# Package 'girafe'

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Type Package

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**Imports** methods, Biobase, Biostrings (>= 2.47.6), graphics, grDevices, stats, utils, IRanges (>= 2.13.12)

Suggests MASS, org.Mm.eg.db, RColorBrewer

Enhances genomeIntervals

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Description The package 'girafe' deals with the genome-level representation of aligned reads from next-generation sequencing data. It contains an object class for enabling a detailed description of genome intervals with aligned reads and functions for comparing, visualising, exporting and working with such intervals and the aligned reads. As such, the package interacts with and provides a link between the packages ShortRead, IRanges and genomeIntervals.

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# Description

This function can be used to assess the significance of sliding-window read counts. The background distribution of read counts in windows is assumed to be a Negative-Binomial (NB) one. The two parameters of the NB distribution, mean 'mu' and dispersion 'size', are estimated using any of the methods described below (see details). The estimated NB distribution is used to assign a *p*-value to each window based on the number of aligned reads in the window. The *p*-values can be corrected for multiple testing using any of the correction methods implemented for p. adjust.

# Usage

```
addNBSignificance(x, estimate="NB.012", correct = "none", max.n=10L)
```

# **Arguments**

x	$\boldsymbol{A}$ data.frame of class slidingWindowSummary, as returned by the function $\ensuremath{\text{perWindow}}.$
estimate	string; which method to use to estimate the parameters of the NB background distribution; see below for details
correct	string; which method to use for $p$ -value adjustment; can be any method that is implemented for $p$ . adjust including "none" if no correction is desired.
max.n	integer; only relevant if estimate=="NB.ML"; in that case specifies that windows with up to this number of aligned reads should be considered for estimating the background distribution.

#### **Details**

The two parameters of the Negative-Binomial (NB) distribution are: mean ' $\lambda$ ' (or 'mu') and size 'r' (or 'size').

The function knows a number of methods to estimate the parameters of the NB distribution.

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"NB.012" Solely the windows with only 0, 1, or 2 aligned reads are used for estimating  $\lambda$  and 'r'. From the probability mass function g(k) = P(X = k) of the NB distribution, it follows that the ratios

$$q_1 = \frac{g(1)}{g(0)} = \frac{\lambda \cdot r}{\lambda + r}$$

and

$$q_2 = \frac{g(2)}{g(1)} = \frac{\lambda \cdot (r+1)}{2 \cdot (\lambda + r)} \ .$$

The observed numbers of windows with 0-2 aligned reads are used to estimate

$$\widehat{q_1} = \frac{n_1}{n_0}$$

and

$$\widehat{q}_2 = \frac{n_2}{n_1}$$

and from these estimates, one can obtain estimates for  $\widehat{\lambda}$  and  $\widehat{r}$ .

"NB.ML" This estimation method uses the function fitdistr from package 'MASS'. Windows with up to n. max aligned reads are considered for this estimate.

**"Poisson"** This estimate also uses the windows the 0-2 aligned reads, but uses these numbers to estimates the parameter  $\lambda$  of a Poisson distribution. The parameter 'r' is set to a very large number, such that the estimated NB distribution actually is a Poisson distribution with mean and variance equal to  $\lambda$ .

#### Value

A data.frame of class slidingWindowSummary, which is the the supplied argument x extended by an additional column p.value which holds the p-value for each window. The attribute NBparams of the result contains the list of the estimated parameters of the Negative-Binomial background distribution.

# Author(s)

Joern Toedling

#### References

Such an estimation of the Negative-Binomial parameters has also been described in the paper: Ji et al.(2008) An integrated system CisGenome for analyzing ChIP-chip and ChIP-seq data. Nat Biotechnol. 26(11):1293-1300.

#### See Also

```
perWindow, p.adjust
```

```
exDir <- system.file("extdata", package="girafe")
exA <- readAligned(dirPath=exDir, type="Bowtie",
    pattern="aravinSRNA_23_no_adapter_excerpt_mm9_unmasked.bwtmap")
exAI <- as(exA, "AlignedGenomeIntervals")
exPX <- perWindow(exAI, chr="chrX", winsize=1e5, step=0.5e5)
exPX <- addNBSignificance(exPX, correct="bonferroni")
str(exPX)
exPX[exPX$p.value <= 0.05,]</pre>
```

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agiFromBam

Create AlignedGenomeIntervals objects from BAM files.

## **Description**

Function to create AlignedGenomeIntervals objects from BAM (binary alignment map format) files. Uses functions from package Rsamtools to parse BAM files.

# Usage

```
agiFromBam(bamfile, ...)
```

# **Arguments**

bamfile File path of BAM file. BAM file should be sorted and have an index in the same

directory (see Details below).

. . . further arguments passed on to function scanBam

#### **Details**

Note: the BAM files must be sorted and must also have an index file (\*.bai) in the same directory. These should be done when creating the BAM. However, the functions sortBam and indexBam can be used for the same purpose, as can the respective modules of the "samtools" library ('samtools sort' and 'samtools index').

The BAM files are parsed chromosome by chromosome to limit the memory footprint of the function. Thus, this function aims to be a less-memory-consuming alternative to first reading in the BAM file using the readAligned function and then converting the AlignedRead object into an AlignedGenomeIntervals object.

# Value

An object of class AlignedGenomeIntervals.

# Author(s)

J Toedling

# References

```
http://samtools.sourceforge.net
```

#### See Also

```
scanBam, AlignedGenomeIntervals-class
```

```
fl <- system.file("extdata", "ex1.bam", package="Rsamtools")
ExGi <- agiFromBam(fl)
head(detail(ExGi))</pre>
```

AlignedGenomeIntervals-class

Class 'AlignedGenomeIntervals'

# **Description**

A class for representing reads from next-generation sequencing experiments that have been aligned to genomic intervals.

#### **Objects from the Class**

Objects can be created either by:

- 1. calls of the form new("AlignedGenomeIntervals", .Data, closed, ...).
- 2. using the auxiliary function AlignedGenomeIntervals and supplying separate vectors of same length which hold the required information: AlignedGenomeIntervals(start, end, chromosome, strand, reads, matches, sequence) If arguments reads or matches are not specified, they are assumed to be '1' for all intervals.
- 3. or, probably the most common way, by coercing from objects of class AlignedRead.

#### **Slots**

.Data: two-column integer matrix, holding the start and end coordinates of the intervals on the chromosomes

sequence: character; sequence of the read aligned to the interval

reads: integer; total number of reads that were aligned to this interval

matches: integer; the total number of genomic intervals that reads which were aligned to this interval were aligned to. A value of '1' thus means that this read sequence matches uniquely to this one genome interval only

organism: string; an identifier for the genome of which organism the intervals are related to. Functions making use of this slot require a specific annotation package org.<organism>.eg.db. For example if organism is 'Hs', the annotation package 'org.Hs.eg.db' is utilised by these functions. The annotation packages can be obtained from the Bioconductor repositories.

annotation: data.frame; see class genome\_intervals for details

closed: matrix; see class genome\_intervals for details

type: character; see class genome\_intervals for details

score: numeric; optional score for each aligned genome interval

id: character; optional identifier for each aligned genome interval

chrlengths: integer; optional named integer vector of chromosome lengths for the respective genome; if present it is used in place of the chromosome lengths retrieved from the annotation package (see slot organism)

#### **Extends**

Class Genome\_intervals-class, directly. Class Intervals\_full, by class "Genome\_intervals", distance 2.

#### Methods

coerce Coercion method from objects of class AlignedRead, which is defined in package ShortRead, to objects of class AlignedGenomeIntervals

**coverage** signature("AlignedGenomeIntervals"): computes the read coverage over all chromosomes. If the organism of the object is set correctly, the chromosome lengths are retrieved from the appropriate annotation package, otherwise the maximum interval end is taken to be the absolute length of that chromosome (strand).

The result of this method is a list and the individual list elements are of class Rle, a class for encoding long repetitive vectors that is defined in package IRanges.

The additional argument byStrand governs whether the coverage is computed separately for each strand. If byStrand=FALSE (default) only one result is returned per chromosome. If byStrand=TRUE, there result is two separate R1e objects per chromosome with the strand appended to the chromosome name.

**detail** signature("AlignedGenomeIntervals"): a more detailed output of all the intervals than provided by show; only advisable for objects containing few intervals

extend signature("AlignedGenomeIntervals") with additional arguments fiveprime=0L and threeprime=0L. These must be integer numbers and greater than or equal to 0. They specify how much is subtracted from the left border of the interval and added to the right side. Which end is 5' and which one is 3' are determined from the strand information of the object. Lastly, if the object has an organism annotation, it is checked that the right ends of the intervals do not exceed the respective chromosome lengths.

**export** export the aligned intervals as tab-delimited text files which can be uploaded to the UCSC genome browser as 'custom tracks'. Currently, there are methods for exporting the data into 'bed' format and 'bedGraph' format, either writing the intervals from both strands into one file or into two separate files (formats 'bedStrand' and 'bedGraphStrand', respectively). Details about these track formats can be found at the UCSC genome browser web pages.

The additional argument writeHeader can be set to FALSE to suppress writing of the track definition header line to the file.

For Genome\_intervals objects, only 'bed' format is supported at the moment and does not need to be specified.

hist signature("AlignedGenomeIntervals"): creates a histogram of the lengths of the reads
 aligned to the intervals

**organism** Get or set the organism that the genome intervals in the object correspond to. Should be a predefined code, such as 'Mm' for mouse and 'Hs' for human. The reason for this code, that, if the organism is set, a corresponding annotation package that is called org.<organism>.eg.db is used, for example for obtaining the chromosome lengths to be used in methods such as coverage. These annotation packages can be obtained from the Bioconductor repository.

plot visualisation method; a second argument of class Genome\_intervals\_stranded can be provided for additional annotation to the plot. Please see below and in the vignette for examples. Refer to the documentation of plotAligned for more details on the plotting function.

**reduce** collapse/reduce aligned genome intervals by combining intervals which are completely included in each other, combining overlapping intervals AND combining immediately adjacent intervals (if method="standard"). Intervals are only combined if they are on the same chromosome, the same strand AND have the same match specificity of the aligned reads.

If you only want to combine intervals that have exactly the same start and stop position (but may have reads of slightly different sequence aligned to them), then use the argument method="exact".

If you only want to combine intervals that have exactly the same 5' or 3' end (but may differ in the other end and in the aligned sequence), then use the argument method="same5" (same 5' end) or method="same3" (same 3' end).

Finally, it's possible to only collapse/reduce aligned genome intervals that overlap each other by at least a certain fraction using the argument min.frac.min.frac is a number between 0.0 and 1.0. For example, if you call reduce with argument min.frac=0.4, only intervals that overlap each other by at least 40 percent are collapsed/merged.

**sample** draw a random sample of n (Argument size) of the aligned reads (without or with replacement) and returns the AlignedGenomeIntervals object defined by these aligned reads.

score access or set a custom score for the object

**sort** sorts the intervals by chromosome name, start and end coordinate in increasing order (unless decreasing=TRUE is specified) and returns the sorted object

subset take a subset of reads, matrix-like subsetting via '\[' can also be used

# Author(s)

Joern Toedling

#### See Also

Genome\_intervals-class, AlignedRead-class, plotAligned

```
######### toy example:
A <- new("AlignedGenomeIntervals",
       .Data=cbind(c(1,3,4,5,8,10), c(5,5,6,8,9,11)),
       annotation=data.frame(
         seq_name=factor(rep(c("chr1","chr2","chr3"), each=2)),
         strand=factor(c("-","-","+","+","+","+") ,levels=c("-","+")),
         inter_base=rep(FALSE, 6)),
       reads=rep(3L, 6), matches=rep(1L,6),
       sequence=c("ACATT","ACA","CGT","GTAA","AG","CT"))
show(A)
detail(A)
## alternative initiation of this object:
A <- AlignedGenomeIntervals(
   start=c(1,3,4,5,8,10), end=c(5,5,6,8,9,11),
  chromosome=rep(c("chr2","chrX","chr1"), each=2),
   strand=c("-","-","+","+","+","+"),
   sequence=c("ACATT","ACA","CGT","GGAA","AG","CT"),
   reads=c(1L, 5L, 2L, 7L, 3L, 3L))
detail(A)
## custom identifiers can be assigned to the intervals
id(A) <- paste("gi", 1:6, sep="")</pre>
## subsetting and combining
detail(A[c(1:4)])
detail(c(A[1], A[4]))
## sorting: always useful
A <- sort(A)
detail(A)
## the 'reduce' method provides a cleaned-up, compact set
```

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```
detail(reduce(A))
## with arguments specifying additional conditions for merging
detail(reduce(A, min.frac=0.8))
## 'sample' to draw a sample subset of reads and their intervals
detail(sample(A, 10))
## biological example
exDir <- system.file("extdata", package="girafe")</pre>
exA <- readAligned(dirPath=exDir, type="Bowtie",
 pattern="aravinSRNA_23_no_adapter_excerpt_mm9_unmasked.bwtmap")
exAI <- as(exA, "AlignedGenomeIntervals")</pre>
organism(exAI) <- "Mm"
show(exAI)
## which chromosomes are the intervals on?
table(chromosome(exAI))
## subset
exAI[is.element(chromosome(exAI), c("chr1","chr2"))]
## compute coverage per chromosome:
coverage(exAI[is.element(chromosome(exAI), c("chr1","chr2"))])
### plotting:
load(file.path(exDir, "mgi_gi.RData"))
if (interactive())
  plot(exAI, mgi.gi, chr="chrX", start=50400000, end=50410000)
### overlap with annotated genome elements:
exOv <- interval_overlap(exAI, mgi.gi)</pre>
## how many elements do read match positions generally overlap:
table(listLen(ex0v))
## what are the 13 elements overlapped by a single match position:
mgi.gi[ex0v[[which.max(listLen(ex0v))]]]
## what kinds of elements are overlapped
(tabOv <- table(as.character(mgi.gi$type)[unlist(exOv)]))</pre>
### display those classes:
my.cols <- rainbow(length(tabOv))</pre>
if (interactive())
   pie(tabOv, col=my.cols, radius=0.85)
```

countReadsAnnotated

Sum up aligned reads per category of genome feature

# **Description**

A function to sum up aligned reads per category of genome feature (i.e. gene, ncRNA, etc.).

# Usage

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#### **Arguments**

GI	object of class AlignedGenomeIntervals
М	Annotation object of class Genome_intervals_stranded or Genome_intervals; describes the genomic coordinates of annotated genome features, such as genes, miRNAs, etc.
typeColumn	string; which column of the annotation object M describes the type of the genome feature
fractionGI	which fraction of the intervals in object GI are required to ovelap with a feature in M in order to be considered to correspond to that feature.
mem.friendly	logical; should a version which requires less memory but takes a bit longer be used
showAllTypes	logical; should a table of genome feature types in M be displayed?

#### **Details**

The read counts are summed up over each type of genome feature, and the read counts are normalised by their number of genomic matches. For example if a read has two matches in the genome, but only one inside a miRNA, it would count 0.5 for miRNAs.

#### Value

A named numeric vector which gives the summed read counts for each supplied type of genome feature.

# Author(s)

J Toedling

```
A <- AlignedGenomeIntervals(
         start=c(1,8,14,20), end=c(5,15,19,25),
         chromosome=rep("chr1", each=4),
         strand=c("+","+","+","+"),
         sequence=c("ACATT","TATCGGAC","TCGGACT","GTAACG"),
         reads=c(7L, 2L, 4L, 5L) )
M2 <- new("Genome_intervals_stranded",</pre>
          rbind(c(2,6), c(1,15), c(20,30)),
          closed = matrix(TRUE, ncol=2, nrow=3),
          annotation = data.frame(
            seq_name= factor(rep("chr1", 3)),
            inter_base= logical(3),
            strand=factor(rep("+", 3), levels=c("+","-")),
            alias=c("miRNA1", "gene1", "tRNA1"),
            type=c("miRNA","gene","tRNA")) )
if (interactive()){
   grid.newpage()
   plot(A, M2, chr="chr1", start=0, end=35,
        nameColum="alias", show="plus")
}
countReadsAnnotated(A, M2, typeColumn="type")
```

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fracOverlap	Retrieve intervals overlapping by fraction of width	

#### **Description**

Function to retrieve overlapping intervals that overlap at least by a specified fraction of their widths.

# Usage

```
fracOverlap(I1, I2, min.frac=0.0, both=TRUE, mem.friendly=FALSE)
```

#### **Arguments**

I1	object that inherits from class Genome_intervals
12	object that inherits from class Genome_intervals

min. frac numeric; minimum required fraction of each of the two interval widths by which

two intervals should overlap in order to be marked as overlapping.

both logical; shall both overlap partners meet the minimum fraction min.frac re-

quirement? If FALSE, then overlaps with only partner involved to at least that

fraction are also reported.

mem.friendly logical; if set to TRUE an older but memory-friendlier version of interval\_overlap

is used inside this function. Note that mem.friendly is only evaluated if I1 or

I2 is of class AlignedGenomeIntervals.

#### Value

An object of class data. frame with one row each for a pair of overlapping elements.

Index1 Index of interval in first interval list
Index2 Index of interval in second interval list
n number of bases that the two intervals overlap
fraction1 fraction of interval 1's width by which the two intervals

fraction1 fraction of interval 1's width by which the two intervals overlap fraction2 fraction of interval 2's width by which the two intervals overlap

# Author(s)

J. Toedling

#### See Also

```
interval_overlap
```

```
data("gen_ints", package="genomeIntervals")
i[4,2] <- 13L
fracOverlap(i, i, 0.5)</pre>
```

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intPhred	Extract integer Phred score values from FastQ data

# **Description**

Function to extract integer Phred score values from FastQ data.

# Usage

```
intPhred(x, method="Sanger", returnType="list")
```

# **Arguments**

x object of class ShortReadQ; which contains read sequences and quality scores;

usually read in from a Fastq files.

method string; one of 'Sanger', 'Solexa' or 'previousSolexa'. See details below.

returnType string; in which format should the result be returned, either as a 'list' or as a

'matrix'.

#### Details

There are different standards for encoding read qualities in Fastq files. The 'Sanger' format encodes a Phred quality score from 0 to 93 using ASCII 33 to 126. The current 'Solexa'/Illumina format (1.3 and higher) encodes a Phred quality score from 0 to 40 using ASCII 64 to 104. The 'previous Solexa'/Illumina format (1.0) encodes a custom Solexa/Illumina quality score from -5 to 40 using ASCII 59 to 104. This custom Solexa quality score is approximately equal to the Phred scores for high qualities, but differs in the low quality range.

#### Value

If returnType is equal to 'list': A list of integer Phred quality values of the same length as the number of reads in the object x.

If returnType is equal to 'matrix': A matrix of integer Phred quality values. The number of rows is the number of reads in the object x. The number of columns is the maximum length (width) over all reads in object x. The last entries for reads that are shorter than this maximum width are 'NA'.

#### Author(s)

Joern Toedling

#### References

```
http://maq.sourceforge.net/fastq.shtml
```

# See Also

```
ShortReadQ-class, readFastq
```

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#### **Examples**

medianByPosition

Compute median quality for each nucleotide position

#### **Description**

This function computes the median quality for each position in a read over all reads in a ShortReadQ object.

# Usage

```
medianByPosition(x, method = "Sanger", batchSize = 100000L)
```

# **Arguments**

x object of class ShortReadQ, such as the result of function readFastq

method string; passed on to function intPhred

batchSize number of rows to process in each iteration; directly influences RAM usage of

this function

#### Details

The quality values are computed for each batch of reads and stored as numeric Rle objects for each postion. In each iteration, the Rle object of the current batch is merged with the previous one in order to keep the RAM usage low.

# Value

A numeric vector of the median values per nucleotide position in the reads. The length of this vector corresponds to the length of the longest read in the data.

# Author(s)

Joern Toedling

#### See Also

intPhred

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# **Examples**

perWindow

Investigate aligned reads in genome intervals with sliding windows

#### **Description**

Investigate aligned reads in genome intervals with sliding windows.

# Usage

# **Arguments**

object of class AlignedGenomeIntervals

chr string; which chromosome to investigate with sliding windows

winsize integer; size of the sliding window in base-pairs

step integer; offset between the start positions of two sliding windows

normaliseByMatches

logical; should the number of reads per AlignedGenomeInterval be normalised by the number of genomic matches of the read sequence before summing them

up in each window? (i.e. derivation a weighted sum of read counts)

mem.friendly logical; argument passed on to function interval\_overlap; if TRUE the less

RAM and, if the parallel package is attached, multiple processors are used for

computing the overlap, on the expense of time

#### **Details**

The windows are constructed from the first base position onto which a read has been mapped until the end of the chromosome.

#### Value

a data. frame with the following information for each sliding window on the chromosome

chr string; which chromosome the interval is on

start integer; start coordinate of the windows on the chromosome end integer; end coordinate of the windows on the chromosome

n. overlap integer; number of read match positions inside the window. Per match position

there can be one or more reads mapped, so this number always is smaller than

n.reads

n.reads numeric; number of reads which match positions inside this window; can be

floating-point numbers if argument normaliseByMatches=TRUE

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n.unique	and for which this position is contained inside this window
max.reads	integer; the maximal number of reads at any single one match position contained inside this window
first	integer; coordinate of the first read alignment found inside the window
last	integer; coordinate of the last read alignment found inside the window

The result is of class data. frame and in addition of the (S3) class slidingWindowSummary, which may be utilized by follow-up functions.

#### Author(s)

Joern Toedling

#### See Also

AlignedGenomeIntervals-class

# **Examples**

```
exDir <- system.file("extdata", package="girafe")
exA <- readAligned(dirPath=exDir, type="Bowtie",
    pattern="aravinSRNA_23_no_adapter_excerpt_mm9_unmasked.bwtmap")
exAI <- as(exA, "AlignedGenomeIntervals")
exPX <- perWindow(exAI, chr="chrX", winsize=1e5, step=0.5e5)
head(exPX[order(exPX$n.overlap, decreasing=TRUE),])</pre>
```

trimAdapter

Remove 3' adapter contamination

#### **Description**

Function to remove 3' adapter contamination from reads

# Usage

# **Arguments**

fq Object of class ShortReadQ; the reads with possible adapter contamination.

adapter object of class DNAString or class character; the sequence of the 3' adapter

which could give rise to the 3' contamination. If of class character, it is con-

verted to a DNAString inside the function.

match.score numeric; alignment score for matching bases mismatch.score numeric; alignment score for mismatches

score.threshold

numeric; minimum total alignment score required for an overlap match between the 3' end of the read and the 5' end of the adapter sequence.

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#### **Details**

Performs an overlap alignment between the ends of the reads and the start of the adapter sequence.

#### Value

An object of class ShortReadQ containing the reads without the 3' adapter contamination.

#### Note

The function trimLRPatterns from package ShortRead may be a faster alternative to this function.

#### Author(s)

J. Toedling

# See Also

```
pairwiseAlignment, narrow, readFastq, writeFastq
```

#### **Examples**

# **Description**

For each genome interval in one set, finds the nearest interval in a second set of genome intervals.

#### Value

a data.frame with a number of rows equal to the number of intervals in argument from. The elements of the data.frame are:

```
distance_to_nearest
```

which\_nearest

numeric; distance to nearest interval from object to. Is 0 if the current interval in object from did overlap one or more intervals in object to

list; each list element are the indices or the intervals in object to that have the

closest distance to the current interval in object from

which\_overlap list; each list element are the indices or the intervals in object to that do overlap

with the current interval in object from

#### Methods

Currently, the package *girafe* contains method implementations for the first object (Argument: from) being of any of the classes "AlignedGenomeIntervals", "Genome\_intervals" or "Genome\_intervals\_stranded". The second object (Argument: to) has be of class "Genome\_intervals\_stranded" or "Genome\_intervals".

#### Note

If the supplied objects are stranded, as it is the case with objects of classes 'AlignedGenomeIntervals' and 'Genome\_intervals\_stranded', then the overlap and distance is solely computed between intervals on the same strand.

For objects of class 'Genome\_intervals', overlap and distances are computed regardless of strand information.

#### Author(s)

Joern Toedling

#### See Also

which\_nearest

```
### process aligned reads
exDir <- system.file("extdata", package="girafe")</pre>
exA <- readAligned(dirPath=exDir, type="Bowtie",</pre>
 pattern="aravinSRNA_23_no_adapter_excerpt_mm9_unmasked.bwtmap")
exAI <- as(exA, "AlignedGenomeIntervals")</pre>
## load annotated genome features
load(file.path(exDir, "mgi_gi.RData"))
## subset for sake of speed:
A <- exAI[is.element(segnames(exAI), c("chrX", "chrY"))]
G <- mgi.gi[is.element(seqnames(mgi.gi), c("chrX","chrY"))]</pre>
## find nearest annotated feature for each AlignedGenomeInterval
WN <- which_nearest(A, G)</pre>
dim(WN); tail(WN)
## notice the difference to:
tail(which_nearest(as(A, "Genome_intervals"), G))
# the last interval in A is located antisense to a gene,
# but not overlapping anything on the same strand
```

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