

# Package ‘CAGEr’

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**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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'ClusteringMethods.R' 'Annotations.R' 'AggregationMethods.R'  
'CorrelationMethods.R' 'CumulativeDistributionFunctions.R'  
'GetMethods.R' 'CumulativeDistributionMethods.R'  
'ExportFunctions.R' 'ExportMethods.R'  
'ExpressionProfilingMethods.R' 'ImportFunctions.R'  
'SetMethods.R' 'ImportMethods.R' 'MergingMethods.R'  
'NormalizationFunctions.R' 'NormalizationMethods.R'  
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CAGEr-package	<i>Analysis of CAGE (Cap Analysis of Gene Expression) sequencing data for precise mapping of transcription start sites and promoterome mining</i>
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### 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	<i>Apply functions to identical CTSSes.</i>
---------	---------------------------------------------

---

### 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(ctssDT, colName, fun)
```

**Arguments**

<code>ctssDT</code>	A <code>data.table</code> representing CTSSes.
<code>colName</code>	The name of the column on which to apply the function.
<code>fun</code>	The function to apply.

**Examples**

```
ctssDT <- 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      ))  
ctssDT  
CAGEr:::byCtss(ctssDT, "tag_count", sum)
```

---

`.clusterAggregateAndSum`*Aggregate identical clusters and sum their scores.*

---

**Description**

Private function using `data.table` objects to perform 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**

```
.clusterAggregateAndSum(clusters, key)  
  
## S4 method for signature 'data.table'  
.clusterAggregateAndSum(clusters, key)  
  
## S4 method for signature 'data.frame'  
.clusterAggregateAndSum(clusters, key)  
  
## S4 method for signature 'GRanges'  
.clusterAggregateAndSum(clusters, key)
```

**Arguments**

<code>clusters</code>	Clusters to be aggregated. <code>data.frame</code> , or <code>GRanges</code> , which will be coerced to <code>data.frame</code> .
<code>key</code>	Name of the column containing the factor used to aggregate the clusters.

---

`.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

<code>cum.sums</code>	Named list of vectors containing cumulative sum for each cluster (returned by the <code>CTSScumulativesTagClusters</code> or <code>CTSScumulativesCC</code> function).
<code>clusters</code>	<a href="#">TagClusters</a> or <a href="#">ConsensusClusters</a> object representing tag clusters or consensus clusters.
<code>q</code>	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))
```

---

`.powerLaw`                      *.powerLaw*

---

### Description

Private funtion 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 reconstructed.

**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**

Balwierz, P. J., Carninci, P., Daub, C. O., Kawai, J., Hayashizaki, Y., Van Belle, W., Beisel, C., et al. (2009). Methods for analyzing deep sequencing expression data: constructing the human and mouse promoterome with deepCAGE data. *Genome Biology*, 10(7), R79.

---

aggregateTagClusters *Aggregate TCs across all samples*

---

**Description**

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

**Usage**

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

```

)

## S4 method for signature 'CAGEr'
aggregateTagClusters(
  object,
  tpmThreshold = 5,
  excludeSignalBelowThreshold = TRUE,
  qLow = NULL,
  qUp = NULL,
  maxDist = 100,
  useMulticore = FALSE,
  nrCores = NULL
)

```

### Arguments

object	A <a href="#">CAGEr</a> 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.
maxDist	Maximal length of the gap (in base-pairs) between two tag clusters for them to be part of the same consensus clusters.
useMulticore	Logical, should multicore be used (supported only on Unix-like platforms).
nrCores	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: [CTSSstoGenes\(\)](#), [CustomConsensusClusters\(\)](#), [annotateCTSS\(\)](#), [clusterCTSS\(\)](#), [cumulativeCTSSdistribution\(\)](#), [getCTSS\(\)](#), [normalizeTagCount\(\)](#), [quantilePositions\(\)](#), [summariseChrExpr\(\)](#)

Other CAGEr clusters functions: [CTSSclusteringMethod\(\)](#), [CTSScumulativesTagClusters\(\)](#), [CustomConsensusClusters\(\)](#), [clusterCTSS\(\)](#), [consensusClustersDESeq2\(\)](#), [consensusClustersGR\(\)](#), [cumulativeCTSSdistribution\(\)](#), [plotInterquartileWidth\(\)](#), [quantilePositions\(\)](#), [tagClustersGR\(\)](#)

**Examples**

```
consensusClustersGR(exampleCAGEexp)
aggregateTagClusters( exampleCAGEexp, tpmThreshold = 50
                      , excludeSignalBelowThreshold = FALSE, maxDist = 100)
consensusClustersGR(exampleCAGEexp)

aggregateTagClusters( exampleCAGEexp, tpmThreshold = 50
                      , excludeSignalBelowThreshold = TRUE, maxDist = 100)
consensusClustersGR(exampleCAGEexp)

aggregateTagClusters( exampleCAGEexp, tpmThreshold = 50
                      , excludeSignalBelowThreshold = TRUE, maxDist = 100
                      , qLow = 0.1, qUp = 0.9)
consensusClustersGR(exampleCAGEexp)
```

---

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)

## S4 method for signature 'CAGEexp,GRanges'
annotateCTSS(object, ranges)

annotateConsensusClusters(object, ranges)

## S4 method for signature 'CAGEexp,GRanges'
annotateConsensusClusters(object, ranges)

```

**Arguments**

object	CAGEexp object.
ranges	A <a href="#">GRanges</a> object, optionally containing gene_name, type and transcript_type metadata.

**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**

[CTSSstoGenes](#), and the [exampleZv9\\_annot](#) example data.

Other CAGEr object modifiers: [CTSSstoGenes\(\)](#), [CustomConsensusClusters\(\)](#), [aggregateTagClusters\(\)](#), [clusterCTSS\(\)](#), [cumulativeCTSSdistribution\(\)](#), [getCTSS\(\)](#), [normalizeTagCount\(\)](#), [quantilePositions\(\)](#), [summariseChrExpr\(\)](#)

Other CAGEr annotation functions: [plotAnnot\(\)](#), [ranges2annot\(\)](#), [ranges2genes\(\)](#), [ranges2names\(\)](#)

**Examples**

```

annotateCTSS(exampleCAGEexp, exampleZv9_annot)
colData(exampleCAGEexp)

annotateConsensusClusters(exampleCAGEexp, exampleZv9_annot)

```

```
consensusClustersGR(exampleCAGEexp)
```

---

bam2CTSS

*bam2CTSS*

---

## Description

Converts from BAM to CTSS

## Usage

```
bam2CTSS(gr, removeFirstG, correctSystematicG, genome)
```

## Arguments

gr	A <a href="#">GRanges</a> object returned by <a href="#">import.bam</a> .
removeFirstG	See <a href="#">getCTSS()</a> .
correctSystematicG	See <a href="#">getCTSS()</a> .
genome	See <a href="#">coerceInBSgenome()</a> .

## 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\(\)](#)



```

, inputFileType = "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$11 <- CTSStagCountDF(exampleCAGEexp) |> sapply ( \ (col) sum(col > 0) )
exampleCAGEexp$11

```

---

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

Haberle V, Forrest ARR, Hayashizaki Y, Carninci P and Lenhard B (2015). “CAGEr: precise TSS data retrieval and high-resolution promoterome mining for integrative analyses.” *Nucleic Acids Research*, 43, pp. e51., <http://nar.oxfordjournals.org/content/43/8/e51>

---

CAGEr_Multicore	<i>Multicore support in CAGEr</i>
-----------------	-----------------------------------

---

**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**

```
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**

```
CAGEr:::CAGEr_Multicore()  
CAGEr:::CAGEr_Multicore(TRUE,)  
CAGEr:::CAGEr_Multicore(TRUE, 3)  
CAGEr:::CAGEr_Multicore(FALSE, 3)
```

---

CCgranges2dataframe	<i>consensus Cluster convertors</i>
---------------------	-------------------------------------

---

**Description**

consensus Cluster convertors

**Usage**

```
CCgranges2dataframe(gr)  
  
CCdataframe2granges(df)
```

**Arguments**

gr                    Consensus clusters in GRanges format.  
df                    Consensus clusters in data.frame format.

---

clusterCTSS                    *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**

```
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'  
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 <a href="#">CAGER</a> object.
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 <a href="#">Details</a> .
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 <i>et al.</i> , 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 <a href="#">GRanges</a> object or a <a href="#">data.frame</a> 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.
nrCores	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

Frith *et al.* (2007) A code for transcription initiation in mammalian genomes, *Genome Research* **18**(1):1-12, (<http://www.cbrc.jp/paraclu/>).

### See Also

`tagClustersGR`, `aggregateTagClusters` and `CTSSclusteringMethod`.

Other CAGEr object modifiers: `CTSSstoGenes()`, `CustomConsensusClusters()`, `aggregateTagClusters()`, `annotateCTSS()`, `cumulativeCTSSdistribution()`, `getCTSS()`, `normalizeTagCount()`, `quantilePositions()`, `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	<i>coerceInBSgenome</i>
------------------	-------------------------

---

**Description**

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

**Usage**

```
coerceInBSgenome(gr, genome)
```

**Arguments**

gr	The genomic ranges to coerce.
genome	The name of a BSgenome package, which must be 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 seqnames of the GRanges are also set accordingly to the BSgenome.

---

ConsensusClusters-class
<i>ConsensusClusters</i>

---

**Description**

The 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.

---

```
consensusClusters<- Set consensus clusters from CAGEr objects
```

---

### Description

Set the information on consensus clusters in a [CAGEr](#) object.

### Usage

```
consensusClustersSE(object) <- value

## S4 replacement method for signature 'CAGEexp,RangedSummarizedExperiment'
consensusClustersSE(object) <- value

consensusClustersGR(object) <- value

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

### Arguments

object	A <a href="#">CAGEr</a> object.
value	A data.frame of consensus clusters

### Details

These setter methods are mostly for internal use, but are exported in case they may be useful to advanced users.

### Author(s)

Vanja Haberle  
Charles Plessy

---

```
consensusClustersDESeq2
```

*Export consensus cluster expression data for DESeq2 analysis*

---

### Description

Creates 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\(\)](#), [plotInterquartileWidth\(\)](#), [quantilePositions\(\)](#), [tagClustersGR\(\)](#)

**Examples**

```
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**

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

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

```

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

consensusClustersSE(object)

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

```

### Arguments

object	A <a href="#">CAGEr</a> object.
sample	Optional. Label of the CAGE dataset (experiment, sample) for which to extract sample-specific information on consensus clusters.
returnInterquantileWidth	Should the interquantile width of consensus clusters in specified sample be returned. Used only when sample argument is specified, otherwise ignored.
qLow, qUp	Position of which quantile should be used as a left (lower) or right (upper) boundary when calculating interquantile width. Used only when sample argument is specified and returnInterquantileWidth = TRUE, otherwise ignored.

### Value

consensusClustersGR returns a [ConsensusClusters](#) object, which wraps the [GRanges](#) class. The score columns indicates 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.

consensusClustersSE returns the [SummarizedExperiment](#) stored in the consensusClusters experiment slot of the CAGEexp object.

### Author(s)

Vanja Haberle  
Charles Plessy

### See Also

[consensusClusters<-\(\)](#)

Other CAGEr accessor methods: [CTSSclusteringMethod\(\)](#), [CTSScoordinatesGR\(\)](#), [CTSScumulativeTagClusters\(\)](#), [CTSSnormalizedTpmDF\(\)](#), [CTSStagCountDF\(\)](#), [GeneExpDESeq2\(\)](#), [GeneExpSE\(\)](#), [expressionClasses\(\)](#),

```
genomeName(), inputFileType(), inputFiles(), librarySizes(), sampleLabels(), seqNameTotalsSE(),
tagClustersGR()
```

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

## Examples

```
consensusClustersGR( exampleCAGEexp, sample = 2
                    , returnInterquartileWidth = 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

```
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
```

## Arguments

<code>object</code>	A <a href="#">CAGEr</a> object.
<code>samples</code>	Sample name(s), number(s) or NULL (default) for all samples.
<code>sample</code>	A single sample name or number, or NULL (default) for all samples.

q	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.

---

consensusClustersTpm *Extracting consensus clusters tpm matrix from CAGEr object*

---

### Description

Extracts a table with normalized CAGE tag values for consensus clusters across all samples from a [CAGEr](#) object.

### Usage

```
consensusClustersTpm(object)

## 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))
```

---

coverage-functions      *Private functions behind* cumulativeCTSSdistribution

---

## Description

`.getCumsum` calculates cumulative sums of tpm along the clusters.

## Usage

```
.getCAGESignalCoverage(ctss.chr, clusters)
.getCumsumChr2(clusters, ctss, chrom, str)
.getCumsum(ctss, clusters, useMulticore = FALSE, nrCores = NULL)
```

## Arguments

<code>ctss.chr</code>	A ‘CTSS.chr’ object (guaranteed to have only one chromosome).
<code>clusters</code>	GRanges as per <code>tagClustersGR()</code> .
<code>ctss</code>	GRanges as per <code>CTSScoordinatesGR</code> , with the score of one sample.
<code>chrom</code>	a chromosome name
<code>str</code>	a strand name
<code>useMulticore, nrCores</code>	See <code>clusterCTSS</code> .

## Details

‘`.getCAGESignalCoverage`’ does... Note that strand is not taken into account.  
`.getCumsumChr2`

## Value

`.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) v.

## Examples

```
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
ctss.chr <- as(ctss, "CTSS.chr")
clusters <- GRanges( seqnames = Rle("chr1")
                   , ranges = IRanges(c(1,12,25,31,32), c(4,14,28,31,33))
```



```

                                , strand = "+")
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 `bgenomeName`, 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, ..., bgenomeName = NULL)

CTSS(
  seqnames = NULL,
  pos = NULL,
  strand = NULL,
  ...,
  seqinfo = NULL,
  seqlengths = NULL,
  bgenomeName = 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)

```

**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**

```
# 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 <- "AAAAAAAAA"
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
ctss.chr <- as(CTSScoordinatesGR(exampleCAGEexp), "CTSS.chr")
```

---

CTSSclusteringMethod *Get/set CTSS clustering method*

---

### Description

Returns or sets the name of the method that was used 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\(\)](#), [CTSStagCountDF\(\)](#), [GeneExpDESeq2\(\)](#), [GeneExpSE\(\)](#), [consensusClustersGR\(\)](#), [expressionClasses\(\)](#), [genomeName\(\)](#), [inputFileType\(\)](#), [inputFiles\(\)](#), [librarySizes\(\)](#), [sampleLabels\(\)](#), [seqNameTotalsSE\(\)](#), [tagClustersGR\(\)](#)

Other CAGER clusters functions: [CTSScumulativesTagClusters\(\)](#), [CustomConsensusClusters\(\)](#), [aggregateTagClusters\(\)](#), [clusterCTSS\(\)](#), [consensusClustersDESeq2\(\)](#), [consensusClustersGR\(\)](#), [cumulativeCTSSdistribution\(\)](#), [plotInterquartileWidth\(\)](#), [quantilePositions\(\)](#), [tagClustersGR\(\)](#)

## Examples

```
CTSSclusteringMethod(exampleCAGEexp)
```

---

CTSScoordinatesGR	<i>Genomic coordinates of TSSs from a CAGEr object</i>
-------------------	--------------------------------------------------------

---

## 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

CTSSstagCountSE(object) <- value

## S4 replacement method for signature 'CAGEexp'
CTSSstagCountSE(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

**See Also**[getCTSS](#)[clusterCTSS](#)

Other CAGEr accessor methods: [CTSSclusteringMethod\(\)](#), [CTSScumulativesTagClusters\(\)](#), [CTSSnormalizedTpmDF\(\)](#), [CTSStagCountDF\(\)](#), [GeneExpDESeq2\(\)](#), [GeneExpSE\(\)](#), [consensusClustersGR\(\)](#), [expressionClasses\(\)](#), [genomeName\(\)](#), [inputFileType\(\)](#), [inputFiles\(\)](#), [librarySizes\(\)](#), [sampleLabels\(\)](#), [seqNameTotalsSE\(\)](#), [tagClustersGR\(\)](#)

**Examples**

```
CTSScoordinatesGR(exampleCAGEexp)
```

```
CTSScoordinatesGR(exampleCAGEexp)
```

---

CTSScumulativesTagClusters

*Get/set CTSS cumulative TC or CC data*

---

**Description**

Accessor function.

**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 <a href="#">CAGEexp</a> object.
samples	One or more valid sample names.
value	CTSScumulativesTagClusters data

**Value**

List of numeric Rle.

**See Also**

Other CAGEr clusters functions: [CTSSclusteringMethod\(\)](#), [CustomConsensusClusters\(\)](#), [aggregateTagClusters\(\)](#), [clusterCTSS\(\)](#), [consensusClustersDESeq2\(\)](#), [consensusClustersGR\(\)](#), [cumulativeCTSSdistribution\(\)](#), [plotInterquartileWidth\(\)](#), [quantilePositions\(\)](#), [tagClustersGR\(\)](#)

Other CAGEr accessor methods: [CTSSclusteringMethod\(\)](#), [CTSScoordinatesGR\(\)](#), [CTSSnormalizedTpmDF\(\)](#), [CTSStagCountDF\(\)](#), [GeneExpDESeq2\(\)](#), [GeneExpSE\(\)](#), [consensusClustersGR\(\)](#), [expressionClasses\(\)](#), [genomeName\(\)](#), [inputFileType\(\)](#), [inputFiles\(\)](#), [librarySizes\(\)](#), [sampleLabels\(\)](#), [seqNameTotalsSE\(\)](#), [tagClustersGR\(\)](#)

---

CTSSnormalizedTpmDF     *Extracting normalized CAGE signal for TSSs from CAGEr objects*

---

**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 <a href="#">sampleLabels(object)</a> , 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	<i>Raw CAGE TSSs expression counts</i>
----------------	----------------------------------------

---

**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 CTSS object (wrapping GRanges) containing a score column indicating expression values for a given sample, or a GRangesList of CTSS objects.

**Author(s)**

Vanja Haberle

Charles Plessy

**See Also**

[getCTSS\(\)](#)

Other CAGEr accessor methods: [CTSSclusteringMethod\(\)](#), [CTSScoordinatesGR\(\)](#), [CTSScumulativeTagClusters\(\)](#), [CTSSnormalizedTpmDF\(\)](#), [GeneExpDESeq2\(\)](#), [GeneExpSE\(\)](#), [consensusClustersGR\(\)](#), [expressionClasses\(\)](#), [genomeName\(\)](#), [inputFileType\(\)](#), [inputFiles\(\)](#), [librarySizes\(\)](#), [sampleLabels\(\)](#), [seqNameTotalsSE\(\)](#), [tagClustersGR\(\)](#)

**Examples**

```
CTSStagCountDF(exampleCAGEexp)
```

```
CTSStagCountDA(exampleCAGEexp)
```

```
CTSStagCountGR(exampleCAGEexp, 1)
```

```
CTSStagCountGR(exampleCAGEexp, "all")
```

```
CTSStagCountSE(exampleCAGEexp)
```



---

CTSSstoGenes	<i>Make a gene expression table.</i>
--------------	--------------------------------------

---

### Description

Add a gene expression table in the GeneExpSE experiment slot of an annotated [CAGEexp](#) object.

### Usage

```
CTSSstoGenes(object)

## S4 method for signature 'CAGEexp'
CTSSstoGenes(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\(\)](#), [summariseChrExpr\(\)](#)

Other CAGEr gene expression analysis functions: [GeneExpDESeq2\(\)](#), [ranges2genes\(\)](#)

**Examples**

```
CTSSstoGenes(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 raw CAGE counts along each tag cluster or consensus cluster in every sample within a CAGER object.

**Usage**

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

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

**Arguments**

object	A <a href="#">CAGER</a> 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 are stored in the metadata slot using the RleList class.

**Author(s)**

Vanja Haberle

**See Also**

Other CAGEr object modifiers: [CTSSstoGenes\(\)](#), [CustomConsensusClusters\(\)](#), [aggregateTagClusters\(\)](#), [annotateCTSS\(\)](#), [clusterCTSS\(\)](#), [getCTSS\(\)](#), [normalizeTagCount\(\)](#), [quantilePositions\(\)](#), [summariseChrExpr\(\)](#)

Other CAGEr clusters functions: [CTSSclusteringMethod\(\)](#), [CTSScumulativesTagClusters\(\)](#), [CustomConsensusClusters\(\)](#), [aggregateTagClusters\(\)](#), [clusterCTSS\(\)](#), [consensusClustersDESeq2\(\)](#), [consensusClustersGR\(\)](#), [plotInterquartileWidth\(\)](#), [quantilePositions\(\)](#), [tagClustersGR\(\)](#)

**Examples**

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

clusterCTSS( exampleCAGEexp, threshold = 50, thresholdIsTpm = TRUE
             , nrPassThreshold = 1, method = "distclu", maxDist = 20
             , removeSingletons = TRUE, keepSingletonsAbove = 100)
cumulativeCTSSdistribution(exampleCAGEexp, clusters = "tagClusters")
aggregateTagClusters( exampleCAGEexp, tpmThreshold = 50
                     , excludeSignalBelowThreshold = FALSE, maxDist = 100)
cumulativeCTSSdistribution(exampleCAGEexp, clusters = "consensusClusters")
```

---

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**

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

## 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  $\geq$  threshold in  $\geq$  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: [CTSSstoGenes\(\)](#), [aggregateTagClusters\(\)](#), [annotateCTSS\(\)](#), [clusterCTSS\(\)](#), [cumulativeCTSSdistribution\(\)](#), [getCTSS\(\)](#), [normalizeTagCount\(\)](#), [quantilePositions\(\)](#), [summariseChrExpr\(\)](#)

Other CAGEr clusters functions: [CTSSclusteringMethod\(\)](#), [CTSScumulativesTagClusters\(\)](#), [aggregateTagClusters\(\)](#), [clusterCTSS\(\)](#), [consensusClustersDESeq2\(\)](#), [consensusClustersGR\(\)](#), [cumulativeCTSSdistribution\(\)](#), [plotInterquantileWidth\(\)](#), [quantilePositions\(\)](#), [tagClustersGR\(\)](#)

### Examples

```

cc <- consensusClustersGR(exampleCAGEexp)
CustomConsensusClusters(exampleCAGEexp, cc)

```

---

distclu-functions      *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

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

**Value**

`.cluster.ctss.strand` returns an `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**

```
# Get example data
library(IRanges)
library(GenomicRanges)

#.cluster.ctss.strand
ctss.ipos.chr <- IPos(c(1,3,4,12,14,25,28))
CAGEr:::.cluster.ctss.strand(ctss.ipos.chr, 5)

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

```

)
# Returns an empty data.table object if given an empty IRanges object.
identical(CAGEr:::.cluster.ctss.strand(IPos(), 20), data.table::data.table())

#.cluster.ctss.chr
ctss.chr <- as(CTSScoordinatesGR(exampleCAGEexp), "CTSS.chr")
CAGEr:::.cluster.ctss.chr(ctss.chr, 20)

#.ctss2clusters
ctss <- CTSScoordinatesGR(exampleCAGEexp)
score(ctss) <- CTSSnormalizedTpmDF(exampleCAGEexp)[[1]]
seqnames(ctss)[rep(c(TRUE,FALSE), length(ctss) / 2)] <- "chr16"
ctss
clusters <- CAGEr:::.ctss2clusters(ctss, 20)
clusters

#.summarize.clusters
CAGEr:::.summarize.clusters(clusters)
CAGEr:::.summarize.clusters(clusters, removeSingletons = TRUE)
CAGEr:::.summarize.clusters(clusters, removeSingletons = TRUE, keepSingletonsAbove = 5)

#.distclu
CAGEr:::.distclu(CTSSstagCountSE(exampleCAGEexp))
## Not run:
CAGEr:::.distclu(CTSSstagCountSE(exampleCAGEexp), useMulticore = TRUE)

## End(Not run)

```

---

exampleCAGEexp

*Example CAGEexp object.*


---

## Description

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

## Usage

```
exampleCAGEexp
```

## Format

A [CAGEexp](#) object.

## Examples

```

## Not run:
pathsToInputFiles <- list.files( system.file("extdata", package = "CAGEr")
                                , "ctss$"
                                , full.names = TRUE)

```

```

sampleLabels <- sub( ".chr17.ctss", "", basename(pathsToInputFiles))
exampleCAGEexp <-
  CAGEexp( genomeName      = "BSgenome.Drerio.UCSC.danRer7"
          , inputFiles     = pathsToInputFiles
          , inputFileType  = "ctss"
          , sampleLabels   = sub( ".chr17.ctss", "", basename(pathsToInputFiles)))
exampleCAGEexp <- getCTSS(exampleCAGEexp)
librarySizes(exampleCAGEexp)
colData(exampleCAGEexp)
exampleCAGEexp$l1 <- NULL
exampleCAGEexp <- exampleCAGEexp[,c(5, 2, 1, 3, 4)] # Non-alphabetic order may help catch bugs
CTSStagCountSE(exampleCAGEexp) <- CTSStagCountSE(exampleCAGEexp)[1:5000,] # Slim the object
exampleCAGEexp$librarySizes <- sapply(CTSStagCountDF(exampleCAGEexp), sum) # Repair metadata
exampleCAGEexp <-
  summariseChrExpr(exampleCAGEexp)           |>
  annotateCTSS(exampleZv9_annot)             |>
  CTSSstoGenes()                           |>
  normalizeTagCount()                      |>
  getExpressionProfiles("CTSS")            |>
  clusterCTSS()                            |>
  cumulativeCTSSdistribution("tagClusters") |>
  quantilePositions("tagClusters")         |>
  aggregateTagClusters()                   |>
  annotateConsensusClusters(exampleZv9_annot) |>
  cumulativeCTSSdistribution("consensusClusters") |>
  quantilePositions("consensusClusters")   |>
  getExpressionProfiles("consensusClusters")
save(exampleCAGEexp, file = "data/exampleCAGEexp.RData", compress = "xz")

## End(Not run)

```

---

exampleZv9\_annot

*Example zebrafish annotation data*

---

## Description

Annotation data for zebrafish's chromosome 17's interval 26000000-54000000 (Zv9/danRer7 genome), to be used in documentation examples.

## Usage

```
exampleZv9_annot
```

## Format

An object of class GRanges of length 7467.



## 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=>

And here it is as XML.

```
<?xml version="1.0" encoding="UTF-8"?>
<!DOCTYPE Query>
<Query virtualSchemaName = "default" formatter = "TSV" header = "0" uniqueRows = "0" count = "" datasetC
  <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.

```
x <- read.delim("~/Downloads/mart_export.txt", stringsAsFactors = FALSE)
e <- GRanges(paste0("chr", x$Chromosome.Name), IRanges(x$Exon.Chr.Start..bp., x$Exon.Chr.End..bp.), i
e$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$Exon.Chr.End..bp.), i
e$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, "+", "-"))
```

```

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) > 260000000 & end(gff) < 540000000]
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 interquartile range and a thick box denoting position of the dominant (most frequently) used TSS.

**Usage**

```

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(
  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 <a href="#">CAGEexp</a> object.
what	Which elements should be exported: CTSS for individual <i>CTSSs</i> , tagClusters for <i>tag clusters</i> or consensusClusters for <i>consensus clusters</i> .
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 |>
```

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

---

expressionClasses	<i>Extract labels of expression classes</i>
-------------------	---------------------------------------------

---

### Description

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

### Usage

```
expressionClasses(object)

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

## S4 method for signature 'ConsensusClusters'
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

```
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](#)

---

`GeneExpDESeq2`*Export gene expression data for DESeq2 analysis*

---

## 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

```
GeneExpDESeq2(object, design)

## S4 method for signature 'CAGEexp'
GeneExpDESeq2(object, design)
```

## Arguments

<code>object</code>	A <code>CAGEexp</code> object.
<code>design</code>	A formula for the DESeq2 analysis.

## Author(s)

Charles Plessy

## See Also

`DESeqDataSet` in the DESeq2 package.

Other CAGEr gene expression analysis functions: `CTSSstoGenes()`, `ranges2genes()`

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

## Examples

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



---

GeneExpSE	<i>Retrieves the SummarizedExperiment containing gene expression levels.</i>
-----------	------------------------------------------------------------------------------

---

**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**

```
GeneExpSE(object)

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

**Arguments**

`object`            A [CAGEexp](#) object.

**Author(s)**

Charles Plessy

**See Also**

Other CAGEr accessor methods: [CTSSclusteringMethod\(\)](#), [CTSScoordinatesGR\(\)](#), [CTSScumulativesTagClusters\(\)](#), [CTSSnormalizedTpmDF\(\)](#), [CTSStagCountDF\(\)](#), [GeneExpDESeq2\(\)](#), [consensusClustersGR\(\)](#), [expressionClasses\(\)](#), [genomeName\(\)](#), [inputFileType\(\)](#), [inputFiles\(\)](#), [librarySizes\(\)](#), [sampleLabels\(\)](#), [seqNameTotalsSE\(\)](#), [tagClustersGR\(\)](#)

**Examples**

```
GeneExpSE(exampleCAGEexp)
```

---

<code>genomeName</code>	<i>Extracting genome name from CAGEr objects</i>
-------------------------	--------------------------------------------------

---

**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\(\)](#), [inputFileType\(\)](#), [inputFiles\(\)](#), [librarySizes\(\)](#), [sampleLabels\(\)](#), [seqNameTotalsSE\(\)](#), [tagClustersGR\(\)](#)

Other CAGEr setter methods: [inputFileType\(\)](#), [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 <a href="#">CAGEexp</a> object.
sequencingQualityThreshold	Only CAGE tags with average sequencing quality $\geq$ sequencingQualityThreshold and mapping quality $\geq$ mappingQualityThreshold are kept. Used only if <code>inputFileType(object) == "bam"</code> or <code>inputFileType(object) == "bamPairedEnd"</code> , <i>i.e.</i> when input files are BAM files of aligned sequenced CAGE tags, otherwise ignored. If there are no sequencing quality values in the BAM file ( <i>e.g.</i> 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.

**Value**

Returns the object, in which the tagCountMatrix experiment will be occupied by a [RangedSummarizedExperiment](#) containing the expression data as a DataFrame of R1e integers, and the CTSS coordinates as genomic ranges in a [CTSS](#) object. The expression data can be retrieved with the [CTSStagCountDF](#) function. In addition, the library sizes are calculated and stored in the object's sample data (see [librarySizes](#)).

**Author(s)**

Vanja Haberle

**References**

Harbers and Carninci (2005) Tag-based approaches for transcriptome research and genome annotation, *Nature Methods* **2**(7):495-502.

Carninci *et al.* (2006) Genome-wide analysis of mammalian promoter architecture and evolution, *Nature Genetics* **38**(7):626-635.

**See Also**

[inputFileType](#), [librarySizes](#).

Other CAGEr object modifiers: [CTSStoGenes\(\)](#), [CustomConsensusClusters\(\)](#), [aggregateTagClusters\(\)](#), [annotateCTSS\(\)](#), [clusterCTSS\(\)](#), [cumulativeCTSSdistribution\(\)](#), [normalizeTagCount\(\)](#), [quantilePositions\(\)](#), [summariseChrExpr\(\)](#)

**Examples**

```
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, inputFileType = "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.

**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 <a href="#">CAGEexp</a> 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) <a href="#">som::som</a> ] function from <i>som</i> package. kmeans uses the K-means algorithm implemented in the <a href="#">stats::kmeans</a> ] 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 x yDim\$ clusters returned by SOM.

## 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 objects'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

Toronen *et al.* (1999) Analysis of gene expression data using self-organizing maps, *FEBS Letters* 451:142-146.

## See Also

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

## Examples

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

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

```
getExpressionProfiles(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

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

## S4 method for signature 'CAGEexp'
getShiftingPromoters(
  object,
  tpmThreshold = 0,
  scoreThreshold = -Inf,
  fdrThreshold = 1
)
```

### Arguments

<code>object</code>	A <code>CAGEexp</code> object.
<code>tpmThreshold</code>	Consensus clusters with total CAGE signal $\geq$ <code>tpmThreshold</code> in each of the compared groups will be returned.
<code>scoreThreshold</code>	Consensus clusters with shifting score $\geq$ <code>scoreThreshold</code> will be returned. The default value <code>-Inf</code> returns all consensus clusters (for which score could be calculated, <i>i.e.</i> the ones that have at least one tag in each of the compared samples).
<code>fdrThreshold</code>	Consensus clusters with adjusted P-value (FDR) from Kolmogorov-Smirnov test $\geq$ <code>fdrThreshold</code> will be returned. The default value <code>1</code> returns all consensus clusters (for which K-S test could be performed, <i>i.e.</i> the ones that have at least one tag in each of the compared samples).

### Value

Returns a 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

**See Also**

Other CAGEr promoter shift functions: [scoreShift\(\)](#)

**Examples**

```
#head(getShiftingPromoters( exampleCAGEexp, tpmThreshold = 100
#                           , scoreThreshold = 0.4, fdrThreshold = 0.01))
```

---

hanabi

*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**

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

## S4 method for signature 'matrix'
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 <code>DataFrame</code> or <code>data.frame</code> format where columns are samples and rows are features such as genes, TSS, etc, or a vector of counts (tag counts, molecule counts, ...), or <code>GRanges</code> or <code>GRangesList</code> 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. <code>useMulticore = TRUE</code> 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 <code>useMulticore = TRUE</code> (set to <code>NULL</code> 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	<i>Hanabi class</i>
--------------	---------------------

---

**Description**

TBD

**Details**

TBD

---

hanabiPlot	<i>hanabiPlot</i>
------------	-------------------

---

**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**

```
h <- hanabi(CTSSStagCountDF(exampleCAGEexp))
hanabiPlot(h, group = 1:5)
hanabiPlot(hanabi(CTSSStagCountDF(exampleCAGEexp), n = 20, step = 0.8, from = 25000), group = 1:5)
hanabiPlot(hanabi(CTSSStagCountDF(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")
```

---

import.bam

*import.bam*

---

**Description**

Imports CTSS data from a BAM file.

**Usage**

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

**Arguments**

filepath	The path to the BAM file.
filetype	bam or bamPairedEnd.
sequencingQualityThreshold	See <a href="#">getCTSS()</a> .
mappingQualityThreshold	See <a href="#">getCTSS()</a> .

**See Also**

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

**Examples**

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

---

```
import.bam.ctss      import.bam.ctss
```

---

**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 <a href="#">getCTSS()</a> .
mappingQualityThreshold	See <a href="#">getCTSS()</a> .
removeFirstG	See <a href="#">getCTSS()</a> .
correctSystematicG	See <a href="#">getCTSS()</a> .
genome	See <a href="#">coerceInBSgenome()</a> .

**Value**

Returns a [CTSS](#) object.

**See Also**

Other loadFileIntoGPos: [bam2CTSS\(\)](#), [import.CTSS\(\)](#), [import.bam\(\)](#), [import.bedCTSS\(\)](#), [import.bedScore\(\)](#), [import.bedmolecule\(\)](#), [loadFileIntoGPos\(\)](#), [moleculesGR2CTSS\(\)](#)

---

import.bedCTSS	<i>import.bedCTSS</i>
----------------	-----------------------

---

**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 exmaple file
# import.BED(system.file("extdata", "example.bed", package = "CAGEr"))
```

---

import.bedmolecule	<i>import.bedmolecule</i>
--------------------	---------------------------

---

**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 exmaple file
# import.BED(system.file("extdata", "example.bed", package = "CAGEr"))
```

---

import.bedScore            *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.

**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 exmaple file
# import.bedScore(system.file("extdata", "example.bed", package = "CAGEr"))
```



---

```
import.CAGEscanMolecule  
    import.CAGEscanMolecule
```

---

### Description

Imports a CAGEscan “molecule” file in a [GRanges](#) object

### 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            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	<i>Extracting paths to input files from CAGEr objects</i>
------------	-----------------------------------------------------------

---

**Description**

Extracts the paths to CAGE data input files from code [CAGEexp](#) objects.

**Usage**

```
inputFiles(object)

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

inputFiles(object) <- value

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

**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\(\)](#), [inputFileType\(\)](#), [librarySizes\(\)](#), [sampleLabels\(\)](#), [seqNameTotalsSE\(\)](#), [tagClustersGR\(\)](#)

Other CAGEr setter methods: [genomeName\(\)](#), [inputFileType\(\)](#), [sampleLabels\(\)](#), [setColors\(\)](#)

**Examples**

```
inputFiles(exampleCAGEexp)
```

---

inputFileType	<i>Input file formats for CAGEr objects</i>
---------------	---------------------------------------------

---

### Description

Get or set the information on the type of CAGE data input files from [CAGEexp](#) objects.

### Usage

```
inputFileType(object)

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

inputFileType(object) <- value

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

### Arguments

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

### Details

The following input file types are supported:

- bam: A single-ended BAM file.
- bamPairedEnd: A paired-ended BAM file.
- 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.
- CTSSstable
- 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\(\)](#), [CTSStagCountDF\(\)](#), [GeneExpDESeq2\(\)](#), [GeneExpSE\(\)](#), [consensusClustersGR\(\)](#), [expressionClasses\(\)](#), [genomeName\(\)](#), [inputFileType\(\)](#), [inputFiles\(\)](#), [sampleLabels\(\)](#), [seqNameTotalsSE\(\)](#), [tagClustersGR\(\)](#)

**Examples**

```
librarySizes(exampleCAGEexp)
```

---

loadFileIntoGPos	<i>loadFileIntoGPos</i>
------------------	-------------------------

---

**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 <a href="#">getCTSS()</a> .
mappingQualityThreshold	See <a href="#">getCTSS()</a> .
removeFirstG	See <a href="#">getCTSS()</a> .
correctSystematicG	See <a href="#">getCTSS()</a> .
genome	See <a href="#">coerceInBSgenome()</a> .

**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**

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

**Arguments**

<code>libs</code>	A data frame with containing columns required by the scope chosen.
<code>scope</code>	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 <code>mapStatsScopes()</code> for details on each scope.
<code>group</code>	A vector of factors defining groups in the data. By default, the “group” column of the “libs” table.
<code>facet</code>	A vector of factors defining facets in the data (in the sense of <code>ggplot2</code> ’s <code>facet_wrap</code> function).
<code>normalise</code>	Whether to normalise or not. Default: TRUE.

**Details**

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

See <http://stackoverflow.com/questions/10417003/stacked-barplot-with-errorbars-using-ggplot2> about stacked barplot.

**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 *promoter*, *exon*, *intron* and otherwise *intergenic* regions.

The mapped scope reports the number of molecules aligning in *promoter*, *exon*, *intron* and otherwise *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 *tag dust*, *rRNA*, *spikes*, plus the *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 *cleaning*, *mapping*, and *deduplication*, plus the number of *unique molecule counts*.

The legacy all 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 extracted tags.

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

<code>cs1</code>	A CAGEr object
<code>cs2</code>	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

**See Also**

[CAGEexp](#)

**Examples**

```
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")

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

ce2 <- CAGEexp(genomeName = "BSgenome.Drerio.UCSC.danRer7",
  inputFiles = pathsToInputFiles[3], inputFileType = "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**

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

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

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

**Arguments**

object	A <a href="#">CAGEr</a> object.
mergeIndex	Integer vector specifying which experiments should be merged. (one value per sample, see <a href="#">Details</a> ).
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"))
exampleCAGEexp
```

---

moleculesGR2CTSS	<i>moleculesGR2CTSS</i>
------------------	-------------------------

---

### Description

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

### Usage

```
moleculesGR2CTSS(gr)
```

### Arguments

`gr` A [GRanges](#) object.

### 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	<i>Normalizing raw CAGE tag count</i>
-------------------	---------------------------------------

---

### 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 <a href="#">CAGEexp</a> 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.
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	$-1 * \alpha$ 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  $y = -1 * \alpha * x + \beta$ , 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: [CTSSstoGenes\(\)](#), [CustomConsensusClusters\(\)](#), [aggregateTagClusters\(\)](#), [annotateCTSS\(\)](#), [clusterCTSS\(\)](#), [cumulativeCTSSdistribution\(\)](#), [getCTSS\(\)](#), [quantilePositions\(\)](#), [summariseChrExpr\(\)](#)

### Examples

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

---

parseCAGEscanBlocksToGrangeTSS

*parseCAGEscanBlocksToGrangeTSS*

---

### 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)
```

---

plot.hanabi

*Plotting Hanabi objects*

---

**Description**

S3 method to plot hanabi objects. Used by the [hanabiPlot](#) function.

**Usage**

```
## 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

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

### Author(s)

Charles Plessy

### See Also

Other CAGEr richness functions: [hanabiPlot\(\)](#), [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)

## S4 method for signature 'data.frame'
plotAnnot(x, scope, title, group = "default", facet = NULL, normalise = TRUE)

## S4 method for signature 'DataFrame'
plotAnnot(x, scope, title, group = "default", facet = NULL, normalise = TRUE)

## S4 method for signature 'CAGExp'
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 <code>mapStatsScopes()</code> 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 <code>facet_wrap</code> function.
normalise	Whether to normalise or not. Default: TRUE.

**Details**

Stacked barplots with error bars inspired from <http://stackoverflow.com/questions/10417003/stacked-barplot-with-errorbars-using-ggplot2>. See <http://www.biomedcentral.com/1471-2164/14/665/figure/F1> for example.

**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", "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")
```



---

plotCorrelation      *Pairwise scatter plots and correlations of CAGE signal*

---

### 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

```
plotCorrelation(  
  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  
)
```

```
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(
  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 <a href="#">CAGEr</a> object or (only for plotCorrelation2) a <a href="#">SummarizedExperiment</a> or an expression table as a <a href="#">DataFrame</a> , <a href="#">data.frame</a> or <a href="#">matrix</a> 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>i.e.</i> 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 <a href="#">sampleLabels</a> function.
method	A character string indicating which correlation coefficient should be computed. Passed to cor function. Can be one of "pearson", "spearman", or "kendall".
tagCountThreshold	Only TSSs with tag count $\geq$ 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\(\)](#), [plotInterquartileWidth\(\)](#), [plotReverseCumulatives\(\)](#)

### 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**

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

```
## S4 method for signature 'CAGEexp'
plotInterquantileWidth(
  object,
  clusters = c("tagClusters", "consensusClusters"),
  tpmThreshold = 5,
  qLow = 0.1,
  qUp = 0.9,
  xlim = c(0, 150)
)
```

### Arguments

object	A <a href="#">CAGEexp</a> 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.

### 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))
```

---

plotReverseCumulatives

*Plot reverse cumulative number of CAGE tags per CTSS*

---

### 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 <a href="#">plot</a> .
xlim, ylim	Axis range parameters passed to <a href="#">plot</a> .

### Details

Number of CAGE tags (X-axis) is plotted against the number of TSSs that are supported by  $\geq$  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  $y = -1 * \alpha * x + \beta$  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.

### 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

[normalizeTagCount](#)

Other CAGER plot functions: [hanabiPlot\(\)](#), [plotAnnot\(\)](#), [plotCorrelation\(\)](#), [plotExpressionProfiles\(\)](#), [plotInterquartileWidth\(\)](#)



**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
)

## 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 R1e 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: [CTSSstoGenes\(\)](#), [CustomConsensusClusters\(\)](#), [aggregateTagClusters\(\)](#), [annotateCTSS\(\)](#), [clusterCTSS\(\)](#), [cumulativeCTSSdistribution\(\)](#), [getCTSS\(\)](#), [normalizeTagCount\(\)](#), [summariseChrExpr\(\)](#)

Other CAGEr clusters functions: [CTSSclusteringMethod\(\)](#), [CTSScumulativesTagClusters\(\)](#), [CustomConsensusClusters\(\)](#), [aggregateTagClusters\(\)](#), [clusterCTSS\(\)](#), [consensusClustersDESeq2\(\)](#), [consensusClustersGR\(\)](#), [cumulativeCTSSdistribution\(\)](#), [plotInterquantileWidth\(\)](#), [tagClustersGR\(\)](#)

**Examples**

```
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))
```

---

ranges2annot

*Hierarchical annotation of CTSSes*

---

**Description**

Assigns region types such as promoter, exon or unknown to CTSSes.

**Usage**

```
ranges2annot(ranges, annot)
```

**Arguments**

ranges	A <a href="#">CTSS</a> object, for example extracted from a <code>RangedSummarizedExperiment</code> object with the <code>rowRanges</code> command.
annot	A <a href="#">GRanges</a> from which promoter positions will be inferred. Typically <code>GENCODE</code> . If the type metadata is present, it should contain gene, exon and transcript among its values. Otherwise, all entries are considered transcripts. If the <code>transcript_type</code> metadata is available, the entries that may not be primary products (for instance 'snoRNA') are discarded.

**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)
```

---

`ranges2genes`*ranges2genes*

---

## Description

Assign gene symbol(s) to Genomic Ranges.

## Usage

```
ranges2genes(ranges, genes)
```

## Arguments

<code>ranges</code>	Genomics Ranges object, for example extracted from a <code>RangedSummarizedExperiment</code> object with the <code>rowRanges</code> command.
<code>genes</code>	A <a href="#">GRanges</a> object containing <code>gene_name</code> 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 [CTSSstoGenes](#).

## Value

A `Rle` character vector of same length as the `GRanges` object, indicating one gene symbol or a semicolon-separated list of gene symbols for each range.

## Author(s)

Charles Plessy

## See Also

[CTSScoordinatesGR](#), [exampleZv9\\_annot](#)

Other CAGEr annotation functions: [annotateCTSS\(\)](#), [plotAnnot\(\)](#), [ranges2annot\(\)](#), [ranges2names\(\)](#)

Other CAGEr gene expression analysis functions: [CTSSstoGenes\(\)](#), [GeneExpDESeq2\(\)](#)

## Examples

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

---

ranges2names	<i>ranges2names</i>
--------------	---------------------

---

## Description

Intersection of genomic ranges

## Usage

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

## Arguments

rangesA      A [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](#) character vector of same length as the rangesA [GRanges](#) object, indicating one name or a semicolon-separated list of names from the each rangesB object.

## 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)
```

---

`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

```
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

<code>object</code>	A <code>CAGEr</code> object.
<code>value</code>	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: `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\(\)](#), [inputFilesType\(\)](#), [inputFiles\(\)](#), [librarySizes\(\)](#), [seqNameTotalsSE\(\)](#), [tagClustersGR\(\)](#)

Other CAGEr setter methods: [genomeName\(\)](#), [inputFilesType\(\)](#), [inputFiles\(\)](#), [setColors\(\)](#)

### Examples

```
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**

```

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

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

```

**Arguments**

object	A <a href="#">CAGEr</a> 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:

$$\text{score} = \max(F1 - F2) / \max(F1)$$

where F1 is a cumulative sum of CAGE signal along consensus cluster in the group of samples with lower total signal in that consensus cluster, and F2 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  $[-\text{Inf}, 1]$ , 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 `cumulativeCTSSdistribution` function.

The slots `shiftingGroupX`, `shiftingGroupY` and `consensusClustersShiftingScores` of the provided `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 `getShiftingPromoters` function.

#### Author(s)

Vanja Haberle

#### See Also

`cumulativeCTSSdistribution`

Other CAGE promoter shift functions: `getShiftingPromoters()`

**Examples**

```
# scoreShift( exampleCAGEexp
#             , groupX = c("sample1", "sample2")
#             , groupY = "sample3"
#             , testKS = TRUE, useTpmKS = FALSE)
# head(getShiftingPromoters(exampleCAGEexp))
```

---

seqNameTotalsSE	<i>Retrieves the SummarizedExperiment containing chromosome expression totals.</i>
-----------------	------------------------------------------------------------------------------------

---

**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**

```
seqNameTotalsSE(object)

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

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\(\)](#), [inputFileType\(\)](#), [inputFiles\(\)](#), [librarySizes\(\)](#), [sampleLabels\(\)](#), [tagClustersGR\(\)](#)

**Examples**

```
seqNameTotalsSE(exampleCAGEexp)
```

---

setColors	<i>Set colors for samples</i>
-----------	-------------------------------

---

### 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 <a href="#">CAGEr</a> object.
colors	A character vector of one valid R color specification per sample (see <a href="#">col2rgb</a> for details). Provided colors are assigned to samples in the order they are returned by the <a href="#">sampleLabels</a> 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\(\)](#), [inputFileType\(\)](#), [inputFiles\(\)](#), [sampleLabels\(\)](#)

### Examples

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

**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 (*CI 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 TATAGGG 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**

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

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

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

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

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

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

```
## S4 method for signature 'CTSS'
```

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

```
## S4 method for signature 'CTSS'
```

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

**Arguments**

<code>object</code>	A <a href="#">CAGEexp</a> object object containing CTSS data and the name of a reference genome.
<code>distance</code>	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.
<code>barcode</code>	A vector of sample barcode sequences, or the name of a column metadata of the CAGEexp object containing this information. ( <i>Not implemented yet</i> )
<code>linker</code>	The sequence of the tail of the template-switching oligonucleotide, that will be matched with the genome sequence (defaults to TATAGGG).

**Value**

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, the input object is modified in place. 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**

Tang *et al.*, “Suppression of artifacts and barcode bias in high-throughput transcriptome analyses utilizing template switching.” *Nucleic Acids Res.* **2013** Feb 1;41(3):e44. PubMed ID: [23180801](#), DOI: [10.1093/nar/gks112](#)

**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.
"BSgenome.Drerio.UCSC.danRer7"

findStrandInvaders(exampleCAGEexp)
removeStrandInvaders(exampleCAGEexp)
```

---

summariseChrExpr	<i>Expression levels by chromosomes</i>
------------------	-----------------------------------------

---

**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: [CTSSstoGenes\(\)](#), [CustomConsensusClusters\(\)](#), [aggregateTagClusters\(\)](#), [annotateCTSS\(\)](#), [clusterCTSS\(\)](#), [cumulativeCTSSdistribution\(\)](#), [getCTSS\(\)](#), [normalizeTagCount\(\)](#), [quantilePositions\(\)](#)

**Examples**

```
summariseChrExpr(exampleCAGEexp)
```

---

TagClusters-class	<i>TagClusters</i>
-------------------	--------------------

---

**Description**

TagClusters

**Details**

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

---

tagClustersGR	<i>Extract tag clusters (TCs) for individual CAGE experiments</i>
---------------	-------------------------------------------------------------------

---

**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(
  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 <a href="#">TagClusters</a> 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.

### Author(s)

Vanja Haberle  
Charles Plessy

### See Also

Other CAGEr accessor methods: [CTSSclusteringMethod\(\)](#), [CTSScoordinatesGR\(\)](#), [CTSScumulativesTagClusters\(\)](#), [CTSSnormalizedTpmDF\(\)](#), [CTSSstagCountDF\(\)](#), [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 )
```



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