Package ‘TCseq’

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Type Package

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Description Quantitative and differential analysis of epigenomic and
transcriptomic time course sequencing data, clustering analysis
and visualization of temporal patterns of time course data.

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License GPL (> = 2)

LazyData TRUE

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Suggests testthat

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R topics documented:

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Description

clust is a S4 class for storing results of a clustering analysis for time course data.

Details

: The clust objects are returned from timeclust and have a show method printing a compact summary of their contents

Slots

Object of this class contains the following slots:

method clustering method that has been used
dist distance method that has been used
data a matrix of original or standardized data that has been used for the analysis
centers a matrix of class centers
cluster an integer vector of length \( n \) \((n\) is the number of data points each integer indicates the cluster a data point belongs to. For the fuzzy cmeans clustering method, a data point is assigned to the closest cluster to which the data point has highest membership value.

membership a matrix with membership values of the data points to all the clusters

Author(s)

Mengjun Wu

See Also

timeclust.@
**clust.accessors**

Accessors to extract slots of a clust class.

### Description

Accessors are provided to extract data, centers, cluster, membership, membership slots of a clust class.

### Usage

```r
clustData(object)

## S4 method for signature 'clust'
clustData(object)

clustCenters(object)

## S4 method for signature 'clust'
clustCenters(object)

clustCluster(object)

## S4 method for signature 'clust'
clustCluster(object)

clustMembership(object)

## S4 method for signature 'clust'
clustMembership(object)
```

### Arguments

- `object` clust object

### Value

- `clustData` returns data matrix.
- `clustCenters` returns a matrix of centers.
- `clustCluster` returns an integer vector.
- `clustMembership` returns a matrix of membership, see `clust` for details.

### Author(s)

Mengjun Wu

### See Also

- `clust`
countReads  

**countReads**  

*count mapped reads overlap genomic intervals*

**Description**

This function counts mapped reads from multiple BAM files that overlap genomic intervals in genomicFeature in a TCA object. The counting result is stored in 'count' slot of the TCA object.

**Usage**

```r
countReads(object, dir, method = "summarizeoverlaps", zero.based = TRUE, ...)
```

**Arguments**

- `object`  
  a TCA object

- `dir`  
  character string giving the directory where BAM files are stored.

- `method`  
  character string giving the counting method. Options are 'summarizeOverlaps' and 'featureCounts'. For Windows system, only 'summarizeOverlaps' can be used, For Linux system, both methods can be used.

- `zero.based`  
  Logical. If TRUE, the start positions of the genomic intervals are 0-based, if FALSE, the start positions will be 1-based.

- `...`  
  additional arguments passed to `summarizeOverlaps` and `featureCounts` in Rsubread package

**Details**

This function provides two options 'summarizeOverlaps' from GenomicAlignments package and `featureCounts` from Rsubread package to count the aligned reads. As Rsubread package is only available for Linux systems, Windows users can only use 'summarizeOverlaps'. The user could specify counting details by passing additional arguments (...), otherwise the default settings of the two methods are used. For counting details, see `summarizeOverlaps`, `featureCounts` in Rsubread package

**Value**

A TCA object with updated 'count' slot.

**Author(s)**

Mengjun Wu

**See Also**

- `summarizeOverlaps`, `featureCounts` in Rsubread package
counts

Extracts counts of a TCA object.

Description

counts extract raw read counts stored in a TCA object or compute normalized counts.

Usage

## S4 method for signature 'TCA'
counts(object, normalization = "none", lib.norm = TRUE, 
    log = FALSE, ...)

## S4 replacement method for signature 'TCA'
counts(object) <- value

Arguments

object a TCA object

normalization character string giving the normalization method. Options are 'none' (original raw counts), 'cpm' (counts per million), 'rpkm' (reads per kilobase per million).

lib.norm logical indicating whether or not use effective library size (see 'Details' below) when normalization is 'cpm' or 'rpkm'.

log logical if TRUE, the returned value will be on a log2 scale.

... additional arguments passed to cpm or rpkm

value an integer matrix

Details

when calculating normalized counts, library size can be rescaled to minimize the log-fold changes between samples for most genomic features (e.g. genes, binding sites) by multiplying a scale factor. The rescaled library size is called effective library size. In this function, the scale factor is calculated using the weighted trimmed mean of M-values (TMM, Robinson et al (2010))

If log2 values are computed, a small count would be added to avoid logarithm of zero. a small count is set proportional to the library size, the average value of such small counts of all libraries counts is set to 0.25 by default.

Value

An integer matrix

Author(s)

Mengjun Wu

References

Examples

```r
data(tca_ATAC)
c <- counts(tca_ATAC)
# normalized counts table
c_norm <- counts(tca_ATAC, normalization='rpkm')
```

countsTable  
An example read Counts table

Description

A dataset of exemplary read counts

Usage

```r
data(countsTable)
```

Format

A data frame containing experiment design information for 12 samples/libraries.

Value

A data frame

Examples

```r
data(countsTable)
```

DBanalysis  
Perform differential binding analysis

Description

This function performs differential analysis by fitting read counts to a negative binomial generalized linear model.

Usage

```r
DBanalysis(object, categories = "timepoint", norm.lib = TRUE,
          filter.type = NULL, filter.value = NULL, samplePassfilter = 2, ...)
```
Arguments

- **object**: a TCA object.
- **categories**: character string indicating levels of which factor (column in the design slot) are compared in the differential analysis. For time course analysis, the default factor is `timepoint`.
- **norm.lib**: logical indicating whether or not use effective library size when perform normalization. See ‘Details’ of `counts`.
- **filter.type**: character string indicating which type of count (raw or normalized) is used when doing filtering. Options are ‘raw’, `cpm`, ‘rpkm’, ‘NULL’. NULL’ means no filtering will be performed.
- **filter.value**: A numeric value; if values of selected `filter.type` (‘raw’, `cpm`, ‘rpkm’) of a genomic feature are larger than the `filter.value` in at least a certain number (samplePassfilter) of samples/libraries for any of the conditions, such genomic feature will be kept; otherwise the genomic feature will be dropped.
- **samplePassfilter**: numeric value indicating the least number of samples/libraries a genomic feature with counts (raw or normalized) more than `filter.value` for all conditions if such genomic feature will be kept.
- ... additional arguments passed to `glmFit` from edgeR package.

Details

The differential event is detected by using the generalized linear model (GLM) methods (McCarthy et al, 2012). This function fits the read counts of each genes to a negative binomial glm by using `glmFit` function from edgeR. To further test the significance of changes, see `DBresult`, `TopDBresult`.

Value

A TCA object

Author(s)

Mengjun Wu, Lei Gu

References


See Also

`DBresult`, `TopDBresult`

Examples

data(tca_ATAC)
tca_ATAC <- DBanalysis(tca_ATAC)
DBresult

This function performs differential analysis by fitting read reads to GLM by DBanalysis. DBresult extracts the differential analysis results of given contrasts for all genomic features or genomic features with significant differential events. DBresult.cluster returns similar results while the results only contain genomic features belong to a given cluster.

Usage

DBresult(object, group1 = NULL, group2 = NULL, contrasts = NULL, 
p.adjust = "fdr", top.sig = FALSE, pvalue = "paj", 
pvalue.threshold = 0.05, abs.fold = 2, direction = "both", 
result.type = "GRangesList")

DBresult.cluster(object, group1 = NULL, group2 = NULL, contrasts = NULL, 
p.adjust = "fdr", top.sig = FALSE, pvalue = "paj", 
pvalue.threshold = 0.05, abs.fold = 2, direction = "both", cluster, 
cmthreshold = NULL, result.type = "GRangesList")

Arguments

object a TCA object, for DBresult, DBanalysis should already be called on the object; for DBresult.cluster, both DBanalysis and timeclust should be already called.

group1 character string giving the level to be compared, that is the denominator in the fold changes.

group2 a character vector giving other levels to compared with group1. that are numerator in the fold changes.

contrasts a character vector, each character string in the vector gives a contrast of two groups with the format group2vsgroup1’. group1 is the denominator level in the fold changes and group2 is the numerator level in the fold changes.


top.sig logical if TRUE, only genomic regions with significant differential events will be returned. Significant differential events are defined by log2-fold changes, p-values or adjusted p-values.

pvalue character string specify the type of p-values used to define significant differential events (‘PValue’ or adjusted p-value ‘paj’) 

pvalue.threshold a numeric value giving threshold of selected p-value. Significant differential events have lower (adjusted) p-values than the threshold.

abs.fold a numeric value, the least absolute log2-fold changes
DBresult

direction character string specify the direction of fold changes ('up' (positive fold changes),
down' (negative fold changes), both'(both positive and negative fold changes)).
Significant events have log2-fold changes exceeding abs.fold in defined directions.

result.type character string giving the data type of return value. Options are "GRangesList" and "list".

cluster an integer, the result tables of genomic features belong to the cluster are extracted.

cmthreshold a numeric value, this argument is applicable only if cmeans' clustering method is selected when calling timeclust function. if not NULL, the result table of genomic features that belong to the defined cluster and the membership values to this cluster exceed cmthreshold are extracted.

Details

This function uses glmLRT from edgeR which perform likelihood ratio tests for testing significance of changes. For more detail, see glmLRT

Value

A list or a GRangesList. If result.type is "GRangesList", a GRangesList is returned containing the differential analysis results for all provided contrasts. Each GRanges object of the list is one contrast, the analysis results are contained in 4 metadata columns:
logFC log2-fold changes of differential event between two tested.
PValue p-values.
paj adjusted p-values
id genomic feature name

If result.type is "list", a List of data frames is returned. Each data frame is one contrast and contains the following columns:
logFC log2-fold changes of differential event between two tested.
PValue p-values.
paj adjusted p-values
chr name of the chromosomes
start starting position of the feature in the chromosome
end ending position of the feature in the chromosome
id genomic feature name

Note

If not NULL group1, group2 and contrasts, result tables are extracted from comparisons in contrasts.

Author(s)

Mengjun Wu, Lei Gu

See Also

glmLRT
Examples

data(tca_ATAC)
tca_ATAC <- DBanalysis(tca_ATAC)
### extract differential analysis of 24h, 72h to 0h
# set the contrasts using the 'group1' and 'group2' parameters
res1 <- DBresult(tca_ATAC, group1 = '0h', group2 = c('24h', '72h'))
# one can get the same result by setting the contrasts using the 'contrasts' parameter
res2 <- DBresult(tca_ATAC, contrasts = c('24hvs0h', '72hvs0h'))
# extract significant differential events
res.sig <- DBresult(tca_ATAC, contrasts = c('24hvs0h', '72hvs0h'),
                     top.sig = TRUE)

# extract differential analysis of 24h, 72h to 0h of a given cluster
tca_ATAC <- timecourseTable(tca_ATAC, filter = TRUE)
tca_ATAC <- timeclust(tca_ATAC, algo = 'cm', k = 6)
res_cluster1 <- DBresult.cluster(tca_ATAC, group1 = '0h',
                                 group2 = c('24h', '72h'),
                                 cluster = 1)

---

**experiment**  
An example experiment design without BAM file information

**Description**

A dataset of exemplary experiment design without BAM file information

**Usage**

data(experiment)

**Format**

A data frame containing experiment design information for 12 samples/libraries.

**Value**

A data frame

**Examples**

data(experiment)
**experiment_BAMfile**

An example experiment design with BAM file information

**Description**

A dataset of exemplary experiment design with BAM file information

**Usage**

`data(experiment_BAMfile)`

**Format**

A data frame containing experiment design information for 12 samples/libraries.

**Value**

A data frame

**Examples**

`data(experiment_BAMfile)`

---

**genomicIntervals**

An example reference genomic regions

**Description**

A dataset of exemplary genomic regions

**Usage**

`data(genomicIntervals)`

**Format**

A data frame containing 2751 genomic regions.

**Value**

A data frame

**Examples**

`data(genomicIntervals)`
Description

This function merges genomic coordinates of a given data frame or reads in BED files (e.g. generated from a peak caller) under given directory and merge genomic regions that have overlapping genomic intervals into a single feature. The single feature represents the widest genomic interval that covers all merged regions.

Usage

peakreference(data = NULL, dir = NULL, pattern = NULL, merge = TRUE, overlap = 1, ratio = NULL)

Arguments

data
   a data frame containing coordinates information of peaks to be merged. Columns of the data frame should be consistent with the BED format where the first column is the name of the chromosome, the second column is the starting position and the third column is the ending position.

dir
   character string giving the directory where BED files are stored. If data is not given, the function will read in the BED files under code.

pattern
   an regular expression, only files that have names match the regular expression will be read in.

merge
   logical indicating whether to merge overlapped regions or not. If False, regions are simply combined.

overlap
   a numeric value giving the least number of base(s) two regions should overlap when merging them.

ratio
   a numeric value giving the threshold of overlapping ratio between two regions to merge them. See 'Details' below for the definition of the overlapping ratio.

Details

The overlapping ratio (OR) is defined as:

\[
OR = \frac{n}{\min(length(a), length(b))}
\]

\(a, b\) are two genomic regions, \(n\) is the number of overlapping bases between region \(a\) and region \(b\).

Value

a data frame with four columns: chr, start, stop, id

Author(s)

Mengjun Wu, Lei Gu
TCA-class

Examples

peaks <- data.frame(chr = c(rep('chr1',4),rep('chr2', 3), rep('chr3',2)),
                      start = c(100,148,230,300,330,480,1000,700,800),
                      end = c(150,220,500,450,600,900,1050,760,900))

merged_peaks <- peakreference(data = peaks, merge = TRUE, overlap = 1)

TCA-class

TCA class and constructor

Description

TCA is a S4 class for storing input data, results of differential binding and clustering analysis. A TCA object can be created by the constructor function from a table of sample information, a table genomic coordinates of features, read counts(optional).

Usage

TCA(design, counts = matrix(0L, 0L, 0L), genomicFeature, zero.based = TRUE)
TCAFromSummarizedExperiment(se, genomicFeature = NULL)

Arguments

design     a data frame containing information about samples/libraries. For time course analysis, design should contain at least three columns (case insensitive): sampleid, timepoint and group providing time point and group information of each sample/library. If counts is not provided when creating TCA object, the column BAMfile can be included in the design data frame, providing corresponding BAM filename of each sample/library, this information can be used for generating count table by using countReads function.

counts     an integer matrix containing read counts. Rows correspond to genomic features and columns to samples/libraries.

genomicFeature     a data frame or a GRanges object containing genomic coordinates of features of interest (e.g. genes in RNA-seq, binding regions in ChIP-seq). If genomicFeature is a data frame, four columns are required in genomicFeature: id, chr, start, end; if genomicFeature is a Granges object, the metadata column "id" is required. For TCAFromSummarizedExperiment, genomicFeature must be provided if se is a SummarizedExperiment object.

zero.based Logical. If TRUE, the start positions of the genomic ranges in the returned TCA object are 0-based, if FALSE, the start positions will be 1-based.

se            A SummarizedExperiment or a RangedSummarizedExperiment object. The object might contain multiple assays (count table) in the assay list, only the first one will be taken to construct TCA object. For SummarizedExperiment object, genomicFeature must be provided while for RangedSummarizedExperiment object, the genomic features will be extracted directly from the object.
Details

A TCA object can be created without providing read counts, read counts can be provided by \code{counts} or generated by \code{countReads}, the number of rows should equal to that in \code{genomicFeature} and the number of columns should equal to number of rows in design. Input data and analysis results in a TCA object can be accessed by using corresponding accessors and functions. The TCA objects also have a show method printing a compact summary of their contents see \code{counts}, \code{TCA.accessors}, \code{DBresult}, \code{tcTable}, \code{timeclust}, \code{clust}

Value

A TCA object

Author(s)

Mengjun Wu
Mengjun Wu

See Also

\code{counts}, \code{TCA.accessors}, \code{DBresult}, \code{timeclust}, \code{clust}

Examples

# create data frame of experiment design: 4 time points and 2 replicates for each time point.
d <- data.frame(sampleID = 1:8, group = rep(c(1, 2, 3, 4), 2),
    timepoint = rep(c('0h','24h','48h','72h'), 2))

# create data frame of genomic intervals of interest
gf <- data.frame(chr = rep(c('chr1', 'chr2', 'chr4'), 2),
    start = seq(100, 2000, by = 300),
    end = seq(100, 2000, by = 300) + 150,
    id = paste0('peak', 1:7))
tca <- TCA(design = d, genomicFeature = gf)
genomicFeature(tca)

# if count table is available
c <- matrix(sample(1000, 56), nrow = 7, dimnames = list(paste0('peak', 1:7), 1:8))
tca <- TCA(design = d, counts = c, genomicFeature = gf)
# replace the count table of a \code{TCA} object

c2 <- matrix(sample(500, 56), nrow = 7, dimnames = list(paste0('peak', 1:7), 1:8))

counts(tca) <- c2

---

\code{TCA.accessors} \hspace{1cm} \textit{Accessors to extract slots of a TCA class.}
Description

Accessors are provided to extract design, genomicFeature, tcTable, clustResults slots of a TCA class. The design slot stores experimental information of samples/libraries, the genomicFeature slot stores genomic coordinates of features, the tcTable slot stores time course data as a matrix, where rows are genomic features and columns time points. The clustResults slot stores results of clustering analysis as a clust object.

Usage

```r
## S4 method for signature 'TCA'
design(object)
genomicFeature(object)
tcTable(object)
## S4 method for signature 'TCA'
tcTable(object)
clustResults(object)
## S4 method for signature 'TCA'
clustResults(object)
```

Arguments

- object: TCA object

Value

- design returns a data frame.
- genomicFeature returns a data frame.
- tcTable returns a numeric matrix.
- clustResults returns a clust object, see clust for details.

Author(s)

Mengjun Wu

See Also

- clust

Examples

```r
data(tca_ATAC)
genomicFeature(tca_ATAC)
tcTable(tca_ATAC)
```
tca_ATAC

An example TCA object

Description
A TCA object storing exemplary ATAC-seq time course data, including the experiment design, counts table, reference genomic regions.

Usage
data(tca_ATAC)

Format
A TCA object of exemplary ATAC-seq time course data

Value
A TCA object

Examples
data(tca_ATAC)

---

timeclust
time course data clustering

Description
This function performs clustering analysis of time course data.

Usage
timeclust(x, algo, k, dist = "euclidean", centers = NULL, standardize = TRUE, ...)

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>x</td>
<td>a TCA object returned from timecourseTable or a matrix</td>
</tr>
<tr>
<td>algo</td>
<td>character string giving a clustering method. Options are km' (kmeans), 'pam' (partitioning around medoids), 'hc' (hierachical clustering), 'cm' (cmeans).</td>
</tr>
<tr>
<td>k</td>
<td>numeric value between 1 and n - 1 (n is the number of data points to be clustered).</td>
</tr>
<tr>
<td>dist</td>
<td>character string specifying method for distance(dissimilarity) calculation. It should be one of 'correlation' or one of the distance measure method in dist function (for example 'euclidean', 'manhattan')</td>
</tr>
<tr>
<td>centers</td>
<td>a numeric matrix giving intial centers for kmeans, pam or cmeans. If given, Number of rows of centers must be equal to k.</td>
</tr>
<tr>
<td>standardize</td>
<td>logical, if TRUE, z-score transformation will performed on the data before clustering. See 'Details' below.</td>
</tr>
</tbody>
</table>

... additional arguments passing to kmeans, pam, hclust, cmeans
Details

two types of clustering methods are provided: hard clustering (kmeans, pam, hclust) and soft clustering(cmeans). In Hard clustering, a data point can only be allocated to exactly one cluster (for hclust, cutree is used to cut a tree into clusters), while in soft clustering (also known as fuzzy clustering), a data point can be assigned to multiple clusters, membership values are used to indicate to what degree a data point belongs to each cluster. For more details, see the help() page of each function.

To avoid the influence of expression level to the clustering analysis, z-score transformation can be applied to covert the expression values to z-scores by performing the following formula:

\[ z = \frac{x - \mu}{\sigma} \]

\(x\) is value to be converted (e.g., a expression value of a genomic feature in one condition), \(\mu\) is the population mean (e.g., average expression value of a genomic feature in different conditions), \(\sigma\) is the standard deviation (e.g., standard deviation of expression of a genomic feature in different conditions).

Value

If \(x\) is a TCA object, a TCA object will be returned. If \(x\) is a matrix, a clust object will be returned.

Author(s)

Mengjun Wu

See Also

clust, kmeans, pam, hclust, cutree

Examples

```r
x <- matrix(rnorm(1600,sd=0.3), nrow = 200,
            dimnames = list(paste0(‘/Var’ peak, 1:200), 1:8))
clust_res <- timeclust(x, algo = ‘cm’, k = 4) # return a clust object
```

timeclustplot

Plot clustering results for time course data.

Description

This function plots the clusters generated from timeclust. For fuzzy cmeans clustering, data points are color-coded according to membership values, the color palettes can be customized.

Usage

timeclustplot(object = NULL, categories = "timepoint",
             value = "expression", cols = NULL, cl.color = "gray50",
             membership.color = rainbow(30, s = 3/4, v = 1, start = 1/6),
             title.size = 18, axis.line.size = 0.6, axis.title.size = 18,
             axis.text.size = 16, legend.title.size = 14, legend.text.size = 14)
timecourseTable

constructs time course table for clustering analysis

Description

This function constructs a time course table of which rows corresponding to genomic features and columns the timepoint. Values can be mean normalized read counts or log2-fold changes compared to the first timepoint. The time course table is used for clustering analysis.

Arguments

- **object**: a TCA object or a clust object
- **categories**: character string giving the x-axis label
- **value**: character string giving the y-axis label
- **cols**: integer value specifying number of columns in the final layout.
- **cl.color**: character string specifying a color for hard clustering.
- **membership.color**: color palettes, a character vector of n colors
- **title.size**: numeric value specifying the font size of title of each plot in the layout
- **axis.line.size**: numeric value specifying the size of both axis lines
- **axis.title.size**: numeric value specifying the font size of titles of both axis
- **axis.text.size**: numeric value specifying the font size of labels of both axis
- **legend.title.size**: numeric value specifying the font size of legend title
- **legend.text.size**: numeric value specifying the font size of legend text

Value

Plot all clusters in one plot and return a list of ggplot objects, each object is for one cluster. The ggplot object can be drawn by calling `print.ggplot`

Author(s)

Mengjun Wu

Examples

```R
x <- matrix(sample(500, 1600, replace = TRUE), nrow = 200,
            dimnames = list(paste0("Var", 1:200), 1:8))
clust_res <- timeclust(x, algo = "cm", k = 4, standardize = TRUE)
p <- timeclustplot(clust_res, cols = 2)
# to plot a individual cluster
print(p[[2]]) # plot cluster 2
print(p[[3]]) # plot cluster 3
```
timecourseTable

Usage
timecourseTable(object, value = "expression", lib.norm = TRUE,
    norm.method = "rpkm", subset = NULL, filter = FALSE, pvalue = "fdr",
    pvalue.threshold = 0.05, abs.fold = 2, direction = "both", ...)

Arguments

object         a TCA object returned by DBanalysis.
value          character string, either 'expression' or 'FC'. 'expression' is the mean normalized read counts of replicates, FC is the log2-fold changes compared to the first time point.
lib.norm       logical indicating whether or not use effective library size (see 'Details' in counts).
norm.method    character string specifying the normalization method if value is 'expression'.
subset         optional character vector giving a subset of genomic features. If not NULL, time course table is generated for only this subset of genomic features.
filter         logical, whether to drop the genomic features shows no significant changes (defined by pvalue, pvalue.threshold, abs.fold and direction) between any two time points.
pvalue         character string specify the type of p-values ('PValue' or adjusted p-value 'paj')
pvalue.threshold a numeric value giving threshold of selected p-value, only features with higher (adjusted) p-values than the threshold are kept.
abs.fold       a numeric value, the least absolute log2-fold changes
direction      character string specify the direction of fold changes ('up' (positive fold changes), 'down' (negative fold changes), 'both' (both positive and negative fold changes)), features changes more than abs.fold in the defined direction are kept.
...             additional arguments passing to rpkm, cpm

Value

A TCA object

Note

If 'expression' in value is chosen, for replicates, the normalized expression value is first calculated for each replicate, then mean value is taken to represent the normalized expression value.

Author(s)

Mengjun Wu

Examples
data(tca_ATAC)
tca_ATAC <- DBanalysis(tca_ATAC)
tca_ATAC <- timecourseTable(tca_ATAC, value = 'expression',
    lib.norm = TRUE, norm.method = 'rpkm')
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