# Package 'BRGenomics'

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```
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BRGer	nomics-package	BRGen nomics		Tool	s fo	r tl	ne I	Effi	cie	nt A	Ana	ılys	is o	of I	Hi	gh	-Re	esc	olu	tio	n	$G\epsilon$	?-
Index																							46
	tas_unio_ciii4		 		•	• •		•			•		•		•	•	• •	•	•	•	•	•	+3
	txs_dm6_chr4																						
	tidyChromosomes .																						
	subsetRegionsBySig																						
	subsampleBySpikeIr subsampleGRanges																						
	PROseq-data																						
	mergeGRangesData																						
	makeGRangesBRG																						
	import_bam																						
	import-functions .																						
	getStrandedCoverage																						
	getSpikeInNFs																						
	getSpikeInCounts .																						
	getPausingIndices .																						
	getMaxPositionsByS	_																					
	getDESeqResults .																						
	getDESeqDataSet .																						
	getCountsByRegions	8	 																				12
	getCountsByPosition	ns	 																				10
	genebodies																						
	bootstrap-signal-by-	osition	 																				5
	binNdimensions		 																				3

# **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>

binNdimensions 3

binNdimensions

Generating and Aggregating Data Within N-dimensional Bins

# **Description**

Divide data along different dimensions into equally spaced bins, and summarize the datapoints that fall into any of these n-dimensional bins.

# Usage

```
binNdimensions(dims.df, nbins = 10, ncores = detectCores())

aggregateByNdimensionalBins(
    X,
    dims.df,
    nbins = 10,
    FUN = mean,
    ...,
    ignore.na = TRUE,
    drop = FALSE,
    empty = NA,
    ncores = detectCores()
)

densityInNdimensionalBins(dims.df, nbins = 10, ncores = detectCores())
```

# **Arguments**

dims.df	A dataframe containing one or more columns of numerical data for which bins will be generated.
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.
ncores	Number of cores to use for computations.
х	The name of the dimension in dims.df to aggregate, or a separate numerical vector or dataframe of data to be aggregated. If x is a numerical vector, each value in x corresponds to a row of dims.df, and so length(x) must be equal to nrow(dims.df). Likewise, if x is a dataframe, nrow(x) must equal nrow(dims.df). Supplying a dataframe for x has the advantage of simultaneously aggregating different sets of data, and returning a single dataframe.
FUN	A function to use for aggregating data within each bin.
	Additional arguments passed to FUN.
ignore.na	Logical indicating if NA values of x should be ignored. Default is TRUE.
drop	A logical indicating if empty bin combinations should be removed from the output. By default (FALSE), all possible combinations of bins are returned, and empty bins contain a value given by empty.
empty	When drop = FALSE, the value returned for empty bins. By default, empty bins

should be 0.

return NA. However, in many circumstances (e.g. if FUN = sum), the empty value

4 binNdimensions

#### **Details**

These functions take 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. For instance, if each row of dims.df were a gene, the columns (the different 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.

binNdimensions returns the bin numbers themselves. The output dataframe has the same dimensions as the input dims.df, but each input data has been replaced by its bin number (an integer).

aggregateByNdimensionalBins summarizes some input data x in each combination of bins, i.e. in each n-dimensional bin. Each row of the output dataframe is a unique combination of the input bins (i.e. each n-dimensional bin), and the output columns are identical to those in dims.df, with the addition of one or more columns containing the aggregated data in each n-dimensional bin. If the input x was a vector, the column is named "value"; if the input x was a dataframe, the column names from x are maintained.

densityInNdimensionalBins returns a dataframe just like aggregateByNdimensionalBins, except the "value" column contains the number of observations that fall into each n-dimensional bin.

#### Value

A dataframe.

#### Author(s)

Mike DeBerardine

```
data("PROseg") # import included PROseg data
data("txs_dm6_chr4") # import included transcripts
# find counts in promoter, early genebody, and near CPS
pr <- promoters(txs_dm6_chr4, 0, 100)</pre>
early_gb <- genebodies(txs_dm6_chr4, 500, 1000, fix.end = "start")</pre>
cps <- genebodies(txs_dm6_chr4, -500, 500, fix.start = "end")</pre>
df <- data.frame(counts_pr = getCountsByRegions(PROseq, pr),</pre>
              counts_gb = getCountsByRegions(PROseq, early_gb),
              counts_cps = getCountsByRegions(PROseq, cps))
#----#
# divide genes into 20 bins for each measurement
#----#
bin3d <- binNdimensions(df, nbins = 20, ncores = 2)</pre>
length(txs_dm6_chr4)
nrow(bin3d)
bin3d[1:6, ]
#----#
```

```
# get number of genes in each bin
bin_counts <- densityInNdimensionalBins(df, nbins = 20,</pre>
                                    ncores = 2)
bin_counts[1:6, ]
#-----#
# get mean cps reads in bins of promoter and genebody reads
bin2d_cps <- aggregateByNdimensionalBins("counts_cps", df,</pre>
                                    nbins = 20, ncores = 2)
bin2d_cps[1:6, ]
subset(bin2d_cps, is.finite(value))[1:6, ]
# get median cps reads for those bins
#-----#
bin2d_cps_med <- aggregateByNdimensionalBins("counts_cps", df, nbins = 20,</pre>
                                       FUN = median, ncores = 2)
bin2d_cps_med[1:6, ]
subset(bin2d_cps_med, is.finite(value))[1:6, ]
```

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,
  blacklist = NULL,
  zero_blacklisted = 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), or a list of such GRanges objects.

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).

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.

blacklist An optional GRanges object containing regions that should be excluded from

signal counting.

zero\_blacklisted

When set to FALSE (the default), signal at blacklisted sites is ignored from computations. If set to TRUE, signal at blacklisted sites will be treated as equal to zero. For bootstrapping, the default behavior of ignoring signal at blacklisted

sites should almost always be kept.

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

A dataframe containing x-values, means, lower quantiles, upper quantiles, and the sample name (as a convenience for row-binding multiple of these dataframes). If a list of GRanges is given as input, or if multiple fields are given, a single, combined dataframe is returned containing data for all fields/datasets.

### Author(s)

Mike DeBerardine

### See Also

```
getCountsByPositions
```

8 genebodies

```
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, ]
```

genebodies

Extract Genebodies

# 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
)
```

genebodies 9

### **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

getCountsByPositions

getCountsByPositions 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.

# Usage

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

# **Arguments**

A GRanges object in which signal is contained in metadata (typically in the "score" field), or a named list of such GRanges objects.

Regions.gr A GRanges object containing regions of interest.

Size of bins (in bp) to use for counting within each range of regions.gr. Note that counts will *not* be length-normalized.

getCountsByPositions 11

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. By default, an error is returned. 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).

blacklist An optional GRanges object containing regions that should be excluded from

signal counting.

NA\_blacklisted A logical indicating if NA values should be returned for blacklisted regions. By

default, signal in the blacklisted sites is ignored, i.e. the reads are excluded. If NA\_blacklisted = TRUE, those positions are set to NA in the final output.

melt A logical indicating if the count matrices should be melted. If set to TRUE, a

dataframe is returned in containing columns for "region", "position", and "signal". If dataset.gr is a list of multiple GRanges, or if length(field) > 1, a single dataframe is returned, which contains an additional column "sample", which contains individual sample names. If used with multi-width regions.gr, the resulting dataframe will only contain positions that are found within each

respective region.

ncores Multiple cores will only be used if dataset.gr is a list of multiple datasets, or

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. If dataset.gr is a list, a parallel list is returned containing a matrix for each input dataset.

# 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**

12

```
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, ]
\ensuremath{\text{\#}} 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",
  NF = NULL,
  blacklist = NULL,
  melt = FALSE,
  region_names = NULL,
```

getCountsByRegions 13

```
ncores = detectCores()
)
```

#### **Arguments**

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

"score" field), or a named list of such GRanges objects. If a list is given, a dataframe is returned containing the counts in each region for each dataset.

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).

blacklist An optional GRanges object containing regions that should be excluded from

signal counting.

melt If melt = TRUE, a dataframe is returned containing a column for regions and

another column for signal. If multiple datasets are given (if dataset.gr is a list or if length(field) > 1), the output dataframe is melted to contain a third column indicating the sample names. (See section on return values below).

region\_names If melt = TRUE, an optional vector of names for the regions in regions.gr. If

left as NULL, indices of regions.gr are used instead.

ncores Multiple cores will only be used if dataset. gr is a list of multiple datasets, or

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. If dataset.gr is a list of multiple GRanges, or if length(field) > 1, a dataframe is returned. If melt = FALSE (the default), dataframes have a column for each dataset and a row for each region. If melt = TRUE, dataframes contain one column to indicate regions (either by their indices, or by region\_names, if given), another column to indicate signal, and a third column containing the sample name (unless dataset.gr is a single GRanges object).

# 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)</pre>
```

14 getDESeqDataSet

```
head(counts)
# Assign as metadata to the transcript GRanges
txs_dm6_chr4$PROseq <- counts
txs_dm6_chr4[1:6]</pre>
```

getDESeqDataSet

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 = NULL,
  gene_names = NULL,
  sizeFactors = NULL,
  field = "score",
  blacklist = NULL,
  ncores = detectCores(),
  quiet = FALSE
)
```

# **Arguments**

 ${\tt dataset.list} \qquad {\tt An object containing GRanges \ datasets \ that \ can \ be \ passed \ to \ {\tt getCountsByRegions},}$ 

typically a list of GRanges objects, or a multiplexed GRanges object (see de-

tails below).

regions.gr A GRanges object containing regions of interest.

sample\_names Names for each dataset in dataset.list are required. By default (sample\_names

= NULL), if dataset.list is a list, the names of the list elements are used; for a multiplexed GRanges object, the field names 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 oth-

erwise 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. Note that DESeq2 sizeFactors are *not* the same as normalization factors

defined elsewhere in this package. See details below.

getDESeqDataSet 15

Argument passed to getCountsByRegions. Can be used to specify fields in a single multiplexed GRanges object, or individual fields for each GRanges object in dataset.list.

An optional GRanges object containing regions that should be excluded from signal counting. Use of this argument is distinct from the use of non-contiguous gene regions (see details below), and the two can be used simultaneously. Black-

signal counting. Use of this argument is distinct from the use of non-contiguous gene regions (see details below), and the two can be used simultaneously. Black-listing doesn't affect the ranges returned as rowRanges in the output DESeq-

DataSet object (unlike the use of non-contiguous regions).

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 = ~condition.

#### Use of non-contiguous gene regions

In DESeq2, genes must be defined by single, contiguous chromosomal locations. In contrast, 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. If the desired use is to blacklist certain sites in a genelist, note that the blacklist argument can be used.

# 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
```

16 getDESeqResults

```
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>
                         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**

dds

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.

getDESeqResults 17

contrast.numer A string naming the condition to use as the numerator in the DESeq2 comparison, typically the perturbative condition. contrast.denom A string naming the condition to use as the denominator in the DESeq2 comparison, 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. A vector containing DESeq2 sizeFactors to apply to each sample. Each samsizeFactors 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. The significance threshold passed to DESegResults, which is used for indepenalpha dent filtering of results (see DESeq2 documentation). Additional arguments passed to DESeq, given as a list of argument-value pairs, args.DESeq e.g. list(fitType = "local", useT = TRUE). All arguments given here will be passed to DESeq except for object and parallel. If no arguments are given, all defaults will be used. Additional arguments passed to DESeq2::results, given as a list of argumentargs.results 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. The number of cores to use for parallel processing. Multicore processing is only ncores 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

```
#-----#
# getDESeqDataSet
#------#
```

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

#### **Arguments**

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 The size of bin in which to calculate signal scores.

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  ${\tt MaxSiteSignal}$  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
```

```
data("PROseq") # load included PROseq data
data("txs_dm6_chr4") # load included transcripts
#-----#
# first 50 bases of transcripts
#-------#
```

20 getPausingIndices

```
pr <- promoters(txs_dm6_chr4, 0, 50)
pr[1:3]

#------#
# max sites
#------#
getMaxPositionsBySignal(PROseq, pr[1:3], keep.signal = TRUE)

#------#
# max sites in 5 bp bins
#-------#
getMaxPositionsBySignal(PROseq, pr[1:3], binsize = 5, keep.signal = TRUE)</pre>
```

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,
  blacklist = NULL,
  melt = FALSE,
  region_names = NULL,
  ncores = detectCores()
)
```

#### **Arguments**

field

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

"score" field), or a named list of such GRanges objects.

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

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

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. If dataset.gr is a list, a single field should be given, or length(field) should be the equal to the number of datasets in

dataset.gr.

getPausingIndices 21

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. If dataset.gr is a list of datasets, or if length(field) > 1, regions are filtered unless they have promoter signal

in all datasets.

blacklist An optional GRanges object containing regions that should be excluded from

signal counting. If length.normalize = TRUE, blacklisted positions will be excluded from length calculations. Users should take care to note if regions of

interest substantially overlap blacklisted positions.

melt If melt = TRUE, a dataframe is returned containing a column for regions and an-

other column for pausing indices. If multiple datasets are given (if dataset.gr is a list or if length(field) > 1), the output dataframe is melted to contain a third column indicating the sample names. (See section on return values below).

region\_names If melt = TRUE, an optional vector of names for the regions in regions.gr. If

left as NULL, indices of regions.gr are used instead.

ncores Multiple cores will only be used if dataset.gr is a list of multiple datasets, or

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 dataset.gr is a list, or if length(field) > 1, a dataframe is returned, containing a column for each field. However, if melt = TRUE, dataframes contain one column to indicate regions (either by their indices, or by region\_names, if given), another column to indicate signal, and a third column containing the sample name (unless dataset.gr is a single GRanges object).

#### Author(s)

Mike DeBerardine

# See Also

getCountsByRegions

22 getSpikeInCounts

```
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)
```

getSpikeInCounts

Filtering and counting spike-in reads

# **Description**

Filtering and counting spike-in reads

# Usage

```
getSpikeInCounts(
  dataset.gr,
  si_pattern = NULL,
  si_names = NULL,
  field = "score",
  sample_names = NULL,
  ncores = detectCores()
)
removeSpikeInReads(
  dataset.gr,
  si_pattern = NULL,
  si_names = NULL,
  field = "score",
  ncores = detectCores()
)
getSpikeInReads(
```

getSpikeInCounts 23

```
dataset.gr,
  si_pattern = NULL,
  si_names = NULL,
  field = "score",
  ncores = detectCores()
)
```

#### **Arguments**

dataset.gr	A GRanges object or a list of GRanges objects.
si_pattern	A regular expression that matches spike-in chromosomes. Can be used in addition to, or as an alternative to si_names.
si_names	A character vector giving the names of the spike-in chromosomes. Can be used in addition to, or as an alternative to si_pattern.
field	The metadata field in dataset.gr that contains readcounts. If each range is an individual read, set field = NULL.
sample_names	An optional character vector used to rename the datasets in dataset.gr
ncores	The number of cores to use for computations.

#### Value

A dataframe containing total readcounts, experimental (non-spike-in) readcounts, and spike-in readcounts.

# Author(s)

Mike DeBerardine

```
#-----#
# Make list of dummy GRanges
#-----#
gr1_rep1 <- GRanges(seqnames = c("chr1", "chr2", "spikechr1", "spikechr2"),</pre>
                  ranges = IRanges(start = 1:4, width = 1),
                  strand = "+")
gr2\_rep2 \leftarrow gr2\_rep1 \leftarrow gr1\_rep2 \leftarrow gr1\_rep1
# set readcounts
score(gr1\_rep1) \leftarrow c(1, 1, 1, 1) \# 2 exp + 2 spike = 4 total
score(gr2\_rep1) \leftarrow c(2, 2, 1, 1) # 4 exp + 2 spike = 6 total
score(gr1\_rep2) \leftarrow c(1, 1, 2, 1) # 2 exp + 3 spike = 5 total
score(gr2\_rep2) \leftarrow c(4, 4, 2, 2) \# 8 exp + 4 spike = 12 total
grl <- list(gr1_rep1, gr2_rep1,</pre>
           gr1_rep2, gr2_rep2)
names(grl) \leftarrow c("gr1\_rep1", "gr2\_rep1",
               "gr1_rep2", "gr2_rep2")
grl
#-----#
```

getSpikeInNFs

Calculating spike-in normalization factors

# **Description**

Use getSpikeInNFs to obtain the spike-in normalization factors, or spikeInNormGRanges to return the input GRanges objects with their readcounts spike-in normalized.

# Usage

```
getSpikeInNFs(
  dataset.gr,
  si_pattern = NULL,
  si_names = NULL,
  method = c("SRPMC", "SNR", "RPM"),
  batch_norm = TRUE,
  ctrl_pattern = NULL,
  ctrl_names = NULL,
  field = "score",
  sample_names = NULL,
  ncores = detectCores()
)
spikeInNormGRanges(
  dataset.gr,
  si_pattern = NULL,
  si_names = NULL,
  method = c("SRPMC", "SNR", "RPM"),
  batch_norm = TRUE,
  ctrl_pattern = NULL,
  ctrl_names = NULL,
  field = "score",
```

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

# **Arguments**

dataset.gr	A GRanges object, or (more typically) a list of GRanges objects.
si_pattern	A regular expression that matches spike-in chromosomes. Can be used in addition to, or as an alternative to si_names.
si_names	A character vector giving the names of the spike-in chromosomes. Can be used in addition to, or as an alternative to si_pattern.
method	One of the shown methods, which generate normalization factors for converting raw readcounts into "Spike-in normalized Reads Per Million mapped in Control" (the default), "Spike-in Normalized Read counts", or "Reads Per Million mapped". See descriptions below.
batch_norm	A logical indicating if batch normalization should be used (TRUE by default). See descriptions below. If batch normalization is used, sample names must end with "rep#", wherein "#" is one or more characters (usually a number) giving the replicate. If this is not the case, users can use the sample_names argument to make the names conform.
ctrl_pattern	A regular expression that matches negative control sample names.
ctrl_names	A character vector giving the names of the negative control samples. Can be used as an alternative to ctrl_pattern.
field	The metadata field in dataset.gr that contains raw readcounts. If each range is an individual read, set field = NULL.
sample_names	An optional character vector that can be used to rename the samples in dataset.gr. Intended use is if dataset.gr is an unnamed list, or if batch_norm = TRUE but the sample names don't conform to the required naming scheme.

#### Value

ncores

A numeric vector of normalization factors for each sample in dataset.gr. Normalization factors are to be applied by multiplication.

The number of cores to use for computations.

# Spike-in normalized Reads Per Million mapped in Control (SRPMC)

This is the default spike-in normalization method, as its meaning is the most portable and generalizable. Experimental Reads Per Spike-in read (RPS) are calculated for each sample, *i*:

$$RPS_i = \frac{experimental\_reads_i}{spikein\_reads_i}$$

RPS for each sample is divided by RPS for the negative control, which measures the change in total material vs. the negative control. This global adjustment is applied to standard RPM normalization for each sample:

$$NF_i = \frac{RPS_i}{RPS_{control}} \cdot \frac{1x10^6}{experimental\_reads_i}$$

Thus, the negative control(s) are simply RPM-normalized, while the other conditions are in equivalent, directly-comparable units ("Reads Per Million mapped reads in a negative control").

If batch\_norm = TRUE (the default), all negative controls will be RPM-normalized, and the global changes in material for all other samples are calculated *within each batch* (vs. the negative control within the same batch).

If batch\_norm = FALSE, all samples are compared to the average RPS of the negative controls. This method can only be justified if batch has less effect on RPS than other sources of variation.

# Spike-in Normalized Reads (SNR)

If batch\_norm = FALSE, these normalization factors act to scale down the readcounts in each sample to make the spike-in read counts match the sample with the lowest number of spike-in reads:

$$NF_i = \frac{min(spikein\_reads)}{spikein\_reads_i}$$

If batch\_norm = TRUE, such normalization factors are calculated within each batch, but a final batch (replicate) adjustment is performed that results in the negative controls having the same normalized readcounts. In this way, the negative controls are used to adjust the normalized readcounts of their entire replicate. Just as when batch\_norm = FALSE, one of the normalization factors will be 1, while the rest will be <1.

One use for these normalization factors is for normalizing-by-subsampling; see subsampleBySpikeIn.

#### Reads Per Million mapped reads (RPM)

A simple convenience wrapper for calculating normalization factors for RPM normalization:

$$NF_i = \frac{1x10^6}{experimental\_reads_i}$$

If spike-in reads are present, they're removed before the normalization factors are calculated.

#### Author(s)

Mike DeBerardine

## See Also

```
getSpikeInCounts
```

```
score(gr2\_rep2) \leftarrow c(4, 4, 2, 2) \# 8 exp + 4 spike = 12 total
grl <- list(gr1_rep1, gr2_rep1,</pre>
          gr1_rep2, gr2_rep2)
grl
#-----#
# Get RPM NFs
#-----#
# can use the names of all spike-in chromosomes
getSpikeInNFs(grl, si_names = c("spikechr1", "spikechr2"), method = "RPM",
            ncores = 2)
# or use a regular expression that matches the spike-in chromosome names
grep("spike", as.vector(seqnames(gr1_rep1)))
getSpikeInNFs(grl, si_pattern = "spike", method = "RPM", ncores = 2)
# Get simple spike-in NFs ("SNR")
#-----#
# without batch normalization, NFs make all spike-in readcounts match
getSpikeInNFs(grl, si_pattern = "spike", ctrl_pattern = "gr1", method = "SNR",
            batch_norm = FALSE, ncores = 2)
# with batch normalization, negative controls will have same normalized reads;
# other samples are normalized to have same spike-in reads as their matched
getSpikeInNFs(grl, si_pattern = "spike", ctrl_pattern = "gr1", method = "SNR",
            batch_norm = TRUE, ncores = 2)
# Get spike-in NFs with more meaningful units ("RPMC")
# compare to raw RPM NFs above; takes into account spike-in reads;
# units are directly comparable to the negative controls
# with batch normalization, these negative controls are the same, as they have
# the same number of non-spike-in readcounts (they're simply RPM)
getSpikeInNFs(grl, si_pattern = "spike", ctrl_pattern = "gr1", ncores = 2)
# batch_norm = FALSE, the average reads-per-spike-in for the negative controls
# are used to calculate all NFs; unless the controls have the exact same
# ratio of non-spike-in to spike-in reads, nothing is precisely RPM
getSpikeInNFs(grl, si_pattern = "spike", ctrl_pattern = "gr1",
            batch_norm = FALSE, ncores = 2)
#----#
# Apply NFs to the GRanges
#-----#
```

28 getStrandedCoverage

```
spikeInNormGRanges(grl, si_pattern = "spike", ctrl_pattern = "gr1", ncores = 2)
```

getStrandedCoverage

Get strand-specific coverage

#### **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). dataset.gr can also be a list of such GRanges objects.

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. For a single dataset, the max

that will be used is 3, one for each possible strand (plus, minus, and unstranded).

More cores can be used if dataset.gr is a list.

### 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
```

```
#-----#
# Using included full-read data
#------#
# -> whole-read coverage sacrifices meaningful readcount
# information, but can be useful for visualization,
# e.g. for looking at RNA-seq data in a genome browser
data("PROseq_paired")
PROseq_paired[1:6]
```

import-functions 29

```
getStrandedCoverage(PROseq_paired, ncores = 2)[1:6]
#----#
# Getting coverage from single bases of single reads
# included PROseq data is already single-base coverage
data("PROseq")
range(width(PROseq))
# 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(
```

30 import-functions

```
plus_file,
 minus_file,
 genome = NULL,
 keep.X = TRUE,
 keep.Y = TRUE,
 keep.M = FALSE,
 keep.nonstandard = FALSE
)
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 31

#### **Examples**

```
# Import PRO-seq bigWigs -> coverage of 3' bases
# get local address for included bigWig files
p.bw <- system.file("extdata", "PROseq_dm6_chr4_plus.bw",</pre>
                  package = "BRGenomics")
m.bw <- system.file("extdata", "PROseq_dm6_chr4_minus.bw",</pre>
                  package = "BRGenomics")
# import bigWigs
PROseq <- import_bigWig(p.bw, m.bw, genome = "dm6")
PROseq
#-----#
# Import PRO-seq bedGraphs -> whole reads (matched 5' and 3' ends)
#-----#
# get local address for included bedGraph files
p.bg <- system.file("extdata", "PROseq_dm6_chr4_plus.bedGraph",</pre>
                  package = "BRGenomics")
\label{lem:mbg} \verb|m.bg| <- system.file("extdata", "PROseq_dm6_chr4_minus.bedGraph",
                  package = "BRGenomics")
# import bedGraphs
PROseq_paired <- import_bedGraph(p.bg, m.bg, genome = "dm6")
PROseq_paired
```

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
)

import_bam_PROseq(
  file,
  mapq = 20,
```

32 import\_bam

```
revcomp = TRUE,
 shift = -1L.
  trim.to = "3p",
  ignore.strand = FALSE,
  field = "score",
 paired_end = NULL,
 yieldSize = 250000
import_bam_PROcap(
 file,
 mapq = 20,
 revcomp = FALSE,
 shift = 0L,
 trim.to = "5p",
  ignore.strand = FALSE,
  field = "score",
 paired_end = NULL,
 yieldSize = 250000
import_bam_ATACseq(
 file,
 mapq = 20,
 revcomp = FALSE,
 shift = c(4, -5),
  trim.to = "whole",
  ignore.strand = TRUE,
 field = "score",
 paired_end = TRUE,
 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.

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.

ignore.strand Logical indicating if the strand information should be discarded. If TRUE, strand information is discarded *after* revcomp, trim.to, and shift options are ap-

plied.

import\_bam 33

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.
paired_end	Logical indicating if reads should be treated as paired end reads. When set to 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

#### 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

Jason D. Buenrostro, Paul G. Giresi, Lisa C. Zaba, Howard Y. Chang, William J. Greenleaf (2013). Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, dna-binding proteins and nucleosome position. *Nature Methods* 10: 1213–1218. https://doi.org/10.1038/nmeth.2688

34 makeGRangesBRG

makeGRangesBRG

Constructing and checking for base-pair resolution GRanges objects

#### **Description**

makeGRangesBRG 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. isBRG checks whether an object is a basepair resolution GRanges object.

# Usage

```
makeGRangesBRG(dataset.gr, ncores = detectCores())
isBRG(x)
```

#### **Arguments**

dataset.gr A disjoint GRanges object, or a list of such objects.

ncores If dataset.gr is a list, the number of cores to use for computations.

x Object to be tested.

#### **Details**

Note that makeGRangesBRG 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.

makeGRangesBRG 35

#### Value

```
\label{eq:makeGRangesBRG} $$\operatorname{Ranges object for which length(output)} == \sup(\operatorname{width(dataset.gr)}),$$ and for which all(\operatorname{width(output)} == 1).
```

isBRG(x) returns TRUE if x is a GRanges object with the above characteristics.

#### 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()
```

36 mergeGRangesData

```
# make basepair-resolution (single-width)
gr <- makeGRangesBRG(bw)</pre>
isBRG(gr)
range(width(gr))
length(gr)
length(gr) == sum(width(bw))
sum(score(gr)) == sum(score(bw) * width(bw))
#-----#
# Reverse using getStrandedCoverage
#-----#
# -> for more examples, see getStrandedCoverage
undo <- getStrandedCoverage(gr, ncores = 2)</pre>
isBRG(undo)
range(width(undo))
length(undo) == length(bw)
all(score(undo) == score(bw))
```

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.

mergeGRangesData 37

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.

#### 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

38 PROseq-data

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

subsampleBySpikeIn 39

subsampleBySpikeIn

Randomly subsample reads according to spike-in normalization

### **Description**

Randomly subsample reads according to spike-in normalization

### Usage

```
subsampleBySpikeIn(
  dataset.gr,
  si_pattern = NULL,
  si_names = NULL,
  ctrl_pattern = NULL,
  ctrl_names = NULL,
  batch_norm = TRUE,
  RPM_units = FALSE,
  field = "score",
  sample_names = NULL,
  ncores = detectCores()
)
```

#### **Arguments**

RPM\_units

If set to TRUE, the final readcount values will be converted to units equivalent to/directly comparable with RPM for the negative control(s). If field = NULL, the GRanges objects will be converted to disjoint "basepair-resolution" GRanges objects, with normalized readcounts contained in the "score" metadata column.

### Value

An object parallel to dataset.gr, but with fewer reads. E.g. if dataset.gr is a list of GRanges, the output is a list of the same GRanges, but in which each GRanges has fewer reads.

#### Author(s)

Mike DeBerardine

#### See Also

getSpikeInCounts, getSpikeInNFs

40 subsampleGRanges

```
strand = "+")
gr2_rep2 <- gr2_rep1 <- gr1_rep2 <- gr1_rep1</pre>
# set readcounts
score(gr1\_rep1) \leftarrow c(1, 1, 1, 1) # 2 exp + 2 spike = 4 total
score(gr2\_rep1) \leftarrow c(2, 2, 1, 1) # 4 exp + 2 spike = 6 total
score(gr1\_rep2) \leftarrow c(1, 1, 2, 1) # 2 exp + 3 spike = 5 total
score(gr2\_rep2) \leftarrow c(4, 4, 2, 2) \# 8 exp + 4 spike = 12 total
grl <- list(gr1_rep1, gr2_rep1,</pre>
           gr1_rep2, gr2_rep2)
grl
#-----#
# (The simple spike-in NFs)
# see examples for getSpikeInNFs for more
getSpikeInNFs(grl, si_pattern = "spike", ctrl_pattern = "gr1", method = "SNR",
             ncores = 2)
#-----#
# Subsample the GRanges according to the spike-in NFs
#-----#
ss <- subsampleBySpikeIn(grl, si_pattern = "spike", ctrl_pattern = "gr1",</pre>
                       ncores = 2)
SS
lapply(ss, function(x) sum(score(x))) # total reads in each
\ensuremath{\text{\#}}\xspace \text{Put} in units of RPM for the negative control
ssr <- subsampleBySpikeIn(grl, si_pattern = "spike", ctrl_pattern = "gr1",</pre>
                        RPM_units = TRUE, ncores = 2)
ssr
lapply(ssr, function(x) sum(score(x))) # total signal in each
```

 $\verb|subsampleGRanges||$ 

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(
```

subsampleGRanges 41

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

#### **Arguments**

dataset.gr A GRanges object in which signal (e.g. readcounts) are contained within meta-

data, or a list of such GRanges objects.

n, prop Either the number of reads to subsample (n), or the proportion of total signal to

subsample (prop). Either n or prop can be given, but not both. If dataset.gr is a list, or if length(field) > 1, users can supply a vector or list of n or prop values to match the individual datasets, but care should be taken to ensure that a

value is given for each and every dataset.

field The metadata field of dataset.gr that contains readcounts for reach position.

If each range represents a single read, set field = NULL. If multiple fields are given, and dataset.gr is not a list, then dataset.gr will be treated as a multiplexed GRanges, and each field will be treated as an indpendent dataset. See

 ${\tt mergeGRangesData}.$ 

ncores Number of cores to use for computations. Multicore only used when dataset.gr

is a list, or if length(field) > 1.

#### 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 containi	ng 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. Re-

gions 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.

tidyChromosomes 43

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))
# 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)
```

44 tidyChromosomes

### **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, or any another object with associated seqinfo (or a Seqinfo object itself). The object should typically have a standard genome associated with it, 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 45

```
tidyChromosomes(gr)
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

# **Index**

```
* datasets
    PROseq-data, 38
    txs_dm6_chr4, 45
 GenomeInfoDb::standardChromosomes, 44
 multiplexed GRanges, 14
aggregateByNdimensionalBins
        (binNdimensions), 3
binNdimensions, 3
bootstrap-signal-by-position, 5
BRGenomics (BRGenomics-package), 2
BRGenomics-package, 2
densityInNdimensionalBins
        (binNdimensions), 3
DESeq, 17
DESeq2::DESeq, 16
DESeq2::DESeqDataSet, 15
DESeq2::results, 16, 17
DESeqDataSet, 15, 16
genebodies, 8
GenomicRanges::coverage, 28
GenomicRanges::promoters, 9
GenomicRanges::resize, 35
GenomicRanges::resize(), 35
getCountsByPositions, 7, 10, 13, 19
getCountsByRegions, 11, 12, 14, 21, 43
getDESeqDataSet, 14, 16, 17
getDESeqResults, 15, 16
{\tt getMaxPositionsBySignal}, 18
getPausingIndices, 20
getSpikeInCounts, 22, 26, 39
getSpikeInNFs, 24, 39
getSpikeInReads (getSpikeInCounts), 22
getStrandedCoverage, 28, 35
GPos, 35
import-functions, 29
import_bam, 31
import_bam_ATACseq(import_bam), 31
import_bam_PROcap(import_bam), 31
import_bam_PROseq(import_bam), 31
```

```
import_bedGraph (import-functions), 29
import_bigWig (import-functions), 29
isBRG (makeGRangesBRG), 34
makeGRangesBRG, 28, 30, 34, 36, 37
mergeGRangesData, 36, 41
metaSubsample
        (bootstrap-signal-by-position),
metaSubsampleMatrix
        (bootstrap-signal-by-position),
PROseq (PROseq-data), 38
PROseq-data, 38
PROseq_paired (PROseq-data), 38
removeSpikeInReads (getSpikeInCounts),
rtracklayer::import, 30
spikeInNormGRanges (getSpikeInNFs), 24
standardChromosomes, 44
subsampleBySpikeIn, 26, 39
subsampleGRanges, 40
subsetRegionsBySignal, 42
tidyChromosomes, 30, 43
txs_dm6_chr4, 45
```