Package 'nucleR'

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Type Package

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Description

Nucleosome positioning from Tiling Arrays and High-Troughput Sequencing Experiments

Details

Package: nucleR
Type: Package
License: LGPL (>= 3)

LazyLoad: yes

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This package provides a convenient pipeline to process and analize nucleosome positioning experiments from High-Troughtput Sequencing or Tiling Arrays.

Despite it's use is intended to nucleosome experiments, it can be also useful for general ChIP experiments, such as ChIP-on-ChIP or ChIP-Seq.

See following example for a brief introduction to the available functions

Author(s)

Oscar Flores Ricard Illa

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```
library(ggplot2)
# Load example dataset:
# some NGS paired-end reads, mapped with Bowtie and processed with R
# it is a GRanges object with the start/end coordinates for each read.
reads <- get(data(nucleosome_htseq))</pre>
# Process the paired end reads, but discard those with length > 200
preads_orig <- processReads(reads, type="paired", fragmentLen=200)</pre>
# Process the reads, but now trim each read to 40bp around the dyad
preads_trim <- processReads(reads, type="paired", fragmentLen=200, trim=40)</pre>
# Calculate the coverage, directly in reads per million (r.p.m.)
cover_orig <- coverage.rpm(preads_orig)</pre>
cover_trim <- coverage.rpm(preads_trim)</pre>
# Compare both coverages, the dyad is much more clear in trimmed version
t1 <- as.vector(cover_orig[[1]])[1:2000]</pre>
t2 <- as.vector(cover_trim[[1]])[1:2000]
t1 <- (t1-min(t1)) / max(t1-min(t1)) # Normalization
t2 \leftarrow (t2-min(t2)) / max(t2-min(t2)) # Normalization
plot_data <- rbind(</pre>
    data.frame(
        x=seq_along(t1),
        y=t1,
        coverage="original"
    ),
    data.frame(
        x=seq_along(t2),
        y=t2,
        coverage="trimmed"
    )
qplot(x=x, y=y, color=coverage, data=plot_data, geom="line",
```

.fftRegion

```
xlab="position", ylab="coverage")
# Let's try to call nucleosomes from the trimmed version
# First of all, let's remove some noise with FFT
# Power spectrum will be plotted, look how with a 2%
# of the components we capture almost all the signal
cover_clean <- filterFFT(cover_trim, pcKeepComp=0.02, showPowerSpec=TRUE)</pre>
# How clean is it now?
plot_data <- rbind(</pre>
    data.frame(
        x=1:4000,
        y=as.vector(cover_trim[[1]])[1:4000],
        coverage="noisy"
   ),
   data.frame(
       x=1:4000,
        y=as.vector(cover_clean[[1]])[1:4000],
        coverage="filtered"
    )
)
qplot(x=x, y=y, color=coverage, data=plot_data, geom="line",
 xlab="position", ylab="coverage")
# And how similar? Let's see the correlation
cor(cover_clean[[1]], as.vector(cover_trim[[1]]))
# Now it's time to call for peaks, first just as points
# See that the score is only a measure of the height of the peak
peaks <- peakDetection(cover_clean, threshold="25%", score=TRUE)</pre>
plotPeaks(peaks[[1]], cover_clean[[1]], threshold="25%")
# Do the same as previously, but now we will create the nucleosome calls:
peaks <- peakDetection(cover_clean, width=147, threshold="25%", score=TRUE)</pre>
plotPeaks(peaks, cover_clean[[1]], threshold="25%")
#This is all. From here, you can filter, merge or work with the nucleosome
#calls using standard IRanges functions and R/Bioconductor manipulation
```

.fftRegion

FFT Region

Description

Define FFT by regions to avoid a large amount of memory use and a drop in performance

```
.fftRegion(data2, pcKeepComp)
```

.getThreshold 5

Description

If threshold is given as a string with percentage, convert it

Usage

```
.getThreshold(threshold, data)
```

Arguments

threshold threshold given as an absolute value or as a string percentage data vector with values from which to derive the threshold if it's relative

Value

a numeric vector

.loadFiles	File loader Higher order function to import BAM or Bowtie files.
	Deals with wether type is single or paired and with the number of input files

Description

File loader Higher order function to import BAM or Bowtie files. Deals with wether type is single or paired and with the number of input files

Usage

.loadFiles(singleLoad, pairedLoad)

.loadPairedBam	Load a paired-end-end BAM

Description

Load a paired-end-end BAM

Usage

.loadPairedBam(file)

6 .processStrand

 $. \\ load Single Bam$

Load a single-end BAM

Description

Load a single-end BAM

Usage

```
.loadSingleBam(exp)
```

.mid

Find midpoints

Description

Simple function for returning the middle point of a of a GRanges (normal mid doesn't work there)

Usage

.mid(x)

 $. {\tt processStrand}$

Process a given strand from a BAM file to read

Description

Process a given strand from a BAM file to read

Usage

```
.processStrand(strand, bam)
```

Arguments

strand either strand "+" or "-"

bam data.frame representing the BAM file as read by Rsamtools::scanBam

Value

GenomicRanges::GRanges object representing the reads in a given strand

.unlist_as_integer 7

.unlist_as_integer

Unlist an IRanges object into a vector

Description

Wrapper to internal function from the IRanges package. Avoids use of ::: and thus prevents a NOTE warning about the use of a non-exported function

Usage

```
.unlist_as_integer(x)
```

Author(s)

H. Pages, P. Aboyoun and M. Lawrence

.vectorizedAll

Vectorized version of all

Description

Helper function that behaves as a vectorized version of the function all

Usage

```
.vectorizedAll(...)
```

Arguments

arbitraty amount of logical vectors, expected to have the same length

Value

logical vector

.xlapply

mclapply warapper

Description

Wrapper to choose between lapply and mclapply accordingly

```
.xlapply(X, FUN, ..., mc.cores = 1)
```

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controlCorrection Correct

Correct experimental profiles with control sample

Description

This function allows the correction of experimental coverage profiles (usually MNase digested nucleosomal DNAs in this library) with control samples (usually naked DNA sample digested with MNase). This is useful to correct MNase biase.

Usage

```
controlCorrection(exp, ctr, ...)
## S4 method for signature 'SimpleRleList'
controlCorrection(exp, ctr, mc.cores = 1)
## S4 method for signature 'Rle'
controlCorrection(exp, ctr)
## S4 method for signature 'list'
controlCorrection(exp, ctr, mc.cores = 1)
## S4 method for signature 'numeric'
controlCorrection(exp, ctr)
```

Arguments

exp, ctr	Comparable experimental and control samples (this means same format and equivalent preprocessment)
	Further arguments to be passed to or from other methods.
mc.cores	Number of cores available for parallel list processing

Details

This substracts the enrichment in the control sample respect it's mean from the experimental profile.

This is useful for examinating the effect of the MNase digestion in nucleosome experiments using a nucleosomal DNA and a genomic (naked) DNA sample. Notice that genomic DNA samples cannot be strand-corrected using single end data, so only paired end controls are useful for this proupose, despite they can be compared against extended nucleosomal DNA single end reads. Furthermore, both datasets must be converted to reads per milion.

This process dificults the nucleosome positioning due the lower sharpness of the peaks, but allows a complementary study of the MNase digestion effect.

Value

Corrected experimental profile

coverage.rpm 9

Author(s)

Oscar Flores <oflores@mmb.pcb.ub.es>

Examples

```
map = syntheticNucMap(as.ratio=TRUE)
exp = coverage.rpm(map$syn.reads)
ctr = coverage.rpm(map$ctr.reads)
corrected = controlCorrection(exp, ctr)
```

coverage.rpm

Coverage calculation and normalization to reads per million (rpm)

Description

Calculates the coverage values from a GenomicRanges::GRanges or IRanges::IRanges object normalized to reads per million, allowing the comparison of experiments with a different absolut number of reads.

Usage

```
coverage.rpm(data, scale = 1e+06, ...)
## S4 method for signature 'GRanges'
coverage.rpm(data, scale = 1e+06, ...)
## S4 method for signature 'CompressedGRangesList'
coverage.rpm(data, scale = 1e+06, ...)
## S4 method for signature 'IRanges'
coverage.rpm(data, scale = 1e+06, ...)
```

Arguments

data	GenomicRanges::GenomicRanges or IRanges::IRanges with the reads information
scale	By default, a million (1e6), but you could change this value for abnormal high or low amount of reads.
	Additional arguments to be passed to coverage function

Value

RleList object with the coverage objects

Author(s)

Oscar Flores <oflores@mmb.pcb.ub.es>

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See Also

```
processReads(), IRanges::coverage()
```

Examples

export.bed

Export ranges in BED format

Description

Export ranges in BED format, compatible with UCSC genome browser, IGB, and others.

```
export.bed(
  ranges,
  score = NULL,
  chrom,
  name,
  desc = name,
  filepath = name,
  splitByChrom = TRUE
)
## S4 method for signature 'IRanges'
export.bed(ranges, score = NULL, chrom, name, desc = name, filepath = name)
## S4 method for signature 'CompressedIRangesList'
export.bed(
  ranges,
  score = NULL,
  name,
  desc = name,
  filepath = name,
  splitByChrom = TRUE
)
```

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```
## S4 method for signature 'GRanges'
export.bed(
  ranges,
  score = NULL,
  name,
  desc = name,
  filepath = name,
  splitByChrom = TRUE
)
```

Arguments

ranges Ranges to export, in IRanges::IRanges, IRanges::IRangesList or GenomicRanges::GRanges

format

score Score data if not included in ranges object. Bed file will put all scores=1000 if

scores are not present

chrom For single IRanges objects, the chromosome they represent. For other data

types, values from names (...) will be used.

name Name of the track

desc Description of the track

filepath Path and prefix of the file(s) to write. Chromosome number and "bed" extension

will be automatically added.

splitByChrom If multiple chromosomes are given, should they be splitted into one file per

chromosome or shall them be saved all together?

Value

(none)

Author(s)

Oscar Flores <oflores@mmb.pcb.ub.es>

References

BED format specification: http://genome.ucsc.edu/FAQ/FAQformat#format1

```
# Generate some ranges with scores
library(GenomicRanges)
ran <- GRanges(
    seqnames="chrX", ranges=IRanges(start=1:100, end=101:200),
    score=(1:100)/100
)
# Export as bed file</pre>
```

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export.wig

Export values in WIG format

Description

Export coverage/intensity values in WIG format, compatible with UCSC genome browser, IGB and others.

Usage

```
export.wig(data, name, chrom = "", filepath = name)
```

Arguments

data Coverage/intensity values (numeric vector)

name Name of the track

chrom Information about chromosome if not inferrable from data (only for numeric

vectors)

filepath Filepath where to save the object. Chromosome name and "wig" extension will

be automatically added

Value

(none)

Author(s)

Oscar Flores <oflores@mmb.pcb.ub.es>

References

WIG format specification: http://genome.ucsc.edu/FAQ/FAQformat#format6

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Examples

```
# Load data
data(nucleosome_htseq)
cover <- coverage.rpm(nucleosome_htseq)

# Create wig file
export.wig(cover, name="example_track")

# This would create the file "example_track.chr1.wig" with:

# track type=wiggle_0 name="example_track"

# fixedStep chrom=chr1 start=1 step=1

# 55.55247

# 55.55247

# 277.7623

# 388.8673

# ...</pre>
```

filterFFT

Clean noise and smoothing for genomic data using Fourier-analysis

Description

Remove noise from genomic data smoothing and cleaning the observed signal. This function doesn't alter the shape or the values of the signal as much as the traditional method of sliding window average does, providing a great correlation within the original and filtered data (>0.99).

```
filterFFT(
  data,
  pcKeepComp = "auto",
  showPowerSpec = FALSE,
  useOptim = TRUE,
  ...
)

## S4 method for signature 'SimpleRleList'
filterFFT(
  data,
  pcKeepComp = "auto",
  showPowerSpec = FALSE,
  useOptim = TRUE,
  mc.cores = 1,
  ...
)
```

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```
## S4 method for signature 'Rle'
filterFFT(
  data,
  pcKeepComp = "auto",
  showPowerSpec = FALSE,
  useOptim = TRUE,
)
## S4 method for signature 'list'
filterFFT(
  data,
  pcKeepComp = "auto",
  showPowerSpec = FALSE,
  useOptim = TRUE,
 mc.cores = 1,
)
## S4 method for signature 'numeric'
filterFFT(
  data,
  pcKeepComp = "auto",
  showPowerSpec = FALSE,
  useOptim = TRUE,
)
```

Arguments

data	Coverage or intensities values representing the results of the NGS of TA exper-

iment. This attribute could be a individual vector representing a chromosome

(Rle or numeric object) or a list of them.

pcKeepComp Number of components to select, in percentage respect total length of the sam-

ple. Allowed values are numeric (in range 0:1) for manual setting or "auto" for

automatic detection. See details.

showPowerSpec Plot the Power Spectrum of the Fast Fourier Transform to visually identify the

selected components (see details).

useOptim This function implements tweaks to a standard fft call to improve (dramatically)

the performance in large genomic data. These optimizations can be bypassed by

setting this parameter to FALSE.

... Other parameters to be passed to pcKeepCompDetect function

mc.cores If multiple cores are available, maximum number of them to use for parallel

processing of data elements (only useful if data is a list of elements)

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Details

Fourier-analysis principal components selection is widely used in signal processing theory for an unbiased cleaning of a signal over the time.

Other procedures, as the traditional sliding window average, can change too much the shape of the results in function of the size of the window, and moreover they don't only smooth the noise without removing it.

With a Fourier Transform of the original signal, the input signal is descomposed in different wavelets and described as a combination of them. Long frequencies can be explained as a function of two ore more periodical shorter frequecies. This is the reason why long, unperiodic sequences are usually identified as noise, and therefore is desireable to remove them from the signal we have to process.

This procedure here is applied to genomic data, providing a novel method to obtain perfectly clean values wich allow an efficient detection of the peaks which can be used for a direct nucleosome position recognition.

This function select a certain number of components in the original power spectrum (the result of the Fast Fourier Transform which can be seen with showPowerSpec=TRUE) and sets the rest of them to 0 (component knock-out).

The amout of components to keep (given as a percentage of the input lenght) can be set by the pcKeepComp. This will select the first components of the signal, knock-outing the rest. If this value is close to 1, more components will be selected and then more noise will be allowed in the output. For an effective filtering which removes the noise keeping almost all relevant peaks, a value between 0.01 and 0.05 is usually sufficient. Lower values can cause merging of adjacent minor peaks.

This library also allows the automatic detection of a fitted value for pcKeepComp. By default, if uses the pcKeepCompDetect function, which looks which is the minimum percentage of components than can reproduce the original signal with a corelation between the filtered and the original one of 0.99. See the help page of pcKeepCompDetect for further details and reference of available parameters.

One of the most powerful features of nucleR is the efficient implementation of the FFT to genomic data. This is achived trought few tweaks that allow an optimum performance of the Fourier Transform. This includes a by-range filtering, an automatic detection of uncovered regions, windowed execution of the filter and padding of the data till nearest power of 2 (this ensures an optimum case for FFT due the high factorization of components). Internal testing showed up that in specific datasets, these optimizations lead to a dramatic improvement of many orders of magnitude (from 3 days to few seconds) while keeping the correlation between the native fft call and our filterFFT higher than 0.99. So, the use of these optimizations is highly recomended.

If for some reason you want to apply the function without any kind of optimizations you can specify the parameter useOptim=FALSE to bypass them and get the pure knockout inverse from native FFT call. All other parameters can be still applyied in this case.

Value

Numeric vector with cleaned/smoothed values

Author(s)

Oscar Flores <oflores@mmb.pcb.ub.es>, David Rosell <david.rossell@irbbarcelona.org>

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References

Smith, Steven W. (1999), The Scientist and Engineer's Guide to Digital Signal Processing (Second ed.), San Diego, Calif.: California Technical Publishing, ISBN 0-9660176-3-3 (availabe online: http://www.dspguide.com/pdfbook.htm)

Examples

```
# Load example data, raw hybridization values for Tiling Array
raw_data <- get(data(nucleosome_tiling))

# Filter data
fft_data <- filterFFT(raw_data, pcKeepComp=0.01)

# See both profiles
library(ggplot2)
plot_data <- rbind(
    data.frame(x=seq_along(raw_data), y=raw_data, intensities="raw"),
    data.frame(x=seq_along(fft_data), y=fft_data, intensities="filtered"))

qplot(x=x, y=y, data=plot_data, geom="line", xlab="position",
    ylab="intensities") + facet_grid(intensities~.)

# The power spectrum shows a visual representation of the components
fft_data <- filterFFT(raw_data, pcKeepComp=0.01, showPowerSpec=TRUE)</pre>
```

fragmentLenDetect

Fragments length detection from single-end sequencing samples

Description

When using single-ended sequencing, the resulting partial sequences map only in one strand, causing a bias in the coverage profile if not corrected. The only way to correct this is knowing the average size of the real fragments. nucleR uses this information when preprocessing single-ended sequences. You can provide this information by your own (usually a 147bp length is a good aproximation) or you can use this method to automatically guess the size of the inserts.

```
fragmentLenDetect(
  reads,
  samples = 1000,
  window = 5000,
  min.shift = 1,
  max.shift = 100,
  mc.cores = 1,
  as.shift = FALSE
)
```

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```
## S4 method for signature 'AlignedRead'
fragmentLenDetect(
  reads,
  samples = 1000,
 window = 1000,
 min.shift = 1,
 max.shift = 100,
 mc.cores = 1,
 as.shift = FALSE
)
## S4 method for signature 'GRanges'
fragmentLenDetect(
  reads,
  samples = 1000,
 window = 1000,
 min.shift = 1,
 max.shift = 100,
 mc.cores = 1,
 as.shift = FALSE
)
```

Arguments

reads	Raw single-end reads ShortRead::AlignedRead or GenomicRanges::GRanges format)		
samples	Number of samples to perform the analysis (more = slower but more accurate)		
window	Analysis window. Usually there's no need to touch this parameter.		
min.shift, max.shift			
	Minimum and maximum shift to apply on the strands to detect the optimal fragment size. If the range is too big, the performance decreases.		
mc.cores	If multicore support, maximum number of cores allowed to use.		
as.shift	If TRUE, returns the shift needed to align the middle of the reads in opposite strand. If FALSE, returns the mean inferred fragment length.		

Details

This function shifts one strand downstream one base by one from min.shift to max.shift. In every step, the correlation on a random position of length window is checked between both strands. The maximum correlation is returned and averaged for samples repetitions.

The final returned length is the best shift detected plus the width of the reads. You can increase the performance of this function by reducing the samples value and/or narrowing the shift range. The window size has almost no impact on the performance, despite a to small value can give biased results.

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Value

Inferred mean lenght of the inserts by default, or shift needed to align strands if as . shift=TRUE.

Author(s)

Oscar Flores <oflores@mmb.pcb.ub.es>

Examples

```
library(GenomicRanges)
library(IRanges)
# Create a sinthetic dataset, simulating single-end reads, for positive and
# negative strands
# Positive strand reads
pos <- syntheticNucMap(nuc.len=40, lin.len=130)$syn.reads</pre>
# Negative strand (shifted 147bp)
neg <- IRanges(end=start(pos)+147, width=40)</pre>
sim <- GRanges(</pre>
    seqnames="chr1",
    ranges=c(pos, neg),
    strand=c(rep("+", length(pos)), rep("-", length(neg)))
)
# Detect fragment lenght (we know by construction it is really 147)
fragmentLenDetect(sim, samples=50)
# The function restricts the sampling to speed up the example
```

mergeCalls

Automatic merging of overlapped nucleosome calls

Description

This function joints close nucleosome calls into one larger, fuzzy nucleosome.

```
mergeCalls(
  calls,
  min.overlap = 50,
  discard.low = 0.2,
  mc.cores = 1,
  verbose = TRUE
)

## S4 method for signature 'GRanges'
mergeCalls(calls, min.overlap = 50, discard.low = 0.2, verbose = TRUE)
```

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Arguments

calls	GenomicRanges::GRanges with scored and ranged nucleosome calls from peakScoring or peakDetection(, score=TRUE).
min.overlap	Minimum overlap between two reads for merge them
discard.low	Discard low covered calls (i.e. calls with score_h < discard.low will be discarded)
mc.cores	Number of cores available to parallel data processing.
verbose	Show progress info?

Details

This functions looks for overlapped calls and join those with more than min.overlap bases overlapped. More than two reads can be joined in one single call if all of them are overlapped at least that distance with almost another read in the range.

Joining is performed in chain, so if nucleosome call A is close to B and B is close to C, the final call will comprise the range A-B-C. The resulting scores (mixed, width, height) of the final joined call will be the average value of the individual scores.

The parameter discard. low allows to ignore the small peaks that could be merged with larger ones, originating large calls. In the case that all of the overlapped reads in a given position have score_h less than discard.low, all of them will be selected instead of deleting that call.

Value

GenomicRanges::GRanges with merged calls and the additional data column nmerge, with the count of how many original ranges are merged in the resulting range.

Author(s)

Oscar Flores <oflores@mmb.pcb.ub.es>

See Also

```
peakScoring()
```

```
# Generate a synthetic coverage map (assuming reads of 40bp and fragments
# of 130)
map <- syntheticNucMap(
     wp.num=20, fuz.num=20, nuc.len=40, lin.len=130, rnd.seed=1
)
cover <- filterFFT(coverage.rpm(map$syn.reads))
# Find peaks over FFT filtered coverage
calls <- peakDetection(filterFFT(
     cover, pcKeepComp=0.02), width=130, score=TRUE
)</pre>
```

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```
# Merge overlapped calls
merged_calls <- mergeCalls(calls)
plotPeaks(merged_calls, cover)</pre>
```

nucleosome_htseq

Example reads from high-troughtput sequencing nucleosome positioning experiment

Description

Few reads from paired-ended MNase-seq experiment in S.cerevisiae where mononucleosomes were sequenced

Format

GRanges with the range of the reads and a data column with the strand information.

Details

This data is obtained from MNase digested nucleosomal DNA and sequenced with Illumina platform. Paired-ended reads where mapped to SacCer1 genome using Bowtie, and imported to R using the package ShortRead and paired ends where merged into a single range.

Reads were sorted by chromosome and starting position and only a few reads from the starting positions of chromosome 1 are presented.

Source

Publication pending

nucleosome_tiling

Example intensities from Tiling Microarray nucleosome positioning experiment

Description

Some bases from S.cerevisiae tiling microarray where mononucleosomes were sequenced and hybridizated with histone-free naked DNA. The intensity is the normalized ratio between the intensities from nucleosomic and naked DNA.

Format

numeric vector with the intensities.

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Details

Due to the difficulty of providing a raw file, this file has been preprocessed. See details.

The raw .CEL files from Affymetrix S.Cerevisiae Tilling 1.0R Array (3 nucleosomal + 3 naked DNA) has been merged using package Starr and the resulting ExpressionSet object has been passed to processTilingArray function from this package as follows:

```
processTilingArray(data, exprName, chrPAttern="Sc:Oct_2003;chr1", closeGaps=50)
```

The first 8000bp of the chr1 have been saved as this example dataset.

Source

Publication pending

pcKeepCompDetect

Auto detection of a fitted pcKeepComp param for filterFFT function

Description

This function tries to obtain the minimum number of components needed in a FFT filter to achieve or get as close as possible to a given correlation value. Usually you don't need to call directly this function, is used in filterFFT by default.

Usage

```
pcKeepCompDetect(
   data,
   pc.min = 0.01,
   pc.max = 0.1,
   max.iter = 20,
   verbose = FALSE,
   cor.target = 0.98,
   cor.tol = 0.001,
   smpl.num = 25,
   smpl.min.size = 2^10,
   smpl.max.size = 2^14
)
```

Arguments

verbose

data

Numeric vector to be filtered

pc.min, pc.max

Range of allowed values for pcKeepComp (minimum and maximum), in the range 0:1.

max.iter

Maximum number of iterations

Extra information (debug)

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cor.target	Target correlation between the filtered and the original profiles. A value around 0.99 is recommeded for Next Generation Sequencing data and around 0.7 for Tiling Arrays.		
cor.tol	Tolerance allowed between the obtained correlation an the target one.		
smpl.num	If data is a large vector, some samples from the vector will be used instead the whole dataset. This parameters tells the number of samples to pick.		
<pre>smpl.min.size, smpl.max.size</pre>			
	Minimum and maximum size of the samples. This is used for selection and subselection of ranges with meaningful values (i,e, different from 0 and NA). Power of 2 values are recommended, despite non-mandatory.		
	Parameters to be pass to autoPcKeepComp		

Details

This function predicts a suitable pcKeepComp value for filterFFT function. This is the recommended amount of components (in percentage) to keep in the filterFFT function to obtain a correlation of (or near of) cor.target.

The search starts from two given values pc.min, pc.max and uses linial interpolation to quickly reach a value that gives a corelation between the filtered and the original near cor.target within the specified tolerance cor.tol.

To allow a quick detection without an exhaustive search, this function uses a subset of the data by randomly sampling those regions with meaningful coverage values (i,e, different from 0 or NA) larger than smpl.min.size. If it's not possible to obtain smpl.max.size from this region (this could be due to flanking 0's, for example) at least smpl.min.size will be used to check correlation. Mean correlation between all sampled regions is used to test the performance of the pcKeepComp parameter.

If the number of meaningful bases in data is less than smpl.min.size * (smpl.num/2) all the data vector will be used instead of using sampling.

Value

Fitted pcKeepComp value

Author(s)

Oscar Flores <oflores@mmb.pcb.ub.es>, David Rosell <david.rosell@irbbarcelona.org>

```
# Load dataset
data(nucleosome_htseq)
data <- as.vector(coverage.rpm(nucleosome_htseq)[[1]])
# Get recommended pcKeepComp value
pckeepcomp <- pcKeepCompDetect(data, cor.target=0.99)
print(pckeepcomp)
# Call filterFFT</pre>
```

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```
f1 <- filterFFT(data, pcKeepComp=pckeepcomp)

# Also this can be called directly
f2 <- filterFFT(data, pcKeepComp="auto", cor.target=0.99)

# Plot
library(ggplot2)
i <- 1:2000
plot_data <- rbind(
    data.frame(x=i, y=data[i], coverage="original"),
    data.frame(x=i, y=f1[i], coverage="two calls"),
    data.frame(x=i, y=f2[i], coverage="one call")
)
qplot(x=x, y=y, color=coverage, data=plot_data, geom="line",
    xlab="position", ylab="coverage")</pre>
```

peakDetection

Detect peaks (local maximum) from values series

Description

This function allows a efficient recognition of the local maximums (peaks) in a given numeric vector.

```
peakDetection(
  data,
  threshold = 0.25,
  chromosome = NULL,
 width = 1,
  score = TRUE,
 min.cov = 2,
 mc.cores = 1
)
## S4 method for signature 'list'
peakDetection(
  data,
  threshold = "25%",
 width = 1,
  score = TRUE,
 min.cov = 2,
 mc.cores = 1
)
## S4 method for signature 'numeric'
```

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```
peakDetection(
  data,
  threshold = "25%",
  chromosome = NULL,
  width = 1,
  score = TRUE,
  min.cov = 2,
  mc.cores = 1
)
```

Arguments

data	Input numeric values, or a list of them
threshold	Threshold value from which the peaks will be selected. Can be given as a percentage string (i.e., "25\\%" will use the value in the 1st quantile of data) or as an absolute coverage numeric value (i.e., 20 will not look for peaks in regions without less than 20 reads (or reads per milion)).
chromosome	Optionally specify the name of the chromosome for input data that doesn't specify it.
width	If a positive integer > 1 is given, the peaks are returned as a range of the given width centered in the local maximum. Useful for nucleosome calling from a coverage peak in the dyad.
score	If TRUE, the results will be scored using peakScoring() function.
min.cov	Minimum coverage that a peak needs in order to be considered as a nucleosome call.
mc.cores	The number of cores to use, i.e. at most how many child processes will be run simultaneously. Parallelization requires at least two cores.

Details

It's recommended to smooth the input with filterFFT prior the detection.

Value

The type of the return depends on the input parameters:

- numeric (or a list of them) if width==1 & score==FALSE containing the position of the peaks.
- data.frame (or list of them) if width==1 & score==TRUE containing a 'peak' column with the position of the peak plus a 'score' column with its score.
- IRanges (or IRangesList) if width>1 & score==FALSE containing the ranges of the peaks.
- GRanges if width>1 & score==TRUE containing the ranges of the peaks and the assigned score.

Note

If width > 1, those ranges outside the range 1:length(data) will be skipped.

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Author(s)

Oscar Flores <oflores@mmb.pcb.ub.es>

See Also

```
filterFFT(), peakScoring()
```

Examples

```
# Generate a random peaks profile
reads <- syntheticNucMap(nuc.len=40, lin.len=130)$syn.reads
cover <- coverage.rpm(reads)

# Filter them
cover_fft <- filterFFT(cover)

# Detect and plot peaks (up a bit the threshold for accounting synthetic
# data)
peaks <- peakDetection(cover_fft, threshold="40%", score=TRUE)
plotPeaks(peaks, cover_fft, threshold="40%", start=10000, end=15000)

# Now use ranges version, which accounts for fuzziness when scoring
peaks <- peakDetection(cover_fft, threshold="40%", score=TRUE, width=147)
plotPeaks(peaks, cover_fft, threshold="40%", start=10000, end=15000)</pre>
```

peakScoring

Peak scoring function

Description

Scores peaks detected with function peakDetection according the height and the sharpness (width) of the peak. This function can be called automatically from peakDetection if score=TRUE.

```
peakScoring(peaks, data, threshold = 0.25, ...)

## S4 method for signature 'list'
peakScoring(peaks, data, threshold = "25%", mc.cores = 1)

## S4 method for signature 'IRangesList'
peakScoring(
   peaks,
   data,
   threshold = "25%",
   weight.width = 1,
   weight.height = 1,
```

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```
dyad.length = 38,
    mc.cores = 1
)

## S4 method for signature 'numeric'
peakScoring(peaks, data, chromosome = NULL, threshold = "25%")

## S4 method for signature 'IRanges'
peakScoring(
    peaks,
    data,
    chromosome = NULL,
    threshold = "25%",
    weight.width = 1,
    weight.height = 1,
    dyad.length = 38
)
```

Arguments

peaks The identified peaks resulting from peakDetection. Could be a numeric vector

with the position of the peaks, or a IRanges object with the extended range of the peak. For both types, list support is implemented as a numeric list or a

IRangesList

data Data of nucleosome coverage or intensites.

threshold The non-default threshold previously used in peakDetection function, if ap-

plicable. Can be given as a percentage string (i.e., "25\\%" will use the value in the 1st quantile of data) or as an absolute coverage numeric value (i.e., 20 will not look for peaks in regions without less than 20 reads (or reads per million)).

.. Further arguments to be passed to or from other methods.

mc.cores If input is a list or IRangeList, and multiple cores support is available, the

maximum number of cores for parallel processing.

weight.height,weight.width

If the score is a range, the height and the widht score (coverage and fuzzynes)

can be defined with different weights with these parameters. See details.

dyad.length How many bases account in the nucleosome dyad for sharpness description. If

working with NGS data, works best with the reads width value for single-ended

data or the trim value given to the processReads function.

chromosome Optionally specify the name of the chromosome for input data that doesn't spec-

ify it.

Details

This function scores each previously identified peak according its height and sharpness.

The height score (score_h) tells how large is a peak, higher means more coverage or intensity, so better positioned nucleosome. This score is obtained by checking the observed peak value in a Normal distribution with the mean and sd of data. This value is between 0 and 1.

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The width score (score_w) is a mesure of how sharp is a peak. With a NGS coverage in mind, a perfect phased (well-positioned) nucleosome is this that starts and ends exactly in the same place many times. The shape of this ideal peak will be a rectangular shape of the lenght of the read. A wider top of a peak could indicate fuzzyness. The parameter dyad.length tells how long should be the "flat" region of an ideal peak. The optimum value for this parameter is the lenght of the read in single-ended data or the trim value of the function processReads. For Tiling Arrays, the default value should be fine.

This score is obtained calculating the ratio between the mean of the nucleosome scope (the one provided by range in the elements of peaks) and the dyad.length central bases. This value is normalized between 0 and 1.

For punctual, single points peaks (provided by numeric vector or list as peaks attribute) the score returned is the height score.

For range peaks the weighted sum of the height and width scores is used. This is: ((score_h * weight.height) / sum.wei) + ((score_w * weight.width) / sum.wei). Note that you can query for only one score by setting its weight to 1 and the other to 0.

Value

In the case of numeric input, the value returned is a data.frame containing a 'peak' and a 'score' column. If the input is a list, the result will be a list of data.frame.

If input is a IRanges or IRangesList, the result will be a data.frame or GenomicRanges::GRanges object with one or multiple spaces respectively and a 3 data column with the mixed, width and heigh score.

Author(s)

Oscar Flores <oflores@mmb.cpb.ub.es>

See Also

```
peakDetection(), processReads(),
```

```
# Generate a synthetic map

# Trimmed length nucleosome map
map <- syntheticNucMap(nuc.len=40, lin.len=130)

# Get the information of dyads and the coverage
peaks <- c(map$wp.starts, map$fz.starts)
cover <- filterFFT(coverage.rpm(map$syn.reads))

# Calculate the scores
scores <- peakScoring(peaks, cover)
plotPeaks(scores$peak, cover, scores=scores$score, start=5000, end=10000)</pre>
```

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plotPeaks

 $Nucleosome\ calling\ plot\ function$

Description

Helper function for a quick and convenient overview of nucleosome calling data.

```
plotPeaks(peaks, data, ...)
## S4 method for signature 'numeric'
plotPeaks(
 peaks,
  data,
  threshold = 0,
  scores = NULL,
  start = 1,
  end = length(data),
  xlab = "position",
  ylab = "coverage",
  type = 1,
  col.points = "red",
  thr.lty = 1,
  thr.lwd = 1,
  thr.col = "darkred",
  scor.col = col.points,
  scor.cex = 2.5,
  scor.digits = 2,
  scor.nudge = 2000
)
## S4 method for signature 'data.frame'
plotPeaks(peaks, data, ...)
## S4 method for signature 'GRanges'
plotPeaks(peaks, data, ...)
## S4 method for signature 'IRanges'
plotPeaks(
  peaks,
  data,
  threshold = 0,
  scores = NULL,
  start = 1,
  end = length(data),
  dyn.pos = TRUE,
```

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```
xlab = "position",
 ylab = "coverage",
  type = 1,
  col.points = "red",
  thr.lty = 1,
  thr.lwd = 1,
  thr.col = "darkred",
  rect.thick = 2,
  rect.lwd = 0.5,
  rect.border = "black",
  scor.col = col.points,
  scor.cex = 2.5,
  scor.digits = 2,
  indiv.scores = FALSE,
  scor.nudge = 2000
)
```

Arguments

indiv.scores

score.

peaks numeric, data. frame, IRanges or GRanges object containing the detected peaks information. See help of peakDetection() or peakScoring() for more details. Coverage or Tiling Array intensities data Arguments to be passed to other methods. threshold Threshold applied in peakDetection scores If peaks is a data. frame or a GRanges it's obtained from 'score' column, otherwise, scores can be given here as a numeric vector. start, end Start and end points defining a subset in the range of data. This is a convenient way to plot only a small region of data, without dealing with subsetting of range or score objects. xlab, ylab, type, col.points Default values with general properties of the plot thr.lty, thr.lwd, thr.col Default values with general properties for threshold representation scor.col, scor.nudge, scor.cex, scor.digits Default values for ggplot2::geom_text() representation for score numbers, if available. dyn.pos If peaks are ranges, should they be positioned dynamicaly on top of the peaks or staticaly at threshold baseline. Spacing of overlapping ranges is automatically applied if FALSE. rect.thick, rect.lwd, rect.border Default values for ggplot2::geom_rect() representation of ranges. rect.thick indicates the thickness of the rectangles.

Show or hide individual scores for width and height in brakets besides the mixed

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Details

This function is intended to plot data previously processed with nucleR pipeline. It shows a coverage/intensity profile toghether with the identified peaks. If available, score of each peak is also shown.

Value

(none)

Author(s)

Ricard Illa < ricard.illa@irbbarcelona.org>

See Also

```
peakDetection(), peakScoring(), ggplot2::ggplot(),
```

Examples

```
# Generate a random peaks profile
reads <- syntheticNucMap(nuc.len=40, lin.len=130)$syn.reads
cover <- coverage.rpm(reads)

# Filter them
cover_fft <- filterFFT(cover)

# Detect peaks
peaks <- peakDetection(cover_fft, threshold="40%", score=TRUE, width=140)

# Plot peaks and coverage profile (show only a window)
plotPeaks(peaks, cover_fft, threshold="40%", start=1000, end=6000)</pre>
```

processReads

Process reads from High-Troughtput Sequencing experiments

Description

This method allows the processment of NGS nucleosome reads from different sources and a basic manipulation of them. The tasks includes the correction of strand-specific single-end reads and the trimming of reads to a given length.

```
processReads(data, type = "single", fragmentLen, trim, ...)
## S4 method for signature 'AlignedRead'
processReads(data, type = "single", fragmentLen, trim, ...)
```

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```
## S4 method for signature 'CompressedGRangesList'
processReads(data, type = "single", fragmentLen, trim, ...)
## S4 method for signature 'GRanges'
processReads(data, type = "single", fragmentLen, trim, ...)
```

Arguments

data Sequence reads objects, probably imported using other packages as ShortRead.

Allowed object types are ShortRead::AlignedRead and GenomicRanges::GRanges

with a strand attribute.

type Describes the type of reads. Values allowed are single for single-ended reads

and paired for paired-ended.

fragmentLen Expected original length of the sequenced fragments. See details.

trim Length to trim the reads (or extend them if trim > read length)

... Other parameters passed to fragmentLenDetect if no fixed fragmentLen is

given.

Details

This function reads a ShortRead::AlignedRead or a GenomicRanges::GRanges object containing the position, length and strand of the sequence reads.

It allows the processment of both paired and single ended reads. In the case of single end reads this function corrects the strand-specific mapping by shifting plus strand reads and minus strand reads towards a middle position where both strands are overlaped. This is done by accounting the expected fragment length (fragmentLen).

For paired end reads, mononucleosomal reads could extend more than expected length due to mapping issues or experimental conditions. In this case, the fragmentLen variable sets the threshold from which reads longer than it should be ignored.

If no value is supplied for fragmentLen it will be calculated automatically (increasing the computing time) using fragmentLenDetect with default parameters. Performance can be increased by tunning fragmentLenDetect parameters in a separated call and passing its result as fragmentLen parameter.

In some cases, could be useful trim the reads to a shorter length to improve the detection of nucleosome dyads, easing its detection and automatic positioning. The parameter trim allows the selection of how many nucleotides select from each read.

A special case for single-ended data is setting the trim to the same value as fragmentLen, so the reads will be extended strand-wise towards the 3' direction, creating an artificial map comparable with paired-ended data. The same but opposite can be performed with paired-end data, setting a trim value equal to the read length from paired ended, so paired-ended data will look like single-ended.

Value

GenomicRanges::GRanges containing the aligned/trimmed individual reads.

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Note

IMPORTANT: this information is only used to correct possible strand-specific mapping, this package doesn't link the two ends of paired reads.

Author(s)

```
Oscar Flores <oflores@mmb.pcb.ub.es>
```

See Also

ShortRead::AlignedRead, GenomicRanges::GRanges, fragmentLenDetect()

Examples

```
# Load data
data(nucleosome_htseq)
# Process nucleosome reads, select only those shorter than 200bp
pr1 <- processReads(nucleosome_htseq, fragmentLen=200)</pre>
# Now process them, but picking only the 40 bases surrounding the dyad
pr2 <- processReads(nucleosome_htseq, fragmentLen=200, trim=40)</pre>
# Compare the results:
library(ggplot2)
cov1 <- as.vector(coverage.rpm(pr1)[["chr1"]])</pre>
cov2 <- as.vector(coverage.rpm(pr2)[["chr1"]])</pre>
plot_data <- rbind(</pre>
    data.frame(x=seq_along(cov1), y=cov1, coverage="original"),
    data.frame(x=seq_along(cov2), y=cov2, coverage="trimmed")
)
qplot(x=x, y=y, geom="line", data=plot_data, xlab="position",
  ylab="coverage") + facet_grid(coverage~.)
```

processTilingArray

Obtain and clean nucleosome positioning data from tiling array

Description

Process and transform the microarray data coming from tiling array nucleosome positioning experiments.

```
processTilingArray(
  data,
  exprName,
  chrPattern,
```

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```
inferLen = 50,
mc.cores = 1,
quiet = FALSE
)
```

Arguments

data ExpressionSet object wich contains the data of the tiling array.

exprName Name of the sample in ExpressionSet which contains the ratio between nucle-

osomal and genomic dna (if using Starr, the description argument supplied to getRatio function). If this name is not provided, it is assumed data has only

one column.

chrPattern Only chromosomes that contain chrPattern string will be selected from Ex-

pressionSet. Sometimes tilling arrays contain control quality information that is imported as a chromosome. This allows filtering it. If no value is supplied, all

chromosomes will be used.

inferLen Maximum length (in basepairs) for allowing data gaps inference. See details

for further information.

mc.cores Number of cores available to parallel data processing.

quiet Avoid printing on going information (TRUE | FALSE)

Details

The processing of tiling arrays could be complicated as many types exists on the market. This function deals ok with Affymetrix Tiling Arrays in yeast, but hasn't been tested on other species or platforms.

The main aim is convert the output of preprocessing steps (supplied by third-parties packages) to a clean genome wide nucleosome occupancy profile.

Tiling arrays doesn't use to provide a one-basepair resolution data, so one gets one value per probe in the array, covering X basepairs and shifted (tiled) Y basepairs respect the surrounding ones. So, one gets a piece of information every Y basepairs.

This function tries to convert this noisy, low resolution data, to a one-basepair signal, which allows a fast recognition of nucleosomes without using large and artificious statistical machinery as Hidden Markov Models using posterion noise cleaning process.

As example, imagine your array has probes of 20mers and a tiling between probes of 10bp. Starting at position 1 (covering coordinates from 1 to 20), the next probe will be in position 10 (covering the coordinates 10 to 29). This can be represented as two hybridization intensity values on coordinates 1 and 10. This function will try to infer (using a lineal distribution) the values from 2 to 9 using the existing values of probes in coordinate 1 and coordinate 10.

The tiling space between adjacent array probes could be not constant, or could be also there are regions not covered in the used microarray. With the function argument inferLen you can specify wich amout of space (in basepairs) you allow to infer the non-present values.

If at some point the range not covered (gap) between two adjacent probes of the array is greater than inferLen value, then the coordinates between these probes will be setted to NA.

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Value

RleList with the observed/inferred values for each coordinate.

Warning

This function could not cover all kind of arrays in the market. This package assumes the data is processed and normalized prior this processing, using standard microarray packages existing for R, like Starr.

Note

This function should be suitable for all data objects of kind ExpressionSet coding the annotations "chr" for chromosome and "pos" for position (accessible by pData(data@featureData)) and a expression value (accessible by exprs(data)).

Author(s)

Oscar Flores <oflores@mmb.pcb.ub.es>

See Also

```
Biobase::ExpressionSet, Starr::getRatio()
```

```
## Not run:
   # Dataset cannot be provided for size restrictions
   # This is the code used to get the hybridization ratio with Starr from
   # CEL files
   library("Starr")
   TA_parsed <- readCelFile(</pre>
        BPMap, CELfiles, CELnames, CELtype, featureData=TRUE, log.it=TRUE
   TA_loess <- normalize.Probes(TA_parsed, method="loess")</pre>
   TA_ratio <- getRatio(</pre>
        TA_loess, TA_loess$type=="IP", TA_loess$type=="CONTROL", "myRatio"
   # From here, we use nucleR:
    # Preprocess the array, using the calculated ratio feature we named
    # "myRatio".
    # This will also select only those chromosomes with the pattern
   # "Sc:Oct_2003; chr", removing control data present in that tiling
   # Finally, we allow that loci not covered by a prove being inferred
    # from adjacent ones, as far as they are separated by 50bp or less
   arr <- processTilingArray(</pre>
        TA_ratio, "myRatio", chrPattern="Sc:Oct_2003;chr", inferLen=50
```

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```
# From here we can proceed with the analysis:
arr_fft <- filterFFT(arr)
arr_pea <- peakDetection(arr_fft)
plotPeaks(arr_pea, arr_fft)
## End(Not run)</pre>
```

readBAM

Import reads from a list of BAM files.

Description

This function allows to load reads from BAM files from both single and paired-end commming from Next Generation Sequencing nucleosome mapping experiments.

Usage

```
readBAM(files, type = "paired")
```

Arguments

files List of input BAM files.

type Describes the type of reads. Values allowed are single for single-ended reads

and paired for pair-ended.

Value

GenomicRanges::GRangesList containing the reads of each input BAM file.

Author(s)

Ricard Illa <ricard.illa@irbbarcelona.org>

```
infile <- system.file(
    "extdata", "cellCycleM_chrII_5000-25000.bam", package="nucleR"
)
reads <- readBAM(infile, type="paired")</pre>
```

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rea	ᄱᇝᇧ	+ -	_

Import reads from a vector of Bowtie files

Description

This function allows to load reads from Bowtie files from both single and paired-end commming from Next Generation Sequencing nucleosome mapping experiments.

Usage

```
readBowtie(files, type = "paired")
```

Arguments

files List of input Bowtie files.

type Describes the type of reads. Values allowed are single for single-ended reads

and paired for pair-ended.

Value

GenomicRanges::GRangesList containing the reads of each input BAM file.

Author(s)

Ricard Illa <ricard.illa@irbbarcelona.org>

syntheticNucMap

Generates a synthetic nucleosome map

Description

This function generates a synthetic nucleosome map using the parameters given by the user and returns the coverage (like NGS experiments) or a pseudo-hybdridization ratio (like Tiling Arrays) toghether with the perfect information about the well positioned and fuzzy nucleosome positions.

```
syntheticNucMap(
   wp.num = 100,
   wp.del = 10,
   wp.var = 20,
   fuz.num = 50,
   fuz.var = 50,
   max.cover = 20,
   nuc.len = 147,
```

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```
lin.len = 20,
rnd.seed = NULL,
as.ratio = FALSE,
show.plot = FALSE)
```

Arguments

wp.num	Number of well-positioned (non overlapped) nucleosomes. They are placed uniformly every nuc.len+lin.len basepairs.
wp.del	Number of well-positioned nucleosomes (the ones generated by wp.num) to remove. This will create an uncovered region.
wp.var	Maximum variance in basepairs of the well-positioned nucleosomes. This will create some variation in the position of the reads describing the same nucleosome.
fuz.num	Number of fuzzy nucleosomes. They are distributed randomly over all the region. They could be overlapped with other well-positioned or fuzzy nucleosomes.
fuz.var	Maximum variance of the fuzzy nucleosomes. This allow to set different variance in well-positioned and fuzzy nucleosome reads (using wp.var and fuz.var).
max.cover	Maximum coverage of a nucleosome, i.e., how many times a nucleosome read can be repeated. The final coverage probably will be higher by the addition of overlapping nucleosomes.
nuc.len	Nucleosome length. It's not recomended change the default 147bp value.
lin.len	Linker DNA length. Usually around 20 bp.
rnd.seed	As this model uses random distributions for the placement, by setting the rnd.seed to a known value allows to reproduce maps in different executions or computers. If you don't need this, just left it in default value.
as.ratio	If as.ratio=TRUE this will create and return a synthetic naked DNA control map and the ratio between it and the nucleosome coverage. This can be used to simulate hybridization ratio data, like the one in Tiling Arrays.
show.plot	If TRUE, will plot the output coverage map, with the nucleosome calls and optionally the calculated ratio.

Value

A list with the following elements:

- wp.starts Start points of well-positioned nucleosomes
- wp.nreads Number of repetitions of each well positioned read
- wp.reads Well positioned nucleosome reads (IRanges format), containing the repetitions
- fuz.starts Start points of the fuzzy nucleosomes
- fuz.nreads Number of repetitions of each fuzzy nucleosome read
- fuz.reads Fuzzy nucleosome reads (IRanges format), containing all the repetitions

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• syn.reads All synthetic nucleosome reads togheter (IRanges format)

The following elements will be only returned if as.ratio=TRUE:

- ctr.reads The pseudo-naked DNA (control) reads (IRanges format)
- syn.ratio The calculated ratio nucleosomal/control (Rle format)

Author(s)

Oscar Flores <oflores@mmb.pcb.ub.es>

```
# Generate a synthetic map with 50wp + 20fuzzy nucleosomes using fixed
# random seed=1
res <- syntheticNucMap(wp.num=50, fuz.num=20, show.plot=TRUE, rnd.seed=1)</pre>
# Increase the fuzzyness
res <- syntheticNucMap(</pre>
    wp.num=50, fuz.num=20, wp.var=70, fuz.var=150, show.plot=TRUE,
)
# Calculate also a random map and get the ratio between random and
# nucleosomal
res <- syntheticNucMap(</pre>
    wp.num=50, wp.del=0, fuz.num=20, as.ratio=TRUE, show.plot=TRUE,
)
print(res)
# Different reads can be accessed separately from results
# Let's use this to plot the nucleosomal + the random map
library(ggplot2)
as <- as.vector(coverage.rpm(res$syn.reads))</pre>
bs <- as.vector(coverage.rpm(res$ctr.reads))</pre>
cs <- as.vector(res$syn.ratio)</pre>
plot_data <- rbind(</pre>
    data.frame(x=seq_along(as), y=as, lab="nucleosomal"),
    data.frame(x=seq_along(bs), y=bs, lab="random"),
    data.frame(x=seq_along(cs), y=cs, lab="ratio")
qplot(x=x, y=y, data=plot_data, geom="area", xlab="position", ylab="") +
    facet_grid(lab~., scales="free_y")
```

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