

# Package ‘htSeqTools’

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**Title** Quality Control, Visualization and Processing for  
High-Throughput Sequencing data

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S4Vectors, IRanges, methods, MASS, BSgenome, GenomeInfoDb (>=  
1.1.3), GenomicRanges (>= 1.17.11)

**Enhances** parallel,multicore

**Description** We provide efficient, easy-to-use tools for  
High-Throughput Sequencing (ChIP-seq, RNAseq etc.). These  
include MDS plots (analogues to PCA), detecting inefficient  
immuno-precipitation or over-amplification artifacts, tools to  
identify and test for genomic regions with large accumulation  
of reads, and visualization of coverage profiles.

**License** GPL (>=2)

**LazyLoad** yes

**Collate** ClassDefinitions.R GenericDefs.R alignPeaks.R extendRanges.R  
filterDuplReads.R mergeRegions.R stdPeakLocation.R  
rowLogRegLRT.R enrichedRegions.R enrichedPeaks.R listOverlap.R  
tabDuplReads.R cmdsFit-class.R cmds.R fdrEnrichedCounts.R  
islandCounts.R countRepeats.R ssdCoverage.R giniCoverage.R  
regionsCoverage.R gridCover-methods.R enrichedChrRegions.R  
plotChrRegions.R coverageDiff.R plotMeanCoverage.R  
findPeakHeight.R

**biocViews** Sequencing, QualityControl

**NeedsCompilation** no

## R topics documented:

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|            |   |
|------------|---|
| alignPeaks | <i>Align peaks in a ChIP-Seq experiment by removing the strand specific bias.</i> |
|------------|---|

---

## Description

Align peaks in a ChIP-Seq experiment by removing the shift between reads aligned to the plus and the minus strands.

## Usage

```
alignPeaks(x, strand, npeaks = 1000, bandwidth = 150, mc.cores=1)
```

## Arguments

|           |   |
|-----------|---|
| x         | A list, RangedData or an IRangesList object containing the aligned reads in each chromosome.  |
| strand    | Strand that each read was aligned to. If x is of class list, strand can be a character vector of length 1 indicating the name of the field in x indicating the strand, i.e. x[[1]][[strand]] contains the strand information. |
| npeaks    | Number of peaks to be used to estimate the shift size.  |
| bandwidth | Only reads with distance less than bandwidth between them and their closest gene are used to estimate the shift size.   |
| mc.cores  | Number of cores to be used for parallel computing (passed on to mclapply). Only used if x is of class list.   |

## Details

The procedure detects the `npeaks` highest peaks (using reads from both strands simultaneously). Then it selects reads which are less than `bandwidth` base pairs away from any of the peaks. Then it computes (a) the average distance between reads on the plus strand and the closest peak, (b) the same distance for reads on the minus strand. The mean difference between (a) and (b) is the estimated shift size. Reads on the plus strand are shifted to the right, whereas reads on the minus strands are shifted to the left.

## Value

A `CompressedIRangesList` object with all reads shifted so that the strand specific bias is no longer present.

## Methods

`signature(x = "IRangesList", strand = "list")` Each element in `x` corresponds to a chromosome, and each range gives the start/end of a sequence. `strand` indicates the strand for the ranges in `x`.

`signature(x = "RangedData", strand = "character")` `x` gives read start and end positions, and `strand` gives the name of the variable in `values(x)` containing the strand information.

`signature(x = "list", strand = "character")` The method for `RangedData` is applied to each element in `x` separately, as each element may have a different strand-specific bias.

## Examples

```
#Generate 1000 reads containing strand-specific bias
st <- runif(1000,1,250)
strand <- rep(c('+','-'),each=500)
st[strand=='-'] <- st[strand=='-'] + runif(500,50,100)
x <- RangedData(IRanges(st,st+38),strand=strand)
#Estimate and remove the bias
xalign <- alignPeaks(x, strand='strand', npeaks=1)
```

---

cmds

*Classical Multi-Dimensional Scaling*

---

## Description

`cmds` obtain the coordinates of the elements in `x` in a `k` dimensional space which best approximate the distances between objects. For high-throughput sequencing data we define the distance between two samples as  $1 - \text{correlation}$  between their respective coverages. This provides PCA analog for sequencing data.

## Usage

```
cmds(x, k=2, logscale=TRUE, mc.cores=1, cor.method='pearson')
```

## Arguments

|            |  |
|------------|--|
| x          | A list object, e.g. each element containing the output of a sequencing run.  |
| k          | Dimensionality of the reconstructed space, typically set to 2 or 3.  |
| logscale   | If set to TRUE correlations are computed for $\log(x+1)$ .   |
| mc.cores   | Number of cores. Setting <code>mc.cores&gt;1</code> allows running computations in parallel. Setting <code>mc.cores</code> to too large a value may require a lot of memory. |
| cor.method | A character string indicating which correlation coefficient (or covariance) is to be computed. One of "pearson" (default), "kendall", or "spearman", can be abbreviated.     |

## Value

The function returns a `mdsFit` object, with slots `points` containing the coordinates, `d` with the distances between elements, `dapprox` with the distances between objects in the approximated space, and `R.square` indicating the percentage of variability in `d` accounted for by `dapprox`.

Since the coverage distribution is typically highly asymmetric, setting `logscale=TRUE` reduces the influence of the highest coverage regions in the distance computation, as this is based on the Pearson correlation coefficient.

## Methods

`signature(x = "list")` Use Classical Multi-Dimensional Scaling to plot each element of the `list` object in a `k`-dimensional space. The coverage is computed for each element in `x`, and the pairwise correlations between elements is used to define distances.

## Examples

```
data(htSample)
cmds1 <- cmds(htSample)

cmds1
plot(cmds1)
```

---

cmdsFit

*Classical Multi-Dimensional Scaling for a distance matrix*

---

## Description

`cmdsFit` obtains coordinates in a `k` dimensional space which best approximate the given distances between objects.

## Usage

```
cmdsFit(d, k=2, type='classic', add=FALSE, cor.method='pearson')
```

**Arguments**

|            |  |
|------------|--|
| d          | Distances between objects  |
| k          | Dimensionality of the reconstructed space, typically set to 2 or 3.  |
| type       | Set to "classic" to perform classical MDS (uses function cmdscale from package stats). Set to "isoMDS" to use Kruskal's non-metric MDS (uses function isoMDS from package MASS). |
| add        | Logical indicating if an additive constant $c^*$ should be computed, and added to the non-diagonal dissimilarities such that all $n-1$ eigenvalues are non-negative in cmdscale  |
| cor.method | A character string indicating which correlation coefficient (or covariance) is to be computed. One of "pearson" (default), "kendall", or "spearman", can be abbreviated.         |

**Value**

The function returns a cmdsFit object. See help("cmdsFit-class") for details.

**Methods**

signature(d = "matrix") Use Classical Multi-Dimensional Scaling to represent points in a k-dimensional space.

**Examples**

```
### Not run
#d <- matrix(c(0,5,10,5,0,15,10,15,0),byrow=TRUE,ncol=3)
#cmdsFit(d,add=TRUE)
```

---

cmdsFit-class

*Class "cmdsFit"*

---

**Description**

Classical Multi-Dimensional Scaling Fit. Function cmds creates object of this class.

**Objects from the Class**

Objects can be created by calls of the form new("cmdsFit", ...).

**Slots**

points: Object of class "matrix" with (x,y) coordinates in the approximated space.

d: Object of class "matrix" with original distances between individuals.

dapprox: Object of class "matrix" with distances between individuals in the approximated space.

R.square: Percentage of variability in d explained by dapprox (object of class "numeric")

**Methods**

There are show and plot methods defined for this class.

**Author(s)**

David Rossell

**See Also**

cmdscales from package base.

**Examples**

```
showClass("cmdsFit")
```

---

|                 |  |
|-----------------|--|
| countHitsWindow | <i>Compute number of hits in a moving window along the chromosome.</i> |
|-----------------|--|

---

**Description**

Computes a smoothed number of hits along the chromosome by using moving windows of user specified size.

**Usage**

```
countHitsWindow(x, chrLength, windowSize = 10^4 - 1)
```

**Arguments**

|            |   |
|------------|---|
| x          | Object containing hits (start, end and chromosome). Currently only RangedData objects are accepted. |
| chrLength  | Named vector indicating the length of each chromosome in base pairs.                                |
| windowSize | Size of the window used to smooth the hit count.  |

**Methods**

signature(x = "RangedData") x contains chromosome, start and end positions for each hit.

**Examples**

```
set.seed(1)
st <- round(rnorm(1000,500,100))
st[st>=10000] <- 10000
strand <- rep(c('+','-'),each=500)
space <- rep('chr1',length(st))
x <- RangedData(IRanges(st,st+38),strand=strand,space=space)
countHitsWindow(x, chrLength=c(chr1=10000), windowSize=99)
```

---

|              |   |
|--------------|---|
| coverageDiff | <i>Compute the difference in coverage between two objects</i> |
|--------------|---|

---

### Description

Computes coverage of sample1 minus coverage of sample2, taking into account that the chromosomes in sample1 and sample2 are not necessarily the same.

### Usage

```
coverageDiff(sample1, sample2, chrLength)
```

### Arguments

|           |  |
|-----------|--|
| sample1   | Object with reads from sample 1. Typically, a RangedData object.   |
| sample2   | Object with reads from sample 2. Typically, a RangedData object.   |
| chrLength | Named vector with chromosome lengths. This can be obtained from the Bioconductor annotation packages, e.g. <code>BSSgenome.Dmelanogaster.UCSC.dm3</code> for <code>drosophila melanogaster</code> , etc. |

### Details

Computation is restricted to chromosomes in `names(chrLength)`.

### Value

SimpleRleList with differences in coverage.

### Examples

```
sample1 <- RangedData(IRanges(1:10,11:20),space='chr1')
sample2 <- RangedData(IRanges(1:10,11:20),space=rep(c('chr1','chr2'),each=5))
chrLength <- c(50,25); names(chrLength) <- c('chr1','chr2')
coverageDiff(sample1,sample2,chrLength)
```

---

|                    |  |
|--------------------|--|
| enrichedChrRegions | <i>Find chromosomal regions with a high concentration of hits.</i> |
|--------------------|--|

---

### Description

This function looks for chromosomal regions where there is a large accumulation of hits, e.g. significant peaks in a chip-seq experiment or differentially expressed genes in an rna-seq or microarray experiment. Regions are found by computing number of hits in a moving window and selecting regions based on a FDR cutoff.

### Usage

```
enrichedChrRegions(hits1, hits2, chrLength, windowSize=10^4-1, fdr=0.05, nSims=10, mc.cores=1)
```

**Arguments**

|            |  |
|------------|--|
| hits1      | Object containing hits (chromosome, start, and end). Can be a GRanges or RangedData object.                    |
| hits2      | Optionally, another object containing hits. If specified, regions will be defined by comparing hits1 vs hits2. |
| chrLength  | Named vector indicating the length of each chromosome in base pairs  |
| windowSize | Size of the window used to smooth the hit count (see details)  |
| fdr        | Desired FDR level (see details)  |
| nSims      | Number of simulations to be used to estimate the FDR   |
| mc.cores   | Number of processors to be used in parallel computations (passed on to mclapply)                               |

**Details**

A smoothed number of hits is computed by counting the number of hits in a moving window of size `windowSize`. Notice that only the mid-point of each hit in `hits1` (and `hits2` if specified) is used. That is, hits are not treated as intervals but as being located at a single base pair.

If `hits2` is missing, regions with large smoothed number of hits are selected. To assess statistical significance, we generate hits (also 1 base pair long) randomly distributed along the genome and compute the smoothed number of hits. The number of simulated hits is set equal to `nrow(hits1)`. The process is repeated `nSims` times, resulting in several independent simulations. To estimate the FDR, several thresholds to define enriched chromosomal regions are considered. For each threshold  $t$ , we count the number of regions above the threshold in the observed data and in the simulations. For each threshold  $t$ , the FDR is estimated as the average number of regions with score  $\geq t$  in the simulations over the number of regions with score  $\geq t$  in the observed data.

If `hits2` is not missing, the difference in smoothed proportion of hits (i.e. the number of hits in the window divided by the overall number of hits) between the two groups is used as a test statistic. To assess statistical significance, we generate randomly scramble hits between sample 1 and sample 2 (maintaining the original number of hits in each sample), and we re-compute the test statistic. The FDR for a given threshold  $t$  is estimated as the number of bases in the simulated data with test statistic  $> t$  divided by number of bases in observed data with test statistic  $> t$ .

The lowest  $t$  with estimated FDR below `fdr` is used to define enriched chromosomal regions.

**Value**

Object of class GRanges (if input is GRanges) or RangedData (if input is RangedData) containing regions with smoothed hit count above the specified FDR level.

**Methods**

```
signature(hits1 = "GRanges", hits2 = "missing"), signature(hits1 = "RangedData", hits2 = "missing")
  Look for chromosome zones with a large number of hits reported in hits1.
```

```
signature(hits1 = "GRanges", hits2 = "GRanges"), signature(hits1 = "RangedData", hits2 = "RangedData")
  Look for chromosomal zones with a different density of hits in hits1 vs hits2.
```

**Examples**

```
set.seed(1)
st <- round(rnorm(100, 500, 100))
st[st > 10000] <- 10000
```



```
strand <- rep(c('+','-'),each=50)
hits1 <- GRanges('chr1', IRanges(st,st+38),strand=strand)
chrLength <- c(chr1=10000)
enrichedChrRegions(hits1,chrLength=chrLength, windowSize=99, nSims=1)
```

---

enrichedPeaks                      *Find peaks in sequencing experiments.*

---

## Description

Find peaks in significantly enriched regions found via enrichedRegions.

## Usage

```
enrichedPeaks(regions, sample1, sample2, minHeight=100, space, mc.cores=1)
```

## Arguments

|           |   |
|-----------|---|
| regions   | list or RangedData indicating the regions in which we wish to find peaks.   |
| sample1   | IRangesList or IRanges object containing start and end of sequences in sample 1.  |
| sample2   | Same for sample 2. May be left missing, in which case only sample1 is used to find peaks.   |
| minHeight | If sample2 is missing, peaks are defined as regions where the coverage in sample1 is greater or equal than minHeight. If sample2 is specified, the difference of coverage in sample1 minus sample2 must be greater or equal than minHeight.   |
| space     | Character text giving the name of the space for the RangedData object. Only used if sample1 and sample2 are of class RangedData, for list this is set up automatically.   |
| mc.cores  | If mc.cores>1 computations for each element in the IRangesList objects are performed in parallel (using the parallel function from package parallel). Notice: this option launches as many parallel processes as there are elements in x, which can place strong demands on the processor and memory. |

## Value

Object of class RangedData indicating peaks higher than minHeight. Only peaks overlapping with regions are reported. The maximum of the coverage in each selected peak is reported in the column height (coverage in sample1 - sample2 when sample2 is specified). The column region.pvalue returns the p-value associated to the region that the peak belongs to (i.e. it is inherited from regions). Therefore, notice that all peaks corresponding to a single region will present the same region.pvalue.

## Methods

signature(regions = "RangedData", sample1 = "IRanges", sample2 = "IRanges") sample1 indicates the start/end of reads in sample 1, and similarly for sample2. Only the subset of regions indicated by the argument space will be used.

signature(regions = "RangedData", sample1 = "IRanges", sample2 = "missing") sample1 indicates the start/end of reads in sample 1, and similarly for sample2. Only the subset of regions indicated by the argument space will be used.

`signature(regions = "RangedData", sample1 = "IRangesList", sample2 = "IRangesList")`  
 regions contains the regions of interest, sample1 and sample2 the reads in sample 1 and sample 2, respectively. `names(sample1)` and `names(sample2)` must correspond to the space names used in regions.

`signature(regions = "RangedData", sample1 = "IRangesList", sample2 = "missing")`  
 regions contains the regions of interest, sample1 the reads in sample 1. `names(sample1)` must correspond to the space names used in regions.

`signature(regions = "RangedData", sample1 = "RangedData", sample2 = "missing")` `space(sample1)` indicates the chromosome, and `start(sample1)` and `end(sample1)` indicate the start/end of the reads in sample 1.

`signature(regions = "RangedData", sample1 = "RangedData", sample2 = "RangedData")`  
`space(sample1)` indicates the chromosome, and `start(sample1)` and `end(sample1)` indicate the start/end of the reads in sample 1. Similarly for sample2.

## See Also

`enrichedRegions`

## Examples

```
set.seed(1)
st <- round(rnorm(1000,500,100))
strand <- rep(c('+','-'),each=500)
space <- rep('chr1',length(st))
sample1 <- RangedData(IRanges(st,st+38),strand=strand,space=space)
st <- round(runif(1000,1,1000))
sample2 <- RangedData(IRanges(st,st+38),strand=strand,space=space)

#Find enriched regions and call peaks
mappedreads <- c(sample1=nrow(sample1),sample2=nrow(sample2))
regions <- enrichedRegions(sample1,sample2,mappedreads=mappedreads,minReads=50)
peaks <- enrichedPeaks(regions,sample1=sample1,sample2=sample2,minHeight=50)
peaks <- peaks[width(peaks)>10,]
peaks

#Compute coverage in peaks
cover <- coverage(sample1)
coverinpeaks <- regionsCoverage(chr=space(peaks),start=start(peaks),end=end(peaks),cover=cover)

#Evaluate coverage in regular grid and plot
#Can be helpful fo clustering of peak profiles
coveringgrid <- gridCoverage(coverinpeaks)
coveringgrid
plot(coveringgrid)

#Standardize peak profiles dividing by max coverage
stdcoveringgrid <- stdGrid(coveringgrid, colname='maxCov')
stdcoveringgrid
```

---

enrichedRegions      *Find significantly enriched regions in sequencing experiments.*

---

### Description

Find regions with a significant accumulation of reads in a sequencing experiment.

### Usage

```
enrichedRegions(sample1, sample2, regions, minReads=10, mappedreads,
pvalFilter=0.05, exact=FALSE, p.adjust.method='none', twoTailed=FALSE,
mc.cores=1)
```

### Arguments

|                 |  |
|-----------------|--|
| sample1         | Either start and end of sequences in sample 1 (IRangesList, RangedData or IRanges object), or list with sequences for all samples (sample2 must be left missing in this case) .  |
| sample2         | Same for sample 2. Can be left missing.  |
| regions         | If specified, the analysis is restricted to the regions indicated in regions, using the value columns featuring the read count for each sample. This is mutually exclusive with the default behaviour where sample1 and/or sample2 are provided.   |
| minReads        | The regions to be tested for enrichment are those with coverage greater or equal than minReads. If sample1 is a list, the overall coverage adding all samples is used. Otherwise, if twoTailed is FALSE, only the reads in sample 1 are counted. If twoTailed is TRUE, the sum of reads in samples 1 and 2 are counted.  |
| mappedreads     | Number of mapped reads for the sample. Has to be of class integer. Will be used to compute RPKM.   |
| pvalFilter      | Only regions with P-value below pvalFilter are reported as being enriched.   |
| exact           | If set to TRUE, an exact test is used whenever some expected cell counts are 5 or less (chi-square test based on permutations if sample1 is a list object, Fisher's exact test otherwise), i.e. when the asymptotic chi-square/likelihood-ratio test calculations break down. Ignored if sample2 is missing, as in this case calculations are always exact.  |
| p.adjust.method | P-value adjustment method, passed on to p.adjust.  |
| twoTailed       | If set to FALSE, only regions with a higher concentration of reads in sample 1 than in sample 2 are reported. If set to TRUE, regions with higher concentration of sample 2 reads are also reported. Ignored if sample2 is missing.  |
| mc.cores        | If mc.cores is greater than 1, computations are performed in parallel for each element in the IRangesList objects. Whenever possible the mclapply function is used, therefore exactly mc.cores are used. For some signatures mclapply cannot be used, in which case the parallel function from package parallel is used. Note: the latter option launches as many parallel processes as there are elements in x, which can place strong demands on the processor and memory. |

## Details

The calculations depend on whether `sample2` is missing or not. Non-missing `sample2` case. First, regions with coverage above `minReads` are selected. Second, the number of reads falling in the selected regions are computed for sample 1 and sample 2. Third, the counts are compared via a chi-square test (with Yates continuity correction), which takes into account the total number of sequences in each sample. Finally, statistically significant regions are selected and returned in `RangedData` or `list` objects.

Missing `sample2`. First, regions with coverage above `minReads` are selected. Second, the number of reads in sample 1 falling in the selected regions is computed. Third, the proportion of reads in each region is tested for enrichment via a one-tailed Binomial exact test.

## Value

Object of class `RangedData` indicating the significantly enriched regions, the number of reads in each sample for those regions, the fold changes (adjusted considering the overall number of sequences in each sample) and the chi-square test P-values.

## Methods

`signature(sample1 = "missing", sample2 = "missing", regions = "RangedData") ranges(regions)` indicates the chromosome, start and end of genomic regions, while `values{regions}` should indicate the observed number of reads for each group in each region. `enrichedRegions` tests the null hypothesis that the proportion of reads in the region is equal across all groups via a likelihood-ratio test (or permutation-based chi-square for regions where the expected counts are below 5 for some group).

`signature(sample1 = "list", sample2 = "missing", regions = "missing")` Each element in `sample1` contains the read start/end of an individual sample. `enrichedRegions` identifies regions with high concentration of reads (across all samples) and then compares the counts across groups using a likelihood-ratio test (or permutation-based chi-square for regions where the expected counts are below 5 for some group).

`signature(sample1 = "RangedData", sample2 = "RangedData", regions = "missing") space(sample1)` indicates the chromosome, `start(sample1)` and `end(sample1)` the start/end position of the reads. Similarly for `sample2`. `enrichedRegions` identifies regions with high concentration of reads (across all samples) and then compares the counts across groups using a likelihood-ratio test (or permutation-based chi-square for regions where the expected counts are below 5 for some group).

`signature(sample1 = "RangedData", sample2 = "missing", regions = "missing") space(sample1)` indicates the chromosome, `start(sample1)` and `end(sample1)` the start/end position of the reads. `enrichedRegions` tests the null hypothesis that an unusually high proportion of reads has been observed in the region using an exact binomial test.

## Examples

```
set.seed(1)
st <- round(rnorm(1000,500,100))
strand <- rep(c('+', '-'),each=500)
space <- rep('chr1',length(st))
sample1 <- RangedData(IRanges(st,st+38),strand=strand,space=space)
st <- round(rnorm(1000,1000,100))
sample2 <- RangedData(IRanges(st,st+38),strand=strand,space=space)
enrichedRegions(sample1,sample2,twoTailed=TRUE)
```

---

|              |   |
|--------------|---|
| extendRanges | <i>Extend reads or sequences by a user-specified number of bases.</i> |
|--------------|---|

---

### Description

This function allows to extend ranges up to a user-specified length, which can be helpful in ChIP-seq analysis.

### Usage

```
extendRanges(x, seqLen = 200, chrLength, mc.cores=1)
```

### Arguments

|           |   |
|-----------|---|
| x         | Object containing reads.  |
| seqLen    | Desired sequence length after extension.  |
| chrLength | Integer vector indicating the length of each chromosome. names(chrLength) must match those in x. This argument is used to ensure that no reads are extended beyond the maximum chromosome length. |
| mc.cores  | Number of cores to use in parallel computations (passed on to mclapply).  |

### Value

A list of IRanges objects with extended sequence length.

### Methods

signature(x = "RangedData") space(x) indicates the chromosome, start(x) and end(x) the start/end positions of each read.

signature(x = "list") Each element in x is assumed to correspond to a different sample.

### Author(s)

David Rossell

### Examples

```
set.seed(1)
st <- round(rnorm(1000,500,100))
st[st>2000] <- 2000
strand <- rep(c('+','-'),each=500)
space <- rep('chr1',length(st))
sample1 <- RangedData(IRanges(st,st+38),strand=strand,space=space)
extendRanges(sample1, seqLen=200, chrLength=c(chr1=2000))
```

---

|                   |   |
|-------------------|---|
| fdrEnrichedCounts | <i>Posterior probability that a certain number of repeats are higher than expected by chance.</i> |
|-------------------|---|

---

### Description

Given a vector of number of repeats (e.g. there are 100 sequences appearing once, 50 sequences appearing twice etc.) the function computes the false discovery rate that each number of repeats is unusually high.

### Usage

```
fdrEnrichedCounts(counts, use=1:10, components=0, mc.cores=1)
```

### Arguments

|            |  |
|------------|--|
| counts     | vector with observed frequencies. The vector must have names. <code>tabDuplReads</code> function can be used for this purpose.   |
| use        | number of repeats to be used when estimating the null distribution. The number of repeats expected if no unusually high repeats are present. The first 10 are used by default.   |
| components | number of negative binomials that will be used to fit the null distribution. The default value is 1. This value has to be between 0 and 4. If 0 is given the optimal number of negative binomials is chosen using the Bayesian information criterion (BIC) |
| mc.cores   | number of cores to be used to compute calculations. This parameter will be passed to <code>mclapply</code>   |

### Details

The null distribution is a combination of  $n$  negative binomials where.  $n$  is assigned through the `components` parameter. If `components` is equal to 0 the optimal number of negative binomials is chosen using the Bayesian information criterion (BIC). The parameters of the null distribution are estimated from the number of observations with as many repeats as told in the `use` parameter. If `use` is 1:10 the null distribution will be estimated using repeats that appear 1 time, 2 times, ... or 10 times.

False discovery rate for usually high number of repeats is done following an empirical Bayes scheme similar to that in Efron et al. Let  $f_0(x)$  be the null distribution,  $f(x)$  be the overall distribution and  $(1-\pi_0)$  the proportion of unusually high repeats. We assume the two component mixture  $f(x) = \pi_0 f_0(x) + (1-\pi_0)f_1(x)$ . Essentially,  $f(x)$  is estimated from the data (imposing that  $f(x)$  must be monotone decreasing after its mode using `isoreg` from `packbase` base, to improve the estimate in the tails). Currently  $\pi_0$  is set to 1, i.e. its maximum possible value, which provides an upper bound for the FDR. The estimated false discovery rate for enrichment is  $1-\pi_0 * (1-\text{cumsum}(f_0(x))) / (1-\text{cumsum}(f(x)))$ . A monotone regression (`isoreg`) is applied to remove small random fluctuations in the estimated FDR and to guarantee that it decreases with  $x$ .

### Value

data.frame with the following columns:

|       |  |
|-------|--|
| pdfH0 | vector with pdf under the null hypothesis of no enrichment |
|-------|--|

pdfOverall      vector with pdf for mixture distribution  
 fdrEnriched     vector with false discovery rate that each count is significantly enriched

## References

Ji et al. An integrated software system for analyzing ChIP-chip and ChIP-seq data. *Nature Biotechnology*, 2008, 26, 1293-1300.

Efron et al. Empirical Bayes Analysis of a Microarray Experiment, *Journal of the American Statistical Association*, 2001, 96, 1151-1160.

## Examples

```
#Generate 1000 sequences repeated once, on the average
nrepeats <- c(rpois(10^4,1),rpois(10,10))
nrepeats <- nrepeats[nrepeats>0]
counts <- table(nrepeats)
barplot(counts) -> xaxis #observe bimodality around 10
fdrest <- fdrEnrichedCounts(counts,use=1:5,components=1)
cutoff <- xaxis[which(fdrest$fdrEnriched<0.95)[1]]
abline(v=cutoff,col=2)
text(cutoff,counts[1]/2,'cut-off',col=2)
head(fdrest)
```

---

filterDuplReads      *Detect and filter duplicated reads/sequences.*

---

## Description

filterDuplReads filters highly repeated sequences, i.e. with the same chromosome, start and end positions. As many such sequences are likely due to over-amplification artifacts, this can be a useful pre-processing step for ultra high-throughput sequencing data. A false discovery rate is computed for each number of repeats being unusually high. The reads with a higher false discovery rate will be removed. For more information on the false discovery rate calculation please read the fdrEnrichment manual.

tabDuplReads counts the number reads with no duplications, duplicated once, twice etc.

## Usage

```
filterDuplReads(x, maxRepeats, fdrOverAmp=0.01, negBinomUse=.999,components=0, mc.cores=1)
```

```
tabDuplReads(x, minRepeats=1, mc.cores=1)
```

## Arguments

x                    Object containing read locations. Currently methods for RangedData and list. Duplication is assessed based only on the space, start, end and x[['strand']], i.e. even if they are different based on other variables stored in values(x), the reads are considered duplicated and only the first appearance is returned.

maxRepeats        Reads appearing maxRepeats or more times will be excluded. If not specified, this is setup automatically based on fdrOverAmp.

|             |  |
|-------------|--|
| fdrOverAmp  | Reads with false discovery rate of being over-amplified greater than fdrOverAmp are excluded.  |
| negBinomUse | Number of counts that will be used to compute the null distribution. Using 1 - 1/1000 would mean that 99.9% of the reads will be used. The ones with higher number of repetitions are the excluded ones.   |
| components  | number of negative binomials that will be used to fit null distribution. The default value is 1. This value has to be between 0 and 4. If 0 is given the optimal number of negative binomials is chosen using the Bayesian information criterion (BIC) |
| mc.cores    | Number of cores to be used in parallel computing (passed on to mclapply).  |
| minRepeats  | The table is only produced for reads with at least minRepeats repeats.   |

### Value

filterDuplReads returns x without highly repetitive sequences, determined by maxRepeats or ppOverAmp.

tabDuplReads returns a table counting the number of sequences repeating 1 times, 2 times, 3 times etc.

### Methods

Methods for filterDuplReads and tabDuplReads

signature(x = "RangedData") Two reads are duplicated if they have the same space, start and end position.

signature(x = "list") The method is applied separately to each RangedData element in the list.

### Author(s)

Evarist Planet, David Rossell, Oscar Flores

### See Also

fdrEnrichedCounts to compute the posterior probability that a certain number of repeats is due to over-amplification.

### Examples

```
set.seed(1)
st <- round(rnorm(1000,500,100))
strand <- rep(c('+','-'),each=500)
space <- sample(c('chr1','chr2'),size=length(st),replace=TRUE)
sample1 <- RangedData(IRanges(st,st+38),strand=strand,space=space)

#Add artificial repeats
st <- rep(400,20)
repeats <- RangedData(IRanges(st,st+38),strand='+',space='chr1')
sample1 <- rbind(sample1,repeats)

filterDuplReads(sample1)
```



---

|                |   |
|----------------|---|
| findPeakHeight | <i>FDR and optimal minHeight value estimation for ChIP-Seq peak calling with enrichedPeaks.</i> |
|----------------|---|

---

### Description

Uses False Discovery Rate to estimate the optimal value of the minHeight parameter in the call to enrichedPeaks. False Discovery Rate is computed by swapping IP and Input samples and calculating the ratio of 'false' peaks identified for a given set of minHeight values.

### Usage

```
findPeakHeight(regions, sample1, sample2, hmin=5, hmax=200, myfdr=0.01, gridSize=25, space, mc.cores,
plotminHeight(x,...))
```

### Arguments

|          |   |
|----------|---|
| regions  | RangedData indicating the regions in which we wish to find peaks.   |
| sample1  | IRangesList or Rle object containing start and end of sequences in sample 1 (IP sample) or their coverage respectively.   |
| sample2  | Same for sample 2 (Control sample).   |
| hmin     | Minimum minHeight value to be considered for FDR estimation. Defaults to 5.   |
| hmax     | Maximum minHeight value to be considered for FDR estimation. Max coverage difference between sample 1 and sample 2 will be also calculated, and the minimum of the two will be used as hmax. This is done to avoid a skewed distribution of minHeight values to test for FDR estimation.              |
| myfdr    | Desired FDR cut-off.  |
| gridSize | Number of intermediate steps of minHeight threshold to consider between hmin and hmax. Since FDR and optimal minHeight estimation is done by actually performing peak calls, selecting a high value for gridSize can come at a big computational cost. Default value of 25 or close is recommended.   |
| space    | Character text giving the name of the space for the RangedData object. Only used if sample1 and sample2 are of class Rle, for RangedData and IRangesList this is set up automatically.  |
| mc.cores | If mc.cores>1 computations for each element in the IRangesList objects are performed in parallel (using the parallel function from package parallel). Notice: this option launches as many parallel processes as there are elements in x, which can place strong demands on the processor and memory. |
| x        | An object of class list as returned by the call to findPeakHeight.  |
| ...      | Other graphical arguments passed to function plot.  |

### Value

Object of class list with slots fdr, npeaks indicating peak calling FDR and number of peaks identified in the IP sample for each considered minHeight value and cut, opt with the desired FDR cut-off and the corresponding minHeight value to be used in the call to enrichedPeaks.

**Methods**

signature(regions = "RangedData", sample1 = "RangedData", sample2 = "RangedData")  
 sample1 indicates the start/end of reads in sample 1 (IP Sample), and similarly for sample2 (Control sample). Only the subset of regions indicated by the argument space will be used.

signature(regions = "RangedData", sample1 = "IRangesList", sample2 = "IRangesList")  
 regions contains the regions of interest, sample1 and sample2 the reads in sample 1 and sample 2, respectively. names(sample1) and names(sample2) must correspond to the space names used in regions.

signature(regions = "RangedData", sample1 = "Rle", sample2 = "Rle") regions contains the regions of interest, sample1 and sample2 the coverage for the reads in sample 1 and sample 2, respectively. names(sample1) and names(sample2) must correspond to the space names used in regions.

**See Also**

enrichedPeaks

**Examples**

```
set.seed(1)
st <- round(rnorm(1000,500,100))
strand <- rep(c('+','-'),each=500)
space <- rep('chr1',length(st))
sample1 <- RangedData(IRanges(st,st+38),strand=strand,space=space)
st <- round(runif(1000,1,1000))
sample2 <- RangedData(IRanges(st,st+38),strand=strand,space=space)

#Find enriched regions and call peaks
mappedreads <- c(sample1=nrow(sample1),sample2=nrow(sample2))
regions <- enrichedRegions(sample1,sample2,mappedreads=mappedreads,minReads=50)
minHeight <- findPeakHeight(regions,sample1=sample1,sample2=sample2)
plotminHeight(minHeight)
peaks <- enrichedPeaks(regions,sample1=sample1,sample2=sample2,minHeight=minHeight$opt)
peaks <- peaks[width(peaks)>10,]
peaks

#Compute coverage in peaks
cover <- coverage(sample1)
coverinpeaks <- regionsCoverage(chr=peaks$space,start=start(peaks),end=end(peaks),cover=cover)

#Evaluate coverage in regular grid and plot
#Can be helpful fo clustering of peak profiles
coveringgrid <- gridCoverage(coverinpeaks)
coveringgrid
plot(coveringgrid)

#Standardize peak profiles dividing by max coverage
stdcoveringgrid <- stdGrid(coveringgrid, colname='maxCov')
stdcoveringgrid
plot(stdcoveringgrid)
```

---

|              |                                  |
|--------------|----------------------------------|
| giniCoverage | <i>Compute Gini coefficient.</i> |
|--------------|----------------------------------|

---

### Description

Calculate Gini coefficient of High-throughput Sequencing aligned reads. The index provides a measure of "inequality" in read coverage which can be used for quality control purposes (see details).

### Usage

```
giniCoverage(sample, mc.cores = 1, mk.plot = FALSE, seqName = "missing", species="missing", chrLen
```

### Arguments

|            |   |
|------------|---|
| sample     | A RangedData or list object   |
| seqName    | If sample is a RangedData, name of sequence to use in plots   |
| mk.plot    | Logical. If TRUE, logarithm of coverage values' histogram and Lorenz Curve plot are plotted.                      |
| mc.cores   | If mc.cores is greater than 1, computations are performed in parallel for each element in the IRangesList object. |
| chrLengths | An integer array with lengths of chromosomes in sample for simulations of uniformly distributed reads.            |
| species    | A BSgenome species to obtain chromosome lengths for simulations of uniformly distributed reads.                   |
| numSim     | Number of simulations to perform in order to find the expected Gini coefficient.                                  |

### Details

The Gini coefficient provides a measure of "inequality" in read coverage. This can be used in any sequencing experiment where the goal is to find peaks, i.e. unusual accumulation of reads in some genomic regions. For instance, Chip-Seq etc. Typically these experiments will consist of samples of interest (e.g. immuno-precipitated) and controls. The samples of interest should exhibit higher peaks, whereas reads in the controls should show a more uniform distribution. Since the Gini coefficient can be seen as a measure of departure from uniformity, the coefficient should present smaller values in the control samples. Since the Gini coefficient depends on the number of reads per sample, a correction is performed by subtracting the Gini index from a sample with uniformly distributed reads.

### Value

If `mk.plot==FALSE`, the Gini index and adjusted Gini index for each element in the `list` or `RangedData` object.

If `mk.plot==TRUE`, a plot is produced showing the logarithm of coverage values' histogram and Lorenz Curve plot.

**Methods**

`signature(sample = "RangedData", mc.cores = "ANY", mk.plot = "ANY", seqName = "ANY", species = "ANY")`  
 Analyze a single `RangeData` object with `'chrLengths'` used for simulations (`'Species'` is ignored).

`signature(sample = "RangedData", mc.cores = "ANY", mk.plot = "ANY", seqName = "ANY", species = "BSgenome")`  
 Analyze a single `RangeData` object with chromosome lengths for simulations taken from `BSgenome` `'species'` (package must be installed).

`signature(sample = "RangedData", mc.cores = "ANY", mk.plot = "ANY", seqName = "ANY", species = "list")`  
 Analyze a single `RangeData` object with `'chrLengths'` used as chromosome lengths in simulations.

`signature(sample = "RangedData", mc.cores = "ANY", mk.plot = "ANY", seqName = "ANY", species = "list", largestEnd = TRUE)`  
 Analyze all `RangeData` objects from `sample` (list) with chromosome lengths for simulations taken as the largest end position of reads in each chromosome of all samples.

`signature(sample = "list", mc.cores = "ANY", mk.plot = "ANY", seqName = "ANY", species = "character")`  
 Analyze all `RangeData` objects from `sample` (list) with `'chrLengths'` used as chromosome lengths in simulations (`'Species'` is ignored).

`signature(sample = "list", mc.cores = "ANY", mk.plot = "ANY", seqName = "ANY", species = "character", largestEnd = TRUE)`  
 Analyze all `RangeData` objects from `sample` (list) with chromosome lengths for simulations taken from `BSgenome` `'species'` (package must be installed).

`signature(sample = "list", mc.cores = "ANY", mk.plot = "ANY", seqName = "ANY", species = "missing")`  
 Analyze all `RangeData` objects from `sample` (list) with `'chrLengths'` used as chromosome lengths in simulations.

`signature(sample = "list", mc.cores = "ANY", mk.plot = "ANY", seqName = "ANY", species = "missing", largestEnd = TRUE)`  
 Analyze all `RangeData` objects from `sample` (list) with chromosome lengths for simulations taken as the largest end position of reads in each chromosome of sample.

**Author(s)**

Camille Stephan-Otto

**References**

See the definition of the Gini coefficient and Lorenz curve at [http://en.wikipedia.org/wiki/Gini\\_coefficient](http://en.wikipedia.org/wiki/Gini_coefficient)

**See Also**

[ssdCoverage](#) for another measure of inequality in coverage.

**Examples**

```
set.seed(1)
peak1 <- round(rnorm(500, 100, 10))
peak1 <- RangedData(IRanges(peak1, peak1+38), space='chr1')
peak2 <- round(rnorm(500, 200, 10))
peak2 <- RangedData(IRanges(peak2, peak2+38), space='chr1')
ip <- rbind(peak1, peak2)
bg <- runif(1000, 1, 300)
bg <- RangedData(IRanges(bg, bg+38), space='chr1')

rd1 <- list(ip, bg)
ssdCoverage(rd1)
giniCoverage(rd1)
```

---

|                 |                   |
|-----------------|-------------------|
| gridCover-class | Class "gridCover" |
|-----------------|-------------------|

---

### Description

Objects of class `gridCover` store coverage information evaluated on a grid on pre-specified genomic regions.

### Objects from the Class

Objects of this class are returned by call to the function `gridCoverage`.

### Slots

**cover:** Object of class "matrix" with one row for each genomic region of interest, and 500 columns. Columns 1-100 contain the coverage in the promoter region (as specified in argument `promoterDistance` to `gridCoverage`). Columns 101-500 contain the coverage between start and end as indicated to `promoterDistance`.

**viewsInfo:** Object of class "DataFrame" with information relative to each region (strand, mean and maximum coverage).

### Methods

**plot** signature(x = "gridCover", y = "ANY"): Plot the coverage.

**lines** signature(x = "gridCover"): Add lines to an existing plot.

**show** signature(object = "gridCover"): Show method.

**stdGrid** signature(x = "gridCover"): Standardize the coverage by dividing by either the mean or the maximum coverage in each region.

**getViewInfo** signature(x = "gridCover"): Accessor for the `viewsInfo` slot.

**getCover** signature(x = "gridCover"): Accessor for the `cover` slot.

### Author(s)

David Rossell

### See Also

[regionsCoverage](#) to compute coverage on pre-specified regions, [gridCoverage](#) to compute coverage on a grid.

### Examples

```
##See help(gridCoverage)
```

---

|          |  |
|----------|--|
| htSample | <i>Example ChIP-sequencing data with 2 replicates per group obtained in two different dates.</i> |
|----------|--|

---

### Description

This `GRangesList` contains a subset of *drosophila melanogaster* ChIP-sequencing data obtained with the Illumina sequencer. An immuno-precipitated and a control input sample were obtained at two experimental dates (details not provided as this is still unpublished data). In order to save space and let the examples run quicker, only reads mapping to the first 500kb of chr2L are included.

### Usage

```
data(htSample)
```

### Format

`GRangesList` where each element contains reads from a different sample. `names(htSample)` indicate the group and batch (experimental date) that each sample corresponds to.

### Details

Data was pre-processed using the Illumina pipeline and mapped to the *drosophila melanogaster* dm3 genome using Bowtie. Only uniquely mapping sequences with at most 2 mismatches in the first 28 bases were kept. See the package vignette for some more details on this dataset.

### Examples

```
data(htSample)
htSample
```

---

|              |   |
|--------------|---|
| islandCounts | <i>Find genomic regions with high coverage and count number of reads overlapping each region in each sample</i> |
|--------------|---|

---

### Description

Finds genomic regions where the coverage is above a user-specified threshold and counts the number of ranges in each sample overlapping each region.

### Usage

```
islandCounts(x, minReads=10, mc.cores=1)
```

### Arguments

|                       |  |
|-----------------------|--|
| <code>x</code>        | RangedData or list containing the reads. If a list is provided, the overall coverage across all its elements is used to find the regions of interest, but individual counts are computed for each element in the list. |
| <code>minReads</code> | Only regions with coverage above <code>minReads</code> are considered.   |
| <code>mc.cores</code> | If <code>mc.cores &gt; 1</code> computations are performed in parallel, using function <code>mclapply</code> from package <code>parallel</code> .  |

**Details**

The output of `islandCounts` can be the input data for a number of downstream analysis methods. Although for a simple-minded analysis one could use `enrichedRegions`, one will usually want to use more sophisticated analyses (e.g. from packages `DEseq`, `BayesPeak`, `limma` etc.)

**Value**

Object of class `RangedData` indicating the regions with coverage above `minReads` and the number of reads overlapping each sample for those regions.

**Methods**

`signature(x = "RangedData")` `x` is assumed to contain the reads from a single sample. Genomic regions with high coverage will be detected and the number of reads overlapping these regions will be computed.

`signature(x = "list")` `x` is assumed to contain the reads for several samples, one sample in each element of the list. The overall coverage across all samples is computed by adding the coverage in the individual samples, and the regions with overall coverage above the user-specified threshold are selected. Then the number of reads overlapping each region is computed.

**Examples**

```
set.seed(1)
st <- round(rnorm(1000,500,100))
strand <- rep(c('+','-'),each=500)
space <- rep('chr1',length(st))
sample1 <- RangedData(IRanges(st,st+38),strand=strand,space=space)
st <- round(rnorm(1000,1000,100))
sample2 <- RangedData(IRanges(st,st+38),strand=strand,space=space)

regions <- islandCounts(list(sample1,sample2),minReads=50)
regions

#Plot coverage
plot(coverage(sample1)[[1]],type='l',xlim=c(0,2000))
lines(coverage(sample2)[[1]],col=2)
```

---

listOverlap

*Assess the overlap between two or three lists.*


---

**Description**

Assess the overlap between two or three lists, e.g. ChIP-Seq peaks vs. genes selected from a microarray, or peaks obtained in different experiments.

**Usage**

```
listOverlap(list1, list2, list3, univ, ...)
```

**Arguments**

|                    |  |
|--------------------|--|
| <code>list1</code> | Vector with elements in the first list. This can either be a character vector indicating the element names, or a named factor vector indicating some classification for the elements in the first list.                |
| <code>list2</code> | Vector with elements in the second list. This should be a character vector indicating the element names.   |
| <code>list3</code> | Vector with elements in the third list. This should be a character vector indicating the element names. The overlap assesment method used depends on whether this argument is specified or not. See details.           |
| <code>univ</code>  | character vector indicating the universe of all elements from which <code>list1</code> and <code>list2</code> were obtained. The overlap assessment depends on whether this argument is specified or not. See details. |
| <code>...</code>   | Further arguments to be passed on to <code>chisq.test</code> in 2 list overlapping.  |

**Details**

For `signature(list1='character', list2='character', list3='missing', univ='character')` the overlap is assessed with respect to the universe of all possible elements `univ`. That is, we count the number of elements that are common to `list1` and `list2`, those appearing only in either `list1` or `list2`, and those not appearing in either (but appearing in `univ`). A typical example: `list1` contains names of genes with a peak in ChIP-Seq experiment 1, `list2` names of genes with a peak in ChIP-Seq experiment 2, and `univ` the names of all genes in the organism.

For `signature(list1='character', list2='character', list3='character', univ='character')` the overlap is assessed by fitting and anova comparison of linear models. This is done to test whether 3-way overlap is significant with respect to the universe of all possible elements `univ` when compared to a model considering just the combination of 2-way overlapping. A typical example: `list1`, `list2` and `list3` contain names of genes with peaks in three different ChIP-Seq experiments, and `univ` the names of all genes in the organism.

For `signature(list1='factor', list2='character', univ='missing')` the distribution of `list1` is compared between elements appearing and not appearing in `list2`. A typical example: `list1` indicates the differential expression status for a number of genes, and `list2` contains the names of the genes which had a peak in a ChIP-Seq experiment.

**Value**

For comparison of 2 lists, an `htest` object from a chi-square test that evaluates if the two lists are statistically independent from each other. This is a named list: the observed overlap is stored in `observed` and the P-value in `p.value`.

For 3 list comparison, a `list` object containing the occurrence and frequency tables (`xtable`, `ftable`), the fitted linear models (`glm1`, `glm2`), and the anova P-value (`pvalue`).

**Methods**

`signature(list1 = "character", list2 = "character", list3 = "character", univ = "character")`  
Studies 3-way associations.

`signature(list1 = "character", list2 = "character", list3 = "missing", univ = "character")`  
Studies bivariate associations.

`signature(list1 = "factor", list2 = "character", list3 = "missing", univ = "missing")`  
Studies bivariate associations.



**Examples**

```
#Overlap between diff expression and chip-seq peaks
deStatus <- factor(c(0,0,0,0,1,1,1))
names(deStatus) <- paste('Gene',1:7)
peaks <- c('Gene 6','Gene 7')
ans <- listOverlap(list1=deStatus,list2=peaks)
ans$observed
ans$p.value

#Overlap between peaks obtained from two different experiments
peaks2 <- c('Gene 1','Gene 2','Gene 7')
univ <- paste('Gene',1:7)
ans <- listOverlap(list1=peaks,list2=peaks2,univ=univ)
ans$observed
ans$p.value
```

---

|              |  |
|--------------|--|
| mergeRegions | <i>Merge nearby chromosomal regions.</i> |
|--------------|--|

---

**Description**

Merges regions that are less than `maxDist` bases apart.

**Usage**

```
mergeRegions(intervals, chromosome, score, annot, aggregateFUN='median', maxDist=300)
```

**Arguments**

|                           |   |
|---------------------------|---|
| <code>intervals</code>    | Object indicating start and end of each region. It can either be a matrix, <code>data.frame</code> , <code>IRanges</code> , <code>RangedData</code> or an <code>RleViews</code> object. If a matrix or <code>data.frame</code> , it must have columns named <code>start</code> and <code>end</code> .                     |
| <code>chromosome</code>   | Chromosome that the region belongs to (optional). If supplied, must be of the same length as <code>start</code> and <code>end</code> .  |
| <code>score</code>        | Numerical score for each interval. Scores in merged intervals are aggregated using function <code>aggregateFUN</code> . If <code>intervals</code> is of class <code>RangedData</code> , this should be a character vector of length 1 indicating the name of the variable in <code>values(x)</code> containing the score. |
| <code>annot</code>        | Character indicating annotation information for each interval. Annotations in merged intervals are pasted in a single string (annotations appearing in more than one interval are only reported once in the merged interval).   |
| <code>aggregateFUN</code> | Function to aggregate score.  |
| <code>maxDist</code>      | Regions less than <code>maxDist</code> apart are merged into a single region  |

**Value**

The result is returned in a `data.frame` indicating the start and end of each merged interval. If the arguments were provided, the information in `chromosome`, `score` and `annot` is provided in additional columns. If the input argument `intervals` was of class `RangedData`, the results are returned in a `RangedData` object.

**Methods**

signature(intervals = "data.frame") intervals\$start and intervals\$end give the interval start/end positions.

signature(intervals = "IRanges") start(intervals) and end(intervals) give the interval start/end positions.

signature(intervals = "matrix") The columns start and end in intervals give the interval start/end positions

signature(intervals = "RangedData") start(intervals) and end(intervals) give the interval start/end positions.

signature(intervals = "RleViews") start(intervals) and end(intervals) give the interval start/end positions.

**Author(s)**

David Rossell

**Examples**

```
st <- c(10,20,1000)
intervals <- RangedData(IRanges(st,st+10),space='chr1')

intervals
mergeRegions(intervals,maxDist=300)
```

---

plot-methods

*Methods for Function plot in Package 'htSeqTools'*

---

**Description**

Methods for function plot in Package 'htSeqTools'

**Methods**

signature(x = "cmdsFit") Produces a Multi-Dimensional scaling plot. See cmds for details.

signature(x = "gridCover") Plots the average coverage for each point in the grid. See gridCover for details.

**Examples**

```
### Not run
#d <- matrix(c(0,5,10,5,0,15,10,15,0),byrow=TRUE,ncol=3)
#rownames(d) <- colnames(d) <- letters[1:3]
#fit1 <- cmdsFit(d,add=TRUE)
#plot(fit1)
```

---

|                |   |
|----------------|---|
| plotChrRegions | <i>Plot chromosomal regions of interest</i> |
|----------------|---|

---

**Description**

Produces a plot with all chromosomes for a given organism, marking regions of interest in a user-specified color.

**Usage**

```
plotChrRegions(regions, chrLength, markColor='red', ...)
```

**Arguments**

|           |   |
|-----------|---|
| regions   | RangedData object with chromosome, start and end positions (chromosome must be stored in space(regions)). |
| chrLength | Named integer vector with chromosome lengths in base pairs.   |
| markColor | Color to be used to mark the regions in the chromosome.   |
| ...       | Further parameters passed on to plot.   |

**Value**

This function produces a plot.

**Examples**

```
set.seed(1)
chr <- rep(c('chr1', 'chr2'), each=10)
chrLength <- c(chr1=10000, chr2=5000)
st <- c(runif(10, 1, 10000), runif(10, 1, 5000))
regions <- RangedData(IRanges(st, st+50), space=chr)

plotChrRegions(regions, chrLength=chrLength)
```

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|                 |  |
|-----------------|--|
| regionsCoverage | <i>Compute coverage on user specified genomic regions.</i> |
|-----------------|--|

---

**Description**

regionsCoverage computes coverage for user specified genomic regions.

gridCoverage evaluates the coverage on a regular grid with the same number of points for each region (facilitating further plotting, clustering etc).

stdGrid standardized the coverage by dividing by the average or maximum coverage at each region.

**Usage**

```
regionsCoverage(chr, start, end, cover)
```

```
gridCoverage(cover)
```

```
stdGrid(cover, colname="maxCov")
```

**Arguments**

|         |   |
|---------|---|
| chr     | Vector with chromosome names.   |
| start   | Vector with start position. start>end indicates that region is on the negative strand.  |
| end     | Vector with end position. start>end indicates that region is on the negative strand.  |
| cover   | For regionsCoverage, cover is an object of class RleList with the genome-wide coverage (typically obtained by a previous call to coverage). For gridCoverage this is the coverage evaluated at user-specified regions, as returned by regionsCoverage. For stdGrid this is the coverage evaluated on a grid, as returned by gridCoverage. |
| colname | Name of the column in cover@viewsInfo to be used for the standardizing. Currently only "meanCov" and "maxCov" are implemented.  |

**Value**

regionsCoverage returns a list with two components

|           |   |
|-----------|---|
| views     | RleViewsList with coverage evaluated at specified regions. Orientation is always so that start<end, i.e. For most practical purposes, regions on the reverse strand will need to be inverted. |
| viewsInfo | SplitDataFrameList containing information about each peak (chromosome, strand, mean and maximum coverage).  |

gridCoverage and stdGrid return an object of class gridCover. The slot cover is a matrix with the coverage evaluated on a grid of 500 equi-spaced points, whereas the slot viewsInfo is the same as that returned by regionsCoverage (see above). For regions between 100bp and 500bp long, a linear interpolation is used to evaluate the coverage on the 500 points grid. For regions less than 100bp long, NAs are returned.

**Methods**

Methods for regionsCoverage:

signature(chr = "ANY", start = "ANY", end = "ANY", cover = "RleList") Evaluates the coverage cover at the genomic positions specified by chr, start, end.

Methods for stdGrid:

signature(cover = "gridCover") Standardizes the coverage evaluated on a grid (typically, as returned by gridCoverage) by dividing by the mean or maximum coverage.

**See Also**

[gridCover-class](#)

**Examples**

```
#See help(enrichedPeaks)
```

---

|              |                                     |
|--------------|-------------------------------------|
| rowLogRegLRT | <i>Row-wise logistic regression</i> |
|--------------|-------------------------------------|

---

### Description

Row-wise logistic regressions are applied to a matrix with counts. For each row, an overall test comparing the column counts across columns is performed. Optionally, chi-square permutation tests are used when the expected counts are below 5 for some column.

### Usage

```
rowLogRegLRT(counts, exact = TRUE, p.adjust.method = "none")
```

### Arguments

|                 |  |
|-----------------|--|
| counts          | Matrix with counts   |
| exact           | If set to TRUE, an exact test is used whenever some expected cell counts are 5 or less |
| p.adjust.method | p-value adjustment method, passed on to p.adjust                                       |

### Details

For each column, the proportion of counts in each row (with respect to the overall counts in that column) is computed. Then a statistical comparison of these proportions across groups is performed via a likelihood-ratio test (if exact==TRUE a permutation based chi-square test is used whenever the expected counts in some column is below 5).

Notice that data from column  $j$  can be viewed as a multinomial distribution with probabilities  $p_j$ , where  $p_j$  is a vector of length  $nrow(x)$ . `rowLogRegLRT` tests the null hypothesis  $p1[i]=...pc[i]$  for  $i=1...nrow(x)$ , where  $c$  is  $ncol(x)$ . This actually ignores the multinomial sampling model and focuses on its binomial margins, which is a reasonable approximation when the number  $nrow(x)$  is large and substantially improves computation speed.

### Examples

```
#The first two rows present different counts across columns
#The last two columns do not
x <- matrix(c(70,10,10,10,35,35,10,10),ncol=2)
x
rowLogRegLRT(x)
```

---

|             |  |
|-------------|--|
| ssdCoverage | <i>Standardized SD of the genomic coverage</i> |
|-------------|--|

---

### Description

Compute variability of the genomic coverage, measured as standardized SD per thousand sequences (see details). For instance, this can measure how pronounced are the peaks in a ChIP-Seq experiments, which can serve as a quality control to detect inefficient immuno-precipitation.

**Usage**

```
ssdCoverage(x, mc.cores=1)
```

**Arguments**

|                       |  |
|-----------------------|--|
| <code>x</code>        | Object with ranges indicating the start and end of each read. Currently, <code>x</code> can be of class <code>list</code> , <code>RangedData</code> and <code>IRangesList</code> . |
| <code>mc.cores</code> | Set <code>mc.cores</code> to a value greater than 1 to perform computations in parallel, using package <code>mclapply</code> .   |

**Details**

`ssdCoverage` first computes the coverage for each sample and computes the standard deviation (SD) of the coverage. However, SD is not an appropriate measure of coverage unevenness, as its expected value is proportional to  $\sqrt{n}$ , where  $n$  is the number of reads (this can be seen with simple algebra).

`ssdCoverage` therefore reports  $1000 \cdot \text{SD} / \sqrt{n}$ , which can be interpreted as the standardized SD per thousand sequences.

**Value**

Numeric vector with coefficients of variation.

**Methods**

`signature(x = "IRangesList")` A single coefficient of variation is returned, as a weighted average of the coefficients of variation for each chromosome (weighted according to the chromosome length).

`signature(x = "RangedData")` The method for `IRangesList` is used on `ranges(x)`.

`signature(x = "list")` A vector with coefficients of variation for each element in `x` are returned, by repeatedly calling the method for `RangedData` objects. Use `mc.cores` to speed up computations with `mclapply`, but be careful as this requires more memory.

**Examples**

```
set.seed(1)
#Simulate IP data
peak1 <- round(rnorm(500,100,10))
peak1 <- RangedData(IRanges(peak1,peak1+38),space='chr1')
peak2 <- round(rnorm(500,200,10))
peak2 <- RangedData(IRanges(peak2,peak2+38),space='chr1')
ip <- rbind(peak1,peak2)

#Generate uniform background
bg <- runif(1000,1,300)
bg <- RangedData(IRanges(bg,bg+38),space='chr1')

rd1 <- list(ip,bg)
ssdCoverage(rd1)
giniCoverage(rd1)
```

---

stdPeakLocation      *Peak density with respect to closest gene.*

---

## Description

stdPeakLocation plots the density of peaks with respect to the genomic feature (e.g. gene) in standardized gene coordinates so that genes with different lengths are comparable.

PeakLocation produces the same plot in non-standardized coordinates (i.e. distances are measured in base pairs).

plotMeanCoverage plots the mean coverage in a series of selected genomic regions.

## Usage

```
stdPeakLocation(x, peakDistance=1000, startpos='start_position', endpos='end_position',
strand='strand', distance, main='', xlab='Distance relative to feature length', xaxt='n',
xlim=c(-1,2), densityType="kernel", nbreaks=10, ...)
```

```
PeakLocation(x, peakDistance=1000, startpos='start_position', endpos='end_position',
strand='strand', distance, main='', xlab='Distance (bp)',
densityType="kernel", breaks, ...)
```

```
plotMeanCoverage(cover, x, upstreambp=1000, downstreambp=5000,
startpos='start_position', endpos='end_position', normalize=FALSE,
smooth=FALSE, span=0.05, main='', xlab='(bp)', ylab='Average coverage', ...)
```

## Arguments

|              |  |
|--------------|--|
| x            | A RangedData or data.frame indicating peak start and end in start and end, and start and end of the closest genomic feature (e.g. gene) in startpos and endpos.                                  |
| cover        | An RleList object containing the coverage, as returned by the function coverage.   |
| peakDistance | Peaks more than peakDistance bases upstream or more than 3*peakDistance downstream of the closest feature are discarded.   |
| startpos     | Name of the variable storing the start position of the closest genomic feature.  |
| endpos       | Name of the variable storing the end position of the closest genomic feature.  |
| strand       | Name of the variable storing the strand for the closest genomic feature.   |
| distance     | Name of the variable indicating the distance between the peak and the closest genomic feature. If left missing the distance between the feature start and the mid-point of the peak is computed. |
| main         | Graphical parameter passed on to plot.   |
| xlab         | Graphical parameter passed on to plot.   |
| ylab         | Graphical parameter passed on to plot.   |
| xaxt         | Graphical parameter passed on to plot.   |
| xlim         | In stdPeakLocation the x-axis limit is set to xlim*peakDistance.   |
| densityType  | If we want a density plot or a histogram. Has to be one of "kernel" (for the density plot) or "hist" for the histogram.  |

|              |   |
|--------------|---|
| nbreaks      | Number of breaks to be used. It will not be used if densityType is different from "hist".   |
| breaks       | This parameter will be passed to the hist plotting function. It will not be used if densityType is different from "hist".   |
| upstreambp   | Number of bp upstream of the TSS where the coverage should be computed  |
| downstreambp | Number of bp downstream of the TSS where the coverage should be computed  |
| normalize    | When set to TRUE the average coverage in each position is divided by the average across all positions. This is useful when trying to super-impose data from several experiments that had different read coverage. |
| smooth       | If set to TRUE, the average coverage is smooth by calling loess.  |
| span         | Parameter controlling smoothing, passed on to loess. Larger values indicate more smoothing.   |
| ...          | Further parameters passed on to plot.   |

### Value

This function produces a density plot.

### Methods

Methods for stdPeakLocation, PeakLocation

signature(x = "data.frame") The data frame should contain columns named start and end indicating the peak location, txStart, txEnd indicating transcription start/end of the closest gene and strand indicating the strand.

signature(x = "RangedData") start(x) and end(x) indicate the peak location. x should contain variables x[['txStart']], x[['txEnd']] indicating the transcription start/end of the closest gene and x[['strand']] indicating the strand.

Methods for plotMeanCoverage

signature(cover="RleList", x="RangedData") cover contains the coverage and x the genomic regions of interest.

### Examples

```
#Generate synthetic peaks
set.seed(1)
st <- runif(100,1,1000)
en <- st+runif(length(st),25,100)
peaks <- RangedData(IRanges(st,en),space='chr1')

#Assign distance to closest gene
#(typically one would call annotatePeakInBatch
#from package ChIPpeakAnno to do this)
peaks[['start_position']] <- start(peaks) + runif(nrow(peaks),-500,1000)
peaks[['end_position']] <- peaks[['start_position']] + 500
peaks[['distance']] <- peaks[['start_position']] - start(peaks)
peaks[['strand']] <- sample(c('+','-'),nrow(peaks),replace=TRUE)
PeakLocation(peaks,peakDistance=1000)
```



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