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Contents

clust-class . . . 2
clust.accessors . . . 3
countReads . . . 4
counts . . . 5
countsTable . . . 6
DBanalysis . . . 7
DBresult . . . 8
experiment . . . 11
experiment_BAMfile . . . 12
genomicIntervals . . . 12
peakreference . . . 13
TCA-class . . . 14
TCA.accessors . . . 16
tca_ATAC . . . 17
timeclust . . . 17
timeclustplot . . . 19
timecourseTable . . . 20

Index **23**

`clust-class` *clust class*

Description

`clust` is a S4 class for storing results of the clustering analysis of 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 `clust` class contains the following slots:

`method` clustering method used

`dist` distance metric used

`data` a matrix of original or standardized data used in the analysis

`centers` a matrix of cluster centers

`cluster` an integer vector of length n (the integers are the indices of clusters the data points belong 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 of membership values of the data points to each 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, and membership slots stored in a clust class.

Usage

```
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 object

Value

clustData returns a 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 *count mapped reads overlap genomic intervals*

Description

This function counts mapped reads from multiple BAM files overlapping genomic intervals in genomicFeature in a TCA object. The resulted count table is stored in count slot of the TCA object.

Usage

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

Arguments

object	a TCA object.
dir	character string giving the directory of BAM files.
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 in GenomicAlignments package or featureCounts in Rsubread package.

Details

This function provides two options to count the mapped reads: "summarizeOverlaps" in the GenomicAlignments package and "featureCounts" in the Rsubread package. As Rsubread package is only avaible for linux systems, Windows users can only choose "summarizeOverlaps". The user could further customize the counting paramters by passing additional arguments (...), otherwise the default settings of the two methods will be used. For details of the counting parameters, see summarizeOverlaps, featureCounts.

Value

A TCA object with updated count slot.

Author(s)

Mengjun Wu

See Also

summarizeOverlaps, featureCounts

---

counts *Extracts counts of a TCA object.*

Description

counts extract raw read counts stored in a TCA object or compute normalized counts from the raw 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 in the edgeR package.
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. The actual added count will be scaled according to the library size, for details see addPriorCount in the edgeR package when not specified, the prior count is set to 0.25 by default.

Value

An integer matrix

Author(s)

Mengjun Wu

References

Robinson, M. D., & Oshlack, A. (2010). A scaling normalization method for differential expression analysis of RNA-seq data. Genome biology, 11(3), 1.

Examples

```
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

```
data(countsTable)
```

Format

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

Value

A data frame

Examples

```
data(countsTable)
```

DBanalysis *Perform differential expression analysis*

Description

This function is a wrapper for the glmFit in edgeR package.

Usage

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

Arguments

object	a TCA object.
categories	character string giving which column in design will be used for differential analysis. For time course analysis, the default column is "timepoint".
norm.lib	logical indicating whether or not use effective library size when perform normalization. See counts for more details.
filter.type	character string indicating which type of count (raw or normalized) is used when performing filtering. Options are "raw", "cpm", "rpkm", "NULL". No filtering will be performed when using "NULL'.
filter.value	a numberic value; minimum values of selected filter.type ("raw", "cpm", "rpkm"). It is used in combination with samplePassfilter.
samplePassfilter	a numberic value indicating the minimum number of samples/libraries in which a genomic feature has counts value (raw or normalized) more than filter.value. Smaller than this number, the genomic feature will be filtered out.
...	additional arguments passed to glmFit from edgeR package.

Details

The differetial 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

McCarthy,D.J.,Chen, Y., & Smyth, G. K.(2012). Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. Nucleic acids research 40, 4288-4297.

See Also

DBresult, TopDBresult

Examples

```
data(tca_ATAC)
tca_ATAC <- DBanalysis(tca_ATAC)
```

DBresult *This function tests for differential expression*

Description

This function is a wrapper for glmLRT in edgeR package. It performs likelihood ratio tests for given coefficinets contrasts after fitting read counts to a negative binomial glm by DBanalysis. DBresult also extracts the diffential analysis results of given contrasts at a chosen significance level. DBresult.cluster returns similar results but 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 group to be compared with, i.e., the denominator in the fold changes. group1 can be set NULL and will be ignored if the comparisons are passed to contrasts
group2	a character vetor giving the other groups to compare with group1, i.e., the numerator in the fold changes. group2 can be set NULL and will be ignored if the comparisons are passed to contrasts
contrasts	a character vector, each 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.
p.adjust	character string specifying a correction method for p-values. Options are "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", and "none".
top.sig	logical if TRUE, only genomic regions with given log2-fold changes and significance levels (p-value) will be returned. Log2-fold changes are defined by abs.fold and direction; significance levels are defined by pvalue and pvalue.threshold
pvalue	character string specify the type of p-values used for defining the significance level(PValue or adjusted p-value paj)
pvalue.threshold	a numeric value giving threshold of selected p-value, Significant changes have lower (adjusted) p-values than the threshold.
abs.fold	a numeric value, the minimum absolute log2-fold changes. The returned genomic regions have changes with absolute log2-fold changes exceeding abs.fold.
direction	character string specify the direction of fold changes. "up": positive fold changes; "down": negative fold changes; "both": both positive and negative fold changes.

result.type	character string giving the data type of return value. Options are "GRangesList" and "list".
cluster	an integer giving the number of cluster from which genomic features 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 the significance of changes. For more deatils, 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 between two groups.

PValue p-values.

paj adjusted p-values

id name of genomic features

If result.type is "list", a list of data frames is returned. Each data frame contains one contrast with the following columns:

logFC log2-fold changes between two groups.

PValue p-values.

paj adjusted p-values

chr name of chromosomes

start starting positions of features in the chromosomes

end ending postitions of features in the chromosomes

id name of genomic features

Note

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

Author(s)

Mengjun Wu, Lei Gu

See Also

glmLRT

Examples

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

# extract differntial 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 infomration*

Description

A dataset of exemplary experiment design without BAM file infomration

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 infomration*

Description

A dataset of exemplary experiment design with BAM file infomration

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)
```

peakreference *combine and merge multiple BED files*

Description

This function merges overlapping genomic regions into a single feature. The merged single feature represents the widest genomic interval that covers all overlapping regions.

Usage

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

Arguments

data	a data frame containg coordinates information of peaks to be merged. Columns of the data frame should be consistent with the BED format where the first column contains chromosome information, the second column the starting position, and the third column the ending position.
dir	a character string giving the directory where BED files are stored. If data is not given, the function will reads 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 numberic value giving the least number of base(s) two regions should overlap when merging them.
ratio	a numberic value giving the thresold 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

Examples

```
peaks <- data.frame(chr = c(rep('chr1',4),rep('chr2', 3), rep('chr3',2)),
                    start = c(100,148,230,300,330,480,1000,700,801),
                    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 analysis and clustering analysis. A TCA object can be created by the constructor function taking a table of sample information, a table of the genomic coordinates of features, and read count table (optional).

Usage

```
TCA(design, counts = matrix(0L, 0L, 0L), genomicFeature, zero.based = TRUE)

TCAFromSummarizedExperiment(se, genomicFeature = NULL)
```

Arguments

design	a data frame containing information of samples/libraries. For time course analysis, design table 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, an optional column bamfile can be used to provide BAM filename of each sample/library and generate count table using countReads function later.
counts	an integer matrix containing read counts. Rows correspond to genomic features and columns to samples/libraries. The name of column s should be the same as the time points in design.
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 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 counts or generated by countReads. For the read counts, the number of rows should equal to that in 'genomicFeature and the number of columns should equal to number of rows in design; in addition, the name of column names should be the same as the time points 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 counts, TCA.accessors, DBresult, tcTable, timeclust. clust

Value

A TCA object

Author(s)

Mengjun Wu

See Also

counts, TCA.accessors, DBresult, timeclust, 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 = c(rep('chr1', 3), rep('chr2', 2), rep('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
```

TCA.accessors *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 couse 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

```
## 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 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

```
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, read counts, 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 couse data clustering*

Description

This function performs clustering analysis of the time course data.

Usage

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

Arguments

x	a TCA object returned from timecourseTable or a matrix
algo	a character string giving a clustering method. Options are "km" (kmeans), "pam" (partitioning around medoids), "hc" (hierachical clustering), "cm" (cmeans).
k	a numeric value between 1 and $n - 1$ (n is the number of data points to be clustered).
dist	a character string specifying either "distance" or "correlation" will be used to measure the distance between data points.
dist.method	a character string. It can be chosen from one of the correlation methods in cor function ("pearson", "spearman", "kendall") if dist is "correlation", or one of the distance measure methods in dist function (for example, "euclidean", "manhattan") if dist is "distance".
centers	a numeric matrix giving intial centers for kmeams, pam or cmeans. If given, number of rows of the matrix must be equal to k.
standardize	logical, if TRUE, z-score transformation will performed on the data before clustering. See 'Details' below.
...	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.

To better capture the differences of temporal patterns rather than expression levels, z-score transformation can be applied to covert the the expression values to z-scores by performing the following formula:

$$z = \frac{x - \mu}{\sigma}$$

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

```
example.mat <- matrix(rnorm(1600,sd=0.3), nrow = 200,
            dimnames = list(paste0('peak', 1:200), 1:8))
clust_res <- timeclust(x = example.mat, 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
)
```

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 drawed by calling `print.ggplot`

Author(s)

Mengjun Wu

Examples

```
x <- matrix(sample(500, 1600, replace = TRUE), nrow = 200,
            dimnames = list(paste0('peak', 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 *constructs time course table for clustering analysis*

Description

This function constructs a time course table of which rows are genomic features and columns time points. values can be normalized expression levels or log2-fold changes compared to a control time point. The time course table is used for clustering analysis.

Usage

```
timecourseTable(
  object,
  value = "expression",
  control.group = NULL,
  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	a 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.
control.group	a character string giving the time point to be compared with, i.e., the denominator in the fold changes. It should match one of the time points in the design table in the TCA object.
lib.norm	logical indicating whether or not use effective library size (see "Details" in counts).
norm.method	a character string specifying the normalization method if value is "expression"
subset	an optinal 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: "none" is unadjusted p-value or one of adjusted p-value "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr".
pvalue.threshold	a numeric value giving threshold of selected p-value, significant changes have lower (adjusted) p-values than the threshold.
abs.fold	a numeric value, the least minimum log2-fold changes. The returned genomic regions have changes with absolute log2-fold changes exceeding abs.fold.
direction	character string specify the direction of fold changes. "up": positive fold changes; "down": negative fold changes; "both": both positive and negative fold changes.
...	additional arguments passing to rpkm, cpm

Value

A TCA object

Note

If "expression" in value is chosen, the average normalized expression values of replicates for each group will be calculated and returned.

Author(s)

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Examples

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

Index

∗ **datasets**
countsTable, 6
experiment, 11
experiment_BAMfile, 12
genomicIntervals, 12
tca_ATAC, 17

addPriorCount, *5*

clust, *3, 4*, *15, 16*, *18*
clust *(*clust-class*)*, 2
clust-class, 2
clust.accessors, 3
clustCenters *(*clust.accessors*)*, 3
clustCenters,clust-method *(*clust.accessors*)*, 3
clustCluster *(*clust.accessors*)*, 3
clustCluster,clust-method *(*clust.accessors*)*, 3
clustData *(*clust.accessors*)*, 3
clustData,clust-method *(*clust.accessors*)*, 3
clustMembership *(*clust.accessors*)*, 3
clustMembership,clust-method *(*clust.accessors*)*, 3
clustResults *(*TCA.accessors*)*, 16
clustResults,TCA-method *(*TCA.accessors*)*, 16
cmeans, *18*
cor, *18*
countReads, 4, *14, 15*
counts, 5, *7*, *15*, *21*
counts,TCA-method *(*counts*)*, 5
counts<-,TCA-method *(*counts*)*, 5
countsTable, 6
cpm, *5*, *21*
cutree, *18*

DBanalysis, 7, *8*
DBresult, 8, *15*
design *(*TCA.accessors*)*, 16
design,TCA-method *(*TCA.accessors*)*, 16
dist, *18*

experiment, 11
experiment_BAMfile, 12

featureCounts, *4, 5*

genomicFeature *(*TCA.accessors*)*, 16
genomicFeature,TCA-method *(*TCA.accessors*)*, 16
genomicIntervals, 12
glmFit, *7*
glmLRT, *8*, *10*

hclust, *18*

kmeans, *18*

pam, *18*
peakreference, 13
print.ggplot, *20*

rpkm, *5*, *21*

summarizeOverlaps, *4, 5*

TCA *(*TCA-class*)*, 14
TCA-class, 14
TCA.accessors, *15*, 16
tca_ATAC, 17
TCAFromSummarizedExperiment *(*TCA-class*)*, 14
tcTable, *15*
tcTable *(*TCA.accessors*)*, 16
tcTable,TCA-method *(*TCA.accessors*)*, 16
timeclust, *2, 3*, *10*, *15*, 17, *19*
timeclustplot, 19
timecourseTable, *18*, 20