

Package ‘CAGEfightR’

October 16, 2019

Type Package

Title Analysis of Cap Analysis of Gene Expression (CAGE) data using
Bioconductor

Version 1.4.0

Author Malte Thodberg

Maintainer Malte Thodberg <maltethodberg@gmail.com>

Description CAGE is a widely used high throughput assay for measuring transcription start site (TSS) activity. CAGEfightR is an R/Bioconductor package for performing a wide range of common data analysis tasks for CAGE and 5'-end data in general.

Core functionality includes: import of CAGE TSSs (CTSSs), tag (or unidirectional) clustering for TSS identification, bidirectional clustering for enhancer identification, annotation with transcript and gene models, correlation of TSS and enhancer expression, calculation of TSS shapes, quantification of CAGE expression as expression matrices and genome browser visualization.

URL <https://github.com/MalteThodberg/CAGEfightR>

BugReports <https://github.com/MalteThodberg/CAGEfightR/issues>

Depends R (>= 3.5), GenomicRanges (>= 1.30.1), rtracklayer (>= 1.38.2), SummarizedExperiment (>= 1.8.1)

Imports pryr(>= 0.1.3), assertthat(>= 0.2.0), methods, Matrix(>= 1.2-12), Matrix.utils(>= 0.9.6), grr(>= 0.9.5), BiocGenerics(>= 0.24.0), S4Vectors(>= 0.16.0), IRanges(>= 2.12.0), GenomeInfoDb(>= 1.14.0), GenomicFeatures(>= 1.29.11), BiocParallel(>= 1.12.0), GenomicFiles(>= 1.14.0), Gviz(>= 1.22.2), InteractionSet(>= 1.9.4), GenomicInteractions(>= 1.15.1)

License GPL-3 + file LICENSE

Encoding UTF-8

LazyData true

RoxygenNote 6.1.1

Suggests knitr, rmarkdown, BiocStyle, org.Mm.eg.db,
TxDb.Mmusculus.UCSC.mm9.knownGene

VignetteBuilder knitr

biocViews Software, Transcription, Coverage, GeneExpression, GeneRegulation, PeakDetection, DataImport, DataRepresentation, Transcriptomics, Sequencing, Annotation, GenomeBrowsers, Normalization, Preprocessing, Visualization

git_url <https://git.bioconductor.org/packages/CAGEfightR>

git_branch RELEASE_3_9

git_last_commit 90e6c25

git_last_commit_date 2019-05-02

Date/Publication 2019-10-15

R topics documented:

assignGeneID	3
assignMissingID	4
assignTxID	5
assignTxType	7
balanceBC	9
balanceD	9
bwCommonGenome	10
bwGenomeCompatibility	11
bwValid	12
calcBidirectionality	13
calcComposition	14
calcPooled	15
calcShape	15
calcSupport	17
calcTotalTags	18
calcTPM	18
checkPeaked	19
checkPooled	20
clusterBidirectionally	20
clusterUnidirectionally	22
combineClusters	23
exampleDesign	24
findLinks	25
findStretches	26
quantifyClusters	27
quantifyCTSSs	28
quantifyGenes	29
quickEnhancers	30
quickTSSs	31
shapeEntropy	32
shapeIQR	32
shapeMultimodality	33
subsetByBidirectionality	34
subsetByComposition	35
subsetBySupport	36
swapRanges	37
swapScores	38
trackBalance	38

trackClusters	40
trackCTSS	41
trackLinks	42
trimToPeak	43
trimToPercentiles	44
tuneTagClustering	46

Index	48
--------------	-----------

assignGeneID	<i>Annotate ranges with gene ID.</i>
--------------	--------------------------------------

Description

Annotate a set of ranges in a GRanges object with gene IDs (i.e. Entrez Gene Identifiers) based on their genic context. Features overlapping multiple genes are resolved by distance to the nearest TSS. Genes are obtained from a TxDb object, or can manually supplied as a GRanges.

Usage

```
assignGeneID(object, geneModels, ...)

## S4 method for signature 'GenomicRanges,GenomicRanges'
assignGeneID(object, geneModels,
  outputColumn = "geneID", swap = NULL, upstream = 1000,
  downstream = 100)

## S4 method for signature 'RangedSummarizedExperiment,GenomicRanges'
assignGeneID(object,
  geneModels, ...)

## S4 method for signature 'GenomicRanges,TxDb'
assignGeneID(object, geneModels,
  outputColumn = "geneID", swap = NULL, upstream = 1000,
  downstream = 100)

## S4 method for signature 'RangedSummarizedExperiment,TxDb'
assignGeneID(object,
  geneModels, ...)
```

Arguments

object	GRanges or RangedSummarizedExperiment: Ranges to be annotated.
geneModels	TxDb or GRanges: Gene models via a TxDb, or manually specified as a GRanges-List.
...	additional arguments passed to methods.
outputColumn	character: Name of column to hold geneID values.
swap	character or NULL: If not NULL, use another set of ranges contained in object to calculate overlaps, for example peaks in the thick column.
upstream	integer: Distance to extend annotated promoter upstream.
downstream	integer: Distance to extend annotated promoter downstream.

Value

object with geneID added as a column in rowData (or mccols).

See Also

Other Annotation functions: [assignMissingID](#), [assignTxID](#), [assignTxType](#)

Examples

```
data(exampleUnidirectional)

# Obtain gene models from a TxDb-object:
library(TxDb.Mmusculus.UCSC.mm9.knownGene)
txdb <- TxDb.Mmusculus.UCSC.mm9.knownGene

# Assign geneIDs
assignGeneID(exampleUnidirectional,
             geneModels=txdb,
             outputColumn='geneID')

# Assign geneIDs using only TC peaks:
assignGeneID(exampleUnidirectional,
             geneModels=txdb,
             outputColumn='geneID',
             swap='thick')
```

assignMissingID

Annotate ranges with missing IDs.

Description

This function can relabel ranges with missing IDs (i.e. returned by `assignTxID` and `assignGeneID`), in case they need to be retained for further analysis.

Usage

```
assignMissingID(object, ...)

## S4 method for signature 'character'
assignMissingID(object, prefix = "Novel")

## S4 method for signature 'GenomicRanges'
assignMissingID(object,
               outputColumn = "geneID", prefix = "Novel")

## S4 method for signature 'RangedSummarizedExperiment'
assignMissingID(object,
               outputColumn = "geneID", prefix = "Novel")
```

Arguments

object	character, GRanges or RangedSummarizedExperiment: IDs to have NAs replaced with new IDs.
...	additional arguments passed to methods.
prefix	character: New name to assign to ranges with missing IDs, in the style prefix1, prefix2, etc.
outputColumn	character: Name of column to hold txID values.

Value

object with NAs replaced in outputColumn

See Also

Other Annotation functions: [assignGeneID](#), [assignTxID](#), [assignTxType](#)

Examples

```
data(exampleUnidirectional)

# Obtain gene models from a TxDb-object:
library(TxDb.Mmusculus.UCSC.mm9.knownGene)
txdb <- TxDb.Mmusculus.UCSC.mm9.knownGene

# Assign geneIDs using only TC peaks:
exampleUnidirectional <- assignGeneID(exampleUnidirectional,
                                     geneModels=txdb,
                                     outputColumn='geneID',
                                     swap='thick')

# Replace NAs with 'Novel'
assignMissingID(exampleUnidirectional)

# Replace NAs with 'NovelTSS'
assignMissingID(exampleUnidirectional, prefix = 'NovelTSS')
```

assignTxID

Annotate ranges with transcript ID.

Description

Annotate a set of ranges in a GRanges object with transcript IDs based on their genic context. All overlapping transcripts are returned. Transcripts are obtained from a TxDb object, or can manually supplied as a GRanges.

Usage

```
assignTxID(object, txModels, ...)

## S4 method for signature 'GenomicRanges,GenomicRanges'
assignTxID(object, txModels,
```

```

outputColumn = "txID", swap = NULL)

## S4 method for signature 'RangedSummarizedExperiment,GenomicRanges'
assignTxID(object,
  txModels, ...)

## S4 method for signature 'GenomicRanges,TxDb'
assignTxID(object, txModels,
  outputColumn = "txID", swap = NULL, upstream = 1000,
  downstream = 0)

## S4 method for signature 'RangedSummarizedExperiment,TxDb'
assignTxID(object, txModels,
  ...)

```

Arguments

object	GRanges or RangedSummarizedExperiment: Ranges to be annotated.
txModels	TxDb or GRanges: Transcript models via a TxDb, or manually specified as a GRanges.
...	additional arguments passed to methods.
outputColumn	character: Name of column to hold txID values.
swap	character or NULL: If not NULL, use another set of ranges contained in object to calculate overlaps, for example peaks in the thick column.
upstream	integer: Distance to extend annotated promoter upstream.
downstream	integer: Distance to extend annotated promoter downstream.

Value

object with txID added as a column in rowData (or mcols)

See Also

Other Annotation functions: [assignGeneID](#), [assignMissingID](#), [assignTxType](#)

Examples

```

data(exampleUnidirectional)

# Obtain transcript models from a TxDb-object:
library(TxDb.Mmusculus.UCSC.mm9.knownGene)
txdb <- TxDb.Mmusculus.UCSC.mm9.knownGene

# Assign txIDs
assignTxID(exampleUnidirectional,
  txModels=txdb,
  outputColumn='geneID')

# Assign txIDs using only TC peaks:
assignTxID(exampleUnidirectional,
  txModels=txdb,
  outputColumn='geneID',
  swap='thick')

```

assignTxType	<i>Annotate ranges with transcript type.</i>
--------------	--

Description

Annotate a set of ranges in a GRanges object with transcript type (txType) based on their genic context. Transcripts are obtained from a TxDb object, but can alternatively be specified manually as a GRangesList.

Usage

```
assignTxType(object, txModels, ...)

## S4 method for signature 'GenomicRanges,GenomicRangesList'
assignTxType(object, txModels,
  outputColumn = "txType", swap = NULL, noOverlap = "intergenic")

## S4 method for signature 'RangedSummarizedExperiment,GenomicRangesList'
assignTxType(object,
  txModels, ...)

## S4 method for signature 'GenomicRanges,TxDb'
assignTxType(object, txModels,
  outputColumn = "txType", swap = NULL, tssUpstream = 100,
  tssDownstream = 100, proximalUpstream = 1000,
  detailedAntisense = FALSE)

## S4 method for signature 'RangedSummarizedExperiment,TxDb'
assignTxType(object, txModels,
  ...)
```

Arguments

object	GRanges or RangedSummarizedExperiment: Ranges to be annotated.
txModels	TxDb or GRangesList: Transcript models via a TxDb, or manually specified as a GRangesList.
...	additional arguments passed to methods.
outputColumn	character: Name of column to hold txType values.
swap	character or NULL: If not NULL, use another set of ranges contained in object to calculate overlaps, for example peaks in the thick column.
noOverlap	character: In case categories are manually supplied with as a GRangesList, what to call regions with no overlap.
tssUpstream	integer: Distance to extend annotated promoter upstream.
tssDownstream	integer: Distance to extend annotated promoter downstream.
proximalUpstream	integer: Maximum distance upstream of promoter to be considered proximal.
detailedAntisense	logical: Whether to mirror all txType categories in the antisense direction (TRUE) or lump them all together (FALSE).

Value

object with txType added as factor column in rowData (or mcols)

See Also

Other Annotation functions: [assignGeneID](#), [assignMissingID](#), [assignTxID](#)

Examples

```
## Not run:
data(exampleUnidirectional)

# Obtain transcript models from a TxDb-object:
library(TxDb.Mmusculus.UCSC.mm9.knownGene)
txdb <- TxDb.Mmusculus.UCSC.mm9.knownGene

# Assign txIDs
assignTxType(exampleUnidirectional,
             txModels=txdb)

# Assign txIDs using only TC peaks:
exampleUnidirectional <- assignTxType(exampleUnidirectional,
                                     txModels=txdb,
                                     swap='thick')

# The 'promoter' and 'proximal' category boundaries can be changed:
assignTxType(exampleUnidirectional,
             txModels=txdb,
             swap='thick',
             tssUpstream=50,
             tssDownstream=50,
             proximalUpstream=100)

# Annotation using complete antisense categories:
exampleUnidirectional <- assignTxType(exampleUnidirectional,
                                     txModels=txdb,
                                     outputColumn='txTypeExtended',
                                     swap='thick',
                                     detailedAntisense=TRUE)

# The output is always a factor added as a column:
summary(rowRanges(exampleUnidirectional)$txType)
summary(rowRanges(exampleUnidirectional)$txTypeExtended)

# To avoid using a TxDb-object, a GRangesList can be supplied:
custom_hierarchy <- GRangesList(promoters=granges(promoters(txdb)),
                               exons=granges(exons(txdb)))
assignTxType(exampleUnidirectional,
             txModels=custom_hierarchy,
             outputColumn='customType',
             swap='thick',
             noOverlap = 'intergenic')

## End(Not run)
```

balanceBC	<i>Balance statistic: Bhattacharyya coefficient (BC)</i>
-----------	--

Description

Calculates the Bhattacharyya coefficient from observed plus/minus upstream/downstream signals to a perfect bidirectional site, where plus-downstream = 50

Usage

```
balanceBC(PD, MD, PU, MU)
```

Arguments

PD	Plus-Downstream signal
MD	Minus-Downstream signal
PU	Plus-Upstream signal
MU	Plus-Upstream signal

Value

Balance score of the same class as inputs.

Examples

```
# Unbalanced
balanceBC(2, 3, 1, 0)

# Balanced
balanceBC(3, 3, 0, 0)
```

balanceD	<i>Balance statistic: Andersson's D.</i>
----------	--

Description

Calculates the D-statistics from Andersson et al the observed plus/minus downstream signals. The D statistics is rescaled from -1:1 to 0:1 so it can be used for slice-reduce identification of bidirectional sites.

Usage

```
balanceD(PD, MD, PU, MU)
```

Arguments

PD	Plus-Downstream signal
MD	Minus-Downstream signal
PU	Plus-Upstream signal
MU	Plus-Upstream signal

Value

Balance score of the same class as inputs.

Examples

```
# Unbalanced
balanceD(2, 3, 1, 0)

# Balanced
balanceD(3, 3, 0, 0)
```

bwCommonGenome	<i>Find a common genome for a series of BigWig files.</i>
----------------	---

Description

Finds a common genome for a series of BigWig-files, either using only levels present in all files (intersect) or in any file (union).

Usage

```
bwCommonGenome(plusStrand, minusStrand, method = "intersect")
```

Arguments

plusStrand	BigWigFileList: BigWig files with plus-strand CTSS data.
minusStrand	BigWigFileList: BigWig files with minus-strand CTSS data.
method	character: Either 'intersect' or 'union'.

Value

Sorted Seqinfo-object.

See Also

Other BigWig functions: [bwGenomeCompatibility](#), [bwValid](#)

Examples

```
# Use the BigWig-files included with the package:
data('exampleDesign')
bw_plus <- system.file('extdata', exampleDesign$BigWigPlus,
                      package = 'CAGEfightR')
bw_minus <- system.file('extdata', exampleDesign$BigWigMinus,
                       package = 'CAGEfightR')

# Create two named BigWigFileList-objects:
bw_plus <- BigWigFileList(bw_plus)
bw_minus <- BigWigFileList(bw_minus)
names(bw_plus) <- exampleDesign$Name
names(bw_minus) <- exampleDesign$Name
```

```
# Find the smallest common genome (intersect) across the BigWigList-objects:
bwCommonGenome(plusStrand=bw_plus, minusStrand=bw_minus, method='intersect')

# Find the most inclusive genome (union) across the BigWigList-objects:
bwCommonGenome(plusStrand=bw_plus, minusStrand=bw_minus, method='union')
```

bwGenomeCompatibility *Check if BigWig-files are compatible with a given genome.*

Description

Given a genome, checks whether a series of BigWig-files are compatible by checking if all common seqlevels have equal seqlengths.

Usage

```
bwGenomeCompatibility(plusStrand, minusStrand, genome)
```

Arguments

plusStrand	BigWigFileList: BigWig files with plus-strand CTSS data.
minusStrand	BigWigFileList: BigWig files with minus-strand CTSS data.
genome	Seqinfo: Genome information.

Value

TRUE, raises an error if the supplied genome is incompatible.

See Also

Other BigWig functions: [bwCommonGenome](#), [bwValid](#)

Examples

```
# Use the BigWig-files included with the package:
data('exampleDesign')
bw_plus <- system.file('extdata', exampleDesign$BigWigPlus,
                      package = 'CAGEfightR')
bw_minus <- system.file('extdata', exampleDesign$BigWigMinus,
                      package = 'CAGEfightR')

# Create two named BigWigFileList-objects:
bw_plus <- BigWigFileList(bw_plus)
bw_minus <- BigWigFileList(bw_minus)
names(bw_plus) <- exampleDesign$Name
names(bw_minus) <- exampleDesign$Name

# Make a smaller genome:
si <- seqinfo(bw_plus[[1]])
si <- si['chr18']

# Check if it is still compatible:
bwGenomeCompatibility(plusStrand=bw_plus, minusStrand=bw_minus, genome=si)
```

bwValid	<i>Check if BigWig-files are valid.</i>
---------	---

Description

Checks if a BigWigFile or BigWigFileList is composed of readable files with the proper .bw extension.

Usage

```
bwValid(object)

## S4 method for signature 'BigWigFile'
bwValid(object)

## S4 method for signature 'BigWigFileList'
bwValid(object)
```

Arguments

object BigWigFile or BigWigFileList

Value

TRUE, if any tests fails an error is raised.

See Also

Other BigWig functions: [bwCommonGenome](#), [bwGenomeCompatibility](#)

Examples

```
# Use the BigWig-files included with the package:
data('exampleDesign')
bw_plus <- system.file('extdata', exampleDesign$BigWigPlus,
                      package = 'CAGEfightR')

# Create a named BigWigFileList-object with names
bw_plus <- BigWigFileList(bw_plus)
names(bw_plus) <- exampleDesign$Name

# Check a single BigWigFile:
bwValid(bw_plus[[1]])

# Check the entire BigWigFileList:
bwValid(bw_plus)
```

calcBidirectionality *Calculate sample-wise bidirectionality of clusters.*

Description

For each cluster, calculate how many individual samples shows transcription in both directions. This is referred to as the 'bidirectionality'. Clusters must be unstranded (*) and have a midpoint stored in the thick column

Usage

```
calcBidirectionality(object, ...)

## S4 method for signature 'GRanges'
calcBidirectionality(object, samples,
  inputAssay = "counts", outputColumn = "bidirectionality")

## S4 method for signature 'GPos'
calcBidirectionality(object, ...)

## S4 method for signature 'RangedSummarizedExperiment'
calcBidirectionality(object, ...)
```

Arguments

object	GenomicRanges or RangedSummarizedExperiment: Unstranded clusters with midpoints stored in the 'thick' column.
...	additional arguments passed to methods.
samples	RangedSummarizedExperiment: Sample-wise CTSSs stored as an assay.
inputAssay	character: Name of assay in samples holding input CTSS values.
outputColumn	character: Name of column in object to hold bidirectionality values.

Value

object returned with bidirectionality scores added in rowData (or mcols).

See Also

Other Calculation functions: [calcComposition](#), [calcPooled](#), [calcShape](#), [calcSupport](#), [calcTPM](#), [calcTotalTags](#), [subsetByBidirectionality](#), [subsetByComposition](#), [subsetBySupport](#)

Examples

```
data(exampleCTSSs)
data(exampleBidirectional)

calcBidirectionality(exampleBidirectional, samples=exampleCTSSs)
```

calcComposition *Calculate composition of CAGE data.*

Description

For every feature, count in how many samples it is expressed above a certain fraction (e.g. 10 percent) within a grouping, usually genes. This count is referred to as the 'composition' value.

Usage

```
calcComposition(object, inputAssay = "counts",
  outputColumn = "composition", unexpressed = 0.1, genes = "geneID")
```

Arguments

object	RangedSummarizedExperiment: CAGE data quantified at CTSS, cluster or gene-level.
inputAssay	character: Name of assay holding input expression values.
outputColumn	character: Name of column in rowRanges to hold composition values.
unexpressed	numeric: Composition will be calculated based on features larger than this cut-off.
genes	character: Name of column in rowData holding genes (NAs are not currently allowed.)

Value

object with composition added as a column in rowData.

See Also

Other Calculation functions: [calcBidirectionality](#), [calcPooled](#), [calcShape](#), [calcSupport](#), [calcTPM](#), [calcTotalTags](#), [subsetByBidirectionality](#), [subsetByComposition](#), [subsetBySupport](#)

Examples

```
data(exampleUnidirectional)

# Annotate clusters with geneIDs:
library(TxDb.Mmusculus.UCSC.mm9.knownGene)
txdb <- TxDb.Mmusculus.UCSC.mm9.knownGene
exampleUnidirectional <- assignGeneID(exampleUnidirectional,
  geneModels=txdb,
  outputColumn='geneID',
  swap='thick')

# Calculate composition values:
exampleUnidirectional <- subset(exampleUnidirectional, !is.na(geneID))
calcComposition(exampleUnidirectional)

# Use a lower threshold
calcComposition(exampleUnidirectional,
  unexpressed=0.05,
  outputColumn='lenientComposition')
```

calcPooled	<i>Calculate pooled expression across all samples.</i>
------------	--

Description

Sum expression of features across all samples to obtain a 'pooled' signal.

Usage

```
calcPooled(object, inputAssay = "TPM", outputColumn = "score")
```

Arguments

object	RangedSummarizedExperiment: CAGE data quantified at CTSS, cluster or gene-level.
inputAssay	character: Name of assay holding input expression values.
outputColumn	character: Name of column in rowRanges to hold pooled expression.

Value

object with pooled expression added as a column in rowRanges.

See Also

Other Calculation functions: [calcBidirectionality](#), [calcComposition](#), [calcShape](#), [calcSupport](#), [calcTPM](#), [calcTotalTags](#), [subsetByBidirectionality](#), [subsetByComposition](#), [subsetBySupport](#)

Examples

```
data(exampleCTSSs)

# Calculate TPM using supplied total number of tags:
exampleCTSSs <- calcTPM(exampleCTSSs, totalTags='totalTags')

# Sum TPM values over samples:
calcPooled(exampleCTSSs)
```

calcShape	<i>Calculate Tag Cluster shapes</i>
-----------	-------------------------------------

Description

Apply a shape-function to the pooled CTSS signal of every Tag Cluster (TC).

Usage

```

calcShape(object, pooled, ...)

## S4 method for signature 'GRanges,GRanges'
calcShape(object, pooled,
  outputColumn = "IQR", shapeFunction = shapeIQR, ...)

## S4 method for signature 'RangedSummarizedExperiment,GRanges'
calcShape(object, pooled,
  ...)

## S4 method for signature 'GRanges,RangedSummarizedExperiment'
calcShape(object, pooled,
  ...)

## S4 method for signature 'GRanges,GPos'
calcShape(object, pooled, ...)

## S4 method for signature
## 'RangedSummarizedExperiment,RangedSummarizedExperiment'
calcShape(object,
  pooled, ...)

```

Arguments

object	GenomicRanges or RangedSummarizedExperiment: TCs.
pooled	GenomicRanges or RangedSummarizedExperiment: Pooled CTSS as the score column.
...	additional arguments passed to shapeFunction.
outputColumn	character: Name of column to hold shape statistics.
shapeFunction	function: Function to apply to each TC (See details).

Value

object with calculated shape statistics added as a column in rowData (or mcols).

See Also

Other Calculation functions: [calcBidirectionality](#), [calcComposition](#), [calcPooled](#), [calcSupport](#), [calcTPM](#), [calcTotalTags](#), [subsetByBidirectionality](#), [subsetByComposition](#), [subsetBySupport](#)
 Other Shape functions: [shapeEntropy](#), [shapeIQR](#), [shapeMultimodality](#)

Examples

```

data(exampleCTSSs)
data(exampleUnidirectional)

# Calculate pooled CTSSs using pre-calculated number of total tags:
exampleCTSSs <- calcTPM(exampleCTSSs, totalTags='totalTags')
exampleCTSSs <- calcPooled(exampleCTSSs)

```

calcTotalTags	<i>Calculate the total number of CAGE tags across samples.</i>
---------------	--

Description

For each CAGE library, calculate the total number of tags.

Usage

```
calcTotalTags(object, inputAssay = "counts",  
              outputColumn = "totalTags")
```

Arguments

object	RangedSummarizedExperiment: CAGE data quantified at CTSS, cluster or gene-level.
inputAssay	character: Name of assay holding input expression values.
outputColumn	character: Name of column in colData to hold number of total tags.

Value

object with total tags per library added as a column in colData.

See Also

Other Calculation functions: [calcBidirectionality](#), [calcComposition](#), [calcPooled](#), [calcShape](#), [calcSupport](#), [calcTPM](#), [subsetByBidirectionality](#), [subsetByComposition](#), [subsetBySupport](#)

Examples

```
data(exampleUnidirectional)  
calcTotalTags(exampleUnidirectional)
```

calcTPM	<i>Calculate CAGE Tags-Per-Million (TPM)</i>
---------	--

Description

Normalize CAGE-tag counts into TPM values.

Usage

```
calcTPM(object, inputAssay = "counts", outputAssay = "TPM",  
        totalTags = NULL, outputColumn = "totalTags")
```

Arguments

object	RangedSummarizedExperiment: CAGE data quantified at CTSS, cluster or gene-level.
inputAssay	character: Name of assay holding input expression values.
outputAssay	character: Name of assay to hold TPM values.
totalTags	character or NULL: Column in colData holding the total number of tags for each samples. If NULL, this will be calculated using calcTotalTags.
outputColumn	character: Name of column in colData to hold number of total tags, only used if totalTags is NULL.

Value

object with TPM-values added as a new assay. If totalTags is NULL, total tags added as a column in colData.

See Also

Other Calculation functions: [calcBidirectionality](#), [calcComposition](#), [calcPooled](#), [calcShape](#), [calcSupport](#), [calcTotalTags](#), [subsetByBidirectionality](#), [subsetByComposition](#), [subsetBySupport](#)

Examples

```
data(exampleUnidirectional)

# Calculate TPM:
calcTPM(exampleUnidirectional)

# Use pre-calculated total number of tags:
calcTPM(exampleUnidirectional,
         outputAssay='TPMsupplied',
         totalTags='totalTags')
```

checkPeaked

Helper for checking cluster with peaks

Description

Checks whether a supplied set of cluster have valid peaks: Whether the thick column contains IRanges all contained within the main ranges.

Usage

```
checkPeaked(object)
```

Arguments

object GRanges or GPos: Clusters with peaks to be checked.

Value

TRUE if object is correct format, otherwise an error is thrown

See Also

Other Checking functions: [checkPooled](#)

Examples

```
data(exampleUnidirectional)
checkPeaked(rowRanges(exampleUnidirectional))
```

checkPooled

Helper for checking pooled signal

Description

Checks whether a supplied pooled signal is valid: Single bp disjoint with signal in the score column with supplied genome information.

Usage

```
checkPooled(object)
```

Arguments

object GRanges or GPos: Pooled signal to be checked

Value

TRUE if object is correct format, otherwise an error is thrown

See Also

Other Checking functions: [checkPeaked](#)

Examples

```
data(exampleCTSSs)
checkPooled(rowRanges(exampleCTSSs))
```

clusterBidirectionally

Bidirectional clustering of pooled CTSSs.

Description

Finds sites with (balanced and divergent) bidirectional transcription using sliding windows of summed coverage: The Bhattacharyya coefficient (BC) is used to quantify departure from a perfectly balanced site, and a slice-reduce is used to identify sites.

Usage

```
clusterBidirectionally(object, ...)

## S4 method for signature 'GRanges'
clusterBidirectionally(object, window = 201,
  balanceThreshold = 0.95, balanceFun = balanceBC)

## S4 method for signature 'GPos'
clusterBidirectionally(object, ...)

## S4 method for signature 'RangedSummarizedExperiment'
clusterBidirectionally(object, ...)
```

Arguments

object	GenomicRanges or RangedSummarizedExperiment: Pooled CTSSs stored in the score column.
...	additional arguments passed to methods.
window	integer: Width of sliding window used for calculating window sums.
balanceThreshold	numeric: Minimum value of the BC to use for slice-reduce, a value of 1 corresponds to perfectly balanced sites.
balanceFun	function: Advanced users may supply their own function for calculating the balance score instead of the the default balanceBC. See details for instructions.

Value

GRanges with bidirectional sites: Minimum width is $1 + 2 * \text{window}$, TPM sum (on both strands) in the score column, maximal bidirectional site in the thick column and maximum balance in the balance column.

See Also

Other Clustering functions: [clusterUnidirectionally](#), [trimToPeak](#), [trimToPercentiles](#), [tuneTagClustering](#)

Examples

```
## Not run:
data(exampleCTSSs)

# Calculate pooledTPM, using supplied number of total tags
exampleCTSSs <- calcTPM(exampleCTSSs,
  inputAssay='counts',
  outputAssay='TPM',
  totalTags='totalTags')
exampleCTSSs <- calcPooled(exampleCTSSs, inputAssay='TPM')

# Cluster using defaults: balance-treshold of 199 and window of 199 bp:
clusterBidirectionally(exampleCTSSs)

# Use custom thresholds:
clusterBidirectionally(exampleCTSSs, balanceThreshold=0.99, window=101)

## End(Not run)
```

 clusterUnidirectionally

Unidirectional Clustering (Tag Clustering) of pooled CTSSs.

Description

Finds unidirectional Tag Clusters (TCs) with a pooled TPM above a certain threshold using a slice-reduce approach. Additionally calculates the sum and peak position of the TCs.

Usage

```
clusterUnidirectionally(object, ...)

## S4 method for signature 'GRanges'
clusterUnidirectionally(object, pooledCutoff = 0,
  mergeDist = 20L)

## S4 method for signature 'RangedSummarizedExperiment'
clusterUnidirectionally(object, ...)

## S4 method for signature 'GPos'
clusterUnidirectionally(object, ...)
```

Arguments

object	GRanges or RangedSummarizedExperiment: Basepair-wise pooled CTSS.
...	additional arguments passed to methods.
pooledCutoff	numeric: Minimum pooled value to be considered as TC.
mergeDist	integer: Merge TCs within this distance.

Value

GRanges with TPM sum as the score column, and TC peak as the thick column.

See Also

Other Clustering functions: [clusterBidirectionally](#), [trimToPeak](#), [trimToPercentiles](#), [tuneTagClustering](#)

Examples

```
data(exampleCTSSs)

# Calculate pooledTPM, using supplied number of total tags
exampleCTSSs <- calcTPM(exampleCTSSs,
  inputAssay='counts',
  outputAssay='TPM',
  totalTags='totalTags')
exampleCTSSs <- calcPooled(exampleCTSSs, inputAssay='TPM')

# Cluster using defaults: slice-threshold of 0 and reduce-distance of 20
clusterUnidirectionally(exampleCTSSs)
```

```
# Use custom thresholds:
clusterUnidirectionally(exampleCTSSs, pooledCutoff=1, mergeDist=25)
```

```
combineClusters      Combine two CAGE experiments.
```

Description

This function can safely combine two CAGE experiments, for example TCs and enhancers, for later analysis, by making sure no ranges in the final object are overlapping.

Usage

```
combineClusters(object1, object2, ...)
```

```
## S4 method for signature
## 'RangedSummarizedExperiment,RangedSummarizedExperiment'
combineClusters(object1,
  object2, removeIfOverlapping = "none")
```

Arguments

```
object1      RangedSummarizedExperiment: First experiment to be combined.
object2      RangedSummarizedExperiment: First experiment to be combined.
...          arguments passed to methods.
removeIfOverlapping
             character: Whether to keep overlapping ranges ('none') or discard from either
             the first ('object1') or second ('object2') experiment.
```

Value

RangedSummarizedExperiment with merged and sorted ranges (colData and metadata are carried over unchanged).

Examples

```
data(exampleUnidirectional)
data(exampleBidirectional)

# Clusters must have identical colData to be combined:
exampleUnidirectional$totalTags <- NULL

# Combine, keeping potential overlaps
combineClusters(object1=exampleUnidirectional, object2=exampleBidirectional)

# If features overlap, keep only from object1
combineClusters(object1=exampleUnidirectional, object2=exampleBidirectional,
  removeIfOverlapping='object2')

# If features overlap, keep only from object2
combineClusters(object1=exampleUnidirectional, object2=exampleBidirectional,
  removeIfOverlapping='object1')
```

`exampleDesign`*Example CAGE Data*

Description

Subset of the CAGE dataset from the paper 'Identification of Gene Transcription Start Sites and Enhancers Responding to Pulmonary Carbon Nanotube Exposure in Vivo'. CTSS data from subsets of chr18 and chr19 across 3 mouse (mm9) samples are included. Datasets can be loaded with the `data` function.

Usage

`exampleDesign``exampleCTSSs``exampleUnidirectional``exampleBidirectional``exampleGenes`

Format

Example data from various stages of CAGEfightR:

exampleDesign `DataFrame`: Description of samples, including .bw filenames

exampleCTSS `RangedSummarizedExperiment`: CTSSs

exampleUnidirectional `RangedSummarizedExperiment`: Unidirectional or Tag Clusters

exampleBidirectionalCluster `RangedSummarizedExperiment`: Bidirectional clusters

exampleGenes `RangedSummarizedExperiment`: Genes

Source

<http://pubs.acs.org/doi/abs/10.1021/acsnano.6b07533>

Examples

```
data(exampleDesign)
data(exampleCTSSs)
data(exampleUnidirectional)
data(exampleBidirectional)
data(exampleGenes)
```

findLinks	<i>Find nearby pairs of clusters and calculate pairwise correlations.</i>
-----------	---

Description

Finds all links or pairs of clusters within a certain distance of each other and then calculates the correlation between them. The links found can be restricted to only be between two classes, for example TSSs to enhancers.

Usage

```
findLinks(object, ...)

## S4 method for signature 'GRanges'
findLinks(object, maxDist = 10000L,
  directional = NULL)

## S4 method for signature 'RangedSummarizedExperiment'
findLinks(object, inputAssay,
  maxDist = 10000L, directional = NULL, corFun = stats::cor.test,
  vals = c("estimate", "p.value"), ...)
```

Arguments

object	GRanges or RangedSummarizedExperiment: Clusters, possibly with expression for calculating correlations.
...	additional arguments passed to methods or ultimately corFun.
maxDist	integer: Maximum distance between links.
directional	character: Name of a column in object holding a grouping of the clusters. This must be a factor with two levels. The first level is used as the basis for calculating orientation (see below).
inputAssay	character: Name of assay holding expression values (if object is a RangedSummarizedExperiment)
corFun	function: Function for calculating pairwise correlations. See notes for supplying custom functions.
vals	character: Statistics extracted from the results produced by corFun. See notes for supplying custom functions.

Details

A custom function for calculation correlations can be supplied by the user. The output of this function must be a named list or vector of numeric values. The names of the vals to be extracted should be supplied to vals.

Value

A GInteractions holding the links, along with the distance between them and correlation estimate and p-value calculated from their expression. If a directional analysis was performed, the two anchors are always connecting members of the two classes and the orientation of the second anchor relative to the first is additionally calculated (e.g. whether an enhancers is upstream or downstream of the TSS).

See Also

Other Spatial functions: [findStretches](#), [trackLinks](#)

Examples

```
library(InteractionSet)

# Subset to highly expressed unidirectional clusters
TCs <- subset(exampleUnidirectional, score > 10)

# Find links within a certain distance
findLinks(TCs, inputAssay="counts", maxDist=10000L)

# To find TSS-to-enhancer type links, first merge the clusters:
exampleBidirectional$totalTags <- TCs$totalTags
rowRanges(TCs)$clusterType <- "TSS"
rowRanges(exampleBidirectional)$clusterType <- "Enhancer"
SE <- combineClusters(TCs, exampleBidirectional, removeIfOverlapping="object1")
rowRanges(SE)$clusterType <- factor(rowRanges(SE)$clusterType, levels=c("TSS", "Enhancer"))

# Calculate kendall correlations of TPM values:
SE <- calcTPM(SE, totalTags="totalTags")
findLinks(SE, inputAssay="TPM", maxDist=10000L, directional="clusterType", method="kendall")
```

findStretches

Find stretches of clusters

Description

Finds stretches or groups of clusters along the genome, where each cluster is within a certain distance of the next. Once stretches have been identified, the average pairwise correlation between all clusters in the stretch is calculated. A typical use case is to look for stretches of enhancers, often referred to as "super enhancers".

Usage

```
findStretches(object, ...)

## S4 method for signature 'GRanges'
findStretches(object, mergeDist = 10000L,
  minSize = 3L)

## S4 method for signature 'RangedSummarizedExperiment'
findStretches(object, inputAssay,
  mergeDist = 10000L, minSize = 3L, corFun = cor, ...)
```

Arguments

object	GRanges or RangedSummarizedExperiment: Clusters, possibly with expression for calculating correlations.
...	additional arguments passed to methods or ultimately corFun.
mergeDist	integer: Maximum distance between clusters to be merged into stretches.

minSize	integer: Minimum number of clusters in stretches.
inputAssay	character: Name of assay holding expression values (if object is a RangedSummarizedExperiment)
corFun	function: Function for calculating correlations. Should behave and produce output similar to cor().

Value

A GRanges containing stretches with number of clusters and average pairwise correlations calculated. The revmap can be used to retrieve the original clusters (see example below.)

See Also

Other Spatial functions: [findLinks](#), [trackLinks](#)

Examples

```
# Calculate TPM values for bidirectional clusters
data(exampleBidirectional)
BCs <- calcTPM(exampleBidirectional)

# Find stretches
pearson_stretches <- findStretches(BCs, inputAssay="TPM")

# Use Kendall instead of pearson and require bigger stretches
kendall_stretches <- findStretches(BCs, inputAssay="TPM",
                                  minSize=5, method="kendall")

# Use the revmap to get stretches as a GRangesList
grl <- extractList(rowRanges(BCs), kendall_stretches$revmap)
names(grl) <- names(kendall_stretches)
```

quantifyClusters	<i>Quantify expression of clusters (TSSs or enhancers) by summing CTSSs within clusters.</i>
------------------	--

Description

Quantify expression of clusters (TSSs or enhancers) by summing CTSSs within clusters.

Usage

```
quantifyClusters(object, clusters, inputAssay = "counts",
                 sparse = FALSE)
```

Arguments

object	RangedSummarizedExperiment: CTSSs.
clusters	GRanges: Clusters to be quantified.
inputAssay	character: Name of assay holding expression values to be quantified (usually counts).
sparse	logical: If the input is a sparse matrix, TRUE will keep the output matrix sparse while FALSE will coerce it into a normal matrix.

Value

RangedSummarizedExperiment with row corresponding to clusters. seqinfo and colData is copied over from object.

See Also

Other Quantification functions: [quantifyCTSSs](#), [quantifyGenes](#)

Examples

```
# CTSSs stored in a RangedSummarizedExperiment:
data(exampleCTSS)

# Clusters to be quantified as a GRanges:
data(exampleUnidirectional)
clusters <- rowRanges(exampleUnidirectional)

# Quantify clusters:
quantifyClusters(exampleCTSSs, clusters)

# For exceptionally large datasets,
# the resulting count matrix can be left sparse:
quantifyClusters(exampleCTSSs, rowRanges(exampleUnidirectional), sparse=TRUE)
```

quantifyCTSSs

Quantify CAGE Transcriptions Start Sites (CTSSs)

Description

This function reads in CTSS count data from a series of BigWig-files and returns a CTSS-by-library count matrix. For efficient processing, the count matrix is stored as a sparse matrix (dgCMatrix).

Usage

```
quantifyCTSSs(plusStrand, minusStrand, design = NULL, genome = NULL,
  tileWidth = 10000000L)
```

Arguments

plusStrand	BigWigFileList: BigWig files with plus-strand CTSS data.
minusStrand	BigWigFileList: BigWig files with minus-strand CTSS data.
design	DataFrame or data.frame: Additional information on samples.
genome	Seqinfo: Genome information. If NULL the smallest common genome will be found using bwCommonGenome.
tileWidth	integer: Size of tiles to parallelize over.

Value

RangedSummarizedExperiment, where assay is a sparse matrix (dgCMatrix) of CTSS counts..

See Also

Other Quantification functions: [quantifyClusters](#), [quantifyGenes](#)

Examples

```
## Not run:
# Load the example data
data('exampleDesign')
# Use the BigWig-files included with the package:
bw_plus <- system.file('extdata', exampleDesign$BigWigPlus,
                      package = 'CAGEfightR')
bw_minus <- system.file('extdata', exampleDesign$BigWigMinus,
                      package = 'CAGEfightR')

# Create two named BigWigFileList-objects:
bw_plus <- BigWigFileList(bw_plus)
bw_minus <- BigWigFileList(bw_minus)
names(bw_plus) <- exampleDesign$Name
names(bw_minus) <- exampleDesign$Name

# Quantify CTSSs, by default this will use the smallest common genome:
CTSSs <- quantifyCTSSs(plusStrand=bw_plus,
                      minusStrand=bw_minus,
                      design=exampleDesign)

# Alternatively, a genome can be specified:
si <- seqinfo(bw_plus[[1]])
si <- si['chr18']
CTSSs <- quantifyCTSSs(plusStrand=bw_plus,
                      minusStrand=bw_minus,
                      design=exampleDesign,
                      genome=si)

# Quantification can be speed up by using multiple cores:
library(BiocParallel)
register(MulticoreParam(workers=3))
CTSSs <- quantifyCTSSs(plusStrand=bw_plus,
                      minusStrand=bw_minus,
                      design=exampleDesign,
                      genome=si)

## End(Not run)
```

quantifyGenes

Quantify expression of genes

Description

Obtain gene-level expression estimates by summing clusters annotated to the same gene. Unannotated transcripts (NAs) are discarded.

Usage

```
quantifyGenes(object, genes, inputAssay = "counts", sparse = FALSE)
```

Arguments

object	RangedSummarizedExperiment: Cluster-level expression values.
genes	character: Name of column in rowData holding gene IDs (NAs will be discarded).
inputAssay	character: Name of assay holding values to be quantified, (usually counts).
sparse	logical: If the input is a sparse matrix, TRUE will keep the output matrix sparse while FALSE will coerce it into a normal matrix.

Value

RangedSummarizedExperiment with rows corresponding to genes. Location of clusters within genes is stored as a GRangesList in rowRanges. seqinfo and colData is copied over from object.

See Also

Other Quantification functions: [quantifyCTSSs](#), [quantifyClusters](#)

Examples

```
data(exampleUnidirectional)

# Annotate clusters with geneIDs:
library(TxDb.Mmusculus.UCSC.mm9.knownGene)
txdb <- TxDb.Mmusculus.UCSC.mm9.knownGene
exampleUnidirectional <- assignGeneID(exampleUnidirectional,
                                     geneModels=txdb,
                                     outputColumn='geneID')

# Quantify counts within genes:
quantifyGenes(exampleUnidirectional, genes='geneID', inputAssay='counts')

# For exceptionally large datasets,
# the resulting count matrix can be left sparse:
quantifyGenes(exampleUnidirectional,
               genes='geneID',
               inputAssay='counts',
               sparse=TRUE)
```

quickEnhancers

Identify and quantify enhancers.

Description

A convenient wrapper around clusterBidirectionally, subsetByBidirectionality and quantifyClusters.

Usage

```
quickEnhancers(object)
```

Arguments

object RangedSummarizedExperiment: Location and counts of CTSSs, usually found by calling `quantifyCTSSs`.

Value

RangedSummarizedExperiment containing location and counts of enhancers.

See Also

Other Wrapper functions: [quickTSSs](#)

Examples

```
# See the CAGEfightR vignette for an overview!
```

quickTSSs	<i>Identify and quantify Transcription Start Sites (TSSs).</i>
-----------	--

Description

A convenient wrapper around `calcTPM`, `calcPooled`, `tuneTagClustering`, `clusterUnidirectionally` and `quantifyClusters`.

Usage

```
quickTSSs(object)
```

Arguments

object RangedSummarizedExperiment: Location and counts of CTSSs, usually found by calling `quantifyCTSSs`.

Value

RangedSummarizedExperiment containing location and counts of TSSs

See Also

Other Wrapper functions: [quickEnhancers](#)

Examples

```
# See the CAGEfightR vignette for an overview!
```

shapeEntropy	<i>Shape statistic: Shannon Entropy</i>
--------------	---

Description

Calculates the Shannon Entropy (base log2) for a vector. Zeros are removed before calculation.

Usage

```
shapeEntropy(x)
```

Arguments

x numeric Rle vector: Coverage series.

Value

Numeric.

See Also

Other Shape functions: [calcShape](#), [shapeIQR](#), [shapeMultimodality](#)

Examples

```
# Hypothetical shard/broad clusters:
x_sharp <- Rle(c(1,1,1,4,5,2,1,1))
x_broad <- Rle(c(1,2,3,5,4,3,2,1))

# Calculate Entropy
shapeEntropy(x_sharp)
shapeEntropy(x_broad)

# See calcShape for more usage examples
```

shapeIQR	<i>Shape statistic: Interquartile range</i>
----------	---

Description

Calculates the interquartile range of a vector.

Usage

```
shapeIQR(x, lower = 0.25, upper = 0.75)
```

Arguments

x numeric Rle vector: Coverage series.
lower numeric: Lower quartile.
upper numeric: Upper quartile.

Value

Numeric

See Also

Other Shape functions: [calcShape](#), [shapeEntropy](#), [shapeMultimodality](#)

Examples

```
# Hypothetical shard/broad clusters:
x_sharp <- Rle(c(1,1,1,4,5,2,1,1))
x_broad <- Rle(c(1,2,3,5,4,3,2,1))

# Calculate IQR
shapeIQR(x_sharp)
shapeIQR(x_broad)

# See calcShape for more usage examples
```

shapeMultimodality *Shape statistic: Multimodality*

Description

Shape statistic: Multimodality

Usage

```
shapeMultimodality(x)
```

Arguments

x numeric Rle vector: Coverage series.

Value

Numeric.

See Also

Other Shape functions: [calcShape](#), [shapeEntropy](#), [shapeIQR](#)

Examples

```
# See calcShape for usage examples
```

```
subsetByBidirectionality
```

Subset by sample-wise bidirectionality of clusters.

Description

A convenient wrapper around `calcBidirectionality` and `subset`.

Usage

```
subsetByBidirectionality(object, ...)

## S4 method for signature 'GRanges'
subsetByBidirectionality(object, samples,
  inputAssay = "counts", outputColumn = "bidirectionality",
  minSamples = 0)

## S4 method for signature 'GPos'
subsetByBidirectionality(object, ...)

## S4 method for signature 'RangedSummarizedExperiment'
subsetByBidirectionality(object,
  ...)
```

Arguments

<code>object</code>	<code>GRanges</code> or <code>RangedSummarizedExperiment</code> : Unstranded clusters with peaks stored in the 'thick' column.
<code>...</code>	additional arguments passed to methods.
<code>samples</code>	<code>RangedSummarizedExperiment</code> : Sample-wise CTSSs stored as an assay.
<code>inputAssay</code>	character: Name of assay in samples holding input CTSS values.
<code>outputColumn</code>	character: Name of column in object to hold bidirectionality values.
<code>minSamples</code>	integer: Only regions with bidirectionality above this value are retained.

Value

object with bidirectionality values added as a column, and low bidirectionality regions removed.

See Also

Other Subsetting functions: [subsetByComposition](#), [subsetBySupport](#)

Other Calculation functions: [calcBidirectionality](#), [calcComposition](#), [calcPooled](#), [calcShape](#), [calcSupport](#), [calcTPM](#), [calcTotalTags](#), [subsetByComposition](#), [subsetBySupport](#)

Examples

```
data(exampleCTSSs)
data(exampleBidirectional)

# Keep only clusters that are bidirectional in at least one sample:
subsetByBidirectionality(exampleBidirectional, samples=exampleCTSSs)
```

subsetByComposition *Subset by composition across samples*

Description

A convenient wrapper around calcComposition and subset.

Usage

```
subsetByComposition(object, inputAssay = "counts",  
  outputColumn = "composition", unexpressed = 0.1, genes = "geneID",  
  minSamples = 1)
```

Arguments

object	RangedSummarizedExperiment: CAGE data quantified at CTSS, cluster or gene-level.
inputAssay	character: Name of assay holding input expression values.
outputColumn	character: Name of column in rowRanges to hold composition values.
unexpressed	numeric: Composition will be calculated based on features larger than this cut-off.
genes	character: Name of column in rowData holding genes (NAs are not allowed.)
minSamples	numeric: Only features with composition in more than this number of samples will be kept.

Value

RangedSummarizedExperiment with composition values added as a column in rowData and features with less composition than minSamples removed.

See Also

Other Subsetting functions: [subsetByBidirectionality](#), [subsetBySupport](#)

Other Calculation functions: [calcBidirectionality](#), [calcComposition](#), [calcPooled](#), [calcShape](#), [calcSupport](#), [calcTPM](#), [calcTotalTags](#), [subsetByBidirectionality](#), [subsetBySupport](#)

Examples

```
data(exampleUnidirectional)  
  
# Annotate clusters with geneIDs:  
library(TxDb.Mmusculus.UCSC.mm9.knownGene)  
txdb <- TxDb.Mmusculus.UCSC.mm9.knownGene  
  
exampleUnidirectional <- assignGeneID(exampleUnidirectional,  
  geneModels=txdb,  
  outputColumn='geneID')  
exampleUnidirectional <- subset(exampleUnidirectional, !is.na(geneID))  
  
# Keep only clusters more than 10% in more than one sample:  
calcComposition(exampleUnidirectional)
```

```
# Keep only clusters more than 5% in more than 2 samples:
subsetByComposition(exampleUnidirectional, unexpressed = 0.05, minSamples=2)
```

subsetBySupport	<i>Subset by support across samples</i>
-----------------	---

Description

A convenient wrapper around calcSupport and subset.

Usage

```
subsetBySupport(object, inputAssay = "counts",
  outputColumn = "support", unexpressed = 0, minSamples = 1)
```

Arguments

object	RangedSummarizedExperiment: CAGE data quantified at CTSS, cluster or gene-level.
inputAssay	character: Name of assay holding input expression values.
outputColumn	character: Name of column in rowRanges to hold support values.
unexpressed	numeric: Support will be calculated based on features larger than this cutoff.
minSamples	numeric: Only features with support in more than this number of samples will be kept.

Value

RangedSummarizedExperiment with support added as a column in rowRanges and features with less support than minSamples removed.

See Also

Other Subsetting functions: [subsetByBidirectionality](#), [subsetByComposition](#)

Other Calculation functions: [calcBidirectionality](#), [calcComposition](#), [calcPooled](#), [calcShape](#), [calcSupport](#), [calcTPM](#), [calcTotalTags](#), [subsetByBidirectionality](#), [subsetByComposition](#)

Examples

```
data(exampleBidirectional)

# Keep clusters with at least one tag in two samples
subsetBySupport(exampleBidirectional)

# Keep clusters with at least two tags in four samples
subsetBySupport(exampleBidirectional, unexpressed=1, minSamples=2)
```

swapRanges	<i>Swap ranges in a GRanges.</i>
------------	----------------------------------

Description

Swap out the range of a GRanges-object with another IRanges-object stored inside the same object. I.e., swapping cluster widths with cluster peaks.

Usage

```
swapRanges(object, ...)  
  
## S4 method for signature 'GenomicRanges'  
swapRanges(object, inputColumn = "thick",  
           outputColumn = NULL)  
  
## S4 method for signature 'RangedSummarizedExperiment'  
swapRanges(object, ...)
```

Arguments

object	GRanges or RangedSummarizedExperiment: Primary ranges to be swapped out.
...	additional arguments passed to methods.
inputColumn	character: Name of column holding IRanges to be swapped in.
outputColumn	character or NULL: Name of column to hold swapped out ranges, if NULL original ranges are not saved.

Value

GRanges with inputColumn swapped in as ranges.

See Also

Other Swapping functions: [swapScores](#)

Examples

```
data(exampleUnidirectional)  
gr <- rowRanges(exampleUnidirectional)  
  
# Swap in peaks as main ranges  
peaks <- swapRanges(gr)  
head(width(gr))  
head(width(peaks))  
  
# swapRanges() can also be directly called on a RangedSummarizedExperiment:  
swapRanges(exampleUnidirectional)  
  
# The original can optionally be saved in the output object  
swapRanges(gr, outputColumn = 'swapped')
```

swapScores	<i>Swap scores in SummarizedExperiment</i>
------------	--

Description

Take scores for a specific sample and a specific assay and put them into rowData.

Usage

```
swapScores(object, outputColumn = "score", inputAssay, sample)
```

Arguments

object	SummarizedExperiment: CAGE-data
outputColumn	character: Column in rowData to hold swapped in scores.
inputAssay	character: Name of assay to take scores from.
sample	character: Name of sample to take scores from.

Value

SummarizedExperiment with sample scores from inputAssay in rowData.

See Also

Other Swapping functions: [swapRanges](#)

Examples

```
data(exampleCTSSs)
sample_names <- colnames(exampleCTSSs)

# Replace scores with values from the first sample:
x <- swapScores(exampleCTSSs, inputAssay='counts', sample=sample_names[1])
rowRanges(x)
```

trackBalance	<i>Create Genome Browser Track of bidirectional balance scores</i>
--------------	--

Description

Visualize balance scores used for detection of bidirectional sites. Mainly intended as diagnostic tools for expert user.

Usage

```
trackBalance(object, ...)

## S4 method for signature 'GRanges'
trackBalance(object, window = 199,
  plusColor = "cornflowerblue", minusColor = "tomato",
  balanceColor = "forestgreen", ...)

## S4 method for signature 'GPos'
trackBalance(object, ...)

## S4 method for signature 'RangedSummarizedExperiment'
trackBalance(object, ...)
```

Arguments

object	GenomicRanges or RangedSummarizedExperiment: Ranges with CTSSs in the score column.
...	additional arguments passed to DataTrack.
window	integer: Width of sliding window used for calculating windowed sums.
plusColor	character: Color for plus-strand coverage.
minusColor	character: Color for minus-strand coverage.
balanceColor	character: Color for bidirectional balance.

Value

list of 3 DataTracks for upstream, downstream and balance.

Note

Potentially consumes a large amount of memory!

See Also

Other Genome Browser functions: [trackCTSS](#), [trackClusters](#), [trackLinks](#)

Examples

```
## Not run:
library(Gviz)
data(exampleCTSSs)
data(exampleBidirectional)

# Calculate pooled CTSSs
exampleCTSSs <- calcTPM(exampleCTSSs, totalTags='totalTags')
exampleCTSSs <- calcPooled(exampleCTSSs)

# Find a bidirectional cluster to plot:
BC <- rowRanges(exampleBidirectional[10,])
start(BC) <- start(BC) - 250
end(BC) <- end(BC) + 250
subsetOfCTSSs <- subsetByOverlaps(exampleCTSSs, BC)
```

```
# Build balance track
balance_track <- trackBalance(subsetOfCTSSs)

# Plot
plotTracks(balance_track, from=start(BC), to=end(BC),
           chromosome=seqnames(BC))

## End(Not run)
```

trackClusters *Create genome browser track of clusters.*

Description

Create a Gviz-track of clusters (unidirectional TCs or bidirectional enhancers), where cluster strand and peak is indicated.

Usage

```
trackClusters(object, ...)

## S4 method for signature 'GRanges'
trackClusters(object, plusColor = "cornflowerblue",
             minusColor = "tomato", unstrandedColor = "hotpink", ...)

## S4 method for signature 'RangedSummarizedExperiment'
trackClusters(object, ...)
```

Arguments

object	GRanges: GRanges with peaks in the thick-column.
...	additional arguments passed on to GeneRegionTrack.
plusColor	character: Color for plus-strand features.
minusColor	character: Color for minus-strand features.
unstrandedColor	character: Color for unstranded features.

Value

GeneRegionTrack-object.

See Also

Other Genome Browser functions: [trackBalance](#), [trackCTSS](#), [trackLinks](#)

Examples

```

library(Gviz)
data(exampleUnidirectional)

# Find some wide unidirectional clusters:
TCs <- subset(exampleUnidirectional, width >= 100)

# Create track
clusters_track <- trackClusters(TCs[1:2,], name='Tag clusters', col=NULL)

# Plot
plotTracks(clusters_track)

# See vignette for examples on how to combine multiple Gviz tracks

```

trackCTSS

Create Genome Browser track of CTSSs.

Description

Create a Gviz-track of CTSSs, where Plus/minus strand signal is shown positive/negative. This representation makes it easy to identify bidirectional peaks.

Usage

```

trackCTSS(object, ...)

## S4 method for signature 'GRanges'
trackCTSS(object, plusColor = "cornflowerblue",
  minusColor = "tomato", ...)

## S4 method for signature 'RangedSummarizedExperiment'
trackCTSS(object, ...)

## S4 method for signature 'GPos'
trackCTSS(object, ...)

```

Arguments

object	GenomicRanges or RangedSummarizedExperiment: Ranges with CTSSs in the score column.
...	additional arguments passed on to DataTrack.
plusColor	character: Color for plus-strand coverage.
minusColor	character: Color for minus-strand coverage.

Value

DataTrack-object.

See Also

Other Genome Browser functions: [trackBalance](#), [trackClusters](#), [trackLinks](#)

Examples

```

library(Gviz)
data(exampleCTSSs)
data(exampleUnidirectional)
data(exampleBidirectional)

# Example uni- and bidirectional clusters
TC <- rowRanges(subset(exampleUnidirectional, width>=100)[3,])
BC <- rowRanges(exampleBidirectional[3,])

# Create pooled track
subsetOfCTSSs <- subsetByOverlaps(exampleCTSSs, c(BC, TC, ignore.mcols=TRUE))
pooledTrack <- trackCTSS(subsetOfCTSSs)

# Plot
plotTracks(pooledTrack, from=start(TC)-100, to=end(TC)+100,
           chromosome=seqnames(TC), name='TC')
plotTracks(pooledTrack, from=start(BC)-100, to=end(BC)+100,
           chromosome=seqnames(BC), name='BC')

# See vignette for examples on how to combine multiple Gviz tracks

```

trackLinks

Create a genome browser track of links.

Description

Create a Gviz-track of links (e.g. between TSSs and enhancers), where arches connect the different pairs of clusters. The height of arches can be set to scale the strength of the interaction (for example indicating higher correlation). This function is a thin wrapper around the InteractionTrack-class from the GenomicInteractions package. Currently, only scaling arch height by p-value is supported.

Usage

```
trackLinks(object, ...)
```

Arguments

object	GInteractions: Links or pairs between clusters.
...	additional arguments passed to InteractionTrack via displayPars.

Value

InteractionTrack-object from the GenomicInteractions package.

See Also

Other Genome Browser functions: [trackBalance](#), [trackCTSS](#), [trackClusters](#)

Other Spatial functions: [findLinks](#), [findStretches](#)

Examples

```

library(InteractionSet)
library(Gviz)
library(GenomicInteractions)

# Links between highly expressed unidirectional clusters
TCs <- subset(exampleUnidirectional, score > 10)
TC_links <- findLinks(TCs, inputAssay="counts", maxDist=10000L)
link_track <- trackLinks(TC_links, name="TSS links", interaction.measure="p.value")

# Plot region
plot_region <- GRanges(seqnames="chr18",
                      ranges = IRanges(start=start(anchors(TC_links[1],
                                                            "first")),
                                       end=end(anchors(TC_links[1],
                                                       "second"))))

# Plot using Gviz
plotTracks(link_track,
           from=start(plot_region),
           to=end(plot_region),
           chromosome = as.character(seqnames(plot_region)))
# See vignette for examples on how to combine multiple Gviz tracks

```

trimToPeak

Trim width of TCs by distance from TC peak

Description

Trim the width of TCs by distance from the TC peaks.

Usage

```

trimToPeak(object, pooled, ...)

## S4 method for signature 'GRanges,GRanges'
trimToPeak(object, pooled, upstream,
           downstream)

## S4 method for signature 'GRanges,GPos'
trimToPeak(object, pooled, ...)

## S4 method for signature 'RangedSummarizedExperiment,GenomicRanges'
trimToPeak(object,
           pooled, ...)

## S4 method for signature 'GRanges,RangedSummarizedExperiment'
trimToPeak(object, pooled,
           ...)

## S4 method for signature
## 'RangedSummarizedExperiment,RangedSummarizedExperiment'

```

```
trimToPeak(object,
           pooled, ...)
```

Arguments

object	GenomicRanges or RangedSummarizedExperiment: Tag clusters.
pooled	GenomicRanges or RangedSummarizedExperiment: Basepair-wise pooled CTSS (stored in the score column).
...	additional arguments passed to methods.
upstream	integer: Maximum upstream distance from TC peak.
downstream	integer: Maximum downstream distance from TC peak.

Value

data.frame with two columns: threshold and nTCs (number of Tag Clusters)

See Also

Other Clustering functions: [clusterBidirectionally](#), [clusterUnidirectionally](#), [trimToPercentiles](#), [tuneTagClustering](#)

Other Trimming functions: [trimToPercentiles](#)

Examples

```
data(exampleCTSSs)
data(exampleBidirectional)

# Calculate pooled CTSSs
exampleCTSSs <- calcTPM(exampleCTSSs, totalTags='totalTags')
exampleCTSSs <- calcPooled(exampleCTSSs)

# Choose a few wide clusters:
TCs <- subset(exampleUnidirectional, width >= 100)

# Trim to +/- 10 bp of TC peak
trimToPeak(TCs, pooled=exampleCTSSs, upstream=10, downstream=10)
```

trimToPercentiles	<i>Trim width of TCs to expression percentiles</i>
-------------------	--

Description

Given a set of TCs and genome-wide CTSS coverage, reduce the width of TC until a certain amount of expression has been removed.

Usage

```

trimToPercentiles(object, pooled, ...)

## S4 method for signature 'GRanges,GRanges'
trimToPercentiles(object, pooled,
  percentile = 0.1, symmetric = FALSE)

## S4 method for signature 'GRanges,GPos'
trimToPercentiles(object, pooled, ...)

## S4 method for signature 'RangedSummarizedExperiment,GenomicRanges'
trimToPercentiles(object,
  pooled, ...)

## S4 method for signature 'GRanges,RangedSummarizedExperiment'
trimToPercentiles(object,
  pooled, ...)

## S4 method for signature
## 'RangedSummarizedExperiment,RangedSummarizedExperiment'
trimToPercentiles(object,
  pooled, ...)

```

Arguments

object	GenomicRanges or RangedSummarizedExperiment: TCs to be trimmed.
pooled	GenomicRanges or RangedSummarizedExperiment: CTSS coverage.
...	additional arguments passed to methods.
percentile	numeric: Fraction of expression to remove from TCs.
symmetric	logical: Whether to trim the same amount from both edges of the TC (TRUE) or always trim from the least expressed end (FALSE).

Value

GRanges with trimmed TCs, including recalculated peaks and scores.

See Also

Other Clustering functions: [clusterBidirectionally](#), [clusterUnidirectionally](#), [trimToPeak](#), [tuneTagClustering](#)

Other Trimming functions: [trimToPeak](#)

Examples

```

data(exampleCTSSs)
data(exampleBidirectional)

# Calculate pooled CTSSs
exampleCTSSs <- calcTPM(exampleCTSSs, totalTags='totalTags')
exampleCTSSs <- calcPooled(exampleCTSSs)

```

```

# Choose a few wide clusters:
TCs <- subset(exampleUnidirectional, width >= 100)

# Symmetric trimming (same percentage from each side):
TCs_sym <- trimToPercentiles(TCs, pooled=exampleCTSSs, symmetric=FALSE)

# Assymmetric trimming (always trim from lowest side):
TCs_asym <- trimToPercentiles(TCs, pooled=exampleCTSSs, symmetric=TRUE)

# Compare the two results sets of widths:
summary(width(TCs_sym) - width(TCs_asym))

```

tuneTagClustering	<i>Determine the optimal pooled threshold for unidirectional tag clustering.</i>
-------------------	--

Description

This function counts the number of Tag Clusters (TCs) for an series of small incremental pooled cutoffs

Usage

```

tuneTagClustering(object, ...)

## S4 method for signature 'GRanges'
tuneTagClustering(object, steps = 10L,
  mergeDist = 20L, searchMethod = "minUnique", maxExponent = 1)

## S4 method for signature 'RangedSummarizedExperiment'
tuneTagClustering(object, ...)

## S4 method for signature 'GPos'
tuneTagClustering(object, ...)

```

Arguments

object	GenomicRanges or RangedSummarizedExperiment: Pooled CTSS.
...	additional arguments passed to methods.
steps	integer: Number of thresholds to analyze (in addition to treshold=0).
mergeDist	integer: Merge TCs within this distance.
searchMethod	character: For advanced user only, see details.
maxExponent	numeric: The maximal threshold to analyse is obtained as $\min(\text{score}) \cdot 2^{\text{maxExponent}}$ (only used if searchMethod='exponential').

Value

data.frame with two columns: threshold and nTCs (number of Tag Clusters)

See Also

Other Clustering functions: [clusterBidirectionally](#), [clusterUnidirectionally](#), [trimToPeak](#), [trimToPercentiles](#)

Examples

```
## Not run:
data(exampleCTSSs)

# Calculate pooledTPM, using supplied number of total tags
exampleCTSSs <- calcTPM(exampleCTSSs,
                        inputAssay='counts',
                        outputAssay='TPM',
                        totalTags='totalTags')
exampleCTSSs <- calcPooled(exampleCTSSs, inputAssay='TPM')

# Set backend
library(BiocParallel)
register(SerialParam())

# Find optimal slice-threshold for reduce distance of 20:
tuneTagClustering(object=exampleCTSSs)

## End(Not run)
```

Index

*Topic **datasets**
 exampleDesign, 24

assignGeneID, 3, 5, 6, 8
 assignGeneID, GenomicRanges, GenomicRanges-method
 (assignGeneID), 3
 assignGeneID, GenomicRanges, TxDb-method
 (assignGeneID), 3
 assignGeneID, RangedSummarizedExperiment, GenomicRanges-method
 (assignGeneID), 3
 assignGeneID, RangedSummarizedExperiment, TxDb-method
 (assignGeneID), 3
 assignMissingID, 4, 4, 6, 8
 assignMissingID, character-method
 (assignMissingID), 4
 assignMissingID, GenomicRanges-method
 (assignMissingID), 4
 assignMissingID, RangedSummarizedExperiment-method
 (assignMissingID), 4
 assignTxID, 4, 5, 5, 8
 assignTxID, GenomicRanges, GenomicRanges-method
 (assignTxID), 5
 assignTxID, GenomicRanges, TxDb-method
 (assignTxID), 5
 assignTxID, RangedSummarizedExperiment, GenomicRanges-method
 (assignTxID), 5
 assignTxID, RangedSummarizedExperiment, TxDb-method
 (assignTxID), 5
 assignTxType, 4–6, 7
 assignTxType, GenomicRanges, GenomicRangesList-method
 (assignTxType), 7
 assignTxType, GenomicRanges, TxDb-method
 (assignTxType), 7
 assignTxType, RangedSummarizedExperiment, GenomicRangesList-method
 (assignTxType), 7
 assignTxType, RangedSummarizedExperiment, TxDb-method
 (assignTxType), 7

balanceBC, 9
 balanced, 9
 bwCommonGenome, 10, 11, 12
 bwGenomeCompatibility, 10, 11, 12
 bwValid, 10, 11, 12
 bwValid, BigWigFile-method (bwValid), 12
 bwValid, BigWigFileList-method
 (bwValid), 12

calcBidirectionality, 13, 14–19, 34–36
 calcBidirectionality, GPos-method
 (calcBidirectionality), 13
 calcBidirectionality, GRanges-method
 (calcBidirectionality), 13
 calcBidirectionality, RangedSummarizedExperiment-method
 (calcBidirectionality), 13
 calcComposition, 13, 14, 15–19, 34–36
 calcPooled, 13, 14, 15, 16–19, 34–36
 calcShape, 13–15, 15, 17–19, 32–36
 calcShape, GRanges, GPos-method
 (calcShape), 15
 calcShape, GRanges, GRanges-method
 (calcShape), 15
 calcShape, GRanges, RangedSummarizedExperiment-method
 (calcShape), 15
 calcShape, RangedSummarizedExperiment, GRanges-method
 (calcShape), 15
 calcShape, RangedSummarizedExperiment, RangedSummarizedExperiment-method
 (calcShape), 15
 calcSupport, 13–16, 17, 18, 19, 34–36
 calcTotalTags, 13–17, 18, 19, 34–36
 calcTPM, 13–18, 18, 34–36
 checkPeaked, 19, 20
 checkPooled, 20, 20
 clusterBidirectionally, 20, 22, 44, 45, 47
 clusterBidirectionally, GPos-method
 (clusterBidirectionally), 20
 clusterBidirectionally, GRanges-method
 (clusterBidirectionally), 20
 clusterBidirectionally, RangedSummarizedExperiment-method
 (clusterBidirectionally), 20
 clusterUnidirectionally, 21, 22, 44, 45,
 47
 clusterUnidirectionally, GPos-method
 (clusterUnidirectionally), 22
 clusterUnidirectionally, GRanges-method
 (clusterUnidirectionally), 22
 clusterUnidirectionally, RangedSummarizedExperiment-method
 (clusterUnidirectionally), 22
 combineClusters, 23

- combineClusters, RangedSummarizedExperiment, RangedSummarizedExperiment-method (combineClusters), 23
- exampleBidirectional (exampleDesign), 24
- exampleCTSSs (exampleDesign), 24
- exampleDesign, 24
- exampleGenes (exampleDesign), 24
- exampleUnidirectional (exampleDesign), 24
- findLinks, 25, 27, 42
- findLinks, GRanges-method (findLinks), 25
- findLinks, RangedSummarizedExperiment-method (findLinks), 25
- findStretches, 26, 26, 42
- findStretches, GRanges-method (findStretches), 26
- findStretches, RangedSummarizedExperiment-method (findStretches), 26
- quantifyClusters, 27, 29, 30
- quantifyCTSSs, 28, 28, 30
- quantifyGenes, 28, 29, 29
- quickEnhancers, 30, 31
- quickTSSs, 31, 31
- shapeEntropy, 16, 32, 33
- shapeIQR, 16, 32, 32, 33
- shapeMultimodality, 16, 32, 33, 33
- subsetByBidirectionality, 13–19, 34, 35, 36
- subsetByBidirectionality, GPos-method (subsetByBidirectionality), 34
- subsetByBidirectionality, GRanges-method (subsetByBidirectionality), 34
- subsetByBidirectionality, RangedSummarizedExperiment-method (subsetByBidirectionality), 34
- subsetByComposition, 13–19, 34, 35, 36
- subsetBySupport, 13–19, 34, 35, 36
- swapRanges, 37, 38
- swapRanges, GenomicRanges-method (swapRanges), 37
- swapRanges, RangedSummarizedExperiment-method (swapRanges), 37
- swapScores, 37, 38
- trackBalance, 38, 40–42
- trackBalance, GPos-method (trackBalance), 38
- trackBalance, GRanges-method (trackBalance), 38
- trackBalance, RangedSummarizedExperiment-method (trackBalance), 38
- trackClusters, RangedSummarizedExperiment, RangedSummarizedExperiment-method (trackClusters), 40
- trackClusters, GRanges-method (trackClusters), 40
- trackClusters, RangedSummarizedExperiment-method (trackClusters), 40
- trackCTSS, 39, 40, 41, 42
- trackCTSS, GPos-method (trackCTSS), 41
- trackCTSS, GRanges-method (trackCTSS), 41
- trackCTSS, RangedSummarizedExperiment-method (trackCTSS), 41
- trackLinks, 26, 27, 39–41, 42
- trimToPeak, 21, 22, 43, 45, 47
- trimToPeak, GRanges, GPos-method (trimToPeak), 43
- trimToPeak, GRanges, GRanges-method (trimToPeak), 43
- trimToPeak, GRanges, RangedSummarizedExperiment-method (trimToPeak), 43
- trimToPeak, RangedSummarizedExperiment, GenomicRanges-method (trimToPeak), 43
- trimToPeak, RangedSummarizedExperiment, RangedSummarizedExperiment-method (trimToPeak), 43
- trimToPercentiles, 21, 22, 44, 44, 47
- trimToPercentiles, GRanges, GPos-method (trimToPercentiles), 44
- trimToPercentiles, GRanges, GRanges-method (trimToPercentiles), 44
- trimToPercentiles, GRanges, RangedSummarizedExperiment-method (trimToPercentiles), 44
- trimToPercentiles, RangedSummarizedExperiment, GenomicRanges-method (trimToPercentiles), 44
- trimToPercentiles, RangedSummarizedExperiment, RangedSummarizedExperiment-method (trimToPercentiles), 44
- tuneTagClustering, 21, 22, 44, 45, 46
- tuneTagClustering, GPos-method (tuneTagClustering), 46
- tuneTagClustering, GRanges-method (tuneTagClustering), 46
- tuneTagClustering, RangedSummarizedExperiment-method (tuneTagClustering), 46