

# Package ‘girafe’

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**Title** Genome Intervals and Read Alignments for Functional Exploration

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**Imports** methods, Biobase, Biostrings (>= 2.47.6), pwalign, 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.

**License** Artistic-2.0

**LazyLoad** yes

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Additional reduce-methods

*Auxiliary methods for Function reduce in Package 'girafe'*

---

### Description

This methods were written to resurrect the functionality of the 'reduce' method of package 'IRanges' for objects belonging to classes defined in 'IRanges'. This functions had been overwritten by the later import of package 'intervals'. See the corresponding help pages of package IRanges for more details on these methods.

### Methods

signature(x = "CompressedIRangesList") see help page in package 'IRanges'

signature(x = "IRanges") see help page in package 'IRanges'

signature(x = "IntegerRanges") see help page in package 'IRanges'

signature(x = "IntegerRangesList") see help page in package 'IRanges'

### See Also

[IntegerRangesList-class](#), [IntegerRanges-class](#), [IRanges-class](#)

---

addNBSignificance      *assess significance of sliding-window read counts*

---

## 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

<code>x</code>	A data.frame of class <code>slidingWindowSummary</code> , as returned by the function <code>perWindow</code> .
<code>estimate</code>	string; which method to use to estimate the parameters of the NB background distribution; see below for details
<code>correct</code>	string; which method to use for $p$ -value adjustment; can be any method that is implemented for <code>p.adjust</code> including “none” if no correction is desired.
<code>max.n</code>	integer; only relevant if <code>estimate=="NB.ML"</code> ; 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.

**“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

$$\hat{q}_1 = \frac{n_1}{n_0}$$

and

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

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

“**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](#)

### 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)
exPX <- addNBSignificance(exPX, correct="bonferroni")
str(exPX)
exPX[exPX$p.value <= 0.05,]
```

---

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](#)

**Examples**

```
f1 <- system.file("extdata", "ex1.bam", package="Rsamtools")
ExGi <- agiFromBam(f1)
head(detail(ExGi))
```

---

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 `R1e`, 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 Rle 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](#)**Examples**

```
##### 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="")

## 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
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")
exA <- readAligned(dirPath=exDir, type="Bowtie",
  pattern="aravinSRNA_23_no_adapter_excerpt_mm9_unmasked.bwtmap")
exAI <- as(exA, "AlignedGenomeIntervals")
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)
## how many elements do read match positions generally overlap:
table(listLen(exOv))
## what are the 13 elements overlapped by a single match position:
mgi.gi[exOv[[which.max(listLen(exOv))]]]
## what kinds of elements are overlapped
(tabOv <- table(as.character(mgi.gi$type)[unlist(exOv)]))
### display those classes:
my.cols <- rainbow(length(tabOv))
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

```
countReadsAnnotated(GI, M, typeColumn="type", fractionGI=0.7,
                    mem.friendly=FALSE, showAllTypes=FALSE)
```

## Arguments

GI	object of class <code>AlignedGenomeIntervals</code>
M	Annotation object of class <code>Genome_intervals_stranded</code> or <code>Genome_intervals</code> ; 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 overlap 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

**Examples**

```
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",
  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,
    nameColumn="alias", show="plus")
}
countReadsAnnotated(A, M2, typeColumn="type")
```

---

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 <code>Genome_intervals</code>
I2	object that inherits from class <code>Genome_intervals</code>
<code>min.frac</code>	numeric; minimum required fraction of each of the two interval widths by which two intervals should overlap in order to be marked as overlapping.
<code>both</code>	logical; shall both overlap partners meet the minimum fraction <code>min.frac</code> requirement? If FALSE, then overlaps with only partner involved to at least that fraction are also reported.
<code>mem.friendly</code>	logical; if set to TRUE an older but memory-friendlier version of <code>interval_overlap</code> is used inside this function. Note that <code>mem.friendly</code> is only evaluated if I1 or I2 is of class <code>AlignedGenomeIntervals</code> .

**Value**

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

<code>Index1</code>	Index of interval in first interval list
<code>Index2</code>	Index of interval in second interval list
<code>n</code>	number of bases that the two intervals overlap
<code>fraction1</code>	fraction of interval 1's width by which the two intervals overlap
<code>fraction2</code>	fraction of interval 2's width by which the two intervals overlap

**Author(s)**

J. Toedling

**See Also**

[interval\\_overlap](#)

**Examples**

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

---

<code>getFeatureCounts</code>	<i>get the read counts for a supplied set of genomic features</i>
-------------------------------	---

---

**Description**

get the read counts for a supplied set of genomic features

**Usage**

```
getFeatureCounts(AI, FG, nameColumn = "Name", fractionIncluded = 1,
returnType = "AlignedGenomeIntervals", mem.friendly = FALSE)
```

**Arguments**

AI	AlignedGenomeIntervals object
FG	Genome\_intervals objects of genomic features
nameColumn	character indicating which column of the object FG holds the identifiers of the genomic features; is used to assess the number of genomic copies per feature
fractionIncluded	double; which fraction of an interval needs to be included in a feature in order to count for the feature
returnType	one of AlignedGenomeIntervals or integer
mem.friendly	logical; passed on to fracOverlap function, determines if overlap should be computed chromosome-wise, optionally distributed over several CPUs (with package parallel)

**Value**

depends on argument returnType: one of AlignedGenomeIntervals or a named integer

**Author(s)**

Joern Toedling

**See Also**

[fracOverlap](#)

---

girafe-internal

*Internal girafe functions*

---

**Description**

Called internally by other girafe functions. Normally need not be called by the user.

**Author(s)**

Wolfgang Huber, Joern Toedling

**See Also**

[AlignedGenomeIntervals-class](#)

---

intPhred	<i>Extract integer Phred score values from FastQ data</i>
----------	---

---

### 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](#)

**Examples**

```

exDir <- system.file("extdata", package="girafe")
ra <- readFastq(dirPath=exDir, pattern=
  "aravinSRNA_23_plus_adapter_excerpt.fastq")
raquals <- intPhred(ra, method="Sanger",
  returnType="matrix")
ra.qmed <- apply(raquals, 2, median)
if (interactive())
  plot(ra.qmed, type="h", ylim=c(0,42), xlab="Base position",
    ylab="Median Phred Quality Score", lwd=2, col="steelblue")

```

---

medianByPosition	<i>Compute median quality for each nucleotide position</i>
------------------	--

---

**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 R1e objects for each position. In each iteration, the R1e 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](#)

**Examples**

```
exDir <- system.file("extdata", package="girafe")
ra <- readFastq(dirPath=exDir, pattern=
  "aravinSRNA_23_plus_adapter_excerpt.fastq")
medianByPosition(ra, batchSize=200)
```

perWindow

*Investigate aligned reads in genome intervals with sliding windows***Description**

Investigate aligned reads in genome intervals with sliding windows.

**Usage**

```
perWindow(object, chr, winsize, step, normaliseByMatches = TRUE,
  mem.friendly = FALSE)
```

**Arguments**

object	object of class <code>AlignedGenomeIntervals</code>
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 <code>AlignedGenomeInterval</code> be normalised by the number of genomic matches of the read sequence before summing them up in each window? ( <i>i.e.</i> derivation a weighted sum of read counts)
mem.friendly	logical; argument passed on to function <code>interval_overlap</code> ; if TRUE the less RAM and, if the <code>parallel</code> 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 <code>n.reads</code>
n.reads	numeric; number of reads which match positions inside this window; can be floating-point numbers if argument <code>normaliseByMatches=TRUE</code>

n.unique	integer; number of reads which each only have one match position in the genome 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),])
```

---

plotAligned

*Visualise reads aligned to genome intervals*

---

### Description

Visualise reads aligned to genome intervals

### Usage

```
plotAligned(x, y, chr, start, end, plus.col = "#00441b",
  minus.col = "#283d78", gff, featureLegend = FALSE,
  gffChrColumn = "seq_name", gffTypeColumn="type",
  gffNameColumn="ID",
  featureExclude = c("chromosome", "nucleotide_match", "insertion"),
  showStrands="both", extraColors=NULL, ylim, highlight, main, ...)
```

### Arguments

x	Object of class <code>AlignedGenomeIntervals</code>
y	This argument is only specified for compatibility with <code>plot.default</code> and not used in the function.
chr	string; on which chromosome is the region to plot
start	integer; start coordinate of the chromosome region to plot
end	integer; end coordinate of the chromosome region to plot



plus.col	which colour to use for the reads on the Plus strand
minus.col	which colour to use for the reads on the Plus strand
gff	Data frame containing annotation for genomic feature to be used to further annotate the plot. Note that it must include a column called “type” that indicates the type of each genomic feature (e.g. miRNA, gene etc.).
featureLegend	logical; should a legend that describes the colour code for the annotated genome features be appended at the bottom of the plot?
gffChrColumn	string; which column of the gff data.frame holds the chromosome identifier of each feature.
gffTypeColumn	string; which column of the gff data.frame holds the type/class identifier of each feature. Used for the colouring of features.
gffNameColumn	what is the column of the gff data.frame called that holds the identifier of the element that should be displayed in the plot; default: “name”
featureExclude	character; which kinds of annotated genome features specified in the gff are to be ignored for the plot
showStrands	string; which strands to show in the plot; defaults to “both”, but users can specify to show only the reads on “plus” or “minus” strand
extraColors	named character vector which allows the user to specify custom colours for feature types; colours must be specified in RGB format as hexadecimal strings starting with “#”, e.g. “#addfff” for light-blue
ylim	range of read numbers to plot (y-axis limits); if not specified they are computed from the data in the specified region
highlight	currently unused
main	string; main title to use for the plot
...	further arguments passed on to the more primitive plotting functions used

### Details

This function implements the plot method for objects of class AlignedGenomeIntervals.

### Value

Returns NULL; this function is called for the side-effect of creating the plot.

### Note

This function was inspired by and borrows source code from the function plotAlongChrom in package tilingArray

### Author(s)

Joern Toedling, Wolfgang Huber

### See Also

[AlignedGenomeIntervals-class](#)

**Examples**

```

A <- AlignedGenomeIntervals(
  start=c(1,8,10,20), end=c(5,15,16,25),
  chromosome=rep("chr1", each=4),
  strand=c("+", "+", "+", "+"),
  sequence=c("ACATT", "TATCGGAC", "TCGGACT", "GTAACG"),
  reads=c(5L, 2L, 4L, 7L) )
M2 <- new("Genome_intervals_stranded",
  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())
  plot(A, M2, chr="chr1", start=0, end=35,
       nameColum="alias", showStrands="plus")

if (interactive())
  ## use 'extraColors' to replace default colours (or to add new ones):
  plot(A, M2, chr="chr1", start=0, end=35, nameColum="alias",
       showStrands="plus", extraColors=c("tRNA"="#6666DD"))

## See also the examples in the vignette and on the manual page
## of the class 'AlignedGenomeIntervals'

```

---

plotNegBinomFit      *Plot Negative Binomial Fit*

---

**Description**

Plot Negative Binomial Fit

**Usage**

```
plotNegBinomFit(x, breaks = c(-0.5:15.5, 1e+08), bar.col=rainbow(2),
  addLegend=TRUE, legend.names=c("data", "background"), ...)
```

**Arguments**

x	data.frame; slidingWindowSummary
breaks	numeric vector of breakpoints
bar.col	colours for the bars
addLegend	logical; should a legend be added in the top-right corner of the plot
legend.names	character; names for the legend
...	further arguments passed on to function barplot

**Value**

returns NULL; only called for its side-effect of producing the plot

**Author(s)**

J Toedling

**See Also**[barplot](#)

plotReads

*Function to plot aligned reads along the chromosome***Description**

Function to plot aligned reads along the chromosome

**Usage**

```
plotReads(dat, ylim, strand = "plus", vpr, sampleColor = NULL,
          zeroLine = FALSE, main, pointSize = unit(1, "mm"),
          cexAxisLabel = 1, cexAxis = 1, ylab, ...)
```

**Arguments**

dat	a list with arguments <b>x.start</b> integer; the genome start coordinates of the data to visualise <b>x.end</b> integer; the genome end coordinates of the data to visualise <b>y</b> numeric; the levels of the data to visualise <b>flag</b> numeric; specifies the category of each value, e.g. marks which data values belong to unique read alignments and which not
ylim	y-axis limits of the plotting window
strand	string; which of the two strands is plotted
vpr	which viewport to use for this plot
sampleColor	which colour to use for the data
zeroLine	logical; should a line at y=0 be drawn?
main	string; main title for the plot
pointSize	width of each dot/bar
cexAxisLabel	numeric; expansion factor for the axis labels
cexAxis	numeric; expansion factor for the axis labels
ylab	y-axis label
...	further arguments passed on to the more primitive plotting functions that are used

**Details**This function is used inside the plotting method for objects of class `AlignedGenomeIntervals`.**Value**returns `Null`; called for plotting single reads inside the function `plotAligned`

**Author(s)**

Joern Toedling

---

`trimAdapter`*Remove 3' adapter contamination*

---

**Description**

Function to remove 3' adapter contamination from reads

**Usage**

```
trimAdapter(fq, adapter, match.score = 1, mismatch.score = -1,  
           score.threshold = 2)
```

**Arguments**

<code>fq</code>	Object of class <code>ShortReadQ</code> ; the reads with possible adapter contamination.
<code>adapter</code>	object of class <code>DNASTring</code> or class <code>character</code> ; the sequence of the 3' adapter which could give rise to the 3' contamination. If of class <code>character</code> , it is converted to a <code>DNASTring</code> inside the function.
<code>match.score</code>	numeric; alignment score for matching bases
<code>mismatch.score</code>	numeric; alignment score for mismatches
<code>score.threshold</code>	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.

**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**

```
exDir <- system.file("extdata", package="girafe")
## load reads containing adapter fragments at the end
ra23.wa <- readFastq(dirPath=exDir, pattern=
  "aravinSRNA_23_plus_adapter_excerpt.fastq")
table(width(ra23.wa))
# adapter sequence obtained from GEO page
# accession number: GSE10364
#adapter <- DNASTring("CTGTAGGCACCATCAAT")
adapter <- "CTGTAGGCACCATCAAT"

# trim adapter
ra23.na <- trimAdapter(ra23.wa, adapter)
table(width(ra23.na))
```

---

```
weightedConsensusMatrix
  compute weighted consensus matrix
```

---

**Description**

computes weighted consensus matrix

**Usage**

```
weightedConsensusMatrix(seqs, weights, shift = NULL,
  baseLetters = c("A", "C", "G", "T", "N"))
```

**Arguments**

seqs	character vector of read sequences
weights	integer; weights (read counts)
shift	integer; shift of each read sequence relative to the first column of the consensus matrix, by default: 0
baseLetters	alphabet

**Value**

A consensus matrix

**Author(s)**

J Toedling

**See Also**

[consensusMatrix](#)

**Examples**

```
## Align following sequences with weights:
##   ACATT      1
##   CGTTA     10
##   TTG        3
##   GACATT     4

dweights <- c(1L, 10L, 3L, 4L)
d <- c("ACATT", "CGTTA", "TTG", "GACATT")
dshifts <- c(0L, 1L, 2L, -1L)
W <- girafe::weightedConsensusMatrix(d, dweights, shift=dshifts)
consensusString(W, ambiguityMap="N")
```

---

which\_nearest-methods *Methods for function 'which\_nearest' and genome intervals*

---

**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	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
which_nearest	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](#)**Examples**

```
### process aligned reads
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")

## load annotated genome features
load(file.path(exDir, "mgi_gi.RData"))

## subset for sake of speed:
A <- exAI[is.element(seqnames(exAI), c("chrX","chrY"))]
G <- mgi.gi[is.element(seqnames(mgi.gi), c("chrX","chrY"))]

## find nearest annotated feature for each AlignedGenomeInterval
WN <- which_nearest(A, G)
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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