Package ‘CAGEr’

April 3, 2024

Title Analysis of CAGE (Cap Analysis of Gene Expression) sequencing data for precise mapping of transcription start sites and promoterome mining

Version 2.8.0

Date 2023-10-07

Imports BiocGenerics, BiocParallel, BSgenome, CAGEfightR, data.table, DelayedArray, DelayedMatrixStats, formula.tools, GenomeInfoDb, GenomicAlignments, GenomicRanges (>= 1.37.16), ggplot2 (>= 2.2.0), gtools, IRanges (>= 2.18.0), KernSmooth, memoise, plyr, Rsamtools, reshape2, rtracklayer, S4Vectors (>= 0.27.5), som, stringdist, stringi, SummarizedExperiment, utils, vegan, VGAM

Depends methods, MultiAssayExperiment, R (>= 4.1.0)

Suggests BSgenome.Drerio.UCSC.danRer7, DESeq2, FANTOM3and4CAGE, BiocStyle, knitr, rmarkdown

Description Preprocessing of CAGE sequencing data, identification and normalization of transcription start sites and downstream analysis of transcription start sites clusters (promoters).

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biocViews Preprocessing, Sequencing, Normalization, FunctionalGenomics, Transcription, GeneExpression, Clustering, Visualization


LazyData true
VignetteBuilder  knitr
RoxygenNote  7.2.1
Roxygen  list(markdown = TRUE)
Encoding  UTF-8
git_url  https://git.bioconductor.org/packages/CAGEr
git_branch  RELEASE_3.18
git_last_commit  6ad953b
git_last_commit_date  2023-10-24
Repository  Bioconductor 3.18
Date/Publication  2024-04-03
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CAGEr-package  

Analysis of CAGE (Cap Analysis of Gene Expression) sequencing data for precise mapping of transcription start sites and promoterome mining

Description

The CAGEr package performs identification of transcription start sites and frequency of their usage from input CAGE sequencing data, normalization of raw CAGE tag count, clustering of TSSs into tag clusters (TC) and their aggregation across multiple CAGE experiments to construct the promoterome. It manipulates multiple CAGE experiments at once, performs expression profiling across experiments both at level of individual TSSs and clusters of TSSs, exports several different types of track files for visualization in the UCSC Genome Browser, performs analysis of promoter width and detects differential usage of TSSs (promoter shifting) between samples. Multicore option for parallel processing is supported on Unix-like platforms.

Author(s)

Vanja Haberle

.byCtss  

Apply functions to identical CTSSes.

Description

.byCTSS is a private function using data.table objects to preform grouping operations at a high performance. These functions use non-standard evaluation in a context that raises warnings in R CMD check. By separating these functions from the rest of the code, I hope to make the workarounds easier to manage.
Usage

`.byCtss(ctssDT, colName, fun)`

## S4 method for signature 'data.table'
`byCtss(data.table, colName, fun)`

Arguments

crssDT A `data.table` representing CTSSes.
colName The name of the column on which to apply the function.
fun The function to apply.

Examples

crssDT <- data.table::data.table(
    chr = c("chr1", "chr1", "chr1", "chr2"),
    pos = c(1, 1, 2, 1),
    strand = c("+", "+", "+", "-" ),
    tag_count = c(1, 1, 1, 1))
crssDT
CAGEr:::byCtss(crssDT, "tag_count", sum)

---

`.get.quant.pos  Get quantile positions

Description

Private function that calculates position of quantiles for CTSS clusters based on distribution of tags within the clusters.

Usage

`.get.quant.pos(cum.sums, clusters, q)`

Arguments

cum.sums Named list of vectors containing cumulative sum for each cluster (returned by the CTSScumulativesTagClusters or CTSScumulativesCC function).
clusters `TagClusters` or `ConsensusClusters` object representing tag clusters or consensus clusters.
q desired quantiles - single value or a vector of values.

Value

Returns the `clusters` object with one more metadata column per value in q, containing Rle integers giving the relative distance of the quantile boundaries to the start position.
Examples

cum.sums <- RleList(`1` = Rle(1), `2` = cumsum(Rle(c(1, 1, 1, 2, 4, 0, 1, 1))))
clusters <- GRanges(c("chr1:100-101", "chr1:120-127"))
CAGEr:::.get.quant.pos(cum.sums, clusters, c(.2, .8))

Description

Private function for normalizing CAGE tag count to a referent power-law distribution.

Usage

.powerLaw(tag.counts, fitInRange = c(10, 1000), alpha = 1.25, T = 10^6)

Arguments

tag.counts          Numerical values whose reverse cumulative distribution will be fitted to power-law (e.g. tag count or signal for regions, peaks, etc.)
fitInRange          Range in which the fitting is done (values outside of this range will not be considered for fitting)
alpha               Slope of the referent power-law distribution (the actual slope has negative sign and will be -1*alpha)
T                    total number of tags (signal) in the referent power-law distribution.

Details

S4 Methods are provided for integer vectors, Rle objects, data.frame objects and DataFrame objects, so that the most complex objects can be deconstructed in simpler parts, normalized and re-constructed.

Value

Normalized values (vector of the same length as input values); i.e. what would be the value of input values in the referent distribution. Output objects are numeric, possibly Rle-encoded or wrapped in data.frames or DataFrames according to the input.

References

aggregateTagClusters

Description

Aggregates tag clusters (TCs) across all CAGE datasets within the CAGEr object to create a referent set of consensus clusters.

Usage

```r
aggregateTagClusters(
  object,
  tpmThreshold = 5,
  excludeSignalBelowThreshold = TRUE,
  qLow = NULL,
  qUp = NULL,
  maxDist = 100,
  useMulticore = FALSE,
  nrCores = NULL
)
```

## S4 method for signature 'CAGEr'

```r
aggregateTagClusters(
  object,
  tpmThreshold = 5,
  excludeSignalBelowThreshold = TRUE,
  qLow = NULL,
  qUp = NULL,
  maxDist = 100,
  useMulticore = FALSE,
  nrCores = NULL
)
```

Arguments

- **object**  
  A CAGEr object

- **tpmThreshold**  
  Ignore tag clusters with normalized signal < tpmThreshold when constructing the consensus clusters.

- **excludeSignalBelowThreshold**  
  When TRUE the tag clusters with normalized signal < tpmThreshold will not contribute to the total CAGE signal of a consensus cluster. When set to FALSE all TCs that overlap consensus clusters will contribute to the total signal, regardless whether they pass the threshold for constructing the clusters or not.

- **qLow, qUp**  
  Set which "lower" (or "upper") quantile should be used as 5' (or 3') boundary of the tag cluster. If NULL the start (for qLow) or end (for qUp) position of the TC is used.
aggregateTagClusters

Maximal length of the gap (in base-pairs) between two tag clusters for them to be part of the same consensus clusters.

Logical, should multicore be used (supported only on Unix-like platforms).

Number of cores to use when useMulticore = TRUE. Default (NULL) uses all detected cores.

Details

Since the tag clusters (TCs) returned by the clusterCTSS function are constructed separately for every CAGE sample within the CAGEr object, they can differ between samples in both their number, genomic coordinates, position of dominant TSS and overall signal. To be able to compare all samples at the level of clusters of TSSs, TCs from all CAGE datasets are aggregated into a single set of consensus clusters. First, TCs with signal \( \geq \) tpmThreshold from all CAGE datasets are selected, and their 5' and 3' boundaries are determined based on provided qLow and qUp parameter (or the start and end coordinates, if they are set to NULL). Finally, the defined set of TCs from all CAGE datasets is reduced to a non-overlapping set of consensus clusters by merging overlapping TCs and TCs \( \leq \) maxDist base-pairs apart. Consensus clusters represent a referent set of promoters that can be further used for expression profiling or detecting "shifting" (differentially used) promoters between different CAGE samples.

Value

Returns the object in which the experiment consensusClusters will be occupied by a RangedSummarizedExperiment containing the cluster coordinates as row ranges, and their expression levels in the counts and normalized assays. These genomic ranges are returned by the consensusClustersGR function and the whole object can be accessed with the consensusClustersSE function. The CTSS ranges of the tagCountMatrix experiment will gain a cluster column indicating which cluster they belong to. Lastly, the number of CTSS outside clusters will be documented in the outOfClusters column data.

Author(s)

Vanja Haberle
Charles Plessy

See Also

Other CAGEr object modifiers: CTSStoGenes(), CustomConsensusClusters(), annotateCTSS(), clusterCTSS(), cumulativeCTSSdistribution(), getCTSS(), normalizeTagCount(), quantilePositions(), quickEnhancers(), resetCAGEexp(), summariseChrExpr()

Other CAGEr clusters functions: CTTSSclusteringMethod(), CTTSScumulativesTagClusters(), CustomConsensusClusters(), clusterCTSS(), consensusClustersDESeq2(), consensusClustersGR(), cumulativeCTSSdistribution(), plotInterquantileWidth(), quantilePositions(), tagClustersGR()

Examples

consensusClustersGR(exampleCAGEexp)
ce <- aggregateTagClusters( exampleCAGEexp, tpmThreshold = 50 , excludeSignalBelowThreshold = FALSE, maxDist = 100)
annotateCTSS

Annotate and compute summary statistics

Description

annotateCTSS annotates the CTSS of a CAGEexp object and computes annotation statistics. annotateConsensusClusters annotates the consensus clusters of a CAGEr object.

Usage

annotateCTSS(object, ranges, upstream = 500, downstream = 500)

## S4 method for signature 'CAGEexp,GRanges'
annotateCTSS(object, ranges, upstream = 500, downstream = 500)

annotateConsensusClusters(object, ranges, upstream = 500, downstream = 500)

## S4 method for signature 'CAGEexp,GRanges'
annotateConsensusClusters(object, ranges, upstream = 500, downstream = 500)

Arguments

object CAGEexp object.
ranges A GRanges object, optionally containing gene_name, type and transcript_type metadata.
upstream Number of bases upstream the start of the transcript models to be considered as part of the promoter region.
downstream Number of bases downstream the start of the transcript models to be considered as part of the promoter region.

Value

annotateCTSS returns the input object with the following modifications:
• The Genomic Ranges of the tagCountMatrix experiment gains an annotation metadata column, with levels such as promoter, exon, intron and unknown. If the annotation has a gene_name metadata, then a genes column is also added, with gene symbols from the annotation.

• The sample metadata gets new columns, indicating total counts in each of the annotation levels. If the annotation has a gene_name metadata, then a genes column is added to indicate the number of different gene symbols detected.

annotateConsensusClusters returns the input object with the same modifications as above.

Author(s)
Charles Plessy

See Also

CTSStoGenes, and the exampleZv9_annot example data.

Other CAGEr object modifiers: CTSStoGenes(), CustomConsensusClusters(), aggregateTagClusters(), clusterCTSS(), cumulativeCTSSdistribution(), getCTSS(), normalizeTagCount(), quantilePositions(), quickEnhancers(), resetCAGEexp(), summariseChrExpr()

Other CAGEr annotation functions: plotAnnot(), ranges2annot(), ranges2genes(), ranges2names()

Examples
annotateCTSS(exampleCAGEexp, exampleZv9_annot)
colData(exampleCAGEexp)

annotateConsensusClusters(exampleCAGEexp, exampleZv9_annot)
consensusClustersGR(exampleCAGEexp)

bam2CTSS

Description
Converts from BAM to CTSS

Usage

bam2CTSS(gr, removeFirstG, correctSystematicG, genome)

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>gr</td>
<td>A GRanges object returned by import.bam.</td>
</tr>
<tr>
<td>removeFirstG</td>
<td>See getCTSS().</td>
</tr>
<tr>
<td>correctSystematicG</td>
<td>See getCTSS().</td>
</tr>
<tr>
<td>genome</td>
<td>See coerceInBSgenome().</td>
</tr>
</tbody>
</table>
Details
Converts genomic ranges representing SAM/BAM alignments into a CTSS object.

Value
Returns a CTSS object.

See Also
Other loadFileIntoGPos: import.CTSS(), import.bam.ctss(), import.bam(), import.bedCTSS(), import.bedScore(), import.bedmolecule(), loadFileIntoGPos(), moleculesGR2CTSS()

CAGEexp-class
CAGEr class to hold all data and metadata about one CAGE experiment.

Description
The CAGEr class is a MultiAssayExperiment object containing all data and metadata about a set of CAGE libraries. It replaced the CAGEset class in 2017. The main difference is that the expression data is stored in DataFrame objects of Rle-encoded expression values, instead of plain data.frames. With large datasets, this saves considerable amounts of memory.

Details
If genomeName is NULL, checks of chromosome names will be disabled and G-correction will not be possible. See https://support.bioconductor.org/p/86437/ for an example on how to create a BSgenome package.

Sample labels must be syntactically valid in the sense of the make.names() function, because they will be used as column names in some tables.

Slots
metadata A list that must at least contain a genomeName member.

See Also
make.names

Examples
pathsToInputFiles <- list.files( system.file("extdata", package = "CAGEr")
   , "ctss$"
   , full.names = TRUE)
sampleLabels <- sub( ".chr17.ctss", ",", basename(pathsToInputFiles))

# The CAGEexp object can be created using specific constructor commands
```
exampleCAGEexp <-
   CAGEexp( genomeName = "BSgenome.Drerio.UCSC.danRer7"
         , inputFiles = pathsToInputFiles
         , inputFilesType = "ctss"
         , sampleLabels = sub( ".chr17.ctss", ",", basename(pathsToInputFiles)))

# Alternatively, it can be created just like another MultiAssayExperiment.
# This is useful when providing pre-existing colData with many columns.

exampleCAGEexp <-
   CAGEexp( metadata = list(genomeName = "BSgenome.Drerio.UCSC.danRer7")
         , colData = DataFrame( inputFiles = pathsToInputFiles
                              , sampleLabels = sampleLabels
                              , inputFilesType = "ctss"
                              , row.names = sampleLabels))

# Expression data is loaded by the getCTSS() function, that also calculates
# library sizes and store them in the object's column data.

exampleCAGEexp <- getCTSS(exampleCAGEexp)
librarySizes(exampleCAGEexp)
colData(exampleCAGEexp)

# CTSS data is stored internally as a SummarizedExperiment that can be retrieved
# as a whole, or as GRanges, or as an expression DataFrame.

CTSStagCountSE(exampleCAGEexp)
CTSScoordinatesGR(exampleCAGEexp)
CTSStagCountDF(exampleCAGEexp)

# Columns of the "colData" table are accessible directly via the "$" operator.

exampleCAGEexp$l1 <- CTSStagCountDF(exampleCAGEexp) |> sapply ( \(col) sum(col > 0) )
exampleCAGEexp$l1
```

---

**CAGEr-class**

**CAGEr objects**

**Description**

The *CAGEr* package provides one class of objects to load, contain and process CAGE data: the *CAGEexp* class, introduced 2017, which is based on the *MultiAssayExperiment* class. In comparison with the original *CAGEset* class (removed in 2021) *CAGEexp* objects benefit from a more efficient data storage, using DataFrames of run-length-encoded (Rle) integers, allowing for the loading and use of much larger transcriptome datasets.
References


---

**CAGEr_Multicore**  
*Multicore support in CAGEr*

---

**Description**

CAGEr is in the transition towards using the BiocParallel for multicore parallelisation. On Windows platforms, the multicore support is disabled transparently, that is, attempts to use multiple cores are silently ignored.

**Usage**

```r
CAGEr_Multicore(useMulticore = FALSE, nrCores = NULL)
```

**Arguments**

- `useMulticore`: TRUE or FALSE
- `nrCores`: number of cores to use (leave NULL to let BiocParallel choose).

**Value**

Returns either a `MulticoreParam` object or a `SerialParam` object.

**Author(s)**

Charles Plessy

**Examples**

```r
CAGEr:::CAGEr_Multicore()
CAGEr:::CAGEr_Multicore(TRUE,)
CAGEr:::CAGEr_Multicore(TRUE, 3)
CAGEr:::CAGEr_Multicore(FALSE, 3)
```
Cluster CTSSs into tag clusters

**Description**

Clusters individual CAGE transcription start sites (CTSSs) along the genome into tag clusters (TCs) using specified *ab initio* method, or assigns them to predefined genomic regions.

**Usage**

```r
clusterCTSS(
  object,
  threshold = 1,
  nrPassThreshold = 1,
  thresholdIsTpm = TRUE,
  method = c("distclu", "paraclu", "custom"),
  maxDist = 20,
  removeSingletons = FALSE,
  keepSingletonsAbove = Inf,
  minStability = 1,
  maxLength = 500,
  reduceToNonoverlapping = TRUE,
  customClusters = NULL,
  useMulticore = FALSE,
  nrCores = NULL
)
```

### S4 method for signature 'CAGEexp'

```r
clusterCTSS(
  object,
  threshold = 1,
  nrPassThreshold = 1,
  thresholdIsTpm = TRUE,
  method = c("distclu", "paraclu", "custom"),
  maxDist = 20,
  removeSingletons = FALSE,
  keepSingletonsAbove = Inf,
  minStability = 1,
  maxLength = 500,
  reduceToNonoverlapping = TRUE,
  customClusters = NULL,
  useMulticore = FALSE,
  nrCores = NULL
)
```

**Arguments**

- **object**
  - A `CAGEr` object.
- **threshold**
  - A non-negative integer.
- **nrPassThreshold**
  - A non-negative integer.
- **thresholdIsTpm**
  - A logical value.
- **method**
  - A character vector.
- **maxDist**
  - A non-negative integer.
- **removeSingletons**
  - A logical value.
- **keepSingletonsAbove**
  - A non-negative integer.
- **minStability**
  - A non-negative integer.
- **maxLength**
  - A non-negative integer.
- **reduceToNonoverlapping**
  - A logical value.
- **customClusters**
  - A `data.frame`
- **useMulticore**
  - A logical value.
- **nrCores**
  - An integer.
threshold, nrPassThreshold
  Ignore CTSSs with signal < threshold in < nrPassThreshold experiments.
thresholdIsTpm
  Logical indicating if threshold is expressed in raw tag counts (FALSE) or normalized signal (TRUE).
method
  Method to be used for clustering: "distclu", "paraclu" or "custom". See Details.
maxDist
  Maximal distance between two neighbouring CTSSs for them to be part of the same cluster. Used only when method = "distclu", otherwise ignored.
removeSingletons
  Logical indicating if tag clusters containing only one CTSS be removed. Ignored when method = "custom".
keepSingletonsAbove
  Controls which singleton tag clusters will be removed. When removeSingletons = TRUE, only singletons with signal < keepSingletonsAbove will be removed. Useful to prevent removing highly supported singleton tag clusters. Default value Inf results in removing all singleton TCs when removeSingletons = TRUE. Ignored when method = "custom".
minStability
  Minimal stability of the cluster, where stability is defined as ratio between maximal and minimal density value for which this cluster is maximal scoring. For definition of stability refer to Frith et al., Genome Research, 2007. Clusters with stability < minStability will be discarded. Used only when method = "paraclu".
maxLength
  Maximal length of cluster in base-pairs. Clusters with length > maxLength will be discarded. Ignored when method = "custom".
reduceToNonoverlapping
  Logical, should smaller clusters contained within bigger cluster be removed to make a final set of tag clusters non-overlapping. Used only method = "paraclu".
customClusters
  Genomic coordinates of predefined regions to be used to segment the CTSSs. The format is either a GRanges object or a data.frame with the following columns: chr (chromosome name), start (0-based start coordinate), end (end coordinate), strand (either "+", or "-"). Used only when method = "custom".
useMulticore
  Logical, should multicore be used. useMulticore = TRUE has no effect on non-Unix-like platforms.
rnCores
  Number of cores to use when useMulticore = TRUE. Default value NULL uses all detected cores.

Details

The "distclu" method is an implementation of simple distance-based clustering of data attached to sequences, where two neighbouring TSSs are joined together if they are closer than some specified distance (see distclu-functions for implementation details).

"paraclu" is an implementation of Paraclu algorithm for parametric clustering of data attached to sequences (Frith et al., Genome Research, 2007). Since Paraclu finds clusters within clusters (unlike distclu), additional parameters (removeSingletons, keepSingletonsAbove, minStability, maxLength and reduceToNonoverlapping) can be specified to simplify the output by discarding
too small (singletons) or too big clusters, and to reduce the clusters to a final set of non-overlapping clusters.

Clustering is done for every CAGE dataset within the CAGEr object separately, resulting in a different set of tag clusters for every CAGE dataset. TCs from different datasets can further be aggregated into a single referent set of consensus clusters by calling the `aggregateTagClusters` function.

**Value**

Returns the `CAGEexp` object, in which, the results will be stored as a GRangesList of `TagClusters` objects in the metadata slot `tagClusters`. The `TagClusters` objects will contain a `filteredCTSSidx` column if appropriate. The clustering method name is saved in the metadata slot of the GRangesList.

**Author(s)**

Vanja Haberle

**References**


**See Also**

tagClustersGR, `aggregateTagClusters` and `CTSSclusteringMethod`.

Other CAGEr object modifiers: `CTSStoGenes()`, `CustomConsensusClusters()`, `aggregateTagClusters()`, `annotateCTSS()`, `cumulativeCTSSdistribution()`, `getCTSS()`, `normalizeTagCount()`, `quantilePositions()`, `quickEnhancers()`, `resetCAGEexp()`, `summariseChrExpr()`

Other CAGEr clusters functions: `CTSSclusteringMethod()`, `CTSScumulativesTagClusters()`, `CustomConsensusClusters()`, `aggregateTagClusters()`, `consensusClustersDESeq2()`, `consensusClustersGR()`, `cumulativeCTSSdistribution()`, `plotInterquantileWidth()`, `quantilePositions()`, `tagClustersGR()`

**Examples**

```
# Using 'distclu', notice argument 'maxDist'
ce <- clusterCTSS( exampleCAGEexp, threshold = 50, thresholdIsTpm = TRUE,
                   nrPassThreshold = 1, method = "distclu", maxDist = 20,
                   removeSingletons = TRUE, keepSingletonsAbove = 100)
tagClustersGR(ce, "Zf.30p.dome")

# Using 'paraclu', notice arguments 'maxLength' and 'minStability'
ce <- clusterCTSS( exampleCAGEexp, threshold = 50, thresholdIsTpm = TRUE,
                   nrPassThreshold = 1, method = "paraclu",
                   removeSingletons = TRUE, keepSingletonsAbove = 100,
                   maxLength = 500, minStability = 1,
                   reduceToNonoverlapping = TRUE)
tagClustersGR(ce, "Zf.30p.dome")
```
**coerceInBSgenome**

**Description**

A private (non-exported) function to discard any range that is not compatible with the CAGEr object's BSgenome.

**Usage**

\[
\text{coerceInBSgenome}(\text{gr}, \text{genome})
\]

**Arguments**

- \text{gr} The genomic ranges to coerce.
- \text{genome} The name of a BSgenome package, which must me installed, or NULL to skip coercion.

**Value**

A GRanges object in which every range is guaranteed to be compatible with the given BSgenome object. The sequnames of the GRanges are also set accordingly to the BSgenome.

---

**ConsensusClusters-class**

\textit{ConsensusClusters}

**Description**

The \texttt{ConsensusClusters} class represents consensus clusters. It is used internally by CAGEr for type safety.

**Details**

Consensus clusters must not overlap, so that a single TSS in the genome can only be attributed to a single cluster.
consensusClustersDESeq2

Description

Create a DESeqDataSet using the consensus cluster expression data in the experiment slot consensusClusters and the sample metadata of the CAGEexp object. The formula must be built using factors already present in the sample metadata.
Usage

consensusClustersDESeq2(object, design)

## S4 method for signature 'CAGEexp'
consensusClustersDESeq2(object, design)

Arguments

object  A CAGEexp object.
design  A formula for the DESeq2 analysis.

Author(s)

Charles Plessy

See Also

DESeqDataSet in the DESeq2 package.

Other CAGEr clusters functions: CTSSclusteringMethod(), CTSScumulativesTagClusters(),
CustomConsensusClusters(), aggregateTagClusters(), clusterCTSS(), consensusClustersGR(),
cumulativeCTSSdistribution(), plotInterquantileWidth(), quantilePositions(), tagClustersGR()

Examples

```r
exampleCAGEexp$group <- c("a", "a", "b", "b", "a")
consensusClustersDEseq2(exampleCAGEexp, ~group)
```

---

**consensusClustersGR**

*Get consensus clusters from CAGEr objects*

Description

Extracts the information on consensus clusters from a CAGEr object.

Usage

```r
consensusClustersGR(
  object,
  sample = NULL,
  returnInterquantileWidth = FALSE,
  qLow = NULL,
  qUp = NULL
)

## S4 method for signature 'CAGEexp'
consensusClustersGR(
```
consensusClustersGR

object,
sample = NULL,
returnInterquantileWidth = FALSE,
qLow = NULL,
qUp = NULL
)

consensusClustersSE(object)

## S4 method for signature 'CAGEexp'

## Arguments

object       A CAGEr object.
sample       Optional. Label of the CAGE dataset (experiment, sample) for which to extract sample-specific information on consensus clusters. When no sample is specified (NULL), sample-agnostic information on consensus clusters is provided. This includes the dominant_ctss and tpm.dominant_ctss for each consensus cluster.

returnInterquantileWidth

Should the interquantile width of consensus clusters be returned? When sample argument is specified, the interquantile widths of the consensus clusters in that specified sample are returned, otherwise, the (sample-agnostic) interquantile width of the consensus cluster itself is returned.

qLow, qUp

Position of which quantile should be used as a left (lower) or right (upper) boundary when calculating interquantile width. Used only when returnInterquantileWidth = TRUE, otherwise ignored.

## Value

consensusClustersGR returns a ConsensusClusters object, which wraps the GRanges class. The score columns indicate the normalised expression value of each cluster, either across all samples (sample = NULL), or for the selected sample. The legacy tpm column may be removed in the future. When sample argument is NOT specified, total CAGE signal across all CAGE datasets (samples) is returned in the tpm column. When sample argument is specified, the tpm column contains CAGE signal of consensus clusters in that specific sample. When returnInterquantileWidth = TRUE, additional sample-specific information is returned, including position of the dominant TSS, and interquantile width of the consensus clusters in the specified sample or otherwise, sample-agnostic information is returned.

consensusClustersSE returns the SummarizedExperiment stored in the consensusClusters experiment slot of the CAGEexp object.

## Author(s)

Vanja Haberle
Charles Plessy
**consensusClustersQuantile**

See Also

- `consensusClusters<-()`

Other CAGEr accessor methods: `CTSSclusteringMethod()`, `CTSScoordinatesGR()`, `CTSScumulativesTagClusters()`, `CTSSnormalizedTpmDF()`, `CTSTagCountDF()`, `GeneExpDEseq2()`, `GeneExpSE()`, `expressionClasses()`, `genomeName()`, `inputFilesType()`, `inputFiles()`, `librarySizes()`, `sampleLabels()`, `seqNameTotalsSE()`, `tagClustersGR()`

Other CAGEr clusters functions: `CTSSclusteringMethod()`, `CTSScumulativesTagClusters()`, `CustomConsensusClusters()`, `aggregateTagClusters()`, `clusterCTSS()`, `consensusClustersDEseq2()`, `cumulativeCTSSdistribution()`, `plotInterquantileWidth()`, `quantilePositions()`, `tagClustersGR()`

Examples

```r
consensusClustersGR(exampleCAGEexp, sample = 2,
                      returnInterquantileWidth = TRUE,
                      qLow = 0.1, qUp = 0.9)
```

---

**consensusClustersQuantile**

Quantile metadata stored in CAGEr objects.

Description

Accessors for consensus cluster quantile data in CAGEr objects.

Usage

```r
consensusClustersQuantileLow(object, samples = NULL)

## S4 method for signature 'CAGEexp'
consensusClustersQuantileLow(object, samples = NULL)

consensusClustersQuantileUp(object, samples = NULL)

## S4 method for signature 'CAGEexp'
consensusClustersQuantileUp(object, samples = NULL)

consensusClustersQuantile(object, sample = NULL, q)

## S4 method for signature 'CAGEexp'
consensusClustersQuantile(object, sample = NULL, q)

consensusClustersQuantileLow(object, samples = NULL) <- value

consensusClustersQuantileUp(object, samples = NULL) <- value
```
consensusClustersTpm

Arguments

object A CAGEr object.
samples Sample name(s), number(s) or NULL (default) for all samples.
sample A single sample name or number, or NULL (default) for all samples.
qu A quantile.
value A list (one entry per sample) of data frames with multiple columns: cluster for the cluster ID, and then q_{0 . n} where \(0 . n\) indicates a quantile.

Description

Extracts a table with normalized CAGE tag values for consensus clusters across all samples from a CAGEr object.

Usage

consensusClustersTpm(object)

```r
## S4 method for signature 'CAGEexp'
consensusClustersTpm(object)
```

Arguments

object A CAGEr object.

Value

Returns the matrix of normalized expression values of CAGE clusters across all samples.

Author(s)

Vanja Haberle

See Also

consensusClustersSE

Examples

`head(consensusClustersTpm(exampleCAGEexp))`
Description

\texttt{.getCumsum} calculates cumulative sums of tpm along the clusters.

Usage

\texttt{.getCAGEsignalCoverage(\texttt{ctss.chr}, \texttt{clusters})}
\texttt{.getCumsumChr2(\texttt{clusters}, \texttt{ctss}, \texttt{chrom}, \texttt{str})}
\texttt{.getCumsum(\texttt{ctss}, \texttt{clusters}, \texttt{useMulticore = FALSE}, \texttt{nrCores = NULL})}

Arguments

- \texttt{ctss.chr} A \texttt{'CTSS.chr'} object (guaranteed to have only one chromosome).
- \texttt{clusters} GRanges as per \texttt{tagClustersGR()}.
- \texttt{ctss} GRanges as per \texttt{CTSScoordinatesGR}, with the score of one sample.
- \texttt{chrom} a chromosome name
- \texttt{str} a strand name
- \texttt{useMulticore}, \texttt{nrCores}
  - See \texttt{clusterCTSS}.

Details

\texttt{.getCAGEsignalCoverage} does... Note that strand is not taken into account.
\texttt{.getCumsumChr2}

Value

\texttt{.getCumsum} returns a list of Rle vectors (IRanges package) containing cumulative sum for each cluster (length of list is equal to number of clusters and names of the list components correspond to the name of the corresponding cluster)\texttt{v}.

Examples

\begin{verbatim}
library(GenomicRanges)
library(IRanges)
ctss <- CTSS( seqnames = "chr1"
  , IRanges(c(1,3,4,12,14,25,28,31,35), w=1)
  , strand = "+"
)
score(ctss) <- 1
tc <- as(ctss, "CTSS.chr")
clusters <- GRanges( seqnames = Rle("chr1")
  , ranges = IRanges(c(1,12,25,31,32), c(4,14,28,31,33))
\end{verbatim}
chrom <- "chr1"
str <- "+
CAGEr:::.getCAGEsignalCoverage(ctss.chr, clusters)
CAGEr:::.getCumsumChr2(clusters, ctss, chrom, str)

ctss <- CTSSnormalizedTpmGR(exampleCAGEexp, "Zf.30p.dome")
ctss <- ctss[ctss$filteredCTSSidx]
clusters <- tagClustersGR(exampleCAGEexp, "Zf.30p.dome")
clusters.cumsum <- CAGEr:::.getCumsum(ctss, head(clusters))
decode(clusters.cumsum[[3]])
ctss[queryHits(findOverlaps(ctss, clusters[3]))]
clusters[3]

CTSS-class  CAGE Transcription Start Sites

Description

The CTSS class represents CAGE transcription start sites (CTSS) at single-nucleotide resolution, using GenomicRanges::UnstitchedGPos as base class. It is used by CAGEr for type safety.

The CTSS constructor takes the same arguments as GenomicRanges::GPos, plus bsgenomeName, and minus stitch, which is hardcoded to FALSE.

The CTSS.chr class represents a CTSS object that is guaranteed to be only on a single chromosome. It is used internally by CAGEr for type-safe polymorphic dispatch.

Usage

## S4 method for signature 'CTSS'
show(object)

## S4 method for signature 'CTSS'
initialize(.Object, ..., bsgenomeName = NULL)

CTSS(
    seqlines = NULL,
    pos = NULL,
    strand = NULL,
    ...
)

## S4 method for signature 'CTSS,GRanges'
coerce(from, to = "GRanges", strict = TRUE)

## S4 method for signature 'GRanges,CTSS'
coerce(from, to = "CTSS", strict = TRUE)
CTSS-class

Arguments

- object: See `methods::show`
- .Object: See `methods::new`
- bsgenomeName: String containing the name of a BSgenome package.
- seqnames, pos, strand, seqinfo, seqlengths, ...
  See the documentation of `GenomicRanges::GPos` for further details.
- from, to, strict
  See `methods::coerce`.

Details

The `genomeName` element of the metadata slot is used to store the name of the BSgenome package used when constructing the CAGEr object.

Coercion from GRanges to CTSS loses information, but it seems to be fine, since other coercions like `as(1.2, "integer")` do the same.

Author(s)

Charles Plessy

Examples

```r
# Convert an UnstitchedGPos object using the new() constructor.
gp <- GPos("chr1:2:-", stitch = FALSE)
ctss <- new("CTSS", gp, bsgenomeName = "BSgenome.Drerio.UCSC.danRer7")
genomeName(ctss)

# Create a new object using the CTSS() constructor.
CTSS("chr1", 2, "-", bsgenomeName = "BSgenome.Drerio.UCSC.danRer7")

# Coerce CTSS to GRanges
as(ctss, "GRanges")

# Coerce a GRanges object to CTSS using the as() method.
gr <- GRanges("chr1:1-10:-")
gr$seq <- "AAAAAAAAAA"
seqlengths(gr) <- 100
genome(gr) <- "foo"
as(gr, "CTSS")
identical(seqinfo(gr), seqinfo(as(gr, "CTSS")))
as(as(gr, "CTSS"), "CTSS") # Make sure it works twice in a row

# (internal use) Transform CTSS to CTSS.chr object
crss.chr <- as(CTSScoordinatesGR(exampleCAGEexp), "CTSS.chr")
```
CTSSclusteringMethod

Get/set CTSS clustering method

Description

Returns or sets the name of the method that was used to make tag clusters from the CTSSs of a CAGEr object.

Usage

CTSSclusteringMethod(object)

## S4 method for signature 'GRangesList'
CTSSclusteringMethod(object)

## S4 method for signature 'CAGEexp'
CTSSclusteringMethod(object)

CTSSclusteringMethod(object) <- value

## S4 replacement method for signature 'GRangesList'
CTSSclusteringMethod(object) <- value

## S4 replacement method for signature 'CAGEexp'
CTSSclusteringMethod(object) <- value

Arguments

object A CAGEr object.
value character

Author(s)

Vanja Haberle
Charles Plessy

See Also

clusterCTSS

Other CAGEr accessor methods: CTSScoordinatesGR(), CTSScumulativesTagClusters(), CTSSnormalizedTpmDF(), CTStagCountDF(), GeneExpDESeq2(), GeneExpSE(), consensusClustersGR(), expressionClasses(), genomeName(), inputFilesType(), inputFiles(), librarySizes(), sampleLabels(), seqNameTotalsSE(), tagClustersGR()

Other CAGEr clusters functions: CTSScumulativesTagClusters(), CustomConsensusClusters(), aggregateTagClusters(), clusterCTSS(), consensusClustersDESeq2(), consensusClustersGR(), cumulativeCTSSdistribution(), plotInterquantileWidth(), quantilePositions(), tagClustersGR()
CTSScoordinatesGR

Examples

CTSSclusteringMethod(exampleCAGEexp)

Description

Extracts the genomic coordinates of all detected TSSs from CAGEexp objects.

Usage

CTSScoordinatesGR(object)

## S4 method for signature 'CAGEexp'
CTSScoordinatesGR(object)

CTSScoordinatesGR(object) <- value

## S4 replacement method for signature 'CAGEexp'
CTSScoordinatesGR(object) <- value

CTSSTagCountSE(object) <- value

## S4 replacement method for signature 'CAGEexp'
CTSSTagCountSE(object) <- value

Arguments

object A CAGEexp object.

value Coordinates to update, in a format according to the function name.

Value

CTSScoordinatesGR returns the coordinates as a CTSS() object wrapping genomic ranges. A filteredCTSSidx column metadata will be present if clusterCTSS() was ran earlier.

Author(s)

Vanja Haberle

Charles Plessy
CTSScumulativesTagClusters

Description

Get/set CTSS cumulative TC or CC data

Usage

CTSScumulativesTagClusters(object, samples = NULL)

## S4 method for signature 'CAGEexp'
CTSScumulativesTagClusters(object, samples = NULL)

CTSScumulativesCC(object, samples = NULL)

## S4 method for signature 'CAGEexp'
CTSScumulativesCC(object, samples = NULL)

CTSScumulativesTagClusters(object) <- value

## S4 replacement method for signature 'CAGEexp'
CTSScumulativesTagClusters(object) <- value

Arguments

object A CAGEexp object.
samples One or more valid sample names.
value CTSScumulativesTagClusters data

See Also

getchCTSS
clusterCTSS

Other CAGEr accessor methods: CTSSclusteringMethod(), CTSScumulativesTagClusters(), CTSSnormalizedTpmDF(), CTStagCountDF(), GeneExpDESeq2(), GeneExpSE(), consensusClustersGR(), expressionClasses(), genomeName(), inputFileType(), inputFiles(), librarySizes(), sampleLabels(), seqNameTotalsSE(), tagClustersGR()
CTSSnormalizedTpmDF

Value

List of numeric Rle.

See Also

Other CAGEr clusters functions: CTSSclusteringMethod(), CustomConsensusClusters(), aggregateTagClusters(), clusterCTSS(), consensusClustersDESeq2(), consensusClustersGR(), cumulativeCTSSdistribution(), plotInterquantileWidth(), quantilePositions(), tagClustersGR()

Other CAGEr accessor methods: CTSSclusteringMethod(), CTSScoordinatesGR(), CTSSnormalizedTpmDF(), CTSStagCountDF(), GeneExpDESeq2(), GeneExpSE(), consensusClustersGR(), expressionClasses(), genomeName(), inputFilesType(), inputFiles(), librarySizes(), sampleLabels(), seqNameTotalsSE(), tagClustersGR()

Description

Extracts the normalized CAGE signal for all detected TSSs in all CAGE datasets from CAGEexp objects.

Usage

CTSSnormalizedTpmDF(object)

## S4 method for signature 'CAGEexp'

CTSSnormalizedTpmDF(object)

CTSSnormalizedTpmGR(object, samples)

## S4 method for signature 'CAGEexp'

CTSSnormalizedTpmGR(object, samples)

Arguments

object A CAGEexp object.
samples The name of sample(s) as reported by sampleLabels(object), or the number identifying the sample(s).

Value

CTSSnormalizedTpmDF returns a DataFrame of normalised expression values.

Author(s)

Vanja Haberle
Charles Plessy
See Also

normalizeTagCount

Other CAGEr accessor methods: CTSSclusteringMethod(), CTSScoordinatesGR(), CTSScumulativesTagClusters(), CTSSTagCountDF(), GeneExpDEseq2(), GeneExpSE(), consensusClustersGR(), expressionClasses(), genomeName(), inputFileType(), inputFiles(), librarySizes(), sampleLabels(), seqNameTotalsSE(), tagClustersGR()

Examples

CTSSnormalizedTpmDF(exampleCAGEexp)

CTSSnormalizedTpmGR(exampleCAGEexp, 1)
exampleCAGEexp |> CTSSnormalizedTpmGR("all")

CTSSTagCountDF

Raw CAGE TSSs expression counts

Description

Extracts the tag count for all detected TSSs in all CAGE datasets from CAGEexp objects.

Usage

CTSSTagCountDF(object)

## S4 method for signature 'CAGEexp'
CTSSTagCountDF(object)

CTSSTagCountDA(object)

## S4 method for signature 'CAGER'
CTSSTagCountDA(object)

CTSSTagCountGR(object, samples)

## S4 method for signature 'CAGEexp'
CTSSTagCountGR(object, samples)

CTSSTagCountSE(object)

## S4 method for signature 'CAGEexp'
CTSSTagCountSE(object)
Arguments

object A CAGEexp object.
samples For CTSStagCountGR only: name(s) or number(s) identifying sample(s) or "all" to return a GRangesList of all the samples.

Value

Returns an object with number of CAGE tags supporting each TSS (rows) in every CAGE dataset (columns). The class of the object depends on the function being called:

- CTSStagCountDF: A DataFrame of Rle integers.
- CTSStagCountDA: A DelayedArray wrapping a DataFrame of Rle integers.
- CTSStagCountSE: A RangedSummarizedExperiment containing a DataFrame of Rle integers.
- CTSStagCountGR: A CTS object (wrapping GRanges) containing a score column indicating expression values for a given sample, or a GRangesList of CTS objects.

Author(s)

Vanja Haberle
Charles Plessy

See Also

getCTSS()

Other CAGEr accessor methods: CTSscClusteringMethod(), CTSscCoordinatesGR(), CTSscCumulativeSTagClusters(), CTSscNormalizedTpmDF(), GeneExpDEseq2(), GeneExpSE(), consensusClustersGR(), expressionClasses(), genomeName(), inputFilesType(), inputFiles(), librarySizes(), sampleLabels(), seqNameTotalsSE(), tagClustersGR()

Examples

CTSStagCountDF(exampleCAGEexp)

CTSStagCountDA(exampleCAGEexp)

CTSStagCountGR(exampleCAGEexp, 1)

CTSStagCountGR(exampleCAGEexp, "all")

CTSStagCountSE(exampleCAGEexp)
CTSStoGenes  Make a gene expression table.

Description
Add a gene expression table in the GeneExpSE experiment slot of an annotated CAGEexp object.

Usage
CTSStoGenes(object)

## S4 method for signature 'CAGEexp'
CTSStoGenes(object)

Arguments
object A CAGEexp object that was annotated with the annotateCTSS() function.

Value
The input object with the following modifications:

- A new geneExpMatrix experiment containing gene expression levels as a SummarizedExperiment object with one assay called counts, which is plain matrix of integers. (This plays better than Rle DataFrames when interfacing with downstream packages like DESeq2, and since the number of genes is limited, a matrix will not cause problems of performance.)
- New genes column data added, indicating total number of gene symbols detected per library.
- New unannotated column data added, indicating for each sample the number of counts that did not overlap with a known gene.

Author(s)
Charles Plessy

See Also
annotateCTSS().

Other CAGEr object modifiers: CustomConsensusClusters(), aggregateTagClusters(), annotateCTSS(), clusterCTSS(), cumulativeCTSSdistribution(), getCTSS(), normalizeTagCount(), quantilePositions(), quickEnhancers(), resetCAGEexp(), summariseChrExpr()

Other CAGEr gene expression analysis functions: GeneExpDESeq2(), ranges2genes()
Examples

```r
CTSToGenes(exampleCAGEexp)
all(librarySizes(exampleCAGEexp) -
    colSums(SummarizedExperiment::assay(GeneExpSE(exampleCAGEexp))) ==
    exampleCAGEexp$unannotated)
```

---

cumulativeCTSSdistribution

*Cumulative sums of CAGE counts along genomic regions*

Description

Calculates the cumulative sum of normalised CAGE counts along each tag cluster or consensus cluster in every sample within a CAGER object.

Usage

```r
cumulativeCTSSdistribution(
    object,
    clusters = c("tagClusters", "consensusClusters"),
    useMulticore = FALSE,
    nrCores = NULL
)
```

```r
## S4 method for signature 'CAGEexp'
cumulativeCTSSdistribution(
    object,
    clusters = c("tagClusters", "consensusClusters"),
    useMulticore = FALSE,
    nrCores = NULL
)
```

Arguments

- **object**: A CAGER object
- **clusters**: tagClusters or consensusClusters.
- **useMulticore**: Logical, should multicore be used. useMulticore = TRUE has no effect on non-Unix-like platforms.
- **nrCores**: Number of cores to use when useMulticore = TRUE (set to NULL to use all detected cores).

Value

In CAGEexp objects, cumulative sums for the tag clusters are stored in the metadata slot using the RleList class. For consensus clusters, they are stored in assays of the consensusClusters experiment slot of the CAGEexp object.
**CustomConsensusClusters**

**Author(s)**
Vanja Haberle
Charles Plessy

**See Also**
Other CAGEr object modifiers: `CTSStoGenes()`, `CustomConsensusClusters()`, `aggregateTagClusters()`, `annotateCTSS()`, `clusterCTSS()`, `getCTSS()`, `normalizeTagCount()`, `quantilePositions()`, `quickEnhancers()`, `resetCAGEexp()`, `summariseChrExpr()`

Other CAGEr clusters functions: `CTSSclusteringMethod()`, `CTSScumulativesTagClusters()`, `CustomConsensusClusters()`, `aggregateTagClusters()`, `clusterCTSS()`, `consensusClustersDESeq2()`, `consensusClustersGR()`, `plotInterquantileWidth()`, `quantilePositions()`, `tagClustersGR()`

**Examples**

```r
cumulativeCTSSdistribution(exampleCAGEexp, clusters = "tagClusters")
CTSScumulativesTagClusters(exampleCAGEexp)[[1]][1:6]
cumulativeCTSSdistribution(exampleCAGEexp, clusters = "consensusClusters")
CTSScumulativesCC(exampleCAGEexp)[[1]][1:6]
```

---

**CustomConsensusClusters**

*Expression levels of consensus cluster*

**Description**

Intersects custom consensus clusters with the CTSS data in a `CAGEexp` object, and stores the result as a expression matrices (raw and normalised tag counts).

**Usage**

```r
CustomConsensusClusters(
  object,
  clusters,
  threshold = 0,
  nrPassThreshold = 1,
  thresholdIsTpm = TRUE
)
```

```r
## S4 method for signature 'CAGEexp,GRanges'
CustomConsensusClusters(
  object,
  clusters,
  threshold = 0,
  nrPassThreshold = 1,
  thresholdIsTpm = TRUE
)
```
Arguments

object     A CAGEexp object
clusters   Consensus clusters in GRanges format.
threshold, nrPassThreshold
          Only CTSSs with signal >= threshold in >= nrPassThreshold experiments
          will be used for clustering and will contribute towards total signal of the cluster.
thresholdIsTpm Logical, is threshold raw tag count value (FALSE) or normalized signal (TRUE).

Details

Consensus clusters must not overlap, so that a single base of the genome can only be attributed to a single cluster. This is enforced by the .ConsensusClusters constructor.

Value

stores the result as a new RangedSummarizedExperiment in the experiment slot of the object. The assays of the new experiment are called counts and normalized. An outOfClusters column is added to the sample metadata to reflect the number of molecules that do not have their TSS in a consensus cluster.

Author(s)

Charles Plessy

See Also

Other CAGEr object modifiers: CTSStoGenes(), aggregateTagClusters(), annotateCTSS(),
clusterCTSS(), cumulativeCTSSdistribution(), getCTSS(), normalizeTagCount(), quantilePositions(),
quickEnhancers(), resetCAGEexp(), summariseChrExpr()

Other CAGEr clusters functions: CTSSclusteringMethod(), CTSScumulativesTagClusters(),
aggregateTagClusters(), clusterCTSS(), consensusClustersDEseq2(), consensusClustersGR(),
cumulativeCTSSdistribution(), plotInterquantileWidth(), quantilePositions(), tagClustersGR()

Examples

cc <- consensusClustersGR(exampleCAGEexp)
CustomConsensusClusters(exampleCAGEexp, cc)
Private functions for distance clustering.

Description

The flow of data is that a **CTSS** object of CTSSes is progressively deconstructed, and data to form the clusters is progressively integrated in a **data.table** object, which is finally converted to **GRanges** at the end. Doing the whole clustering with **GRanges** is more elegant, but looping on a **GRangesList** was just too slow. Maybe the operation on the **data.table** is more efficient because it is vectorised.

*cluster.ctss.strand* does the strandless distance clustering of strandless CTSS positions from a single chromosome. Input does not need to be sorted, but **pay attention that the output is sorted.**

*cluster.ctss.chr* does the stranded distance clustering of CTSS on a single chromosome, by dispatching both strands to *cluster.ctss.strand* and merging the results, taking care keep the cluster IDs unique. Be careful that this function does not look at the score.

*ctss2clusters* does the stranded distance clustering of CTSS.

*summarize.clusters* calculates the number of CTSS, and the position and score of a main peak, for each cluster.

*distclu* receives the data from the main **clusterCTSS** and dispatches each for (possibly parallel) processing.

Usage

```r
.cluster.ctss.strand(ctss.ipos.chr, max.dist)
.cluster.ctss.chr(ctss.chr, max.dist)
.ctss2clusters(ctss, max.dist = 20, useMulticore = FALSE, nrCores = NULL)
.summarize.clusters(
  ctss.clustered,
  removeSingletons = FALSE,
  keepSingletonsAbove = Inf
)
.distclu(
  se,
  max.dist = 20,
  removeSingletons = FALSE,
  keepSingletonsAbove = Inf,
  useMulticore = FALSE,
  nrCores = NULL
)
```
Arguments

- **ctss.ipos.chr**: A IPos object.
- **max.dist**: See `clusterCTSS()`.
- **ctss.chr**: A CTSS.chr object.
- **ctss**: A CTSS object with a score column.
- **useMulticore, nrCores**: See `clusterCTSS()`.
- **ctss.clustered**: A `data.table` object representing the cluster ID (id), chromosome coordinates (chr, pos, strand) and the score of each CTSS.
- **removeSingletons**: Remove “singleton” clusters that span only a single nucleotide? (default = FALSE).
- **keepSingletonsAbove**: Even if `removeSingletons = TRUE`, keep singletons when their score is above threshold (default = Inf).
- **se**: A `SummarizedExperiment` object representing the CTSSes and their expression in each sample.

Value

- `.cluster.ctss.strand` returns a `data.table` object containing arbitrary cluster IDs (as integers) for each CTSS.
- `.cluster.ctss.chr` returns a `data.table` object representing the chromosome coordinates (chr, pos, strand) of each CTSS, with their cluster ID (id).
- `.ctss2clusters` returns a `data.table` object representing the cluster ID (id), chromosome coordinates (chr, pos, strand) and the score of each CTSS.
- `.summarize.clusters` returns GRanges describing the clusters.
- `.distclu` returns GRanges describing the clusters.

Examples

```r
# Get example data
library(IRanges)
library(GenomicRanges)
#.cluster.ctss.strand
c Ges:::.cluster.ctss.strand(ctss.ipos.chr, 5)

cff.chr <- CTSScoordinatesGR(exampleCAGEexp)
cff.chr <- cff.chr[strand(cff.chr) == "+"]
cff.ipos.chr <- ranges(cff.chr)
# Same result if not sorted
identical(
  CAGEx:::.cluster.ctss.strand(cff.ipos.chr, 20),
  CAGEx:::.cluster.ctss.strand(cff.ipos.chr[sample(seq_along(cff.ipos.chr))], 20)
```
exampleCAGEexp

Example CAGE exp object.

Description

Lazy-loaded example CAGE exp object, containing most of the CAGEr data structures created with the CAGEr modifier functions.

Usage

eexampleCAGEexp

Format

A CAGE exp object.

Examples

## Not run:
pathsToInputFiles <- list.files( system.file("extdata", package = "CAGEr")
                           , "ctss$"
                           , full.names = TRUE)
exampleZv9_annot <- GRanges()
exampleCAGEexp <- CAGEexp(genomeName = "BSgenome.Drerio.UCSC.danRer7", inputFiles = pathsToInputFiles, inputFilesType = "ctss", sampleLabels = sub("\.chr17.ctss", ",", basename(pathsToInputFiles)))

## End(Not run)
Details

Data was retrieved from ENSEMBL’s Biomart server using a query to extract gene, transcripts and exon coordinates. For the record, here it is as URL (long, possibly overflowing).

http://mar2015.archive.ensembl.org/biomart/martview/78d86c1d6b4ef51568ba6d46f7d8b254?VIRTUALSCHEMANAME=default&ATTRIBUTES=...ne_ensembl.default.structure.is_constitutive|drerio_gene_ensembl.default.structure.rank&FILTERS=&VISIBLEPANEL=resultspanel

And here it is as XML.

```xml
<?xml version="1.0" encoding="UTF-8"?>
<!DOCTYPE Query>
<Query virtualSchemaName = "default" formatter = "TSV" header = "0" uniqueRows = "0" count = "" datasetConfigVersion = "0.6" >
<Dataset name = "drerio_gene_ensembl" interface = "default" >
  <Attribute name = "ensembl_gene_id" />
  <Attribute name = "ensembl_transcript_id" />
  <Attribute name = "start_position" />
  <Attribute name = "end_position" />
  <Attribute name = "transcript_start" />
  <Attribute name = "transcript_end" />
  <Attribute name = "strand" />
  <Attribute name = "chromosome_name" />
  <Attribute name = "external_gene_name" />
  <Attribute name = "gene_biotype" />
  <Attribute name = "exon_chrom_start" />
  <Attribute name = "exon_chrom_end" />
  <Attribute name = "is_constitutive" />
  <Attribute name = "rank" />
</Dataset>
</Query>
```

The downloaded file was then transformed as follows.

```r
x <- read.delim("~/Downloads/mart_export.txt", stringsAsFactors = FALSE)
e <- GRanges(paste0("chr", x$Chromosome.Name), IRanges(x$Exon.Chr.Start..bp., x$Exon.Chr.End..bp.), ifelse(x$Strand + 1, "+", "-"))
e$gene_name <- Rle(x$Associated.Gene.Name)
e$transcript_type <- Rle(x$Gene.type)
e$type <- "exon"
e$type <- Rle(e$type)

e <- GRanges(paste0("chr", x$Chromosome.Name), IRanges(x$Exon.Chr.Start..bp., x$Exon.Chr.End..bp.), ifelse( x$Strand + 1, "+", "-"))
e$gene_name <- Rle(x$Associated.Gene.Name)
e$transcript_type <- Rle(x$Gene.type)
e$type <- "exon"
e$type <- Rle(e$type)
e <- sort(unique(e))

g <- GRanges( paste0("chr", x$Chromosome.Name)
  , IRanges(x$Gene.Start..bp., x$Gene.End..bp.)
  , ifelse( x$Strand + 1, "+", "-"))
```
```r
 g$gene_name <- Rle(x$Associated.Gene.Name)
 g$transcript_type <- Rle(x$Gene.type)
 g$type <- "gene"
 g$type <- Rle(g$type)
 g <- sort(unique(g))

t <- GRanges(paste0("chr", x$Chromosome.Name),
            IRanges(x$Transcript.Start..bp., x$Transcript.End..bp.),
            ifelse(x$Strand + 1, "+", "-"))

t$gene_name <- Rle(x$Associated.Gene.Name)
 t$transcript_type <- Rle(x$Gene.type)
 t$type <- "transcript"
 t$type <- Rle(t$type)
 t <- sort(unique(t))

gff <- sort(c(g, t, e))
gff <- gff[seqnames(gff) == "chr17"]
gff <- gff[start(gff) > 26000000 & end(gff) < 54000000]
seqlevels(gff) <- seqlevelsInUse(gff)

save(gff, "data/exampleZv9_annot.RData", compress = "xz")
```

**Author(s)**

Prepared by Charles Plessy <plessy@riken.jp> using archive ENSEMBL data.

**References**

http://mar2015.archive.ensembl.org/biomart/

---

**exportToTrack**

Converts TSSs and clusters of TSSs to a genome browser track format

**Description**

Converts CTSS, tag clusters or consensus clusters to the UCSCData format of the rtracklayer package, that can be exported to BED file(s) with track information for genome browsers. CTSSes and consensus clusters are optionally colored by their expression class. Tag clusters and consensus clusters can be displayed in a whiskerplot-like representation with a line showing full span on the cluster, filled block showing interquantile range and a thick box denoting position of the dominant (most frequently) used TSS.

**Usage**

```r
exportToTrack(
          object,
```
what = c("CTSS", "tagClusters", "consensusClusters"),
qLow = NULL,
qUp = NULL,
colorByExpressionProfile = FALSE,
oneTrack = TRUE)

## S4 method for signature 'CAGEexp'
exportToTrack(
  object,
  what = c("CTSS", "tagClusters", "consensusClusters"),
  qLow = NULL,
  qUp = NULL,
colorByExpressionProfile = FALSE,
oneTrack = TRUE)

## S4 method for signature 'GRangesList'
exportToTrack(
  object,
  what = c("CTSS", "tagClusters", "consensusClusters"),
  qLow = NULL,
  qUp = NULL,
colorByExpressionProfile = FALSE,
oneTrack = TRUE)

## S4 method for signature 'GRanges'
exportToTrack(
  object,
  what = c("CTSS", "tagClusters", "consensusClusters"),
  qLow = NULL,
  qUp = NULL,
colorByExpressionProfile = FALSE,
oneTrack = TRUE)

## S4 method for signature 'CTSS'
exportToTrack(
  object,
  what = c("CTSS", "tagClusters", "consensusClusters"),
  qLow = NULL,
  qUp = NULL,
colorByExpressionProfile = FALSE,
oneTrack = TRUE)

## S4 method for signature 'TagClusters'

exportToTrack

exportToTrack(
  object,
  what = c("CTSS", "tagClusters", "consensusClusters"),
  qLow = NULL,
  qUp = NULL,
  colorByExpressionProfile = FALSE,
  oneTrack = TRUE
)

## S4 method for signature 'ConsensusClusters'
exportToTrack(
  object,
  what = c("CTSS", "tagClusters", "consensusClusters"),
  qLow = NULL,
  qUp = NULL,
  colorByExpressionProfile = FALSE,
  oneTrack = TRUE
)

Arguments

object A CAGEexp object.
what Which elements should be exported: CTSS for individual CTSSs, tagClusters for tag clusters or consensusClusters for consensus clusters.
qLow, qUp Position of which "lower" (resp. "upper") quantile should be used as 5' (resp. 3') boundary of the filled block in whiskerplot-like representation of the cluster. Default: NULL (plain line representation). Ignored when what = "CTSS".
colorByExpressionProfile Logical, should blocks be colored in the color of their corresponding expression class. Ignored when what equals "tagClusters".
oneTrack Logical, should the data be converted in an individual object or a list of objects?

Details

The BED representations of CTSSs, tag cluster and consensus clusters can be directly visualised in the ZENBU or UCSC Genome Browsers.

When what = "CTSS", one UCSCData object with single track of 1 bp blocks representing all detected CTSSs (in all CAGE samples) is created. CTSSs can be colored according to their expression class (see getExpressionProfiles and plotExpressionProfiles). For colorByExpressionProfile = FALSE, CTSSs included in the clusters are shown in black and CTSSs that were filtered out in gray.

When what = "tagClusters", one track per CAGE dataset is created, which can be exported to a single UCSCData object (by setting oneFile = TRUE) or separate ones (FALSE). If no quantile boundaries were provided (qLow and qUp are NULL, TCs are represented as simple blocks showing the full span of TC from the start to the end. Setting qLow and/or qUp parameters to a value of the desired quantile creates a gene-like representation with a line showing full span of the TC, filled block showing specified interquantile range and a thick 1 bp block denoting position of the dominant (most frequently used) TSS. All TCs in one track (one CAGE dataset) are shown in the same color.
When `what = "consensusClusters"` consensus clusters are exported. Since there is only one set of consensus clusters common to all CAGE datasets, only one track is created in case of a simple representation. This means that when `qLow = NULL` and `qUp = NULL` one track with blocks showing the full span of consensus cluster from the start to the end is created. However, the distribution of the CAGE signal within consensus cluster can be different in different CAGE samples, resulting in different positions of quantiles and dominant TSS. Thus, when `qLow` and/or `qUp` parameters are set to a value of the desired quantile, a separate track with a gene-like representation is created for every CAGE dataset. These tracks can be exported to a single UCSCData object (by setting `oneFile = TRUE`) or separate ones (by setting `oneFile = FALSE`). The gene-like representation is analogous to the one described above for the TCs. In all cases consensus clusters can be colored according to their expression class (provided the expression profiling of consensus clusters was done by calling `getExpressionProfiles` function). Colors of expression classes match the colors in which they are shown in the plot returned by the `plotExpressionProfiles` function. For `colorByExpressionProfile = FALSE` all consensus clusters are shown in black.

**Value**

Returns either a `rtracklayer` UCSCData object, or a `GRangesList` of them.

**Author(s)**

Vanja Haberle
Charles Plessy

**Examples**

# You can export from a CAGEexp object or from a cluster object directly:
`exportToTrack(exampleCAGEexp, what = "CTSS")` # Is same as:
`exportToTrack(CTSScoordinatesGR(exampleCAGEexp))` # Or:
`exampleCAGEexp |> CTSScoordinatesGR() |> exportToTrack()

# Export a single sample,
`exampleCAGEexp |> CTSStagCountGR(2) |> exportToTrack()`
`exampleCAGEexp |> CTSSnormalizedTpmGR(2) |> exportToTrack()`

# Exporting multiple samples results in a GRangesList of UCSCData objects.
`exportToTrack(exampleCAGEexp, what = "CTSS", oneTrack = FALSE)`
`exampleCAGEexp |> CTSStagCountGR("all") |> exportToTrack()`
`exampleCAGEexp |> CTSSnormalizedTpmGR("all") |> exportToTrack()`

### exporting CTSSs colored by expression class
# Temporarily disabled
# `exportToTrack(exampleCAGEexp, what = "CTSS", colorByExpressionProfile = TRUE)`

### exporting tag clusters in gene-like representation
`exportToTrack(exampleCAGEexp, what = "tagClusters", qLow = 0.1, qUp = 0.9)`
`tagClustersGR(exampleCAGEexp, 1) |> exportToTrack(qLow = 0.1, qUp = 0.9)`

### exporting consensus clusters
`exportToTrack( exampleCAGEexp, what = "consensusClusters")`
`exampleCAGEexp |>`
expressionClasses

```r
expressionClasses(CTSScoordinatesGR("Zf.high", qLow = .1, qUp = .9) |> exportToTrack(qLow = .1, qUp = .9)
```

---

**expressionClasses**  
*Extract labels of expression classes*

---

### Description

Retrieves labels of *expression classes* of individual CTSSs or consensus clusters from a CAGEr object.

### Usage

```r
expressionClasses(object)
```

#### S4 method for signature 'CTSS'
```r
expressionClasses(object)
```

#### S4 method for signature 'ConsensusClusters'
```r
expressionClasses(object)
```

### Arguments

- **object**  
  A CAGEr object.

### Value

Returns a Rle-encoded vector of labels of *expression classes*. The number of labels matches the number of expression clusters returned by `getExpressionProfiles` function.

### See Also

Other CAGEr expression clustering functions:  
- `getExpressionProfiles()`  
- `plotExpressionProfiles()`  

Other CAGEr accessor methods:  
- `CTSSclusteringMethod()`, `CTSScoordinatesGR()`, `CTSScumulativesTagClusters()`,  
- `CTSSnormalizedTpmDF()`, `CTSstagCountDF()`, `GeneExpDESeq2()`, `GeneExpSE()`, `consensusClustersGR()`,  
- `genomeName()`, `inputFileType()`, `inputFiles()`, `librarySizes()`, `sampleLabels()`, `seqNameTotalsSE()`,  
- `tagClustersGR()`

### Examples

```r
expressionClasses(CTSScoordinatesGR(exampleCAGEexp))  
exampleCAGEexp |> consensusClustersGR() |> expressionClasses()
```
FANTOM5humanSamples  FANTOM5 human samples

Description
Lazy-loaded data.frame object, containing information about FANTOM5 libraries. Its use is described in more details in the vignette “Use of CAGE resources with CAGEr”.

Usage
FANTOM5humanSamples

Format
A data.frame with sample, type, description, library_id and data_url columns.

See Also
Other FANTOM data: FANTOM5mouseSamples

FANTOM5mouseSamples  FANTOM5 mouse samples

Description
Lazy-loaded data.frame object, containing information about FANTOM5 libraries. Its use is described in more details in the vignette “Use of CAGE resources with CAGEr”.

Usage
FANTOM5mouseSamples

Format
A data.frame with sample, type, description, library_id and data_url columns.

See Also
Other FANTOM data: FANTOM5humanSamples
Description

Looks up the bases directly upstream provided genomic ranges and searches for a gapless match with a target sequence within a given edit distance.

Usage

flagByUpstreamSequences(object, target, distance = 0)

## S4 method for signature 'CTSS'
flagByUpstreamSequences(object, target, distance = 0)

## S4 method for signature 'TagClusters'
flagByUpstreamSequences(object, target, distance = 0)

## S4 method for signature 'ConsensusClusters'
flagByUpstreamSequences(object, target, distance = 0)

## S4 method for signature 'GRanges'
flagByUpstreamSequences(object, target, distance = 0)

Arguments

object A CTSS, a TagClusters, ConsensusClusters or a GenomicRanges::GRanges object from which a BSgenome object can be reached.
target A target sequence.
distance The maximal edit distance between the genome and the target sequence (default: 0).

Details

If the provided object represents tag clusters or consensus clusters, the search will be done upstream its dominant peak. Convert the object to the GRanges class if this is not the behaviour you want.

Value

A logical-RLe vector indicating if ranges matched the target.

Author(s)

Charles Plessy
### Description

Creates a `DESeqDataSet` using the gene expression data in the experiment slot `geneExpMatrix` and the sample metadata of the `CAGEexp` object. The formula must be built using factors already present in the sample metadata.

### Usage

```r
GeneExpDESeq2(object, design)
```

#### S4 method for signature 'CAGEexp'

```r
GeneExpDESeq2(object, design)
```

### Arguments

- `object`: A `CAGEexp` object.
- `design`: A formula for the DESeq2 analysis.

### Author(s)

Charles Plessy

### See Also

- `DESeqDataSet` in the DESeq2 package.
- Other CAGEr gene expression analysis functions: `CTSstoGenes()`, `ranges2genes()`.
- Other CAGEr accessor methods: `CTSSclusteringMethod()`, `CTSScoordinatesGR()`, `CTSScumulativesTagClusters()`, `CTSSnormalizedTpmDF()`, `CTSstagCountDF()`, `GeneExpSE()`, `consensusClustersGR()`, `expressionClasses()`, `genomeName()`, `inputFilesType()`, `inputFiles()`, `librarySizes()`, `sampleLabels()`, `seqNameTotalsSE()`, `tagClustersGR()`.

### Examples

```r
exampleCAGEexp$group <- factor(c("a", "a", "b", "b", "a"))
GeneExpDESeq2(exampleCAGEexp, ~group)
```
**GeneExpSE**

Retrieves the SummarizedExperiment containing gene expression levels.

**Description**

Get or set a SummarizedExperiment using the gene expression data in the experiment slot geneExpMatrix and the sample metadata of the CAGEexp object.

**Usage**

```r
GeneExpSE(object)
```

```r
## S4 method for signature 'CAGEexp'
GeneExpSE(object)
```

**Arguments**

- `object`: A CAGEexp object.

**Author(s)**

Charles Plessy

**See Also**

Other CAGEr accessor methods: CTSSclusteringMethod(), CTSScoordinatesGR(), CTSScumulativeTagsClusters(), CTSSNormalizedTpmDF(), CTSStagCountDF(), GeneExpDESeq2(), consensusClustersGR(), expressionClasses(), genomeName(), inputFilesType(), inputFiles(), librarySizes(), sampleLabels(), seqNameTotalsSE(), tagClustersGR()  

**Examples**

```r
GeneExpSE(exampleCAGEexp)
```

---

**genomeName**

Extracting genome name from CAGEr objects

**Description**

Extracts the name of a referent genome from a CAGEexp or a CTSS object.
Usage

genomeName(object)

## S4 method for signature 'CAGEexp'
genomeName(object)

## S4 method for signature 'CTSS'
genomeName(object)

genomeName(object) <- value

## S4 replacement method for signature 'CAGEexp'
genomeName(object) <- value

## S4 replacement method for signature 'CTSS'
genomeName(object) <- value

Arguments

object A CAGEexp or a CTSS object.
value The name of a BSgenome package.

Details

CAGEexp objects constructed with NULL in place of the genome name can not run some commands that need access to genomic data, such as BigWig export or G-correction.

Value

Returns a name of a BSgenome package used as a referent genome.

Author(s)

Vanja Haberle
Charles Plessy

See Also

Other CAGEr accessor methods: CTSSclusteringMethod(), CTSScoordinatesGR(), CTSScumulativesTagClusters(), CTSSnormalizedTpmDF(), CTSStagCountDF(), GeneExpDEseq2(), GeneExpSE(), consensusClustersGR(), expressionClasses(), inputFilesType(), inputFiles(), librarySizes(), sampleLabels(), seqNameTotalsSE(), tagClustersGR()

Other CAGEr setter methods: inputFilesType(), inputFiles(), sampleLabels(), setColors()

Examples

genomeName(exampleCAGEexp)
getCTSS

Reading CAGE data from input file(s) and detecting TSSs

Description

Reads input CAGE datasets into CAGEr object, constructs CAGE transcriptions start sites (CTSSs) and counts number of CAGE tags supporting every CTSS in each input experiment. See inputFileType for details on the supported input formats. Preprocessing and quality filtering of input CAGE tags, as well as correction of CAGE-specific ‘G’ nucleotide addition bias can be also performed before constructing TSSs.

Usage

getCTSS(
  object,
  sequencingQualityThreshold = 10,
  mappingQualityThreshold = 20,
  removeFirstG = TRUE,
  correctSystematicG = TRUE,
  useMulticore = FALSE,
  nrCores = NULL
)

## S4 method for signature 'CAGEexp'
getCTSS(
  object,
  sequencingQualityThreshold = 10,
  mappingQualityThreshold = 20,
  removeFirstG = TRUE,
  correctSystematicG = TRUE,
  useMulticore = FALSE,
  nrCores = NULL
)

Arguments

object A CAGEexp object.
sequencingQualityThreshold Only CAGE tags with average sequencing quality \(\geq\) sequencingQualityThreshold and mapping quality \(\geq\) mappingQualityThreshold are kept. Used only if inputFileType(object) == "bam" or inputFileType(object) == "bamPairedEnd", i.e. when input files are BAM files of aligned sequenced CAGE tags, otherwise ignored. If there are no sequencing quality values in the BAM file (e.g. Heliscope single molecule sequencer does not return sequencing qualities) all reads will by default have this value set to -1. Since the default value of sequencingQualityThreshold is 10, all the reads will consequently be discarded. To avoid this behaviour and
keep all sequenced reads set sequencingQualityThreshold to -1 when processing data without sequencing qualities. If there is no information on mapping quality in the BAM file (e.g. software used to align CAGE tags to the referent genome does not provide mapping quality) the mappingQualityThreshold parameter is ignored. In case of paired-end sequencing BAM file (i.e. inputFileType(object) == "bamPairedEnd") only the first mate of the properly paired reads (i.e. the five prime end read) will be read and subject to specified thresholds.

mappingQualityThreshold
See sequencingQualityThreshold.

removeFirstG Logical, should the first nucleotide of the CAGE tag be removed in case it is a G and it does not map to the referent genome (i.e. it is a mismatch). Used only if inputFileType(object) == "bam" or inputFileType(object) == "bamPairedEnd", i.e. when input files are BAM files of aligned sequenced CAGE tags, otherwise ignored. See Details.

correctSystematicG Logical, should the systematic correction of the first G nucleotide be performed for the positions where there is a G in the CAGE tag and G in the genome. This step is performed in addition to removing the first G of the CAGE tags when it is a mismatch, i.e. this option can only be used when removeFirstG = TRUE, otherwise it is ignored. The frequency of adding a G to CAGE tags is estimated from mismatch cases and used to systematically correct the G addition for positions with G in the genome. Used only if inputFileType(object) == "bam" or inputFileType(object) == "bamPairedEnd", i.e. when input files are BAM files of aligned sequenced CAGE tags, otherwise ignored. See Details.

useMulticore Logical, should multicore be used. useMulticore = TRUE has no effect on non-Unix-like platforms.

nrCores Number of cores to use when useMulticore = TRUE (set to NULL to use all detected cores).

Details
In the CAGE experimental protocol an additional G nucleotide is often attached to the 5' end of the tag by the template-free activity of the reverse transcriptase used to prepare cDNA (Harbers and Carninci, Nature Methods 2005). In cases where there is a G at the 5' end of the CAGE tag that does not map to the corresponding genome sequence, it can confidently be considered spurious and should be removed from the tag to avoid misannotating actual TSS. Thus, setting removeFirstG = TRUE is highly recommended.

However, when there is a G both at the beginning of the CAGE tag and in the genome, it is not clear whether the original CAGE tag really starts at this position or the G nucleotide was added later in the experimental protocol. To systematically correct CAGE tags mapping at such positions, a general frequency of adding a G to CAGE tags can be calculated from mismatch cases and applied to estimate the number of CAGE tags that have G added and should actually start at the next nucleotide/position. The option correctSystematicG is an implementation of the correction algorithm described in Carninci et al., Nature Genetics 2006, Supplementary Information section 3-e.
getExpressionProfiles

Value
Returns the object, in which the tagCountMatrix experiment will be occupied by a `RangedSummarizedExperiment` containing the expression data as a `DataFrame` of Rle integers, and the CTSS coordinates as genomic ranges in a `CTSS` object. The expression data can be retrieved with the `CTSTagCountDF` function. In addition, the library sizes are calculated and stored in the object’s sample data (see `librarySizes`).

Author(s)
Vanja Haberle

References

See Also
`inputFilesType`, `librarySizes`.
Other CAGEr object modifiers: `CTSstoGenes()`, `CustomConsensusClusters()`, `aggregateTagClusters()`, `annotateCTSS()`, `clusterCTSS()`, `cumulativeCTSSdistribution()`, `normalizeTagCount()`, `quantilePositions()`, `quickEnhancers()`, `resetCAGEexp()`, `summariseChrExpr()`

Examples
```r
library(BSgenome.Drerio.UCSC.danRer7)
pathsToInputFiles <- system.file("extdata", c("Zf.unfertilized.egg.chr17.ctss", "Zf.30p.dome.chr17.ctss", "Zf.prim6.rep1.chr17.ctss"), package="CAGEr")
labels <- paste("sample", seq(1,3,1), sep = "")
myCAGEexp <- new("CAGEexp", genomeName = "BSgenome.Drerio.UCSC.danRer7", inputFiles = pathsToInputFiles, inputFilesType = "ctss", sampleLabels = labels)
myCAGEexp <- getCTSS(myCAGEexp)
```

getExpressionProfiles  **CAGE data based expression clustering**

Description
Clusters CAGE expression across multiple experiments, both at level of individual TSSs or entire clusters of TSSs.
getExpressionProfiles

Usage

getExpressionProfiles(
  object,
  what = c("CTSS", "consensusClusters"),
  tpmThreshold = 5,
  nrPassThreshold = 1,
  method = c("som", "kmeans"),
  xDim = 5,
  yDim = 5
)

## S4 method for signature 'CAGEexp'
getExpressionProfiles(
  object,
  what = c("CTSS", "consensusClusters"),
  tpmThreshold = 5,
  nrPassThreshold = 1,
  method = c("som", "kmeans"),
  xDim = 5,
  yDim = 5
)

## S4 method for signature 'DelayedArray'
getExpressionProfiles(
  object,
  what = c("CTSS", "consensusClusters"),
  tpmThreshold = 5,
  nrPassThreshold = 1,
  method = c("som", "kmeans"),
  xDim = 5,
  yDim = 5
)

Arguments

object: A CAGEexp object
what: At which level the expression clustering is done (CTSS or consensusClusters)
tpmThreshold, nrPassThreshold: Ignore clusters when their normalized CAGE signal is lower than tpmThreshold in at least nrPassThreshold experiments.
method: Method to be used for expression clustering. som uses the self-organizing map (SOM) algorithm of Toronen and coll., FEBS Letters (1999) som::som function from som package. kmeans uses the K-means algorithm implemented in the stats::kmeans function.
xDim, yDim: With method = "kmeans", xDim specifies number of clusters that will be returned by K-means algorithm and yDim is ignored. With method = "som", xDim specifies the the first and yDim the second dimension of the self-organizing map, which results in total $xDim \times yDim$ clusters returned by SOM.
getExpressionProfiles

Details

Expression clustering can be done at level of individual CTSSs, in which case the feature vector used as input for clustering algorithm contains log-transformed and scaled (divided by standard deviation) normalized CAGE signal at individual TSS across multiple experiments. Only TSSs with normalized CAGE signal $\geq$ tpmThreshold in at least nrPassThreshold CAGE experiments are used for expression clustering. However, CTSSs along the genome can be spatially clustered into tag clusters for each experiment separately using the clusterCTSS function, and then aggregated across experiments into consensus clusters using aggregateTagClusters function. Once the consensus clusters have been created, expression clustering at the level of these wider genomic regions (representing entire promoters rather than individual TSSs) can be performed. In that case the feature vector used as input for clustering algorithm contains normalized CAGE signal within entire consensus cluster across multiple experiments, and threshold values in tpmThreshold and nrPassThreshold are applied to entire consensus clusters.

Value

Returns a modified CAGEexp object. If what = "CTSS" the object's metadata elements CTSSexpressionClusteringMethod and CTSSexpressionClasses will be set accordingly, and if what = "consensusClusters" the elements consensusClustersExpressionClusteringMethod and consensusClustersExpressionClasses will be set. Labels of expression classes (clusters) can be retrieved using expressionClasses function.

Author(s)

Vanja Haberle
Charles Plessy

References


See Also

Other CAGEr expression clustering functions: expressionClasses(), plotExpressionProfiles()

Examples

getExpressionProfiles( exampleCAGEexp, "CTSS" , tpmThreshold = 50, nrPassThreshold = 1 , method = "som", xDim = 3, yDim = 3)

getAddressExpressionProfiles( exampleCAGEexp, "CTSS" , tpmThreshold = 50, nrPassThreshold = 1 , method = "kmeans", xDim = 3)

getAddressExpressionProfiles( exampleCAGEexp, "consensusClusters")
getShiftingPromoters  Select consensus clusters with shifting score above threshold

Description

Extracts consensus clusters with shifting score and/or FDR (adjusted P-value from Kolmogorov-Smirnov test) above specified threshold. Returns their genomic coordinates, total CAGE signal and the position of dominant TSS in the two compared groups of CAGE samples, along with the value of the shifting score, P-value and FDR. Scores and P-values/FDR have to be calculated beforehand by calling `scoreShift` function.

Usage

```r
getShiftingPromoters(
  object,
  groupX,
  groupY,
  tpmThreshold = 0,
  scoreThreshold = -Inf,
  fdrThreshold = 1
)
```

Arguments

- `object` A `CAGEexp` object.
- `groupX, groupY` Character vector of the one or more CAGE dataset labels in the first (`groupX`) and in the second group (`groupY`). Shifting promoters for the specified group pair are returned.
- `tpmThreshold` Consensus clusters with total CAGE signal \(\geq tpmThreshold\) in each of the compared groups will be returned.
- `scoreThreshold` Consensus clusters with shifting score \(\geq scoreThreshold\) will be returned. The default value \(-Inf\) returns all consensus clusters (for which score could be calculated, i.e. the ones that have at least one tag in each of the compared samples).
fdrThreshold Consensus clusters with adjusted P-value (FDR) from Kolmogorov-Smirnov test \( \geq fdrThreshold \) will be returned. The default value 1 returns all consensus clusters (for which K-S test could be performed, \textit{i.e.} the ones that have at least one tag in each of the compared samples).

Value

Returns a \texttt{data.frame} of shifting promoters with genomic coordinates and positions of dominant TSS and CAGE signal in the two compared (groups of) samples, along with shifting score and adjusted P-value (FDR).

Author(s)

Vanja Haberle
Sarvesh Nikumbh

See Also

Other CAGEr promoter shift functions: \texttt{scoreShift()}

Examples

```r
getShiftingPromoters( exampleCAGEexp
    , groupX = "Zf.unfertilized.egg"
    , groupY = "Zf.30p.dome" ) |> head()
```

---

**hanabi**

\textit{Calculate richness in preparation for plotting}

**Description**

Rarefy data at multiple sample sizes using the vegan package and return a ‘hanabi’ object that can be passed to plot functions.

The computation can be long, so the steps of rarefaction and plotting are kept separate.

**Usage**

```r
hanabi(
  x,
  n = 20,
  step = 0.75,
  from = NULL,
  useMulticore = FALSE,
  nrCores = NULL
)
```
## S4 method for signature 'Rle'
hanabi(
  x,
  n = 20,
  step = 0.75,
  from = NULL,
  useMulticore = FALSE,
  nrCores = NULL
)

## S4 method for signature 'numeric'
hanabi(
  x,
  n = 20,
  step = 0.75,
  from = NULL,
  useMulticore = FALSE,
  nrCores = NULL
)

## S4 method for signature 'integer'
hanabi(
  x,
  n = 20,
  step = 0.75,
  from = NULL,
  useMulticore = FALSE,
  nrCores = NULL
)

## S4 method for signature 'GRanges'
hanabi(
  x,
  n = 20,
  step = 0.75,
  from = NULL,
  useMulticore = FALSE,
  nrCores = NULL
)

## S4 method for signature 'List'
hanabi(
  x,
  n = 20,
  step = 0.75,
  from = NULL,
  useMulticore = FALSE,
  nrCores = NULL
)
### S4 method for signature 'list'

```r
hanabi(
  x,
  n = 20,
  step = 0.75,
  from = NULL,
  useMulticore = FALSE,
  nrCores = NULL
)
```

### S4 method for signature 'matrix'

```r
hanabi(
  x,
  n = 20,
  step = 0.75,
  from = NULL,
  useMulticore = FALSE,
  nrCores = NULL
)
```

#### Arguments

- **x**
  - An object contained expression counts on which richness scores can be calculated. For example an expression table in `DataFrame` or `data.frame` format where columns are samples and rows are features such as genes, TSS, etc, or a vector of counts (tag counts, molecule counts, ...), or GRanges or GRangesList objects, etc.

- **n**
  - The maximum number of rarefactions per sample.

- **step**
  - Subsample sizes are calculated by taking the largest sample and multiplying it by the step "n" times.

- **from**
  - Add one sample size (typically "0") in order to extend the plot on the left-hand side.

- **useMulticore**
  - Logical, should multicore be used. `useMulticore = TRUE` has no effect on non-Unix-like platforms. At the moment, it also has only effects on lists and list-derived classes (data frames but not matrices).

- **nrCores**
  - Number of cores to use when `useMulticore = TRUE` (set to NULL to use all detected cores).

#### Details

This function does not take directly CAGEr objects as input, because hanabi plots can be made from CTSS, clustered or gene-level data, therefore it is not possible to guess which one to use.

#### Value

A list-based object of class "hanabi".
Author(s)

Charles Plessy

See Also

vegan::rarecurve.
Other CAGEr richness functions: hanabiPlot(), plot.hanabi()

Examples

h <- hanabi(CTSStagCountDF(exampleCAGEexp))
h
plot(h)
hanabi(CTSStagCountGR(exampleCAGEexp, 2))

hanabi-class  Hanabi class

Description

TBD

Details

TBD

hanabiPlot  hanabiPlot

Description

Plot feature discovery curves

Usage

hanabiPlot(x, group, col = NULL, legend.pos = "topleft", pch = 1, ...)

Arguments

x  A hanabi object.
group  A character vector or a factor grouping the samples.
col  A character vector colors (at most one per group).
legend.pos  Position of the legend, passed to the legend function.
pch  Plot character at the tip of the lines and in the legend.
...  Further arguments to be passed to the plot.hanabi function.
**Details**

Plots the number of features (genes, transcripts, ...) detected for a given number of counts (reads, unique molecules, ...). Each library is sub-sampled by rarefaction at various sample sizes, picked to provide enough points so that the curves look smooth. The final point is plotted as an open circle, hence the name "hanabi", which means fireworks in Japanese.

The rarefactions take time to do, so this step is done by a separate function, so that the result is easily cached.

**Author(s)**

Charles Plessy

**See Also**

Other CAGEr richness functions: `hanabi`, `plot.hanabi()`

Other CAGEr richness functions: `hanabi`, `plot.hanabi()`

Other CAGEr plot functions: `plotAnnot()`, `plotCorrelation()`, `plotExpressionProfiles()`, `plotInterquantileWidth()`, `plotReverseCumulatives()`

**Examples**

```r
h <- hanabi(CTSStagCountDF(exampleCAGEexp))
hanabiPlot(h, group = 1:5)
hanabiPlot(hanabi(CTSStagCountDF(exampleCAGEexp), n = 20, step = 0.8, from = 25000), group = 1:5)
hanabiPlot(hanabi(CTSStagCountDF(exampleCAGEexp), n = 10, step = 0.98), group = 1:5)
hanabiPlot(h, group=c("A", "A", "B", "C", "B"), col=c("red", "green", "blue"))
hanabiPlot(h, group = 1:5, pch=1:5, col="purple")
```

---

**Description**

Imports CTSS data from a BAM file.

**Usage**

```r
import.bam(
  filepath,
  filetype,
  sequencingQualityThreshold = 10,
  mappingQualityThreshold = 20
)```
import.bam.ctss

Arguments

filepath The path to the BAM file.
filetype bam or bamPairedEnd.
sequencingQualityThreshold See getCTSS().
mappingQualityThreshold See getCTSS().
removeFirstG See getCTSS().

See Also

Other loadFileIntoGPos: bam2CTSS(), import.CTSS(), import.bam.ctss(), import.bedCTSS(), import.bedScore(), import.bedmolecule(), loadFileIntoGPos(), moleculesGR2CTSS()

Examples

# TODO: add example file
# import.bam(system.file("extdata", "example.bam", package = "CAGEr"))

Description

Imports CTSS data from a BAM file.

Usage

import.bam.ctss(
  filepath,
  filetype,
  sequencingQualityThreshold,
  mappingQualityThreshold,
  removeFirstG,
  correctSystematicG,
  genome
)

Arguments

filepath The path to the BAM file.
filetype bam or bamPairedEnd.
sequencingQualityThreshold See getCTSS().
mappingQualityThreshold See getCTSS().
removeFirstG See getCTSS().
import.bedCTSS

correctSystematicG
    See getCTSS().

genome
    See coerceInBSgenome().

Value

Returns a CTSS object.

See Also

Other loadFileIntoGPos: bam2CTSS(), import.CTSS(), import.bam(), import.bedCTSS(), import.bedScore(), import.bedmolecule(), loadFileIntoGPos(), moleculesGR2CTSS()

Description

Imports a BED file where each line represents a single base, with a score counting the number of CAGE transcription start sites (CTSS).

Usage

import.bedCTSS(filepath)

Arguments

filepath
    The path to the BED file.

Value

A GRanges object where each line represents one nucleotide.

See Also

Other loadFileIntoGPos: bam2CTSS(), import.CTSS(), import.bam.ctss(), import.bam(), import.bedScore(), import.bedmolecule(), loadFileIntoGPos(), moleculesGR2CTSS()

Examples

# TODO: add example file
# import.BED(system.file("extdata", "example.bed", package = "CAGEr"))
import.bedmolecule

Description
 Imports a BED file where each line counts for one molecule in a GRanges object where each line represents one nucleotide.

Usage
 import.bedmolecule(filepath)

Arguments
 filepath The path to the BED file.

Value
 Returns a CTSS object.

See Also
 Other loadFileIntoGPos: bam2CTSS(), import.CTSS(), import.bam.ctss(), import.bam(), import.bedCTSS(), import.bedScore(), loadFileIntoGPos(), moleculesGR2CTSS()

Examples
 # TODO: add example file
 # import.BED(system.file("extdata", "example.bed", package = "CAGEr"))

import.bedScore

Description
 Imports a BED file where the score indicates a number of counts for a given alignment.

Usage
 import.bedScore(filepath)

Arguments
 filepath The path to the BED file.
import.CAGEscanMolecule

Value

A GRanges object where each line represents one nucleotide.

See Also

Other loadFileIntoGPos: bam2CTSS(), import.CTSS(), import.bam.ctss(), import.bam(), import.bedCTSS(), import.bedmolecule(), loadFileIntoGPos(), moleculesGR2CTSS()

Examples

# TODO: add example file
# import.bedScore(system.file("extdata", "example.bed", package = "CAGER"))

import.CAGEscanMolecule(filepath)

Usage

import.CAGEscanMolecule(filepath)

Arguments

filepath The path to the “molecule” file.

See Also

parseCAGEscanBlocksToGrangeTSS

Examples

# TODO import.CAGEscanMolecule(system.file("extdata", "example.molecule.txt", package = "CAGER"))
**import.CTSS**

**Description**
Imports a "CTSS" file in a GPos object

**Usage**
import.CTSS(filepath)

**Arguments**
- filepath: The path to the "CTSS" file.

Note that the format of the "CTSS" files handled in this function is not the same as the FANTOM5 "CTSS" files (which are plain BED).

**See Also**
Other loadFileIntoGPos: bam2CTSS(), import.bam.ctss(), import.bam(), import.bedCTSS(), import.bedScore(), import.bedmolecule(), loadFileIntoGPos(), moleculesGR2CTSS()

**Examples**
CAGEr:::import.CTSS(system.file("extdata", "Zf.high.chr17.ctss", package = "CAGEr"))

**inputFiles**

**Description**
Extracts the paths to CAGE data input files from codeCAGEexp objects.

**Usage**
inputFiles(object)

## S4 method for signature 'CAGEexp'
inputFiles(object)

inputFiles(object) <- value

## S4 replacement method for signature 'CAGEexp'
inputFiles(object) <- value
**inputFilesType**

*Input file formats for CAGEr objects*

**Arguments**

object A CAGEexp object.

value A character vector with one file path per sample.

**Value**

Returns a character vector of paths to CAGE data input files.

**Author(s)**

Vanja Haberle

Charles Plessy

**See Also**

Other CAGEr accessor methods: `CTSSclusteringMethod()`, `CTSScoordinatesGR()`, `CTSScumulativesTagClusters()`, `CTSSnormalizedTpmDF()`, `CTSStagCountDF()`, `GeneExpDESeq2()`, `GeneExpSE()`, `consensusClustersGR()`, `expressionClasses()`, `genomeName()`, `inputFilesType()`, `librarySizes()`, `sampleLabels()`, `seqNameTotalsSE()`, `tagClustersGR()`

Other CAGEr setter methods: `genomeName()`, `inputFilesType()`, `sampleLabels()`, `setColors()`

**Examples**

```r
inputFiles(exampleCAGEexp)
```

```r
inputFilesType <- value
```

```r
## S4 replacement method for signature 'CAGEexp'
inputFilesType(object) <- value
```
inputFilesType

Arguments

object A CAGEexp object.
value A character vector with one file type per sample.

Details

The following input file types are supported:

- bed: A BED file where each line counts for one molecule.
- bedScore: A BED file where the score indicates a number of counts for a given alignment
- CAGEscanMolecule: Experimental. For the CAGEscan 3.0 pipeline.
- ctss: A tabulation-delimited file describing CAGE Transcription Start Sites (CTSS) with four columns indicating chromosome, 1-based coordinate, strand and score respectively.
- CTSStable
- FANTOM5
- ENCODE
- FANTOM3and4
- ZebrafishDevelopment

Value

Returns the type of the file format of CAGE data input files, e.g. "bam" or "ctss". In the case of CAGEexp objects, the return value is character vector with one member per sample.

Author(s)

Vanja Haberle
Charles Plessy

See Also

getCTSS
Other CAGEr accessor methods: CTSSclusteringMethod(), CTSScoordinatesGR(), CTSScumulativesTagClusters(), CTSSnormalizedTpmDF(), CTSStagCountDF(), GeneExpDEseq2(), GeneExpSE(), consensusClustersGR(), expressionClasses(), genomeName(), inputFiles(), librarySizes(), sampleLabels(), seqNameTotalsSE(), tagClustersGR()
Other CAGEr setter methods: genomeName(), inputFiles(), sampleLabels(), setColors()

Examples

inputFilesType(exampleCAGEexp)
librarySizes

Extracting library sizes from CAGEr objects

Description

Extracts the library sizes (total number of CAGE tags) for all CAGE datasets from CAGEexp objects.

Usage

librarySizes(object)

## S4 method for signature 'CAGEexp'
librarySizes(object)

Arguments

object A CAGEexp object.

Details

Library sizes are calculated when loading data with the getCTSS function and stored in the librarySizes column of the colData of CAGEexp objects.

Value

Returns an integer vector of total number of CAGE tags (library size) for all CAGE datasets in the CAGEr object.

Author(s)

Vanja Haberle

See Also

getCTSS

Other CAGEr accessor methods: CTSSclusteringMethod(), CTSScoordinatesGR(), CTSScumulativesTagClusters(), CTSSnormalizedTpmDF(), CTSTagCountDF(), GeneExpDESeq2(), GeneExpSE(), consensusClustersGR(), expressionClasses(), genomeName(), inputFilesType(), inputFiles(), sampleLabels(), seqNameTotalsSE(), tagClustersGR()

Examples

librarySizes(exampleCAGEexp)
loadFileIntoGPos

Description

A private (non-exported) function to load from each file format supported by CAGEr

Usage

loadFileIntoGPos(
  filepath,
  filetype = c("bam", "bamPairedEnd", "bed", "bedctss", "bedScore", "CAGEscanMolecule", "ctss"),
  sequencingQualityThreshold,
  mappingQualityThreshold,
  removeFirstG,
  correctSystematicG,
  genome
)

Arguments

filepath The path to the file to load.
filetype The type of the file
sequencingQualityThreshold See getCTSS().
mappingQualityThreshold See getCTSS().
removeFirstG See getCTSS().
correctSystematicG See getCTSS().
genome See coerceInBSgenome().

Value

A GPos() object where the score represents the number of CAGE tags starting on that nucleotide.

See Also

import.CTSS

Other loadFileIntoGPos: bam2CTSS(), import.CTSS(), import.bam.ctss(), import.bam(), import.bedCTSS(), import.bedScore(), import.bedmolecule(), moleculesGR2CTSS()
**mapStats**

**Process mapping statistics**

**Description**

Using a data frame containing mapping statistics in counts, transform the data in percentages that can be used for stacked barplots.

**Usage**

```r
mapStats(libs, scope, group = "default", facet = NULL, normalise = TRUE)
```

**Arguments**

- **libs**: A data frame with containing columns required by the scope chosen.
- **scope**: The name of a “scope”, that defines which data is plotted and how it is normalised, or a function that implements a custom scope. See `mapStatsScopes()` for details on each scope.
- **group**: A vector of factors defining groups in the data. By default, the “group” column of the “libs” table.
- **facet**: A vector of factors defining facets in the data (in the sense of ggplot2’s `facet_wrap` function).
- **normalise**: Whether to normalise or not. Default: `TRUE`.

**Details**

See the plotAnnot vignette and the `mapStatsScopes()` help page for details on what the scopes are.


**Value**

Returns a data frame with mean and standard deviation of normalised mapping statistics, plus absolute positions for the error bars. The first column, group, is a vector of factors sorted with the `gtools::mixedorder()` function. The facet column, if any, is always called facet.

**Author(s)**

Charles Plessy

**See Also**

`plotAnnot`, `mapStatsScopes`
Examples

CAGEr:::mapStats(as.data.frame(colData(exampleCAGEexp)), "counts", sampleLabels(exampleCAGEexp))
CAGEr:::mapStats(as.data.frame(colData(exampleCAGEexp)), "counts", c("A", "A", "B", "B", "C"))

mapStatsScopes  mapStats scopes

Description

Functions implementing the scope parameter of the \link{mapStats} function.

Usage

msScope_counts(libs)
msScope_mapped(libs)
msScope_qc(libs)
msScope_steps(libs)
msScope_all(libs)
msScope_annotation(libs)

Arguments

libs  A data frame containing metadata describing samples in sequence libraries.

Details

The counts scope reports the number of molecules aligning in \textit{promoter, exon, intron} and otherwise \textit{intergenic} regions.

The mapped scope reports the number of molecules aligning in \textit{promoter, exon, intron} and otherwise \textit{intergenic}, plus the number of PCR duplicates (mapped tags minus molecule counts), plus the number of non-properly paired mapped tags.

The qc scope reports the number of tags removed as \textit{tag dust, rRNA, spikes}, plus the \textit{unmapped} tags, plus the number of non-properly paired mapped tags, plus the number of PCR duplicates (mapped tags minus molecule counts), plus the number of unique molecule counts.

The steps scope reports the number of tags removed by \textit{cleaning, mapping, and deduplication}, plus the number of unique molecule counts.

The legacy all scope reports the number of tags in \textit{promoters, exons, introns, or mapped} elsewhere, or removed because they match rRNA or are likely primer artefacts, normalised by the total number of extracted tags.
mergeCAGEsets

The legacy annotation scope reports the number of tags in promoters, exons, introns, or mapped elsewhere, or removed because they match rRNA or are likely primer artefacts, normalised by the total number of mapped tags.

Value

Returns a list with three elements: libs contains a modified version of the input data frame where columns have been reorganised as needed, columns contains the names of the columns to use for plotting and provides the order of the stacked bars of the plotAnnot function, total indicates the total counts used for normalising the data.

mergeCAGEsets  Merge two CAGEr objects into one

Description

Merges two CAGEr objects into one by combining the CTSS genomic coordinates and raw tag counts. The resulting object will contain a union of TSS positions present in the two input objects and raw tag counts for those TSSs in all samples from both input objects.

Usage

mergeCAGEsets(cs1, cs2)

## S4 method for signature 'CAGEexp,CAGEexp'
mergeCAGEsets(cs1, cs2)

Arguments

cs1  A CAGEr object
cs2  A CAGEr object

Value

Note that merging discards all other information present in the two CAGEr objects, that is, the merged object will not contain any normalised tag counts, CTSS clusters, quantile positions, etc., so these have to be calculated again by calling the appropriate functions on the merged object. Also, it is only possible to merge two objects that contain TSS information for the same reference genome and do not share any sample names.

Returns a CAGEexp object, which contains a union of TSS positions present in the two input objects and raw tag counts for those TSSs in all samples from both input objects.

Author(s)

Vanja Haberle
Charles Plessy
mergeSamples

See Also

CAGEexp

Examples

```r
library(BSgenome.Drerio.UCSC.danRer7)

pathsToInputFiles <- system.file("extdata", c("Zf.unfertilized.egg.chr17.ctss", "Zf.30p.dome.chr17.ctss", "Zf.prim6.repl.chr17.ctss"), package="CAGEr")

ce1 <- CAGEexp(genomeName = "BSgenome.Drerio.UCSC.danRer7", inputFiles = pathsToInputFiles[1:2], inputFilesType = "ctss", sampleLabels = c("sample1", "sample2"))
ce1 <- getCTSS(ce1)

ce2 <- CAGEexp(genomeName = "BSgenome.Drerio.UCSC.danRer7", inputFiles = pathsToInputFiles[3], inputFilesType = "ctss", sampleLabels = "sample3")
ce2 <- getCTSS(ce2)

ce <- mergeCAGEsets(ce1, ce2)
```

mergeSamples

Merge CAGE samples

Description

Merges individual CAGE samples (datasets, experiments) within the CAGEr object into specified groups.

Usage

```r
mergeSamples(object, mergeIndex, mergedSampleLabels)
```

## S4 method for signature 'CAGEexp'
mergeSamples(object, mergeIndex, mergedSampleLabels)

Arguments

- **object**: A CAGEr object.
- **mergeIndex**: Integer vector specifying which experiments should be merged. (one value per sample, see Details).
- **mergedSampleLabels**: Labels for the merged datasets (same length as the number of unique values in mergeIndex).
Details

The samples within the CAGEr object are merged by adding the raw tag counts of individual CTSS that belong to the same group. After merging, all other slots in the CAGEr object will be reset and any previous data for individual experiments will be removed.

mergeIndex controls which samples will be merged. It is an integer vector that assigns a group identifier to each sample, in the same order as they are returned by sampleLabels(object). For example, if there are 8 CAGE samples in the CAGEr object and mergeIndex = c(1,1,2,2,3,2,4,4), this will merge a) samples 1 and 2, b) samples 3, 4 and 6, c) samples 7 and 8, and d) it will leave sample 5 as it is, resulting in 4 final merged datasets.

Labels provided in mergedSampleLabels will be assigned to merged datasets in the ascending order of mergeIndex values, i.e. first label will be assigned to a dataset created by merging datasets labeled with lowest mergeIndex value (in this case 1), etc.

Value

The slots sampleLabels, librarySizes and tagCountMatrix of the provided CAGEr object will be updated with the information on merged CAGE datasets and will replace the previous information on individual CAGE datasets. All further slots with downstream information will be reset.

Author(s)

Vanja Haberle
Charles Plessy

Examples

mergeSamples( exampleCAGEexp, mergeIndex = c(3,2,4,4,1), mergedSampleLabels = c("zf_unfertilized", "zf_high", "zf_30p_dome", "zf_prim6"))

moleculesGR2CTSS

Description

Calculates CTSS positions from a GenomicRanges object where each element represents a single molecule.

Usage

moleculesGR2CTSS(gr)

Arguments

gr A GRanges object.
normalizeTagCount

Value

Returns a GRanges object.

See Also

Other loadFileIntoGPos: bam2CTSS(), import.CTSS(), import.bam.ctss(), import.bam(), import.bedCTSS(), import.bedScore(), import.bedmolecule(), loadFileIntoGPos()

Examples

gr <- GenomicRanges::GRanges("chr1", IRanges::IRanges(1, 10), c("+", "-", "+")
CAGEr:::moleculesGR2CTSS(gr)

normalizeTagCount Normalizing raw CAGE tag count

Description

Normalizes raw CAGE tag count per CTSS in all experiments to a same referent distribution. A simple tag per million normalization or normalization to a referent power-law distribution (Balwierz et al., Genome Biology 2009) can be specified.

Usage

normalizeTagCount(
  object,
  method = c("powerLaw", "simpleTpm", "none"),
  fitInRange = c(10, 1000),
  alpha = 1.25,
  T = 10^6
)

## S4 method for signature 'CAGEexp'
normalizeTagCount(
  object,
  method = c("powerLaw", "simpleTpm", "none"),
  fitInRange = c(10, 1000),
  alpha = 1.25,
  T = 10^6
)

Arguments

  object A CAGEexp object

  method Method to be used for normalization. Can be either "simpleTpm" to convert tag counts to tags per million or "powerLaw" to normalize to a referent power-law distribution, or "none" to keep using the raw tag counts in downstream analyses.
**normalizeTagCount**

fitInRange: An integer vector with two values specifying a range of tag count values to be used for fitting a power-law distribution to reverse cumulatives. Used only when method = "powerLaw", otherwise ignored. See Details.

alpha: \(-1 \times \alpha\) will be the slope of the referent power-law distribution in the log-log representation. Used only when method = "powerLaw", otherwise ignored. See Details.

T: Total number of CAGE tags in the referent power-law distribution. Setting \(T = 10^6\) results in normalized values that correspond to tags per million in the referent distribution. Used only when method = "powerLaw", otherwise ignored. See Details.

**Details**

It has been shown that many CAGE datasets follow a power-law distribution (Balwierz et al., Genome Biology 2009). Plotting the number of CAGE tags (X-axis) against the number of TSSs that are supported by \(\geq\) of that number of tags (Y-axis) results in a distribution that can be approximated by a power-law. On a log-log scale this theoretical referent distribution can be described by a monotonically decreasing linear function \(y = -1 \times \alpha \times x + \beta\), which is fully determined by the slope \(\alpha\) and total number of tags \(T\) (which together with \(\alpha\) determines the value of \(\beta\)). Thus, by specifying parameters \(\alpha\) and \(T\) a desired referent power-law distribution can be selected. However, real CAGE datasets deviate from the power-law in the areas of very low and very high number of tags, so it is advisable to discard these areas before fitting a power-law distribution. fitInRange parameter allows to specify a range of values (lower and upper limit of the number of CAGE tags) that will be used to fit a power-law. Plotting reverse cumulatives using plotReverseCumulatives function can help in choosing the best range of values. After fitting a power-law distribution to each CAGE dataset individually, all datasets are normalized to a referent distribution specified by \(\alpha\) and \(T\). When \(T = 10^6\), normalized values are expressed as tags per million (tpm).

**Value**

The slot normalizedTpmMatrix of the provided CAGEexp object will be occupied by normalized CAGE signal values per CTSS across all experiments, or with the raw tag counts (in case method = "none").

**Author(s)**

Vanja Haberle

**References**

Balwierz et al. (2009) Methods for analyzing deep sequencing expression data: constructing the human and mouse promoterome with deepCAGE data, Genome Biology 10(7):R79.

**See Also**

plotReverseCumulatives, CTSSnormalizedTpmDF
Other CAGEr object modifiers: CTSStoGenes(), CustomConsensusClusters(), aggregateTagClusters(), annotateCTSS(), clusterCTSS(), cumulativeCTSSdistribution(), getCTSS(), quantilePositions(), quickEnhancers(), resetCAGEexp(), summariseChrExpr()

Examples

ce1 <- normalizeTagCount(exampleCAGEexp, method = "simpleTpm")
ce2 <- normalizeTagCount(exampleCAGEexp, method = "powerLaw")

Description

Parse a string describing a block in a CAGEscan molecule, as output by the "CAGEscan 3.0" pipeline.

Usage

parseCAGEscanBlocksToGrangeTSS(blocks)

Arguments

blocks A character string representing a block in a CAGEscan molecule.

Value

A GRanges object representing a TSS.

In CAGEscan molecules, blocks are separated by ‘|’, ‘;’ or ‘:’ for gap of coverage, splice junction (confident) and splice junction (maybe) respectively. Strand is “+” if first coordinate is lower than the second one, and “-” otherwise.

See Also

import.CAGEscanMolecule

Examples

myMolecule <- paste0("chr11:66268633-66268693, ", "chr11:66271796-66271869; ", "chr11:66272156-66272252| ", "chr11:66272364-66272460")
myFirstBlock <- sub("[,;|].*", "", myMolecule)
CAGEr:::parseCAGEscanBlocksToGrangeTSS(myFirstBlock)
Description

S3 method to plot hanabi objects. Used by the \texttt{hanabiPlot} function.

Usage

```r
## S3 method for class 'hanabi'
plot(
x, 
alpha = 0.5, 
col = "black", 
xlab = "Total counts", 
ylab = "Unique features", 
main = "Hanabi plot", 
pch = 1, 
...
)
## S3 method for class 'hanabi'
points(x, ...)
## S3 method for class 'hanabi'
lines(x, ...)
```

Arguments

- \texttt{x}  
The hanabi object to plot.
- \texttt{alpha}  
The alpha transparency of the plot lines.
- \texttt{col}  
A vector indicating a color per sample (or a vector that can be recycled that way).
- \texttt{xlab}  
Horizontal axis label.
- \texttt{ylab}  
Vertical axis label.
- \texttt{main}  
Plot title.
- \texttt{pch}  
Plot character at the tip of the lines.
- \texttt{...}  
Other parameters passed to the generic plot, points or lines functions.

Author(s)

Charles Plessy

See Also

Other CAGEr richness functions: \texttt{hanabiPlot()}, \texttt{hanabi}
plotAnnot  

Plot annotation statistics

Description
Plot mapping statistics of an object containing mapping statistics in counts as percentages in stacked barplots.

Usage
plotAnnot(x, scope, title, group = "default", facet = NULL, normalise = TRUE)

Arguments
x  An object from which can be extracted a table with columns named promoter, exon, intron, mapped, extracted, rdna, and tagdust, that will be passed to the mapStats function.
scope  The name of a “scope”, that defines which data is plotted and how it is normalised, or a function implementing that scope. See mapStatsScopes() for details on each scope.
title  The title of the plot.
group  A factor to group the samples, or the name of a colData column of a CAGEexp object, or a formula giving the names of columns to be pasted together.
facet  A factor or the name of a colData column of a CAGEexp object, to facet the samples in the sense of ggplot2’s facet_wrap function.
normalise  Whether to normalise or not. Default: TRUE.

Details

Value
Returns invisibly a ggplot2 object of class c("gg", "ggplot").
Author(s)

Charles Plessy

See Also

mapStats() for a list of scopes.

Other CAGEr annotation functions: annotateCTSS(), ranges2annot(), ranges2genes(), ranges2names()

Other CAGEr plot functions: hanabiPlot(), plotCorrelation(), plotExpressionProfiles(), plotInterquantileWidth(), plotReverseCumulatives()

Examples

p <- plotAnnot(exampleCAGEexp, 'counts', 'Here is the title')
print(p)
p + ggplot2::theme_bw()
ggplot2::theme_set(ggplot2::theme_bw()); p
plotAnnot(exampleCAGEexp, 'counts', 'Same, non-normalised', normalise = FALSE)
exampleCAGEexp$myGroups <- factor(c("A", "B", "B", "C"))
plotAnnot(exampleCAGEexp, 'counts', group = "myGroups")
plotAnnot(exampleCAGEexp, 'counts', group = "myGroups")
plotAnnot(exampleCAGEexp, 'counts', group = ~sampleLabels + myGroups)
plotAnnot(exampleCAGEexp, CAGEr:::msScope_counts, group = "myGroups")

---

plotAnnot

Plotting Function

Description

Calculates the pairwise correlation between samples and creates a plot matrix showing the correlation coefficients in the upper triangle, the sample names in the diagonal, and the scatter plots in the lower triangle.

Usage

plotAnnot(
  object,
  what = c("CTSS", "consensusClusters"),
  values = c("raw", "normalized"),
  samples = "all",
  method = "pearson",
  tagCountThreshold = 1,
  applyThresholdBoth = FALSE,
  plotSize = 800
)

## S4 method for signature 'CAGEr'
plotCorrelation(
  object,
  what = c("CTSS", "consensusClusters"),
  values = c("raw", "normalized"),
  samples = "all",
  method = "pearson",
  tagCountThreshold = 1,
  applyThresholdBoth = FALSE,
  plotSize = 800
)

plotCorrelation2(
  object,
  what = c("CTSS", "consensusClusters"),
  values = c("raw", "normalized"),
  samples = "all",
  method = "pearson",
  tagCountThreshold = 1,
  applyThresholdBoth = FALSE,
  digits = 3
)

## S4 method for signature 'CAGEexp'
plotCorrelation2(
  object,
  what = c("CTSS", "consensusClusters"),
  values = c("raw", "normalized"),
  samples = "all",
  method = "pearson",
  tagCountThreshold = 1,
  applyThresholdBoth = FALSE,
  digits = 3
)

## S4 method for signature 'SummarizedExperiment'
plotCorrelation2(
  object,
  what = c("CTSS", "consensusClusters"),
  values = c("raw", "normalized"),
  samples = "all",
  method = "pearson",
  tagCountThreshold = 1,
  applyThresholdBoth = FALSE,
  digits = 3
)

## S4 method for signature 'DataFrame'
plotCorrelation2(
```r
plotCorrelation

object,
what = c("CTSS", "consensusClusters"),
values = c("raw", "normalized"),
samples = "all",
method = "pearson",
tagCountThreshold = 1,
applyThresholdBoth = FALSE,
digits = 3
)

## S4 method for signature 'data.frame'
plotCorrelation2(
  object,
  what = c("CTSS", "consensusClusters"),
  values = c("raw", "normalized"),
samples = "all",
  method = "pearson",
tagCountThreshold = 1,
  applyThresholdBoth = FALSE,
digits = 3
)

## S4 method for signature 'matrix'
plotCorrelation2(
  object,
  what = c("CTSS", "consensusClusters"),
  values = c("raw", "normalized"),
samples = "all",
  method = "pearson",
tagCountThreshold = 1,
  applyThresholdBoth = FALSE,
digits = 3
)

Arguments

object A CAGEr object or (only for plotCorrelation2) a SummarizedExperiment or an expression table as a DataFrame, data.frame or matrix object.
what The clustering level to be used for plotting and calculating correlations. Can be either "CTSS" to use individual TSSs or "consensusClusters" to use consensus clusters, i.e. entire promoters. Ignored for anything else than CAGEr objects.
values Use either "raw" (default) or "normalized" CAGE signal. Ignored for plain expression tables.
samples Character vector indicating which samples to use. Can be either "all" to select all samples in a CAGEr object, or a subset of valid sample labels as returned by the sampleLabels function.
method A character string indicating which correlation coefficient should be computed. Passed to cor function. Can be one of "pearson", "spearman", or "kendall".
```
plotCorrelation

tagCountThreshold
Only TSSs with tag count >= tagCountThreshold in either one (applyThresholdBoth = FALSE) or both samples (applyThresholdBoth = TRUE) are plotted and used to calculate correlation.

applyThresholdBoth
See tagCountThreshold above.

plotSize
Size of the individual comparison plot in pixels - the total size of the resulting png will be length(samples) * plotSize in both dimensions. Ignored in plotCorrelation2.

digits
The number of significant digits for the data to be kept in log scale. Ignored in plotCorrelation. In plotCorrelation2, the number of points plotted is considerably reduced by rounding the point coordinates to a small number of significant digits before removing duplicates. Chose a value that makes the plot visually indistinguishable with non-deduplicated data, by making tests on a subset of the data.

Details
In the scatter plots, a pseudo-count equal to half the lowest score is added to the null values so that they can appear despite logarithmic scale.

SummarizedExperiment objects are expected to contain raw tag counts in a “counts” assay and the normalized expression scores in a “normalized” assay.

Avoid using large matrix objects as they are coerced to DataFrame class without special care for efficiency.

plotCorrelation2 speeds up the plotting by a) deduplicating that data: no point is plot twice at the same coordinates, b) rounding the data so that indistinguishable positions are plotted only once, c) using a black square glyph for the points, d) caching some calculations that are made repeatedly (to determine where to plot the correlation coefficients), and e) preventing coercion of DataFrames to data.frames.

Value
Displays the plot and returns a matrix of pairwise correlations between selected samples. The scatterplots of plotCorrelation are colored according to the density of points, and in plotCorrelation2 they are just black and white, which is much faster to plot. Note that while the scatterplots are on a logarithmic scale with pseudocount added to the zero values, the correlation coefficients are calculated on untransformed (but thresholded) data.

Author(s)
Vanja Haberle
Charles Plessy

See Also
Other CAGEr plot functions: hanabiPlot(), plotAnnot(), plotExpressionProfiles(), plotInterquantileWidth(), plotReverseCumulatives()
plotExpressionProfiles

Examples

plotCorrelation2(exampleCAGEexp, what = "consensusClusters", value = "normalized")

plotExpressionProfiles

Plot CAGE expression profiles

Description

Beanplot of distribution of normalized expression across CAGE experiments for individual expression classes, colored and labeled according to the information set when expression clustering was performed.

Usage

plotExpressionProfiles(object, what)

## S4 method for signature 'CAGEexp'
plotExpressionProfiles(object, what = c("CTSS", "consensusClusters"))

Arguments

object A CAGEr object.
what CTSS or consensusClusters.

Details

The beanplots are shown in one labeled box per expression class. Each beanplot represents one CAGE experiment. The vertical axis represents scaled normalized expression. The color of each class is determined by the labels returned by expression clustering.

Author(s)

Vanja Haberle
Charles Plessy

See Also

Other CAGEr plot functions: hanabiPlot(), plotAnnot(), plotCorrelation(), plotInterquantileWidth(), plotReverseCumulatives()
Other CAGEr expression clustering functions: expressionClasses(), getExpressionProfiles()

Examples

plotExpressionProfiles(exampleCAGEexp, what = "CTSS")
exampleCAGEexp |> plotExpressionProfiles("consensusClusters")
plotInterquantileWidth

*Plot cluster widths*

Description

Histograms of the interquantile width of tag clusters or consensus clusters in each CAGE experiment.

Usage

```r
plotInterquantileWidth(
  object,
  clusters = c("tagClusters", "consensusClusters"),
  tpmThreshold = 5,
  qLow = 0.1,
  qUp = 0.9,
  xlim = c(0, 150)
)
```

## S4 method for signature 'CAGEexp'

```r
plotInterquantileWidth(
  object,
  clusters = c("tagClusters", "consensusClusters"),
  tpmThreshold = 5,
  qLow = 0.1,
  qUp = 0.9,
  xlim = c(0, 150)
)
```

Arguments

- **object**: A `CAGEexp` object
- **clusters**: tagClusters or consensusClusters.
- **tpmThreshold**: Exclude clusters with normalized signal lower than tpmThreshold.
- **qLow**, **qUp**: Quantile defining the 5' ("lower") and 3' ("upper") boundaries of the clusters.
- **xlim**: Range of width to be plotted.

Details

Interquantile width is a more robust measure of the promoter width than the total span of the region, because it takes into account the magnitude of the expression in the region. Positions of specified quantiles within each cluster have to be calculated beforehand by calling `quantilePositions`.

Value

Plots the histograms with the ggplot2 engine and returns the plot object invisibly.
plotReverseCumulatives

Author(s)
Vanja Haberle
Charles Plessy

See Also
Other CAGEr plot functions: hanabiPlot(), plotAnnot(), plotCorrelation(), plotExpressionProfiles(), plotReverseCumulatives()
Other CAGEr clusters functions: CTSSclusteringMethod(), CTSScumulativesTagClusters(), CustomConsensusClusters(), aggregateTagClusters(), clusterCTSS(), consensusClustersDESeq2(), consensusClustersGR(), cumulativeCTSSdistribution(), quantilePositions(), tagClustersGR()

Examples

plotInterquantileWidth( exampleCAGEexp, clusters = "tagClusters"
    , tpmThreshold = 50, qLow = 0.1, qUp = 0.9
    , xlim = c(2,200))

plotInterquantileWidth( exampleCAGEexp, clusters = "consensusClusters"
    , tpmThreshold = 50, qLow = 0.1, qUp = 0.9
    , xlim = c(2,200))

Description

Plots the reverse cumulative distribution of the number of CAGE tags per CTSS for all CAGE datasets present in the CAGER object. The plots should be used as help in choosing the parameters for power-law normalization: the range of values to fit the power-law and the slope of the referent power-law distribution (Balwierz et al., Genome Biology 2009).

Usage

plotReverseCumulatives(
    object,
    values = c("raw", "normalized"),
    fitInRange = c(10, 1000),
    onePlot = FALSE,
    main = NULL,
    legend = TRUE,
    xlab = "number of CAGE tags",
    ylab = "number of CTSSs (>= nr tags)",
    xlim = c(1, 1e+05),
    ylim = c(1, 1e+06)
## S4 method for signature 'CAGEr'
plotReverseCumulatives(
    object,
    values = c("raw", "normalized"),
    fitInRange = c(10, 1000),
    onePlot = FALSE,
    main = NULL,
    legend = TRUE,
    xlab = "number of CAGE tags",
    ylab = "number of CTSSs (>= nr tags)",
    xlim = c(1, 1e+05),
    ylim = c(1, 1e+06)
)

### Arguments

- **object**
  - A CAGEr object

- **values**
  - Which values should be plotted: raw (default) for raw CAGE tag counts or normalized for normalized tag count values.

- **fitInRange**
  - An integer vector with two values specifying a range of tag count values to be used for fitting a power-law distribution to reverse cumulatives. Ignored is set to NULL. See Details.

- **onePlot**
  - Logical, should all CAGE datasets be plotted in the same plot (TRUE) or in separate plots (FALSE).

- **main**
  - Main title for the plot.

- **legend**
  - Set to NULL to prevent the display of the sample legend.

- **xlab, ylab**
  - Axis labels passed to `plot`.

- **xlim, ylim**
  - Axis range parameters passed to `plot`.

### Details

Number of CAGE tags (X-axis) is plotted against the number of TSSs that are supported by >= of that number of tags (Y-axis) on a log-log scale for each sample. In addition, a power-law distribution is fitted to each reverse cumulative using the values in the range specified by `fitInRange` parameter. The fitted distribution is defined by \( y = -1 \times \alpha \times x + \beta \) on the log-log scale, and the value of \( \alpha \) for each sample is shown on the plot. In addition, a suggested referent power-law distribution to which all samples should be normalized is drawn on the plot and corresponding parameters (slope \( \alpha \) and total number of tags \( T \)) are denoted on the plot. Referent distribution is chosen so that its slope (\( \alpha \)) is the median of slopes fitted to individual samples and its total number of tags (\( T \)) is the power of 10 nearest to the median number of tags of individual samples. Resulting plots are helpful in deciding whether power-law normalization is appropriate for given samples and reported \( \alpha \) values aid in choosing optimal \( \alpha \) value for referent power-law distribution to which all samples will be normalized. For details about normalization see `normalizeTagCount` function.
quantilePositions

Value

Plots of reverse cumulative number of CAGE tags per CTSS for each CAGE dataset within CAGEr object. Alpha values of fitted power-laws and suggested referent power-law distribution are reported on the plot in case values = "raw".

Author(s)

Vanja Haberle

References

Balwierz et al. (2009) Methods for analyzing deep sequencing expression data: constructing the human and mouse promoterome with deepCAGE data, Genome Biology 10(7):R79.

See Also

distributeTagCount

Other CAGEr plot functions: hanabiPlot(), plotAnnot(), plotCorrelation(), plotExpressionProfiles(), plotInterquantileWidth()

Examples

plotReverseCumulatives( exampleCAGEexp, xlim = c(1, 1e4), ylim = c(1, 1e5)
  , fitInRange = c(5,100), onePlot = TRUE)
plotReverseCumulatives( exampleCAGEexp, values = "normalized"
  , fitInRange = c(200, 2000), onePlot = TRUE)
plotReverseCumulatives( exampleCAGEexp[,4:5], fitInRange = c(5,100)
  , onePlot = TRUE, main = "prim6 replicates")

quantilePositions

Determine CTSS quantile positions within clusters

Description

Calculates the positions of “upper” and “lower” quantiles of CAGE signal along tag clusters or consensus clusters in each sample of a CAGEexp object.

Usage

quantilePositions(
  object,
  clusters = c("tagClusters", "consensusClusters"),
  qLow = 0.1,
  qUp = 0.9,
  useMulticore = FALSE,
  nrCores = NULL
)
```r
## S4 method for signature 'CAGEexp'
quantilePositions(
  object,
  clusters = c("tagClusters", "consensusClusters"),
  qLow = 0.1,
  qUp = 0.9,
  useMulticore = FALSE,
  nrCores = NULL
)
```

**Arguments**

- `object`: A CAGEExp object.
- `clusters`: Either `tagClusters` or `consensusClusters`.
- `qLow`, `qUp`: Which “lower” or “upper” quantiles should be calculated. Numeric vector of values in range `[0,1]`.
- `useMulticore`: Logical, should multicore be used. `useMulticore = TRUE` has only effect on Unix-like platforms.
- `nrCores`: Number of cores to use when `useMulticore = TRUE`. Default value `NULL` uses all detected cores.

**Details**

From the 5’ end the position, the position of a quantile `q` is determined as the first base in which of the cumulative expression is higher or equal to `q%` of the total CAGE signal of that cluster. Promoter *interquantile width* is defined as the distance (in base pairs) between a “lower” and an “upper” quantile position.

**Value**

Returns the objects, in which the positions of the quantiles are defined relatively to the start point of their cluster, for more efficient Rle compression. The quantile data for tag clusters are stored in the `TagClusters` objects directly. The quantile data for consensus clusters are stored in integer matrices named “q_x”, where `x` represents the quantile (for instance, q_0.1), and these matrices are assays of the `consensusClusters RangedSummarizedExperiment`.

**Author(s)**

Vanja Haberle
Charles Plessy

**See Also**

Other CAGEr object modifiers: `CTSStoGenes()`, `CustomConsensusClusters()`, `aggregateTagClusters()`, `annotateCTSS()`, `clusterCTSS()`, `cumulativeCTSSdistribution()`, `getCTSS()`, `normalizeTagCount()`, `quickEnhancers()`, `resetCAGEexp()`, `summariseChrExpr()`
quickEnenhancers

Other CAGEr clusters functions: `CTSSclusteringMethod()`, `CTSScumulativesTagClusters()`, `CustomConsensusClusters()`, `aggregateTagClusters()`, `clusterCTSS()`, `consensusClustersDESeq2()`, `consensusClustersGR()`, `cumulativeCTSSdistribution()`, `plotInterquantileWidth()`, `tagClustersGR()

Examples

```r
quantilePositions(exampleCAGEexp, "tagClusters", qLow = c(0.1, 0.2), qUp = c(0.8, 0.9))
tagClustersGR(exampleCAGEexp)
quantilePositions(exampleCAGEexp, "consensusClusters", qLow = c(0.1, 0.2), qUp = c(0.8, 0.9))
```

quickEnenhancers  Identify and quantify enhancers.

Description

A convenient wrapper to the function `CAGEfightR::quickEnhencers()`.

Usage

```r
quickEnhancers(object)
```

## S4 method for signature 'CAGEexp'
quickEnhancers(object)

Arguments

object  A CAGEexp object

Details

The CAGEr object will be converted to a format similar to the output of `CAGEfightR::quantifyCTSSs()`, and then passed to the quickEnhancers function.

Value

A RangedSummarizedExperiment object. See the example below on how to attach it to the experiment list of a CAGEexp object.

Note

At the moment the conversion is expensive as it goes from DataFrame of Rle to data.frame to matrix.

See Also

Other CAGEr object modifiers: `CTSStoGenes()`, `CustomConsensusClusters()`, `aggregateTagClusters()`, `annotateCTSS()`, `clusterCTSS()`, `cumulativeCTSSdistribution()`, `getCTSS()`, `normalizeTagCount()`, `quantilePositions()`, `resetCAGEexp()`, `summariseChrExpr()`
Examples

```r
# Can not run as long as the test data has nothing on the minus strand!
## Not run:
quickEnhancers(exampleCAGEexp)
## End(Not run)
```

ranges2annot  *Hierarchical annotation of genomic regions.*

Description

Assigns region types such as promoter, exon or unknown to genomic regions such as CTSS, tag clusters, or consensus clusters.

Usage

```r
ranges2annot(ranges, annot, upstream = 500, downstream = 500)
```

Arguments

- `ranges`: A `GenomicRanges::GRanges` object, for example extracted from a `RangedSummarizedExperiment` object with the `rowRanges` command.
- `annot`: A `GRanges` from which promoter positions will be inferred. Typically GENCODE. If the `type` metadata is present, it should contain gene, exon and transcript among its values. Otherwise, all entries are considered transcripts. If the `transcript_type` metadata is available, the entries that may not be primary products (for instance ‘snoRNA’) are discarded.
- `upstream`: Number of bases upstream the start of the transcript models to be considered as part of the `promoter region`.
- `downstream`: Number of bases downstream the start of the transcript models to be considered as part of the `promoter region`.

Details

Only the biotypes that are likely to have a pol II promoter will be filtered in. This is currently hardcoded in the function; see its source code. Example of biotypes without a pol II promoter: VDJ segments, miRNA, but also snoRNA, etc. Thus, the *Intergenic* category displayed in output of the `plotAnnot` may include counts overlapping with real exons of discarded transcribed regions: be careful that large percentages do not necessarily suggest abundance of novel promoters.

Value

A Run-length-encoded (Rle) factor of same length as the CTSS object, indicating if the interval is promoter, exon, intron or unknown, or just promoter, gene, unknown if the type metadata is absent.
Author(s)
Charles Plessy

See Also
CTSScoordinatesGR, exampleZv9_annot

Other CAGEr annotation functions: annotateCTSS(), plotAnnot(), ranges2genes(), ranges2names()

Examples
CAGEr:::ranges2annot(CTSScoordinatesGR(exampleCAGEexp), exampleZv9_annot)

ctss <- GenomicRanges::GRanges("chr1", IRanges::IPos(c(1,100,200,1500)), "+")
ctss <- GenomicRanges::GPos(ctss, stitch = FALSE)
ctss <- as(ctss, "CTSS")
gr1 <- GenomicRanges::GRanges("chr1"
                           , IRanges::IRanges(c(650, 650, 1400), 2000), "+")
CAGEr:::ranges2annot(ctss, gr1)
gr2 <- gr1
gr2$type <- c("transcript", "exon", "transcript")
gr2$transcript_type <- c("protein_coding", "protein_coding", "miRNA")
CAGEr:::ranges2annot(ctss, gr2, up=500, down=20)

Description
Assign gene symbol(s) to Genomic Ranges.

Usage
ranges2genes(ranges, genes)

Arguments

ranges GenomicRanges::GRanges object, for example extracted from a SummarizedExperiment::RangedSummarizedExperiment
object with the SummarizedExperiment::rowRanges command.

genes A GRanges object containing gene_name metadata.

Details
This private (non-exported) function is used to assign gene symbols to genomic ranges. It is run by
annotateCTSS, which has to be run before CTSSToGenes.
Value

A `S4Vectors::Rle` factor of same length as the `GRanges` object, indicating one gene symbol or a semicolon-separated list of gene symbols for each range. The levels are alphabetically sorted.

Author(s)

Charles Plessy

See Also

`CTSScoordinatesGR`, `exampleZv9_annot`

Other CAGEr annotation functions: `annotateCTSS()`, `plotAnnot()`, `ranges2annot()`, `ranges2names()`

Other CAGEr gene expression analysis functions: `CTSstoGenes()`, `GeneExpDESeq2()

Examples

```r
CAGEr:::ranges2genes(CTSScoordinatesGR(exampleCAGEexp), exampleZv9_annot)
```

---

### ranges2names

#### Description

Intersection of genomic ranges

#### Usage

```r
ranges2names(rangesA, rangesB)
```

#### Arguments

- `rangesA` : A `GenomicRanges::GRanges` object.
- `rangesB` : A second `GRanges` object.

#### Details

This private (non-exported) function intersects two genomic ranges and for each element of the first object returns the name of the elements of the second object that it intersects with.

#### Value

A `Rle` factor of same length as the `rangesA` `GRanges` object, indicating one name or a semicolon-separated list of names from each `rangesB` object. The levels are in order of appearance to maintain genomic coordinate sort order when the names are cluster names.
resetCAGEexp

Author(s)
Charles Plessy

See Also
Other CAGEr annotation functions: annotateCTSS(), plotAnnot(), ranges2annot(), ranges2genes()

Examples
names(exampleZv9_annot) <- exampleZv9_annot$gene_name
cAGER:::ranges2names(CTSScoordinatesGR(exampleCAGEexp), exampleZv9_annot)

resetCAGEexp

Reset a CAGEexp object

Description
Removes all data but the raw CTSS counts and coordinates from a CAGEexp object. Useful after removing samples.

Usage
resetCAGEexp(object)

## S4 method for signature 'CAGEexp'
resetCAGEexp(object)

Arguments
object A CAGEexp object

Value
Returns a CAGEexp object, which contains a non-normalised tagCountMatrix experiment.

Author(s)
Charles Plessy

See Also
Other CAGEr object modifiers: CTSStoGenes(), CustomConsensusClusters(), aggregateTagClusters(), annotateCTSS(), clusterCTSS(), cumulativeCTSSdistribution(), getCTSS(), normalizeTagCount(), quantilePositions(), quickEnhancers(), summariseChrExpr()

Examples
resetCAGEexp(exampleCAGEexp)
rowsum.RleDataFrame  

**rowsum function for Rle DataFrames**

### Description

Drop-in replacement for the `rowsum` function, which does not work natively on `S4Vectors::DataFrame` objects containing `S4Vectors::Rle`-encoded numerical values.

### Usage

```r
rowsum.RleDataFrame(x, group, reorder = TRUE, na.rm = FALSE, ...)
```

### Arguments

- **x**: A `DataFrame` containing only numerical `Rle` columns.
- **group**: a vector or factor giving the grouping, with one element per row of `x`. Missing values will be treated as another group and a warning will be given.
- **reorder**: If TRUE, then the result will be in order of `sort(unique(group))`, if FALSE, it will be in the order that groups were encountered.
- **na.rm**: Logical (TRUE or FALSE). Should NA (including NaN) values be discarded?
- **...**: Other arguments to be passed to or from methods.

### Details

See the file `benchmarks/rowsum_on_Rle_DF.md` in the source Git repository of `CAGEr` for the alternatives that were considered.

### Author(s)

Charles Plessy

### See Also

Other `Rle` DataFrames: `rowSums.RleDataFrame()`

### Examples

```r
exampleCAGEexp |> CTSTagCountDF() |> 
CAGEr:::rowsum.RleDataFrame(decode(CTSScoordinatesGR(exampleCAGEexp)$cluster), reorder = FALSE)
```
rowSums.RleDataFrame  

rowSums function for Rle DataFrames

Description

Drop-in replacement for the rowSums function, which does not work natively on S4Vectors::DataFrame objects containing S4Vectors::Rle-encoded numerical values.

Usage

rowSums.RleDataFrame(x, na.rm = FALSE)

Arguments

x
A DataFrame containing only numerical Rle columns.

na.rm
logical. Should missing values (including NaN) be omitted from the calculations?

Details

See the file benchmarks/rowSums_on_Rle_DF.md in the source Git repository of CAGEr for the alternatives that were considered.

Value

A Rle-encoded numerical vector of the same class as in the DataFrame.

Author(s)

Charles Plessy

See Also

Other Rle DataFrames: rowsum.RleDataFrame()

Examples

eampleCAGEexp | CTStagCountDF() | CAGEr:::rowSums.RleDataFrame(na.rm = TRUE)
sampleLabels

Get and set sample labels

Description

sampleLabels gets or sets the labels and colors of CAGE datasets (samples) from CAGEr objects. sampleList is an accessory function for convenience iteration in functions such as lapply or mapply. There is no set method for sampleList.

Usage

```r
sampleLabels(object)
## S4 method for signature 'CAGEexp'
sampleLabels(object)
## S4 method for signature 'CTSS'
sampleLabels(object)

sampleList(object)
## S4 method for signature 'CAGEr'
sampleList(object)

sampleLabels(object) <- value
## S4 replacement method for signature 'CAGEexp'
sampleLabels(object) <- value
## S4 replacement method for signature 'CTSS'
sampleLabels(object) <- value
```

Arguments

- `object`: A CAGEr object.
- `value`: A character vector with a unique and valid name for each sample. The names attributes indicate the colors.

Details

In CAGEexp objects, renaming samples is possible only before data is loaded.

Value

sampleLabels returns a named character vector representing labels of all CAGE datasets present in the CAGEr object. The vector values are the labels and the vector names are the colors.
sampleList returns a named list where elements and their names are the sample names, for instance:

```r
list(sampleA = "sampleA", sampleB = "sampleB")
```

Thus, after iterating on it with `lapply`, the element names will be sample names.

**Note**

If no colors are supplied, then default colors will be assigned using the `rainbow` function. Assigned colors are not guaranteed to be stable.

**Author(s)**

Vanja Haberle
Charles Plessy

**See Also**

`setColors`

Other CAGEr accessor methods: `CTSSclusteringMethod()`, `CTSScoordinatesGR()`, `CTSScumulativesTagClusters()`, `CTSSnormalizedTpmDF()`, `CTSStagCountDF()`, `GeneExpDESeq2()`, `GeneExpSE()`, `consensusClustersGR()`, `expressionClasses()`, `genomeName()`, `inputFileType()`, `inputFiles()`, `librarySizes()`, `seqNameTotalsSE()`, `tagClustersGR()`

Other CAGEr setter methods: `genomeName()`, `inputFileType()`, `inputFiles()`, `setColors()`

**Examples**

```r
sampleLabels(exampleCAGEexp)
sampleList(exampleCAGEexp)
```

---

**scoreShift**

*Calculate promoter shifting score*

**Description**

Calculates the shifting score for all consensus clusters (promoters) between two specified (groups of) CAGE datasets. Shifting score is a measure of differential usage of TSSs within consensus cluster between two samples, which indicates the degree of physical separation of TSSs used in these samples within given consensus cluster. In addition to shifting score, a statistical significance (P-value and FDR) of differential TSS usage is calculated for each consensus cluster using Kolmogorov-Smirnov test.
Usage

```r
scoreShift(
  object,
  groupX,
  groupY,
  testKS = TRUE,
  useTpmKS = TRUE,
  useMulticore = F,
  nrCores = NULL
)
```

## S4 method for signature 'CAGEexp'
```r
scoreShift(
  object,
  groupX,
  groupY,
  testKS = TRUE,
  useTpmKS = TRUE,
  useMulticore = F,
  nrCores = NULL
)
```

Arguments

- **object**
  A CAGEr object.

- **groupX, groupY**
  Character vector of the one or more CAGE dataset labels in the first (groupX) and in the second group (groupY). Shifting score for each consensus cluster will be calculated by comparing CAGE signal in the samples from groupX against the signal in the samples from groupY. If there is more than one CAGE dataset in the group, the datasets within that group will be merged together before comparison with the other group. See Details.

- **testKS**
  Logical, should Kolomogorov-Smirnov test for statistical significance of differential TSS usage be performed, and P-values and FDR returned. See Details.

- **useTpmKS**
  Logical, should normalized (tpm) values (TRUE) or raw tag counts (FALSE) be used to derive sample sizes for Kolomogorov-Smirnov test. Used only when testKS = TRUE, otherwise ignored. See Details.

- **useMulticore**
  Logical, should multicore be used. useMulticore = TRUE is supported only on Unix-like platforms.

- **nrCores**
  Number of cores to use when useMulticore = TRUE. Default value NULL uses all detected cores.

Details

TSSs within one consensus cluster (promoter) can be used differently in different samples (cell types, tissues, developmental stages), with respect to their position and frequency of usage detected.
by CAGE. This function calculates shifting scores of all consensus clusters between two specified (groups of) CAGE samples to detect promoters that are used differently in these two samples. Shifting score is a measure of differential TSS usage defined as:

\[
\text{score} = \max(F_1 - F_2) / \max(F_1)
\]

where \(F_1\) is a cumulative sum of CAGE signal along consensus cluster in the group of samples with lower total signal in that consensus cluster, and \(F_2\) in the opposite group. Since cumulative sum can be calculated in both forward (5' -> 3') and reverse (3' -> 5') direction, shifting score is calculated for both cases and the bigger value is selected as final shifting score. Value of the shifting score is in the range \([-\infty, 1]\), where value of 1 means complete physical separation of TSSs used in the two samples for given consensus cluster. In general, any non-negative value of the shifting score can be interpreted as the proportion of transcription initiation in the sample with lower expression that is happening "outside" (either upstream or downstream) of the region used for transcription initiation in the other sample. Negative values indicate no physical separation, i.e. the region used for transcription initiation in the sample with lower expression is completely contained within the region used for transcription initiation in the other sample.

In addition to shifting score which indicates only physical separation (upstream or downstream shift of TSSs), a more general assessment of differential TSS usage can be obtained by performing a two-sample Kolmogorov-Smirnov test on cumulative sums of CAGE signal along the consensus cluster. In that case, cumulative sums in both samples are scaled to range \([0, 1]\) and are considered to be empirical cumulative distribution functions (ECDF) reflecting sampling of TSS positions during transcription initiation. Kolmogorov-Smirnov test is performed to assess whether the two underlying probability distributions differ. To obtain P-value (i.e. the level at which the null-hypothesis can be rejected), sample sizes that generated the ECDFs are required, in addition to actual K-S statistics calculated from ECDFs. These are derived either from raw tag counts, i.e. exact number of times each TSS in the cluster was sampled during sequencing (when useTpmKS = FALSE), or from normalized tpm values (when useTpmKS = TRUE). P-values obtained from K-S tests are further adjusted for multiple testing using Benjamini & Hochberg (BH) method and for each P-value a corresponding false-discovery rate (FDR) is also reported.

Since calculation of shifting scores and Kolmogorov-Smirnov test require cumulative sums along consensus clusters, they have to be calculated beforehand by calling \texttt{cumulativeCTSSdistribution} function.

The slots \texttt{shiftingGroupX}, \texttt{shiftingGroupY} and \texttt{consensusClustersShiftingScores} of the provided \texttt{CAGEexp} object will be occupied by the information on the groups of CAGE datasets that have been compared and shifting scores of all consensus clusters. Consensus clusters (promoters) with shifting score and/or FDR above specified threshold can be extracted by calling \texttt{getShiftingPromoters} function.

\textbf{Author(s)}

Vanja Haberle

Sarvesh Nikumbh

\textbf{See Also}

\texttt{cumulativeCTSSdistribution}

Other CAGEr promoter shift functions: \texttt{getShiftingPromoters()}

Examples

```r
scoreShift(exampleCAGEexp,
  groupX = c("Zf.unfertilized.egg"),
  groupY = "Zf.30p.dome",
  testKS = TRUE, useTpmKS = FALSE)
```

### Description

Get or set a `SummarizedExperiment` summarising whole-chromosome expression levels in the experiment slot `seqNameTotals` and the sample metadata of the `CAGEexp` object.

#### Usage

```r
seqNameTotalsSE(object)
```
```r
seqNameTotalsSE(object) <- value
```

#### Arguments

- `object`: A `CAGEexp` object.
- `value`: A `SummarizedExperiment` object where rows represent reference sequences such as chromosomes.

#### Author(s)

Charles Plessy

#### See Also

- `summariseChrExpr`

Other CAGEr accessor methods: `CTSSclusteringMethod()`, `CTSScoordinatesGR()`, `CTSScumulativesTagClusters()`, `CTSSnormalizedTpmDF()`, `CTSStagCountDF()`, `GeneExpDESeq2()`, `GeneExpSE()`, `consensusClustersGR()`, `expressionClasses()`, `genomeName()`, `inputFilesType()`, `inputFiles()`, `librarySizes()`, `sampleLabels()`, `tagClustersGR()`

#### Examples

```r
seqNameTotalsSE(exampleCAGEexp)
```
setColors

Set colors for samples

Description

Assigns one color to each sample in the CAGEr object. These colors are used in various plots and exported tracks to consistently represent corresponding samples.

Usage

setColors(object, colors = NULL)

## S4 method for signature 'CAGEr'
setColors(object, colors = NULL)

Arguments

- **object**: A CAGEr object.
- **colors**: A character vector of one valid R color specification per sample (see col2rgb for details). Provided colors are assigned to samples in the order they are returned by the sampleLabels function.

Value

Assigns one color to each sample in the CAGEr object and modifies it in place.

Author(s)

Vanja Haberle

See Also

Other CAGEr setter methods: `genomeName()`, `inputFilesType()`, `inputFiles()`, `sampleLabels()`

Examples

```r
sampleLabels(exampleCAGEexp)
sampleLabels(exampleCAGEexp, 5)
sampleLabels(exampleCAGEexp)
sampleLabels(exampleCAGEexp, c("#ff0000ff", "#CCFF00", "blue", "grey", 1))
sampleLabels(exampleCAGEexp)
sampleLabels(exampleCAGEexp, c("red", "darkgreen", "blue", "grey", "black"))
sampleLabels(exampleCAGEexp)
```
Strand invaders

Detect and remove strand invasion artefacts

**Description**

*findStrandInvaders* detects strand invasion artefacts in the CTSS data. *removeStrandInvaders* removes them.

*Strand invaders* are artefacts produced by template switching reactions used in methods such as *nanoCAGE* and its derivatives (*C1 CAGE*, ...). They are described in details in Tang *et al*., 2013. Briefly, these artefacts create CAGE-like signal downstream of genome sequences highly similar to the tail of template-switching oligonucleotides, which is TATAGG in recent (2017) nanoCAGE protocols. Since these artefacts represent truncated cDNAs, they do not indicate promoter regions. It is therefore advisable to remove these artefacts. Moreover, when a sample barcode is near the linker sequence (which is not the case in recent nanoCAGE protocols), the strand-invasion artefacts can produce *sample-specific biases*, which can be confounded with biological effects depending on how the barcode sequences were chosen. A barcode parameter is provided to incorporate this information.

**Usage**

```r
findStrandInvaders(object, distance = 1, barcode = NULL, linker = "TATAGGG")

removeStrandInvaders(object, distance = 1, barcode = NULL, linker = "TATAGGG")
```

**Arguments**

- **object**
  A *CAGEexp* object object containing CTSS data and the name of a reference genome.

- **distance**
  The maximal edit distance between the genome and linker sequences. Regardless this parameter, only a single mismatch is allowed in the last three bases of the linker.

- **barcode**
  A vector of sample barcode sequences, or the name of a column metadata of the *CAGEexp* object containing this information. *(Not implemented yet)*

- **linker**
  The sequence of the tail of the template-switching oligonucleotide, that will be matched with the genome sequence (defaults to TATAGG).
findStrandInvaders returns a logical-Rle vector indicating the position of the strand invaders in the input ranges. With CTSS objects as input removeStrandInvaders returns the object after removing the CTSS positions identified as strand invaders. In the case of CAGEexp objects, a modified object is returned. Its sample metadata is also updated by creating a new strandInvaders column that indicates the number of molecule counts removed. This value is subtracted from the counts column so that the total number of tags is still equal to librarySizes.

References


Examples

# Note that these examples do not do much on the example data since it was not constructed using a protocol based using the template-switching method.

findStrandInvaders(exampleCAGEexp)
removeStrandInvaders(exampleCAGEexp)

summariseChrExpr

Expression levels by chromosomes

Description

Counts the number of molecules detected per chromosome, normalises by library size and stores the raw and normalised results in the CAGEr object.

Usage

summariseChrExpr(object)

## S4 method for signature 'CAGEexp'
summariseChrExpr(object)

Arguments

object A CAGEexp object objects are not supported).

Value

Modifies the CAGEexp by adding a “seqNameTotals” experiment containing matrices where rows represent chromosomes and columns represent samples.
Author(s)

Charles Plessy

See Also

seqNameTotals

Other CAGEr object modifiers: CTSStoGenes(), CustomConsensusClusters(), aggregateTagClusters(), annotateCTSS(), clusterCTSS(), cumulativeCTSSdistribution(), getCTSS(), normalizeTagCount(), quantilePositions(), quickEnhancers(), resetCAGEexp()

Examples

summariseChrExpr(exampleCAGEexp)

tagClustersGR

TagClusters-class

TagClusters

Description

TagClusters

Details

The TagClusters class represents tag clusters. It is used internally by CAGEr for type safety.

Example

Extract tag clusters (TCs) for individual CAGE experiments

description

Extracts tag clusters (TCs) produced by clusterCTSS function for a specified CAGE experiment from a CAGEexp object.

Usage

tagClustersGR(
  object,
  sample = NULL,
  returnInterquantileWidth = FALSE,
  qLow = NULL,
  qUp = NULL
)

## S4 method for signature 'CAGEexp'
`tagClustersGR`

`tagClustersGR`

```
tagClustersGR(
  object,
  sample = NULL,
  returnInterquantileWidth = FALSE,
  qLow = NULL,
  qUp = NULL
)
tagClustersGR(object, sample = NULL) <- value
```

## S4 replacement method for signature 'CAGEexp,ANY,TagClusters'
tagClustersGR(object, sample = NULL) <- value

## S4 replacement method for signature 'CAGEexp,missing,GRangesList'
tagClustersGR(object, sample = NULL) <- value

### Arguments

- **object**: A CAGEexp object.
- **sample**: Label of the CAGE dataset (experiment, sample) for which to extract tag clusters. If `samples = NULL`, a list of all the clusters for each sample is returned.
- **returnInterquantileWidth**: Return the interquantile width for each tag cluster.
- **qLow, qUp**: Position of which quantile should be used as a left (lower) or right (upper) boundary (for `qLow` and `qUp` respectively) when calculating interquantile width. Default value `NULL` results in using the start coordinate of the cluster. Used only when `returnInterquantileWidth = TRUE`, otherwise ignored.
- **value**: A `TagClusters` object.

### Value

Returns a `GRangesList` or a `GRanges` object with genomic coordinates, position of dominant TSS, total CAGE signal and additional information for all TCs from specified CAGE dataset (sample). If `returnInterquantileWidth = TRUE`, interquantile width for each TC is also calculated using provided quantile positions. The `S4Vectors::metadata` slot of the object contains a copy of the CAGEexp object’s `column data`, as well as information on the clustering method in a `clusteringMethod` element.

### Author(s)

- Vanja Haberle
- Charles Plessy

### See Also

Other CAGEr accessor methods: `CTSSclusteringMethod()`, `CTSScoordinatesGR()`, `CTSScumulatesTagClusters()`, `CTSSnormalizedTpmsDF()`, `CTSStagCountDF()`, `GeneExpDESeq2()`, `GeneExpSE()`, `consensusClustersGR()`.
expressionClasses(), genomeName(), inputFileType(), inputFiles(), librarySizes(), sampleLabels(), seqNameTotalsSE()

Other CAGEr clusters functions: CTSSclusteringMethod(), CTSScumulativesTagClusters(), CustomConsensusClusters(), aggregateTagClusters(), clusterCTSS(), consensusClustersDESeq2(), consensusClustersGR(), cumulativeCTSSdistribution(), plotInterquantileWidth(), quantilePositions()

Examples

tagClustersGR( exampleCAGEexp, "Zf.high", TRUE, 0.1, 0.9 )
tagClustersGR( exampleCAGEexp, 1
    , returnInterquantileWidth = TRUE, qLow = 0.1, qUp = 0.9 )
tagClustersGR( exampleCAGEexp )@metadata$colData
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