

Package ‘csaw’

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Description Detection of differentially bound regions in ChIP-seq data
with sliding windows, with methods for normalization and proper
FDR control.

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checkBimodality	<i>Check bimodality of regions</i>
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Description

Compute the maximum bimodality score across all base pairs in each region.

Usage

```
checkBimodality(bam.files, regions, width=100, param=readParam(),
  prior.count=2, invert=FALSE)
```

Arguments

bam.files	a character vector containing paths to sorted and indexed BAM files
regions	a GRanges object specifying the regions over which bimodality is to be calculated
width	an integer scalar or list indicating the span with which to compute bimodality
param	a readParam object containing read extraction parameters
prior.count	a numeric scalar specifying the prior count to compute bimodality scores
invert	a logical scalar indicating whether bimodality score should be inverted

Details

Consider a base position x . This function counts the number of forward- and reverse-strand reads within the interval $[x-width+1, x]$. It then calculates the forward:reverse ratio after adding `prior.count` to both counts. This is repeated for the interval $[x, x+width-1]$, and the reverse:forward ratio is then computed. The smaller of these two ratios is used as the bimodality score.

Sites with high bimodality scores will be enriched for forward- and reverse-strand enrichment on the left and right of the site, respectively. Given a genomic region, this function will treat each base position as a site. The largest bimodality score across all positions will be reported for each region.

The idea is to assist with the identification of transcription factor binding sites, which exhibit strong strand bimodality. The function will be less useful for broad targets like histone marks.

If multiple `bam.files` are specified, they are effectively pooled so that counting uses all reads in all files. A separate value of `width` can be specified for each library, to account for differences in fragmentation – see the `ext` argument for [windowCounts](#) for more details. In practice, this is usually unnecessary. Setting `width` to the average fragment length yields satisfactory results in most cases.

If `invert` is set, the bimodality score will be flipped around, i.e., it will be maximized when reverse-strand coverage dominates on the left, and forward-strand coverage dominates on the right. This is designed for use in CAGE analyses where this inverted bimodality is symptomatic of enhancer RNAs.

Value

A numeric vector containing the maximum bimodality score across all bases in each region.

Author(s)

Aaron Lun

Examples

```
bamFiles <- system.file("exdata", c("rep1.bam", "rep2.bam"), package="csaw")
incoming <- GRanges(c('chrA', 'chrA', 'chrB', 'chrC'),
  IRanges(c(1, 500, 100, 1000), c(100, 580, 500, 1500)))

checkBimodality(bamFiles, incoming)
checkBimodality(bamFiles, incoming, width=200)
checkBimodality(bamFiles, incoming, param=readParam(minq=20, dedup=TRUE))
checkBimodality(bamFiles, incoming, prior.count=5)

# Works on PE data; scores are computed from paired reads.
bamFile <- system.file("exdata", "pet.bam", package="csaw")
checkBimodality(bamFile, incoming[1:3], param=readParam(pe="both"))
checkBimodality(bamFile, incoming[1:3], param=readParam(pe="both", max.frag=100))
```

clusterFDR

Compute the cluster-level FDR

Description

Compute the FDR across clusters based on the test-level FDR threshold

Usage

```
clusterFDR(ids, threshold, weight=NULL)
controlClusterFDR(target, adjp, FUN, ..., weight=NULL, grid.param=NULL)
```

Arguments

<code>ids</code>	an integer vector of cluster IDs for each significant test below <code>threshold</code>
<code>threshold</code>	a numeric scalar, specifying the FDR threshold used to define the significant tests
<code>target</code>	a numeric scalar specifying the desired cluster-level FDR threshold
<code>adjp</code>	a numeric vector of window-level adjusted p-values
<code>FUN</code>	a clustering function that takes a logical vector indicating which windows are significant, and returns an integer vector of cluster IDs (see below)
<code>...</code>	additional arguments to be passed to <code>FUN</code>
<code>weight</code>	a numeric vector of frequency weights, for internal use
<code>grid.param</code>	a named list of grid search parameters, see Details

Value

For `clusterFDR`, a numeric scalar is returned as the cluster-level FDR.

For `controlClusterFDR`, a list is returned containing two numeric scalars – `threshold`, the window-level FDR threshold to control the cluster-level FDR near `target`; and `FDR`, the estimate of the cluster-level FDR corresponding to `threshold`.

Definition of the cluster-level FDR

The `clusterFDR` function computes an informal estimate of the cluster-level FDR, where each cluster is formed by aggregating only significant tests. In the context of ChIP-seq, each significant test refers to a DB window that is detected at a FDR below `threshold`. The idea is to obtain an error rate while reporting the precise coordinates of a DB subinterval in a complex region.

The cluster-level FDR is defined as the proportion of reported clusters that have no true positives. Simply using `threshold` is not appropriate, as the cluster- and window-level FDRs are not equivalent. This function also differs from the standard pipeline that is based on `combineTests`. Specifically, region definition in the standard pipeline must be independent of DB so that precise coordinates of the DB subinterval cannot be reported. This is overcome by clustering directly on DB windows and applying post-hoc control of the cluster-level FDR.

Note that the calculation of the cluster-level FDR here is not statistically rigorous. In particular, the observed number of false positive tests is estimated based on `threshold` and the total number of significant tests. This is not guaranteed to be an upper bound, especially when the observed window-level FDR is variable. Thus, users should use the standard `combineTests`-based pipeline wherever possible. Clustering on significant windows should only be performed where the precise coordinates of the DB subinterval are important for interpretation.

Searching for the best threshold

`controlClusterFDR` will identify the window-level FDR threshold required to control the cluster-level FDR at `target`. This is necessary as the latter is not a simple function of the former. In fact, the discontinuous nature of the cluster-level FDR means that the returned value may not be below `target`. Rather, the closest estimate is returned, e.g., for a `target` of 0.05, a value of 0.051 is preferred to a value of 0.02, even though only the latter is less than the `target`.

A grid search is used to identify clusters of significant windows at each window-level threshold, and the corresponding cluster-level FDR is computed with `clusterFDR`. At each iteration, the grid point with the closest cluster-level FDR to `target` is chosen and the grid is recentered around that point. The size of the grid is also scaled down to provide greater resolution. Users can tune the settings of the grid search by specifying elements in `grid.param` as:

length: an integer scalar specifying the length of the grid, defaults to 21

range: a numeric scalar indicating the range of the grid in logit units, defaults to 20

iter: an integer scalar indicating the number of iterations of the grid search

scale: a numeric scalar specifying how the range of the grid should be downscaled at each iteration

The FUN argument should be a function that accepts a logical vector specifying significance, and returns an integer vector of cluster IDs. If, for example, it accepts an input vector `ix`, then the output should contain cluster IDs corresponding to the entries of `which(ix)`. This is because cluster IDs are only defined for significant tests, given that only those tests are used for clustering.

Note about weights

In both functions, the `weight` argument is assumed to contain frequency weights of significant tests/windows. For example, a weight of 2 for a test would be equivalent to repeating that test (i.e., repeating the same window so it shows up twice in your analysis). These weights should be the same as those used during weighted FDR control to compute adjusted p-values. In general, users should not set this argument unless you know what you're doing.

Author(s)

Aaron Lun

See Also

[mergeWindows](#), [combineTests](#), [clusterWindows](#)

Examples

```
# Setting up the windows and p-values.
set.seed(100)
windows <- GRanges("chrA", IRanges(1:1000, 1:1000))
test.p <- runif(1000)
test.p[c(1:10, 100:110, 220:240)] <- 0 # 3 significant subintervals.

# Defining significant windows.
threshold <- 0.05
is.sig <- p.adjust(test.p, method="BH") <= threshold

# Assuming that we only cluster significant windows.
merged <- mergeWindows(windows[is.sig], tol=0)
clusterFDR(merged$id, threshold)

# Setting up another example with more subintervals.
test.p <- runif(1000)
test.p[rep(1:2, 50) + rep(0:49, each=2) * 20] <- 0
adj.p <- p.adjust(test.p, method="BH")
clusterFUN <- function(x) { mergeWindows(windows[x], tol=0)$id }
controlClusterFDR(0.05, adj.p, clusterFUN)
```

`clusterWindows`*Cluster DB windows into clusters*

Description

Clusters significant windows into clusters while controlling the cluster-level FDR.

Usage

```
clusterWindows(regions, tab, target, pval.col=NULL,  
              fc.col=NA, tol, ..., weight=NULL, grid.param=NULL)
```

Arguments

<code>regions</code>	a <code>GRanges</code> or <code>RangedSummarizedExperiment</code> object containing window coordinates
<code>tab</code>	a dataframe of results with a <code>PValue</code> field for each window
<code>target</code>	a numeric scalar indicating the desired cluster-level FDR
<code>pval.col</code>	a string or integer scalar specifying the column of <code>tab</code> with the p-values
<code>fc.col</code>	a string or integer scalar specifying the column of <code>tab</code> with the log-fold changes
<code>tol, ...</code>	arguments to be passed to mergeWindows
<code>weight, grid.param</code>	arguments to be passed to controlClusterFDR

Details

Windows are identified as DB based on the adjusted p-values in `tab`. Only these DB windows are then used directly for clustering via [mergeWindows](#). This identifies DB regions consisting solely of DB windows. If `tol` is not specified, it is set to 100 bp by default and a warning is raised. If `fc.col` is used to specify the column of log-fold changes, clusters are formed according to the sign of the log-fold change in [mergeWindows](#).

DB-based clustering is obviously not blind to the DB status, so standard methods for FDR control are not valid. Instead, post-hoc control of the cluster-level FDR is applied by using [controlClusterFDR](#). This aims to control the cluster-level FDR at `target` (which is set to 0.05 if not specified). The aim is to provide some interpretable results when DB-blind clustering is not appropriate, e.g., for diffuse marks involving long stretches of the genome. Reporting each marked stretch in its entirety would be cumbersome, so this method allows the DB subintervals to be identified directly.

Value

A named list similar to that reported by [mergeWindows](#) with an ID vector in `id` and region coordinates of each cluster in `region`. Non-significant windows are marked with NA values in `ids`. An additional element `FDR` is also included, representing the estimate of the cluster-level FDR for the returned regions.

Author(s)

Aaron Lun

See Also

[mergeWindows](#), [controlClusterFDR](#)

Examples

```
set.seed(10)
x <- round(runif(100, 100, 1000))
gr <- GRanges("chrA", IRanges(x, x+5))
tab <- data.frame(PValue=rbeta(length(x), 1, 50), logFC=rnorm(length(x)))

clusterWindows(gr, tab, target=0.05, tol=10)
clusterWindows(gr, tab, target=0.05, tol=10, fc.col="logFC")
```

combineTests

Combine statistics across multiple tests

Description

Combines p-values across clustered tests using Simes' method to control the cluster FDR.

Usage

```
combineTests(ids, tab, weight=NULL, pval.col=NULL, fc.col=NULL)
```

Arguments

ids	an integer vector containing the cluster ID for each test
tab	a dataframe of results with PValue and at least one logFC field for each test
weight	a numeric vector of weights for each window, defaults to 1 for each test
pval.col	an integer scalar specifying the column of tab containing the p-values, or a character string containing the name of that column
fc.col	an integer vector specifying the columns of tab containing the log-fold changes, or a character vector containing the names of those columns

Details

This function uses Simes' procedure to compute the combined p-value for each cluster of tests with the same value of ids. Each combined p-value represents evidence against the global null hypothesis, i.e., all individual nulls are true in each cluster. This may be more relevant than examining each test individually when multiple tests in a cluster represent parts of the same underlying event, e.g., genomic regions consisting of clusters of windows. The BH method is also applied to control the FDR across all clusters.

The importance of each test within a cluster can be adjusted by supplying different relative weight values. This may be useful for downweighting low-confidence tests, e.g., those in repeat regions. In Simes' procedure, weights are interpreted as relative frequencies of the tests in each cluster. Note that these weights have no effect between clusters and will not be used to adjust the computed FDR.

By default, the relevant fields in tab are identified by matching the column names to their expected values. Multiple fields in tab containing the logFC substring are allowed, e.g., to accommodate ANOVA-like contrasts. If the column names are different from what is expected, specification of

the correct columns can be performed using `pval.col` and `fc.col`. This will overwrite any internal selection of the appropriate fields.

This function will report the number of windows with log-fold changes above 0.5 and below -0.5, to give some indication of whether binding increases or decreases in the cluster. If a cluster contains non-negligible numbers of up and down windows, this indicates that there may be a complex DB event within that cluster. Similarly, complex DB may be present if the total number of windows is larger than the number of windows in either category (i.e., change is not consistent across the cluster). Note that the threshold of 0.5 is arbitrary and has no impact on the significance calculations.

A simple clustering approach for windows is provided in [mergeWindows](#). However, anything can be used so long as it does not compromise type I error control, e.g., promoters, gene bodies, independently called peaks. Any tests with NA values for `ids` will be ignored.

Value

A dataframe with one row per cluster and the numeric fields `PValue`, the combined p-value; and `FDR`, the q-value corresponding to the combined p-value. An integer field `nWindows` specifies the total number of windows in each cluster. There are also two integer fields `*.up` and `*.down` for each log-FC column in `tab`, containing the number of windows with log-FCs above 0.5 or below -0.5, respectively. The name of each row represents the ID of the corresponding cluster.

Author(s)

Aaron Lun

References

- Simes RJ (1986). An improved Bonferroni procedure for multiple tests of significance. *Biometrika* 73, 751-754.
- Benjamini Y and Hochberg Y (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Series B* 57, 289-300.
- Benjamini Y and Hochberg Y (1997). Multiple hypotheses testing with weights. *Scand. J. Stat.* 24, 407-418.
- Lun ATL and Smyth GK (2014). De novo detection of differentially bound regions for ChIP-seq data using peaks and windows: controlling error rates correctly. *Nucleic Acids Res.* 42, e95

See Also

[mergeWindows](#)

Examples

```
ids <- round(runif(100, 1, 10))
tab <- data.frame(logFC=rnorm(100), logCPM=rnorm(100), PValue=rbeta(100, 1, 2))
combined <- combineTests(ids, tab)
head(combined)

# With window weighting.
w <- round(runif(100, 1, 5))
combined <- combineTests(ids, tab, weight=w)
head(combined)

# With multiple log-FCs.
```



```
tab$logFC.whee <- rnorm(100, 5)
combined <- combineTests(ids, tab)
head(combined)

# Manual specification of column IDs.
combined <- combineTests(ids, tab, fc.col=c(1,4), pval.col=3)
head(combined)

combined <- combineTests(ids, tab, fc.col="logFC.whee", pval.col="PValue")
head(combined)
```

consolidateClusters *Consolidate DB clusters*

Description

Consolidate DB results from multiple analyses with cluster-level FDR control.

Usage

```
consolidateClusters(data.list, result.list, equiweight=TRUE, ...)
```

Arguments

<code>data.list</code>	a list of <code>RangedSummarizedExperiment</code> and/or <code>GRanges</code> objects
<code>result.list</code>	a list of data frames containing the DB test results for each entry of <code>data.list</code>
<code>equiweight</code>	a logical scalar indicating whether equal weighting from each analysis should be enforced
<code>...</code>	arguments to be passed to clusterWindows

Details

This function consolidates DB results from multiple analyses, typically involving different window sizes. The aim is to provide comprehensive detection of DB at a range of spatial resolutions. Significant windows from each analysis are identified and used for clustering with [clusterWindows](#). This represents the post-hoc counterpart to [consolidateSizes](#).

Some effort is required to equalize the contribution of the results from each analysis. This is done by setting `equiweight=TRUE`, where the weight of each window is inversely proportional to the number of windows from that analysis. These weights are used as frequency weights for window-level FDR control (to identify DB windows prior to clustering). Otherwise, the final results would be dominated by large number of small windows.

Users can cluster by the sign of log-fold changes, to obtain clusters of DB windows of the same sign. However, note that nested windows with opposite signs may give unintuitive results - see [mergeWindows](#) for details.

Value

A named list is returned containing:

<code>id</code>	a list of integer vectors indicating the cluster ID for each window in <code>data.list</code>
<code>region</code>	a <code>GRanges</code> object containing the coordinates for each cluster
<code>FDR</code>	a numeric scalar containing the cluster-level FDR estimate

Author(s)

Aaron Lun

See Also[clusterWindows](#), [consolidateSizes](#)**Examples**

```
# Making up some GRanges.
low <- GRanges("chrA", IRanges(runif(100, 1, 1000), width=5))
med <- GRanges("chrA", IRanges(runif(40, 1, 1000), width=10))
high <- GRanges("chrA", IRanges(runif(10, 1, 1000), width=20))

# Making up some DB results.
dbl <- data.frame(logFC=rnorm(length(low)), PValue=rbeta(length(low), 1, 20))
dbm <- data.frame(logFC=rnorm(length(med)), PValue=rbeta(length(med), 1, 20))
dbh <- data.frame(logFC=rnorm(length(high)), PValue=rbeta(length(high), 1, 20))
result.list <- list(dbl, dbm, dbh)

# Consolidating.
cons <- consolidateClusters(list(low, med, high), result.list, tol=20)
cons$region
cons$id
cons$FDR

# Without weights.
cons <- consolidateClusters(list(low, med, high), result.list, tol=20, equiweight=FALSE)
cons$FDR

# Using the signs.
cons <- consolidateClusters(list(low, med, high), result.list, tol=20, fc.col="logFC")
```

<code>consolidateSizes</code>	<i>Consolidate window sizes</i>
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Description

Consolidate DB results from multiple window sizes.

Usage

```
consolidateSizes(data.list, result.list, equiweight=TRUE, merge.args=list(),
  combine.args=list(), region=NULL, overlap.args=list())
```

Arguments

<code>data.list</code>	a list of <code>RangedSummarizedExperiment</code> and/or <code>GRanges</code> objects
<code>result.list</code>	a list of data frames containing the DB test results for each entry of <code>data.list</code>
<code>equiweight</code>	a logical scalar indicating whether equal weighting from each window size should be enforced
<code>merge.args</code>	a named list of parameters to pass to mergeWindows

`combine.args` a named list of parameters to pass to [combineTests](#)
`region` a GRanges object specifying regions of interest for overlapping with windows
`overlap.args` a named list of parameters to pass to [findOverlaps](#)

Details

This function consolidates DB results from multiple window sizes, to provide comprehensive detection of DB at a range of spatial resolutions. RangedSummarizedExperiment objects can be generated by running [windowCounts](#) at a range of window sizes. Windows of all sizes are clustered together through [mergeWindows](#), and the p-values from all windows in each cluster are combined using [combineTests](#). If `merge.args$tol` is not specified, it is automatically set to 100 bp and a warning is raised.

Some effort is required to equalize the contribution of each window size to the combined p-value of each cluster. This is done by setting `equiweight=TRUE`, where the weight of each window is inversely proportional to the number of windows of that size. Otherwise, the combined p-value would be determined by numerous small windows in each cluster.

If `region` is specified, each entry of `region` is defined as a cluster. Windows in each cluster are identified using [findOverlaps](#), and consolidation is performed across multiple window sizes like before. Note that the returned `id` will be a list of [Hits](#) objects rather than integer vectors, as one window (subject) may overlap more than one region (query).

Value

A named list is returned containing:

`id` a list of integer vectors, where each vector corresponds to an object in `data.list`; the entries of the vector specify the cluster to which each row of that object is assigned
`region` a GRanges object containing the genomic coordinates of the clusters of merged windows (or other regions, if `region` is specified)
`table` a data frame containing the combined DB results for each region

Author(s)

Aaron Lun

See Also

[windowCounts](#), [mergeWindows](#), [findOverlaps](#), [combineTests](#)

Examples

```

bamFiles <- system.file("exdata", c("rep1.bam", "rep2.bam"), package="csaw")
low <- windowCounts(bamFiles, width=1, filter=1)
med <- windowCounts(bamFiles, width=100, filter=1)
high <- windowCounts(bamFiles, width=500, filter=1)

# Making up some DB results.
dbl <- data.frame(logFC=rnorm(nrow(low)), PValue=runif(nrow(low)), logCPM=0)
dbm <- data.frame(logFC=rnorm(nrow(med)), PValue=runif(nrow(med)), logCPM=0)
dbh <- data.frame(logFC=rnorm(nrow(high)), PValue=runif(nrow(high)), logCPM=0)

# Consolidating.

```

```

cons <- consolidateSizes(list(low, med, high), list(dbl, dbm, dbh),
merge.args=list(tol=100, max.width=300))
cons$region
cons$id
cons$table

# Without weights.
cons <- consolidateSizes(list(low, med, high), list(dbl, dbm, dbh),
merge.args=list(tol=100, max.width=300), equiweight=FALSE)
cons$table

# Trying with a custom region.
of.interest <- GRanges(c('chrA', 'chrA', 'chrB', 'chrC'),
IRanges(c(1, 500, 100, 1000), c(200, 1000, 700, 1500)))
cons <- consolidateSizes(list(low, med, high), list(dbl, dbm, dbh),
region=of.interest)
cons$table
cons$id

# Trying with limited numbers of overlaps; empty regions are ignored.
cons <- consolidateSizes(list(low[1,], med[1,], high[1,]),
list(dbl[1,], dbm[1,], dbh[1,]), region=of.interest)
cons$region
cons$table

```

correlateReads

Compute correlation coefficients between reads

Description

Computes the auto- or cross-correlation coefficients between read positions across a set of delay intervals.

Usage

```
correlateReads(bam.files, max.dist=1000, cross=TRUE, param=readParam())
```

Arguments

bam.files	a character vector containing paths to sorted and indexed BAM files
max.dist	integer scalar specifying the maximum delay distance over which correlation coefficients will be calculated
cross	a logical scalar specifying whether cross-correlations should be computed
param	a readParam object containing read extraction parameters

Details

If cross=TRUE, reads are separated into those mapping on the forward and reverse strands. Positions on the forward strand are shifted forward by a delay interval. The chromosome-wide correlation coefficient between the shifted forward positions and the original reverse positions are computed. This is repeated for all delay intervals less than max.dist. A weighted mean for the cross-correlation is taken across all chromosomes, with weighting based on the number of reads.

Cross-correlation plots can be used to check the quality of immunoprecipitation for ChIP-Seq experiments involving transcription factors or punctate histone marks. Strong immunoprecipitation should result in a peak at a delay corresponding to the fragment length. A spike may also be observed at the delay corresponding to the read length. This is probably an artefact of the mapping process where unique mapping occurs to the same sequence on each strand.

By default, marked duplicate reads are removed from each BAM file prior to calculation of coefficients. This is strongly recommended, even if the rest of the analysis will be performed with duplicates retained. Otherwise, the read length spike will dominate the plot, such that the fragment length peak will no longer be easily visible.

If `cross=FALSE`, auto-correlation coefficients are computed without use of strand information. This is designed to guide estimation of the average width of enrichment for diffuse histone marks. For example, the width can be defined as the delay distance at which the autocorrelations become negligible. However, this tends to be ineffective in practice as diffuse marks tend to have very weak correlations to begin with.

If multiple BAM files are specified in `bam.files`, the reads from all libraries are pooled prior to calculation of the correlation coefficients. This is convenient for determining the average correlation profile across an entire dataset. Separate calculations for each file will require multiple calls to `correlateReads`.

Paired-end data is also supported, whereby correlations are computed using only those reads in proper pairs. This may be less meaningful as the presence of proper pairs will inevitably result in a strong peak at the fragment length. Instead, IP efficiency can be diagnosed by treating paired-end data as single-end, e.g., with `pe="first"` in [readParam](#).

Value

A numeric vector of length `max.dist+1` containing the correlation coefficients for each delay interval from 0 to `max.dist`.

Author(s)

Aaron Lun

References

Kharchenko PV, Tolstorukov MY and Park, PJ (2008). Design and analysis of ChIP-seq experiments for DNA-binding proteins. *Nat. Biotechnol.* 26, 1351-1359.

See Also

[ccf](#)

Examples

```
n <- 20
bamFile <- system.file("exdata", "rep1.bam", package="csaw")
par(mfrow=c(2,2))

x <- correlateReads(bamFile, max.dist=n)
plot(0:n, x, xlab="delay (bp)", ylab="ccf")

x <- correlateReads(bamFile, max.dist=n, param=readParam(dedup=TRUE))
plot(0:n, x, xlab="delay (bp)", ylab="ccf")
```

```
x <- correlateReads(bamFile, max.dist=n, cross=FALSE)
plot(0:n, x, xlab="delay (bp)", ylab="acf")

# Also works on paired-end data.
bamFile <- system.file("exdata", "pet.bam", package="csaw")
x <- correlateReads(bamFile, param=readParam(pe="both"))
head(x)
```

csawUsersGuide

View csaw user's guide

Description

Finds the location of the user's guide and opens it for viewing.

Usage

```
csawUsersGuide(view=TRUE)
```

Arguments

`view` logical scalar specifying whether the document should be opened

Details

The `csaw` package is designed for de novo detection of differentially bound regions from CHIP-seq data. It provides methods for window-based counting, normalization, filtering and statistical analyses via `edgeR`. The user guide for this package can be obtained by running this function.

For non-Windows operating systems, the PDF viewer is taken from `Sys.getenv("R_PDFVIEWER")`. This can be changed to `x` by using `Sys.putenv(R_PDFVIEWER=x)`. For Windows, the default viewer will be selected to open the file.

Note that the user's guide is not a true vignette as it is not generated using [Sweave](#) when the package is built. This is due to the time-consuming nature of the code when run on realistic case studies.

Value

A character string giving the file location. If `view=TRUE`, the system's default PDF document reader is started and the user's guide is opened.

Author(s)

Aaron Lun

See Also

[system](#)

Examples

```
# To get the location:
csawUsersGuide(view=FALSE)
# To open in pdf viewer:
## Not run: csawUsersGuide()
```

detailRanges	<i>Add annotation to ranges</i>
--------------	---------------------------------

Description

Add detailed exon-based annotation to specified genomic regions.

Usage

```
detailRanges(incoming, txdb, orgdb, dist=5000, promoter=c(3000, 1000),
             max.intron=1e6, key.field="ENTREZID", name.field="SYMBOL",
             ignore.strand=TRUE)
```

Arguments

<code>incoming</code>	a GRanges object containing the ranges to be annotated
<code>txdb</code>	a TranscriptDb object for the genome of interest
<code>orgdb</code>	a genome wide annotation object for the genome of interest
<code>dist</code>	an integer scalar specifying the flanking distance to annotate
<code>promoter</code>	an integer vector of length 2, where first and second values define the promoter as some distance upstream and downstream from the TSS, respectively
<code>max.intron</code>	an integer scalar indicating the maximum distance between exons
<code>key.field</code>	a character scalar specifying the keytype for name extraction
<code>name.field</code>	a character scalar or vector specifying the column(s) to use as the gene name
<code>ignore.strand</code>	a logical scalar indicating whether strandedness in <code>incoming</code> should be ignored

Details

This function adds exon-based annotations to a given set of genomic regions, in the form of compact character strings specifying the features overlapping and flanking each region. The aim is to determine the genic context of empirically identified regions. This allows some basic biological interpretation of binding/markings in those regions. All neighboring genes within a specified range are reported, rather than just the closest gene to the region. If a region in `incoming` is stranded and `ignore.strand=FALSE`, annotated features will only be reported if they lie on the same strand as that region.

If `incoming` is missing, then the annotation will be provided directly to the user in the form of a GRanges object. This may be more useful when further work on the annotation is required. Exon numbers are provided in the metadata with promoters and gene bodies labelled as 0 and -1, respectively. Overlaps to introns can be identified by finding those regions that overlap with gene bodies but not with any of the corresponding exons.

Value

If `incoming` is not provided, a GRanges object will be returned containing ranges for the exons, promoters and gene bodies. Gene keys (e.g., Entrez IDs) are stored as row names. Gene symbols, exon numbers and internal groupings (for exons of genes with multiple genomic locations) are also stored as metadata.

If incoming is a GRanges object, a list will be returned with overlap, left and right elements. Each element is a character vector of length equal to the number of ranges in incoming. Each non-empty string records the gene symbol, the overlapped exons and the strand. For left and right, the gap between the range and the annotated feature is also included.

Explanation of fields

For annotated features overlapping a region, the character string in the overlap output vector will be of the form GENE|EXONS|STRAND. GENE is the gene symbol by default, but reverts to <XXX> if no symbol is defined for a gene with the Entrez ID XXX. The EXONS indicate the exon or range of exons that are overlapped. The STRAND is, obviously, the strand on which the gene is coded. For annotated regions flanking the region within a distance of dist, the character string in the left or right output vectors will have an additional [DIST] value. This represents the gap between the edge of the region and the closest exon for that gene.

Exons are numbered in order of increasing start or end position for genes on the forward or reverse strands, respectively. Promoters are defined as the region of length promoter upstream of the gene TSS, itself defined as the start of the first exon (for genes on the forward strand) or the end of the last exon (otherwise). All promoters are marked as exon 0 for simplicity. Exon ranges in EXON are reported from as a comma-separated list where stretches of consecutive exons are summarized into a range. If the region overlaps an intron, it is labelled with I in EXON. No intronic overlaps are reported if there is an exonic overlap.

Note that promoter and intronic annotations are only reported for the overlap vector to reduce redundancy in the output. For example, it makes little sense to report that the region is both flanking and overlapping an intron. Similarly, the value of DIST is more relevant when it is reported to the nearest exon rather than to an intron (in which case, the distance would be zero if the intron overlaps the region). In cases where the distance is reported to the first exon, it can be used to refine the choice of promoter.

Other options

The max.intron value is necessary to deal with genes that have ambiguous locations on the genome. If a gene has exons on different chromosomes, its location is uncertain and the gene is partitioned into two sets of exons for separate processing. However, this is less obvious when the ambiguous locations belong to the same chromosome. The max.intron value protects against excessively large genes that may occur from considering those locations as a single transcriptional unit. Exons are partitioned into two (or more) internal groupings for further processing.

The default settings for key.field and name.field will work for human and mouse genomes, but may not work for other organisms. The key.field should refer to the key type in the OrgDb object, and also correspond to the GENEID of the TxDb object. For example, in S. cerevisiae, key.field is set to "ORF" while name.field is set to "GENENAME". If multiple entries are supplied in name.field, the value of GENE is defined as a semicolon-separated list of each of those entries.

Author(s)

Aaron Lun

Examples

```
require(org.Mm.eg.db)
require(TxDb.Mmusculus.UCSC.mm10.knownGene)
```



```

current <- readRDS(system.file("exdata", "exrange.rds", package="csaw"))
output <- detailRanges(current, orgdb=org.Mm.eg.db,
  txdb=TxDB.Mmusculus.UCSC.mm10.knownGene)
head(output$overlap)
head(output$right)
head(output$left)

detailRanges(txdb=TxDB.Mmusculus.UCSC.mm10.knownGene, orgdb=org.Mm.eg.db)

## Not run:
output <- detailRanges(current, txdb=TxDB.Mmusculus.UCSC.mm10.knownGene,
  orgdb=org.Mm.eg.db, name.field=c("ENTREZID"))
head(output$overlap)

output <- detailRanges(current, txdb=TxDB.Mmusculus.UCSC.mm10.knownGene,
  orgdb=org.Mm.eg.db, name.field=c("SYMBOL", "ENTREZID"))
head(output$overlap)

## End(Not run)

```

extractReads	<i>Extract reads from a BAM file</i>
--------------	--------------------------------------

Description

Extract reads from a BAM file with the specified parameter settings.

Usage

```
extractReads(bam.file, region, ext=NA, param=readParam(), as.reads=FALSE)
```

Arguments

bam.file	a character string containing the path to a sorted and indexed BAM file
region	a GRanges object of length 1 describing the region of interest
ext	an integer scalar or list specifying the fragment length for directional read extension
param	a readParam object specifying how reads should be extracted
as.reads	a logical scalar indicating whether reads should be returned instead of fragments for paired-end data

Details

This function extracts the reads from a BAM file overlapping a given genomic interval. The interpretation of the values in `param` is the same as that throughout the package. The aim is to supply the raw data for visualization, in a manner that maintains consistency with the rest of the analysis.

If `pe!="both"` in `param`, stranded intervals corresponding to the reads will be reported. If `ext` is not NA, directional read extension will also be performed – see [windowCounts](#) for more details. If `pe="both"`, intervals are unstranded and correspond to fragments from proper pairs.

If `as.reads=TRUE` and `pe="both"`, the reads in each proper pair are returned directly as a `GRanges-List` of length 2. The two internal `GRanges` are of the same length and contain the forward and reverse reads for each proper pair in parallel. In other words, the `n`th elements of the first and second `GRanges` represent the `n`th proper pair.

Any strandedness of region is ignored. If strand-specific extraction is desired, this can be done by setting `param$forward` via [reform](#). Alternatively, the returned `GRanges` can be filtered to retain only the desired strand.

Value

If `pe!="both"` or `as.reads=FALSE`, a `GRanges` object is returned containing the read (for single-end data) or fragment intervals (for paired-end data). If `pe="both"` and `as.reads=TRUE`, a `GRanges-List` is returned containing the paired reads – see [Details](#).

Author(s)

Aaron Lun

See Also

[readParam](#), [windowCounts](#)

Examples

```
bamFile <- system.file("exdata", "rep1.bam", package="csaw")
extractReads(bamFile, GRanges("chrA", IRanges(100, 500)))
extractReads(bamFile, GRanges("chrA", IRanges(100, 500)),
  param=readParam(dedup=TRUE))
extractReads(bamFile, GRanges("chrB", IRanges(100, 500)))

bamFile <- system.file("exdata", "pet.bam", package="csaw")
extractReads(bamFile, GRanges("chrB", IRanges(100, 500)),
  param=readParam(pe="both"))
extractReads(bamFile, GRanges("chrB", IRanges(100, 500)),
  param=readParam(pe="first"))

# Extracting as reads.
extractReads(bamFile, GRanges("chrB", IRanges(100, 500)),
  param=readParam(pe="both"), as.reads=TRUE)

# Dealing with the extension length.
bamFile <- system.file("exdata", "rep1.bam", package="csaw")
my.reg <- GRanges("chrA", IRanges(10, 200))
extractReads(bamFile, my.reg)
extractReads(bamFile, my.reg, ext=100)
```

Description

Convenience function to compute filter statistics for windows, based on proportions or using enrichment over background.

Usage

```
filterWindows(data, background, type="global", prior.count=2, norm.fac=NULL)
```

Arguments

<code>data</code>	a <code>RangedSummarizedExperiment</code> object containing window- or bin-level counts
<code>background</code>	another <code>RangedSummarizedExperiment</code> object, containing counts for background regions when <code>type!="proportion"</code>
<code>type</code>	a character string specifying the type of filtering to perform; can be any of <code>c("global", "local", "control", "proportion")</code>
<code>prior.count</code>	a numeric scalar, specifying the prior count to use in <code>aveLogCPM</code>
<code>norm.fac</code>	a numeric scalar representing the normalization factor between ChIP and control samples, or a list of two <code>RangedSummarizedExperiment</code> objects; only used when <code>type="control"</code>

Details

Proportion-based filtering supposes that a certain percentage of the genome is genuinely bound. If `type="proportion"`, the filter statistic is defined as the ratio of the rank to the total number of windows. Rank is in ascending order, i.e., higher abundance windows have higher ratios. Windows are retained that have rank ratios above a threshold, e.g., 0.99 if 1% of the genome is assumed to be bound.

All other values of `type` will perform background-based filtering, where abundances of the windows are compared to those of putative background regions. The filter statistic are generally defined as the difference between window and background abundances, i.e., the log-fold increase in the counts. Windows can be filtered to retain those with large filter statistics, to select those that are more likely to contain genuine binding sites. The differences between the methods center around how the background abundances are obtained for each window.

If `type="global"`, the median average abundance across the genome is used as a global estimate of the background abundance. This should be used when background contains unfiltered counts for large (2 - 10 kbp) genomic bins, from which the background abundance can be computed. The filter statistic for each window is defined as the difference between the window abundance and the global background. If background is not supplied, the background abundance is directly computed from entries in data.

If `type="local"`, the counts of each row in data are subtracted from those of the corresponding row in background. The average abundance of the remaining counts is computed and used as the background abundance. The filter statistic is defined by subtracting the background abundance from the corresponding window abundance for each row. This is designed to be used when background contains counts for expanded windows, to determine the local background estimate.

If `type="control"`, the background abundance is defined as the average abundance of each row in background. The filter statistic is defined as the difference between the average abundance of each row in data and that of the corresponding row in background. This is designed to be used when background contains read counts for each window in the control sample(s). Unlike `type="local"`, there is no subtraction of the counts in background prior to computing the average abundance.

Value

A list is returned with abundances, the average abundance of each entry in data; `filter`, the filter statistic for the given type; and, for `type!="proportion"`, `back.abundances`, the average abundance of each entry in background.

Additional details

Proportion and global background filtering are dependent on the total number of windows/bins in the genome. However, empty windows or bins are automatically discarded in `windowCounts` (exacerbated if `filter` is set above unity). This will result in underestimation of the rank or overestimation of the global background. To avoid this, the total number of windows or bins is inferred from the spacing.

For background-based methods, the abundances of large bins or regions in background must be rescaled for comparison to those of smaller windows - see `getWidths` and `scaledAverage` for more details. In particular, the effective width of the window is often larger than `width`, due to the counting of fragments rather than reads. The fragment length is extracted from `data$ext` and `background$ext`, though users will need to set `data$rlen` or `background$rlen` for unextended reads (i.e., `ext=NA`).

The `prior.count` protects against inflated log-fold increases when the background counts are near zero. A low prior is sufficient if background has large counts, which is usually the case for wide regions. Otherwise, `prior.count` should be increased to a larger value like 5. This may be necessary in `type="control"`, where background contains counts for small windows in the control sample.

Normalization for composition bias

When `type=="control"`, ChIP samples will be compared to control samples to compute the filter statistic. Composition biases are likely to be present, where increased binding at some loci reduces coverage of other loci in the ChIP samples. This incorrectly results in smaller filter statistics for the latter loci, as the fold-change over the input is reduced. To correct for this, a normalization factor between ChIP and control samples can be computed from `norm.fac`.

Users should supply a list containing two `RangedSummarizedExperiment` objects, each containing the counts for large (~10 kbp) bins. The first and second objects should contain counts for the libraries in `data` and `background`, respectively. The median difference in the average abundance between the two objects is then computed across all bins. This is used as a normalization factor to correct the filter statistics for each window.

The idea is that most bins represent background regions, such that a systematic difference in abundance between ChIP and control should represent the composition bias. Alternatively, a normalization factor can be specified manually in `norm.fac`. This should represent the scaling factor for the library sizes of the control samples relative to the ChIP samples, i.e., the “average” fold increase in coverage of the control over ChIP for the background regions. However, if the value is left as `NULL`, a warning will be issued.

See Also

[windowCounts](#), [aveLogCPM](#), [getWidths](#), [scaledAverage](#)

Examples

```
bamFiles <- system.file("exdata", c("rep1.bam", "rep2.bam"), package="csaw")
data <- windowCounts(bamFiles, filter=1)

# Proportion-based (keeping top 1%)
stats <- filterWindows(data, type="proportion")
head(stats$filter)
keep <- stats$filter > 0.99
new.data <- data[keep,]

# Global background-based (keeping fold-change above 3).
```

```

background <- windowCounts(bamFiles, bin=TRUE, width=300)
stats <- filterWindows(data, background, type="global")
head(stats$filter)
keep <- stats$filter > log2(3)

# Local background-based.
locality <- regionCounts(bamFiles, resize(rowRanges(data), fix="center", 300))
stats <- filterWindows(data, locality, type="local")
head(stats$filter)
keep <- stats$filter > log2(3)

# Control-based (pretend "rep.2" is a control library).
stats <- filterWindows(data[,1], data[,2], type="control", prior.count=5)
head(stats$filter)
keep <- stats$filter > log2(3)

# Control-based with binning for normalization.
binned <- windowCounts(bamFiles, width=10000, bin=TRUE)
stats <- filterWindows(data[,1], data[,2], type="control", prior.count=5,
norm.fac=list(binned[,1], binned[,2]))

```

findMaxima

Find local maxima

Description

Find the local maxima for a given set of genomic regions.

Usage

```
findMaxima(regions, range, metric, ignore.strand=TRUE)
```

Arguments

regions	a GRanges object
range	an integer scalar specifying the range of surrounding regions to consider as local
metric	a numeric vector of values for which the local maxima is found
ignore.strand	a logical scalar indicating whether to consider the strandedness of regions

Details

For each region in `regions`, this function will examine all regions within `range` on either side. It will then determine if the current region has the maximum value of `metric` across this range. A typical metric to maximize might be the sum of counts or the average abundance across all libraries.

Preferably, regions should contain regularly sized and spaced windows or bins, e.g., from [windowCounts](#). The sensibility of using this function for arbitrary regions is left to the user. In particular, the algorithm will not support nested regions and will fail correspondingly if any are detected.

If `ignore.strand=FALSE`, the entries in `regions` are split into their separate strands. The function is run separately on the entries for each strand, and the results are collated into a single output. This may be useful for strand-specific applications.

Value

A logical vector indicating whether each region in regions is a local maxima.

Author(s)

Aaron Lun

See Also

[windowCounts](#), [aveLogCPM](#)

Examples

```
bamFiles <- system.file("exdata", c("rep1.bam", "rep2.bam"), package="csaw")
data <- windowCounts(bamFiles, filter=1)
regions <- rowRanges(data)
metric <- edgeR::aveLogCPM(asDGEList(data))
findMaxima(regions, range=10, metric=metric)
findMaxima(regions, range=50, metric=metric)
findMaxima(regions, range=100, metric=metric)

findMaxima(regions, range=10, metric=runif(length(regions)))
findMaxima(regions, range=50, metric=runif(length(regions)))
findMaxima(regions, range=100, metric=runif(length(regions)))
```

getBestTest

Get the best test in a cluster

Description

Find the test with the strongest evidence for rejection of the null in each cluster.

Usage

```
getBestTest(ids, tab, by.pval=TRUE, weight=NULL, pval.col=NULL, cpm.col=NULL)
```

Arguments

ids	an integer vector containing the cluster ID for each test
tab	a table of results with a PValue field for each test
by.pval	a logical scalar, indicating whether selection should be performed on corrected p-values
weight	a numeric vector of weights for each window, defaults to 1 for each test
pval.col	an integer scalar specifying the column of tab containing the p-values, or a character string containing the name of that column
cpm.col	an integer scalar specifying the column of tab containing the log-CPM values, or a character string containing the name of that column

Details

Clusters are identified as those tests with the same value of `ids` (any NA values are ignored). If `by.pval=TRUE`, this function identifies the test with the lowest p-value as that with the strongest evidence against the null in each cluster. The p-value of the chosen test is adjusted using the Bonferroni correction, based on the total number of tests in the parent cluster. This is necessary to obtain strong control of the family-wise error rate such that the best test can be taken from each cluster for further consideration.

The importance of each window in each cluster can be adjusted by supplying different relative weight values. Each weight is interpreted as a different threshold for each test in the cluster. Larger weights correspond to lower thresholds, i.e., less evidence is needed to reject the null for tests deemed to be more important. This may be useful for upweighting particular tests, e.g., windows containing a motif for the TF of interest.

Note the difference between this function and `combineTests`. The latter presents evidence for any rejections within a cluster. This function specifies the exact location of the rejection in the cluster, which may be more useful in some cases but at the cost of conservativeness. In both cases, clustering procedures such as `mergeWindows` can be used to identify the cluster.

If `by.pval=FALSE`, the best test is defined as that with the highest log-CPM value. This should be independent of the p-value so no adjustment is necessary. Weights are not applied here. This mode may be useful when abundance is correlated to rejection under the alternative hypothesis, e.g., picking high-abundance regions that are more likely to contain peaks.

By default, the relevant fields in `tab` are identified by matching the column names to their expected values. If the column names are different from what is expected, specification of the correct column can be performed using `pval.col` and `cpm.col`.

Value

A dataframe with one row per cluster and the numeric fields `best`, the index for the best test in the cluster; `PValue`, the (possibly adjusted) p-value for that test; and `FDR`, the q-value corresponding to the adjusted p-value. Note that the p-value column may be named differently if `pval.col` is specified. Other fields in `tab` corresponding to the best test in the cluster are also returned. Cluster IDs are stored as the row names.

Author(s)

Aaron Lun

References

Wasserman, L, and Roeder, K (2006). Weighted hypothesis testing. *arXiv preprint math/0604172*.

See Also

[combineTests](#), [mergeWindows](#)

Examples

```
ids <- round(runif(100, 1, 10))
tab <- data.frame(logFC=rnorm(100), logCPM=rnorm(100), PValue=rbeta(100, 1, 2))
best <- getBestTest(ids, tab)
head(best)
```

```

best <- getBestTest(ids, tab, cpm.col="logCPM", pval.col="PValue")
head(best)

# With window weighting.
w <- round(runif(100, 1, 5))
best <- getBestTest(ids, tab, weight=w)
head(best)

# By logCPM.
best <- getBestTest(ids, tab, by.pval=FALSE)
head(best)

best <- getBestTest(ids, tab, by.pval=FALSE, cpm.col=2, pval.col=3)
head(best)

```

getPESizes

Compute fragment lengths for paired-end tags

Description

Compute the length of the sequenced fragment for each read pair in paired-end tag (PE) data.

Usage

```
getPESizes(bam.file, param=readParam(pe="both"))
```

Arguments

bam.file	a character string containing the file path to a sorted and indexed BAM file
param	a readParam object containing read extraction parameters

Details

This function assembles a number of paired-end diagnostics. For starters, a read is only mapped if it is not removed by dedup, minq, restrict or discard in `readParam`. Otherwise, the alignment is not considered to be reliable. Any read pair with exactly one unmapped read is discarded, and the number of read pairs lost in this manner is recorded. Obviously, read pairs with both reads unmapped will be ignored completely.

Of the mapped pairs, the valid (i.e., proper) read pairs are identified. These refer to intrachromosomal read pairs where the reads with the lower and higher genomic coordinates map to the forward and reverse strand, respectively. The distance between the positions of the mapped 5' ends of the two reads must also be equal to or greater than the read lengths. Any intrachromosomal read pair that fails these criteria will be considered as improperly oriented. If the reads are on different chromosomes, the read pair will be recorded as being interchromosomal.

Each valid read pair corresponds to a DNA fragment where both ends are sequenced. The size of the fragment can be determined by calculating the distance between the 5' ends of the mapped reads. The distribution of sizes is useful for assessing the quality of the library preparation, along with all of the recorded diagnostics. Note that any `max.frag` specification in `param` will be ignored; sizes for all valid pairs will be returned.

Value

A list containing:

sizes	an integer vector of fragment lengths for all valid read pairs in the library
diagnostics	an integer vector containing the total number of reads, the number of mapped reads, number of mapped singleton reads, pairs with exactly one unmapped read, number of improperly orientated read pairs and interchromosomal pairs

Author(s)

Aaron Lun

See Also

[readParam](#)

Examples

```
bamFile <- system.file("exdata", "pet.bam", package="csaw")
out <- getPESizes(bamFile, param=readParam(pe="both"))
out <- getPESizes(bamFile, param=readParam(pe="both", restrict="chrA"))
out <- getPESizes(bamFile, param=readParam(pe="both", discard=GRanges("chrA", IRanges(1, 50))))
```

getWidths

Get region widths

Description

Get the widths of the read counting interval for each region.

Usage

```
getWidths(data)
```

Arguments

data a RangedSummarizedExperiment object, produced by [windowCounts](#) or [regionCounts](#)

Details

Widths of all regions are increased by the average fragment length during the calculations. This is because each count represents the number of (imputed) fragments overlapping each region. Thus, a 1 bp window has an effective width that includes the average length of each fragment.

The fragment length is taken from `metadata(data)$final.ext`, if it is not NA. Otherwise, it is taken from `data$ext`. If the fragment lengths are different between libraries, the average is used to compute the effective width of the window. For paired-end data, `data$ext` should be an average of the inferred fragment sizes, e.g., obtained with [getPESizes](#).

If `final.ext` is NA and any of `ext` are NA, the function will extract the read lengths in `data$rlen`. This is because NA values of `ext` correspond to the use of unextended reads in [windowCounts](#) and [regionCounts](#). The likely read lengths are automatically computed in each function but can also be set manually.

Value

An integer vector containing the effective width, in base pairs, of each region.

Author(s)

Aaron Lun

See Also

[windowCounts](#), [regionCounts](#)

Examples

```
bamFiles <- system.file("exdata", c("rep1.bam", "rep2.bam"), package="csaw")
data <- windowCounts(bamFiles, filter=1)
getWidths(data)

# Average is used when multiple fragment lengths are present.
data <- windowCounts(bamFiles, ext=list(c(50, 100), NA), filter=1)
getWidths(data)

# Using the automatically computed 'rlen'.
data <- windowCounts(bamFiles, ext=NA, filter=1)
getWidths(data)
data$rlen <- 200 # Manually defining it, if required.
getWidths(data)

# Paired-end data also takes the fragment length from 'ext'.
bamFile <- system.file("exdata", "pet.bam", package="csaw")
data <- windowCounts(bamFile, param=readParam(pe="both"), filter=1)
getWidths(data)
data$ext <- 200 # Again, manual definition is accepted.
getWidths(data)
```

maximizeCcf

Find the delay at the maximal CCF

Description

Estimate the average fragment length by maximizing the cross-correlations.

Usage

```
maximizeCcf(profile, ignore=100)
```

Arguments

profile a numeric vector containing a coverage profile, as produced by [correlateReads](#)
ignore an integer scalar specifying the distances to ignore

Details

This function identifies the delay distance at which the cross-correlations are maximized. This distance can then be used as an estimate of the average fragment length, for use in directional extension during read counting.

In some datasets, identification of the maxima is confounded by a phantom peak at the read length. This can be overcome by ignoring the first ignore delay distances, such that the distance corresponding to the true peak is used.

Obviously, this only works in TF experiments with moderate to strong enrichment, where a strong peak in the CCF profile is present. The function may not perform sensibly in the presence of noisy profiles containing multiple local maxima.

Value

The average fragment length is returned as an integer scalar.

Author(s)

Aaron Lun

References

Landt SG, Marinov GK, Kundaje A, et al. (2012). ChIP-seq guidelines and practices of the ENCODE and modENCODE consortia. *Genome Res.* 22, 1813-31.

See Also

[correlateReads](#)

Examples

```
x <- dnorm(-200:200/100) # Mocking up a profile.
maximizeCcf(x)

x2 <- x + dnorm(-50:250/10) # Adding a phantom peak
maximizeCcf(x2)
maximizeCcf(x2, ignore=0)
```

mergeWindows

Merge windows into clusters

Description

Uses a simple single-linkage approach to merge adjacent or overlapping windows into clusters.

Usage

```
mergeWindows(regions, tol, sign=NULL, max.width=NULL, ignore.strand=TRUE)
```

Arguments

<code>regions</code>	a GRanges or RangedSummarizedExperiment object containing window coordinates
<code>tol</code>	a numeric scalar specifying the maximum distance between adjacent windows
<code>sign</code>	a logical vector specifying whether each window has a positive log-FC
<code>max.width</code>	a numeric scalar specifying the maximum size of merged intervals
<code>ignore.strand</code>	a logical scalar indicating whether to consider the strandedness of regions

Details

Windows in `regions` are merged if the gap between the end of one window and the start of the next is no greater than `tol`. Adjacent windows can then be chained together to build a cluster of windows across the linear genome. A value of zero for `tol` means that the windows must be contiguous whereas negative values specify minimum overlaps.

If `sign!=NULL`, windows are only merged if they have the same sign of the log-FC and are not separated by intervening windows with opposite log-FC values. This can be useful to ensure consistent changes when summarizing adjacent DB regions. However, it is not recommended for routine clustering in differential analyses as the resulting clusters will not be independent of the p-value.

Specification of `max.width` prevents the formation of excessively large clusters when many adjacent regions are present. Any cluster that is wider than `max.width` is split into multiple subclusters of (roughly) equal size. Specifically, the cluster interval is partitioned into the smallest number of equally-sized subintervals where each subinterval is smaller than `max.width`. Windows are then assigned to each subinterval based on the location of the window midpoints. Suggested values range from 2000 to 10000 bp, but no limits are placed on the maximum size if it is `NULL`.

The tolerance should reflect the minimum distance at which two regions of enrichment are considered separate. If two windows are more than `tol` apart, they *will* be placed into separate clusters. In contrast, the `max.width` value reflects the maximum distance at which two windows can be considered part of the same region.

Arbitrary regions can also be used in this function. However, caution is required if any fully nested regions are present. Clustering with `sign!=NULL` will lead to a warning as splitting by sign becomes undefined. This is because any genomic region involving the parent window must contain the nested window, such that the cluster will always contain opposite log-fold changes. Splitting with `max.width!=NULL` will not fail, but cluster sizes may not be reduced if very large regions are present.

If `ignore.strand=FALSE`, the entries in `regions` are split into their separate strands. The function is run separately on the entries for each strand, and the results collated. The region returned in the output will be stranded to reflect the strand of the contributing input regions. This may be useful for strand-specific applications.

Note that, in the output, the cluster ID reported in `id` corresponds to the index of the cluster coordinates in the input `region`.

Value

A list containing `id`, an integer vector containing the cluster ID for each window; and `region`, a GRanges object containing the start/stop coordinates for each cluster of windows.

Author(s)

Aaron Lun

See Also

[combineTests](#), [windowCounts](#)

Examples

```
x <- round(runif(10, 100, 1000))
gr <- GRanges(rep("chrA", 10), IRanges(x, x+40))
mergeWindows(gr, 1)
mergeWindows(gr, 10)
mergeWindows(gr, 100)
mergeWindows(gr, 100, sign=rep(c(TRUE, FALSE), 5))
```

normOffsets

Normalize counts between libraries

Description

Calculate normalization factors or offsets using count data from multiple libraries.

Usage

```
## S4 method for signature 'matrix'
normOffsets(object, lib.sizes=NULL, type=c("scaling", "loess"),
            weighted=FALSE, ...)
```

Arguments

object	a matrix of integer counts with one column per library
lib.sizes	a numeric vector specifying the total number of reads per library
type	a character string indicating what type of normalization is to be performed
weighted	a logical scalar indicating whether precision weights should be used for TMM normalization
...	other arguments to be passed to calcNormFactors for type="scaling", or loessFit for type="loess"

Details

If type="scaling", this function provides a convenience wrapper for the [calcNormFactors](#) function in the edgeR package. Specifically, it uses the trimmed mean of M-values (TMM) method to perform normalization. Precision weighting is turned off by default so as to avoid upweighting high-abundance regions. These are more likely to be bound and thus more likely to be differentially bound. Assigning excessive weight to such regions will defeat the purpose of trimming when normalizing the coverage of background regions.

If type="loess", this function performs non-linear normalization similar to the fast loess algorithm in [normalizeCyclicLoess](#). For each sample, a lowess curve is fitted to the log-counts against the log-average count. The fitted value for each bin pair is used as the generalized linear model offset for that sample. The use of the average count provides more stability than the average log-count when low counts are present for differentially bound regions.

If lib.sizes is not specified, a warning is issued and the column sums of counts are used instead. Note that the same lib.sizes should be used throughout the analysis if [normOffsets](#) is called multiple times on the same libraries, e.g., with different bin or window sizes or after different filtering steps. This ensures that the normalization factors or offsets are comparable between calls.

Value

For type="scaling", a numeric vector containing the relative normalization factors for each library.

For type="loess", a numeric matrix of the same dimensions as counts, containing the log-based offsets for use in GLM fitting.

Author(s)

Aaron Lun

References

Robinson MD, Oshlack A (2010). A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biology* 11, R25.

Ballman KV, Grill DE, Oberg AL, Therneau TM (2004). Faster cyclic loess: normalizing RNA arrays via linear models. *Bioinformatics* 20, 2778-86.

See Also

[calcNormFactors](#), [loessFit](#), [normalizeCyclicLoess](#)

Examples

```
# A trivial example
counts <- matrix(rnbinom(400, mu=10, size=20), ncol=4)
normOffsets(counts)
normOffsets(counts, lib.sizes=rep(400, 4))

# Adding undersampling
n <- 1000L
mu1 <- rep(10, n)
mu2 <- mu1
mu2[1:100] <- 100
mu2 <- mu2/sum(mu2)*sum(mu1)
counts <- cbind(rnbinom(n, mu=mu1, size=20), rnbinom(n, mu=mu2, size=20))
actual.lib.size <- rep(sum(mu1), 2)
normOffsets(counts, lib.sizes=actual.lib.size)
normOffsets(counts, logratioTrim=0.4, lib.sizes=actual.lib.size)
normOffsets(counts, sumTrim=0.3, lib.size=actual.lib.size)

# With and without weighting, for high-abundance spike-ins.
n <- 100000
blah <- matrix(rnbinom(2*n, mu=10, size=20), ncol=2)
tospike <- 10000
blah[1:tospike,1] <- rnbinom(tospike, mu=1000, size=20)
blah[1:tospike,2] <- rnbinom(tospike, mu=2000, size=20)
full.lib.size <- colSums(blah)

normOffsets(blah, weighted=TRUE, lib.sizes=full.lib.size)
normOffsets(blah, lib.sizes=full.lib.size)
true.value <- colSums(blah[(tospike+1):n,])/colSums(blah)
true.value <- true.value/exp(mean(log(true.value)))
true.value

# Using loess-based normalization, instead.
```

```
offsets <- normOffsets(counts, type="loess", lib.size=full.lib.size)
head(offsets)
offsets <- normOffsets(counts, type="loess", span=0.4, lib.size=full.lib.size)
offsets <- normOffsets(counts, type="loess", iterations=1, lib.size=full.lib.size)
```

overlapStats *Compute overlap statistics*

Description

Compute assorted statistics for overlaps between windows and regions in a Hits object.

Usage

```
combineOverlaps(olap, tab, o.weight=NULL, i.weight=NULL, ...)
getBestOverlaps(olap, tab, o.weight=NULL, i.weight=NULL, ...)
summitOverlaps(olap, region.best, o.summit=NULL, i.summit=NULL)
```

Arguments

olap	a Hits object produced by findOverlaps , containing overlaps between regions (query) and windows (subject)
tab	a dataframe of DE results for each window
o.weight	a numeric vector specifying weights for each overlapped window
i.weight	a numeric vector specifying weights for each individual window
...	other arguments to be passed to the wrapped functions
region.best	an integer vector specifying the window index that is the summit for each region
o.summit	a logical vector specifying the overlapped windows that are summits, or a corresponding integer vector of indices for such windows
i.summit	a logical vector specifying whether an individual window is a summit, or a corresponding integer vector of indices

Details

These functions provide convenient wrappers around [combineTests](#), [getBestTest](#) and [upweightSummit](#). They accept Hits objects produced by running [findOverlaps](#) between windows and some pre-specified regions. Each set of windows overlapping a region is defined as a cluster to compute the various statistics.

A wrapper is necessary as a window may overlap multiple regions. If so, the multiple instances of that window are defined as distinct “overlapped” windows, where each overlapped window is assigned to a different region. Each overlapped window is represented by a row of `olap`. In contrast, the “individual” window just refers to the window itself, regardless of what it overlaps. This is represented by each row of the `RangedSummarizedExperiment` object and the `tab` derived from it.

The distinction between these two definitions is required to describe the weight arguments. The `o.weight` argument refers to the weights for each region-window relationship. This allows for different weights to be assigned to the same window in different regions. The `i.weight` argument is the weight of the window itself, and is the same regardless of the region. If both are specified, `o.weight` takes precedence.

For `summitOverlaps`, the `region.best` argument is designed to accept the best field in the output of `getBestOverlaps` (run with `by.pval=FALSE`). This contains the index for the individual window that is the summit within each region. In contrast, the `i.summit` argument indicates whether an individual window is a summit, e.g., from `findMaxima`. The `o.summit` argument does the same for overlapped windows, though this has no obvious input within the `csaw` pipeline.

Value

For `combineOverlaps` and `getBestOverlaps`, a dataframe is returned from their respective wrapped functions. Each row of the dataframe corresponds to a region, where regions without overlapped windows are assigned NA values.

For `summitOverlaps`, a numeric vector of weights is produced. This can be used as `o.weight` in the other two functions.

Author(s)

Aaron Lun

See Also

[combineTests](#), [getBestTest](#), [upweightSummit](#)

Examples

```
bamFiles <- system.file("exdata", c("rep1.bam", "rep2.bam"), package="csaw")
data <- windowCounts(bamFiles, width=1, filter=1)
of.interest <- GRanges(c('chrA', 'chrA', 'chrB', 'chrC'),
  IRanges(c(1, 500, 100, 1000), c(200, 1000, 700, 1500)))

# Making some mock results.
N <- nrow(data)
mock <- data.frame(logFC=rnorm(N), PValue=runif(N), logCPM=rnorm(N))

olap <- findOverlaps(of.interest, rowRanges(data))
combineOverlaps(olap, mock)
getBestOverlaps(olap, mock)

# See what happens when you don't get many overlaps.
getBestOverlaps(olap[1,], mock)
combineOverlaps(olap[2,], mock)

# Weighting example, with window-specific weights.
window.weights <- runif(N)
comb <- combineOverlaps(olap, mock, i.weight=window.weights)

# Weighting example, with relation-specific weights.
best.by.ave <- getBestOverlaps(olap, mock, by.pval=FALSE)
w <- summitOverlaps(olap, region.best=best.by.ave$best)
head(w)
stopifnot(length(w)==length(olap))
combineOverlaps(olap, mock, o.weight=w)

# Running summitOverlaps for window-specific summits
# (output is still relation-specific weights, though).
is.summit <- findMaxima(rowRanges(data), range=100, metric=mock$logCPM)
w <- summitOverlaps(olap, i.summit=is.summit)
```



```
head(w)
```

Parameter list methods

Get or modify readParam lists

Description

Extract and modify lists of library-specific readParam objects

Usage

```
reformList(paramlist, ...)  
checkList(paramlist)
```

Arguments

paramlist	a list of readParam objects
...	other arguments, to be passed to reform

Details

The `reformList` function sets the parameters specified in `...` across all elements of `paramlist`. This standardizes all of the internal `readParam` objects, e.g., for the `restrict` parameter. If `paramlist` is a single `readParam` object, then `reformList` is equivalent to [reform](#).

The `checkList` function checks which parameter settings are identical between elements of `paramlist`. This is useful when some parameters must be variable, while others must be the same.

Value

For `reformList`, a list of library-specific `readParam` objects is returned.

For `checkList`, a character vector is returned containing all non-identical parameters across the list.

Author(s)

Aaron Lun

See Also

[windowCounts](#), [regionCounts](#), [reform](#)

Examples

```
bamFiles <- system.file("exdata", c("rep1.bam", "rep2.bam"), package="csaw")  
data <- windowCounts(bamFiles, width=100, filter=1)  
data$param  
  
data <- windowCounts(bamFiles, width=100, filter=1, param=list(readParam(), readParam(minq=100)))  
data$param  
data[,1]$param  
data[,2]$param
```

```

plist <- data$param
reformList(plist, restrict="chrA")
reformList(plist, minq=10)

checkList(plist)
checkList(reformList(plist, minq=10))

```

profileSites

Profile binding sites

Description

Get the coverage profile around potential binding sites.

Usage

```

profileSites(bam.files, regions, range=5000, ext=100, average=TRUE,
             weight=1, param=readParam(), strand=c("ignore", "use", "match"))

```

Arguments

bam.files	a character vector containing paths to one or more BAM files
regions	a GRanges object over which profiles are to be aggregated
range	an integer scalar specifying the range over which the profile will be collected
ext	an integer scalar or list specifying the average fragment length for single-end data
average	a logical scalar specifying whether the profiles should be averaged across regions
weight	a numeric vector indicating the relative weight to be assigned to the profile for each region
param	a readParam object containing read extraction parameters
strand	a string indicating how stranded regions should be handled

Details

This function aggregates the coverage profile around the specified regions. The shape of this profile can guide an intelligent choice of the window size in [windowCounts](#), or to determine if region expansion is necessary in [regionCounts](#). For the former, restricting the regions to locally maximal windows with [findMaxima](#) is recommended prior to use of `profileSites`. The function can be also used to examine average coverage around known features of interest, like genes.

The profile records the number of fragments overlapping each base within range of the start of all regions. Single-end reads are directionally extended to `ext` to impute the fragment (see [windowCounts](#) for more details). For paired-end reads, the interval between each pair is used as the fragment. If multiple `bam.files` are specified, reads are pooled across files for counting into each profile.

Direct aggregation will favor high-abundance regions as these have higher counts. If this is undesirable, high-abundance regions can be downweighted using the `weight` argument. For example, this

can be set to the inverse of the sum of counts across all libraries for each region in regions. This will ensure that each region contributes equally to the final profile.

Aggregation can be turned off by setting `average=FALSE`. In such cases, a separate profile will be returned for each region, instead of the profiles being averaged across all regions. This may be useful, e.g., for constructing heatmaps of enrichment across many regions. Note that `weight` has no effect when aggregation is turned off.

Value

If `average=TRUE`, a numeric vector of average coverages for each base position within `range` is returned, where the average is taken over all regions. The vector is named according to the relative position of each base to the start of the region. If `weight` is set as described above for each region, then the vector will represent average relative coverages, i.e., relative to the number of fragments counted in the region itself.

If `average=FALSE`, an integer matrix of coverage values is returned. Each row of the matrix corresponds to an entry in `regions`, while each column corresponds to a base position with `range`. Column names are set to the relative position of each base to the start of each region.

Comments on strand specificity

If `strand="use"`, the function is called separately on the reverse-stranded regions. The profile for these regions is computed such that the left side of the profile corresponds to the upstream flank on the reverse strand (i.e., the profile is flipped). The center of the profile corresponds to the 5' end of the region on the reverse strand. This may be useful for features where strandedness is important, e.g., TSS's. Otherwise, if `strand="ignore"`, no special treatment is given to reverse-stranded features.

By default, the strandedness of the region has no effect on read extraction. If `strand="match"`, the profile for reverse-strand regions is made with reverse-strand reads only (this profile is also flipped, as described for `strand="use"`). Similarly, only forward-strand reads are used for forward- or unstranded regions. Note that `param$forward` must be set to `NULL` for this to work.

Author(s)

Aaron Lun

See Also

[findMaxima](#), [windowCounts](#), [wwhm](#)

Examples

```
bamFile <- system.file("exdata", "rep1.bam", package="csaw")
data <- windowCounts(bamFile, filter=1)
rwsms <- rowSums(assay(data))
maxed <- findMaxima(rowRanges(data), range=100, metric=rwsms)

x <- profileSites(bamFile, rowRanges(data)[maxed], range=200)
plot(as.integer(names(x)), x)

x <- profileSites(bamFile, rowRanges(data)[maxed], range=500)
plot(as.integer(names(x)), x)

x <- profileSites(bamFile, rowRanges(data)[maxed], range=500, weight=1/rwsms)
plot(as.integer(names(x)), x)
```

```

# Introducing some strandedness.
regs <- rowRanges(data)[maxed]
strand(regs) <- sample(c("-", "+", "*"), sum(maxed), replace=TRUE)
x <- profileSites(bamFile, regs, range=500)
plot(as.integer(names(x)), x)
x2 <- profileSites(bamFile, regs, range=500, strand="use")
points(as.integer(names(x2)), x2, col="red")
x3 <- profileSites(bamFile, regs, range=500, strand="match",
  param=readParam(forward=NULL))
points(as.integer(names(x3)), x3, col="blue")

# Returning separate profiles.
y <- profileSites(bamFile, rowRanges(data)[maxed], range=500, average=FALSE)
dim(y)

```

readParam

readParam class and methods

Description

Class to specify read loading parameters

Details

Each readParam object stores a number of parameters, each pertaining to the extraction of reads from a BAM file. Slots are defined as:

pe: a character string indicating whether paired-end data is present; set to "none", "both", "first" or "second"

max.frag: an integer scalar, specifying the maximum fragment length corresponding to a read pair

dedup: a logical scalar indicating whether marked duplicate reads should be ignored

minq: an integer scalar, specifying the minimum mapping quality score for an aligned read

forward: a logical scalar indicating whether only forward reads should be extracted

restrict: a character vector containing the names of allowable chromosomes from which reads will be extracted

discard: a GRanges object containing intervals in which any alignments will be discarded

BPPARAM: a BiocParallelParam object specifying if and how parallelization is performed

Removing low-quality or irrelevant reads

Marked duplicate reads will be removed with dedup=TRUE. This may be necessary when many rounds of PCR have been performed during library preparation. However, it is not recommended for routine counting as it will interfere with the downstream statistical methods. Note that the duplicate field must be set beforehand in the BAM file for this argument to have any effect.

Reads can also be filtered by their mapping quality scores if minq is specified at a non-NA value. This is generally recommended to remove low-confidence alignments. The exact threshold for minq will depend on the range of scores provided by the aligner. If minq=NA, no filtering on the score will be performed.

If restrict is supplied, reads will only be extracted for the specified chromosomes. This is useful to restrict the analysis to interesting chromosomes, e.g., no contigs/scaffolds or mitochondria. Conversely, if discard is set, a read will be removed if the corresponding alignment is wholly contained within the supplied ranges. This is useful for removing reads in repeat regions.

Parameter settings for paired-end data

For `pe="both"`, reads are extracted with the previously described filters, i.e., `discard`, `minq`, `dedup`. Extracted reads are then grouped into proper pairs. Proper pairs are those where the two reads are close together (on the same chromosome, obviously) and in an inward-facing orientation. The fragment interval is defined as that bounded by the 5' ends of the two reads in a proper pair.

The fragment size is defined as the length of the interval bounded by the 5' ends of two inward-facing reads. Those pairs with fragment sizes above `max.frag` are removed, as they are more likely to be the result of mapping errors than genuinely large fragment sizes. Users should run `getPESizes` to pick an appropriate value for their data sets, though thresholds of around 500-1000 bp are usually fine.

Paired-end data can also be treated as single-end data by only using one read from each pair with `pe="first"` or `"second"`. This is useful for poor-quality data where the paired-end procedure has obviously failed, e.g., with many interchromosomal read pairs or pairs with large fragment lengths. Treating the data as single-end may allow the analysis to be salvaged.

In all cases, users should ensure that each BAM file containing paired-end data is properly synchronized prior to count loading. This can be done using standard tools like `FixMateInformation` from the Picard suite (<http://broadinstitute.github.io/picard>).

Parameter settings for single-end data

If `pe="none"`, reads are assumed to be single-end. Read extraction from BAM files is performed with the same quality filters described above. If `forward` is `NA`, reads are extracted from all strands. Otherwise, reads are only extracted from the forward or reverse strands for `TRUE` or `FALSE`, respectively. This may be useful for applications requiring strand-specific counting. A special case is `forward=NULL` - see `strandedCounts` for more details.

Any soft clipping in alignments are ignored during extraction (this is also true for paired-end data). Soft clips are presumed to be sequencing artifacts, e.g., when the adaptor or barcodes are not properly removed from the read sequence. They are not relevant to computing genomic coverage. Thus, in this package, any references to the length or 5'/3' ends of the read will actually refer to that of the *alignment*. This is often more appropriate, e.g., the 5' end of the alignment represents the end of the fragment after clipping of the artifacts.

Parallelization options

Parallelization can be turned on in several functions by setting `BPPARAM` appropriately. This will usually extract reads from multiple files simultaneously to speed up processing. Users are referred to `?BiocParallelParam` for more details on how to set `BPPARAM`. By default, a `SerialParam` object is used, i.e., no parallelization is performed. This is because it provides little benefit for small files or on systems with I/O bottlenecks.

Constructor

```
readParam(pe="none", max.frag=500, dedup=FALSE, minq=NA, forward=NA, restrict=NULL, discard=GRAn)
  Creates a readParam object. Each argument is placed in the corresponding slot, with coercion
  into the appropriate type.
```

Subsetting

In the code snippets below, `x` is a `readParam` object.

```
x$name: Returns the value in slot name.
```

Other methods

In the code snippets below, `x` is a `readParam` object.

`show(x)`: Describes the parameter settings in plain English.

`reform(x, ...)`: Creates a new `readParam` object, based on the existing `x`. Any named arguments in `...` are used to modify the values of the slots in the new object, with type coercion as necessary.

Author(s)

Aaron Lun

See Also

[windowCounts](#), [regionCounts](#), [correlateReads](#), [getPESizes](#), [BiocParallelParam](#)

Examples

```
blah <- readParam()
blah <- readParam(discard=GRanges("chrA", IRanges(1, 10)))
blah <- readParam(restrict='chr2')
blah$pe
blah$dedup

# Use 'reform' if only some arguments need to be changed.
blah
reform(blah, dedup=TRUE)
reform(blah, pe="both", max.frag=212.0)
```

regionCounts

Count reads overlapping each region

Description

Count the number of extended reads overlapping pre-specified regions

Usage

```
regionCounts(bam.files, regions, ext=100, param=readParam())
```

Arguments

<code>bam.files</code>	a character vector containing paths to sorted and indexed BAM files
<code>regions</code>	a <code>GRanges</code> object containing the regions over which reads are to be counted
<code>ext</code>	an integer scalar or list describing the average length of the sequenced fragment in each library, see ?windowCounts
<code>param</code>	a <code>readParam</code> object containing read extraction parameters, or a list of such objects (one for each BAM file)

Details

This function simply provides a wrapper around [countOverlaps](#) for read counting into specified regions. It is provided so as to allow for counting with awareness of the other parameters, e.g., `ext`, `pe`. This allows users to coordinate region-based counts with those from [windowCounts](#). Checking that the output totals are the same between the two calls is strongly recommended.

Note that the strandedness of regions will not be considered when computing overlaps. In other words, both forward and reverse reads will be counted into each region, regardless of the strandedness of that region. This can be altered by setting the `forward` slot in the `param` object to only count reads from one strand or the other. The strandedness of the output `rowRanges` will depend on the strand(s) from which reads were counted.

See [windowCounts](#) for more details on read extension.

Value

A `RangedSummarizedExperiment` object is returned containing one integer matrix. Each entry of the matrix contains the count for each library (column) at each region (row). The coordinates of each region are stored as the `rowRanges`. The total number of reads, read length and extension length used in each library are stored in the `colData`. Other parameters (e.g., `param`) are stored in the `metadata`.

Author(s)

Aaron Lun

See Also

[countOverlaps](#), [windowCounts](#), [readParam](#)

Examples

```
# A low filter is only used here as the examples have very few reads.
bamFiles <- system.file("exdata", c("rep1.bam", "rep2.bam"), package="csaw")
incoming <- GRanges(c('chrA', 'chrA', 'chrB', 'chrC'),
  IRanges(c(1, 500, 100, 1000), c(200, 1000, 700, 1500)))
regionCounts(bamFiles, regions=incoming)
regionCounts(bamFiles, regions=incoming, param=readParam(restrict="chrB"))

# Loading PE data.
bamFile <- system.file("exdata", "pet.bam", package="csaw")
regionCounts(bamFile, regions=incoming, param=readParam(pe="both"))
regionCounts(bamFile, regions=incoming, param=readParam(max.frag=100,
  pe="first", restrict="chrA"))
```

scaledAverage

Scaled average abundance

Description

Compute the scaled average abundance for each feature.

Usage

```
scaledAverage(y, scale=1, prior.count=NULL, ...)
```

Arguments

<code>y</code>	a <code>DGEList</code> object
<code>scale</code>	a numeric vector indicating the magnitude with which each abundance is to be downscaled
<code>prior.count</code>	a numeric scalar specifying the prior count to add
<code>...</code>	other arguments, to be passed to aveLogCPM

Details

This function computes the average abundance of each feature in `y`, and downscales it according to `scale`. For example, if `scale=2`, the average count is halved, i.e., the returned abundances are decreased by 1 (as they are log2-transformed values). The aim is to set `scale` based on the relative width of regions, to allow abundances to be compared between regions of different size. Widths can be obtained using the [getWidths](#) function.

Some subtlety is necessary regarding the treatment of the `prior.count`. Specifically, the prior used in [aveLogCPM](#) is automatically increased when `scale` is larger. This ensures that the effective prior is the same after the abundance is scaled down. Otherwise, the use of the same prior would incorrectly result in a smaller abundance for larger regions.

Note that the adjustment for width assumes that reads are uniformly distributed throughout each region. This is reasonable for most background regions, but may not be for enriched regions. When the distribution is highly heterogeneous, the downscaled abundance of a large region will not be an accurate representation of the abundance of the smaller regions nested within.

For consistency, the `prior.count` is set to the default value of [aveLogCPM.DGEList](#), if it is not otherwise specified. If a non-default value is used, make sure that it is the same for all calls to `scaledAverage`. This ensures that comparisons between the returned values are valid.

Value

A numeric vector of scaled abundances, with one entry for each row of `y`.

Author(s)

Aaron Lun

See Also

[getWidths](#), [aveLogCPM](#)

Examples

```
bamFiles <- system.file("exdata", c("rep1.bam", "rep2.bam"), package="csaw")
size1 <- 50
data1 <- windowCounts(bamFiles, width=size1, filter=1)
size2 <- 500
data2 <- windowCounts(bamFiles, width=size2, filter=1)

# Adjusting by `scale`, based on median sizes.
head(scaledAverage(asDGEList(data1)))
```



```

relative <- median(getWidths(data2))/median(getWidths(data1))
head(scaledAverage(asDGEList(data2), scale=relative))

# Need to make sure the same prior is used, if non-default.
pc <- 5
head(scaledAverage(asDGEList(data1), prior.count=pc))
head(scaledAverage(asDGEList(data2), scale=relative, prior.count=pc))

# Different way to compute sizes, for 1-to-1 relations.
data3 <- regionCounts(bamFiles, regions=resize(rowRanges(data1),
  fix="center", width=size2))
head(scaledAverage(asDGEList(data1)))
relative.2 <- getWidths(data1)/getWidths(data2)
head(scaledAverage(asDGEList(data3), scale=relative.2))

```

Description

Convenience wrappers for statistical routines operating on `SummarizedExperiment` objects.

Usage

```

## S4 method for signature 'SummarizedExperiment'
normOffsets(object, lib.sizes, assay=1, ...)
## S4 method for signature 'SummarizedExperiment'
normalize(object, lib.sizes, type="scaling", assay=1, ...)
## S4 method for signature 'SummarizedExperiment'
asDGEList(object, lib.sizes, norm.factors, ...)

```

Arguments

<code>object</code>	a <code>SummarizedExperiment</code> object or its derived classes, like that produced by windowCounts
<code>lib.sizes</code>	an (optional) integer vector of library sizes
<code>assay</code>	a numeric scalar indicating which assay in object contains the count matrix
<code>type</code>	a character scaling indicating the type of normalization, see normOffsets
<code>norm.factors</code>	an (optional) numeric vector of normalization factors
<code>...</code>	other arguments to be passed to the function being wrapped

Details

For `normalize` and `normOffset`, counts are extracted from the matrix corresponding to the first assay in the `SummarizedExperiment` object (different assays can be specified with the `assay` argument). For `asDGEList`, counts are extracted from the assay named "counts", with the first assay used if no such assay exists with that name. If not specified in `lib.sizes`, library sizes are taken from the `totals` field in the column data of object. Warnings will be generated if this field is not present.

In the `normOffsets` and `normalize` methods, the extracted counts and library sizes are supplied to `normOffsets, matrix-method`. Similarly, the `asDGEList` method wraps the `DGEList` constructor. In both cases, any arguments in `...` are also passed to the wrapped functions.

If `type="scaling"` in `normalize`, a vector of normalization factors is stored in the `norm.factors` field of the `colData` in the output object. If `type="loess"`, an offset matrix is instead stored as a named entry in the `assays` of the output object.

If `norm.factors` is not specified, `asDGEList` will attempt to extract normalization factors from `object$norm.factors`. If this is not available, factors will be set to the default (all unity). If `assays(object)$offset` is present, this will be assigned to the `offset` field of the output `DGEList` object.

Value

For `normOffsets`, either a numeric matrix or vector is returned; see `normOffsets, matrix-method`.

For `normalize`, an object of the same class as `object` is returned with normalization data stored in various fields.

For `asDGEList`, a `DGEList` object is returned.

Author(s)

Aaron Lun

See Also

`normOffsets`, `DGEList`, `windowCounts`

Examples

```
bamFiles <- system.file("exdata", c("rep1.bam", "rep2.bam"), package="csaw")
data <- windowCounts(bamFiles, width=100, filter=1)

normOffsets(data)
normOffsets(data, lib.sizes=c(10, 100))
head(normOffsets(data, type="loess"))

asDGEList(data)
asDGEList(data, lib.sizes=c(10, 100))
asDGEList(data, norm.factors=c(1.11, 2.23), group=c("a", "b"))

norm.data <- normalize(data)
norm.data$norm.factors
asDGEList(norm.data)$samples$norm.factors
asDGEList(norm.data, norm.factors=c(1,2))$samples$norm.factors # gets priority
norm.data <- normalize(data, type="loess")
head(asDGEList(norm.data)$offset)
```

strandedCounts	<i>Get strand-specific counts</i>
----------------	-----------------------------------

Description

Obtain strand-specific counts for each genomic window or region.

Usage

```
strandedCounts(bam.files, param=readParam(forward=NULL), regions=NULL, ...)
```

Arguments

bam.files	a character vector containing paths to sorted and indexed BAM files
param	a readParam object containing read extraction parameters, where the forward slot must be set to NULL
regions	a GRanges object specifying the regions over which reads are to be counted
...	other arguments to be passed to windowCounts or regionCounts

Details

Some applications require strand-specific counts for each genomic region. This function calls [windowCounts](#) after setting `param$forward` to TRUE and FALSE. Any existing value of `param$forward` is ignored. If `regions` is specified, [regionCounts](#) is used instead of [windowCounts](#).

The function then concatenates the two `RangedSummarizedExperiment` objects (one from each strand). The total numbers of reads are added together to form the new totals. However, the total numbers of reads for each strand are also stored for future reference. Count loading parameters are also stored in the metadata.

Each row in the concatenated object corresponds to a stranded genomic region, where the strand of the region indicates the strand of the reads that were counted in that row. Note that there may not be two rows for each genomic region. This is because any empty rows, or those with counts below `filter`, will be removed within each call to [windowCounts](#).

Value

A `RangedSummarizedExperiment` object containing strand-specific counts for genomic regions.

Warnings

Users should be aware that many of the downstream range-processing functions are not strand-aware by default, e.g., [mergeWindows](#). Any strandedness of the ranges will be ignored in these functions. If strand-specific processing is desired, users must manually set `ignore.strand=FALSE`.

The input `param$forward` should be set to NULL, as a safety measure. This is because the returned object is a composite of two separate calls to the relevant counting function. If the same `param` object is supplied to other functions, an error will be thrown if `param$forward` is NULL. This serves to remind users that such functions should instead be called twice, i.e., separately for each strand after setting `param$forward` to TRUE or FALSE.

Author(s)

Aaron Lun

See Also

[windowCounts](#), [regionCounts](#)

Examples

```

bamFiles <- system.file("exdata", c("rep1.bam", "rep2.bam"), package="csaw")
xparam <- readParam(forward=NULL)
out <- strandedCounts(bamFiles, filter=1, param=xparam)
out

strandedCounts(bamFiles, filter=1, width=100, param=xparam)
strandedCounts(bamFiles, filter=1, param=reform(xparam, minq=20))

incoming <- GRanges(c('chrA', 'chrA', 'chrB', 'chrC'),
  IRanges(c(1, 500, 100, 1000), c(200, 1000, 700, 1500)))
strandedCounts(bamFiles, regions=incoming, param=xparam)
strandedCounts(bamFiles, regions=incoming, param=reform(xparam, dedup=TRUE))

# Throws an error, as the same reads are not involved.
try(windowCounts(bamFiles, filter=1, width=100, param=xparam))

# Library sizes should be the same as that without strand-specificity.
colData(out)
out.ref <- windowCounts(bamFiles, param=reform(xparam, forward=NA))
stopifnot(identical(out.ref$totals, out$totals))

# Running assorted functions on strandedCounts output.
mergeWindows(rowRanges(out), tol=100)
mergeWindows(rowRanges(out), tol=100, ignore.strand=FALSE)

rwsms <- rowSums(assay(out))
summary(findMaxima(rowRanges(out), range=100, metric=rwsms))
summary(findMaxima(rowRanges(out), range=100, metric=rwsms, ignore.strand=FALSE))

```

upweightSummit

Upweight summits

Description

Upweight the highest-abundance window(s) in a cluster.

Usage

```
upweightSummit(ids, summits)
```

Arguments

<code>ids</code>	an integer vector of cluster IDs
<code>summits</code>	a logical vector indicating whether each window is a summit, or an integer vector containing the indices of summit windows

Details

This function computes weights for each window in a cluster, where the highest-abundance windows are upweighted. These weights are intended for use in `combineTests`, such that the summits of a cluster have a greater influence on the combined p-value. This is more graduated than simply using the summits alone, as potential DB between summits can still be detected. Summits can be obtained through `findMaxima` or by running `getBestTest` with `by.pval=FALSE`.

The exact value of the weight is arbitrary. Greater weight represents a stronger belief that DB occurs at the most abundant window. Here, the weighting scheme is designed such that the maximum Simes correction is not more than twice that without weighting. It will also be no more than twice that from applying Simes' method on the summits alone. This (restrained) conservativeness is an acceptable cost for considering DB events elsewhere in the cluster, while still focusing on the most abundant site.

Value

A numeric vector of weights, where the highest-abundance window in each cluster is assigned a greater weight. Any windows with NA values for `ids` or `summits` will have a weight of zero.

Author(s)

Aaron Lun

References

Benjamini Y and Hochberg Y (1997). Multiple hypotheses testing with weights. *Scand. J. Stat.* 24, 407-418.

See Also

[combineTests](#), [findMaxima](#), [getBestTest](#)

Examples

```
nwin <- 20
set.seed(20)
ids <- sample(5, nwin, replace=TRUE)
summits <- sample(5, nwin, replace=TRUE)==1L
weights <- upweightSummit(ids, summits)

# Checking that the summit is upweighted in each cluster.
split(data.frame(summits, weights), ids)
```

windowCounts

Count reads overlapping each window

Description

Count the number of extended reads overlapping a sliding window at spaced positions across the genome.

Usage

```
windowCounts(bam.files, spacing=50, width=spacing, ext=100, shift=0,
             filter=10, bin=FALSE, param=readParam())
```

Arguments

<code>bam.files</code>	a character vector containing paths to sorted and indexed BAM files
<code>spacing</code>	an integer scalar specifying the distance between consecutive windows
<code>width</code>	an integer scalar specifying the width of the window
<code>ext</code>	an integer scalar or a list of two integer scalars/vectors, containing the average length(s) of the sequenced fragments in each library
<code>shift</code>	an integer scalar specifying how much the start of each window should be shifted to the left
<code>filter</code>	an integer scalar for the minimum count sum across libraries for each window
<code>bin</code>	a logical scalar indicating whether binning should be performed
<code>param</code>	a <code>readParam</code> object containing read extraction parameters

Value

A `RangedSummarizedExperiment` object is returned containing one integer matrix. Each entry of the matrix contains the count for each library (column) at each window (row). The coordinates of each window are stored as the `rowRanges`. The total number of reads in each library are stored as `totals` in the `colData`, along with the read (`rLen`) and extension lengths (`ext`) for each library. Other window counting parameters (e.g., `spacing`, `width`, `param`) are stored in the `metadata`.

Defining the sliding windows

A window is defined as a genomic interval of size equal to `width`. The value of `width` can be interpreted as the width of the contact area between the DNA and protein. In practical terms, it determines the spatial resolution of the analysis. Larger windows count reads over a larger region which results in larger counts. This results in greater detection power at the cost of resolution.

By default, the first window on a chromosome starts at base position 1. This can be shifted to the left by specifying an appropriate value for `shift`. New windows are defined by sliding the current window to the right by the specified `spacing`. Increasing `spacing` will reduce the frequency at which counts are extracted from the genome. This results in some loss of resolution but it may be necessary when machine memory is limited.

If `bin` is set, settings are internally adjusted so that all reads are counted into non-overlapping adjacent bins of size `width`. Specifically, `spacing` is set to `width` and `filter` is capped at a maximum value of 1 (empty bins can be retained with `filter=0`). Only the 5' end of each read or the midpoint of each fragment (for paired-end data) is used in counting.

Read extraction and counting

Read extraction from the BAM files is governed by the `param` argument. This specifies whether reads are to be read in single- or paired-end mode, whether to apply a threshold to the mapping quality, and so on – see `?readParam` for details. The strandedness of the output `rowRanges` is set based on the strand(s) from which the reads are extracted and counted. This is determined by the value of the forward slot in the input `param` object.

Fragments are inferred from reads by directional extension (single-end; see below) or by identifying proper pairs (paired-end; see `readParam` for more details). The number of fragments overlapping

the window for each library is then counted for each window position. Windows will be removed if the count sum across all libraries is below `filter`. This reduces the memory footprint of the output by not returning empty or near-empty windows, which are usually uninteresting anyway.

Elaborating on directional extension

For single-end reads, directional extension is performed whereby each read is extended from its 3' end to the average fragment length, i.e., `ext`. This obtains a rough estimate of the interval of the fragment from which the read was derived. It is particularly useful for TF data, where extension specifically increases the coverage of peaks that exhibit strand bimodality. No extension is performed if `ext` is set to NA, such that the read length is used as the fragment length in that library.

If libraries have different fragment lengths, this can be accommodated by supplying a list of 2 elements to `ext`. The first element (named `init.ext` here, for convenience) should be an integer vector specifying the extension length for each library. The second element (`final.ext`) should be an integer scalar specifying the final fragment length. All reads are directionally extended by `init.ext`, and the resulting fragment is resized to `final.ext` by shrinking or expanding from the fragment midpoint. For a bimodal peak, scaling effectively aligns the subpeaks on a given strand across all libraries to a common location. This removes the most obvious differences in widths.

If any element of `init.ext` is NA, no extension is performed for the corresponding library. If `final.ext` is set to NA, no rescaling is performed from the library-specific fragment lengths. Values of `init.ext` are stored as the `ext` field in the `colData` of the output object, while `final.ext` is stored in the metadata.

Comments on ext for paired-end data

Directional extension is not performed for paired-end data, so the values in `ext` are not used directly. However, rescaling can still be performed to standardize fragment lengths across libraries by resizing each fragment from its midpoint. This will use the second element of `ext` as `final.ext`, if `ext` is specified as a list of length 2.

On a similar note, some downstream functions will use the extension length in the output `colData` as the average fragment length. Thus, to maintain compatibility, the `ext` field in `colData` is set to the average of the inferred fragment lengths for valid read pairs. These values will not be used in `windowCounts`, but instead, in functions like [getWidths](#).

Author(s)

Aaron Lun

See Also

[correlateReads](#), [readParam](#)

Examples

```
# A low filter is only used here as the examples have very few reads.
bamFiles <- system.file("exdata", c("rep1.bam", "rep2.bam"), package="csaw")
windowCounts(bamFiles, filter=1)
windowCounts(bamFiles, width=100, filter=1)

# Multiple extension lengths.
windowCounts(bamFiles, ext=list(c(50, 100), NA), filter=1)
windowCounts(bamFiles, ext=list(c(50, 100), 80), filter=1)
```

```
# Loading PE data.
bamFile <- system.file("exdata", "pet.bam", package="csaw")
windowCounts(bamFile, param=readParam(pe="both"), filter=1)
windowCounts(bamFile, param=readParam(pe="first"), filter=1)
windowCounts(bamFile, param=readParam(max.frag=100, pe="both"), filter=1)
windowCounts(bamFile, param=readParam(max.frag=100, pe="both", restrict="chrA"), filter=1)
```

wwhm

Window width at half maximum

Description

Get the width of the window from the half-maximum of the coverage profile.

Usage

```
wwhm(profile, regions, ext=100, proportion=0.5, rlen=NULL)
```

Arguments

profile	a numeric vector containing a coverage profile, as produced by profileSites
regions	the GRanges object with which the profile was constructed
ext	an integer scalar specifying the average fragment length for single-end data
proportion	a numeric scalar specifying the proportion of the maximum coverage at which to compute the window width
rlen	a numeric scalar or vector containing read lengths, if any ext=NA, i.e., fragments are unextended reads

Details

This function computes the ideal window size, based on the width of the peak in the coverage profile at the specified proportion of the maximum. Obviously, the values of `regions` and `ext` should be the same as those used in [profileSites](#) (set `ext` to the median fragment length for paired-end data). The `regions` should contain windows of a constant size.

Some subtleties are involved in obtaining the window width. First, twice the average fragment length must be subtracted from the peak width, as the profile is constructed from (inferred) fragments. The size of the viewpoints in `regions` must also be subtracted, to account for the inflated peak width when spatial resolution is lost after aggregation across many windows.

Value

An integer scalar is returned, specifying the ideal window width.

Author(s)

Aaron Lun

See Also

[profileSites](#), [getWidths](#)

Examples

```
x <- dnorm(-200:200/100) # Mocking up a profile.
windows <- GRanges("chrA", IRanges(1, 50)) # Making up some windows.

wwhm(x, windows)
wwhm(x, windows, ext=50)
wwhm(x, windows, proportion=0.2)

# Need to set 'rlen' if ext=NA.
wwhm(x, windows, ext=NA, rlen=10)
```

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