Package 'BRGenomics'

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```
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Description

BRGenomics provides useful functions for analyzing genomics data at base-pair resolution, and for doing so in a way that maximizes compatibility with the wide array of packages available through Bioconductor.

For interactive documentation with code examples, see the online documentation: https://mdeber.github.io/

Author(s)

Mike DeBerardine <mike.deberardine@gmail.com>

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binNdimensions

N-dimensional binning

Description

This function takes in data along 1 or more dimensions, and for each dimension the data is divided into evenly-sized bins from the minimum value to the maximum value, and bin numbers are returned. For instance, if each index of the input data were a gene, the input dimensions would be various quantitative measures of that gene, e.g. expression level, number of exons, length, etc. If plotted in cartesian coordinates, each gene would be a single datapoint, and each measurement would be a separate dimension. The bin numbers for each datapoint in each dimension are returned in a dataframe, with a column for each dimension and a row for each index.

Usage

```
binNdimensions(..., nbins = 10)
```

Arguments

. . .

A single dataframe, or any number of lists or vectors containing different measurements across the same datapoints. If a dataframe is given, columns should correspond to measurements (dimensions). If lists or vectors are given, they must all have the same lengths. Other input classes will be coerced into a single dataframe.

nbins

Either a number giving the number of bins to use for all dimensions (default = 10), or a vector containing the number of bins to use for each dimension of input data given.

Value

A dataframe containing indices in 1:nbins for each datapoint in each dimension.

Author(s)

Mike DeBerardine

```
#-----#
# divide genes into 20 bins for each measurement
#------#

count_bins <- binNdimensions(counts_pr, counts_gb, counts_cps, nbins = 20)
length(txs_dm6_chr4)
nrow(count_bins)
count_bins[1:10, ]</pre>
```

bootstrap-signal-by-position

Bootstrapping Mean Signal by Position for Metaplotting

Description

These functions perform bootstrap subsampling of mean readcounts at different positions within regions of interest (metaSubsample), or, in the more general case of metaSubsampleMatrix, column means of a matrix are bootstrapped by sampling the rows. Mean signal counts can be calculated at base-pair resolution, or over larger bins.

Usage

```
metaSubsample(
  dataset.gr,
  regions.gr,
  binsize = 1,
  first.output.xval = 1,
  sample.name = deparse(substitute(dataset.gr)),
  n.iter = 1000,
  prop.sample = 0.1,
  lower = 0.125,
  upper = 0.875,
  field = "score",
  NF = NULL
  remove.empty = FALSE,
  ncores = detectCores()
)
metaSubsampleMatrix(
  counts.mat,
  binsize = 1,
  first.output.xval = 1,
  sample.name = NULL,
  n.iter = 1000,
  prop.sample = 0.1,
  lower = 0.125,
  upper = 0.875,
  NF = 1,
  remove.empty = FALSE,
  ncores = detectCores()
)
```

Arguments

dataset.gr A GRanges object in which signal is contained in metadata (typically in the

"score" field).

regions.gr A GRanges object containing intervals over which to metaplot. All ranges must

have the same width.

binsize The size of bin (in basepairs, or number of columns for metaSubsampleMatrix)

to use for counting signal. Especially important for counting signal over large

or sparse regions.

first.output.xval

The relative start position of the first bin, e.g. if regions.gr begins at 50 bases upstream of the TSS, set first.output.xval = -50. This number only affects

the x-values that are returned, which are provided as a convenience.

sample.name Defaults to the name of the input dataset. This is included in the output as

a convenience, as it allows row-binding outputs from different samples. If length(field) > 1 and the default sample.name is left, the sample names will

be inferred from the field names.

n.iter Number of random subsampling iterations to perform. Default is 1000.

prop.sample The proportion of the ranges in regions.gr (e.g. the proportion of genes) or

the proportion of rows in counts.mat to sample in each iteration. The default

is 0.1 (10 percent).

lower, upper The lower and upper quantiles of subsampled signal means to return. The de-

faults, 0.125 and 0.875 (i.e. the 12.5th and 85.5th percentiles) return a 75

percent confidence interval about the bootstrapped mean.

field One or more metadata fields of dataset.gr to be counted.

NF An optional normalization factor by which to multiply the counts. If given,

length(NF) must be equal to length(field).

remove.empty A logical indicating whether regions (metaSubsample) or rows (metaSubsampleMatrix)

without signal should be removed from the analysis. Not recommended if using

multiple fields, as the gene lists will no longer be equivalent.

ncores Number of cores to use for computations.

counts.mat A matrix over which to bootstrap column means by subsampling its rows. Typ-

ically, a matrix of readcounts with rows for genes and columns for positions

within those genes.

Value

Dataframe containing x-values, means, lower quantiles, upper quantiles, and the sample name (as a convenience for row-binding multiple of these dataframes). If multiple fields are given, a single dataframe is still returned, but will contain data for all fields.

Author(s)

Mike DeBerardine

See Also

getCountsByPositions

```
data("PROseq") # import included PROseq data
data("txs_dm6_chr4") # import included transcripts
# for each transcript, use promoter-proximal region from TSS to +100
pr <- promoters(txs_dm6_chr4, 0, 100)</pre>
#-----#
# Bootstrap average signal in each 5 bp bin across all transcripts,
# and get confidence bands for middle 30% of bootstrapped means
#----#
set.seed(11)
df <- metaSubsample(PROseq, pr, binsize = 5,</pre>
               lower = 0.35, upper = 0.65,
               ncores = 2)
df[1:10, ]
#-----#
# Plot bootstrapped means with confidence intervals
#-----#
plot(mean \sim x, df, type = "l", main = "PROseq Signal",
   ylab = "Mean + 30% CI", xlab = "Distance from TSS")
polygon(c(df\$x,\ rev(df\$x)),\ c(df\$lower,\ rev(df\$upper)),
      col = adjustcolor("black", 0.1), border = FALSE)
#=========#
# Using a matrix as input
# generate a matrix of counts in each region
countsmat <- getCountsByPositions(PROseq, pr)</pre>
dim(countsmat)
#-----#
# bootstrap average signal in 10 bp bins across all transcripts
#-----#
set.seed(11)
df <- metaSubsampleMatrix(countsmat, binsize = 10,</pre>
                     sample.name = "PROseq",
                     ncores = 2)
df[1:10, ]
# the same, using a normalization factor, and changing the x-values
#-----#
set.seed(11)
df <- metaSubsampleMatrix(countsmat, binsize = 10,</pre>
                    first.output.xval = 0, NF = 0.75,
                    sample.name = "PROseq", ncores = 2)
df[1:10, ]
```

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|--|

Description

This function returns ranges that are defined relative to the strand-specific start and end sites of regions of interest (usually genes).

Usage

```
genebodies(
  genelist,
  start = 300,
  end = -300,
  fix.start = "start",
  fix.end = "end",
  min.window = 0
)
```

Arguments

genelist	A GRanges object containing genes of interest.
start	Depending on fix.start, the distance from either the strand-specific start or end site to begin the returned ranges. If positive, the returned range will begin downstream of the reference position; negative numbers are used to return sites upstream of the reference. Set start = 0 to return the reference position.
end	Identical to the start argument, but defines the strand-specific end position of returned ranges. end must be downstream of start.
fix.start	The reference point to use for defining the strand-specific start positions of returned ranges, either "start" or "end".
fix.end	The reference point to use for defining the strand-specific end positions of returned ranges, either "start" or "end". Cannot be set to "start" if fix.start = "end".
min.window	When fix.start = "start" and fix.end = "end", min.window defines the minimum size (width) of a returned range. However, when fix.end = fix.start, all returned ranges have the same width, and min.window simply size-filters the input ranges.

Details

Unlike GenomicRanges::promoters, distances can be defined to be upstream or downstream by changing the sign of the argument, and both the start and end of the returned regions can be defined in terms of the strand-specific start or end site of the input ranges. For example, genebodies(txs,-50,150,fix.end = "start") is equivalent to promoters(txs,50,151) (the downstream edge is off by 1 because promoters keeps the downstream interval closed). The default arguments return ranges that begin 300 bases downstream of the original start positions, and end 300 bases upstream of the original end positions.

Value

A GRanges object that may be shorter than genelist due to filtering of short ranges. For example, using the default arguments, genes shorter than 600 bp would be removed.

Author(s)

Mike DeBerardine

See Also

```
intra-range-methods
```

Examples

 ${\tt getCountsByPositions} \quad \textit{Get signal counts at each position within regions of interest}$

Description

Get the sum of the signal in dataset.gr that overlaps each position within each range in regions.gr. If binning is used (i.e. positions are wider than 1 bp), any function can be used to summarize the signal overlapping each bin.

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Usage

```
getCountsByPositions(
  dataset.gr,
  regions.gr,
  binsize = 1,
  FUN = sum,
  simplify.multi.widths = c("list", "pad 0", "pad NA"),
  field = "score",
  NF = NULL,
  ncores = detectCores()
)
```

Arguments

dataset.gr A GRanges object in which signal is contained in metadata (typically in the

"score" field).

regions.gr A GRanges object containing regions of interest.

binsize Size of bins (in bp) to use for counting within each range of regions.gr. Note

that counts will not be length-normalized.

FUN If binsize > 1, the function used to aggregate the signal within each bin. By

default, the signal is summed, but any function operating on a numeric vector

can be used.

simplify.multi.widths

A string indicating the output format if the ranges in regions.gr have variable

widths. Default is "list". See details below.

field The metadata field of dataset.gr to be counted. If length(field) > 1, the

output is a list whose elements contain the output for generated each field. If field not found in names(mcols(dataset.gr)), will default to using all fields

found in dataset.gr.

NF An optional normalization factor by which to multiply the counts. If given,

length(NF) must be equal to length(field).

ncores Multiple cores can only be used if length(field) > 1.

Value

If the widths of all ranges in regions. gr are equal, a matrix is returned that contains a row for each region of interest, and a column for each position (each base if binsize = 1) within each region.

Use of multi-width regions of interest

If the input regions.gr contains ranges of varying widths, setting simplify.multi.widths = "list" will output a list of variable-length vectors, with each vector corresponding to an individual input region. If simplify.multi.widths = "pad 0" or "pad NA", the output is a matrix containing a row for each range in regions.gr, but the number of columns is determined by the largest range in regions.gr. For each region of interest, columns that correspond to positions outside of the input range are set, depending on the argument, to 0 or NA.

Author(s)

Mike DeBerardine

See Also

```
getCountsByRegions
```

Examples

```
data("PROseq") # load included PROseq data
data("txs_dm6_chr4") # load included transcripts
#-----#
# counts from 0 to 50 bp after the TSS
#-----#
txs_pr <- promoters(txs_dm6_chr4, 0, 50) # first 50 bases</pre>
countsmat <- getCountsByPositions(PROseq, txs_pr)</pre>
countsmat[10:15, 41:50] # show only 41-50 bp after TSS
# redo with 10 bp bins from 0 to 100
#-----#
# column 5 is sums of rows shown above
txs_pr <- promoters(txs_dm6_chr4, 0, 100)</pre>
countsmat <- getCountsByPositions(PROseq, txs_pr, binsize = 10)</pre>
countsmat[10:15, ]
#-----#
# same as the above, but with the average signal in each bin
#-----#
countsmat <- getCountsByPositions(PROseq, txs_pr, binsize = 10, FUN = mean)</pre>
countsmat[10:15, ]
# standard deviation of signal in each bin
#-----#
countsmat <- getCountsByPositions(PROseq, txs_pr, binsize = 10, FUN = sd)</pre>
round(countsmat[10:15, ], 1)
```

getCountsByRegions

Get signal counts in regions of interest

Description

Get the sum of the signal in dataset.gr that overlaps each range in regions.gr.

Usage

```
getCountsByRegions(
  dataset.gr,
  regions.gr,
  field = "score",
```

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```
NF = NULL,
ncores = detectCores()
)
```

Arguments

dataset.gr A GRanges object in which signal is contained in metadata (typically in the

"score" field).

regions.gr A GRanges object containing regions of interest.

field The metadata field of dataset.gr to be counted. If length(field) > 1, a

dataframe is returned containing the counts for each region in each field. If field not found in names(mcols(dataset.gr)), will default to using all fields

found in dataset.gr.

NF An optional normalization factor by which to multiply the counts. If given,

length(NF) must be equal to length(field).

ncores Multiple cores can only be used if length(field) > 1.

Value

An atomic vector the same length as regions.gr containing the sum of the signal overlapping each range of regions.gr.

Author(s)

Mike DeBerardine

See Also

```
getCountsByPositions
```

```
data("PROseq") # load included PROseq data
data("txs_dm6_chr4") # load included transcripts

counts <- getCountsByRegions(PROseq, txs_dm6_chr4)
length(txs_dm6_chr4)
length(counts)
head(counts)

# Assign as metadata to the transcript GRanges
txs_dm6_chr4$PROseq <- counts

txs_dm6_chr4[1:6]</pre>
```

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 ${\tt getDESeqDataSet} \qquad \qquad {\tt Get\ DESeqDataSet\ objects\ for\ downstream\ analysis}$

Description

This is a convenience function for generating DESeqDataSet objects, but this function also adds support for counting reads across non-contiguous regions.

Usage

```
getDESeqDataSet(
  dataset.list,
  regions.gr,
  sample_names = names(dataset.list),
  gene_names = NULL,
  sizeFactors = NULL,
  field = "score",
  ncores = detectCores(),
  quiet = FALSE
)
```

Arguments

dataset.list	A list of GRanges datasets that can be individually passed to getCountsByRegions. Alternatively, a multiplexed GRanges object can also be used (see details below).
regions.gr	A GRanges object containing regions of interest.
sample_names	Names for each dataset in dataset.list are required, and by default the names of the list elements are used. The names must each contain the string "_rep#", where "#" is a single character (usually a number) indicating the replicate. Sample names across different replicates must be otherwise identical.
gene_names	An optional character vector giving gene names, or any other identifier over which reads should be counted. Gene names are required if counting is to be performed over non-contiguous ranges, i.e. if any genes have multiple ranges. If supplied, gene names are added to the resulting DESeqDataSet object.
sizeFactors	DESeq2 sizeFactors can be optionally applied in to the DESeqDataSet object in this function, or they can be applied later on, either by the user or in a call to getDESeqResults. Applying the sizeFactors later is useful if multiple sets of factors will be explored, although sizeFactors can be overwritten at any time.
field	Argument passed to getCountsByRegions. Optional if dataset.list is a multiplexed GRanges object (see details below).
ncores	Number of cores to use for read counting across all samples. By default, all available cores are used.
quiet	If TRUE, all output messages from call to DESeqDataSet will be suppressed.

Value

A DESeqData object in which rowData are given as rowRanges, which are equivalent to regions.gr, unless there are non-contiguous gene regions (see note below). Samples (as seen in colData) are factored so that samples are grouped by replicate and condition, i.e. all non-replicate samples are treated as distinct, and the DESeq2 design = \sim condition.

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Use of non-contiguous gene regions

In DESeq2, genes must be defined by single, contiguous chromosomal locations. This function allows individual genes to be encompassed by multiple distinct ranges in regions.gr. To use non-contiguous gene regions, provide gene_names in which some names are duplicated. For each unique gene in gene_names, this function will generate counts across all ranges for that gene, but be aware that it will only keep the largest range for each gene in the resulting DESeqDataSet object's rowRanges.

Using multiplexed GRanges as input

If a single multiplexed GRanges object is used in lieu of a list of GRanges, the default arguments for field and sample_names can be left, and sample_names will match the names of the fields in the input GRanges. If provided, field will be used to access/subset the multiplexed GRanges object, and thus all elements of field must be found in names(mcols(dataset.list)).

A note on DESeq2 sizeFactors

DESeq2 sizeFactors are sample-specific normalization factors that are applied by division, i.e. $counts_{norm,i} = counts_i/sizeFactor_i$. This is in contrast to normalization factors as defined in this package (and commonly elsewhere), which are applied by multiplication. Also note that DESeq2's "normalizationFactors" are not sample specific, but rather gene specific factors used to correct for ascertainment bias across different genes (e.g. as might be relevant for GSEA or Go analysis).

Author(s)

Mike DeBerardine

See Also

```
DESeq2::DESeqDataSet, getDESeqResults
```

```
suppressPackageStartupMessages(require(DESeq2))
data("PROseq") # import included PROseq data
data("txs_dm6_chr4") # import included transcripts
# divide PROseq data into 6 toy datasets
ps_a_rep1 <- PROseq[seq(1, length(PROseq), 6)]</pre>
ps_b_rep1 <- PROseq[seq(2, length(PROseq), 6)]</pre>
ps_c_rep1 <- PROseq[seq(3, length(PROseq), 6)]</pre>
ps_a_rep2 <- PROseq[seq(4, length(PROseq), 6)]</pre>
ps_b_rep2 <- PROseq[seq(5, length(PROseq), 6)]</pre>
ps_c_rep2 <- PROseq[seq(6, length(PROseq), 6)]</pre>
ps_list <- list(A_rep1 = ps_a_rep1, A_rep2 = ps_a_rep2,</pre>
                 B_rep1 = ps_b_rep1, B_rep2 = ps_b_rep2,
                 C_rep1 = ps_c_rep1, C_rep2 = ps_c_rep2)
# make flawed dataset (ranges in txs_dm6_chr4 not disjoint)
     this means there is double-counting
# also using discontinuous gene regions, as gene_ids are repeated
dds <- getDESeqDataSet(ps_list,</pre>
```

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```
txs_dm6_chr4,
gene_names = txs_dm6_chr4$gene_id,
quiet = TRUE,
ncores = 2)
```

dds

getDESeqResults

Get DESeq2 results using reduced dispersion matrices

Description

This function calls DESeq2::DESeq and DESeq2::results on a pre-existing DESeqDataSet object and returns a DESeqResults table for one or more pairwise comparisons. However, unlike a standard call to DESeq2::results using the contrast argument, this function subsets the dataset so that DESeq2 only estimates dispersion for the samples being compared, and not for all samples present.

Usage

```
getDESeqResults(
   dds,
   contrast.numer,
   contrast.denom,
   comparisons = NULL,
   sizeFactors = NULL,
   alpha = 0.1,
   args.DESeq = NULL,
   args.results = NULL,
   ncores = detectCores(),
   quiet = FALSE
)
```

Arguments

A DESeqDataSet object, produced using either getDESeqDataSet from this

package or DESeqDataSet from DESeq2. If dds was not created using getDESeqDataSet, dds must be made with design = ~condition such that a unique condition

level exists for each sample/treatment condition.

contrast.numer A string naming the condition to use as the numerator in the DESeq2 compar-

ison, typically the perturbative condition.

contrast.denom A string naming the condition to use as the denominator in the DESeq2 com-

parison, typically the control condition.

comparisons As an optional alternative to supplying a single contrast.numer and contrast.denom,

users can supply a list of character vectors containing numerator-denominator pairs, e.g. list(c("B","A"),c("C","A"),c("C","B")). comparisons can also be a dataframe in which each row is a comparison, the first column contains the numerators, and the second column contains the denominators.

sizeFactors A vector containing DESeq2 sizeFactors to apply to each sample. Each sam-

ple's readcounts are *divided* by its respective DESeq2 sizeFactor. A warning will be generated if the DESeqDataSet already contains sizeFactors, and the

previous sizeFactors will be over-written.

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alpha	The significance threshold passed to DESeqResults. This won't affect the output results, but is used as a performance optimization by DESeq2.
args.DESeq	Additional arguments passed to DESeq, given as a list of argument-value pairs, e.g. list(test = "LRT", fitType = "local"). All arguments given here will be passed to DESeq except for object and parallel. If no arguments are given, all defaults will be used.
args.results	Additional arguments passed to DESeq2::results, given as a list of argument-value pairs, e.g. list(altHypothesis = "greater",lfcThreshold = 1.5). All arguments given here will be passed to results except for object, contrast, alpha, and parallel. If no arguments are given, all defaults will be used.
ncores	The number of cores to use for parallel processing. Multicore processing is only used if more than one comparison is being made (i.e. argument comparisons is used), and the number of cores utilized will not be greater than the number of comparisons being performed.
quiet	If TRUE, all output messages from calls to DESeq and results will be suppressed, although passing option quiet in args. DESeq will supersede this option for the call to DESeq.

Value

For a single comparison, the output is the DESeqResults result table. If a comparisons is used to make multiple comparisons, the output is a named list of DESeqResults objects, with elements named following the pattern "X_vs_Y", where X is the name of the numerator condition, and Y is the name of the denominator condition.

Author(s)

Mike DeBerardine

See Also

```
getDESeqDataSet, DESeq2::results
```

```
# make flawed dataset (ranges in txs_dm6_chr4 not disjoint)
# this means there is double-counting
# also using discontinuous gene regions, as gene_ids are repeated
dds <- getDESeqDataSet(ps_list,</pre>
                    txs_dm6_chr4,
                    gene_names = txs_dm6_chr4$gene_id,
                    ncores = 2)
dds
#-----#
# getDESeqResults
#-----#
res <- getDESeqResults(dds, "B", "A")</pre>
res
reslist <- getDESeqResults(dds, comparisons = list(c("B", "A"), c("C", "A")),
                        ncores = 1)
names(reslist)
reslist$B_vs_A
# or using a dataframe
reslist <- \ getDESeqResults(dds, \ comparisons = \ data.frame(num = c("B", "C"),
                                                   den = c("A", "A")),
                        ncores = 1)
reslist$B_vs_A
```

getMaxPositionsBySignal

Find sites with max signal in regions of interest

Description

For each signal-containing region of interest, find the single site with the most signal. Sites can be found at base-pair resolution, or defined for larger bins.

Usage

```
getMaxPositionsBySignal(
  regions.gr,
  dataset.gr,
  binsize = 1,
  bin.centers = FALSE,
  field = "score",
  keep.signal = FALSE
)
```

Arguments

regions.gr A GRanges object containing regions of interest.

dataset.gr A GRanges object in which signal is contained in metadata (typically in the

"score" field).

binsize The size of bin in which to calculate signal scores.

bin.centers Logical indicating if the centers of bins are returned, as opposed to the entire

bin. By default, entire bins are returned.

field The metadata field of dataset.gr to be counted.

keep.signal Logical indicating if the signal value at the max site should be reported. If set

to TRUE, the values are kept as a new MaxSiteSignal metadata column in the

output GRanges.

Value

Output is a GRanges object with regions.gr metadata, but each range only contains the site within each regions.gr range that had the most signal. If binsize > 1, the entire bin is returned, unless bin.centers = TRUE, in which case a single-base site is returned. The site is set to the center of the bin, and if the binsize is even, the site is rounded to be closer to the beginning of the range.

The output may not be the same length as regions.gr, as regions without signal are not returned. If no regions have signal (e.g. as could happen if running this function on single regions), the function will return an empty GRanges object with intact metadata columns.

If keep.signal = TRUE, the output will also contain metadata for the signal at the max site, named MaxSiteSignal.

Author(s)

Mike DeBerardine

See Also

getCountsByPositions

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```
#-----#
getMaxPositionsBySignal(pr[1:3], PROseq, binsize = 5, keep.signal = TRUE)
```

getPausingIndices

Calculate pausing indices from user-supplied promoters & genebodies

Description

Pausing index (PI) is calculated for each gene (within matched promoters.gr and genebodies.gr) as promoter-proximal (or pause region) signal counts divided by genebody signal counts. If length.normalize = TRUE (recommended), the signal counts within each range in promoters.gr and genebodies.gr are divided by their respective range widths (region lengths) before pausing indices are calculated.

Usage

```
getPausingIndices(
  dataset.gr,
  promoters.gr,
  genebodies.gr,
  field = "score",
  length.normalize = TRUE,
  remove.empty = FALSE,
  ncores = detectCores()
)
```

Arguments

dataset.gr A GRanges object in which signal is contained in metadata (typically in the

"score" field).

promoters.gr A GRanges object containing promoter-proximal regions of interest.

genebodies.gr A GRanges object containing genebody regions of interest.

field The metadata field of dataset.gr to be counted. If length(field) > 1, a

dataframe is returned containing the pausing indices for each region in each field. If field not found in names(mcols(dataset.gr)), will default to using

all fields found in dataset.gr.

length.normalize

A logical indicating if signal counts within regions of interest should be length normalized. The default is TRUE, which is recommended, especially if input

regions don't all have the same width.

remove.empty A logical indicating if genes without any signal in promoters.gr should be

removed. No genes are filtered by default.

ncores Multiple cores can only be used if length(field) > 1.

Value

A vector parallel to the input genelist, unless remove.empty = TRUE, in which case the vector may be shorter. If length(field) > 1, a dataframe is returned, containing a column for each field.

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Author(s)

Mike DeBerardine

See Also

getCountsByRegions

```
data("PROseg") # load included PROseg data
data("txs_dm6_chr4") # load included transcripts
#-----#
# Get promoter-proximal and genebody regions
# genebodies from +300 to 300 bp before the poly-A site
gb <- genebodies(txs_dm6_chr4, 300, -300, min.window = 400)</pre>
# get the transcripts that are large enough (>1kb in size)
txs <- subset(txs_dm6_chr4, tx_name %in% gb$tx_name)</pre>
# for the same transcripts, promoter-proximal region from 0 to +100
pr <- promoters(txs, 0, 100)</pre>
#-----#
# Calculate pausing indices
#-----#
pidx <- getPausingIndices(PROseq, pr, gb)</pre>
length(txs)
length(pidx)
head(pidx)
#-----#
# Without length normalization
head( getPausingIndices(PROseq, pr, gb, length.normalize = FALSE) )
# Removing empty means the values no longer match the genelist
#----#
pidx_signal <- getPausingIndices(PROseq, pr, gb, remove.empty = TRUE)</pre>
length(pidx_signal)
```

20 getStrandedCoverage

Description

Computes strand-specific coverage signal, and returns a GRanges object. Function also works for non-strand-specific data.

Usage

```
getStrandedCoverage(dataset.gr, field = "score", ncores = detectCores())
```

Arguments

dataset.gr A GRanges object either containing ranges for each read, or one in which read-

counts for individual ranges are contained in metadata (typically in the "score"

field).

field The name of the metadata field that contains readcounts. If no metadata field

contains readcounts, and each range represents a single read, set to NULL.

ncores Number of cores to use for calculating coverage. The max that will be used is 3,

one for each possible strand (plus, minus, and unstranded).

Value

A GRanges object with signal in the "score" metadata column. Note that the output is *not* automatically converted into a "basepair-resolution" GRanges object.

Author(s)

Mike DeBerardine

See Also

```
makeGRangesBRG, GenomicRanges::coverage
```

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```
# undo coverage for the first 100 positions
ps <- PROseq[1:100]</pre>
ps_reads <- rep(ps, times = ps$score)</pre>
mcols(ps_reads) <- NULL</pre>
ps_reads[1:6]
# re-create coverage
getStrandedCoverage(ps_reads, field = NULL, ncores = 2)[1:6]
#-----#
# Reversing makeGRangesBRG
#-----#
# -> getStrandedCoverage doesn't return single-width
    GRanges, which is useful because getting coverage
    will merge adjacent bases with equivalent scores
# included PROseq data is already single-width
range(width(PROseq))
isDisjoint(PROseq)
ps_cov <- getStrandedCoverage(PROseq, ncores = 2)</pre>
range(width(ps_cov))
sum(score(PROseq)) == sum(score(ps_cov) * width(ps_cov))
# -> Look specifically at ranges that could be combined
neighbors <- c(shift(PROseq, 1), shift(PROseq, -1))</pre>
hits <- findOverlaps(PROseq, neighbors)</pre>
idx <- unique(from(hits)) # indices for PROseq with neighbor</pre>
PROseq[idx]
getStrandedCoverage(PROseq[idx], ncores = 2)
```

import-functions

Import basepair-resolution files

Description

Import functions for plus/minus pairs of bigWig or bedGraph files.

Usage

```
import_bigWig(
  plus_file,
  minus_file,
  genome = NULL,
  keep.X = TRUE,
  keep.Y = TRUE,
  keep.M = FALSE,
  keep.nonstandard = FALSE
)
```

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```
import_bedGraph(
  plus_file,
  minus_file,
  genome = NULL,
  keep.X = TRUE,
  keep.Y = TRUE,
  keep.M = FALSE,
  keep.nonstandard = FALSE
)
```

Arguments

plus_file, minus_file

Paths for strand-specific input files.

genome

Optional string for UCSC reference genome, e.g. "hg38". If given, non-standard chromosomes are trimmed, and options for sex and mitochondrial chromosomes are applied.

keep.X, keep.Y, keep.M, keep.nonstandard

Logicals indicating which non-autosomes should be kept. By default, sex chromosomes are kept, but mitochondrial and non-standard chromosomes are removed.

Details

For import_bigWig, the output GRanges is formatted by makeGRangesBRG, such that all ranges are disjoint and have width = 1, and the score is single-base coverage, i.e. the number of reads for each position.

import_bedGraph is useful for when both 5'- and 3'-end information is to be maintained for each sequenced molecule. It effectively imports the entire read, and the score represents the number of reads sharing identical 5' and 3' ends.

Value

Imports a GRanges object containing base-pair resolution data, with the score metadata column indicating the number of reads represented by each range.

Author(s)

Mike DeBerardine

See Also

```
tidyChromosomes, rtracklayer::import
```

import_bam 23

import_bam

Import bam files

Description

Import single-end or paired-end bam files as GRanges objects, with various processing options.

Usage

```
import_bam(
  file,
  mapq = 20,
  revcomp = FALSE,
  shift = 0L,
  trim.to = c("whole", "5p", "3p", "center"),
  ignore.strand = FALSE,
  field = "score",
  paired_end = NULL,
  yieldSize = 250000
)
```

Arguments

file Path of a bam file.

mapq Filter reads by a minimum MAPQ score. This is the correct way to filter multi-

aligners.

revcomp Logical indicating if aligned reads should be reverse-complemented.

shift Either an integer giving the number of bases by which to shift the entire read up-

stream or downstream, or a pair of integers indicating shifts to be applied to the 5' and 3' ends of the reads, respectively. Shifting is strand-specific, with negative numbers shifting the reads upstream, and positive numbers shiftem them downstream. This option is applied *after* the revcomp, but before trim. to and

ignore.strand options are applied.

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trim.to Option for selecting specific bases from the reads, applied after the revcomp and shift options. By default, the entire read is maintained. Other options are to take only the 5' base, only the 3' base, or the only the center base of the read. Logical indicating if the strand information should be discarded. If TRUE, strand ignore.strand information is discarded after revcomp, trim.to, and shift options are applied. field Metadata field name to use for readcounts, usually "score". If set to NULL, identical reads (or identical positions if trim. to options applied) are not combined, and the length of the output GRanges will be equal to the number of input reads. Logical indicating if reads should be treated as paired end reads. When set to paired_end NULL (the default), a test is performed to determine if the bam file contains any paired-end reads. yieldSize The number of bam file records to process simultaneously, e.g. the "chunk size". Setting a higher chunk size will use more memory, which can increase speed if there is enough memory available. If chunking is not necessary, set to NA.

Details

If function produces an error, make the paired_end parameter explicit, i.e. TRUE or FALSE.

Value

A GRanges object.

Author(s)

Mike DeBerardine & Nate Tippens

```
# get local address for included bam file
ps.bam <- system.file("extdata", "PROseq_dm6_chr4.bam",</pre>
                package = "BRGenomics")
#----#
# Import entire reads
#-----#
# Note that PRO-seq reads are sequenced as reverse complement
import_bam(ps.bam, revcomp = TRUE, paired_end = FALSE)
# Import entire reads, 1 range per read
#-----#
import_bam(ps.bam, revcomp = TRUE, field = NULL,
        paired_end = FALSE)
# Import PRO-seq reads at basepair-resolution
#-----#
# the typical manner to import PRO-seq data:
import_bam(ps.bam, revcomp = TRUE, trim.to = "3p",
```

makeGRangesBRG 25

makeGRangesBRG

Make base-pair resolution GRanges object

Description

Splits up all ranges in dataset.gr to be each 1 basepair wide. For any range that is split up, all metadata information belonging to that range is inherited by its daughter ranges, and therefore the transformation is non-destructive.

Usage

```
makeGRangesBRG(dataset.gr)
```

Arguments

```
dataset.gr A disjoint GRanges object
```

Details

Note that this function doesn't perform any transformation on the metadata in the input. This function assumes that for an input GRanges object, any metadata for each range is equally correct when inherited by each individual base in that range. In other words, the dataset's "signal" (usually readcounts) fundamentally belongs to a single basepair position.

Value

```
A GRanges object for which length(output) == sum(width(dataset.gr)), and for which all(width(output) == 1).
```

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Motivation

The motivating case for this function is a bigWig file (e.g. one imported by rtracklayer), as big-Wig files typically use run-length compression on the data signal (the 'score' column), such that adjacent bases sharing the same signal are combined into a single range. As basepair-resolution genomic data is typically sparse, this compression has a minimal impact on memory usage, and removing it greatly enhances data handling as each index (each range) of the GRanges object corresponds to a single genomic position.

Generating basepair-resolution GRanges from whole reads

If working with a GRanges object containing whole reads, one can obtain base-pair resolution information by using the strand-specific function GenomicRanges::resize to select a single base from each read: set width = 1 and use the fix argument to choose the strand-specific 5' or 3' end. Then, strand-specific coverage can be calculated using getStrandedCoverage.

On the use of GRanges instead of GPos

The GPos class is a more suitable container for data of this type, as the GPos class is specific to 1-bp-wide ranges. However, in early testing, we encountered some kind of compatibility limitations with the newer GPos class, and have not re-tested it since. If you have feedback on switching to this class, please contact the author. Users can readily coerce a basepair-resolution GRanges object to a GPos object via gp <-GPos(gr, score = score(gr)).

Author(s)

Mike DeBerardine

See Also

```
getStrandedCoverage, GenomicRanges::resize()
```

```
#----#
# Make a bigWig file single width
#-----#
# get local address for an included bigWig file
bw_file <- system.file("extdata", "PROseq_dm6_chr4_plus.bw",</pre>
                    package = "BRGenomics")
# BRGenomics::import_bigWig automatically applies makeGRangesBRG;
# therefore will import using rtracklayer
bw <- rtracklayer::import.bw(bw_file)</pre>
strand(bw) <- "+"
range(width(bw))
length(bw)
# make basepair-resolution (single-width)
gr <- makeGRangesBRG(bw)</pre>
range(width(gr))
length(gr)
length(gr) == sum(width(bw))
```

mergeGRangesData 27

mergeGRangesData

Merge basepair-resolution GRanges objects

Description

Merges 2 or more basepair-resolution (single-width) GRanges objects by combining all of their ranges and associated signal (e.g. readcounts). If multiplex = TRUE, the input datasets are reversibly combined into a multiplexed GRanges containing a field for each input dataset.

Usage

```
mergeGRangesData(
    ...,
    field = "score",
    multiplex = FALSE,
    ncores = detectCores()
)
```

Arguments

... Any number of GRanges objects in which signal (e.g. readcounts) are contained

within metadata. GRanges not single-width will be coerced using makeGRangesBRG. Lists of GRanges can also be passed, but they must be named lists if multiplex = TRUE. Multiple lists can be passed, but if any inputs are lists, then all inputs

must be lists.

field One or more *input* metadata fields to be combined, typically the "score" field.

Fields typically contain coverage information. If only a single field is given (i.e. all input GRanges use the same field), that same field will be used for the output. Otherwise, the score metadata field will be used by default. The output

metadata fields are different if multiplex is enabled.

multiplex When set to FALSE (the default), input GRanges are merged irreversibly into a

single new GRange, effectively combining the reads from different experiments. When multiplex = TRUE, the input GRanges data are reversibly combined into a multiplexed GRanges object, such that each input GRanges object has its own

metadata field in the output.

ncores Number of cores to use for computations.

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Value

A disjoint, basepair-resolution (single-width) GRanges object comprised of all ranges found in the input GRanges objects.

If multiplex = FALSE, single fields from each input are combined into a single field in the output, the total signal of which is the sum of all input GRanges.

If multiplex = TRUE, each field of the output corresponds to an input GRanges object.

Subsetting a multiplexed GRanges object

If multiplex = TRUE, the datasets are only combined into a single object, but the data themselves are not combined. To subset field_i, corresponding to input dataset_i:

```
multi.gr <-mergeGRangesData(gr1,gr2,multiplex = TRUE) subset(multi.gr,gr1 != 0,select
= gr1) # select gr1
```

Author(s)

Mike DeBerardine

See Also

makeGRangesBRG

```
data("PROseq") # load included PROseq data
# divide & recombine PROseq (no overlapping positions)
thirds <- floor( (1:3)/3 * length(PROseq) )
ps_1 <- PROseq[1:thirds[1]]</pre>
ps_2 <- PROseq[(thirds[1]+1):thirds[2]]</pre>
ps_3 <- PROseq[(thirds[2]+1):thirds[3]]</pre>
# re-merge
length(PROseq)
length(ps_1)
length(mergeGRangesData(ps_1, ps_2, ncores = 2))
length(mergeGRangesData(ps_1, ps_2, ps_3, ncores = 2))
# combine PRO-seq with overlapping positions
#-----#
gr1 <- PROseq[10:13]</pre>
gr2 <- PROseq[12:15]</pre>
PR0seq[10:15]
mergeGRangesData(gr1, gr2, ncores = 2)
#-----#
# multiplex separate PRO-seq experiments
```

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PROseq-data

PRO-seq data from Drosophila S2 cells

Description

PRO-seq data from chromosome 4 of Drosophila S2 cells.

Usage

```
data(PROseq)
data(PROseq_paired)
```

Format

A disjoint GRanges object with 47533 ranges with 1 metadata column:

```
score coverage of PRO-seq read 3'-ends ...
```

Source

GEO Accession GSM1032758, run SRR611828.

References

Hojoong Kwak, Nicholas J. Fuda, Leighton J. Core, John T. Lis (2013). Precise Maps of RNA Polymerase Reveal How Promoters Direct Initiation and Pausing. Science, 339(6122), 950–953. https://doi.org/10.1126/science.1229386

30 subsampleGRanges

subsampleGRanges	Randomly subsample reads from GRanges dataset	

Description

Random subsampling is not performed on ranges, but on reads. Readcounts should be given as a metadata field (usually "score"). This function can also subsample ranges directly if field = NULL, but the sample function can be used in this scenario.

Usage

```
subsampleGRanges(dataset.gr, n = NULL, prop = NULL, field = "score")
```

Arguments

dataset.gr	A GRanges object in which signal (e.g. readcounts) are contained within metadata.
n	Number of reads to subsample. Either n or prop can be given.
prop	Proportion of total signal to subsample.
field	The metadata field of dataset.gr that contains readcounts for reach position.

If each range represents a single read, set field = NULL

Value

A GRanges object identical in format to dataset.gr, but containing a random subset of its data. If field != NULL, the length of the output cannot be known *a priori*, but the sum of its score can.

Use with normalized readcounts

If the metadata field contains normalized readcounts, an attempt will be made to infer the normalization factor based on the lowest signal value found in the specified field.

Author(s)

Mike DeBerardine

```
data("PROseq") # load included PROseq data
#------#
# sample 10% of the reads of a GRanges with signal coverage
#------#

ps_sample <- subsampleGRanges(PROseq, prop = 0.1)
# cannot predict number of ranges (positions) that will be sampled length(PROseq)
length(ps_sample)
# 1/10th the score is sampled
sum(score(PROseq))</pre>
```

```
sum(score(ps_sample))
#-----#
# Sample 10% of ranges (e.g. if each range represents one read)
#------#
ps_sample <- subsampleGRanges(PROseq, prop = 0.1, field = NULL)
length(PROseq)
length(ps_sample)
# Alternatively
ps_sample <- sample(PROseq, 0.1 * length(PROseq))
length(ps_sample)</pre>
```

subsetRegionsBySignal Subset regions of interest by quantiles of overlapping signal

Description

A convenience function to subset regions of interest by the amount of signal they contain, according to their quantile (i.e. their signal ranks).

Usage

```
subsetRegionsBySignal(
  regions.gr,
  dataset.gr,
  quantiles = c(0.5, 1),
  field = "score",
  order.by.rank = FALSE,
  density = FALSE,
  keep.signal = FALSE
)
```

Arguments

regions.gr	A GRanges object containing regions of interest.
dataset.gr	A GRanges object in which signal is contained in metadata (typically in the "score" field).
quantiles	A value pair giving the lower quantile and upper quantile of regions to keep. Regions with signal quantiles below the lower quantile are removed, and likewise for regions with signal quantiles above the upper quantile. Quantiles must be in range (0,1). An empty GRanges object is returned if the lower quantile is set to 1 or if the upper quantile is set to 0.
field	The metadata field of dataset.gr to be counted, typically "score".
order.by.rank	If TRUE, the output regions are sorted based on the amount of overlapping signal (in decreasing order). If FALSE (the default), genes are sorted by their positions.
density	A logical indicating whether signal counts should be normalized to the width (chromosomal length) of ranges in regions.gr. By default, no length normal-

ization is performed.

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keep.signal

Logical indicating if signal counts should be kept. If set to TRUE, the signal for each range (length-normalized if density = TRUE) are kept as a new Signal metadata column in the output GRanges object.

Value

A GRanges object of length length(regions.gr) * (upper_quantile -lower_quantile).

Author(s)

Mike DeBerardine

See Also

getCountsByRegions

```
data("PROseg") # load included PROseg data
data("txs_dm6_chr4") # load included transcripts
txs_dm6_chr4
# get the top 50% of transcripts by signal
subsetRegionsBySignal(txs_dm6_chr4, PR0seq)
# get the middle 50% of transcripts by signal
#-----#
subsetRegionsBySignal(txs_dm6_chr4, PROseq, quantiles = c(0.25, 0.75))
\mbox{\#} get the top 10% of transcripts by signal, and sort them by highest signal
#-----#
subsetRegionsBySignal(txs_dm6_chr4, PROseq, quantiles = c(0.9, 1),
                  order.by.rank = TRUE)
#-----#
# remove the most extreme 10% of regions, and keep scores
subsetRegionsBySignal(txs_dm6_chr4, PROseq, quantiles = c(0.05, 0.95),
                  keep.signal = TRUE)
```

tidyChromosomes 33

Description

This convenience function removes non-standard, mitochondrial, and/or sex chromosomes from any GRanges object.

Usage

```
tidyChromosomes(
  gr,
  keep.X = TRUE,
  keep.Y = TRUE,
  keep.M = FALSE,
  keep.nonstandard = FALSE
)
```

Arguments

```
gr Any GRanges object, however the object should have a standard genome set, e.g. genome(gr) <-"hg38" keep.X, keep.Y, keep.M, keep.nonstandard
```

Logicals indicating which non-autosomes should be kept. By default, sex chromosomes are kept, but mitochondrial and non-standard chromosomes are removed.

Details

Standard chromosomes are defined using the standardChromosomes function from the GenomeInfoDb package.

Value

A GRanges object in which both ranges and seqinfo associated with trimmed chromosomes have been removed.

Author(s)

Mike DeBerardine

See Also

```
GenomeInfoDb::standardChromosomes
```

 txs_dm6_chr4

```
tidyChromosomes(gr, keep.M = TRUE)
tidyChromosomes(gr, keep.M = TRUE, keep.Y = FALSE)
tidyChromosomes(gr, keep.nonstandard = TRUE)
```

txs_dm6_chr4

Ensembl transcripts for Drosophila melanogaster, dm6, chromosome 4.

Description

Transcripts obtained from annotation package TxDb.Dmelanogaster.UCSC.dm6.ensGene, which was in turn made by the Bioconductor Core Team from UCSC resources on 2019-04-25. Metadata columns were obtained from "TXNAME" and "GENEID" columns. Data exported from the TxDb package using GenomicFeatures version 1.35.11 on 2019-12-19.

Usage

```
data(txs_dm6_chr4)
```

Format

A GRanges object with 339 ranges and 2 metadata columns:

tx_name Flybase unique identifiers for transcriptsgene_id FLybase unique identifiers for the associated genes

Source

TxDb.Dmelanogaster.UCSC.dm6.ensGene version 3.4.6

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