

Package ‘CAGEfightR’

July 23, 2025

Type Package

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

Version 1.28.0

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(>= 3.6.3), Matrix(>= 1.2-12), BiocGenerics(>= 0.24.0), S4Vectors(>= 0.16.0), IRanges(>= 2.12.0), GenomeInfoDb(>= 1.14.0), GenomicFeatures(>= 1.29.11), GenomicAlignments(>= 1.22.1), 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 7.1.2

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_21

git_last_commit f30b594

git_last_commit_date 2025-04-15

Repository Bioconductor 3.21

Date/Publication 2025-07-23

Contents

assignGeneID	3
assignMissingID	5
assignTxID	6
assignTxType	8
balanceBC	10
balanceD	11
bwCommonGenome	11
bwGenomeCompatibility	12
bwValid	13
calcBidirectionality	14
calcComposition	16
calcPooled	17
calcShape	18
calcSupport	19
calcTotalTags	20
calcTPM	21
checkCTSSs	22
checkPeaked	23
checkPooled	24
clusterBidirectionally	24
clusterUnidirectionally	26
combineClusters	27
convertBAM2BigWig	28
convertBED2BigWig	29
convertGRanges2GPos	31
exampleDesign	32
findLinks	33
findStretches	34
quantifyClusters	36

quantifyCTSSs	37
quantifyCTSSs2	39
quantifyGenes	40
quickEnhancers	41
quickGenes	42
quickTSSs	43
shapeEntropy	43
shapeIQR	44
shapeMean	45
shapeMultimodality	46
subsetByBidirectionality	46
subsetByComposition	47
subsetBySupport	49
swapRanges	50
swapScores	51
trackBalance	51
trackClusters	53
trackCTSS	54
trackLinks	55
trimToPeak	56
trimToPercentiles	58
tuneTagClustering	59
utilsAggregateRows	61
utilsDeStrand	62
utilsScoreOverlaps	63
utilsSimplifyTxDb	64

Index	66
--------------	-----------

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 mcols).

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	<i>Annotate ranges with missing IDs.</i>
-----------------	--

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 replaces 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

Balance statistic: Bhattacharyya coefficient (BC)

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

```
if (.Platform$OS.type != "windows") {
  # Use the BigWig-files included with the package:
  data('exampleDesign')
  bw_plus <- system.file('extdata', exampleDesign$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

```
if (.Platform$OS.type != "windows") {
  # Use the BigWig-files included with the package:
  data('exampleDesign')
  bw_plus <- system.file('extdata', exampleDesign$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

Check if BigWig-files are valid.

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 bidirectionally 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)
```



```

                                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\(\)](#), [shapeMean\(\)](#)

Examples

```

data(exampleCTSSs)
data(exampleUnidirectional)

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

# Calculate shape statistics
calcShape(exampleUnidirectional, pooled=exampleCTSSs,
  outputColumn='entropy', shapeFunction=shapeEntropy)
calcShape(exampleUnidirectional, pooled=exampleCTSSs, outputColumn='IQR',
  shapeFunction=shapeIQR, lower=0.2, upper=0.8)

# See the vignette for how to implement custom shape functions!

```

calcSupport	<i>Calculate support of CAGE data.</i>
-------------	--

Description

Calculate the number of samples expression a feature above a certain level. This number is referred to as the 'support'.

Usage

```

calcSupport(
  object,
  inputAssay = "counts",
  outputColumn = "support",
  unexpressed = 0
)

```

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.

Value

object with support added as a column in rowRanges.

See Also

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

Examples

```
data(exampleUnidirectional)

# Count samples with at least a single tags
exampleUnidirectional <- calcSupport(exampleUnidirectional,
                                     inputAssay='counts',
                                     unexpressed=0)

# Count number of samples with more than 1 TPM and save as a new column.
exampleUnidirectional <- calcTPM(exampleUnidirectional,
                                 totalTags = 'totalTags')
exampleUnidirectional <- calcSupport(exampleUnidirectional,
                                     inputAssay='TPM',
                                     unexpressed=1,
                                     outputColumn='TPMSupport')
```

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='TPMsSupplied',
        totalTags='totalTags')
```

checkCTSSs

Helper for checking files containing CTSSs

Description

Checks whether a file (or GRanges/GPos) contains data formatted in the same manner as CAGE Transcription Start Sites (CTSSs): Each basepair of the genome is associated with a single integer count.

Usage

```
checkCTSSs(object)

## S4 method for signature 'ANY'
checkCTSSs(object)

## S4 method for signature 'GRanges'
checkCTSSs(object)

## S4 method for signature 'character'
checkCTSSs(object)

## S4 method for signature 'GPos'
checkCTSSs(object)

## S4 method for signature 'BigWigFile'
checkCTSSs(object)
```

Arguments

object	BigWigFile, character, GRanges or GPos: Path to the file storing CTSSs, or an already imported GRanges/GPos.
--------	--

Value

TRUE if CTSSs are correctly formatted, otherwise a (hopefully) informative error is thrown.

Note

In the case that a character is supplied pointing to a file, checkCTSSs will not check any extensions, but simply try to read it using `rtracklayer::import`. This means that checkCTSSs can technically analyze BED-files, although CAGEfightR can only import CTSSs from BigWig or bedGraph files.

Examples

```
if (.Platform$OS.type != "windows") {
  # Load example data
  data('exampleDesign')
  bw_plus <- system.file('extdata',
                        exampleDesign$BigWigPlus,
                        package = 'CAGEfightR')
  bw_plus <- BigWigFileList(bw_plus)

  # Check raw file
  checkCTSSs(bw_plus[[1]])

  # Import first, then check
  gr <- import(bw_plus[[1]])
  checkCTSSs(gr)
}
```

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	<i>Helper for checking pooled signal</i>
-------------	--

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	<i>Bidirectional clustering of pooled CTSSs.</i>
------------------------	--

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 $1 + 2 \times \text{window}$, 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-threshold 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	<i>Combine two CAGE experiments.</i>
-----------------	--------------------------------------

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')
```

convertBAM2BigWig	<i>Extract CTSSs from BAM-files (EXPERIMENTAL)</i>
-------------------	--

Description

Function for converting mapped reads in BAM-files to CAGE Transcription Start Sites (CTSSs) in BigWig-files. Currently, this function will simply load a (single-end) BAM-file (respecting a supplied ScanBamParam), optionally remove short tags, and count the number of 5'-ends at each bp. Note, the BAM-file is loaded as a single object, so you must be able to keep at least one complete BAM-file in RAM.

Usage

```
convertBAM2BigWig(input, outputPlus, outputMinus, minLength = 1L, ...)
```

Arguments

input	character: Path to input BAM-file
outputPlus	character: Path to output BigWig-file holding CTSSs on the plus strand.
outputMinus	character: Path to output BigWig-file holding CTSSs on the minus strand.
minLength	integer: Minimum length of mapped reads.
...	Additional arguments passed to rtracklayer::import. This will often include a ScanBamParam

Value

Number of CTSSs/Tags returned invisibly.

Note

WARNING: This function is experimental, has not been thoroughly tested, and will most likely significantly change in upcoming CAGEfightR version. For comments/question please go to the CAGEfightR github page.

Examples

```
# TBA
```

convertBED2BigWig	<i>Convert CTSSs stored in different file formats.</i>
-------------------	--

Description

Collection of functions for converting CTSSs/CTSSs-like data stored in BigWig, bedGraph or BED file formats. BigWig and bedGraph files use a file for each strand, while BED-files stores both strands in a single file. As BigWig files stores info about the chromosome lengths, conversion from bedGraph/BED to BigWig requires a genome. Note that CAGEfightR will only import BigWig or bedGraph files!

Usage

```
convertBED2BigWig(input, outputPlus, outputMinus, genome)

convertBED2BedGraph(input, outputPlus, outputMinus)

convertBedGraph2BigWig(input, output, genome)

convertBigWig2BedGraph(input, output)

convertBigWig2BED(inputPlus, inputMinus, output)

convertBedGraph2BED(inputPlus, inputMinus, output)
```

Arguments

input	character: Path to input files holding CTSSs on both strands.
outputPlus	character: Path to output files holding CTSSs on plus strand.
outputMinus	character: Path to output files holding CTSSs on minus strand.
genome	Seqinfo or character: Genome info passed to rtracklayer::import (see note).
output	character: Path to output files holding CTSSs on both strands.
inputPlus	character: Path to input files holding CTSSs on plus strand.
inputMinus	character: Path to input files holding CTSSs on minus strand.

Value

TRUE returned invisibly if conversion(s) was succesful, otherwise an error is raised.

Note

These functions will warn if input files do not have the correct extensions (.bw, .bedGraph, .bed), but otherwise simply pass input to `rtracklayer::import`. This makes them able to handle compressed files (like .gz). The same applies to the genome argument, which can also be the name of a UCSC genome.

Examples

```
## Not run:
# Find paths to BigWig files
data('exampleDesign')
bw_plus <- system.file('extdata', exampleDesign$BigWigPlus,
                      package = 'CAGEfightR')
bw_minus <- system.file('extdata', exampleDesign$BigWigMinus,
                      package = 'CAGEfightR')

# Designate paths to new files
n_samples <- length(bw_plus)
beds <- replicate(n=n_samples, tempfile(fileext=".bed"))
bg_plus <- replicate(n=n_samples, tempfile(fileext="_plus.bedGraph"))
bg_minus <- replicate(n=n_samples, tempfile(fileext="_minus.bedGraph"))
conv_plus <- replicate(n=n_samples, tempfile(fileext="_plus.bw"))
conv_minus <- replicate(n=n_samples, tempfile(fileext="_minus.bw"))

# Convert BigWig to BED
convertBigWig2BED(inputPlus=bw_plus,
                 inputMinus=bw_minus,
                 output=beds)

# Convert BED to bedGraph
convertBED2BedGraph(input=beds,
                  outputPlus=bg_plus,
                  outputMinus=bg_minus)

# Convert BED to bedGraph
mm9 <- SeqinfoForUCSCGenome("mm9")
convertBED2BigWig(input=beds,
                 outputPlus=conv_plus,
                 outputMinus=conv_minus,
                 genome=mm9)

# Check it's still the same data
x <- import(bw_plus[1])
y <- import(bg_plus[1])
z <- import(conv_plus[1])
all(x == y)
all(x == z)
sum(score(x)) == sum(score(y))
```

```
sum(score(x)) == sum(score(z))

## End(Not run)
```

convertGRanges2GPos	<i>Convert GRanges with scores to GPos</i>
---------------------	--

Description

Converts a GRanges to a GPos, correctly expanding the score column. This is useful is nearby CTSSs with the same count are grouped in the same range (see example).

Usage

```
convertGRanges2GPos(object)
```

Arguments

object	GRanges object with a score column
--------	------------------------------------

Value

GPos with score column

Examples

```
# Example GRanges
gr <- GRanges(Rle(c("chr2", "chr2", "chr3", "chr4")),
              IRanges(start=c(1, 10, 5, 3),
                      end=c(5L, 10L, 5L, 4L)),
              strand="+",
              score=c(2, 1, 3, 11))

# Expand to proper GPos / CTSS format:
gp <- convertGRanges2GPos(gr)

# Double check that the total number of counts remains the same
stopifnot(sum(score(gr) * width(gr)) == sum(score(gp)))
```

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

An object of class RangedSummarizedExperiment with 41256 rows and 3 columns.

An object of class RangedSummarizedExperiment with 21008 rows and 3 columns.

An object of class RangedSummarizedExperiment with 377 rows and 3 columns.

An object of class RangedSummarizedExperiment with 127 rows and 3 columns.

Source

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

Examples

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


findLinks

*Find nearby pairs of clusters and calculate pairwise correlations.***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:
colData(exampleBidirectional) <- colData(TCs)
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: [quantifyCTSSs2\(\)](#), [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 (or bedGraph-files) and returns a CTSS-by-library count matrix. For efficient processing, the count matrix is stored as a sparse matrix (dgCMatrix from the Matrix package), and CTSSs are compressed to a GPos object if possible.

Usage

```
quantifyCTSSs(plusStrand, minusStrand, design = NULL, genome = NULL, ...)

## S4 method for signature 'BigWigFileList,BigWigFileList'
quantifyCTSSs(
  plusStrand,
  minusStrand,
  design = NULL,
  genome = NULL,
  nTiles = 1L
)

## S4 method for signature 'character,character'
quantifyCTSSs(plusStrand, minusStrand, design = NULL, genome = NULL)
```

Arguments

plusStrand	BigWigFileList or character: BigWig/bedGraph files with plus-strand CTSS data.
minusStrand	BigWigFileList or character: BigWig/bedGraph files with minus-strand CTSS data.
design	DataFrame or data.frame: Additional information on samples which will be added to the output
genome	Seqinfo: Genome information. If NULL the smallest common genome will be found using bwCommonGenome when BigWig-files are analyzed.
...	additional arguments passed to methods.
nTiles	integer: Number of genomic tiles to parallelize over.

Value

RangedSummarizedExperiment, where assay is a sparse matrix (dgCMatrix) of CTSS counts and design stored in colData.

See Also

Other Quantification functions: [quantifyCTSSs2\(\)](#), [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_subset <- 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_subset <- quantifyCTSSs(plusStrand=bw_plus,
                             minusStrand=bw_minus,
                             design=exampleDesign,
                             genome=si)

# CAGEfightR also support bedGraph files, first BigWig is converted
bg_plus <- replicate(n=length(bw_plus), tempfile(fileext="_plus.bedGraph"))
bg_minus <- replicate(n=length(bw_minus), tempfile(fileext="_minus.bedGraph"))
names(bg_plus) <- names(bw_plus)
names(bg_minus) <- names(bw_minus)

convertBigWig2BedGraph(input=sapply(bw_plus, resource), output=bg_plus)
convertBigWig2BedGraph(input=sapply(bw_minus, resource), output=bg_minus)

# Then analyze: Note a genome MUST be supplied here!
si <- bwCommonGenome(bw_plus, bw_minus)
```

```

CTSSs_via_bg <- quantifyCTSSs(plusStrand=bg_plus,
                              minusStrand=bg_minus,
                              design=exampleDesign,
                              genome=si)

# Confirm that the two approaches yield the same results
all(assay(CTSSs_via_bg) == assay(CTSSs))

## End(Not run)

```

quantifyCTSSs2

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

```

quantifyCTSSs2(
  plusStrand,
  minusStrand,
  design = NULL,
  genome = NULL,
  tileWidth = 100000000L
)

```

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: [quantifyCTSSs\(\)](#), [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: [quantifyCTSSs2\(\)](#), [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: [quickGenes\(\)](#), [quickTSSs\(\)](#)

Examples

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

quickGenes	<i>Identify and quantify genes.</i>
------------	-------------------------------------

Description

A convenient wrapper around `assignGeneID`, and `quantifyGenes`. Also removes unstranded features

Usage

```
quickGenes(object, geneModels = NULL, ...)
```

Arguments

object RangedSummarizedExperiment: Location and counts of clusters, usually found by calling `quantifyClusters`.

geneModels TxDb or GRanges: Gene models via a TxDb, or manually specified as a GRanges-List.

... additional arguments passed to `assignGeneID`.

Value

RangedSummarizedExperiment containing gene expression and clusters assigned within each gene.

See Also

Other Wrapper functions: [quickEnhancers\(\)](#), [quickTSSs\(\)](#)

Examples

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

quickTSSs

Identify and quantify Transcription Start Sites (TSSs).

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\(\)](#), [quickGenes\(\)](#)

Examples

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

shapeEntropy

Shape statistic: Shannon Entropy

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\(\)](#), [shapeMean\(\)](#)

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

Shape statistic: Interquartile range

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\(\)](#), [shapeMean\(\)](#)

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
```

shapeMean	<i>Shape statistic: Mean</i>
-----------	------------------------------

Description

Calculates the mean of a vector.

Usage

```
shapeMean(x)
```

Arguments

x numeric Rle vector: Coverage series.

Value

Numeric

See Also

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

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 mean
shapeMean(x_sharp)
shapeMean(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.

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

object	GRanges or RangedSummarizedExperiment: Unstranded clusters with peaks 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.
minSamples	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	<i>Subset by composition across samples</i>
---------------------	---

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(rowRanges(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)
```

utilsAggregateRows	<i>Utility: Aggregate rows</i>
--------------------	--------------------------------

Description

Used by quantifyClusters and quantifyGenes. Wrapper around rowsum with a few improvements: 1) Handles dgCMatix 2) Suppresses warnings from and discards NAs in grouping 3) Checks if output can be coerced to integer (useful when aggregating a dgCMatix), 4) For the dgCMatix case, has the option to keep unused levels and output a sparse matrix.

Usage

```
utilsAggregateRows(x, group, drop = TRUE, sparse = FALSE)
```

```
## S4 method for signature 'matrix'
utilsAggregateRows(x, group, drop = TRUE, sparse = FALSE)
```

```
## S4 method for signature 'dgCMatix'
utilsAggregateRows(x, group, drop = TRUE, sparse = FALSE)
```

Arguments

x	matrix or dgCMatix: Matrix to be aggregated.
group	factor: Grouping, can cannot NAs which will be discarded.
drop	logical: Whether to drop unused levels (TRUE) or keep assign them 0 (FALSE).
sparse	logical: Whether output should be coerced to a dense matrix.

Value

matrix (or dgCMatix if sparse=TRUE)

See Also

Other Utility functions: [utilsDeStrand\(\)](#), [utilsScoreOverlaps\(\)](#), [utilsSimplifyTxDb\(\)](#)

Examples

```
library(Matrix)
data("exampleCTSSs")
data("exampleUnidirectional")

# Sparse and dense examples
sparse_matrix <- assay(exampleCTSSs)
dense_matrix <- as(sparse_matrix, "matrix")

# Groupings
grp <- findOverlaps(query = exampleCTSSs,
```

```

        subject = exampleUnidirectional,
        select="arbitrary")

# Aggregate rows and compare
sparse_res <- utilsAggregateRows(sparse_matrix, grp)
dense_res <- utilsAggregateRows(dense_matrix, grp)
all(sparse_res == dense_res)

# Note that storage type was converted to integers!
storage.mode(sparse_res)
storage.mode(dense_res)

# You can also elect to keep a sparse representation
utilsAggregateRows(sparse_matrix, grp, sparse = TRUE)

#### Examples with unused levels ####

# Silly example
dense_mat <- replicate(5, runif(10))
sparse_mat <- as(dense_mat, "dgCMatrix")
fct_unused <- factor(c(1, 1, NA, NA, 3, 3, NA, NA, 5, 5), levels=1:5)

# The default is to drop unused levels
utilsAggregateRows(dense_mat, fct_unused, drop=TRUE)
utilsAggregateRows(sparse_mat, fct_unused, drop=TRUE)

# For dgCMatrix, one can elect to retain these:
utilsAggregateRows(sparse_mat, fct_unused, drop=FALSE)

# For matrix, a warning is produced if either drop or sparse is requested
utilsAggregateRows(dense_mat, fct_unused, drop=FALSE)
utilsAggregateRows(dense_mat, fct_unused, sparse=TRUE)

```

utilsDeStrand

Utility: Split Genomic Ranges by strand

Description

Utility function that attempts to split genomic ranges by strand with `split(object, strand(object))`

Usage

```
utilsDeStrand(object)
```

Arguments

object Any object with a split and strand method, e.g. GRanges/GPos

Value

Object split by strand, e.g. GRangesList.

See Also

Other Utility functions: [utilsAggregateRows\(\)](#), [utilsScoreOverlaps\(\)](#), [utilsSimplifyTxDb\(\)](#)

Examples

```
gp <- GPos(seqnames=Rle(c("chr1", "chr2", "chr1"), c(10, 6, 4)),
           pos=c(44:53, 5:10, 2:5),
           strand=c(rep("+", 10), rep("-", 10)))
gr <- as(gp, "GRanges")
utilsDeStrand(gp)
utilsDeStrand(gr)
```

utilsScoreOverlaps	<i>Utility: Counting overlaps taking into account scores</i>
--------------------	--

Description

Similar to countOverlaps, but takes the score column into account.

Usage

```
utilsScoreOverlaps(query, subject, ...)
```

Arguments

query	same as findOverlaps/countOverlaps
subject	same as findOverlaps/countOverlaps
...	additional arguments passed to findOverlaps

Value

vector of number of overlaps weighed by score column.

See Also

<https://support.bioconductor.org/p/87736/#87758>

Other Utility functions: [utilsAggregateRows\(\)](#), [utilsDeStrand\(\)](#), [utilsSimplifyTxDb\(\)](#)

Examples

```
gr1 <- GRanges(seqnames="chr1",
               ranges=IRanges(start = c(4, 9, 10, 30),
                              end = c(4, 15, 20, 31)),
               strand="+")
gr2 <- GRanges(seqnames="chr1",
               ranges=IRanges(start = c(1, 4, 15, 25),
                              end = c(2, 4, 20, 26)),
               strand=c("+"),
```

```

        score=c(10, 20, 15, 5))
countOverlaps(gr1, gr2)
utilsScoreOverlaps(gr1, gr2)

```

utilsSimplifyTxDb *Utility: Extract annotation hierarchy from a TxDb.*

Description

Used by assignTxType. This function extracts the hierarchical annotations used by assignTxType from a TxDb object. If you are annotating many ranges, it can be time saving to build the hierarchy first, to avoid processing the TxDb for every assignTxDb call.

Usage

```

utilsSimplifyTxDb(
  object,
  tssUpstream = 100,
  tssDownstream = 100,
  proximalUpstream = 1000,
  detailedAntisense = FALSE
)

```

Arguments

object	TxDb: Transcript database
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

GRangesList of annotation hierarchy

See Also

assignTxType

Other Utility functions: [utilsAggregateRows\(\)](#), [utilsDeStrand\(\)](#), [utilsScoreOverlaps\(\)](#)

Examples

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

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

# Simplify txdb
hierachy <- utilsSimplifyTxDb(txdb)

# Standard way of calling
x <- assignTxType(exampleUnidirectional,
                   txModels=txdb)

# Calling with premade hierachy
y <- assignTxType(exampleUnidirectional, txModels=hierachy)

# These are identical
stopifnot(all(rowRanges(x)$txType == rowRanges(y)$txType))

## End(Not run)
```

Index

* Annotation functions

assignGeneID, [3](#)
assignMissingID, [5](#)
assignTxID, [6](#)
assignTxType, [8](#)

* BigWig functions

bwCommonGenome, [11](#)
bwGenomeCompatibility, [12](#)
bwValid, [13](#)

* Calculation functions

calcBidirectionality, [14](#)
calcComposition, [16](#)
calcPooled, [17](#)
calcShape, [18](#)
calcSupport, [19](#)
calcTotalTags, [20](#)
calcTPM, [21](#)
subsetByBidirectionality, [46](#)
subsetByComposition, [47](#)
subsetBySupport, [49](#)

* Checking functions

checkPeaked, [23](#)
checkPooled, [24](#)

* Clustering functions

clusterBidirectionally, [24](#)
clusterUnidirectionally, [26](#)
trimToPeak, [56](#)
trimToPercentiles, [58](#)
tuneTagClustering, [59](#)

* Genome Browser functions

trackBalance, [51](#)
trackClusters, [53](#)
trackCTSS, [54](#)
trackLinks, [55](#)

* Quantification functions

quantifyClusters, [36](#)
quantifyCTSSs, [37](#)
quantifyCTSSs2, [39](#)
quantifyGenes, [40](#)

* Shape functions

calcShape, [18](#)
shapeEntropy, [43](#)
shapeIQR, [44](#)
shapeMean, [45](#)

* Spatial functions

findLinks, [33](#)
findStretches, [34](#)
trackLinks, [55](#)

* Subsetting functions

subsetByBidirectionality, [46](#)
subsetByComposition, [47](#)
subsetBySupport, [49](#)

* Swapping functions

swapRanges, [50](#)
swapScores, [51](#)

* Trimming functions

trimToPeak, [56](#)
trimToPercentiles, [58](#)

* Utility functions

utilsAggregateRows, [61](#)
utilsDeStrand, [62](#)
utilsScoreOverlaps, [63](#)
utilsSimplifyTxDb, [64](#)

* Wrapper functions

quickEnhancers, [41](#)
quickGenes, [42](#)
quickTSSs, [43](#)

* datasets

exampleDesign, [32](#)

assignGeneID, [3](#), [5](#), [7](#), [9](#)

assignGeneID, GenomicRanges, GenomicRanges-method
(assignGeneID), [3](#)

assignGeneID, GenomicRanges, TxDb-method
(assignGeneID), [3](#)

assignGeneID, RangedSummarizedExperiment, GenomicRanges-meth
(assignGeneID), [3](#)

assignGeneID, RangedSummarizedExperiment, TxDb-method
(assignGeneID), [3](#)

- assignMissingID, [4](#), [5](#), [7](#), [9](#)
- assignMissingID, character-method (assignMissingID), [5](#)
- assignMissingID, GenomicRanges-method (assignMissingID), [5](#)
- assignMissingID, RangedSummarizedExperiment-method (assignMissingID), [5](#)
- assignTxID, [4](#), [5](#), [6](#), [9](#)
- assignTxID, GenomicRanges, GenomicRanges-method (assignTxID), [6](#)
- assignTxID, GenomicRanges, TxDb-method (assignTxID), [6](#)
- assignTxID, RangedSummarizedExperiment, GenomicRanges-method (assignTxID), [6](#)
- assignTxID, RangedSummarizedExperiment, TxDb-method (assignTxID), [6](#)
- assignTxType, [4](#), [5](#), [7](#), [8](#)
- assignTxType, GenomicRanges, GenomicRangesList-method (assignTxType), [8](#)
- assignTxType, GenomicRanges, TxDb-method (assignTxType), [8](#)
- assignTxType, RangedSummarizedExperiment, GenomicRangesList-method (assignTxType), [8](#)
- assignTxType, RangedSummarizedExperiment, TxDb-method (assignTxType), [8](#)

- balanceBC, [10](#)
- balanceD, [11](#)
- bwCommonGenome, [11](#), [13](#), [14](#)
- bwGenomeCompatibility, [12](#), [12](#), [14](#)
- bwValid, [12](#), [13](#), [13](#)
- bwValid, BigWigFile-method (bwValid), [13](#)
- bwValid, BigWigFileList-method (bwValid), [13](#)

- calcBidirectionality, [14](#), [16–18](#), [20](#), [21](#), [47–49](#)
- calcBidirectionality, GPos-method (calcBidirectionality), [14](#)
- calcBidirectionality, GRanges-method (calcBidirectionality), [14](#)
- calcBidirectionality, RangedSummarizedExperiment-method (calcBidirectionality), [14](#)
- calcComposition, [15](#), [16](#), [17](#), [18](#), [20](#), [21](#), [47–49](#)
- calcPooled, [15](#), [16](#), [17](#), [18](#), [20](#), [21](#), [47–49](#)
- calcShape, [15–17](#), [18](#), [20](#), [21](#), [44](#), [45](#), [47–49](#)
- calcShape, GRanges, GPos-method (calcShape), [18](#)
- calcShape, GRanges, GRanges-method (calcShape), [18](#)
- calcShape, GRanges, RangedSummarizedExperiment-method (calcShape), [18](#)
- calcShape, RangedSummarizedExperiment, GRanges-method (calcShape), [18](#)
- calcShape, RangedSummarizedExperiment, RangedSummarizedExperiment-method (calcShape), [18](#)
- calcSupport, [15–18](#), [19](#), [20](#), [21](#), [47–49](#)
- calcTotalTags, [15–18](#), [20](#), [20](#), [21](#), [47–49](#)
- calcTPM, [15–18](#), [20](#), [21](#), [47–49](#)
- checkCTSSs, [22](#)
- checkCTSSs, ANY-method (checkCTSSs), [22](#)
- checkCTSSs, BigWigFile-method (checkCTSSs), [22](#)
- checkCTSSs, character-method (checkCTSSs), [22](#)
- checkCTSSs, GPos-method (checkCTSSs), [22](#)
- checkCTSSs, GRanges-method (checkCTSSs), [22](#)
- checkPeaked, [23](#), [24](#)
- checkPooled, [23](#), [24](#)
- clusterBidirectionally, [24](#), [26](#), [57](#), [59](#), [60](#)
- clusterBidirectionally, GPos-method (clusterBidirectionally), [24](#)
- clusterBidirectionally, GRanges-method (clusterBidirectionally), [24](#)
- clusterBidirectionally, RangedSummarizedExperiment-method (clusterBidirectionally), [24](#)
- clusterUnidirectionally, [25](#), [26](#), [57](#), [59](#), [60](#)
- clusterUnidirectionally, GPos-method (clusterUnidirectionally), [26](#)
- clusterUnidirectionally, GRanges-method (clusterUnidirectionally), [26](#)
- clusterUnidirectionally, RangedSummarizedExperiment-method (clusterUnidirectionally), [26](#)
- combineClusters, [27](#)
- combineClusters, RangedSummarizedExperiment, RangedSummarizedExperiment-method (combineClusters), [27](#)
- convertBAM2BigWig, [28](#)
- convertBED2BedGraph (convertBED2BigWig), [29](#)
- convertBED2BigWig, [29](#)
- convertBedGraph2BED (convertBED2BigWig), [29](#)
- convertBedGraph2BigWig (convertBED2BigWig), [29](#)

- convertBigWig2BED (convertBED2BigWig), 29
- convertBigWig2BedGraph (convertBED2BigWig), 29
- convertGRanges2GPos, 31
- exampleBidirectional (exampleDesign), 32
- exampleCTSSs (exampleDesign), 32
- exampleDesign, 32
- exampleGenes (exampleDesign), 32
- exampleUnidirectional (exampleDesign), 32
- findLinks, 33, 35, 56
- findLinks, GRanges-method (findLinks), 33
- findLinks, RangedSummarizedExperiment-method (findLinks), 33
- findStretches, 34, 34, 56
- findStretches, GRanges-method (findStretches), 34
- findStretches, RangedSummarizedExperiment-method (findStretches), 34
- quantifyClusters, 36, 38, 39, 41
- quantifyCTSSs, 36, 37, 39, 41
- quantifyCTSSs, BigWigFileList, BigWigFileList-method (quantifyCTSSs), 37
- quantifyCTSSs, character, character-method (quantifyCTSSs), 37
- quantifyCTSSs2, 36, 38, 39, 41
- quantifyGenes, 36, 38, 39, 40
- quickEnhancers, 41, 42, 43
- quickGenes, 42, 42, 43
- quickTSSs, 42, 43
- shapeEntropy, 18, 43, 44, 45
- shapeIQR, 18, 44, 44, 45
- shapeMean, 18, 44, 45
- shapeMultimodality, 46
- subsetByBidirectionality, 15–18, 20, 21, 46, 48, 49
- subsetByBidirectionality, GPos-method (subsetByBidirectionality), 46
- subsetByBidirectionality, GRanges-method (subsetByBidirectionality), 46
- subsetByBidirectionality, RangedSummarizedExperiment-method (subsetByBidirectionality), 46
- subsetByComposition, 15–18, 20, 21, 47, 47, 49
- subsetBySupport, 15–18, 20, 21, 47, 48, 49
- swapRanges, 50, 51
- swapRanges, GenomicRanges-method (swapRanges), 50
- swapRanges, RangedSummarizedExperiment-method (swapRanges), 50
- swapScores, 50, 51
- trackBalance, 51, 54–56
- trackBalance, GPos-method (trackBalance), 51
- trackBalance, GRanges-method (trackBalance), 51
- trackBalance, RangedSummarizedExperiment-method (trackBalance), 51
- trackClusters, 52, 53, 55, 56
- trackClusters, GRanges-method (trackClusters), 53
- trackClusters, RangedSummarizedExperiment-method (trackClusters), 53
- trackCTSS, 52, 54, 54, 56
- trackCTSS, GPos-method (trackCTSS), 54
- trackCTSS, GRanges-method (trackCTSS), 54
- trackCTSS, RangedSummarizedExperiment-method (trackCTSS), 54
- trackLinks, 34, 35, 52, 54, 55, 55
- trimToPeak, 25, 26, 56, 59, 60
- trimToPeak, GRanges, GPos-method (trimToPeak), 56
- trimToPeak, GRanges, GRanges-method (trimToPeak), 56
- trimToPeak, GRanges, RangedSummarizedExperiment-method (trimToPeak), 56
- trimToPeak, RangedSummarizedExperiment, GenomicRanges-method (trimToPeak), 56
- trimToPeak, RangedSummarizedExperiment, RangedSummarizedExperiment-method (trimToPeak), 56
- trimToPercentiles, 25, 26, 57, 58, 60
- trimToPercentiles, GRanges, GPos-method (trimToPercentiles), 58
- trimToPercentiles, GRanges, GRanges-method (trimToPercentiles), 58
- trimToPercentiles, GRanges, RangedSummarizedExperiment-method (trimToPercentiles), 58
- trimToPercentiles, RangedSummarizedExperiment, GenomicRanges-method (trimToPercentiles), 58
- trimToPercentiles, RangedSummarizedExperiment, RangedSummarizedExperiment-method (trimToPercentiles), 58
- tuneTagClustering, 25, 26, 57, 59, 59

tuneTagClustering,GPos-method
 (tuneTagClustering), [59](#)
tuneTagClustering,GRanges-method
 (tuneTagClustering), [59](#)
tuneTagClustering,RangedSummarizedExperiment-method
 (tuneTagClustering), [59](#)

utilsAggregateRows, [61](#), [63](#), [64](#)
utilsAggregateRows,dgCMatrix-method
 (utilsAggregateRows), [61](#)
utilsAggregateRows,matrix-method
 (utilsAggregateRows), [61](#)
utilsDeStrand, [61](#), [62](#), [63](#), [64](#)
utilsScoreOverlaps, [61](#), [63](#), [63](#), [64](#)
utilsSimplifyTxDb, [61](#), [63](#), [64](#)