

# 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

**VignetteBuilder** knitr

**Author** Federico Comoglio and Cem Sievers

**Maintainer** Federico Comoglio <federico.comoglio@gmail.com>

**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).

**License** GPL-2

**biocViews** Sequencing, Technology, RIPSeq, RNASeq, Bayesian

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wavClusteR-package . . . . .	2
annotateClusters . . . . .	3
estimateFDR . . . . .	5
exportClusters . . . . .	6
exportCoverage . . . . .	7
exportHighConfSub . . . . .	7
exportSequences . . . . .	8
filterClusters . . . . .	9
fitMixtureModel . . . . .	11
getAllSub . . . . .	12
getClusters . . . . .	13
getExpInterval . . . . .	15
getHighConfSub . . . . .	16
getMetaCoverage . . . . .	18
getMetaGene . . . . .	19
getMetaTSS . . . . .	21
model . . . . .	23
plotSizeDistribution . . . . .	23
plotStatistics . . . . .	24
plotSubstitutions . . . . .	26
readSortedBam . . . . .	27

<b>Index</b>	<b>28</b>
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wavClusteR-package	<i>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).</i>
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**Description**

Package: wavClusteR  
 Type: Package  
 Version: 2.5.0  
 Date: 2016-01-09  
 License: GPL-2

**Author(s)**

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**References**

Comoglio F, Sievers C and Paro R (2015) Sensitive and highly resolved identification of RNA-protein interaction sites in PAR-CLIP data, BMC Bioinformatics 16, 32.  
 Sievers C, Schlumpf T, Sawarkar R, Comoglio F and Paro R. (2012) Mixture models and wavelet transforms reveal high confidence RNA-protein interaction sites in MOV10 PAR-CLIP data, Nucleic Acids Res. 40(20):e160. doi: 10.1093/nar/gks697

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 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
"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

Comoglio F, Sievers C and Paro R (2015) Sensitive and highly resolved identification of RNA-protein interaction sites in PAR-CLIP data, BMC Bioinformatics 16, 32.

**See Also**

[getClusters](#)

**Examples**

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

data( model, package = "wavClusterR" )

filename <- system.file( "extdata", "example.bam", package = "wavClusterR" )
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,
                        method = 'mrn',
                        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 )
```

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estimateFDR	<i>Estimate False Discovery Rate within the relative substitution frequency support by integrating PAR-CLIP data and RNA-Seq data</i>
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### 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

```
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 <a href="#">getAllSub</a> function
RNASeq	GRanges object containing aligned RNA-Seq reads as returned by <a href="#">readSortedBam</a>
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.

**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](#) Comoglio F, Sievers C and Paro R (2015) Sensitive and highly resolved identification of RNA-protein interaction sites in PAR-CLIP data, BMC Bioinformatics 16, 32.

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exportClusters	<i>Export clusters as BED track</i>
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**Description**

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

**Usage**

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

**Arguments**

clusters	GRanges object containing individual clusters as identified by the <a href="#">filterClusters</a> 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

**See Also**

[filterClusters](#)

---

exportCoverage	<i>Export coverage as BigWig track</i>
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---

**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	<i>Export high-confidence substitutions as BED track</i>
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---

**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 <a href="#">getHighConfSub</a> 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**

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 <a href="#">filterClusters</a> 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](#)



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filterClusters	<i>Merge clusters and compute all relevant cluster statistics</i>
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### 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 <a href="#">getClusters</a> function
highConfSub	GRanges object containing high-confidence substitution sites as returned by the <a href="#">getHighConfSub</a> function
coverage	An Rle object containing the coverage at each genomic position as returned by a call to <a href="#">coverage</a>
model	List of 5 items containing the estimated mixture model as returned by the <a href="#">fit-MixtureModel</a> 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

Sievers C, Schlumpf T, Sawarkar R, Comoglio F and Paro R. (2012) Mixture models and wavelet transforms reveal high confidence RNA-protein interaction sites in MOV10 PAR-CLIP data, *Nucleic Acids Res.* 40(20):e160. doi: 10.1093/nar/gks697

Comoglio F, Sievers C and Paro R (2015) Sensitive and highly resolved identification of RNA-protein interaction sites in PAR-CLIP data, *BMC Bioinformatics* 16, 32.

### 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,
                        sortedBam = example,
                        method = 'mrn',
                        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

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

### Arguments

countTable	A GRanges object, corresponding to a count table as returned by the <a href="#">getAllSub</a> 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](#)

## Examples

```
## 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	<i>Identify all substitutions observed across genomic positions exhibiting a specified minimum coverage</i>
-----------	---

---

## 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

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

## Arguments

sortedBam	GRanges object containing aligned reads as returned by <a href="#">readSortedBam</a>
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.

**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**

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

---

getClusters	<i>Identify clusters containing high-confidence substitutions and resolve boundaries at high resolution</i>
-------------	---

---

**Description**

Identifies clusters using either the mini-rank norm (MRN) algorithm (default and recommended to achieve highest sensitivity) or via a continuous wavelet transform (CWT) based approach. The former 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. The latter, maintained for compatibility with wavClusteR, computes the CWT on a 1 kb window of the coverage function centered at a high-confidence substitution site, and identifies cluster boundaries by extending away from peak positions.

**Usage**

```
getClusters(highConfSub, coverage, sortedBam, method = 'mrn', cores = 1, threshold, step = 1, snr = 3)
```

**Arguments**

highConfSub	GRanges object containing high-confidence substitution sites as returned by the <a href="#">getHighConfSub</a> function
coverage	An Rle object containing the coverage at each genomic position as returned by a call to <a href="#">coverage</a>
sortedBam	a GRanges object containing all aligned reads, including read sequence (qseq) and MD tag (MD), as returned by the <a href="#">readSortedBam</a> function
method	a character, either set to "mrn" or to "cwt" to compute clusters using the mini-rank norm or the wavelet transform-based algorithm, respectively. Default is "mrn" (recommended).
cores	integer, the number of cores to be used for parallel evaluation. Default is 1.
threshold	numeric, if method = "mrn", 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 <a href="#">getHighConfSub</a> 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.
step	numeric, if method = "cwt", step size of window shift. If two high-confidence substitution sites are located within a distance less than step, the wavelet transform is computed only once. Default: 1, i.e. each high-confidence substitution site is considered independently.
snr	numeric, if method = "cwt", signal-to-noise ratio controlling the peak calling as performed by wavCWTpeaks implemented in the wmtsa package. Default: 3.

**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**

William Constantine and Donald Percival (2011), wmtsa: Wavelet Methods for Time Series Analysis, <http://CRAN.R-project.org/package=wmtsa>

Sievers C, Schlumpf T, Sawarkar R, Comoglio F and Paro R. (2012) Mixture models and wavelet transforms reveal high confidence RNA-protein interaction sites in MOV10 PAR-CLIP data, *Nucleic Acids Res.* 40(20):e160. doi: 10.1093/nar/gks697

Comoglio F, Sievers C and Paro R (2015) Sensitive and highly resolved identification of RNA-protein interaction sites in PAR-CLIP data, *BMC Bioinformatics* 16, 32.

**See Also**

[getHighConfSub](#), [filterClusters](#)

**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" )
coverage <- coverage( example )
clusters <- getClusters( highConfSub = highConfSub,
                        coverage = coverage,
                        sortedBam = example,
                        method = 'mrn',
                        cores = 1,
                        threshold = 2 )
```

---

getExpInterval	<i>Identify the interval of relative substitution frequencies dominated by experimental induction.</i>
----------------	--

---

**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 <code>fitMixtureModel</code>
bayes	Logical, if TRUE the Bayes classifier (cutoff at posterior class probabilities $\geq 0.5$ ) is applied. If FALSE, custom cutoff values should be provided through <code>leftProb</code> and <code>rightProb</code> . 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

**References**

Sievers C, Schlumpf T, Sawarkar R, Comoglio F and Paro R. (2012) Mixture models and wavelet transforms reveal high confidence RNA-protein interaction sites in MOV10 PAR-CLIP data, *Nucleic Acids Res.* 40(20):e160. doi: 10.1093/nar/gks697

Comoglio F, Sievers C and Paro R (2015) Sensitive and highly resolved identification of RNA-protein interaction sites in PAR-CLIP data, *BMC Bioinformatics* 16, 32.

**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	<i>Classify substitutions based on identified RSF interval and return high confidence transitions</i>
----------------	---

---

**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 <a href="#">getAllSub</a> 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 <a href="#">getExpInterval</a> 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	<i>Compute and plot distribution of average coverage or relative log-odds as metagene profile using identified clusters</i>
-----------------	---

---

### 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.

### 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 <a href="#">getClusters</a> function
txDB	TranscriptDb object obtained through a call to the <code>makeTxDbFromUCSC</code> function in the <code>GenomicFeatures</code> 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 <code>ucscGenomes()[, "db"]</code> . 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 <code>supportedUCSCtables()</code> . 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, wavClusterR: an R package for PAR-CLIP data analysis, submitted

**See Also**

[getClusters](#)

**Examples**

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

data( model, package = "wavClusterR" )

filename <- system.file( "extdata", "example.bam", package = "wavClusterR" )
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,
                        method = 'mrn',
                        cores = 1,
                        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**

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

**Arguments**

clusters	GRanges object containing individual clusters as identified by the <a href="#">getClusters</a> function
txDB	TranscriptDb object obtained through a call to the <code>makeTxDbFromUCSC</code> function in the <code>GenomicFeatures</code> 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 <code>ucscGenomes()[, "db"]</code> . 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 <code>supportedUCSCTables()</code> . 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

Comoglio F, Sievers C and Paro R (2015) Sensitive and highly resolved identification of RNA-protein interaction sites in PAR-CLIP data, *BMC Bioinformatics* 16, 32.

**References**

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

**See Also**[getClusters](#)**Examples**

```

require(BSgenome.Hsapiens.UCSC.hg19)

data( model, package = "wavClusterR" )

filename <- system.file( "extdata", "example.bam", package = "wavClusterR" )
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,
                        method = 'mrn',
                        cores = 1,
                        threshold = 2 )

fclusters <- filterClusters( clusters = clusters,
                            highConfSub = highConfSub,
                            coverage = coverage,
                            model = model,
                            genome = Hsapiens,
                            refBase = 'T',
                            minWidth = 12 )
## 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
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 <code>ucscGenomes()[ , "db"]</code> . 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 <code>supportedUCSCTables()</code> . 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 plot function

**Value**

A numeric vector of the same length as nBins containing normalized counts. If 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**

[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 )
```

---

model	<i>Components of the non-parametric mixture model fitted on Ago2 PAR-CLIP data</i>
-------	--

---

### 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

Kishore et al. A quantitative analysis of CLIP methods for identifying binding sites of RNA-binding proteins. *Nat Methods* (2011) vol. 8 (7) pp. 559-64 <http://www.nature.com/nmeth/journal/v8/n7/full/nmeth.1608.html>

### See Also

[fitMixtureModel](#)

---

plotSizeDistribution	<i>Plot the distribution of cluster sizes</i>
----------------------	---

---

### Description

Produce an histogram of cluster sizes

### Usage

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

### Arguments

clusters	GRanges object containing individual clusters as identified by the <a href="#">getClusters</a> 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 <code>hist</code> function

**Value**

Called for its effect, returns a histogram.

**Author(s)**

Federico Comoglio

**See Also**

[getClusters](#)

**Examples**

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

data( model, package = "wavClusterR" )

filename <- system.file( "extdata", "example.bam", package = "wavClusterR" )
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,
                        method = 'mrn',
                        cores = 1,
                        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 <a href="#">getClusters</a> 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**

```
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,
                        method = 'mrn',
                        cores = 1,
                        threshold = 2 )

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

---

plotSubstitutions	<i>Barplot visualization of the number of genomic positions exhibiting a given substitution and, if model provided, additional diagnostic plots.</i>
-------------------	--

---

### 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 <a href="#">getAllSub</a> 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 <code>fitMixtureModel</code>

### 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	<i>Load a sorted BAM file</i>
---------------	-------------------------------

---

**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**

Martin Morgan and Herve Pages, Rsamtools: Binary alignment (BAM), variant call (BCF), or tabix file import, <http://bioconductor.org/packages/release/bioc/html/Rsamtools.html>

**Examples**

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

# Index

## \*Topic **Import**

readSortedBam, 27

## \*Topic **core**

estimateFDR, 5  
filterClusters, 9  
fitMixtureModel, 11  
getAllSub, 12  
getClusters, 13  
getExpInterval, 15  
getHighConfSub, 16

## \*Topic **datasets**

model, 23

## \*Topic **graphics**

annotateClusters, 3  
estimateFDR, 5  
getExpInterval, 15  
getMetaCoverage, 18  
getMetaGene, 19  
getMetaTSS, 21  
plotSizeDistribution, 23  
plotStatistics, 24  
plotSubstitutions, 26

## \*Topic **model**

fitMixtureModel, 11

## \*Topic **package**

wavClusterR-package, 2

## \*Topic **postprocessing**

annotateClusters, 3  
exportClusters, 6  
exportCoverage, 7  
exportHighConfSub, 7  
exportSequences, 8  
getMetaCoverage, 18  
getMetaGene, 19  
getMetaTSS, 21  
plotSizeDistribution, 23  
plotStatistics, 24

annotateClusters, 3

coverage, 9, 14

estimateFDR, 5, 16

exportClusters, 6

exportCoverage, 7

exportHighConfSub, 7

exportSequences, 8

filterClusters, 6–8, 9, 15

fitMixtureModel, 9, 10, 11, 16, 23

getAllSub, 5, 6, 11, 12, 17, 26

getClusters, 3, 4, 9, 10, 13, 18–21, 23–25

getExpInterval, 11, 15, 17

getHighConfSub, 7–10, 14–16, 16

getMetaCoverage, 18

getMetaGene, 19

getMetaTSS, 21

model, 23

plotSizeDistribution, 23

plotStatistics, 24

plotSubstitutions, 26

readSortedBam, 5, 6, 12–14, 21, 22, 27

wavClusterR (wavClusterR-package), 2

wavClusterR-package, 2