Package ‘wavClusteR’

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Type Package

Title Sensitive and highly resolved identification of RNA-protein interaction sites in PAR-CLIP data

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Suggests BiocStyle, knitr, rmarkdown, BSgenome.Hsapiens.UCSC.hg19

Enhances doMC

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Author Federico Comoglio and Cem Sievers

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Description The package provides an integrated pipeline for the analysis of PAR-CLIP data. PAR-CLIP-induced transitions are first discriminated from sequencing errors, SNPs and additional non-experimental sources by a non-parametric mixture model. The protein binding sites (clusters) are then resolved at high resolution and cluster statistics are estimated using a rigorous Bayesian framework. Post-processing of the results, data export for UCSC genome browser visualization and motif search analysis are provided. In addition, the package allows to integrate RNA-Seq data to estimate the False Discovery Rate of cluster detection. Key functions support parallel multicore computing. Note: while wavClusteR was designed for PAR-CLIP data analysis, it can be applied to the analysis of other NGS data obtained from experimental procedures that induce nucleotide substitutions (e.g. BisSeq).

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wavClusteR-package

A comprehensive pipeline for the analysis of PAR-CLIP data. PAR-CLIP-induced transitions are first discriminated from sequencing errors, SNPs and additional non-experimental sources by a non-parametric mixture model. The protein binding sites (clusters) are then resolved at high resolution and cluster statistics are estimated using a rigorous Bayesian framework. Post-processing of the results, data export for UCSC genome browser visualization and motif search analysis are provided. In addition, the package allows to integrate RNA-Seq data to estimate the False Discovery Rate of cluster detection. Key functions support parallel multicore computing. Note: while wavClusteR was designed for PAR-CLIP data analysis, it can be applied to the analysis of other NGS data obtained from experimental procedures that induce nucleotide substitutions (e.g. BisSeq).

Description

Package: wavClusteR
Type: Package
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Author(s)

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References


annotateClusters  
Annotate clusters with respect to transcript features

Description

Carries out strand-specific annotation of clusters with respect to distinct transcript features, particularly introns, coding sequences, 3'-UTRs, 5'-UTRs. Mapping to multiple features and to those outside the above mentioned ones are reported. Unmapped clusters are then further further analyzed and annotated with respect to features localizing on the anti-sense strand. Results can be plotted as dotchart and annotations are returned as clusters metadata.

Usage

annotateClusters(clusters, txDB = NULL, genome = "hg19", tablename = "ensGene", plot = TRUE, verbose = TRUE)

Arguments

clusters  GRanges object containing individual clusters as identified by the getClusters function

(txDB  TranscriptDb object obtained through a call to the makeTxDbFromUCSC function in the GenomicFeatures package. Default is NULL, namely the object will be fetched internally

genome  A character specifying the genome abbreviation used by UCSC. Available abbreviations are returned by a call to ucscGenomes()[,"db"]. Default is "hg19" (human genome)

tablename  A character specifying the name of the UCSC table containing the transcript annotations to retrieve. Available table names are returned by a call to supportedUCSCtables(). Default is "ensGene", namely ensembl gene annotations

plot  Logical, if TRUE a dotchart with cluster annotations is produced

verbose  Logical, if TRUE processing steps are printed

Value

Same as the input GRanges object, with an additional metadata column containing the following character encoding of the genomic feature each cluster maps to:

"CDS ss"  Coding Sequence Sense Strand

"Introns ss"  Intron Sense Strand

"3' UTR ss"  3' UTR Sense Strand

"5' UTR ss"  5' UTR Sense Strand

"Multiple"  More than one of the above

"CDS as"  Coding Sequence Antisense Strand

"Introns as"  Intron Antisense Strand
annotateClusters

"3' UTR as"  3' UTR Antisense Strand
"5' UTR as"  5' UTR Antisense Strand
"Other"     None of the above

If plot=TRUE, a dotchart is produced in addition.

Author(s)

Federico Comoglio

References

M. Carlson and H. Pages and P. Aboyoun and S. Falcon and M. Morgan and D. Sarkar and M. Lawrence, GenomicFeatures: Tools for making and manipulating transcript centric annotations, R package version 1.12.4

See Also

getClusters

Examples

require(BSgenome.Hsapiens.UCSC.hg19)
data( model, package = "wavClusteR" )
filename <- system.file( "extdata", "example.bam", package = "wavClusteR" )
exmaple <- readSortedBam( filename = filename )
countTable <- getAllSub( example, minCov = 10, cores = 1 )
highConfSub <- getHighConfSub( countTable, supportStart = 0.2, supportEnd = 0.7, substitution = "TC" )
coverage <- coverage( example )
clusters <- getClusters( highConfSub = highConfSub, coverage = coverage, sortedBam = example, cores = 1, threshold = 2 )
fclusters <- filterClusters( clusters = clusters, highConfSub = highConfSub, coverage = coverage, model = model, genome = Hsapiens, refBase = 'T', minWidth = 12 )
## Not run: fclusters <- annotateClusters( clusters = fclusters )
estimateFDR

**Description**

Estimate upper and lower bounds for the False Discovery Rate within the relative substitution frequency (RSF) support by integrating PAR-CLIP data and RNA-Seq data (current version makes use of unstranded RNA-Seq)

**Usage**

```r
estimateFDR(countTable, RNASeq, substitution = 'TC', minCov = 20, span = 0.1, cores = 1, plot = TRUE, verbose = TRUE, ...)
```

**Arguments**

- `countTable`: A GRanges object, corresponding to a count table as returned by the `getAllSub` function
- `RNASeq`: GRanges object containing aligned RNA-Seq reads as returned by `readSortedBam`
- `substitution`: A character indicating which substitution is induced by the experimental procedure (e.g. 4-SU treatment - a standard in PAR-CLIP experiments - induces T to C transitions and hence substitution = 'TC' in this case.)
- `minCov`: An integer defining the minimum coverage required at a genomic position exhibiting a substitution. Genomic positions of coverage less than `minCov` are discarded. Default is 20 (see Details).
- `span`: A numeric indicating the width of RSF intervals to be considered for FDR computation. Defaults is 0.1 (i.e. 10 intervals are considered spanning the RSF support (0,1])
- `cores`: An integer defining the number of cores to be used for parallel processing, if available. Default is 1.
- `plot`: Logical, if TRUE a dotchart with cluster annotations is produced
- `verbose`: Logical, if TRUE processing steps are printed
- `...`: Additional parameters to be passed to the `plot` function

**Details**

For details on the FDR computation, please see Comoglio, Sievers and Paro.

**Value**

A list with three slots, containing upper and lower FDR bounds, and the total number of positive instances each RSF interval. If `plot`, these three vectors are depicted as a line plot.
**exportClusters**

**Note**

The approach used to compute the upper bound for the FDR is very conservative. See supplementary information in Comoglio et al. for details.

**Author(s)**

Federico Comoglio and Cem Sievers

**See Also**

readSortedBam, getAllSub


---

**Description**

Export clusters as BED track, compatible with the UCSC genome browser

**Usage**

```r
exportClusters(clusters, filename = 'wavClusters.bed', trackname = 'wavClusters', description = 'wavClusters')
```

**Arguments**

- `clusters`  
  GRanges object containing individual clusters as identified by the filterClusters function
- `filename`  
  A character defining the BED file name. Default to "wavClusters.bed"
- `trackname`  
  A character defining the track.name of the BED file. Default to "wavClusters"
- `description`  
  A character defining the description of the BED file. Default to "wavClusters"

**Value**

A BED file of the exported GRanges object

**Note**

Clusters are color coded according to their strand information (red for the plus strand, blue for the minus strand).

**Author(s)**

Federico Comoglio
exportCoverage

Export coverage as BigWig track

Description
Export coverage as BigWig track, compatible with the UCSC genome browser

Usage
exportCoverage(coverage, filename = 'wavClusters.BigWig')

Arguments
- coverage: An Rle object containing the coverage at each genomic position as returned by a call to coverage
- filename: A character defining the BED file name. Default to "wavClusters.BigWig"

Value
A BigWig file of the exported Rle object

Author(s)
Federico Comoglio

exportHighConfSub

Export high-confidence substitutions as BED track

Description
Export high-confidence substitutions as BED track, compatible with the UCSC genome browser

Usage
exportHighConfSub(highConfSub, filename = 'highConfSub.bed', trackname = 'highConfSub', description = 'highConfSub')

Arguments
- highConfSub: GRanges object containing high-confidence substitution sites as returned by the getHighConfSub function
- filename: A character defining the BED file name. Default to "wavClusters.bed"
- trackname: A character defining the track.name of the BED file. Default to "wavClusters"
- description: A character defining the description of the BED file. Default to "wavClusters"
exportSequences

Value
A BED file of the exported GRanges object

Note
Substitutions are color coded according to their strand information (red for the plus strand, blue for the minus strand).

Author(s)
Federico Comoglio

See Also
getHighConfSub

exportSequences
Export cluster sequences for motif search analysis

Description
Export cluster sequences for motif search analysis (FASTA format), e.g. using MEME-ChIP

Usage
exportSequences(clusters, filename = 'wavClusters.fasta')

Arguments
clusters GRanges object containing individual clusters as identified by the filterClusters function
filename A character defining the BED file name. Default to "wavClusters.fasta"

Value
A FASTA file containing the cluster sequences

Author(s)
Federico Comoglio

See Also
filterClusters
filterClusters

Merge clusters and compute all relevant cluster statistics

Description

If clusters have been identified using the mini-rank norm algorithm, cluster statistics are computed. In contrast, if the CWT-based cluster identification algorithm was used, clusters are first filtered to retain only those instances containing a wavelet peak and a high-confidence substitution site within their cluster boundaries.

Usage

filterClusters(clusters, highConfSub, coverage, model, genome, refBase = 'T', minWidth = 12, verbose = TRUE)

Arguments

clusters  GRanges object containing individual clusters as identified by the getClusters function
highConfSub  GRanges object containing high-confidence substitution sites as returned by the getHighConfSub function
coverage  An Rle object containing the coverage at each genomic position as returned by a call to coverage
model  List of 5 items containing the estimated mixture model as returned by the fitMixtureModel function
genome  BSgenome object of the relevant reference genome (e.g. Hsapiens for the human genome hg19)
refBase  A character specifying the base in the reference genome for which transitions are experimentally induced (e.g. 4-SU treatment - a standard in PAR-CLIP experiments - induces T to C transitions and hence refBase = "T" in this case). Default is "T"
minWidth  An integer corresponding to the minimum width of reported clusters. Shorter clusters are extended to minWidth starting from the cluster center
verbose  Logical, if TRUE processing steps are printed

Value

GRanges object containing the transcriptome-wide identified clusters, having metadata:

Ntransitions  The number of high-confidence transitions within the cluster
MeanCov  The mean coverage within the cluster
NbasesInRef  The number of genomic positions within the cluster corresponding to refBase
CrossLinkEff  The crosslinking efficiency within the cluster, estimated as the ratio between the number of high-confidence transitions within the cluster and the total number of genomic positions therein corresponding to refBase
Sequence: The genomic sequence underlying the cluster (plus strand)

SumLogOdds: The sum of the log-odd values within the cluster

RelLogOdds: The sum of the log-odds divided by the number of high-confidence transitions within the cluster. This variable can be regarded as a proxy for statistical significance and can be therefore used to rank clusters. See Comoglio, Sievers and Paro for details.

Note

1) This function calls the appropriate processing function according to the method used to compute clusters. This information is stored in the metadata(ranges(clusters)) slot as an object of type list.

2) Notice that genome corresponds to the according reference genome matching the organism in which experiments have been carried out. For example genome = Hsapiens is used for the human reference genome (assembly 19), where Hsapiens is provided by BSgenome.Hsapiens.UCSC.hg19.

Author(s)

Federico Comoglio and Cem Sievers

References

Herve Pages, BSgenome: Infrastructure for Biostrings-based genome data packages


See Also

getClusters, getHighConfSub, fitMixtureModel

Examples

require(BSgenome.Hsapiens.UCSC.hg19)

data( model, package = "wavClusteR"

filename <- system.file( "extdata", "example.bam", package = "wavClusteR"
example <- readSortedBam( filename = filename)
countTable <- getAllSub( example, minCov = 10, cores = 1)
highConfSub <- getHighConfSub( countTable, supportStart = 0.2, supportEnd = 0.7, substitution = "TC"
coverage <- coverage( example)
clusters <- getClusters( highConfSub = highConfSub,
coverage = coverage,
thenBam = example,cores = 1,
threshold = 2 )
fclusters <- filterClusters( clusters = clusters,  
  highConfSub = highConfSub,  
  coverage = coverage,  
  model = model,  
  genome = Hsapiens,  
  refBase = 'T',  
  minWidth = 12 )
fclusters

**fitMixtureModel**  
*Fit a non-parametric mixture model from all identified substitutions*

**Description**  
Estimates the two-component mixture model consisting of the mixing coefficients and the density functions.

**Usage**  

```r
fitMixtureModel(countTable, substitution = "TC")
```

**Arguments**

- **countTable**: A GRanges object, corresponding to a count table as returned by the `getAllSub` function
- **substitution**: A character indicating which substitution is induced by the experimental procedure (e.g. 4-SU treatment - a standard in PAR-CLIP experiments - induces T to C transitions and hence substitution = 'TC' in this case.)

**Value**

A list containing:

- **l1**: The first mixing coefficient
- **l2**: The second mixing coefficient
- **p**: The mixture model
- **p1**: The first component of the mixture
- **p2**: The second component of the mixture

**Author(s)**

Federico Comoglio and Cem Sievers

**See Also**

`getAllSub` `getExpInterval`
getAllSub

Examples

```r
## Not run:
filename <- system.file( "extdata", "example.bam", package = "wavClusteR" )
example <- readSortedBam(filename = filename)
countTable <- getAllSub( example, minCov = 10, cores = 1 )

fitMixtureModel( countTable, substitution = "TC" )

## End(Not run)

#load and inspect the model
data( model )
str( model )

#plot densities and estimate the relative substitution frequency support
dominated by PAR-CLIP induction
getExpInterval( model, bayes = TRUE, plot = TRUE )
```

---

### getAllSub

**Identify all substitutions observed across genomic positions exhibiting a specified minimum coverage**

**Description**

All substitutions observed across genomic positions exhibiting user-defined minimum coverage are extracted and a count table is returned. This function supports parallel computing.

**Usage**

```r
getAllSub(sortedBam, minCov = 20, cores = 1)
```

**Arguments**

- `sortedBam`: GRanges object containing aligned reads as returned by `readSortedBam`
- `minCov`: An integer defining the minimum coverage required at a genomic position exhibiting a substitution. Genomic positions of coverage less than `minCov` are discarded. Default is 20 (see Details).
- `cores`: An integer defining the number of cores to be used for parallel processing, if available. Default is 1.

**Details**

The choice of the minimum coverage influences the variance of the relative substitution frequency estimates, which in turn affect the mixture model fit. A conservative value depending on the library size is recommended for a first analysis. Values smaller than 10 have not been tested and are therefore not recommended.
getClusters

Value

A GRanges object containing a count table, where each range correspond to a substitution. The metadata correspond to the following information:

- substitutions: observed substitution, e.g. AT, i.e. A in the reference sequence and T in the mapped read.
- coverage: strand-specific coverage.
- count: number of strand-specific substitutions.

Author(s)

Federico Comoglio and Cem Sievers, with contributions from Martin Morgan

See Also

readSortedBam

Examples

```r
filename <- system.file( "extdata", "example.bam", package = "wavClusteR" )
example <- readSortedBam(filename = filename)
countTable <- getAllSub( example, minCov = 10, cores = 1 )
countTable
```

---

**getClusters**

Identify clusters containing high-confidence substitutions and resolve boundaries at high resolution

Description

Identifies clusters using the mini-rank norm (MRN) algorithm, which employs thresholding of background coverage differences and finds the optimal cluster boundaries by exhaustively evaluating all putative clusters using a rank-based approach. This method has higher sensitivity and an approximately 10-fold faster running time than the CWT-based cluster identification algorithm.

Usage

```r
getClusters(highConfSub, coverage, sortedBam, cores = 1, threshold)
```
getClusters

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>highConfSub</td>
<td>GRanges object containing high-confidence substitution sites as returned by the <code>getHighConfSub</code> function</td>
</tr>
<tr>
<td>coverage</td>
<td>An Rle object containing the coverage at each genomic position as returned by a call to <code>coverage</code></td>
</tr>
<tr>
<td>sortedBam</td>
<td>a GRanges object containing all aligned reads, including read sequence (qseq) and MD tag (MD), as returned by the <code>readSortedBam</code> function</td>
</tr>
<tr>
<td>cores</td>
<td>integer, the number of cores to be used for parallel evaluation. Default is 1.</td>
</tr>
<tr>
<td>threshold</td>
<td>numeric, the difference in coverage to be considered noise. If not specified, a Gaussian mixture model is used to learn a threshold from the data. Empirically, 10% of the minimum coverage required at substitutions (see argument minCov in the <code>getHighConfSub</code> function) might suffice to provide highly resolved clusters. However, if minCov is much lower than the median strand-specific coverage at substitutions $m$, which can be computed using <code>summary(elementMetadata(highConfSub)[, 'coverage'][, 'Median'])</code>, 10% of $m$ might represent an optimal choice.</td>
</tr>
</tbody>
</table>

Value

GRanges object containing the identified cluster boundaries.

Note

Clusters returned by this function need to be further merged by the function `filterClusters`, which also computes all relevant cluster statistics.

Author(s)

Federico Comoglio and Cem Sievers

References


See Also

`getHighConfSub`, `filterClusters`

Examples

```r
filename <- system.file( "extdata", "example.bam", package = "wavClusteR" )
example <- readSortedBam( filename = filename )
countTable <- getAllSub( example, minCov = 10, cores = 1 )
highConfSub <- getHighConfSub( countTable, supportStart = 0.2, supportEnd = 0.7, substitution = "TC" )
coverage <- coverage( example )
```
getExpInterval <- getClusters( highConfSub = highConfSub, 
coverage = coverage, 
sortedBam = example, 
cores = 1, 
threshold = 2 )

getExpInterval

Identify the interval of relative substitution frequencies dominated by
experimental induction.

Description

Identifies the interval/support of relative substitution frequencies (RSFs) dominated by the second
model component, i.e. by the probability of being induced by the experimental procedure. In
addition, this function can be used to generate diagnostic plots of the model fit, representing (i)
model densities and log odds ratio (ii) the posterior class probability, i.e. the probability of a given
observation being generated by experimental induction.

Usage

getExpInterval(model, bayes = TRUE, leftProb, rightProb, plot = TRUE)

Arguments

model A list containing the model as returned by the function fitMixtureModel
bayes Logical, if TRUE the Bayes classifier (cutoff at posterior class probabilities >=
0.5) is applied. If FALSE, custom cutoff values should be provided through
leftProb and rightProb. Default is TRUE.
leftProb Numeric, the posterior probability corresponding to the left boundary (start) of
the high confidence RSF interval.
rightProb Numeric, the posterior probability corresponding to the right boundary (end) of
the high confidence RSF interval.
plot Logical, if TRUE diagnostics plot showing the model components, log odds and
the computed posterior with highlighted identified RSF interval are returned.

Value

A list with two numeric slots, corresponding to the extremes of the RSF interval (RSF support).
supportStart start of the high confidence RSF interval
supportEnd end of the high confidence RSF interval

Author(s)

Federico Comoglio and Cem Sievers
getHighConfSub  

References


See Also

fitMixtureModel getHighConfSub estimateFDR

Examples

data( model )

#default
support <- getExpInterval( model = model, bayes = TRUE, plot = TRUE )
support

#custom interval (based, e.g. on visual inspection of posterior class probability # or evaluation of FDR using the estimateFDRF function)
support <- getExpInterval( model = model, leftProb = 0.2, rightProb = 0.7, plot = TRUE )
support

getHighConfSub

Classify substitutions based on identified RSF interval and return high confidence transitions

Description

Classify genomic positions exhibiting a substitution based on the relative substitution frequency (RSF) interval. The latter is returned by the getExpInterval function, but can be user-specified through visual inspection of the posterior class probability returned by the same function.

Usage

getHighConfSub(countTable, support, supportStart = NA, supportEnd = NA, substitution = "TC")

Arguments

countTable A GRanges object, corresponding to a count table as returned by the getAllSub function

support List, consisting of two numeric slots defining the left and right boundaries (start and end values, respectively) of the RSF interval, as returned by the getExpInterval function.
supportStart Numeric, if support not provided, the RSF value determining the left boundary (start) of the RSF interval. Use this argument to specify a user-defined RSF interval.

supportEnd Numeric, if support not provided, the RSF value determining the right boundary (end) of the RSF interval. Use this argument to specify a user-defined RSF interval.

substitution A character indicating which substitution is induced by the experimental procedure (e.g. 4-SU treatment - a standard in PAR-CLIP experiments - induces T to C transitions and hence substitution = 'TC' in this case.)

Value

a GRanges object containing high confidence substitutions, with strand-specific coverage, counts and RSF values as metadata.

Note

In the example below, left and right boundaries were arbitrarily chosen as showcase.

Author(s)

Federico Comoglio and Cem Sievers

See Also

getAllSub, getExpInterval

Examples

filename <- system.file( "extdata", "example.bam", package = "wavClusteR" )
example <- readSortedBam( filename = filename )
countTable <- getAllSub( example, minCov = 10, cores = 1 )
highConfSub <- getHighConfSub( countTable, supportStart = 0.2, supportEnd = 0.7, substitution = "TC" )
highConfSub

getMetaCoverage

Compute and plot distribution of average coverage or relative log-odds as metagene profile using identified clusters

Description

Transcriptome-wide identified clusters are used to generate a metagene profile by binning gene bodies. Within each bin, the distribution of the average cluster coverage or of the relative log-odds is computed.
getMetaCoverage

Usage

getMetaCoverage(clusters, txDB = NULL, upstream = 1e3, downstream = 1e3, nBins = 40, nBinsUD = 10, minLength = 1, genome = 'hg19', tablename = 'ensGene', odds = FALSE, plot = TRUE, verbose = TRUE, ...)

Arguments

clusters GRanges object containing individual clusters as identified by the getClusters function
txDB TranscriptDb object obtained through a call to the makeTxDbFromUCSC function in the GenomicFeatures package. Default is NULL, namely the object will be fetched internally
upstream An integer corresponding to the number of bases to be considered upstream the gene. Default is 1000
downstream An integer corresponding to the number of bases to be considered downstream the gene. Default is 1000
nBins An integer corresponding to the number of bins to be used to partition the genes. Default is 40
nBinsUD An integer corresponding to the number of bins to be used to partition upstream and downstream regions. Defaults is 10, i.e. the bin size is 100 bases for the default extension
minLength An integer indicating the the minimum required length of a gene in order for it to be considered. Default is 1, i.e. all genes are considered
genome A character specifying the genome abbreviation used by UCSC. Available abbreviations are returned by a call to ucscGenomes()[, "db"]. Default is "hg19" (human genome)
tablename A character specifying the name of the UCSC table containing the transcript annotations to retrieve. Available table names are returned by a call to supportedUCSCtables(). Default is "ensGene", namely ensembl gene annotations
odds Logical, if TRUE relative log-odds distributions are shown instead of mean coverage
plot Logical, if TRUE a dotchart with cluster annotations is produced
verbose Logical, if TRUE processing steps are printed
... Additional parameters to be passed to the plot function

Value

Called for its effects.

Author(s)

Federico Comoglio
References

Comoglio F*, Sievers C* and Paro R, wavClusteR: an R package for PAR-CLIP data analysis, submitted

See Also

getClusters

Examples

```r
require(BSgenome.Hsapiens.UCSC.hg19)
data( model, package = "wavClusteR" )

filename <- system.file( "extdata", "example.bam", package = "wavClusteR" )
example <- readSortedBam( filename = filename )
countTable <- getAllSub( example, minCov = 10, cores = 1 )
highConfSub <- getHighConfSub( countTable, supportStart = 0.2, supportEnd = 0.7, substitution = "TC" )
coverage <- coverage( example )
clusters <- getClusters( highConfSub = highConfSub,
coverage = coverage,
sortedBam = example,
threshold = 2 )
fclusters <- filterClusters( clusters = clusters,
highConfSub = highConfSub,
coverage = coverage,
model = model,
genome = Hsapiens,
refBase = 'T',
minWidth = 12 )
## Not run: getMetaCoverage( clusters = fclusters, odds = FALSE )
```

---

getMetaGene  

**Compute and plot metagene profile using identified clusters**

Description

Transcriptome-wide identified clusters are used to generate a metagene profile by binning gene bodies, upstream and downstream regions.

Usage

```r
getMetaGene(clusters, txDB = NULL, upstream = 1e3, downstream = 1e3, nBins = 40, nBinsUD = 10, minLength = 1, genome = 'hg19', tablename = 'ensGene', plot = TRUE, verbose = TRUE, ...)
```
getMetaGene

Arguments

clusters
   GRanges object containing individual clusters as identified by the getClusters function

txDB
   TranscriptDb object obtained through a call to the makeTxDbFromUCSC function in the GenomicFeatures package. Default is NULL, namely the object will be fetched internally

upstream
   An integer corresponding to the number of bases to be considered upstream the gene. Default is 1000

downstream
   An integer corresponding to the number of bases to be considered downstream the gene. Default is 1000

nBins
   An integer corresponding to the number of bins to be used to partition the genes. Default is 40

nBinsUD
   An integer corresponding to the number of bins to be used to partition upstream and downstream regions. Defaults is 10, i.e. the bin size is 100 bases for the default extension

minLength
   An integer indicating the the minimum required length of a gene in order for it to be considered. Default is 1, i.e. all genes are considered

genome
   A character specifying the genome abbreviation used by UCSC. Available abbreviations are returned by a call to ucscGenomes()[, "db"]. Default is "hg19" (human genome)

tablename
   A character specifying the name of the UCSC table containing the transcript annotations to retrieve. Available table names are returned by a call to supportedUCSCtables(). Default is "ensGene", namely ensembl gene annotations

plot
   Logical, if TRUE a dotchart with cluster annotations is produced

verbose
   Logical, if TRUE processing steps are printed

... Additional parameters to be passed to the plot function

Value

A numeric vector of the same length as nBins + 2 * nBinsUD containing normalized counts. If plot, the metagene profile is also depicted as a line plot.

Author(s)

Federico Comoglio


References

Comoglio F*, Sievers C* and Paro R, wavClusteR: an R package for PAR-CLIP data analysis, submitted

See Also

getClusters
getMetaTSS

Examples

```
require(BSgenome.Hsapiens.UCSC.hg19)

data( model, package = "wavClusteR" )

filename <- system.file( "extdata", "example.bam", package = "wavClusteR" )
example <- readSortedBam( filename = filename )
countTable <- getAllSub( example, minCov = 10, cores = 1 )
highConfSub <- getHighConfSub( countTable, supportStart = 0.2, supportEnd = 0.7, substitution = "TC" )
coverage <- coverage( example )
clusters <- getClusters( highConfSub = highConfSub, coverage = coverage, sortedBam = example, threshold = 2 )
fclusters <- filterClusters( clusters = clusters, highConfSub = highConfSub, coverage = coverage, sortedBam = example, threshold = 2 )
```

```
## Not run: meta <- getMetaGene( clusters = fclusters )
```

getMetaTSS

Compute and plot read densities in genomic regions around transcription start sites

Description

Aligned reads are used generate a metaTSS profile across genomic regions containing transcription start sites (TSSs).

Usage

```
getMetaTSS(sortedBam, txDB = NULL, upstream = 1e3, downstream = 1e3, nBins = 40, genome = 'hg19', tablename = 'ensGene', unique = FALSE, plot = TRUE, verbose = TRUE, ...)
```

Arguments

- **sortedBam**: GRanges object containing aligned reads as returned by `readSortedBam`
- **txDB**: TranscriptDb object obtained through a call to the `makeTxDbFromUCSC` function in the GenomicFeatures package. Default is NULL, namely the object will be fetched internally
- **upstream**: An integer corresponding to the number of bases to be considered upstream the TSS. Default is 1000
getMetaTSS

downstream An integer corresponding to the number of bases to be considered downstream
the TSS. Default is 1000

nBins An integer corresponding to the number of bins to be used to partition the genes.
Default is 40, i.e. bin size 50 bases

genome A character specifying the genome abbreviation used by UCSC. Available abbreviations
are returned by a call to \texttt{ucscGenomes(\_\_\_\_\_\_\_\_\[ 1, "db"\)}. Default is "hg19"
(human genome)

tablename A character specifying the name of the UCSC table containing the transcript annotations

to retrieve. Available table names are returned by a call to \texttt{supportedUCSCtables()}. Default is "ensGene",
namely ensembl gene annotations

unique Logical, if TRUE only non-overlapping TSSs extended by upstream/downstream
are considered. Default is FALSE, i.e. all TSSs are considered

plot Logical, if TRUE a dotchart with cluster annotations is produced

verbose Logical, if TRUE processing steps are printed

... Additional parameters to be passed to the \texttt{plot} function

Value

A numeric vector of the same length as \texttt{nBins} containing normalized counts. If \texttt{plot}, the metaTSS
profile is also depicted as a line plot.

Author(s)

Federico Comoglio

References

Comoglio F*, Sievers C* and Paro R, wavClusteR: an R package for PAR-CLIP data analysis, submitted

See Also

\texttt{readSortedBam}

Examples

require(BSgenome.Hsapiens.UCSC.hg19)

filename <- system.file("extdata", "example.bam", package = "wavClusteR")
example <- readSortedBam( filename = filename )
## Not run: tss <- getMetaTSS( sortedBam = example )
Components of the non-parametric mixture model fitted on Ago2 PAR-CLIP data

Description
The non-parametric mixture model was fit on the entire Ago2 public available PAR-CLIP dataset (Kishore et al.) using the fitMixtureModel function.

Usage
data(model)
model

Format
List of 5 items containing the estimated mixing coefficients and model densities. See the help page of the fitMixtureModel function for a detailed description of the output.

References

See Also
fitMixtureModel

plotSizeDistribution  Plot the distribution of cluster sizes

Description
Produce an histogram of cluster sizes

Usage
plotSizeDistribution( clusters, showCov = FALSE, ... )

Arguments
clusters  GRanges object containing individual clusters as identified by the getClusters function
showCov  logical, if TRUE a scatter plot of average cluster coverage vs. cluster size is shown along with a loess fit. Default is FALSE.
...  Additional parameters to be passed to the hist function
plotStatistics

Value

Called for its effect, returns a histogram.

Author(s)

Federico Comoglio

See Also

getClusters

Examples

```r
require(BSgenome.Hsapiens.UCSC.hg19)

data( model, package = "wavClusteR" )

filename <- system.file( "extdata", "example.bam", package = "wavClusteR" )
example <- readSortedBam( filename = filename )
countTable <- getAllSub( example, minCov = 10, cores = 1 )
highConfSub <- getHighConfSub( countTable, supportStart = 0.2, supportEnd = 0.7, substitution = "TC" )
coverage <- coverage( example )
clusters <- getClusters( highConfSub = highConfSub,
                         coverage = coverage,
                         sortedBam = example,
                         threshold = 2 )

fclusters <- filterClusters( clusters = clusters,
                             highConfSub = highConfSub,
                             coverage = coverage,
                             model = model,
                             genome = Hsapiens,
                             refBase = 'T',
                             minWidth = 12 )

plotSizeDistribution(fclusters, breaks = 30, col = 'skyblue2')
```

plotStatistics

Pairs plot visualization of clusters statistics

Description

Graphical representation of cluster statistics, featuring pairwise correlations in the upper panel.

Usage

plotStatistics(clusters, corMethod = 'spearman', lower = panel.smooth, ...)
Arguments

clusters  GRanges object containing individual clusters as identified by the `getClusters` function

corMethod A character defining the correlation coefficient to be computed. See the help page of the `cor` function for possible options. Default is "spearman". Hence, rank-based Spearman's correlation coefficients are computed

lower A function compatible with the `lower` panel argument of the `pairs` function

... Additional parameters to be passed to the `pairs` function

Value
called for its effect

Author(s)
Federico Comoglio

See Also
getClusters

Examples

```r
require(BSgenome.Hsapiens.UCSC.hg19)
data( model, package = "wavClusteR" )

filename <- system.file( "extdata", "example.bam", package = "wavClusteR" )
example <- readSortedBam( filename = filename )
countTable <- getAllSub( example, minCov = 10, cores = 1 )
highConfSub <- getHighConfSub( countTable, supportStart = 0.2, supportEnd = 0.7, substitution = "TC" )
coverage <- coverage( example )
clusters <- getClusters( highConfSub = highConfSub, 
coverage = coverage, 
sortedBam = example, 
threshold = 2 )

fclusters <- filterClusters( clusters = clusters, 
highConfSub = highConfSub, 
coverage = coverage, 
model = model, 
genome = Hsapiens, 
refBase = 'T', 
minWidth = 12 )
plotStatistics( clusters = fclusters )
```
plotSubstitutions

Barplot visualization of the number of genomic positions exhibiting a given substitution and, if model provided, additional diagnostic plots.

Description

Graphical representation of the total number of genomic positions exhibiting one or more substitutions of a given type. This information is used to estimate the mixing coefficients of the non-parametric mixture model. If the mixture model fit is provided, returns additional diagnostic plots such as the total number of reads exhibiting a given substitution and relative substitution frequency-dependent representations of the total number of genomic positions with substitutions of a given type.

Usage

plotSubstitutions(countTable, highlight = "TC", model)

Arguments

countTable: A GRanges object, corresponding to a count table as returned by the getAllSub function

highlight: A character indicating which substitution should be highlighted in the barplot. A standard PAR-CLIP experiment employing 4-SU treatment induces T to C transitions, encoded as "TC". Default is "TC".

model: A list containing the model as returned by the function fitMixtureModel

Value
called for its effect

Author(s)
Federico Comoglio and Cem Sievers

See Also
getAllSub

Examples

filename <- system.file( "extdata", "example.bam", package = "wavClusteR" )
example <- readSortedBam( filename = filename )
countTable <- getAllSub( example, minCov = 10, cores = 1 )
plotSubstitutions(countTable = countTable, highlight = "TC")
readSortedBam

Load a sorted BAM file

Description
Load a sorted BAM file. Optionally, only reads mapping to a specific set of genomics coordinates are loaded. Only fields strictly necessary to run a wavClusteR analysis are loaded.

Usage
readSortedBam(filename, which)

Arguments
filename Name of the sorted BAM file, including full path to file if it is located outside the current working directory.
which a GRanges or IntegerRangesList specifying the regions on the reference sequence for which matches are desired. See the documentation of the Rsamtools package for details.

Value
a GRanges object containing aligned reads, including read sequence (qseq) and MD tag (MD)

Note
The input BAM file must be sorted and indexed. Alignment with bowtie or bowtie2, conversion from SAM to BAM output, sorting and indexing using SAMtools is recommended.

Author(s)
Federico Comoglio

References

Examples
library(Rsamtools)
filename <- system.file("extdata", "example.bam", package = "wavClusteR")
sortedBam <- readSortedBam( filename = filename )
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