Package 'ChIPseqR'

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Type Package

Title Identifying Protein Binding Sites in High-Throughput Sequencing Data

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Description ChIPseqR identifies protein binding sites from ChIP-seq and nucleosome positioning experiments. The model used to describe binding events was developed to locate nucleosomes but should flexible enough to handle other types of experiments as well.

License GPL (>= 2)

LazyLoad yes

Collate classes.R generics.R initialize.R methods.R access.R package.R

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Contents

ChIPseqR-package . . . 2
acessors . . . 4
alignFeature . . . 5
BindScore-class . . . 6
callBindingSites-methods . . . 10
compress-BindScore . . . 13
compress-methods . . . 14
compress-ReadCounts . . . 14
decompress . . . 15
decompress-methods . . . 15
exportBindSequence . . . 16
getBindCor . . . 17
getBindLen . . . 18
getCutoff . . . 19
internal . . . 20
pickPeak . . . 20
plot,ReadCounts,missing-method . . . 21
plot-BindScore . . . 22
plotReads . . . 23
plotWindow . . . 24
pos2gff . . . 25
ReadCounts-class . . . 26
RLEBindScore-class . . . 27
RLEReadCounts-class . . . 29
simpleNucCall . . . 30
startScore . . . 32
strandPileup . . . 33
windowCounts . . . 34

Index **36**

`ChIPseqR-package` *Identifying Protein Binding Sites in High-Throughput Sequencing Data*

Description

ChIPseqR provides a set of functions for the analysis of ChIP-seq data. Protein binding sites are located by identifying a characteristic pattern of peaks in read counts on both DNA strands.

Details

Package:	ChIPseqR
Type:	Package
Version:	1.13.1
Date:	2012-12-11
License:	GPL (>=2)
LazyLoad:	yes

The easiest way to obtain binding site predictions for nucleosomes is to use `simpleNucCall`. This provides a simple interface to `callBindingSites`. This function operates on `AlignedRead` objects and provides useful defaults for nucleosome analysis. Parameters can be adjusted to detect the presence of other DNA binding proteins, e.g. transcription factors. If more fine control is desired the following steps will produce binding site predictions:

`strandPileup`**:** Turn mapped reads into read counts along the genome.

`startScore`**:** Score potential binding sites.

`getCutoff`**:** Determine cutoff required to achieve desired false discovery rate.

`pickPeak`**:** Find all peaks in the binding site score that exceed the significance threshold determined by `getCutoff`. These are the predicted binding sites.

Author(s)

Peter Humburg

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References

Humburg, P., Helliwell, C., Bulger, D. & Stone, G. ChIPseqR: Analysis of ChIP-seq Experiments. BMC Bioinformatics 12, 39+ (2011).

See Also

`ShortRead`

Examples

```
## See 'simpleNucCall' for examples of how to obtain nucleosome predictions.
```

acessors *Access slots of S4 classes*

Description

Accessor functions for S4 classes in package "ChIPseqR".

Usage

```
 ## S4 method for signature 'ANY'
binding(x, ...)
 ## S4 method for signature 'ANY'
cutoff(x, ...)
 ## S4 replacement method for signature 'ANY'
cutoff(x, ...) <- value
 ## S4 method for signature 'ANY'
nullDist(x, ...)
 ## S4 replacement method for signature 'ANY'
nullDist(x, ...) <- value
 ## S4 method for signature 'ANY'
peaks(x, ...)
 ## S4 method for signature 'ANY'
pvalue(x, ...)
 ## S4 method for signature 'ANY'
support(x, ...)
```

Arguments

x	An S4 object.
...	Further arguments, ignored by default method.
value	New value for slot.

Details

These methods allow safe read (and in some cases write) access to slots of S4 classes and should be used for this purpose rather than modifying slots manually.

Value

The current value of the interrogated slot.

Methods

x = "ANY" Default method for accessor function.

Note

This page documents the generics and their default behaviour. See the help page of each class for class specific implementations.

Author(s)

Peter Humburg

See Also

ReadCounts, BindScore

---

alignFeature *Read counts relative to annotated features*

Description

Creates a set of (strand specific) read counts centred at the genomic features provided.

Usage

```
alignFeature(data, anno, offset = 1000)
```

Arguments

data	List with read counts as returned by strandPileup.
anno	Data frame with annotation data in GFF format.
offset	Half width of window around start point of annotated features.

Value

List with one component for each feature in anno.

Author(s)

Peter Humburg

References

The GFF file format specification: http://www.sanger.ac.uk/Software/formats/GFF/GFF_Spec.shtml

Examples

```
set.seed(1)

## determine binding site locations
b <- sample(1:8.5e5, 500)

## sample read locations
fwd <- unlist(lapply(b, function(x) sample((x-83):(x-73), 20, replace=TRUE)))
rev <- unlist(lapply(b, function(x) sample((x+73):(x+83), 20, replace=TRUE)))

## add some background noise
fwd <- c(fwd, sample(1:(1e6-25), 5000))
rev <- c(rev, sample(25:1e6, 5000))

## create data.frame with read positions as input to strandPileup
reads <- data.frame(chromosome="chr1", position=c(fwd, rev),
 length=25, strand=factor(rep(c("+", "-"), times=c(15000, 15000))))

## create object of class ReadCounts
readPile <- strandPileup(reads, chrLen=1e6, extend=1, plot=FALSE)

## convert binding site locations into GFF format
gff <- data.frame(chromosome="chr1", source="test", feature="binding", start=b-73, end=b+73,
 score=".", strand=".", frame=".")

## align read counts relative to binding site location
aligned <- alignFeature(readPile, gff, offset=500)
```

BindScore-class *Class "BindScore"*

Description

This class provides the infrastructure to store results of ChIP-seq analysis.

Usage

```
 ## S4 method for signature 'BindScore'
binding(x)
 ## S4 method for signature 'BindScore'
chrLength(x, subset)
 ## S4 method for signature 'BindScore'
cutoff(x, type=c("score", "pvalue"))
 ## S4 replacement method for signature 'BindScore'
cutoff(x, type=c("score", "pvalue")) <- value
 ## S4 method for signature 'BindScore'
head(x, n=6, by=c("score", "position"), ...)
 ## S4 method for signature 'BindScore'
lapply(X, FUN, ...)
```

```
 ## S4 method for signature 'BindScore'
length(x)
 ## S4 replacement method for signature 'BindScore'
length(x) <- value
 ## S4 method for signature 'BindScore'
max(x, ..., na.rm=TRUE)
 ## S4 method for signature 'BindScore'
min(x, ..., na.rm=TRUE)
 ## S4 method for signature 'BindScore'
names(x)
 ## S4 replacement method for signature 'BindScore,ANY'
names(x) <- value
 ## S4 method for signature 'BindScore'
nullDist(x)
 ## S4 replacement method for signature 'BindScore'
nullDist(x) <- value
 ## S4 method for signature 'BindScore'
peaks(x, ...)
 ## S4 method for signature 'BindScore'
range(x, ..., na.rm=TRUE)
 ## S4 method for signature 'BindScore'
score(x)
 ## S4 method for signature 'BindScore'
support(x)
 ## S4 method for signature 'BindScore'
tail(x, n=6, by=c("score", "position"), ...)
 BindScore(call, score=list(), pvalue=list(), peaks=list(), cutoff=c(-Inf, 1), nullDist=c(0, 1), names
```

Arguments

x	Object of class BindScore.
X	Object of class BindScore.
subset	Index vector indicating a subset of x. If subset is missing everything is selected.
type	A string indicating which type of cut-off should be returned or changed. Either "score" or "pvalue"
n	Number of entries to show.
by	A string indicating whether the output should be sorted by score or by position in the genome.
na.rm	Logical indication whether NAs should be ignored.
FUN	Function to apply to results for each chromosome.
value	Replacement value.
call	Function call used to generate the values of the other slots.
score	List of binding site scores. One component per chromosome.
pvalue	List of binding site p-values. One component per chromosome.
peaks	List of significant peaks in binding site score. One component per chromosome.

cutoff	Numeric vector of length two indicating the significance cut-off in terms of score and p-value.
nullDist	Parameters of the null distribution.
names	Character vector providing names for chromosomes.
start	Integer indicating position of first binding site score.
compress	Logical indicating whether scores and p-values should be compressed.
digits	The number of decimal places to retain for compression.
...	Further arguments.

Objects from the Class

Objects can be created by calls of the form `new("BindScore", functionCall, score, pvalue, peaks, cutoff, nullDist, names, ...)`. Objects of this class are typically created (and returned) by functions that perform peak calling on ChIP-seq data. Usually there should be no need to create them directly.

Slots

`functionCall`**:** Object of class `"call"` storing the function call used to initiate the analysis.

`score`**:** Object of class `"list"`. The binding site score. One numeric vector per chromosome.

`pvalue`**:** Object of class `"list"`. The (adjusted) p-values corresponding to the scores in slot `score`.

`peaks`**:** Object of class `"list"` giving the location of significant peaks in the binding site score. These correspond to the location of predicted binding sites.

`cutoff`**:** Object of class `"numeric"` with entries 'pvalue' and 'score' giving the significance threshold used for peak calling in terms of p-value and score.

`nullDist`**:** Object of class `"numeric"` providing the parameters of the null distribution used to determine p-values.

`start`**:** Object of class `"integer"` indicating the index corresponding to the first entry in `score` (assumed to be the same for all chromosomes).

Methods

as.data.frame `signature(x = "BindScore")`: Convert results into a `data.frame` giving the location, score and p-value of significant peaks.

[`signature(x = "BindScore", i = "ANY", j = "missing", drop = "missing")`: Restrict results to a subset of chromosomes. Chromosomes can either be identified by name or by numerical index.

[[`signature(x = "BindScore", i = "ANY", j = "missing")`: Restrict results to a single chromosome. Note that `x[["chr1"]]` is identical to `x["chr1"]`.

[[`signature(x = "BindScore", i = "ANY", j = "numeric")`: subset results to restrict them to a region on a single chromosome.

binding `signature(x = "BindScore")`: Returns length of binding site used during analysis.

chrLength `signature(x = "BindScore", subset = "ANY")`: Returns length of all chromosomes represented in `x`.

cutoff<- `signature(x = "BindScore")`: Sets the significance cut-off. Argument `type=c("score", "pvalue")` determines which cut-off is to be set, the other is adjusted accordingly. This recalculates the significance of peaks in the binding site score and may be slow.

cutoff `signature(x = "BindScore")`: Returns significance threshold used for analysis.

head `signature(x = "BindScore")`: Returns the first n peaks. Argument `by = c("score", "position")` determines whether results are sorted by score or by genomic location.

lapply `signature(X = "BindScore")`: Applies a function to results for each chromosome.

length<- `signature(x = "BindScore")`: Reduces the number of chromosomes for which results are stored, i.e., `length(x) <- 3` only retains the first three chromosomes.

length `signature(x = "BindScore")`: Returns the number of binding sites identified by the analysis.

max `signature(x = "BindScore")`: Returns maximum score.

min `signature(x = "BindScore")`: Returns minimum score.

names<- `signature(x = "BindScore", value = "ANY")`: Sets the chromosome names.

names `signature(x = "BindScore")`: Returns the chromosome names.

nullDist<- `signature(x = "BindScore")`: Sets the parameters of the null distribution adjusting the significance cut-off in the process and predicts binding sites using the new null distribution.

peaks `signature(x = "BindScore")`: Returns list of predicted binding sites.

range `signature(x = "BindScore")`: Range of binding site scores.

score `signature(x = "BindScore")`: Returns list of binding site scores.

support `signature(x = "BindScore")`: Returns length of support region used during analysis.

tail `signature(x = "BindScore")`: Returns the last n peaks. Argument `by = c("score", "position")` determines whether results are sorted by score or by genomic location.

Author(s)

Peter Humburg

References

~put references to the literature/web site here ~

See Also

`ReadCounts` for the data structure used as input for the analysis and `callBindingSites`

Examples

```
showClass("BindScore")

set.seed(1)

## determine binding site locations
b <- sample(1:1e6, 5000)
```

```
## sample read locations
fwd <- unlist(lapply(b, function(x) sample((x-83):(x-73), 20, replace=TRUE)))
rev <- unlist(lapply(b, function(x) sample((x+73):(x+83), 20, replace=TRUE)))

## add some background noise
fwd <- c(fwd, sample(1:(1e6-25), 50000))
rev <- c(rev, sample(25:1e6, 50000))

## create data.frame with read positions as input to strandPileup
reads <- data.frame(chromosome="chr1", position=c(fwd, rev),
 length=25, strand=factor(rep(c("+", "-"), times=c(150000, 150000))))

## create object of class ReadCounts
readPile <- strandPileup(reads, chrLen=1e6, extend=1, plot=FALSE)

## predict binding site locations
## the artificial dataset is very small so predictions may not be very reliable
bindScore <- simpleNucCall(readPile, bind=147, support=20, plot=FALSE, compress=FALSE)

## number of binding sites found
length(bindScore)

## the first few predictions, by score
head(bindScore)

## score and p-value cut-off used
cutoff(bindScore)
```

`callBindingSites-methods`

Predict protein binding sites from high-throughput sequencing data

---

Description

Methods for function `callBindingSites` in Package 'ChIPseqR'. These methods are used to identify protein binding sites from ChIP-seq data.

Usage

```
## S4 method for signature 'ANY'
callBindingSites(data, chrLen, plot=TRUE, verbose=TRUE, ..., plotTo)
## S4 method for signature 'character'
callBindingSites(data, type, minQual=70, ...)
## S4 method for signature 'matrix'
callBindingSites(data, chrName="chr", ...)
## S4 method for signature 'ReadCounts'
callBindingSites(data, bind, support, background, bgCutoff=0.9, supCutoff=0.9,
fdr = 0.05, extend=1, tailCut=0.95, piLambda=0.5, adapt=FALSE, corSummary=median, compress = TRUE,
digits = 16, plot=TRUE, verbose=TRUE, ask=FALSE, plotTo, ...)
```

Arguments

data	Either an object containing information about mapped reads or a list. See below for details.
bind	Length of binding region to use (see Details).
support	Length of support region to use (see Details).
background	Length of background window. If this is missing it will be set to 10*(bind+2*support).
chrLen	Numeric vector indicating the length of all chromosomes. Only needed when data is an AlignedRead object. readBfaToc may be used to supply this information.
bgCutoff	Numeric value between 0.5 and 1. This determines how much estimates of the background read density are allowed to vary for adjacent windows. Set to 1 to disable cutoff.
supCutoff	Numeric value between 0.5 and 1. This determines how much estimates of the support region read density are allowed to vary for forward and reverse strand. Set to 1 to disable cutoff.
fdr	Target false discovery rate.
extend	Numeric value indicating how far mapped reads should be extended when calculating read counts.
type	Format of alignment file (see readAligned forr details).
minQual	Minimum alignment quality to use. All reads with lower alignment quality are discarded.
tailCut	Truncation point used to exclude outliers when estimating null distribution.
chrName	Name to use for the single chromosome.
piLambda	If adapt=TRUE this parameter is used to estimate the proportion of scores not related to binding sites.
adapt	Logical indicating whether an adaptive false discovery rate should be used. If this is FALSE (the default) the usual Benjamini-Hochberg procedure is used to control the FDR.
corSummary	Function used to summarise cross-correlation across chromosomes. See the Details section on binding and support region.
compress	Logical indicating whether the return value should be compressed.
digits	Number of decimal places to retain for binding site score for compression.
plot	Logical. If plot=TRUE (the default) some diagnostic plots are produced during the analysis.
verbose	Logical. If verbose=TRUE (the default) status messages are printed to indicate progress.
ask	Logical. Setting this to TRUE causes the system to wait for user input before displaying a new plot. See devAskNewPage.
plotTo	Character string giving the name of a file that should be used to store plots generated during the analysis. If this is not missing a pdf file with the given name will be created.
...	Additional arguments. Most methods pass them on to the ReadCounts method.

Details

The length of binding and support regions can either be given as a single value or as a range of possible values (by providing the minimum and maximum). In the latter case the cross-correlation between read counts on forward and reverse strand will be used to determine a value within that range. Note that this may lead sub-optimal choices of binding and support region length.

Value

An object of class BindScore if compress = FALSE, otherwise an object of class RLEBindScore

Methods

data = "ANY" Default method to handle all forms of input not explicitly handled by their own method. In particular this will be used for objects of class AlignedRead and data.frame but it will handle class for which a strandPileup method is available.

data = "character" Allows to use a file name referring to a file of mapped sequence reads as input.

data = "matrix" Uses a matrix of read counts (for a single chromosome) as input.

data = "ReadCounts" This methods implements the peak calling algorithm. Other methods will typically reformat their input and pass it on to this method.

See Also

simpleNucCall for an interface with nucleosome specific defaults. This function uses strandPileup, startScore, getCutoff and pickPeak. See the help pages of these functions for additional detail on the individual steps involved. See getBindLen for details on the estimation of binding and support region length.

Examples

```
set.seed(1)

## determine binding site locations
b <- sample(1:1e6, 5000)

## sample read locations
fwd <- unlist(lapply(b, function(x) sample((x-83):(x-73), 20, replace=TRUE)))
rev <- unlist(lapply(b, function(x) sample((x+73):(x+83), 20, replace=TRUE)))

## add some background noise
fwd <- c(fwd, sample(1:(1e6-25), 50000))
rev <- c(rev, sample(25:1e6, 50000))

## create data.frame with read positions as input to strandPileup
reads <- data.frame(chromosome="chr1", position=c(fwd, rev),
 length=25, strand=factor(rep(c("+", "-"), times=c(150000, 150000))))

## create object of class ReadCounts
readPile <- strandPileup(reads, chrLen=1e6, extend=1, plot=FALSE)

## predict binding site locations
```

```
## the artificial dataset is very small so predictions may not be very reliable
bindScore <- callBindingSites(readPile, bind=147, support=20, background=2000, plot=FALSE)
```

---

compress-BindScore *Compress BindScore Objects*

Description

Generates a compressed representation of binding site scores.

Usage

```
## S4 method for signature 'BindScore'
compress(x, digits=16)
```

Arguments

x	An object of class BindScore.
digits	Integer indicating the number of decimal places to retain.

Details

Binding site scores are compressed by first rounding them to digits decimal places and then applying run-length encoding.

Value

An object of class RLEBindScore.

Note

Compression reduces the precision of binding site scores and may affect results, especially for small values of digits.

Author(s)

Peter Humburg

See Also

Rle, RleList, compress-BindScore

compress-methods *Methods for Function compress in Package 'ChIPseqR'*

Description

Methods to obtain compressed versions of data structures.

Methods

`signature(x = "BindScore")` Converts `x` into an object of class `RLEBindScore`.

`signature(x = "ReadCounts")` Converts `x` into an object of class `RLEReadCounts`.

`signature(x = "RLEBindScore")` Returns (the already compressed) `x`.

`signature(x = "RLEReadCounts")` Returns (the already compressed) `x`.

compress-ReadCounts *Compress ReadCount Objects*

Description

Generates a compressed representation of read counts.

Usage

```
## S4 method for signature 'ReadCounts'
compress(x)
```

Arguments

`x`	An object of class `ReadCounts`

Details

Run-length encoding is used to obtain a compressed representation of read counts.

Value

An object of class `RLEReadCounts`

Author(s)

Peter Humburg

See Also

`Rle`, `RleList`, `compress-BindScore`

decompress *Extract Read Count and Binding Site Score Representations*

Description

These methods extract read count and binding site sores from compressed representations.

Usage

```
## S4 method for signature 'RLEReadCounts'
decompress(x)
## S4 method for signature 'RLEBindScore'
decompress(x)
```

Arguments

x	An object of class RLEBindScore or RLEReadCounts.

Value

An object of class BindScore or ReadCounts respectively.

Author(s)

Peter Humburg

See Also

compress

decompress-methods *Methods for Function decompress in Package 'ChIPseqR'*

Description

Methods to extract compressed data structures.

Methods

signature(x = "ANY") The default method simply returns x.

signature(x = "Rle") Restores the atomic vector encoded in x.

signature(x = "RLEBindScore") Returns an object of BindScore.

signature(x = "RleList") Extracts the components of x and restores them to atomic vectors.

signature(x = "RLEReadCounts") Returns an object of ReadCounts.

`exportBindSequence` *Export sequence of predicted binding sites*

Description

Extracts sequence of predicted binding sites from reference genome and exports them in FASTA format.

Usage

```
exportBindSequence(prediction, reference, bind, overlap = FALSE, file = "")
```

Arguments

`prediction`	Object of class `BindScore`.
`reference`	Reference genome sequence (as `XStringSet` object).
`bind`	Length of binding site to assume for sequence extraction. This may be missing in which case the value is derived from `'prediction'`.
`overlap`	Logical indicating whether overlapping predictions should be allowed.
`file`	Name of output file.

Value

An `XStringViews` object containing the sequences. If a file name is provided this is returned invisibly.

Author(s)

Peter Humburg

References

Package `Biostrings`

See Also

`XStringViews`, `XStringSet`, `BindScore`

getBindCor *Calculate cross-correlation between read counts*

Description

This function calculates the cross-correlation between read counts on forward and reverse strand.

Usage

```
getBindCor(data, max.lag, summary, plot = TRUE, ...)
```

Arguments

data	An object of class ReadCounts
max.lag	Maximum lag to use in cross-correlation calculation.
summary	Function to use to summarise cross-correlation across chromosomes.
plot	Logical indicating whether to plot cross-correlation.
...	Further arguments, currently ignored.

Details

Function fttcor in package "timsac" is used to carry out the computation.

Value

The (summarised) cross-correlation. If summary is missing a list of cross-correlations for each chromosome is returned.

Author(s)

Peter Humburg

See Also

fftcor, ReadCounts, getBindLen

getBindLen *Estimate length of binding and support region*

Description

The cross-correlation between forward and reverse strand read counts is used to estimate the distance between peaks on both strands. This is then used to derive suitable values for the length of binding and support regions.

Usage

```
getBindLen(data, bind, support, summary = median, verbose = FALSE, plot = TRUE, ...)
```

Arguments

data	An object of class ReadCounts.
bind	Either known length of binding region or minimum and maximum of binding region length to consider.
support	Either known length of support region or minimum and maximum of support region length to consider.
summary	Function to use to summarise cross-correlation across chromosomes.
verbose	Logical indicating whether progress messages should be printed.
plot	Logical indicating whether cross-correlation should be plotted.
...	Further arguments to getBindCor.

Details

This assumes that the first peak in cross-correlation corresponds to the length of the binding site. Note that this is not accurate. The peak is closer to bind + 2*m where m is the median of the read distribution in the support region ('read distribution in the support region' means the read density as a function of distance to binding site start/end). Consequently this method will overestimate the length of the binding site. If either bind or support are of length 1 this is assumed to be the known value and a more accurate estimate for the remaining parameter is used.

Value

A numeric vector giving the estimated lengths of binding and support regions.

Author(s)

Peter Humburg

getCutoff *Determine significance threshold for binding site scores*

Description

Given a vector of observed binding site scores and a desired false discovery rate, this function returns the lowest score that should be considered significant to achieve the given false discovery rate.

Usage

```
getCutoff(score, alpha = 0.05, tailCut = 0.95, adapt = FALSE, lambda, plot = TRUE, returnPval = TRUE)
```

Arguments

score	Numeric vector with binding site scores.
alpha	Desired false discovery rate.
tailCut	Truncation point used to exclude outliers when fitting the null distribution.
adapt	Logical indicating whether an adaptive false discovery rate should be used.
lambda	If adapt is TRUE this is used in estimating the proportion of scores that is unrelated to binding sites.
plot	If this is TRUE (the default) a plot of the observed score distribution and estimated null distribution is generated.
returnPval	Indicates whether or not the corrected p-values for all scores should be returned.

Value

A list with components

cutoff	A numeric vector with the score and nominal false discovery rate corresponding to the determined cutoff.
h0	A numeric vector with the mean and standard deviation of the estimated null distribution.
pvalue	If returnPval is TRUE, the p-values corresponding to the scores in score. Note that all missing values are removed.
pi0	If adapt is TRUE, the estimated proportion of scores not related to binding sites.

Note

This function is used by callBindingSites to determine the significance threshold for peak-calling.

Author(s)

Peter Humburg

References

For the adaptive false discovery rate procedure used if `adapt=TRUE` see JD Storey, JE Taylor and D Siegmund. Strong control, conservative point estimation and simultaneous conservative consistency of false discovery rates: a unified approach. Journal of the Royal Statistical Society B, 66(1):187-205, 2004.

See Also

`callBindingSites`

`internal` *Internal and undocumented functions*

Description

These functions for internal use or currently unsupported.

Author(s)

Peter Humburg

`pickPeak` *Identify peaks above a given threshold*

Description

Given a vector of scores and a threshold, this function finds all peaks that exceed the threshold.

Usage

```
pickPeak(score, threshold, offset = 0, sub = FALSE)
```

Arguments

`score`	Numeric vector.
`threshold`	All values in `score` below this value are ignored.
`offset`	Offset to add to the determined peak locations.
`sub`	Logical. If this is `FALSE` (the default) for each region that exceeds the threshold only the global maximum is returned. Otherwise local maxima are returned as well.

Value

If sub = FALSE a numeric vector giving the location of all peaks. Otherwise a list with components

peaks	The same peak locations that are returned for sub = FALSE.
subPeaks	A list with one component for each entry in 'peaks' giving the location of local maxima.

Note

This function is used by callBindingSites for peak-calling.

Author(s)

Peter Humburg

See Also

callBindingSites, startScore, getCutoff

---

plot,ReadCounts,missing-method

Diagnostic Plots for Read Counts

Description

Produces plots to assess the distribution of reads, either for an entire chromosome or within a (small) window.

Usage

```
## S4 method for signature 'ReadCounts,missing'
plot(x, chr, center, score, width=2000, type=c("hilbert", "window"), ...)
## S4 method for signature 'RLEReadCounts,missing'
plot(x, chr, center, score, width=2000, type=c("hilbert", "window"), ...)
```

Arguments

x	Object of class ReadCounts or RLEReadCounts.
chr	Index or name of chromosome for which read counts should be plotted.
center	For type 'window', the center coordinate of the window to plot.
score	For type 'window', an object of type BindScore (or BindScore) that should be used to include information about the score and predicted binding sites into the plot.
width	For type 'window', the width of the window.
type	Character string indicating the type of plot that should be produced (see details).
...	Further arguments (see details).

Details

Type 'window' produces a plot that shows read counts as vertical bars. If `score` is not missing, it is used to plot the score and predicted binding sites (if any) as well. Any additional arguments are passed on to `.plotWindow`.

Type 'hilbert' produces a Hilbert curve plot of read counts for an entire chromosome. Additional arguments are passed on to `.plotReads`.

Value

Called for its side effect.

Author(s)

Peter Humburg

See Also

`hilbertImage`

---

plot-BindScore *Diagnostic Plots for Binding Site Scores*

Description

Generates plots to assess the fit of the estimated null distribution.

Usage

```
## S4 method for signature 'BindScore,missing'
plot(x, npoints = 10000, type=c("density", "qqplot"), ...)
```

Arguments

`x`	An object of class `BindScore`.
`npoints`	Maximum number of points to plot in a QQ-plot.
`type`	Character string indicating the plot type.
`...`	Further arguments (currently ignored).

Details

Type 'density' produces a histogram of binding site scores with overlaid null distribution. Type 'qqplot' produces a normal QQ-plot comparing the observed binding site scores to the null distribution.

Value

Called for its side effect.

Author(s)

Peter Humburg

`plotReads` *Plot compact representation of read counts on a chromosome*

Description

Creates an image of all read counts for a chromosome.

Usage

```
.plotReads(x, scale = c("total", "ratio"), log = TRUE, ...)
```

Arguments

`x`	A two column matrix with read counts (as produced by `strandPileup`).
`scale`	Character string indicating whether to plot the total number of reads on both strands or the ratio between them.
`log`	Logical indicating whether to use log read counts.
`...`	Further plotting arguments.

Details

The read counts (or read count ratios) are plotted as a Hilbert curve using `hilbertImage`.

Value

Called for its side effect.

Author(s)

Peter Humburg

References

Anders, Simon, 'Visualization of genomic data with the Hilbert curve', Bioinformatics , vol. 25, no. 10, 1231-1235 (2009).

See Also

`strandPileup`, `hilbertImage`

plotWindow *Plot read counts within a genomic region*

Description

Read count within a selected region of the genome are plotted, optionally together with binding site score and location of predicted binding sites.

Usage

```
.plotWindow(data, chr, center, score, width=2000, bind, start, end, bind.col=3, score.type='l',
 xlab=NULL, ylab="Read count", cutoff=TRUE, offset = 1, ...)
```

Arguments

data	Object of class ReadCounts or a list of read counts.
chr	A character string or numeric index identifying the the chromosome on which the region is located.
center	Numeric value giving the center of the region on chromosome 'chr' that should be plotted.
score	An object of class BindScore, may be missing.
width	Width of the window to plot. The plotted region will be [center - width/2, center + width/2].
bind	Length of binding site, ignored if 'score' is missing.
start	Start of plotting window (may be used together with end instead of center).
end	End of plotting window (may be used together with start instead of center).
bind.col	Color used to indicate location of binding sites, ignored if 'score' is missing.
score.type	Plotting type to use for score, ignored if 'score' is missing.
xlab	X-axis label. This defaults to a description of the genomic location constructed from 'chr', 'center' and 'width'
ylab	Y-axis label.
cutoff	Logical indicating whether the significance threshold used to predict binding site locations should be indicated by a horizontal line in the plot.
offset	Position on chromosome chr corresponding to the first read count in data.
...	Further arguments to plot.

Details

If 'score' is present the binding site score is plotted on top of read counts. Scores are rescaled to lie between 0 and the maximum number of reads in the window. A corresponding scale is added to the right hand axis.

Value

Called for its side effect.

Author(s)

Peter Humburg

`pos2gff` *Convert genome coordinates into GFF format*

Description

Provides facility to export the location of genomic features to a GFF formatted file.

Usage

```
pos2gff(pos, method, feature, len, strand, score, name)
```

Arguments

`pos`	Named list with one component per chromosome giving the start position of features on that chromosome.
`method`	Entry for method field in GFF file. Recycled as necessary
`feature`	Entry for feature field in GFF file. Recycled as necessary
`len`	Length of fetures. This is used to calculate matching end positions for each start position given in `pos`
`strand`	Entry for feature field in GFF file. Recycled as necessary
`score`	Entry for feature field in GFF file. Recycled as necessary
`name`	Entry for feature field in GFF file. Recycled as necessary

Value

A `data.frame` with columns `'chromosome'`, `'method'`, `'feature'`, `'start'`, `'end'`, `'score'`, `'strand'`. Writing this data frame to a text file produces a GFF formatted file.

Author(s)

Peter Humburg

References

The GFF specification: http://www.sanger.ac.uk/Software/formats/GFF/GFF_Spec.shtml

Examples

```
pos <- list(chr1=c(10, 50, 60), chr2=c(22, 200, 500))
pos2gff(pos, "test", "foo", 25, c("+", "+", "-", "+", "-", "-"), 0, "test")
```

`ReadCounts-class` *Class "ReadCounts"*

Description

Represents counts of (possibly extended) reads for each strand of the genome.

Usage

```
ReadCounts(counts=list(), names=NULL, compress=TRUE)
```

Arguments

`counts`	A list of read counts. Each component is a two column matrix of strand specific read counts for a chromosome.
`names`	Character vector of chromosome names. If this is `NULL` the names of `counts` are used instead.
`compress`	Logical indicating whether read counts should be compressed.

Objects from the Class

Objects can be created by calls of the form `ReadCounts(counts, names, compress=FALSE)` or by calls to `strandPileup`.

Slots

`counts`**:** Object of class `"list"` with one component per chromosome, containing a matrix of read counts (one column per strand).

Methods

[<- `signature(x = "ReadCounts", i = "ANY", j = "missing")`: Replace read counts for chromosomes indicated by `i`.

[`signature(x = "ReadCounts", i = "ANY", j = "missing", drop = "missing")`: Returns list of read counts for chromosomes indicated by `i`.

[[<- `signature(x = "ReadCounts", i = "ANY", j = "missing")`: Replace read counts for chromosome `i`.

[[`signature(x = "ReadCounts", i = "ANY", j = "missing")`: Returns read counts for chromosome `i`.

$<- `signature(x = "ReadCounts")`: Replace read counts for chromosome `i` (by name).

$ `signature(x = "ReadCounts")`: Returns read counts for chromosome `i` (by name).

callBindingSites `signature(data = "ReadCounts")`: Predict bindingsites from read counts.

chrLength `signature(x = "ReadCounts", subset = "ANY")`: Returns length of all chromosomes represented in `x`.

lapply `signature(X = "ReadCounts")`: Apply function to read counts for each chromosome.

length<- `signature(x = "ReadCounts")`: Change the number of chromosomes represented by `x` to `value`.

length `signature(x = "ReadCounts")`: Number of chromosomes represented by `x`.

names<- `signature(x = "ReadCounts", value = "ANY")`: Change names of chromosomes.

names `signature(x = "ReadCounts")`: Chromosome names.

nreads `signature(x = "ReadCounts", byStrand = "Logical", subset = "ANY")`: Returns the number of reads on each chromosome, split by strand (if `byStrand` is `TRUE`).

sapply `signature(X = "ReadCounts")`: Apply function to read counts for each chromosome.

Author(s)

Peter Humburg

See Also

`BindScore`, `strandPileup` , `compress`, `decompress`

Examples

```
showClass("ReadCounts")

## generate some very simple artificial read data
set.seed(1)
fwd <- sample(c(50:70, 250:270), 30, replace=TRUE)
rev <- sample(c(197:217, 347:417), 30, replace=TRUE)
## create data.frame with read positions as input to strandPileup
reads <- data.frame(chromosome="chr1", position=c(fwd, rev),
 length=25, strand=factor(rep(c("+", "-"), times=c(30, 30))))

## create object of class ReadCounts
readPile <- strandPileup(reads, chrLen=500, extend=1, plot=FALSE, compress=FALSE)

names(readPile)
length(readPile)
sapply(readPile, sum)
```

RLEBindScore-class *Run-length Encoded Binding Site Scores*

Description

This class provides a memory efficient representation of binding site scores.

Objects from the Class

Objects can be created by calls of the form `BindScore(functionCall, score, pvalue, peaks, cutoff, nullDist, names, start, digits, compress=TRUE)` or through calls to `callBindingSites`.

Slots

`functionCall`: Object of class `"call"` storing the function call used to initiate the analysis.

`score`: Object of class `"list"`. The binding site score. One run-length encoded numeric vector per chromosome.

`pvalue`: Object of class `"list"`. The (adjusted and run-length encoded) p-values corresponding to the scores in slot `score`.

`peaks`: Object of class `"list"` giving the location of significant peaks in the binding site score. These correspond to the location of predicted binding sites.

`cutoff`: Object of class `"numeric"` with entries 'pvalue' and 'score' giving the significance threshold used for peak calling in terms of p-value and score.

`nullDist`: Object of class `"numeric"` providing the parameters of the null distribution used to determine p-values.

`start`: Object of class `"integer"` indicating the index corresponding to the first entry in `score` (assumed to be the same for all chromosomes).

Extends

Class `"BindScore"`, directly.

Methods

decompress `signature(x = "RLEBindScore")`: conversion to `BindScore` object.

Author(s)

Peter Humburg

See Also

`BindScore`, `Rle`

Examples

```
showClass("RLEBindScore")

set.seed(1)

## determine binding site locations
b <- sample(1:1e6, 5000)

## sample read locations
fwd <- unlist(lapply(b, function(x) sample((x-83):(x-73), 20, replace=TRUE)))
rev <- unlist(lapply(b, function(x) sample((x+73):(x+83), 20, replace=TRUE)))

## add some background noise
fwd <- c(fwd, sample(1:(1e6-25), 50000))
rev <- c(rev, sample(25:1e6, 50000))
```

```
## create data.frame with read positions as input to strandPileup
reads <- data.frame(chromosome="chr1", position=c(fwd, rev),
 length=25, strand=factor(rep(c("+", "-"), times=c(150000, 150000))))

## create object of class ReadCounts
readPile <- strandPileup(reads, chrLen=1e6, extend=1, plot=FALSE)

## predict binding site locations
## the artificial dataset is very small so predictions may not be very reliable
bindScore <- simpleNucCall(readPile, bind=147, support=20, plot=FALSE, compress=TRUE)

## number of binding sites found
length(bindScore)

## the first few predictions, by score
head(bindScore)

## score and p-value cut-off used
cutoff(bindScore)
```

RLEReadCounts-class *Run-length Encoded Read Counts*

Description

This class provides a memory efficient representation of strand specific read counts.

Objects from the Class

Objects can be created by calls of the form `ReadCounts(counts, names, compress = TRUE)` or by calls to `strandPileup`.

Slots

`counts`: Object of class `"list"` with one component per chromosome, containing a read counts encoded in an object of class `RleList`.

Extends

Class `"ReadCounts"`, directly.

Methods

chrLength `signature(x = "RLEReadCounts")`: Returns length of all chromosomes represented in `x`.

decompress `signature(x = "RLEReadCounts")`: Expands read counts and returns object of class `ReadCounts`.

nreads `signature(x = "RLEReadCounts")`: Returns the number of reads on each chromosome, split by strand (if `byStrand` is `TRUE`)

plot `signature(x = "RLEReadCounts", y = "missing")`: Generates plots of read counts.

Author(s)

Peter Humburg

See Also

`ReadCounts`, `RleList`

Examples

```
showClass("RLEReadCounts")

## generate some very simple artificial read data
set.seed(1)
fwd <- sample(c(50:70, 250:270), 30, replace=TRUE)
rev <- sample(c(197:217, 347:417), 30, replace=TRUE)
## create data.frame with read positions as input to strandPileup
reads <- data.frame(chromosome="chr1", position=c(fwd, rev),
 length=25, strand=factor(rep(c("+", "-"), times=c(30, 30))))

## create object of class ReadCounts
readPile <- strandPileup(reads, chrLen=500, extend=1, plot=FALSE, compress=TRUE)

names(readPile)
length(readPile)
sapply(readPile, sum)
```

---

simpleNucCall *Predict nucleosome positions from high-throughput sequencing data*

Description

This function provides a simplified interface to `callBindingSites` with defaults suitable for the detection of nucleosomes.

Usage

```
simpleNucCall(data, bind=128, support=17, background=2000, chrLen, ...)
```

Arguments

`data`	Either an object of class `AlignedRead` or a list. See below for details.
`bind`	Length of binding region to use (see Details).
`support`	Length of support region to use (see Details).

background	Length of background window. If this is missing it will be set to 10*(bind+2*support).
chrLen	Numeric vector indicating the length of all chromosomes. Only needed when data is an AlignedRead object. readBfaToc may be used to supply this information.
...	Further arguments to callBindingSites

Value

A list with components

binding	A data.frame with columns 'chromosome', 'position', 'score' and 'pvalue' indicating the centre of predicted binding sites together with their score and associated p-value.
score	A list with all calculated scores. One numeric vector per chromosome.
pval	A list with all corrected p-values. One numeric vector per chromosome.

Author(s)

Peter Humburg

References

~put references to the literature/web site here ~

See Also

callBindingSites for additional parameters.

Examples

```
## generate some simple artificial read data
set.seed(1)

## determine binding site locations
b <- sample(1:1e6, 5000)

## sample read locations
fwd <- unlist(lapply(b, function(x) sample((x-83):(x-73), 20, replace=TRUE)))
rev <- unlist(lapply(b, function(x) sample((x+73):(x+83), 20, replace=TRUE)))

## add some background noise
fwd <- c(fwd, sample(1:(1e6-25), 50000))
rev <- c(rev, sample(25:1e6, 50000))

## create data.frame with read positions as input to strandPileup
reads <- data.frame(chromosome="chr1", position=c(fwd, rev),
 length=25, strand=factor(rep(c("+", "-"), times=c(150000, 150000))))

## create object of class ReadCounts
readPile <- strandPileup(reads, chrLen=1e6, extend=1, plot=FALSE)
```

```
## predict binding site locations
bindScore <- simpleNucCall(readPile, bind=147, support=20, plot=FALSE)
```

`startScore` *Score potential protein binding sites*

Description

For each position in the genome this function computes a score indicating the likelihood that a protein binding site starts at that position.

Usage

```
startScore(data, b, support, background, bgCutoff, supCutoff)
```

Arguments

`data`	A two column matrix with read counts. The two columns correspond to reads on the forward and reverse strand respectively.
`b`	Length of binding region.
`support`	Length of support region.
`background`	Length of background window.
`bgCutoff`	Cutoff for the change in read rates between adjacent windows (see Details).
`supCutoff`	Cutoff for the change in read rates between support regions on forward and reverse strand (see Details).

Details

Robust estimates of read rates in background windows and support regions are obtained by limiting the difference between related estimates. Consider a forward support region of length 10 containing 20 reads. The maximum likelihood estimate for the rate parameter of the (assumed) underlying Poisson distribution is $\hat{\lambda} = \frac{20}{10} = 2$. If there are 50 reads in the reverse support region a robust estimate of the rate parameter is obtained as `max(50/10, qpois(supCutoff, lambda=lambda_hat))`

Value

Numeric vector with binding site scores.

Note

Instead of calling this function directly use `callBindingSites`.

Author(s)

Peter Humburg

See Also

callBindingSites

---

strandPileup *Strand specific read counts*

Description

Given a set of aligned reads this function computes the number of reads starting at each position in the genome.

Usage

```
## S4 method for signature 'AlignedRead'
strandPileup(aligned, chrLen, extend,  coords=c("leftmost", "fiveprime"),
  compress = TRUE, plot = TRUE, ask = FALSE, ...)
## S4 method for signature 'data.frame'
strandPileup(aligned, chrLen, extend, coords=c("leftmost", "fiveprime"),
  compress = TRUE, plot = TRUE, ask = FALSE, ...)
```

Arguments

aligned	An object containing information about aligned reads (see Details).
chrLen	A numeric vector giving the length of each chromosome.
extend	A numeric value indicating how far reads should be extended.
coords	A character value indicating the coordinate system to use. See coverage for details.
compress	Logical indicating whether read counts should be compressed.
plot	If this is TRUE (the default) read coverage is plotted for all chromosomes.
ask	Logical. Setting this to TRUE causes the system to wait for user input before displaying a new plot. See devAskNewPage.
...	Further arguments to coverage.

Details

The method for data.frame requires the column names to follow a strict naming scheme. Required columns are

'chromosome' A factor with chromosome names.

'strand' A factor with levels "-" and "+" indicating which strand the read mapped to.

'start' or 'position' Start position of read on chromosome.

'end' or 'length' End position of read or length of read respectively.

Value

An object of class ReadCounts.

Author(s)

Peter Humburg

See Also

coverage, AlignedRead, callBindingSites

Examples

```
## generate some very simple artificial read data
set.seed(1)
fwd <- sample(c(50:70, 250:270), 30, replace=TRUE)
rev <- sample(c(197:217, 347:417), 30, replace=TRUE)
## create data.frame with read positions as input to strandPileup
reads <- data.frame(chromosome="chr1", position=c(fwd, rev),
 length=25, strand=factor(rep(c("+", "-"), times=c(30, 30))))

readPile <- strandPileup(reads, chrLen=500, extend=1, plot=FALSE)
```

---

windowCounts *Summarize read counts in a sliding window*

Description

Read counts are summarized in a sliding window of variable size with variable overlap between windows.

Usage

```
windowCounts(reads, window = 1000, shift = 500, method = sum)
```

Arguments

reads	Numeric vector of read counts.
window	Width of window.
shift	Distance between consecutive window start positions.
method	Function used to produce a summary for each window. It should accept a single numeric vector as argument.

Value

If method returns a single value a vector of all window summaries is returned, otherwise the return value is a list with one component for each window.

Author(s)

Peter Humburg

Examples

```
## generate some very simple artificial read data
set.seed(1)
fwd <- sample(c(50:70, 250:270), 30, replace=TRUE)
rev <- sample(c(197:217, 347:417), 30, replace=TRUE)
## create data.frame with read positions as input to strandPileup
reads <- data.frame(chromosome="chr1", position=c(fwd, rev),
 length=25, strand=factor(rep(c("+", "-"), times=c(30, 30))))

## create object of class ReadCounts
readPile <- strandPileup(reads, chrLen=501, extend=1, plot=FALSE, compress=FALSE)

## get number of reads in sliding window
wdwCount <- windowCounts(apply(readPile[[1]], 1, sum), window=10, shift=5)
```

Index

∗ **classes**
BindScore-class, 6
ReadCounts-class, 26
RLEBindScore-class, 27
RLEReadCounts-class, 29
∗ **hplot**
plot,ReadCounts,missing-method, 21
plot-BindScore, 22
plotReads, 23
plotWindow, 24
∗ **htest**
callBindingSites-methods, 10
getCutoff, 19
simpleNucCall, 30
∗ **internal**
internal, 20
plotReads, 23
plotWindow, 24
∗ **methods**
callBindingSites-methods, 10
compress-methods, 14
decompress-methods, 15
∗ **misc**
alignFeature, 5
pickPeak, 20
windowCounts, 34
∗ **models**
callBindingSites-methods, 10
simpleNucCall, 30
startScore, 32
∗ **package**
ChIPseqR-package, 2
∗ **utilities**
acessors, 4
compress-BindScore, 13
compress-ReadCounts, 14
decompress, 15
exportBindSequence, 16
getBindCor, 17
getBindLen, 18
pos2gff, 25
strandPileup, 33
.plotReads, *22*
.plotReads *(*plotReads*)*, 23
.plotWindow, *22*
.plotWindow *(*plotWindow*)*, 24
[,BindScore,ANY,missing,missing-method *(*BindScore-class*)*, 6
[,ReadCounts,ANY,missing,missing-method *(*ReadCounts-class*)*, 26
[<-,ReadCounts,ANY,missing,ANY-method *(*ReadCounts-class*)*, 26
[<-,ReadCounts,ANY,missing-method *(*ReadCounts-class*)*, 26
[[,BindScore,ANY,missing-method *(*BindScore-class*)*, 6
[[,BindScore,ANY,numeric-method *(*BindScore-class*)*, 6
[[,ReadCounts,ANY,missing-method *(*ReadCounts-class*)*, 26
[[<-,ReadCounts,ANY,missing,ANY-method *(*ReadCounts-class*)*, 26
[[<-,ReadCounts,ANY,missing-method *(*ReadCounts-class*)*, 26
$,ReadCounts-method *(*ReadCounts-class*)*, 26
$<-,ReadCounts,ANY-method *(*ReadCounts-class*)*, 26
$<-,ReadCounts-method *(*ReadCounts-class*)*, 26

acessors, 4
AlignedRead, *3*, *11*, *12*, *30*, *31*, *34*
alignFeature, 5
allGroups *(*internal*)*, 20
allWords *(*internal*)*, 20
as.data.frame,BindScore-method *(*BindScore-class*)*, 6

binding (acessors), 4
binding,ANY-method (acessors), 4
binding,BindScore-method
(BindScore-class), 6
binding-methods (acessors), 4
BindScore, *5*, *12, 13*, *15, 16*, *21, 22*, *24*, *27, 28*
BindScore (BindScore-class), 6
BindScore-class, 6

callBindingSites, *3*, *9*, *19–21*, *27*, *30–34*
callBindingSites
(callBindingSites-methods), 10
callBindingSites,ANY-method
(callBindingSites-methods), 10
callBindingSites,character-method
(callBindingSites-methods), 10
callBindingSites,matrix-method
(callBindingSites-methods), 10
callBindingSites,ReadCounts-method
(callBindingSites-methods), 10
callBindingSites-methods, 10
ChIPseqR (ChIPseqR-package), 2
ChIPseqR-package, 2
chrLength (ReadCounts-class), 26
chrLength,BindScore-method
(BindScore-class), 6
chrLength,ReadCounts-method
(ReadCounts-class), 26
chrLength,RLEReadCounts-method
(RLEReadCounts-class), 29
compress, *15*, *27*
compress (compress-methods), 14
compress,BindScore-method
(compress-BindScore), 13
compress,ReadCounts-method
(compress-ReadCounts), 14
compress,RLEBindScore-method
(compress-methods), 14
compress,RLEReadCounts-method
(compress-methods), 14
compress-BindScore, 13
compress-methods, 14
compress-ReadCounts, 14
coverage, *33, 34*
cutoff (acessors), 4
cutoff,ANY-method (acessors), 4
cutoff,BindScore-method
(BindScore-class), 6
cutoff-methods (acessors), 4
cutoff<- (acessors), 4
cutoff<-,ANY-method (acessors), 4
cutoff<-,BindScore-method
(BindScore-class), 6
cutoff<--methods (acessors), 4

decompress, 15, *27*
decompress,ANY-method
(decompress-methods), 15
decompress,Rle-method
(decompress-methods), 15
decompress,RLEBindScore-method
(decompress), 15
decompress,RleList-method
(decompress-methods), 15
decompress,RLEReadCounts-method
(decompress), 15
decompress-methods, 15
devAskNewPage, *11*, *33*
diNucTest (internal), 20

exportBindSequence, 16

fftcor, *17*
fttcor, *17*

getBindCor, 17, *18*
getBindLen, *12*, *17*, 18
getCutoff, *3*, *12*, 19, *21*

head,BindScore-method
(BindScore-class), 6
hilbertImage, *22, 23*

internal, 20

lapply,BindScore-method
(BindScore-class), 6
lapply,ReadCounts-method
(ReadCounts-class), 26
length,BindScore-method
(BindScore-class), 6
length,ReadCounts-method
(ReadCounts-class), 26
length<-,BindScore-method
(BindScore-class), 6
length<-,ReadCounts-method
(ReadCounts-class), 26

max,BindScore-method (BindScore-class),
6

min,BindScore-method *(BindScore-class)*, 6

names,BindScore-method *(BindScore-class)*, 6
names,ReadCounts-method *(ReadCounts-class)*, 26
names<-,BindScore,ANY-method *(BindScore-class)*, 6
names<-,ReadCounts,ANY-method *(ReadCounts-class)*, 26
nreads *(ReadCounts-class)*, 26
nreads,ReadCounts-method *(ReadCounts-class)*, 26
nreads,RLEReadCounts-method *(RLEReadCounts-class)*, 29
nullDist *(acessors)*, 4
nullDist,ANY-method *(acessors)*, 4
nullDist,BindScore-method *(BindScore-class)*, 6
nullDist-methods *(acessors)*, 4
nullDist<- *(acessors)*, 4
nullDist<-,ANY-method *(acessors)*, 4
nullDist<-,BindScore-method *(BindScore-class)*, 6
nullDist<--methods *(acessors)*, 4

oligoNucTest *(internal)*, 20

peaks *(acessors)*, 4
peaks,ANY-method *(acessors)*, 4
peaks,BindScore-method *(BindScore-class)*, 6
peaks-methods *(acessors)*, 4
pickPeak, *3*, *12*, 20
plot,BindScore,missing-method *(plot-BindScore)*, 22
plot,ReadCounts,missing-method, 21
plot,RLEReadCounts,missing-method *(plot,ReadCounts,missing-method)*, 21
plot-BindScore, 22
plotBind *(internal)*, 20
plotFreq *(internal)*, 20
plotReads, 23
plotWindow, 24
pos2gff, 25
pvalue *(acessors)*, 4
pvalue,ANY-method *(acessors)*, 4
pvalue-methods *(acessors)*, 4

range,BindScore-method *(BindScore-class)*, 6
readAligned, *11*
readBfaToc, *11*, *31*
ReadCounts, *5*, *9*, *14*, *15*, *17*, *18*, *21*, *24*, *29*, *30*, *34*
ReadCounts *(ReadCounts-class)*, 26
ReadCounts-class, 26
Rle, *13*, *14*, *28*
RLEBindScore, *12–15*
RLEBindScore-class, 27
RleList, *13*, *14*, *29*, *30*
RLEReadCounts, *14*, *15*, *21*
RLEReadCounts-class, 29

sapply,ReadCounts-method *(ReadCounts-class)*, 26
score *(acessors)*, 4
score,BindScore-method *(BindScore-class)*, 6
ShortRead, *3*
simpleNucCall, *3*, *12*, 30
startScore, *3*, *12*, *21*, 32
strandPileup, *3*, *5*, *12*, *23*, *26*, *27*, *29*, 33
strandPileup,AlignedRead-method *(strandPileup)*, 33
strandPileup,data.frame-method *(strandPileup)*, 33
strandPileup-methods *(strandPileup)*, 33
support *(acessors)*, 4
support,ANY-method *(acessors)*, 4
support,BindScore-method *(BindScore-class)*, 6
support-methods *(acessors)*, 4

tail,BindScore-method *(BindScore-class)*, 6
triNucTest1 *(internal)*, 20
triNucTest2 *(internal)*, 20

views2nucFreq *(internal)*, 20

windowCounts, 34

XStringSet, *16*
XStringViews, *16*