennBarplotGroup

Venn barplot of DE genes across pairs of biological conditions.

### Description

The function takes as input a binary matrix or data.frame with  $N_g$  rows and  $((N_{bc} - 1) \times N_{bc})/2$  columns with  $N_g$  the number of genes and  $N_{bc}$  the number of biological conditions. The number of 1 in the n-th row gives the number of pairs of biological conditions where the gene n is DE. We consider that a set of pairs of biological conditions forms an intersection if there is at least one gene which is DE for each of these pairs of biological conditions, but not for the others.

The function calls the UpSetR::upset() function in order to plot the number of genes for each possible intersection in an UpSet plot (Venn diagram displayed as a barplot).

### Usage

```
DEplotVennBarplotGroup(Mat.DE.pair.group)
```

# Arguments

Mat.DE.pair.group

Binary matrix or data.frame with  $N_g$  rows and  $((N_{bc} - 1) * N_{bc})/2$  columns with  $N_{bc}$  the number of biological conditions.

#### Value

The function plots the number of genes for each possible intersection in a UpSet plot.

#### See Also

The function

- calls the function UpSetR::upset() in order to plot the UpSet plot.
- is called by the functions DEanalysisGroup() and DEanalysisTimeAndGroup().

### Examples

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DEplotVennBarplotTime Venn barplot of DE genes across time.

### Description

The function takes as input two matrix or data.frame

- a binary matrix or data.frame with  $N_g$  rows corresponding to genes and T-1 columns corresponding to times (with T the number of time points). A '1' in the n-th row and i-th column means that the n-th gene is differentially expressed (DE) at time ti, compared with the reference time t0.
- a numeric matrix or data.frame with  $N_g$  rows corresponding to genes and T-1 columns corresponding to times. The element in n-th row and i-th column corresponds to the  $log_2$  fold change between the time ti and the reference time t0 for the n-th gene. If the gene is DE and the sign is positive, then the gene n will be considered as over-expressed (up-regulated) at time ti. If the gene is DE and the sign is negative, then the gene n will be considered as under-expressed (down-regulated) at time ti.

# Usage

DEplotVennBarplotTime(table.DE.time, Log2.FC.matrix)

### Arguments

table.DE.time	Binary matrix or data.frame (table filled with 0 and 1) with $N_g$ rows and $T-1$
	columns with $N_g$ the number of genes and $T$ the number of time points.
Log2.FC.matrix	Numeric matrix or data frame with $N_q$ rows and $T-1$ columns.

#### Value

The function plots

- the number of genes per time patterns in an UpSet plot (Venn diagram displayed as a barplot) with the R function UpSetR::upset(). By temporal pattern, we mean the set of times ti such that the gene is DE between ti and the reference time t0.
- a similar UpSet plot where each bar is split in different colors corresponding to all possible numbers of DE times where genes are over expressed in a given temporal pattern.

#### See Also

The function

- calls the function UpSetR::upset() in order to plot the UpSet plot.
- is called by the functions DEanalysisTime() and DEanalysisTimeAndGroup().

# Examples

```
set.seed(1994)
Nb.Time <- 4 ## Number of time measurement
##------##
table.DE.time.ex <- matrix(sample(c(0,1), replace=TRUE,</pre>
                           size=40*(Nb.Time-1), c(0.2, 0.8)),
                     ncol=Nb.Time-1)
colnames(table.DE.time.ex) <- paste0("t", 1:(Nb.Time-1))</pre>
##------##
Log2FC.mat.ex <- matrix(round(rnorm(n=40*(Nb.Time-1), mean=0, sd=1),</pre>
                       digits=2),
                  ncol=(Nb.Time-1))
colnames(Log2FC.mat.ex) <- paste0("t", 1:(Nb.Time-1))</pre>
##------##
res.test.VennBarplot <- DEplotVennBarplotTime(table.DE.time=table.DE.time.ex,</pre>
                                    Log2.FC.matrix=Log2FC.mat.ex)
print(res.test.VennBarplot$Upset.graph)
print(res.test.VennBarplot$Upset.graph.with.nb.over)
res.test.VennBarplot$DE.pattern.t.01.sum
```

DEplotVolcanoMA Volcano and MA graphs

## Description

The function returns Volcano plots and MA plots from the results of our function DEanalysisGlobal().

#### Usage

```
DEplotVolcanoMA(
   SEresDE,
   NbGene.plotted = 2,
   SizeLabel = 3,
   Display.plots = FALSE,
   Save.plots = FALSE
)
```

#### Arguments

SEresDE	A SummarizedExperiment class object. Output from DEanalysisGlobal() (see Examples).	
NbGene.plotted	Non negative integer. The algorithm computes the sum of all the absolute $log_2$ fold change present in the element DE.results of Res.DE.analysis for each gene. Only the highest NbGene.plotted genes are plotted in the volcano and MA plots. By default, NbGene.plotted=2.	
SizeLabel	Numeric. Give the size of the names of plotted genes. By default, $SizeLabel=3$ .	
Display.plots	TRUE or FALSE. FALSE as default. If TRUE, all graphs will be plotted. Otherwise no graph will be plotted.	

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Save.plots TRUE or FALSE or a Character. FALSE as default. Path to save the Volcano and MA plots. If NULL, the Volcano and MA plots will not be saved in a sub folder in path.result.
If path.result contains a sub folder entitled "VolcanoPlots", all the Volcano plots will be saved in the sub folder "VolcanoPlots". Otherwise, a sub folder entitled "VolcanoPlots" will be created in path.result and all the Volcano plots will be saved in the sub folder created.
If path.result contains a sub folder entitled "MAplots", all the MA plots will be saved in the sub folder "MAplots". Otherwise, a sub folder entitled "MAplots" will be created in path.result and all the MA plots will be saved in the sub folder "MAplots".

#### Details

- If data belong to different time points only, the function returns T 1 volcano and MA plots (with T the number of time measurements), corresponding to the  $log_2$  fold change between each time ti and the reference time t0, for all i > 0.
- If data belong to different biological conditions only, the function returns  $(N_{bc} * (N_{bc} 1))/2$  volcano and MA plots (with  $N_{bc}$  the number of biological conditions), corresponding to the  $log_2$  fold change between each pair of biological condition.
- If data belong to different biological conditions and time points, the function returns
  - $(T-1) * N_{bc}$  volcano and MA plots, corresponding to the  $log_2$  fold change between each time ti and the reference time t0, for all biological condition.
  - $((T-1) * N_{bc} * (N_{bc} 1))/2$  volcano and MA plots, corresponding to the  $log_2$  fold change between each pair of biological conditions, for all fixed time point.

#### Value

The function returns the same SummarizedExperiment class object SEresDE with Volcano plots and MA plots from the results of our function DEanalysisGlobal(), all saved in the metadata Results[[2]][[3]] of SEresDE.

#### See Also

The function calls the output of DEanalysisGlobal().

in the sub folder created.

```
##------##
## DE analysis
resDET1wt <- DEanalysisGlobal(SEres=resDATAprepSE,</pre>
                      pval.min=0.05,
                      pval.vect.t=NULL,
                      log.FC.min=1,
                      LRT.supp.info=FALSE,
                      Plot.DE.graph=FALSE,
                      path.result=NULL,
                      Name.folder.DE=NULL)
##------##
## Volcano MA
resVolcanoMA <- DEplotVolcanoMA(SEresDE=resDET1wt,</pre>
                       NbGene.plotted=5,
                       Display.plots=TRUE,
                       Save.plots=FALSE)
```

DEresultGroup

Intermediate analysis when samples belong to different biological conditions

# Description

This function realizes the intermediary steps of the analysis of the function DEanalysisGroup().

# Usage

```
DEresultGroup(
   DESeq.result,
   LRT.supp.info = TRUE,
   pval.min = 0.05,
   log.FC.min = 1
)
```

# Arguments

DESeq.result	Output from the function DESeq2::DESeq().
LRT.supp.info	TRUE or FALSE. If TRUE, the algorithm realizes another statistical test in order to detect if, among all biological conditions and/or times, at least one has a different behavior than the others (see the input test in DESeq2::DESeq()).
pval.min	Numeric value between 0 and 1. A gene will be considered as differentially expressed (DE) between two biological conditions if its Benjamini-Hochberg adjusted p-value (see stats::p.adjust()) is below the threshold pval.min. Default value is 0.05.
log.FC.min	Non negative numeric value. If the log2 fold change between biological con- ditions or times has an absolute value below the threshold log.FC.min, then the gene is not selected even if is considered as DE. Default value is 1. If log.FC.min=0, all DE genes will be kept.

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#### DEresultGroup

#### Value

The function returns the same DESeqDataSet class object DESeq.result with the following results, saved in the metadata DEresultsGroup of DESeq.result:

- a data.frame (output DEsummary of DEresultsGroup) which contains
  - gene names
  - pvalues, log2 fold change and DE genes between each pairs of biological conditions.
  - a binary column (1 and 0) where 1 means the gene is DE between at least one pair of biological conditions.
  - N<sub>bc</sub> binary columns, where N<sub>bc</sub> is the number of biological conditions, which gives the specific genes for each biological condition. A '1' in one of these columns means the gene is specific to the biological condition associated to the given column. 0 otherwise. A gene is called specific to a given biological condition BC1, if the gene is DE between BC1 and any other biological conditions, but not DE between any pair of other biological conditions.
  - $N_{bc}$  columns filled with -1, 0 and 1, one per biological condition. A '1' in one of these columns means the gene is up-regulated (or over-expressed) for the biological condition associated to the given column. A gene is called up-regulated for a given biological condition BC1 if the gene is specific to the biological condition BC1 and expressions in BC1 are higher than in the other biological conditions. A '-1' in one of these columns means the gene is down-regulated (or under-expressed) for the biological condition BC1 are higher than in the other biological conditions. A '-1' in one of these columns means the gene is down-regulated (or under-expressed) for the biological condition BC1 if the gene is specific to the biological condition BC1 and expressions in BC1 if the gene is specific to the biological condition BC1 and expressions in BC1 are lower than in the other biological conditions. A '0' in one of these columns means the gene is not specific to the biological condition associated to the given column.
- a data.frame (output DE.per.pair.G of DEresultsGroup) with  $N_g$  rows and  $((N_{bc} 1) \times N_{bc})/2$  columns with  $N_g$  the number of genes and  $N_{bc}$  the number of biological conditions. The number of 1 in the n-th row gives the number of pairs of biological conditions where the gene n is DE. The output DE.per.pair.G will be the input of the function DEplotVennBarplotGroup().
- a contingency matrix (output Contingence.per.group of DEresultsGroup) which gives for each biological condition the number of genes categorized as "Upregulated", "DownRugulated" and "Other". A gene is categorized as 'Other', for a given biological condition BC1, if the gene is not specific to the biological condition BC1. The category 'Other' does not exist when there are only two biological conditions.

The output Contingence.per.group will be the input of the function DEplotBarplot().

#### See Also

The output of the function are used by the main function DEanalysisGroup().

DEresultGroupPerTime Intermediate analysis when samples belong to different biological conditions and different time points.

### Description

This function realizes the intermediate steps of the analysis of the function DEanalysisTimeAndGroup().

### Usage

```
DEresultGroupPerTime(
   DESeq.result,
   LRT.supp.info = TRUE,
   pval.min = 0.05,
   log.FC.min = 1
)
```

#### Arguments

DESeq.result	Output from the function DESeq2::DESeq().
LRT.supp.info	TRUE or FALSE. If TRUE, the algorithm realizes another statistical test in order to detect if, among all biological conditions and/or times, at least one has a different behavior than the others (see the input test in DESeq2::DESeq()).
pval.min	Numeric value between 0 and 1. A gene will be considered as differentially expressed (DE) between two biological conditions if its Benjamini-Hochberg adjusted p-value (see stats::p.adjust()) is below the threshold pval.min. Default value is 0.05.

log.FC.min Non negative numeric value. If the log2 fold change between biological conditions or times has an absolute value below the threshold log.FC.min, then the gene is not selected even if is considered as DE. Default value is 1. If log.FC.min=0, all DE genes will be kept.

### Value

The function returns the same DESeqDataSet class object DESeq.result with the following results, saved in the metadata DEresultsTimeGroup of DESeq.result:

- a data.frame (output DEsummary of DEresultsTimeGroup) which contains
  - pvalues, log2 fold change and DE genes between each pairs of biological conditions for a fixed time ti (except the reference time t0).
  - DE specific genes per biological condition for a fixed time ti (except the reference time t0).
- inputs for the functions: DEplotBarplot(), DEplotBarplotTime(), DEplotVennBarplotGroup(), DEplotVennBarplotTime(), DEplotBarplotFacetGrid(), DEplotAlluvial().

## See Also

The output of the function are used by the main function DEanalysisTimeAndGroup().

```
data("RawCounts_Schleiss2021_CLLsub500")
## We take only the first three times (both group) for the speed of
## the example
Index3t<-c(2:4,11:13,20:22, 29:31,38:40,47:49)
RawCounts_3t<-RawCounts_Schleiss2021_CLLsub500[seq_len(200), c(1,Index3t)]</pre>
## Preprocessing step
resDATAprepSEleuk <- DATAprepSE(RawCounts=RawCounts_3t,</pre>
                              Column.gene=1,
                              Group.position=2,
                              Time.position=4,
                              Individual.position=3)
DESeq2preprocess <- S4Vectors::metadata(resDATAprepSEleuk)$DESeq2obj</pre>
DESeq2obj <- DESeq2preprocess$DESeq2preproceesing</pre>
dds.DE <- DESeq2::DESeq(DESeq2obj)</pre>
##
res.G.T.2 <- DEresultGroupPerTime(DESeq.result=dds.DE,</pre>
                                LRT.supp.info=FALSE,
                                log.FC.min=1,
                                pval.min=0.05)
```

GSEApreprocessing

### Description

The function returns, from the output of DEanalysisGlobal(), specific files designed to be used as input for several online online tools and software given in the section Value.

### Usage

```
GSEApreprocessing(
  SEresDE,
  ColumnsCriteria,
  Set.Operation,
  Rnk.files = TRUE,
  Save.files = FALSE
)
```

# Arguments

SEresDE	A SummarizedExperiment class object. Output from DEanalysisGlobal() (see Examples).
ColumnsCriteria	3
	A vector of integers where each integer indicates a column of SummarizedExperiment::rowData(SErest These columns should either contain only binary values, or may contain other numerical value, in which case extracted outputs from SEresDE will be those with >0 values (see Details).
Set.Operation	A character. The user must choose between "union" (default), "intersect", "set- diff" (see Details).
Rnk.files	TRUE or FALSE. TRUE as default. If TRUE, the rnk files generated by the function (used by the GSEA software) will be saved if Save.files=TRUE and path.result of DEanalysisGlobal() is not NULL. Otherwise the rnk files will not be generated.
Save.files	TRUE or FALSE or a Character. If Save.files=TRUE and the path.result of DEanalysisGlobal() is not NULL, all files will be saved in "2_Supervised- Analysis_Name.folder.DE/ 2-5_Enrichment_analysis_Name.folder.DE/ 2-5- 2_EnrichmentGO_software_preprocessing". If Save.files is a character, it must be a path and all files will be saved in the sub-folder "EnrichmentGO_software_preprocessing". Otherwise, the different files will not be saved.

# Details

We have the following three cases:

• If Set.Operation="union" then the rows extracted from the different datasets (raw counts, normalized data and SummarizedExperiment::rowData(SEresDE)) included in the SummarizedExperiment class object SEresDE are those such that the sum of the selected columns of

SummarizedExperiment::rowData(SEresDE) given in ColumnsCriteria is >0. This means that the selected genes are those having at least one '1' in one of the selected columns.

- If Set.Operation="intersect" then the rows extracted from the different datasets (raw counts, normalized data and SummarizedExperiment::rowData(SEresDE)) included in the SummarizedExperiment class object SEresDE are those such that the product of the selected columns of SummarizedExperiment::rowData(SEresDE) given in ColumnsCriteria is >0. This means that the selected genes are those having '1' in all of the selected columns.
- If Set.Operation="setdiff" then the rows extracted from the different datasets (raw counts, normalized data and SummarizedExperiment::rowData(SEresDE)) included in the SummarizedExperiment class object SEresDE are those such that only one element of the selected columns of SummarizedExperiment::rowData(SEresDE) given in ColumnsCriteria is >0. This means that the selected genes are those having '1' in only one of the selected columns.

#### Value

The function returns

- A vector of character containing gene names specified by ColumnsCriteria and Set.Operation.
- · A vector of character containing all gene names
- And, in case where Save.files=TRUE and the path.result of DEanalysisGlobal() is not NULL, specific files designed to be used as input for the following online tools and software :
  - GSEA: https://www.gsea-msigdb.org/gsea/index.jsp
  - DAVID: https://david.ncifcrf.gov/tools.jsp
  - WebGestalt: http://www.webgestalt.org
  - gProfiler:https://biit.cs.ut.ee/gprofiler/gost
  - Panther: http://www.pantherdb.org
  - ShinyGO: http://bioinformatics.sdstate.edu/go/
  - Enrichr: https://maayanlab.cloud/Enrichr/
  - GOrilla: http://cbl-gorilla.cs.technion.ac.il.

```
data(RawCounts_Antoszewski2022_MOUSEsub500)
## No time points. We take only two groups for the speed of the example
RawCounts_T1Wt <- RawCounts_Antoszewski2022_MOUSEsub500[, seq_len(7)]</pre>
## Preprocessing
resDATAprepSE <- DATAprepSE(RawCounts=RawCounts_T1Wt,</pre>
                     Column.gene=1,
                     Group.position=1,
                     Time.position=NULL,
                     Individual.position=2)
## DE analysis
resDET1wt <- DEanalysisGlobal(SEres=resDATAprepSE,</pre>
                       pval.min=0.05,
                       pval.vect.t=NULL,
                       log.FC.min=1,
```

```
LRT.supp.info=FALSE,

Plot.DE.graph=TRUE,

path.result=NULL,

Name.folder.DE=NULL)

##-------##

resGp <- GSEApreprocessing(SEresDE=resDET1wt,

ColumnsCriteria=2,

Set.Operation="union",

Rnk.files=TRUE,

Save.files=FALSE)
```

# GSEAQuickAnalysis GSEA analysis with gprofiler2

# Description

The function realizes, from the outputs of DEanalysisGlobal(), an enrichment analysis (GSEA) of a subset of genes with the R package gprofiler2.

### Usage

```
GSEAQuickAnalysis(
    Internet.Connection = FALSE,
    SEresDE,
    ColumnsCriteria = 1,
    ColumnsLog2ordered = NULL,
    Set.Operation = "union",
    Organism = "hsapiens",
    MaxNumberGO = 20,
    Background = FALSE,
    Display.plots = TRUE,
    Save.plots = FALSE
)
```

### Arguments

Internet.Connection

 TRUE or FALSE. FALSE by default. If the user is sure to have an internet connection, the user must set Internet.Connection=TRUE, otherwise, the algorithm will not run.

 SEresDE
 A SummarizedExperiment class object. Output from DEanalysisGlobal() (see Examples).

ColumnsCriteria

A vector of integers where each integer indicates a column of SummarizedExperiment::rowData(SErest These columns should either contain only binary values, or may contain other numerical value, in which case extracted outputs from SEresDE will be those with >0 values (see Details). ColumnsLog2ordered

NULL or a vector of integers. If ColumnsLog2ordered is a vector of integers, it corresponds to the columns number of Res.DE.analysis\$DE.results, the output of DEanalysisGlobal(), which must contains  $log_2$  fold change values (see Details).

- Set.Operation A character. The user must choose between "union" (default), "intersect", "setdiff" (see Details).
- Organism A character indicating the organism where data were taken from. See vignette of the R package gprofiler2 for supported organisms. See gprofiler2::gost().
- MaxNumberGO An integer. The user can select the MaxNumberGO most important Gene Ontology (GO) names to be plotted in a lollipop graph. By default, MaxNumberGO=20.
- BackgroundTRUE or FALSE. If TRUE, the statistical enrichment analysis to find over-representation<br/>of functions from Gene Ontology (GO) and biological pathways (e.g. KEGG)<br/>will be done by comparing the functions and biological pathways among the se-<br/>lected DE genes with those associated with all genes in Res.DE.analysis\$DE.results.<br/>If FALSE, the statistical enrichment analysis will be done by comparing the func-<br/>tions and biological pathways among the selected DE genes with all functions<br/>and biological pathways included in the database of gprofiler2 (link in See<br/>Also). See also gprofiler2::gost().
- Display.plots TRUE or FALSE. TRUE as default. If TRUE, all graphs will be plotted. Otherwise no graph will be plotted.
- Save.plots TRUE or FALSE or a Character. If Save.plots=TRUE and the output path.result of DEanalysisGlobal() is not NULL, all files will be saved in "2\_Supervised-Analysis\_Name.folder.DE/ 2-5\_Enrichment\_analysis\_Name.folder.DE/ 2-5-1\_gprofiler2\_results\_Name.folder.DE", with Name.folder.DE an input of DEanalysisGlobal(). If Save.plots is a character, it must be a path and all files will be saved in the sub-folder "gprofiler2\_results\_Name.folder.DE". Otherwise, the different files will not be saved.

### Details

If ColumnsLog2ordered is a vector of integers, the rows of Res.DE.analysis\$DE.results (corresponding to genes) will be decreasingly ordered according to the sum of absolute  $log_2$  fold change (the selected columns must contain  $log_2$  fold change values) before the enrichment analysis. The enrichment analysis will take into account the genes order as the first genes will be considered to have the highest biological importance and the last genes the lowest. See the input ordered\_query of gprofiler2::gost() and the vignette of gprofiler2 for more details.

We have the following three cases:

- If Set.Operation="union" then the rows extracted from the different datasets (raw counts, normalized data and SummarizedExperiment::rowData(SEresDE)) included in the SummarizedExperiment class object SEresDE are those such that the sum of the selected columns of SummarizedExperiment::rowData(SEresDE) given in ColumnsCriteria is >0. This means that the selected genes are those having at least one '1' in one of the selected columns.
- If Set.Operation="intersect" then the rows extracted from the different datasets (raw counts, normalized data and SummarizedExperiment::rowData(SEresDE)) included in the SummarizedExperiment class object SEresDE are those such that the product of the selected

columns of SummarizedExperiment::rowData(SEresDE) given in ColumnsCriteria is >0. This means that the selected genes are those having '1' in all of the selected columns.

• If Set.Operation="setdiff" then the rows extracted from the different datasets (raw counts, normalized data and SummarizedExperiment::rowData(SEresDE)) included in the SummarizedExperiment class object SEresDE are those such that only one element of the selected columns of SummarizedExperiment::rowData(SEresDE) given in ColumnsCriteria is >0. This means that the selected genes are those having '1' in only one of the selected columns.

### Value

The function returns the same SummarizedExperiment class object SEresDE with

- a data.frame which contains the outputs of gprofiler2::gost()
- a Manhattan plot showing all GO names according to their pvalue
- a lollipop graph showing the MaxNumberGO most important GO.

saved in the metadata Results[[2]][[5]] of SEresDE.

The Manhattan plot and the lollipop graph are plotted if Display.plots=TRUE.

### See Also

The function uses the R package gprofiler2 https://cran.r-project.org/web/packages/ gprofiler2/vignettes/gprofiler2.html.

The R package gprofiler2 provides an R interface to the web toolset g:Profiler https://biit.cs.ut.ee/gprofiler/gost.

```
## data importation
data(RawCounts_Antoszewski2022_MOUSEsub500)
## No time points. We take only two groups for the speed of the example
dataT1wt <- RawCounts_Antoszewski2022_MOUSEsub500[seg_len(200), seg_len(7)]</pre>
## Preprocessing with Results of DEanalysisGlobal()
resDATAprepSE <- DATAprepSE(RawCounts=dataT1wt,</pre>
                          Column.gene=1,
                          Group.position=1,
                          Time.position=NULL,
                          Individual.position=2)
## Internet is needed in order to run the following lines of code because
## gprofileR2 needs an internet connection
## DE analysis
# resDET1wt <- DEanalysisGlobal(SEres=resDATAprepSE,</pre>
#
                             pval.min=0.05,
                             pval.vect.t=NULL,
#
#
                             log.FC.min=1,
#
                             LRT.supp.info=FALSE,
#
                             Plot.DE.graph=FALSE,
```

# **HCPC**analysis

#	path.result=NULL,
#	Name.folder.DE=NULL)
#########	
<pre># resGs &lt;- GSEAQuickAnalysis</pre>	(Internet.Connection=TRUE,
#	SEresDE=resDET1wt,
#	ColumnsCriteria=3,
#	ColumnsLog2ordered=NULL,
#	<pre>Set.Operation="union",</pre>
#	Organism="mmusculus",
#	MaxNumberGO=20,
#	Background=FALSE,
#	Display.plots=TRUE,
#	Save.plots=FALSE)

HCPCanalysis

Hierarchical clustering analysis with HCPC (Main function)

# Description

The functions performs a hierarchical clustering on results from a factor analysis with the R function FactoMineR::HCPC().

# Usage

```
HCPCanalysis(
  SEresNorm,
  DATAnorm = TRUE,
  gene.deletion = NULL,
  sample.deletion = NULL,
  Plot.HCPC = FALSE,
  Color.Group = NULL,
  Phi = 25,
  Theta = 140,
  epsilon = 0.2,
  Cex.point = 0.7,
  Cex.label = 0.7,
  motion3D = FALSE,
  path.result = NULL,
  Name.folder.hcpc = NULL
)
```

# Arguments

SEresNorm	Results of the function DATAnormalization().
DATAnorm	TRUE or FALSE. TRUE as default. TRUE means the function uses the normalized
	data. FALSE means the function uses the raw counts data.

gene.deletion	NULL or a vector of characters or a vector of integers. NULL as default. If
	gene.deletion is a vector of characters, all genes with names in gene.deletion
	will be deleted from the data set as input RawCounts of our function DATAprepSE().
	If gene.deletion is a vector of integers, all the corresponding row numbers will
	be deleted from the data set as input RawCounts of our function DATAprepSE().
	If gene.deletion=NULL all genes will be used in the construction of the PCA.
sample.deletio	n
	NULL or a vector of characters or a vector of integers. NULL as default. If
	sample.deletion is a vector of characters, all samples with names in sample.deletion
	will not be used in the construction of the PCA. If sample.deletion is a vec-
	tor of integers, all the corresponding column numbers will not be used in the
	construction of the PCA from the data set as input RawCounts of our function
	DATAprepSE(). If sample.deletion=NULL all samples will be used in the con-
	struction of the PCA.
Plot.HCPC	TRUE or FALSE. FALSE as default. If TRUE, all graphs will be plotted. Otherwise
	no graph will be plotted.
Color Group	NULL or a data frame with $N_1$ rows and two columns where $N_2$ is the number
	of biological conditions. If Color, Group is a data frame, the first column must
	contain the name of each biological condition and the second column must con-
	tain the colors associated to each biological condition. If Color Group=NUL
	the function will automatically attribute a color for each biological condition. If
	samples belong to different time points only. Color, Group will not be used.
Phi	Angle defining the collatitude direction for the 3D PCA plot (see Details in
1 111	graphics::persp())
Thata	Angle defining the azimuthal direction for the 2D DCA plot (see Details in
meta	angle defining the azimuthar direction for the 5D FCA plot (see Details in
	graphicspersp()).
epsilon	Non negative numeric value giving the length between points and their labels in
	all PCA plots which are not automatically plotted by Factominer::PCA().
Cex.point	Non negative numeric value giving the size of points in all PCA plots which are
	not automatically plotted by FactoMineR::PCA().
Cex.label	Non negative numeric value giving the size of the labels associated to each point
	of the all PCA graphs which are not automatically plotted by FactoMineR::PCA().
motion3D	TRUE or FALSE. If TRUE, the 3D PCA plots will also be plotted in a rgl window
	(see plot3Drgl::plotrgl()) allowing to interactively rotate and zoom.
path.result	Character or NULL. Path to save all results. If path.result contains a sub folder
	entitled "1_UnsupervisedAnalysis_Name.folder.hcpc" and a sub sub folder,
	"1-3_HCPCanalysis_Name.folder.hcpc" all results will be saved in the sub
	folder "1_UnsupervisedAnalysis_Name.folder.hcpc/1-3_HCPCanalysis_Name.folder.hcpc".
	Otherwise, a sub folder entitled "1_UnsupervisedAnalysis_Name.folder.hcpc"
	and/or a sub sub folder "1-3_HCPCanalysis_Name.folder.hcpc" will be cre-
	ated in path.result and all results will be saved in "1_UnsupervisedAnaly-
	sis_Name.folder.hcpc/1-3_HCPCanalysis_Name.folder.hcpc". If NULL, the
	results will not be saved in a folder. NULL as default.
Name.folder.hc	pc
	Character or NULL. If Name.folder.hcpc is a character, the folder and sub folder
	names which will contain the PCA graphs will respectively be "1_Unsupervised-
	Analysis_Name.folder.hcpc" and "1-3_HCPCanalysis_Name.folder.hcpc".

#### **HCPC**analysis

Otherwise, the folder and sub folder names will respectively be "1\_Unsuper-visedAnalysis" and "1-3\_HCPCanalysis".

### Details

All results are built from the results of our function DATAnormalization().

The number of clusters is automatically selected by FactoMineR::HCPC() and is described in the section Details of FactoMineR::HCPC().

### Value

The function returns the same SummarizedExperiment class object SEresNorm with the outputs from the function FactoMineR::HCPC(), (saved in the metadata Results[[1]][[3]] of SEresNorm)

- a dendrogram (also called hierarchical tree) using the function factoextra::fviz\_dend()
- one 2D PCA and two 3D PCA produced by the function PCAgraphics() where samples are colored with different colors for different clusters. The two 3D PCA graphs are identical but one of them will be opened in a rgl window (see plot3Drgl::plotrgl()) allowing to interactively rotate and zoom. The interactive 3D graph will be plotted only if motion3D=TRUE.
- A graph indicating for each sample, its cluster and the time and/or biological condition associated to the sample.
- the outputs of FactoMineR::HCPC().

#### See Also

The function calls the functions PCArealization() and FactoMineR::HCPC(). The function FactoMineR::HCPC() will take as input the output of PCArealization().

```
## Simulation raw counts
resSIMcount <- RawCountsSimulation(Nb.Group=2, Nb.Time=3, Nb.per.GT=4,
                                Nb.Gene=10)
## Preprocessing step
resDATAprepSE <- DATAprepSE(RawCounts=resSIMcount$Sim.dat,</pre>
                          Column.gene=1,
                          Group.position=1,
                          Time.position=2,
                          Individual.position=3)
## Normalization
resNorm <- DATAnormalization(SEres=resDATAprepSE,</pre>
                          Normalization="rle",
                          Plot.Boxplot=FALSE,
                          Colored.By.Factors=FALSE)
##-----##
resHCPCanalysis <- HCPCanalysis(SEresNorm=resNorm,</pre>
                             DATAnorm=TRUE,
                             sample.deletion=NULL,
                             gene.deletion=NULL,
                             Plot.HCPC=TRUE,
```

```
Color.Group=NULL,
Phi=25, Theta=140,
Cex.point=1, Cex.label=0.6, epsilon=0.4,
motion3D=FALSE,
path.result=NULL,
Name.folder.hcpc=NULL)
```

MFUZZanalysis

Clustering of temporal patterns (Main function).

# Description

The function performs a soft clustering of temporal patterns based on the fuzzy c-means algorithm using the R package Mfuzz.

### Usage

```
MFUZZanalysis(
   SEresNorm,
   DATAnorm = TRUE,
   DataNumberCluster = NULL,
   Method = "hcpc",
   Max.clust = 6,
   Membership = 0.5,
   Min.std = 0.1,
   Plot.Mfuzz = TRUE,
   path.result = NULL,
   Name.folder.mfuzz = NULL
)
```

# Arguments

SEresNorm	Results of the function DATAnormalization().
DATAnorm	TRUE or FALSE. TRUE as default. TRUE means the function uses the normalized data. FALSE means the function uses the raw counts data.
DataNumberClus	ter
	Data.frame or NULL. NULL as default. If DataNumberCluster is a data.frame where the first column contains the name of the biological conditions and the second the number of cluster selected for each biological condition. If DataNumberCluster=NULL, a number of clusters will be automatically computed for each biological condi- tion (see MFUZZclustersNumber()).
Method	"kmeans" or "hcpc". The method used for selecting the number of cluster to be used for the temporal cluster analysis (see Details). Only used if DataNumberCluster is not NULL.
Max.clust	Integer strictly superior to 1 indicating the maximum number of clusters. Max.clust will be used only if DataNumberCluster=NULL

Membership	Numeric value between 0 and 1. For each cluster, genes with membership values below the threshold Membership will not be displayed. The membership values correspond to the probability of gene to belong to each cluster.
Min.std	Numeric positive value. All genes where their standard deviations are smaller than the threshold Min.std will be excluded.
Plot.Mfuzz	TRUE or FALSE. TRUE as default. If TRUE, all graphs will be plotted. Otherwise no graph will be plotted.
path.result	Character or NULL. Path to save all results. If path.result contains a sub folder entitled "1_UnsupervisedAnalysis_Name.folder.mfuzz" and a sub sub folder, "1-4_MFUZZanalysis_Name.folder.mfuzz" all results will be saved in the sub folder "1_UnsupervisedAnalysis_Name.folder.mfuzz/1-4_MFUZZanalysis_Name.folder.mfuzz. Otherwise, a sub folder entitled "1_UnsupervisedAnalysis_Name.folder.mfuzz" and/or a sub sub folder "1-4_MFUZZanalysis_Name.folder.mfuzz" will be created in path.result and all results will be saved in "1_UnsupervisedAnaly- sis_Name.folder.mfuzz/1-4_MFUZZanalysis_Name.folder.mfuzz". If NULL, the results will not be saved in a folder. NULL as default.
Name.folder.mfu	ZZ
	Character or NULL. If Name.folder.mfuzz is a character, the folder and sub folder names which will contain the PCA graphs will respectively be "1_Unsu- pervisedAnalysis_Name.folder.mfuzz" and "1-4_MFUZZanalysis_Name.folder.mfuzz". Otherwise, the folder and sub folder names will respectively be "1_Unsuper- visedAnalysis" and "1-4_MFUZZanalysis".

### Details

All results are built from the results of our function DATAnormalization().

The Mfuzz package works with datasets where rows correspond to genes and columns correspond to times. If RawCounts (input of our function DATAprepSE()) contains several replicates per time, the algorithm computes the mean of replicates for each gene before using Mfuzz::mfuzz(). When there are several biological conditions, the algorithm realizes the Mfuzz::mfuzz() analysis for each biological condition.

# Value

The function returns the same SummarizedExperiment class object SEresNorm with the different elements below (saved in the metadata Results[[1]][[4]] of SEresNorm)

- the final data used for the Mfuzz analysis (see Details).
- the cluster associated to each gene.
- plots generated by MFUZZclustersNumber() and Mfuzz::mfuzz.plot2() for each biological condition.

### See Also

The function uses the function MFUZZclustersNumber() to compute the optimal number of cluster for each biological condition with the kmeans method.

### Examples

```
## Data simulation
 set.seed(33)
 DATAclustSIM <- matrix(rnorm(12*10*3, sd=0.2,</pre>
                            mean=rep(c(rep(c(1, 6, 9, 4, 3, 1,
                                           6.5, 0.7, 10), times=2),
                                      rep(c(2, 3.6, 3.7, 5, 7.9, 8,
                                           7.5, 3.5, 3.4), times=2)),
                                    each=10)),
                      nrow=30, ncol=12)
 DATAclustSIM <- floor(DATAclustSIM*100)</pre>
 ##
 colnames(DATAclustSIM) <- c("G1_t0_r1", "G1_t1_r1", "G1_t2_r1",</pre>
                           "G1_t0_r2", "G1_t1_r2", "G1_t2_r2",
                           "G2_t0_r3", "G2_t1_r3", "G2_t2_r3",
                           "G2_t0_r4", "G2_t1_r4", "G2_t2_r4")
 ##-----
                                                             ----##
 ## Plot the temporal expression of each individual
 graphics::matplot(t(rbind(DATAclustSIM[, 1:3], DATAclustSIM[, 4:6],
                         DATAclustSIM[, 7:9], DATAclustSIM[, 10:12])),
                  col=rep(c("black", "red"), each=6*10),
                  xlab="Time", ylab="Gene expression", type=c("b"), pch=19)
 ## Preprocessing step
 DATAclustSIM <- data.frame(DATAclustSIM)</pre>
 resDATAprepSE <- DATAprepSE(RawCounts=DATAclustSIM,</pre>
                           Column.gene=NULL,
                           Group.position=1,
                           Time.position=2,
                           Individual.position=3)
 ## Normalization
 resNorm <- DATAnormalization(SEres=resDATAprepSE,</pre>
                           Normalization="rle",
                           Plot.Boxplot=FALSE,
                           Colored.By.Factors=FALSE)
 resMFUZZ <- MFUZZanalysis(SEresNorm=resNorm,</pre>
                         DATAnorm=TRUE,
                         DataNumberCluster=NULL,
                         Membership=0.5,
                         Min.std=0.1,
                         Plot.Mfuzz=TRUE,
                         path.result=NULL)
MFUZZclustersNumber
                       Automatic choice of the number of clusters to use for the Mfuzz anal-
```

ysis

### Description

The function uses stats::kmeans() or FactoMineR::HCPC() in order to compute the number of cluster for the Mfuzz::mfuzz() analysis.

## Usage

```
MFUZZclustersNumber(
   SEresNorm,
   DATAnorm = TRUE,
   Method = "hcpc",
   Max.clust = 3,
   Min.std = 0.1,
   Plot.Cluster = TRUE,
   path.result = NULL
)
```

### Arguments

SEresNorm	Results of the function DATAnormalization().
DATAnorm	TRUE or FALSE. TRUE as default. TRUE means the function uses the normalized data. FALSE means the function uses the raw counts data.
Method	"kmeans" or "hcpc". The method used for selecting the number of cluster to be used for the temporal cluster analysis (see Details). Method="kmeans" is advised for large number of genes.
Max.clust	Integer strictly superior to 1 indicating the maximum number of clusters. The default is Max.clust=10.
Min.std	Numeric positive value. All genes where their standard deviations are smaller than the threshold Min.std will be excluded.
Plot.Cluster	TRUE or FALSE. TRUE as default. If TRUE, the output graph will be plotted. Otherwise the graph will be plotted.
path.result	Character or NULL. Path to save the plot described in the section Value. If NULL, the graph will not be saved in a folder. NULL as default.

#### Details

All results are built from the results of our function DATAnormalization().

The Mfuzz package works with datasets where rows correspond to genes and columns correspond to times. If RawCounts (input of our function DATAprepSE()) contains several replicates per time, the algorithm computes the mean of replicates for each gene before using Mfuzz::mfuzz(). When there are several biological conditions, the algorithm realizes the Mfuzz::mfuzz() analysis for each biological condition.

The kmeans method or the hierarchical clustering method, respectively included in stats::kmeans() and FactoMineR::HCPC(), is used in order to compute the optimal number of clusters. If there are several biological conditions, the algorithm computes one optimal number of clusters per biological condition.

#### Value

The function returns the same SummarizedExperiment class object SEresNorm with the different elements below, saved in the metadata Results[[1]][[4]] of SEresNorm,

- the optimal number of clusters for each biological condition (between 2 and Max.clust).
- a data.frame with  $(N_{bc} + 1)$  columns and Max.clust rows with  $N_{bc}$  the number of biological conditions.
  - If Method="kmeans", the ith rows and the jth column correspond to the within-cluster intertia (see tot.withinss from stats::kmeans()) dividing by the sum of the variance of each row of ExprData of the (j-1)th biological condition computed by stats::kmeans() with i clusters. When there is only one cluster, the within-cluster intertia corresponds to the sum of the variance of each row of ExprData (see Details). The first column contains integers between 1 and Max.clust which corresponds to the number of clusters selected for the stats::kmeans() analysis.
  - If Method="hcpc", the jth column correspond to the clustering heights (see the output height from FactoMineR::HCPC()) dividing by the maximum value of height. The first column contains integers between 1 and Max.clust which corresponds to the number of clusters selected for the stats::kmeans() analysis.
- a plot which gives
  - If Method="kmeans", the evolution of the weighted within-cluster intertia per number of clusters (from 1 to Max.clust) for each biological condition. The optimal number of cluster for each biological condition will be colored in blue.
  - If Method="hcpc", the evolution of the scaled height per number of clusters (from 1 to Max.clust) for each biological condition. The optimal number of cluster for each biological condition will be colored in blue.

## See Also

The function is called by MFUZZanalysis().

### Examples

```
## Data simulation
set.seed(33)
DATAclustSIM <- matrix(rnorm(12*10*3, sd=0.2,</pre>
                              mean=rep(c(rep(c(1, 6, 9, 4, 3, 1,
                                                 6.5, 0.7, 10), times=2),
                                          rep(c(2, 3.6, 3.7, 5, 7.9, 8,
                                                 7.5, 3.5, 3.4), times=2)),
                                        each=10)),
                        nrow=30, ncol=12)
DATAclustSIM <- floor(DATAclustSIM*100)</pre>
##
colnames(DATAclustSIM) <- c("G1_t0_r1", "G1_t1_r1", "G1_t2_r1",</pre>
                              "G1_t0_r2", "G1_t1_r2", "G1_t2_r2"
                              "G2_t0_r3", "G2_t1_r3", "G2_t2_r3",
                              "G2_t0_r4", "G2_t1_r4", "G2_t2_r4")
                                                                     ----##
```

## Plot the temporal expression of each individual

# **PCA**analysis

```
graphics::matplot(t(rbind(DATAclustSIM[, 1:3], DATAclustSIM[, 4:6],
                      DATAclustSIM[, 7:9], DATAclustSIM[, 10:12])),
               col=rep(c("black", "red"), each=6*10),
               xlab="Time", ylab="Gene expression", type=c("b"), pch=19)
##------##
## Preprocessing step
DATAclustSIM <- data.frame(DATAclustSIM)</pre>
resDATAprepSE <- DATAprepSE(RawCounts=DATAclustSIM,</pre>
                        Column.gene=NULL,
                        Group.position=1,
                        Time.position=2,
                        Individual.position=3)
## Normalization
resNorm <- DATAnormalization(SEres=resDATAprepSE,</pre>
                         Normalization="rle",
                         Plot.Boxplot=FALSE,
                         Colored.By.Factors=FALSE)
##------##
resMFUZZcluster <- MFUZZclustersNumber(SEresNorm=resNorm,</pre>
                                  DATAnorm=FALSE,
                                  Method="hcpc",
                                  Max.clust=5,
                                  Plot.Cluster=TRUE,
                                  path.result=NULL)
```

PCAanalysis

Automatic PCA analysis (Main function)

### Description

The functions performs an automatic principal component analysis (PCA) from a gene expression dataset where samples can belong to different biological conditions and/or time points.

### Usage

```
PCAanalysis(
  SEresNorm,
  DATAnorm = TRUE,
  gene.deletion = NULL,
  sample.deletion = NULL,
  Plot.PCA = TRUE,
  Mean.Accross.Time = FALSE,
  Color.Group = NULL,
  Phi = 25,
  Theta = 140,
  epsilon = 0.2,
```

```
Cex.point = 0.7,
Cex.label = 0.7,
motion3D = FALSE,
path.result = NULL,
Name.folder.pca = NULL
)
```

# Arguments

SEresNorm	Results of the function DATAnormalization().
DATAnorm	TRUE or FALSE. TRUE as default. TRUE means the function uses the normalized data. FALSE means the function uses the raw counts data.
gene.deletion	NULL or a vector of characters or a vector of integers. NULL as default. If gene.deletion is a vector of characters, all genes with names in gene.deletion will be deleted from the data set as input RawCounts of our function DATAprepSE(). If gene.deletion is a vector of integers, all the corresponding row numbers will be deleted from the data set as input RawCounts of our function DATAprepSE(). If gene.deletion=NULL all genes will be used in the construction of the PCA.
<pre>sample.deletion</pre>	1
	NULL or a vector of characters or a vector of integers. NULL as default. If sample.deletion is a vector of characters, all samples with names in sample.deletion will not be used in the construction of the PCA. If sample.deletion is a vector of integers, all the corresponding column numbers will not be used in the construction of the PCA from the data set as input RawCounts of our function DATAprepSE(). If sample.deletion=NULL all samples will be used in the construction of the PCA.
Plot.PCA	TRUE or FALSE. TRUE as default. If TRUE, PCA graphs will be plotted. Otherwise no graph will be plotted.
Mean.Accross.Ti	me
	TRUE or FALSE. FALSE as default. If FALSE and if Time.position (input of DATAprepSE()) is not set as NULL, consecutive time points within a sample are linked to help visualization of temporal patterns. If TRUE and if Time.position is not set as NULL, the mean per time of all genes is computed for each biological condition and the means of consecutive time points within biological condition are linked to help visualization of temporal patterns.
Color.Group	NULL or a data.frame with $N_{bc}$ rows and two columns where $N_{bc}$ is the number of biological conditions. If Color . Group (input of DATAprepSE()) is a data.frame, the first column must contain the name of each biological condition and the second column must contain the colors associated to each biological condition. If Color . Group=NULL, the function will automatically attribute a color for each biological condition. If samples belong to different time points only, Color . Group will not be used.
Phi	Angle defining the colatitude direction for the 3D PCA plot (see Details in graphics::persp()).
Theta	Angle defining the azimuthal direction for the 3D PCA plot (see Details in graphics::persp()).

64

epsi

Cex.

Cex.

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path

lon	Non negative numeric value giving the length between points and their labels in all PCA plots which are not automatically plotted by FactoMineR::PCA().
point	Non negative numeric value giving the size of points in all PCA plots which are not automatically plotted by FactoMineR::PCA().
label	Non negative numeric value giving the size of the labels associated to each point of the all PCA graphs which are not automatically plotted by FactoMineR::PCA().
on3D	TRUE or FALSE. If TRUE, the 3D PCA plots will also be plotted in a rgl window (see plot3Drgl::plotrgl()) allowing to interactively rotate and zoom.
.result	Character or NULL. Path to save all results. If path.result contains a sub folder entitled "1_UnsupervisedAnalysis_Name.folder.pca" and a sub sub folder, "1- 2_PCAanalysis_Name.folder.pca" all results will be saved in the sub folder "1_UnsupervisedAnalysis_Name.folder.pca/1-2_PCAanalysis_Name.folder.pca". Otherwise, a sub folder entitled "1_UnsupervisedAnalysis_Name.folder.pca" and/or a sub sub folder "1-2_PCAanalysis_Name.folder.pca" will be created in path.result and all results will be saved in "1_UnsupervisedAnalysis_Name.folder.pca/ 1-2_PCAanalysis_Name.folder.pca". If NULL, the results will not be saved in a folder.NULL as default.

Name.folder.pca

Character or NULL. If Name.folder.pca is a character, the folder and sub folder names which will contain the PCA graphs will respectively be "1\_Unsupervised-Analysis\_Name.folder.pca" and "1-2\_PCAanalysis\_Name.folder.pca". Otherwise, the folder and sub folder names will respectively be "1\_Unsupervised-Analysis" and "1-2\_PCAanalysis".

#### Details

All results are built from the results of our function DATAnormalization().

#### Value

The function returns the same SummarizedExperiment class object SEresNorm with the outputs from the function FactoMineR::PCA(), and several 2D and 3D PCA graphs depending on the experimental design (if Plot.PCA=TRUE), saved in the metadata Results[[1]][[2]] of SEresNorm,

- When samples belong only to different biological conditions, the function returns a 2D and two 3D PCA graphs. In each graph, samples are colored with different colors for different biological conditions. The two 3D PCA graphs are identical but one of them will be opened in a rgl window (see plot3Drgl::plotrgl()) and it allows to interactively rotate and zoom.
- When samples belong only to different time points, the function returns
  - One 2D PCA graph, one 3D PCA graph and the same 3D PCA graph in a rgl window where samples are colored with different colors for different time points. Furthermore, lines are drawn between each pair of consecutive points for each sample (if Mean.Accross.Time=FALSE, otherwise it will be only between means).
  - The same graphs describe above but without lines.
- When samples belong to different time points and different biological conditions, the function returns

- One 2D PCA graph, one 3D PCA graph and the same 3D PCA graph in a rgl window where samples are colored with different colors for different time points. Furthermore, lines are drawn between each pair of consecutive points for each sample (if Mean.Accross.Time=FALSE, otherwise it will be only between means).
- The same graphs describe above but without lines.
- The same six following graphs for each biological condition (one PCA analysis per biological condition). One 2D PCA graph, one 3D PCA graph and the same 3D PCA graph in a rgl window where samples belong to only one biological condition and are colored with different colors for different time points. Furthermore, lines are drawn between each pair of consecutive points for each sample (if Mean.Accross.Time=FALSE, otherwise it will be only between means). The three others graphs are identical to the three previous ones but without lines.

The interactive 3D graphs will be plotted only if motion3D=TRUE.

# See Also

The function calls the R functions PCAgraphics() and ColnamesToFactors().

```
## Simulation raw counts
resSIMcount <- RawCountsSimulation(Nb.Group=2, Nb.Time=3, Nb.per.GT=4,</pre>
                                 Nb.Gene=10)
## Preprocessing step
resDATAprepSE <- DATAprepSE(RawCounts=resSIMcount$Sim.dat,</pre>
                           Column.gene=1,
                           Group.position=1,
                           Time.position=2,
                           Individual.position=3)
## Normalization
resNorm <- DATAnormalization(SEres=resDATAprepSE,</pre>
                           Normalization="rle",
                           Plot.Boxplot=FALSE,
                           Colored.By.Factors=FALSE)
## Color for each group
GROUPcolor <- data.frame(Name=c("G1", "G2"), Col=c("black", "red"))</pre>
----##
resPCAanalysis <- PCAanalysis(SEresNorm=resNorm,</pre>
                            DATAnorm=TRUE,
                             gene.deletion=c("Gene1", "Gene5"),
                             sample.deletion=c(2, 6),
                            Plot.PCA=TRUE,
                            Mean.Accross.Time=FALSE,
                             Color.Group=GROUPcolor,
                             motion3D=FALSE,
                            Phi=25, Theta=140,
                             Cex.label=0.7, Cex.point=0.7, epsilon=0.2,
                             path.result=NULL, Name.folder.pca=NULL)
```

### Description

The function plots 2D and 3D PCA using the function PCArealization() which realizes a PCA analysis. This function is called repeatedly by the function PCAanalysis() if samples belong to different biological conditions and time points.

## Usage

```
PCAgraphics(
  SEresNorm,
  DATAnorm = TRUE,
  gene.deletion = NULL,
  sample.deletion = NULL,
  Plot.PCA = TRUE,
 Mean.Accross.Time = FALSE,
  Color.Group = NULL,
  motion3D = FALSE,
 Phi = 25,
  Theta = 140,
  epsilon = 0.2,
  Cex.point = 0.7,
  Cex.label = 0.7,
  path.result = NULL,
 Name.file.pca = NULL
)
```

# Arguments

SEresNorm	Results of the function DATAnormalization().	
DATAnorm	TRUE or FALSE. TRUE as default. TRUE means the function uses the normalized data. FALSE means the function uses the raw counts data.	
gene.deletion	NULL or a vector of characters or a vector of integers. NULL as default. If gene.deletion is a vector of characters, all genes with names in gene.deletion will be deleted from the data set as input RawCounts of our function DATAprepSE(). If gene.deletion is a vector of integers, all the corresponding row numbers will be deleted from the data set as input RawCounts of our function DATAprepSE(). If gene.deletion=NULL all genes will be used in the construction of the PCA.	
sample.deletion		
	NULL or a vector of characters or a vector of integers. NULL as default. If sample.deletion is a vector of characters, all samples with names in sample.deletion will not be used in the construction of the PCA. If sample.deletion is a vector of integers, all the corresponding column numbers will not be used in the construction of the PCA from the data set as input RawCounts of our function	

	DATAprepSE(). If sample.deletion=NULL all samples will be used in the con- struction of the PCA.
Plot.PCA	TRUE or FALSE. TRUE as default. If TRUE, PCA graphs will be plotted. Otherwise no graph will be plotted.
Mean.Accross.Ti	me
	TRUE or FALSE. FALSE as default. If FALSE and if Time.position (input of DATAprepSE()) is not set as NULL, consecutive time points within a sample are linked to help visualization of temporal patterns. If TRUE and if Time.position is not set as NULL, the mean per time of all genes is computed for each biological condition and the means of consecutive time points within biological condition are linked to help visualization of temporal patterns.
Color.Group	NULL or a data.frame with $N_{bc}$ rows and two columns where $N_{bc}$ is the number of biological conditions. If Color.Group (input of DATAprepSE()) is a data.frame, the first column must contain the name of each biological condition and the second column must contain the colors associated to each biological condition. If Color.Group=NULL, the function will automatically attribute a color for each biological condition. If samples belong to different time points only, Color.Group will not be used.
motion3D	TRUE or FALSE. If TRUE, the 3D PCA plots will also be plotted in a rgl window (see plot3Drgl::plotrgl()) allowing to interactively rotate and zoom.
Phi	Angle defining the colatitude direction for the 3D PCA plot (see Details in graphics::persp()).
Theta	Angle defining the azimuthal direction for the 3D PCA plot (see Details in graphics::persp()).
epsilon	Non negative numeric value giving the length between points and their labels in all PCA plots which are not automatically plotted by FactoMineR::PCA().
Cex.point	Non negative numeric value giving the size of points in all PCA plots which are not automatically plotted by FactoMineR::PCA().
Cex.label	Non negative numeric value giving the size of the labels associated to each point of the all PCA graphs which are not automatically plotted by FactoMineR::PCA().
path.result	Character or NULL. Path to save the different PCA graphs. If NULL, the different PCA graphs will not be saved in a folder. NULL as default.
Name.file.pca	Character or NULL. If Name.file.pca is a character, Name.file.pca will be added at the beginning of all names of the saved graphs.

# Details

All results are built from the results of our function DATAnormalization().

### Value

The function returns the same SummarizedExperiment class object SEresNorm with the outputs from the function FactoMineR::PCA(), and plots several 2D and 3D PCA graphs depending on the experimental design (if Plot.PCA=TRUE), saved in the metadata Results[[1]][[2]] of SEresNorm,

#### **PCAgraphics**

- When samples belong only to different biological conditions, the function returns a 2D and two 3D PCA graphs. In each graph, samples are colored with different colors for different biological conditions. The two 3D PCA graphs are identical but one of them will be opened in a rgl window (see plot3Drgl::plotrgl()) and it allows to interactively rotate and zoom.
- When samples belong only to different time points, the function returns
  - One 2D PCA graph, one 3D PCA graph and the same 3D PCA graph in a rgl window where samples are colored with different colors for different time points. Furthermore, lines are drawn between each pair of consecutive points for each sample (if Mean.Accross.Time=FALSE, otherwise it will be only between means).
  - The same graphs describe above but without lines.
- When samples belong to different time points and different biological conditions, the function returns
  - One 2D PCA graph, one 3D PCA graph and the same 3D PCA graph in a rgl window where samples are colored with different colors for different time points. Furthermore, lines are drawn between each pair of consecutive points for each sample (if Mean.Accross.Time=FALSE, otherwise it will be only between means).
  - The same graphs describe above but without lines.
  - The same six following graphs for each biological condition (one PCA analysis per biological condition). One 2D PCA graph, one 3D PCA graph and the same 3D PCA graph in a rgl window where samples belong to only one biological condition and are colored with different colors for different time points. Furthermore, lines are drawn between each pair of consecutive points for each sample (if Mean.Accross.Time=FALSE, otherwise it will be only between means). The three others graphs are identical to the three previous ones but without lines.

The interactive 3D graphs will be plotted only if motion3D=TRUE.

### See Also

This function is called by our function PCAanalysis() and calls our function PCArealization().

```
## Simulation raw counts
resSIMcount <- RawCountsSimulation(Nb.Group=2, Nb.Time=3, Nb.per.GT=4,</pre>
                                     Nb.Gene=10)
## Preprocessing step
resDATAprepSE <- DATAprepSE(RawCounts=resSIMcount$Sim.dat,</pre>
                              Column.gene=1,
                              Group.position=1,
                              Time.position=2,
                              Individual.position=3)
## Normalization
resNorm <- DATAnormalization(SEres=resDATAprepSE,</pre>
                              Normalization="rle",
                              Plot.Boxplot=FALSE,
                              Colored.By.Factors=FALSE)
## Color for each group
GROUPcolor <- data.frame(Name=c("G1", "G2"), Col=c("black", "red"))</pre>
```

```
##------##
```

```
resPCAgraph <- PCAgraphics(SEresNorm=resNorm,
DATAnorm=TRUE,
gene.deletion=c("Gene1", "Gene5"),
sample.deletion=c(2,6),
Plot.PCA=TRUE,
Mean.Accross.Time=FALSE,
Color.Group=GROUPcolor,
motion3D=FALSE,
Phi=25, Theta=140, Cex.label=0.7,
Cex.point=0.7, epsilon=0.2,
path.result=NULL, Name.file.pca=NULL)
```

PCApreprocessing Reshaped dataset for factorial analysis.

#### Description

The function generates a SummarizedExperiment class object containing the dataset reshaped from the original dataset, to be used by the function FactoMineR::PCA(), which performs the Principal Component Analysis (PCA). This function is called by the function PCArealization(), which also calls the function FactoMineR::PCA().

### Usage

```
PCApreprocessing(SEresNorm, DATAnorm = TRUE)
```

#### Arguments

SEresNorm	Results of the function DATAnormalization().
DATAnorm	TRUE or FALSE. TRUE as default. TRUE means the function uses the normalized
	data. FALSE means the function uses the raw counts data.

# Details

All results are built from the results of our function DATAnormalization().

#### Value

The function returns the same SummarizedExperiment class object SEresNorm with the different elements below

- information for the functions PCArealization() and PCAgraphics()
- a reshape of the originally dataset for the PCA analysis (realized by the function PCArealization())

saved in the metadata Results[[1]][[2]] of SEresNorm.

The reshaped dataset which corresponds to a data.frame with  $(N_g + k)$  columns and  $N_s$  rows, where  $N_q$  is the number of genes,  $N_s$  is the number of samples and

```
70
```

# PCArealization

- k = 1 if samples belong to different biological condition or time points. In that case, the first column will contain the biological condition or the time point associated to each sample.
- k = 2 if samples belong to different biological condition and time points. In that case, the first column will contain the biological condition and the second column the time point associated to each sample.

The other  $N_g$  columns form a sub data.frame which is a transpose of the data.frame composed of the  $N_s$  numeric columns of ExprData.

### See Also

The function is called by our function PCArealization() and uses our function DATAnormalization().

### Examples

```
## Simulation raw counts
resSIMcount <- RawCountsSimulation(Nb.Group=2, Nb.Time=3, Nb.per.GT=4,
                              Nb.Gene=10)
## Preprocessing step
resDATAprepSE <- DATAprepSE(RawCounts=resSIMcount$Sim.dat,</pre>
                        Column.gene=1,
                        Group.position=1,
                        Time.position=2,
                        Individual.position=3)
## Normalization
resNorm <- DATAnormalization(SEres=resDATAprepSE,
                         Normalization="rle",
                         Plot.Boxplot=FALSE,
                         Colored.By.Factors=FALSE)
resPCAdata <- PCApreprocessing(SEresNorm=resNorm,</pre>
                           DATAnorm=TRUE)
```

PCArealization PCA realization

#### Description

From a gene expression dataset, the functions performs the Principal Component Analysis (PCA) through the R function FactoMineR::PCA().

#### Usage

```
PCArealization(
  SEresNorm,
  DATAnorm = TRUE,
  gene.deletion = NULL,
  sample.deletion = NULL,
  Supp.del.sample = FALSE
)
```

### Arguments

SEresNorm	Results of the function DATAnormalization().
DATAnorm	TRUE or FALSE. TRUE as default. TRUE means the function uses the normalized data. FALSE means the function uses the raw counts data.
gene.deletion	NULL or a vector of characters or a vector of integers. NULL as default. If gene.deletion is a vector of characters, all genes with names in gene.deletion will be deleted from the data set as input RawCounts of our function DATAprepSE(). If gene.deletion is a vector of integers, all the corresponding row numbers will be deleted from the data set as input RawCounts of our function DATAprepSE(). If gene.deletion=NULL all genes will be used in the construction of the PCA.
sample.deletion	
	NULL or a vector of characters or a vector of integers. NULL as default. If sample.deletion is a vector of characters, all samples with names in sample.deletion will not be used in the construction of the PCA. If sample.deletion is a vector of integers, all the corresponding column numbers will not be used in the construction of the PCA from the data set as input RawCounts of our function DATAprepSE(). If sample.deletion=NULL all samples will be used in the construction of the PCA.
Supp.del.sample	
	TRUE or FALSE. FALSE by default. If FALSE, the samples selected with sample.deletion will be deleted. If TRUE, the samples selected with sample.deletion will be plotted. These individuals are called supplementary individuals in FactoMineR::PCA().

## Details

All results are built from the results of our function DATAnormalization().

# Value

The function returns the same SummarizedExperiment class object SEresNorm but with the output of the FactoMineR::PCA() function (see FactoMineR::PCA()) saved in the metadata Results[[1]][[2]] of SEresNorm.

### See Also

The PCArealization() function

- is used by the following functions of our package : PCAanalysis() and HCPCanalysis().
- calls the R function PCApreprocessing() for reshaping the data and uses its output for performing a Principal Component (PCA) with FactoMineR::PCA().
## **RawCountsSimulation**

```
Column.gene=1,
                      Group.position=1,
                      Time.position=2,
                      Individual.position=3)
## Normalization
resNorm <- DATAnormalization(SEres=resDATAprepSE,</pre>
                       Normalization="rle",
                       Plot.Boxplot=FALSE,
                       Colored.By.Factors=FALSE)
##------##
resPCAex <- PCArealization(SEresNorm=resNorm,</pre>
                     DATAnorm=TRUE,
                     gene.deletion=c(3, 5),
                     sample.deletion=c("G1_t0_Ind2", "G1_t1_Ind3"),
                     Supp.del.sample=FALSE)
resPCAex2 <- PCArealization(SEresNorm=resNorm,</pre>
                      DATAnorm=TRUE,
                      gene.deletion=c("Gene3", "Gene5"),
                      sample.deletion=c(3, 8),
                      Supp.del.sample=TRUE)
```

RawCountsSimulation RNA-seq raw counts data simulation

## Description

The function simulates an in silico RNA-seq raw counts data inspired from the model used in the DESeq2 package. It is used in some examples of other functions.

# Usage

RawCountsSimulation(Nb.Group, Nb.Time, Nb.per.GT, Nb.Gene)

# Arguments

Nb.Group	Non negative integer. Number of biological condition (minimum 1).
Nb.Time	Non negative integer. Number of time points (minimum 1).
Nb.per.GT	Non negative integer. Number of sample for each condition and time (minimum 1).
Nb.Gene	Non negative integer. Number of genes (minimum 1)

# Value

A simulated RNA-seq raw counts data.

#### Examples

```
RawCountsSimulation(Nb.Group=3, Nb.Time=5, Nb.per.GT=7, Nb.Gene=50)
## RawCountsSimulation(Nb.Group=1, Nb.Time=5, Nb.per.GT=7, Nb.Gene=50)
## RawCountsSimulation(Nb.Group=3, Nb.Time=1, Nb.per.GT=7, Nb.Gene=50)
```

RawCounts\_Antoszewski2022\_MOUSEsub500 Mouse raw counts data

### Description

There are 4 groups : samples with or without hyper activation of the gene NOTTCH1 (N1ha versus N1wt) and with or without knock out of the gene TCF1 (T1ko versus T1wt). The original dataset has 39017 genes but we kept only 500 genes in order to increase the speed of each function in our algorithm.

# Usage

data(RawCounts\_Antoszewski2022\_MOUSEsub500)

#### Format

A data frame with 500 rows (genes) and 13 columns (samples). The column names are as follow

Gene ENSEMBL gene names.

N1wtT1wt\_r1 The sample is the first replica (r1) of the biological condition N1wt and T1wt. N1wtT1wt\_r2 The sample is the second replica (r2) of the biological condition N1wt and T1wt. N1wtT1wt\_r3 The sample is the third replica (r3) of the biological condition N1wt and T1wt. N1haT1wt\_r4 The sample is the first replica (r4) of the biological condition N1ha and T1wt. N1haT1wt\_r5 The sample is the second replica (r5) of the biological condition N1ha and T1wt. N1haT1wt\_r6 The sample is the third replica (r6) of the biological condition N1ha and T1wt. N1haT1wt\_r7 The sample is the first replica (r6) of the biological condition N1ha and T1wt. N1haT1ko\_r7 The sample is the first replica (r7) of the biological condition N1ha and T1ko. N1haT1ko\_r7 The sample is the second replica (r8) of the biological condition N1ha and T1ko. N1haT1ko\_r7 The sample is the third replica (r9) of the biological condition N1ha and T1ko. N1haT1ko\_r10 The sample is the first replica (r10) of the biological condition N1ha and T1ko. N1wtT1ko\_r11 The sample is the second replica (r11) of the biological condition N1wt and T1ko. N1wtT1ko\_r12 The sample is the third replica (r12) of the biological condition N1wt and T1ko.

The following is quoted from the GEO series GSE169116 (link in source):

Summary : "NOTCH1 is a well-established lineage specifier for T cells and among the most frequently mutated genes throughout all subclasses of T cell acute lymphoblastic leukemia (T-ALL). How oncogenic NOTCH1 signaling launches a leukemia-prone chromatin landscape during T-ALL initiation is unknown. Here we demonstrate an essential role for the high-mobility-group transcription factor Tcf1 in orchestrating chromatin accessibility and topology allowing for aberrant Notch1 signaling to convey its oncogenic function. Although essential, Tcf1 is not sufficient to initiate leukemia. The formation of a leukemia-prone landscape at the distal Notch1-regulated Myc enhancer, which is fundamental to this disease, is Tcf1-dependent and occurs within the earliest progenitor stage even before cells adopt a T lymphocyte or leukemic fate. Moreover, we discovered an additional evolutionarily conserved Tcf1-regulated enhancer element, in the distal Myc-enhancer, which is important for the transition of pre-leukemic cells to full-blown disease."

Overall design: "Expression profile comparisons of sorted LSK derived from C57BL/6J; Sv/129 compound mice with Notch1 induced or Tcf1 knocked-down."

We kept 500 genes only in order to increase the speed for each example.

## Value

Mouse dataset with four biological conditions.

#### Source

This dataset comes from Gene Expression Omnibus (GEO) https://www.ncbi.nlm.nih.gov/ geo/query/acc.cgi?acc=GSE169116. The name of the samples was renamed in order to be used with our package.

## References

Antoszewski M, Fournier N, Ruiz Buendía GA, Lourenco J et al. 'Tcf1 is essential for initiation of oncogenic Notch1-driven chromatin topology in T-ALL'. Blood 2022 Jan 12. PMID:35020836. GEO:GSE169116.

## Examples

data(RawCounts\_Antoszewski2022\_MOUSEsub500)

RawCounts\_Leong2014\_FISSIONsub500wt Yeast times series raw counts data after stimulation with and without silencing

#### Description

Raw counts data for fission yeast RNA-Seq experiment with two groups (wt and mut), 6 times (0, 15min, 30min, 60min, 120min, 180min) and 3 replicates for each group and time. The original dataset has 7039 genes but we kept only 500 genes in order to increase the speed of each function in our algorithm.

#### Usage

data(RawCounts\_Leong2014\_FISSIONsub500wt)

#### Format

A data frame with 500 rows (genes) and 37 columns (samples). The column names are as follow

Gene Gene name

- wt\_t0\_r1 The sample is the first replica (r1) of the biological condition control (wt) at time t0 (0
  min)
- wt\_t0\_r2 The sample is the second replica (r2) of the biological condition control (wt) at time t0
   (0 min)
- wt\_t0\_r3 The sample is the third replica (r3) of the biological condition control (wt) at time t0 (0
  min)
- wt\_t1\_r1 The sample is the first replica (r1) of the biological condition control (wt) at time t1 (15
  min)
- wt\_t1\_r2 The sample is the second replica (r2) of the biological condition control (wt) at time t1
   (15 min)
- wt\_t1\_r3 The sample is the third replica (r3) of the biological condition control (wt) at time t1 (15
  min)
- wt\_t2\_r1 The sample is the first replica (r1) of the biological condition control (wt) at time t2 (30
  min)
- wt\_t2\_r3 The sample is the third replica (r3) of the biological condition control (wt) at time t2 (30 min)
- wt\_t3\_r1 The sample is the first replica (r1) of the biological condition control (wt) at time t3 (60
  min)
- wt\_t3\_r2 The sample is the second replica (r2) of the biological condition control (wt) at time t3 (60 min)
- wt\_t3\_r3 The sample is the third replica (r3) of the biological condition control (wt) at time t3 (60 min)
- wt\_t4\_r1 The sample is the first replica (r1) of the biological condition control (wt) at time t4 (120 min)
- wt\_t4\_r2 The sample is the second replica (r2) of the biological condition control (wt) at time t4 (120 min)
- wt\_t4\_r3 The sample is the third replica (r3) of the biological condition control (wt) at time t4 (120 min)
- wt\_t5\_r1 The sample is the first replica (r1) of the biological condition control (wt) at time t5 (180
  min)
- wt\_t5\_r2 The sample is the second replica (r2) of the biological condition control (wt) at time t5 (180 min)
- wt\_t5\_r3 The sample is the third replica (r3) of the biological condition control (wt) at time t5
   (180 min)

The following is quoted from the GEO series GSE56761 (link in source):

Summary: "Mitogen Activated Protein Kinase (MAPK) signaling cascades transduce information arising from events external to the cell, such as environmental stresses, to a variety of downstream effectors and transcription factors. The fission yeast stress activated MAP kinase (SAPK) pathway is conserved with the p38 and JNK pathways in humans, and comprises the MAPKKKs Win1, Wis4, the MAPKK Wis1, and the MAPK, Sty1. Sty1 and its main downstream effector Atf1 regulate a large set of core environmental stress response genes. The fission yeast genome encodes three other ATF proteins: Atf21, Atf31 and Pcr1. Among these, atf21 is specifically induced under conditions of high osmolarity. We have therefore instigated a programme to investigate the role played by non coding RNAs (ncRNAs) in response to osmotic stress challenge in wild type and atf21Delta cells. By integrating global proteomics and RNA sequencing data, we identified a systematic program in which elevated antisense RNAs arising both from ncRNAs and from 3'-overlapping convergent gene-pairs is directly associated with substantial reductions in protein levels throughout the fission yeast genome. We also found an xtensive array of ncRNAs with trans associations that have the potential to influence different biological processes and stress responses in fission yeast, suggesting ncRNAs comprise additional components of the SAPK regulatory system".

Overall design: "Global transcription profiles of fission yeast wild type (WT) and atf21del strains over an osmotic stress time course following treatment with 1M sorbitol at 0, 15, 30, 60, 120 and 180 mins. Strand-specific single end sequencing of total RNA was performed in biological triplicates on the Applied Biosystems SOLiD 5500xl Genetic Analyzer System".

We kept 500 genes only in order to increase the speed for each example.

#### Value

Yeast dataset with 6 time measurements.

#### Source

This dataset can be found in the R Package fission. https://bioconductor.org/packages/ release/data/experiment/html/fission.html Link of GEO series GSE56761: https://www. ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE56761. The name of the samples was renamed in order to be used with our package.

#### References

Leong HS, Dawson K, Wirth C, Li Y et al. 'A global non-coding RNA system modulates fission yeast protein levels in response to stress'. Nat Commun 2014 May 23;5:3947. PMID:24853205. GEO:GSE56761.

#### Examples

data(RawCounts\_Leong2014\_FISSIONsub500wt)

RawCounts\_Schleiss2021\_CLLsub500

Human CCL times series raw counts data after stimulation with and without silencing

## Description

This time series count data (read counts) represents the temporal transcriptional response of primary human chronic lymphocytic leukemia (CLL)-cells after B-cell receptor stimulation. There are 9 time points (before stimulation (0h) and at the time points 1h, 1h30, 3h30, 6h30, 12h, 24h, 48h and 96h after cell stimulation) and samples are divided in two groups : Proliferating (P) and Non Proliferating (NP). There are also 3 replicates for a time and biological condition. The original dataset has 25369 genes but we kept only 500 genes in order to increase the speed of each function in our algorithm.

## Usage

data(RawCounts\_Schleiss2021\_CLLsub500)

#### Format

A data frame with 500 rows (genes) and 55 columns (samples). The column names are as follow

Genes Symbol gene name.

- CLL\_P\_r1\_t0 The sample is the first replica (r1) of the biological condition control (P) at time t0 (0h)
- CLL\_P\_r1\_t1 The sample is the first replica (r1) of the biological condition control (P) at time t1 (1h)
- CLL\_P\_r1\_t2 The sample is the first replica (r1) of the biological condition control (P) at time t2 (1h30)
- **CLL\_P\_r1\_t3** The sample is the first replica (r1) of the biological condition control (P) at time t3 (3h30)
- **CLL\_P\_r1\_t4** The sample is the first replica (r1) of the biological condition control (P) at time t4 (6h30)
- **CLL\_P\_r1\_t5** The sample is the first replica (r1) of the biological condition control (P) at time t5 (12h)
- **CLL\_P\_r1\_t6** The sample is the first replica (r1) of the biological condition control (P) at time t6 (24h)
- CLL\_P\_r1\_t7 The sample is the first replica (r1) of the biological condition control (P) at time t7 (48h)
- **CLL\_P\_r1\_t8** The sample is the first replica (r1) of the biological condition control (P) at time t8 (96h)
- **CLL\_P\_r2\_t0** The sample is the second replica (r2) of the biological condition control (P) at time t0 (0h)

- **CLL\_P\_r2\_t1** The sample is the second replica (r2) of the biological condition control (P) at time t1 (1h)
- **CLL\_P\_r2\_t2** The sample is the second replica (r2) of the biological condition control (P) at time t2 (1h30)
- **CLL\_P\_r2\_t3** The sample is the second replica (r2) of the biological condition control (P) at time t3 (3h30)
- CLL\_P\_r2\_t4 The sample is the second replica (r2) of the biological condition control (P) at time t4 (6h30)
- **CLL\_P\_r2\_t5** The sample is the second replica (r2) of the biological condition control (P) at time t5 (12h)
- **CLL\_P\_r2\_t6** The sample is the second replica (r2) of the biological condition control (P) at time t6 (24h)
- **CLL\_P\_r2\_t7** The sample is the second replica (r2) of the biological condition control (P) at time t7 (48h)
- **CLL\_P\_r2\_t8** The sample is the second replica (r2) of the biological condition control (P) at time t8 (96h)
- CLL\_P\_r3\_t0 The sample is the third replica (r3) of the biological condition control (P) at time t0 (0h)
- CLL\_P\_r3\_t1 The sample is the third replica (r3) of the biological condition control (P) at time t1 (1h)
- **CLL\_P\_r3\_t2** The sample is the third replica (r3) of the biological condition control (P) at time t2 (1h30)
- **CLL\_P\_r3\_t3** The sample is the third replica (r3) of the biological condition control (P) at time t3 (3h30)
- **CLL\_P\_r3\_t4** The sample is the third replica (r3) of the biological condition control (P) at time t4 (6h30)
- **CLL\_P\_r3\_t5** The sample is the third replica (r3) of the biological condition control (P) at time t5 (12h)
- **CLL\_P\_r3\_t6** The sample is the third replica (r3) of the biological condition control (P) at time t6 (24h)
- CLL\_P\_r3\_t7 The sample is the third replica (r3) of the biological condition control (P) at time t7 (48h)
- **CLL\_P\_r3\_t8** The sample is the third replica (r3) of the biological condition control (P) at time t8 (96h)
- **CLL\_NP\_r4\_t0** The sample is the first replica (r4) of the biological condition control (NP) at time t0 (0h)
- CLL\_NP\_r4\_t1 The sample is the first replica (r4) of the biological condition control (NP) at time t1 (1h)
- CLL\_NP\_r4\_t2 The sample is the first replica (r4) of the biological condition control (NP) at time t2 (1h30)
- CLL\_NP\_r4\_t3 The sample is the first replica (r4) of the biological condition control (NP) at time t3 (3h30)

- CLL\_NP\_r4\_t4 The sample is the first replica (r4) of the biological condition control (NP) at time t4 (6h30)
- CLL\_NP\_r4\_t5 The sample is the first replica (r4) of the biological condition control (NP) at time t5 (12h)
- CLL\_NP\_r4\_t6 The sample is the first replica (r4) of the biological condition control (NP) at time t6 (24h)
- CLL\_NP\_r4\_t7 The sample is the first replica (r4) of the biological condition control (NP) at time t7 (48h)
- CLL\_NP\_r4\_t8 The sample is the first replica (r4) of the biological condition control (NP) at time t8 (96h)
- CLL\_NP\_r5\_t0 The sample is the second replica (r5) of the biological condition control (NP) at time t0 (0h)
- CLL\_NP\_r5\_t1 The sample is the second replica (r5) of the biological condition control (NP) at time t1 (1h)
- CLL\_NP\_r5\_t2 The sample is the second replica (r5) of the biological condition control (NP) at time t2 (1h30)
- CLL\_NP\_r5\_t3 The sample is the second replica (r5) of the biological condition control (NP) at time t3 (3h30)
- CLL\_NP\_r5\_t4 The sample is the second replica (r5) of the biological condition control (NP) at time t4 (6h30)
- **CLL\_NP\_r5\_t5** The sample is the second replica (r5) of the biological condition control (NP) at time t5 (12h)
- CLL\_NP\_r5\_t6 The sample is the second replica (r5) of the biological condition control (NP) at time t6 (24h)
- CLL\_NP\_r5\_t7 The sample is the second replica (r5) of the biological condition control (NP) at time t7 (48h)
- CLL\_NP\_r5\_t8 The sample is the second replica (r5) of the biological condition control (NP) at time t8 (96h)
- **CLL\_NP\_r6\_t0** The sample is the third replica (r6) of the biological condition control (NP) at time t0 (0h)
- CLL\_NP\_r6\_t1 The sample is the third replica (r6) of the biological condition control (NP) at time t1 (1h)
- CLL\_NP\_r6\_t2 The sample is the third replica (r6) of the biological condition control (NP) at time t2 (1h30)
- CLL\_NP\_r6\_t3 The sample is the third replica (r6) of the biological condition control (NP) at time t3 (3h30)
- CLL\_NP\_r6\_t4 The sample is the third replica (r6) of the biological condition control (NP) at time t4 (6h30)
- CLL\_NP\_r6\_t5 The sample is the third replica (r6) of the biological condition control (NP) at time t5 (12h)
- **CLL\_NP\_r6\_t6** The sample is the third replica (r6) of the biological condition control (NP) at time t6 (24h)

- CLL\_NP\_r6\_t7 The sample is the third replica (r6) of the biological condition control (NP) at time t7 (48h)
- CLL\_NP\_r6\_t8 The sample is the third replica (r6) of the biological condition control (NP) at time t8 (96h)

The following is quoted from the GEO series GSE130385 (link in source):

Summary: "The B-cell receptor (BCR) signaling is crucial for the pathophysiology of most leukemias and lymphomas originated from mature B lymphocytes and has emerged as a new therapeutic target, especially for chronic lymphocytic leukemia (CLL). However, the precise mechanisms by which BCR signaling controls neoplastic B-cell proliferation are ill characterized. This work was performed using primary leukemic cells of untreated patients at initial stage of CLL (Binet stage A / Rai 0) presenting biological characteristics of aggressive form of the disease (unmutated IGHV genes and ZAP70 protein expression). In order to mimic the primary leukemogenic step occurring in vivo, this study focused on the BCR-dependent proliferation of CLL cells induced ex vivo using anti-IgM, together with mandatory co-stimulating factors (CD40L, IL-4 and IL-21) (Schleiss, Sci Rep, 2019). Cell proliferation was objectivized by the emergence of proliferative clusters and the presence of more than 25% of CLL cells that did undergo division within the cell culture at day 6. To capture the specific actors of the proliferative response in these samples, we also included non-proliferating control CLL samples. Gene expression was analyzed by RNA-seq before stimulation (T0) and at the time points 1h, 1h30, 3h30, 6h30, 12h, 24h, 48h and 96h after cell stimulation clusters."

Overall design: "Temporal transcriptional response (T0 + 8 time points) of primary chronic lymphocytic leukemia (CLL) cells after BCR engagement ex vivo (anti-IgM, IL-4, CD40Ligand and IL-21) of 3 Proliferating (P1, P2, P3) and 3 Non Proliferating samples (NP1, NP2, NP3)".

# Value

Human CCL times series dataset with two biological conditions and with 9 time measurements.

#### Source

This dataset comes from Gene Expression Omnibus (GEO) https://www.ncbi.nlm.nih.gov/ geo/query/acc.cgi?acc=GSE130385. I rewrite the name of the sample in order to be used with my package.

# References

Schleiss C, Carapito R, Fornecker LM, Muller L et al. 'Temporal multiomic modeling reveals a B-cell receptor proliferative program in chronic lymphocytic leukemia'. Leukemia 2021 May;35(5):1463-1474. PMID:33833385. GEO:GSE130385

#### Examples

data(RawCounts\_Schleiss2021\_CLLsub500)

RawCounts\_Weger2021\_MOUSEsub500

Mouse count data with four biological conditions, six time measurements and 500 genes.

## Description

This time series count data (read counts) represents the temporal transcriptional response (six time measurements across the course of a day) of Bmal1 wild-type (WT) and Cry1/2 WT, Bmal1 KO and Cry1/2 WT, Bmal1 (WT) and Cry1/2 KO, and Bmal1 KO and Cry1/2 KO mice under an ad libitum (AL) or night restricted feeding (RF) regimen. Therefore, there are eight biological conditions. As there are only two mice per biological condition, we will not consider the effect of the regimen. The original dataset has 40327 genes but we kept only 500 genes in order to increase the speed of each function in our algorithm.

## Usage

data(RawCounts\_Weger2021\_MOUSEsub500)

#### Format

A data frame with 500 rows (genes) and 97 columns (samples). The column names are as follow

Gene ENSEMBL gene names.

- **BmKo\_t0\_r1** The sample is the first replica (r1) of the biological condition Bmal1 and KO at time t0 (00h).
- **BmKo\_t1\_r1** The sample is the first replica (r1) of the biological condition Bmal1 and KO at time t1 (04h).
- **BmKo\_t2\_r1** The sample is the first replica (r1) of the biological condition Bmal1 and KO at time t2 (08h).
- **BmKo\_t3\_r1** The sample is the first replica (r1) of the biological condition Bmal1 and KO at time t3 (12h).
- **BmKo\_t4\_r1** The sample is the first replica (r1) of the biological condition Bmal1 and KO at time t4 (16h).
- **BmKo\_t5\_r1** The sample is the first replica (r1) of the biological condition Bmal1 and KO at time t5 (20h).
- **BmKo\_t0\_r2** The sample is the first replica (r2) of the biological condition Bmal1 and KO at time t0 (00h).
- **BmKo\_t1\_r2** The sample is the first replica (r2) of the biological condition Bmal1 and KO at time t1 (04h).
- **BmKo\_t2\_r2** The sample is the first replica (r2) of the biological condition Bmal1 and KO at time t2 (08h).
- **BmKo\_t3\_r2** The sample is the first replica (r2) of the biological condition Bmal1 and KO at time t3 (12h).

- **BmKo\_t4\_r2** The sample is the first replica (r2) of the biological condition Bmal1 and KO at time t4 (16h).
- **BmKo\_t5\_r2** The sample is the first replica (r2) of the biological condition Bmal1 and KO at time t5 (20h).
- **BmKo\_t0\_r3** The sample is the first replica (r3) of the biological condition Bmal1 and KO at time t0 (00h).
- **BmKo\_t1\_r3** The sample is the first replica (r3) of the biological condition Bmal1 and KO at time t1 (04h).
- **BmKo\_t2\_r3** The sample is the first replica (r3) of the biological condition Bmal1 and KO at time t2 (08h).
- **BmKo\_t3\_r3** The sample is the first replica (r3) of the biological condition Bmal1 and KO at time t3 (12h).
- **BmKo\_t4\_r3** The sample is the first replica (r3) of the biological condition Bmal1 and KO at time t4 (16h).
- **BmKo\_t5\_r3** The sample is the first replica (r3) of the biological condition Bmal1 and KO at time t5 (20h).
- **BmKo\_t0\_r4** The sample is the first replica (r4) of the biological condition Bmal1 and KO at time t0 (00h).
- **BmKo\_t1\_r4** The sample is the first replica (r4) of the biological condition Bmal1 and KO at time t1 (04h).
- **BmKo\_t2\_r4** The sample is the first replica (r4) of the biological condition Bmal1 and KO at time t2 (08h).
- **BmKo\_t3\_r4** The sample is the first replica (r4) of the biological condition Bmal1 and KO at time t3 (12h).
- **BmKo\_t4\_r4** The sample is the first replica (r4) of the biological condition Bmal1 and KO at time t4 (16h).
- **BmKo\_t5\_r4** The sample is the first replica (r4) of the biological condition Bmal1 and KO at time t5 (20h).
- **BmWt\_t0\_r5** The sample is the first replica (r5) of the biological condition Bmal1 and wild-type at time t0 (00h).
- **BmWt\_t1\_r5** The sample is the first replica (r5) of the biological condition Bmal1 and wild-type at time t1 (04h).
- **BmWt\_t2\_r5** The sample is the first replica (r5) of the biological condition Bmal1 and wild-type at time t2 (08h).
- **BmWt\_t3\_r5** The sample is the first replica (r5) of he biological condition Bmal1 and wild-type at time t3 (12h).
- **BmWt\_t4\_r5** The sample is the first replica (r5) of the biological condition Bmal1 and wild-type at time t4 (16h).
- **BmWt\_t5\_r5** The sample is the first replica (r5) of the biological condition Bmal1 and wild-type at time t5 (20h).
- **BmWt\_t0\_r6** The sample is the first replica (r6) of the biological condition Bmal1 and wild-type at time t0 (00h).

- **BmWt\_t1\_r6** The sample is the first replica (r6) of the biological condition Bmal1 and wild-type at time t1 (04h).
- **BmWt\_t2\_r6** The sample is the first replica (r6) of the biological condition Bmal1 and wild-type at time t2 (08h).
- **BmWt\_t3\_r6** The sample is the first replica (r6) of the biological condition Bmal1 and wild-type at time t3 (12h).
- **BmWt\_t4\_r6** The sample is the first replica (r6) of the biological condition Bmal1 and wild-type at time t4 (16h).
- **BmWt\_t5\_r6** The sample is the first replica (r6) of the biological condition Bmal1 and wild-type at time t5 (20h).
- **BmWt\_t0\_r7** The sample is the first replica (r7) of the biological condition Bmal1 and wild-type at time t0 (00h).
- **BmWt\_t1\_r7** The sample is the first replica (r7) of the biological condition Bmal1 and wild-type at time t1 (04h).
- **BmWt\_t2\_r7** The sample is the first replica (r7) of the biological condition Bmal1 and wild-type at time t2 (08h).
- **BmWt\_t3\_r7** The sample is the first replica (r7) of the biological condition Bmal1 and wild-type at time t3 (12h).
- **BmWt\_t4\_r7** The sample is the first replica (r7) of the biological condition Bmal1 and wild-type at time t4 (16h).
- **BmWt\_t5\_r7** The sample is the first replica (r7) of the biological condition Bmal1 and wild-type at time t5 (20h).
- **BmWt\_t0\_r8** The sample is the first replica (r8) of the biological condition Bmal1 and wild-type at time t0 (00h).
- **BmWt\_t1\_r8** The sample is the first replica (r8) of the biological condition Bmal1 and wild-type at time t1 (04h).
- **BmWt\_t2\_r8** The sample is the first replica (r8) of the biological condition Bmal1 and wild-type at time t2 (08h).
- **BmWt\_t3\_r8** The sample is the first replica (r8) of the biological condition Bmal1 and wild-type at time t3 (12h).
- **BmWt\_t4\_r8** The sample is the first replica (r8) of the biological condition Bmal1 and wild-type at time t4 (16h).
- **BmWt\_t5\_r8** The sample is the first replica (r8) of the biological condition Bmal1 and wild-type at time t5 (20h).
- **CrKo\_t0\_r9** The sample is the first replica (r9) of the biological condition Cry1/2 and KO at time t0 (00h).
- **CrKo\_t1\_r9** The sample is the first replica (r9) of the biological condition Cry1/2 and KO at time t1 (04h).
- **CrKo\_t2\_r9** The sample is the first replica (r9) of the biological condition Cry1/2 and KO at time t2 (08h).
- **CrKo\_t3\_r9** The sample is the first replica (r9) of the biological condition Cry1/2 and KO at time t3 (12h).

- **CrKo\_t4\_r9** The sample is the first replica (r9) of the biological condition Cry1/2 and KO at time t4 (16h).
- **CrKo\_t5\_r9** The sample is the first replica (r9) of the biological condition Cry1/2 and KO at time t5 (20h).
- **CrKo\_t0\_r10** The sample is the first replica (r10) of the biological condition Cry1/2 and KO at time t0 (00h).
- **CrKo\_t1\_r10** The sample is the first replica (r10) of the biological condition Cry1/2 and KO at time t1 (04h).
- **CrKo\_t2\_r10** The sample is the first replica (r10) of the biological condition Cry1/2 and KO at time t2 (08h).
- **CrKo\_t3\_r10** The sample is the first replica (r10) of the biological condition Cry1/2 and KO at time t3 (12h).
- **CrKo\_t4\_r10** The sample is the first replica (r10) of the biological condition Cry1/2 and KO at time t4 (16h).
- **CrKo\_t5\_r10** The sample is the first replica (r10) of the biological condition Cry1/2 and KO at time t5 (20h).
- **CrKo\_t0\_r11** The sample is the first replica (r11) of the biological condition Cry1/2 and KO at time t0 (00h).
- **CrKo\_t1\_r11** The sample is the first replica (r11) of the biological condition Cry1/2 and KO at time t1 (04h).
- **CrKo\_t2\_r11** The sample is the first replica (r11) of the biological condition Cry1/2 and KO at time t2 (08h).
- **CrKo\_t3\_r11** The sample is the first replica (r11) of the biological condition Cry1/2 and KO at time t3 (12h).
- **CrKo\_t4\_r11** The sample is the first replica (r11) of the biological condition Cry1/2 and KO at time t4 (16h).
- **CrKo\_t5\_r11** The sample is the first replica (r11) of the biological condition Cry1/2 and KO at time t5 (20h).
- **CrKo\_t0\_r12** The sample is the first replica (r12) of the biological condition Cry1/2 and KO at time t0 (00h).
- **CrKo\_t1\_r12** The sample is the first replica (r12) of the biological condition Cry1/2 and KO at time t1 (04h).
- **CrKo\_t2\_r12** The sample is the first replica (r12) of the biological condition Cry1/2 and KO at time t2 (08h).
- **CrKo\_t3\_r12** The sample is the first replica (r12) of the biological condition Cry1/2 and KO at time t3 (12h).
- **CrKo\_t4\_r12** The sample is the first replica (r12) of the biological condition Cry1/2 and KO at time t4 (16h).
- **CrKo\_t5\_r12** The sample is the first replica (r12) of the biological condition Cry1/2 and KO at time t5 (20h).
- CrWt\_t0\_r13 The sample is the first replica (r13) of the biological condition Cry1/2 and wild-type at time t0 (00h).

- CrWt\_t1\_r13 The sample is the first replica (r13) of the biological condition Cry1/2 and wild-type at time t1 (04h).
- CrWt\_t2\_r13 The sample is the first replica (r13) of the biological condition Cry1/2 and wild-type at time t2 (08h).
- CrWt\_t3\_r13 The sample is the first replica (r13) of the biological condition Cry1/2 and wild-type at time t3 (12h).
- CrWt\_t4\_r13 The sample is the first replica (r13) of the biological condition Cry1/2 and wild-type at time t4 (16h).
- CrWt\_t5\_r13 The sample is the first replica (r13) of the biological condition Cry1/2 and wild-type at time t5 (20h).
- CrWt\_t0\_r14 The sample is the first replica (r14) of the biological condition Cry1/2 and wild-type at time t0 (00h).
- CrWt\_t1\_r14 The sample is the first replica (r14) of the biological condition Cry1/2 and wild-type at time t1 (04h).
- CrWt\_t2\_r14 The sample is the first replica (r14) of the biological condition Cry1/2 and wild-type at time t2 (08h).
- CrWt\_t3\_r14 The sample is the first replica (r14) of the biological condition Cry1/2 and wild-type at time t3 (12h).
- CrWt\_t4\_r14 The sample is the first replica (r14) of the biological condition Cry1/2 and wild-type at time t4 (16h).
- CrWt\_t5\_r14 The sample is the first replica (r14) of the biological condition Cry1/2 and wild-type at time t5 (20h).
- **CrWt\_t0\_r15** The sample is the first replica (r15) of the biological condition Cry1/2 and wild-type at time t0 (00h).
- CrWt\_t1\_r15 The sample is the first replica (r15) of the biological condition Cry1/2 and wild-type at time t1 (04h).
- CrWt\_t2\_r15 The sample is the first replica (r15) of the biological condition Cry1/2 and wild-type at time t2 (08h).
- **CrWt\_t3\_r15** The sample is the first replica (r15) of the biological condition Cry1/2 and wild-type at time t3 (12h).
- CrWt\_t4\_r15 The sample is the first replica (r15) of the biological condition Cry1/2 and wild-type at time t4 (16h).
- **CrWt\_t5\_r15** The sample is the first replica (r15) of the biological condition Cry1/2 and wild-type at time t5 (20h).
- **CrWt\_t0\_r16** The sample is the first replica (r16) of the biological condition Cry1/2 and wild-type at time t0 (00h).
- CrWt\_t1\_r16 The sample is the first replica (r16) of the biological condition Cry1/2 and wild-type at time t1 (04h).
- **CrWt\_t2\_r16** The sample is the first replica (r16) of the biological condition Cry1/2 and wild-type at time t2 (08h).
- CrWt\_t3\_r16 The sample is the first replica (r16) of the biological condition Cry1/2 and wild-type at time t3 (12h).

- CrWt\_t4\_r16 The sample is the first replica (r16) of the biological condition Cry1/2 and wild-type at time t4 (16h).
- CrWt\_t5\_r16 The sample is the first replica (r16) of the biological condition Cry1/2 and wild-type at time t5 (20h).

The data is used in order to describe our algorithm in the case where samples belong to different time points.

We kept 500 genes only in order to increase the speed for each example.

## Value

Mouse times series dataset with four biological conditions and with 6 time measurements.

#### Source

This dataset comes from Gene Expression Omnibus (GEO) https://www.ncbi.nlm.nih.gov/ geo/query/acc.cgi?acc=GSE135898. The name of the samples was renamed in order to be used with our package.

#### References

Weger BD, Gobet C, David FPA, Atger F et al. 'Systematic analysis of differential rhythmic liver gene expression mediated by the circadian clock and feeding rhythms'. Proc Natl Acad Sci USA 2021 Jan 19;118(3). PMID:33452134. GEO:GSE135898.

## Examples

data(RawCounts\_Weger2021\_MOUSEsub500)

Results\_DEanalysis\_sub500

DE results of three dataset

## Description

The list Results\_DEanalysis\_sub500 contains the results of DEanalysisGlobal() for each of the following raw counts : RawCounts\_Weger2021\_MOUSEsub500, RawCounts\_Leong2014\_FISSIONsub500wt and RawCounts\_Schleiss2021\_CLLsub500

## Usage

data(Results\_DEanalysis\_sub500)

#### Format

A list of 3 SummarizedExperiment class object

Each list in Results\_DEanalysis\_sub500 contains only the necessary outputs of DEanalysisGlobal(), needed for the functions: DEplotVolcanoMA(), DEplotHeatmaps(), GSEApreprocessing(), and GSEAQuickAnalysis(), for each of the following raw counts : RawCounts\_Weger2021\_MOUSEsub500, RawCounts\_Leong2014\_FISSIONsub500wt and RawCounts\_Schleiss2021\_CLLsub500

## Value

Results\_DEanalysis\_sub500 contains the outputs of DEanalysisGlobal() of: RawCounts\_Weger2021\_MOUSEsub500, RawCounts\_Leong2014\_FISSIONsub500wt and RawCounts\_Schleiss2021\_CLLsub500

# Examples

data(Results\_DEanalysis\_sub500)

Transcript\_HomoSapiens\_Database Homo sapiens transcript database

## Description

The database is a data.frame which contains transcript length of homo sapiens genes (40452 genes).

#### Usage

data(Transcript\_HomoSapiens\_Database)

#### Format

A data frame with 500 rows (genes) and 13 columns (samples). The column names are as follow

## symbol ENSEMBL gene names.

Median.length.RNA The sample is the first replica (r1) of the biological condition N1wt and T1wt.

### Details

The first column contains genes symbol of the homo sapiens organism and the second column contains the median of transcript length for each gene of the first column.

## Value

Mouse dataset with four biological conditions.

# Source

HGNC, ENSEMBL and NCBI database.

## Examples

data(Transcript\_HomoSapiens\_Database)

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