

# Package ‘NADfinder’

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**Type** Package

**Title** Call wide peaks for sequencing data

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**Encoding** UTF-8

**Author** Jianhong Ou, Haibo Liu, Jun Yu, Hervé Pagès, Paul Kaufman, Lihua Julie Zhu

**Maintainer** Jianhong Ou <jianhong.ou@duke.edu>,  
Lihua Julie Zhu <julie.zhu@umassmed.edu>

**Description** Nucleolus is an important structure inside the nucleus in eukaryotic cells. It is the site for transcribing rDNA into rRNA and for assembling ribosomes, aka ribosome biogenesis. In addition, nucleoli are dynamic hubs through which numerous proteins shuttle and contact specific non-rDNA genomic loci. Deep sequencing analyses of DNA associated with isolated nucleoli (NAD-seq) have shown that specific loci, termed nucleolus-associated domains (NADs) form frequent three-dimensional associations with nucleoli. NAD-seq has been used to study the biological functions of NAD and the dynamics of NAD distribution during embryonic stem cell (ESC) differentiation.

Here, we developed a

Bioconductor package NADfinder for bioinformatic analysis of the NAD-seq data, including baseline correction, smoothing, normalization, peak calling, and annotation.

**License** GPL ( $\geq 2$ )

**Depends** R ( $\geq 3.4$ ), BiocGenerics, IRanges, GenomicRanges, S4Vectors, SummarizedExperiment

**Imports** graphics, methods, baseline, signal, GenomicAlignments, GenomeInfoDb, rtracklayer, limma, trackViewer, stats, utils, Rsamtools, metap, EmpiricalBrownsMethod, ATACseqQC, corrplot, csaw

**Suggests** RUnit, BiocStyle, knitr, BSgenome.Mmusculus.UCSC.mm10, testthat, BiocManager

**biocViews** Sequencing, DNaseq, GeneRegulation, PeakDetection

**LazyData** TRUE

**VignetteBuilder** knitr

**RoxygenNote** 7.0.2

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## R topics documented:

|                                    |           |
|------------------------------------|-----------|
| NADfinder-package . . . . .        | 2         |
| backgroundCorrection . . . . .     | 3         |
| butterFilter . . . . .             | 3         |
| callPeaks . . . . .                | 4         |
| computeLibSizeChrom . . . . .      | 6         |
| cumulativePercentage . . . . .     | 6         |
| exportSignals . . . . .            | 7         |
| getCorrelations . . . . .          | 8         |
| groupZscores . . . . .             | 9         |
| IntersectionNotStrict . . . . .    | 10        |
| log2se . . . . .                   | 10        |
| peakdet . . . . .                  | 11        |
| plotSig . . . . .                  | 12        |
| single.count . . . . .             | 13        |
| smoothRatiosByChromosome . . . . . | 13        |
| tileCount . . . . .                | 14        |
| tileCount2 . . . . .               | 15        |
| transformData . . . . .            | 17        |
| trimPeaks . . . . .                | 18        |
| triplicate.count . . . . .         | 19        |
| zscoreOverBck . . . . .            | 20        |
| <b>Index</b>                       | <b>21</b> |

---

|                   |  |
|-------------------|--|
| NADfinder-package | <i>Identify nucleolus-associated domains (NADs) from NAD-seq</i> |
|-------------------|--|

---

## Description

Sliding-window based peak calling algorithm using whole genome sequences as control

---

backgroundCorrection *Correct ratios for background*

---

**Description**

Correct ratios of read counts per sliding window for background.

**Usage**

```
backgroundCorrection(ratios, degree = 3, ...)
```

**Arguments**

|        |   |
|--------|---|
| ratios | A vector of numeric. It is log2-transformed ratios, CPMRatios or OddRatios of counts for each window. |
| degree | Degree of polynomial. default 3.  |
| ...    | parameters could be passed to <a href="#">baseline.modpolyfit</a> .                                   |

**Details**

This function implements the background correction methods of algorithm for polynomial fitting. See details via [baseline.modpolyfit](#). This function expects the tendency of decreasing of the ratios from 5' end to 3' end.

**Value**

A vector of numeric. It is the background corrected log2-transformed ratios, CPMRatios or OddRatios.

**Examples**

```
x <- runif(200)
background <- rep(c(20:1)/100, each=10)
backgroundCorrection(x)
```

---

butterFilter *Low pass filter on ratios by butterworth filter*

---

**Description**

The Butterworth filter is a type of signal processing filter designed to have as flat a frequency response as possible in the passband.

**Usage**

```
butterFilter(ratios, N = ceiling(length(ratios)/200))
```

**Arguments**

|        |  |
|--------|--|
| ratios | A vector of numeric. It is log2-transformed ratios, CPMRatios or OddRatios in each window.   |
| N      | numeric(1) or integer(1). Critical frequencies of the low pass filter will be set as 1/N. 1/N is a cutoff at 1/N-th of the Nyquist frequency. By default, it is suppose there are about 200 peaks in the inputs. |

**Value**

A vector of numeric with same length of input ratios. The vector indicates smoothed ratios.

**Examples**

```
ratios <- runif(20000)
butterFilter(ratios)
```

---

|           |   |
|-----------|---|
| callPeaks | <i>Call peaks using transformed, background corrected, and smoothed ratios with biological replicates</i> |
|-----------|---|

---

**Description**

Use limma to calculate p-values for NADs

**Usage**

```
callPeaks(
  se,
  backgroundCorrectedAssay = "bcRatio",
  normalization.method = "quantile",
  N = 100,
  cutoffAdjPvalue = 1e-04,
  countFilter = 1000,
  combineP.method = "minimump",
  smooth.method = "loess",
  lfc = log2(1.5),
  ...
)
```

**Arguments**

|                          |   |
|--------------------------|---|
| se                       | An object of <a href="#">RangedSummarizedExperiment</a> with assays of raw counts, transformed ratios, background corrected ratios, smoothed ratios and z-scores. It should be an element of output of <a href="#">smoothRatiosByChromosome</a> |
| backgroundCorrectedAssay | character(1). Assays names for background corrected log2-transformed ratios, CPMRatios or OddRatios.  |
| normalization.method     | character(1) specifying the normalization method to be used. Choices are "none", "scale", "quantile" or "cyclicloess". See <a href="#">normalizeBetweenArrays</a> for details.  |

|                 |   |
|-----------------|---|
| N               | numeric(1) or integer(1). The number of neighboring windows used for loess smoothing or the inverse of the critical frequencies of the low pass filter for butterworth filter. 1/N is a cutoff at 1/N-th of the Nyquist frequency. Default 100. |
| cutoffAdjPvalue | numeric(1). Cutoff adjust p-value.  |
| countFilter     | numeric(1). Cutoff value for mean of raw reads count in each window.  |
| combineP.method | A method used to combine P-values. Default minimump   |
| smooth.method   | A method used to smooth the ratios. Choices are "loess", "none" and "butterworthfilter".  |
| lfc             | the minimum log2-fold-change that is considered scientifically meaningful   |
| ...             | Parameter not used.   |

## Details

By default, use the mean smoothed ratio for each peak region to calculate p-values

## Value

An object of GRanges of peak list with metadata "AveSig", "P.Value", and "adj.P.Val", where "AveSig" means average signal such as average log2OddsRatio, log2CPMRatio or log2Ratio.

## Author(s)

Jianhong Ou, Haibo Liu and Julie Zhu

## Examples

```
data(triplicate.count)
se <- triplicate.count
se <- log2se(se, transformation = "log2CPMRatio",
            nucleolusCols = c("N18.subsampled.srt-2.bam",
                              "N18.subsampled.srt-3.bam",
                              "N18.subsampled.srt.bam"),
            genomeCols = c("G18.subsampled.srt-2.bam",
                           "G18.subsampled.srt-3.bam",
                           "G18.subsampled.srt.bam"))
se<- smoothRatiosByChromosome(se, chr="chr18")
#add some variability to the data since the triplicate.count data was created using one sample only
assays(se[[1]])$bcRatio[,2] <- assays(se[[1]])$bcRatio[,2] + 0.3
assays(se[[1]])$bcRatio[,3] <- assays(se[[1]])$bcRatio[,3] - 0.3
peaks <- callPeaks(se[[1]],
                  cutoffAdjPvalue=0.001, countFilter=10)
```

---

`computeLibSizeChrom` *Perform overlap queries between reads and genome by sliding windows Count reads over sliding windows.*

---

### Description

Perform overlap queries between reads and genome by sliding windows Count reads over sliding windows.

### Usage

```
computeLibSizeChrom(aln_list)
```

### Arguments

`aln_list` a list.

### Value

A [RangedSummarizedExperiment](#) object with chromosome-level depth The assays slot holds the counts, `rowRanges` holds the annotation from the sliding widows of genome. `metadata` contains `lib.size.chrom` for holding chromosome-level sequence depth

### Author(s)

Jun Yu, Hervé Pagès and Julie Zhu

---

`cumulativePercentage` *Plot the cumulative percentage of tag allocation*

---

### Description

Plot the difference between the cumulative percentage of tag allocation in paired samples.

### Usage

```
cumulativePercentage(
  se,
  binWidth = 1e+05,
  backgroundCorrectedAssay = "bcRatio",
  ...
)
```

### Arguments

`se` An object of [RangedSummarizedExperiment](#) with assays of raw counts, transformed ratios, background correct ratios, smoothed ratios and z-scores. It should be an element of the output of [smoothRatiosByChromosome](#).

`binWidth` `numeric(1)` or `integer(1)`. The width of each bin.

`backgroundCorrectedAssay` `character(1)`. Assays names for background correction ratios.

`...` Parameter not used.

**Value**

A list of data.frame with the cumulative percentages.

**References**

Normalization, bias correction, and peak calling for ChIP-seq Aaron Diaz, Kiyoub Park, Daniel A. Lim, Jun S. Song Stat Appl Genet Mol Biol. Author manuscript; available in PMC 2012 May 3. Published in final edited form as: Stat Appl Genet Mol Biol. 2012 Mar 31; 11(3): 10.1515/1544-6115.1750 /j/sagmb.2012.11.issue-3/1544-6115.1750/1544-6115.1750.xml. Published online 2012 Mar 31. doi: 10.1515/1544-6115.1750 PMID: PMC3342857

**Examples**

```
library(SummarizedExperiment)
data(triplicate.count)
se <- triplicate.count
se <- log2se(se, transformation = "log2CPMRatio",
            nucleolusCols = c("N18.subsampled.srt-2.bam",
                              "N18.subsampled.srt-3.bam",
                              "N18.subsampled.srt.bam"),
            genomeCols = c("G18.subsampled.srt-2.bam",
                           "G18.subsampled.srt-3.bam",
                           "G18.subsampled.srt.bam"))
se <- smoothRatiosByChromosome(se, chr="chr18")
cumulativePercentage(se[["chr18"]])
```

---

exportSignals

*Output signals for visualization*

---

**Description**

Output signals to bedgraph, bed, wig, etc, for track viewer

**Usage**

```
exportSignals(dat, assayName, colName, con, format = "bedGraph", ...)
```

**Arguments**

|           |  |
|-----------|--|
| dat       | An object of <a href="#">GRanges</a> , or <a href="#">RangedSummarizedExperiment</a> with assays of raw counts, ratios, background correct ratios, smoothed ratios and z-scores. It should be an element of output of <a href="#">smoothRatiosByChromosome</a> |
| assayName | character(1). Assay name for <a href="#">RangedSummarizedExperiment</a>  |
| colName   | character(1). Column name of metadata of dat or assay of dat for coverage weight, see <a href="#">coverage</a> , <a href="#">RangedSummarizedExperiment</a> .  |
| con       | The connection to which data is saved. If this is a character vector, it is assumed to be a filename and a corresponding file connection is created and then closed after exporting the object. If missing, a <a href="#">SimpleRleList</a> will be returned.  |
| format    | The format of the output. see <a href="#">export</a> .   |
| ...       | Parameters to be passed to <a href="#">export</a>  |

**Value**

If con is missing, a [SimpleRleList](#) will be returned. Otherwise, nothing is returned.

**Examples**

```
gr <- GRanges("chr1", IRanges(seq_len(100), 201:300), reads=rep(1, 100))
myTrackLine <- new("TrackLine", name="my track",
                  description="description of my track",
                  color=col2rgb("red")[, 1],
                  visibility="full")
exportSignals(gr, colName="reads",
              con="test.bedGraph", trackLine=myTrackLine)
data(triplicate.count)
exportSignals(triplicate.count, "counts",
              "G18.subsampled.srt.bam", "test.bw", format="bigWig")
```

---

|                              |  |
|------------------------------|--|
| <code>getCorrelations</code> | <i>Get correlation coefficients and p-values between biological replicates</i> |
|------------------------------|--|

---

**Description**

Get correlations and p-values between biological replicates based on coverage signal for peak regions. The signals will be filtered by the background cutoff value before calculated correlations. This function also output a correlation plots using the [corrplot](#).

**Usage**

```
getCorrelations(
  se,
  chr = paste0("chr", seq_len(19)),
  ratioAssay = "ratio",
  window = 10000L,
  cutoff = 1,
  method = c("spearman", "pearson", "kendall"),
  file_name = "Correlation plots.pdf",
  ...
)
```

**Arguments**

|                         |  |
|-------------------------|--|
| <code>se</code>         | A <a href="#">RangedSummarizedExperiment</a> object. The output from <a href="#">log2se</a> .        |
| <code>chr</code>        | A vector of character. Filter for seqnames. It should be the chromosome names to be kept.            |
| <code>ratioAssay</code> | character(1). Column name of ratio for correlation calculation.                                      |
| <code>window</code>     | numeric(1) or integer(1). The window size for summary of the ratios.                                 |
| <code>cutoff</code>     | numeric(1). All the coverage signals lower than cutoff value in a given window will be filtered out. |
| <code>method</code>     | character(1) indicating which correlation coefficient is to be computed. See <a href="#">cor</a> .   |
| <code>file_name</code>  | A file name for output correlation plots   |
| <code>...</code>        | Parameters not used.   |



**Value**

A list of matrixes of correlation coefficients and p-values.

**Author(s)**

Jianhong Ou, Haibo Liu

**Examples**

```
data(triplicate.count)
se <- triplicate.count
se <- log2se(se, transformation = "log2CPMRatio",
            nucleolusCols = c("N18.subsampled.srt-2.bam",
                              "N18.subsampled.srt-3.bam",
                              "N18.subsampled.srt.bam"),
            genomeCols = c("G18.subsampled.srt-2.bam",
                           "G18.subsampled.srt-3.bam",
                           "G18.subsampled.srt.bam"))
getCorrelations(se, chr="chr18")
```

---

groupZscores

*Calculate z-scores for each peak*

---

**Description**

Detect peaks and calculate z-scores for each peak

**Usage**

```
groupZscores(zscore)
```

**Arguments**

zscore            A vector of numeric. It is the z-scores of ratios for each window.

**Value**

A data.frame with column names as "zscore", "group", "grp.zscore", and "pvalue".

**Examples**

```
x <- seq_len(500)
a <- 2 * 2*pi/length(x)
y <- 20 * sin(x*a)
noise1 <- 20 * 1/10 * sin(x*a*10)
zscore <- y+noise1
groupZscores(zscore)
```

---

IntersectionNotStrict *Count reads overlapping genomic ranges*

---

### Description

Count reads overlapping a set of genomic features represented as genomic ranges. This function does not work for parallel.

### Usage

```
IntersectionNotStrict(
  features,
  reads,
  ignore.strand = TRUE,
  inter.feature = FALSE
)
```

### Arguments

|               |  |
|---------------|--|
| features      | A object of <a href="#">GRanges</a> representing the feature regions to be counted.  |
| reads         | An object that represents the data to be counted. See <a href="#">summarizeOverlaps</a> . If reads are more than 1 bam files, it should be a vector of character with full path, otherwise current working directory is the default directory. For paired end reads, |
| ignore.strand | logical(1). ignore strand?   |
| inter.feature | not used. This parameter is required by <a href="#">summarizeOverlaps</a> .  |

### Value

return a summarized experiment object with chromosome-level depth information for each input sample as metadata.

---

log2se *calculate the log2 transformed ratios for SummarizedExperiment class*

---

### Description

Calculate the log2 transformed ratios for nucleolus vs genome. pseudo-count will be used to avoid x/0 or log(0).

### Usage

```
log2se(
  se,
  nucleolusCols,
  genomeCols,
  pseudocount = 1L,
  transformation = c("log2OddsRatio", "log2CPMRatio", "log2Ratio"),
  chrom.level.lib = TRUE
)
```

**Arguments**

`se` A [RangedSummarizedExperiment](#) object. The output of `tileCount`.

`nucleolusCols`, `genomeCols` column Names of counts for nucleolus and genome. They should be the column names in the assays of `se`. Ratios will be calculated as  $\log_2(\text{transformed nucleolusCols}/\text{transformed genomeCols})$ .

`pseudocount` default to 1, pseudo-count used to avoid  $x/0$  or  $\log(0)$ .

`transformation` transformation type

`chrom.level.lib` indicating whether calculating CPM or odds using sequence depth of the whole genome or the corresponding chromosome

**Value**

A [RangedSummarizedExperiment](#) object with  $\log_2$  transformed ratios. Assays will be named as `nucleolus`, `genome` and `ratio`.

**Author(s)**

Jianhong Ou and Julie Zhu

**Examples**

```
library(SummarizedExperiment)
se <- SummarizedExperiment(assays=list(counts=DataFrame(A=seq_len(3),
  B=rep(1, 3), C=rep(4, 3), D=rep(2, 3))),
  rowRanges=GRanges(c("chr1", "chr1", "chr2"),
    IRanges(c(1, 10, 20),
      width=9)))
metadata(se)$lib.size.chrom <- data.frame(c(1000, 1000), c(2000, 2000), c(200, 200), c(300, 300))
colnames(metadata(se)$lib.size.chrom) <- c("A", "B", "C", "D")
rownames(metadata(se)$lib.size.chrom) <- c("chr1", "chr2")
log2se(se, nucleolusCols = c("A", "C"), genomeCols = c("B", "D"), transformation = "log2Ratio")
log2se(se, nucleolusCols = c("A", "C"), genomeCols = c("B", "D"), transformation = "log2CPMRatio")
log2se(se, nucleolusCols = c("A", "C"), genomeCols = c("B", "D"),
  transformation = "log2OddsRatio")
```

---

peakdet

*Detect peak positions*

---

**Description**

Detect the peak positions and valley positions leveraging `github::dgromer/peakdet`

**Usage**

```
peakdet(y, delta = 0, silence = TRUE)
```

**Arguments**

|         |  |
|---------|--|
| y       | A numeric vector for searching peaks   |
| delta   | A numeric vector of length 1, defining the minimum absolute changes required for local maximum or minimum detection when slope sign changes. If it is set to 0, the delta will be set to 1/10 of the range of y. |
| silence | logical(1). If false, echo the delta value when delta is set as 0.   |

**Value**

A list with peakpos and valleypos. Both peakpos and valleypos are numeric vectors storing the positions of peaks or valleys.

**Examples**

```
y <- runif(200)
peakdet(y)
y <- sin(seq(0,20))
peakdet(y)
```

---

plotSig

*Plot signals with ideograms*


---

**Description**

Plot signals with ideograms for [GRangesList](#).

**Usage**

```
plotSig(ideo, grList, mcolName, ...)
```

**Arguments**

|          |  |
|----------|--|
| ideo     | Output of <a href="#">loadIdeogram</a> .                             |
| grList   | A <a href="#">GRangesList</a> of data to plot.                       |
| mcolName | Column name of metadata of <a href="#">GRangesList</a> for plotting. |
| ...      | Parameters to pass to <a href="#">ideogramPlot</a>                   |

**Value**

Invisible argument list for [ideogramPlot](#).

**Examples**

```
library(trackViewer)
#ideo <- loadIdeogram("mm10")
ideo <- readRDS(system.file("extdata", "ideo.mm10.rds",
                           package = "NADfinder"))
gr1 <- gr2 <- ideo
mcols(gr1) <- DataFrame(score=runif(length(gr1)))
mcols(gr2) <- DataFrame(score=runif(length(gr2)))
```

```
grList <- GRangesList(gr1, gr2)
plotSig(ideo, grList, mcolName="score", layout=list("chr1"))
```

---

|              |  |
|--------------|--|
| single.count | <i>Counts data for chromosome 18 for an experiment of a single pair of samples</i> |
|--------------|--|

---

### Description

Counts data for chromosome 18 for an experiment of a single pair of samples

---

|                          |  |
|--------------------------|--|
| smoothRatiosByChromosome | <i>Background correction and signal smoothing per chromosome</i> |
|--------------------------|--|

---

### Description

Split the ratios by chromosome and do background correction and signal smoothing.

### Usage

```
smoothRatiosByChromosome(
  se,
  chr = paste0("chr", c(seq_len(21), "X", "Y")),
  ratioAssay = "ratio",
  backgroundCorrectedAssay = "bcRatio",
  smoothedRatioAssay = "smoothedRatio",
  zscoreAssay = "zscore",
  backgroundPercentage = 0.25,
  chrom.level.background = TRUE,
  ...
)
```

### Arguments

|   |   |
|---|---|
| se  | An object of <a href="#">RangedSummarizedExperiment</a> with log2-transformed ratios, CPM-Ratios or OddRatios. Output of <a href="#">log2se</a>   |
| chr   | A character vector, used to filter out seqnames. It should be the chromosome names to be kept.  |
| ratioAssay  | The name of assay in se, which store the values (log2-transformed ratios, CPM-Ratios or OddRatios) to be smoothed.  |
| backgroundCorrectedAssay, smoothedRatioAssay, zscoreAssay | character(1). Assays names for background corrected ratios, smoothed ratios and z-scores based on background corrected ratios.  |
| backgroundPercentage                                      | numeric(1). Percentage of values for background, see <a href="#">zscoreOverBck</a> . The percentage of values lower than this threshold will be treated as background, with 25 percentile as default. |

```
chrom.level.background
      logical(1): TRUE or FALSE, default to TRUE, use chromosome-level back-
      ground to calculate z-score
...      Parameters could be passed to butterFilter.
```

### Value

A [SimpleList](#) of [RangedSummarizedExperiment](#) with smoothed ratios.

### Author(s)

Jianhong Ou, Haibo Liu and Julie Zhu

### Examples

```
data(single.count)
se <- single.count
dat <- log2se(se, nucleolusCols="N18.subsampled.srt.bam", genomeCols="G18.subsampled.srt.bam",
transformation="log2CPMRatio")
dat1 <- smoothRatiosByChromosome(dat, N=100, chr = c("chr18", "chr19"))
dat2 <- smoothRatiosByChromosome(dat, N=100, chr = c("chr18", "chr19"),
      chrom.level.background = FALSE)
```

---

tileCount

*Perform overlap queries between reads and genome by windows*

---

### Description

tileCount extends [summarizeOverlaps](#) by finding coverage for each fixed window in the whole genome

### Usage

```
tileCount(
  reads,
  genome,
  excludeChrs = c("chrM", "M", "Mt", "MT"),
  windowSize = 50000,
  step = 10000,
  mode = IntersectionNotStrict,
  dataOverSamples = FALSE,
  ...
)
```

### Arguments

reads      A [GRanges](#), [GRangesList](#) (should be one read per list element), [GAlignments](#), [GAlignmentsList](#), [GAlignmentPairs](#) or [BamFileList](#) object that represents the data to be counted by [summarizeOverlaps](#). If reads are more than 1 bam files, it should be a vector of character with full path, otherwise current working directory is the default directory.

|                 |  |
|-----------------|--|
| genome          | A BSgenome object from/on which to get/set the sequence and metadata information.  |
| excludeChrs     | A vector of string: chromosomes/scaffolds of no interest for NAD analysis. see <a href="#">summarizeOverlaps</a> . default is countByOverlaps, alia of countOverlaps(features, reads, ignore.strand=ignore.strand) |
| windowSize      | numeric(1) or integer(1). Size of the windows.   |
| step            | numeric(1) or integer(1). Step of generating silding windows.  |
| mode            | One of the pre-defined count methods.  |
| dataOverSamples | logical(1). Data over several samples when use <a href="#">GRangesList</a> as input.   |
| ...             | Additional arguments passed to <a href="#">summarizeOverlaps</a> .   |

**Value**

A [RangedSummarizedExperiment](#) object. The assays slot holds the counts, rowRanges holds the annotation from the sliding widows of genome. metadata contains lib.size.chrom for holding chromosome-level sequence depth

**Author(s)**

Jianhong Ou, Haibo Liu, Herve Pages and Julie Zhu

**Examples**

```
if (interactive())
{
  fls <- list.files(system.file("extdata", package="NADfinder"),
    recursive=FALSE, pattern="*bam$", full=TRUE)
  names(fls) <- basename(fls)
  if (!require(BSgenome.Mmusculus.UCSC.mm10))
  {
    if (!requireNamespace("BiocManager", quietly=TRUE))
      install.packages("BiocManager")
    BiocManager::install("BSgenome.Mmusculus.UCSC.mm10")
    library(BSgenome.Mmusculus.UCSC.mm10)
  }
  se <- tileCount(reads = fls,
    genome = Mmusculus,
    excludeChrs = c("chrM", paste0("chr", c(1:17,19)),
      "chrX", "chrY"),
    windowSize=50000, step=10000)
}
```

**Description**

Perform overlap queries between reads and genome by sliding windows Count reads over sliding windows.

```
if (interactive())
```

```
fls <- list.files(system.file("extdata", package="NADfinder"), recursive=FALSE, pattern="*bam$",
full=TRUE) names(fls) <- basename(fls)
```

```
se <- tileCount2(reads = fls, windowSize=50000, step=10000)
```

**Usage**

```
tileCount2(
  reads,
  fragment.length = 100,
  windowSize = 50000,
  restrict = paste0("chr", c(1:19, "X", "Y")),
  step = 1000,
  filter = 0,
  pe = "both"
)
```

```
tileCount2(
  reads,
  fragment.length = 100,
  windowSize = 50000,
  restrict = paste0("chr", c(1:19, "X", "Y")),
  step = 1000,
  filter = 0,
  pe = "both"
)
```

**Arguments**

|                 |  |
|-----------------|--|
| reads           | An object that represents the names and path of the bam files to be counted. If reads are more than 1 bam files, it should be a vector of character with full path. This function now works for paired end reads |
| fragment.length | integer(1). An integer scalar or a list of two integer scalars/vectors, containing the average length(s) of the sequenced fragments in each library.   |
| windowSize      | numeric(1) or integer(1). Size of the windows.   |
| restrict        | restrict to a set of chromosomes, default to mouse chromosomes.  |
| step            | numeric(1) or integer(1). Step of generating silding windows.  |
| filter          | default to 0 without filtering. An integer scalar for the minimum count sum across libraries for each window   |
| pe              | a character string indicating whether paired-end data is present; set to "none", "both", "first" or "second"   |

**Value**

A [RangedSummarizedExperiment](#) object with chromosome-level depth The assays slot holds the counts, rowRanges holds the annotation from the sliding widows of genome. metadata contains lib.size.chrom for holding chromosome-level sequence depth



**Author(s)**

Jun Yu, Hervé Pagès and Julie Zhu

**Examples**

```

if (interactive())
{
  fls <- list.files(system.file("extdata", package="NADfinder"),
    recursive=FALSE, pattern="*bam$", full=TRUE)
  names(fls) <- basename(fls)

  se <- tileCount2(reads = fls,
    windowSize=50000, step=10000)
}

```

transformData

*transform counts to log2 cpm ratios, log2 ratios or log2 odds ratios***Description**

calculate the log2 ratios, log2 cpm (count per million) ratios, or log2 odds ratios for nucleolus vs genome. pseudo-count will be used to avoid x/0 or log(0).

**Usage**

```

transformData(
  A,
  B,
  seqnames.A,
  seqnames.B,
  pseudo.count = 1L,
  transformation = c("log2OddsRatio", "log2CPMRatio", "log2Ratio"),
  chrom.level.lib = TRUE,
  lib.size.A,
  lib.size.B
)

```

**Arguments**

A, B window-level counts for nucleolus and genome, extracted from the assays of the output of the tileCounts function

seqnames.A, seqnames.B seqnames, extracted from the rowRanges of the output of the tileCounts function

pseudo.count pseudo-count will be used to avoid x/0 or log0, default to 1.

transformation transformation type

chrom.level.lib indicating whether calculating CPM or odds using sequence depth of the whole genome or the corresponding chromosome

lib.size.A, lib.size.B

library size for A and B. these two dataframes contain chromosome-level sequence depth for the chromosomes, which can be extracted from the metadata of the output of the tileCounts function

### Value

a numeric vector of log2 ratios, log2 CPM ratios or log2 odds ratios.

### Author(s)

Julie Zhu

### Examples

```
transformData(seq_len(10), 10:1, seqnames.A = Rle(c("chr1", "chr2" ) , c(5,5)),
Rle(c("chr1", "chr2" ) , c(5,5)), transformation = "log2OddsRatio",
chrom.level.lib = FALSE, lib.size.A = cbind(c("chr1", "chr2"), c(10000, 12000)),
lib.size.B = cbind(c("chr1", "chr2"), c(10000, 12000)))
transformData(seq_len(10), 10:1, seqnames.A = Rle(c("chr1", "chr2" ) , c(5,5)),
Rle(c("chr1", "chr2" ) , c(5,5)), transformation = "log2CPMRatio",
chrom.level.lib = FALSE, lib.size.A = cbind(c("chr1", "chr2"), c(10000, 12000)),
lib.size.B = cbind(c("chr1", "chr2"), c(10000, 12000)))
transformData(seq_len(10), 10:1, seqnames.A = Rle(c("chr1", "chr2" ) , c(5,5)),
Rle(c("chr1", "chr2" ) , c(5,5)), transformation = "log2CPMRatio",
chrom.level.lib = TRUE, lib.size.A = cbind(c("chr1", "chr2"), c(100, 12000)),
lib.size.B = cbind(c("chr1", "chr2"), c(10000, 200)))
transformData(seq_len(10), 10:1, seqnames.A = Rle(c("chr1", "chr2" ) , c(5,5)),
Rle(c("chr1", "chr2" ) , c(5,5)), transformation = "log2OddsRatio",
chrom.level.lib = TRUE, lib.size.A = cbind(c("chr1", "chr2"), c(100, 12000)),
lib.size.B = cbind(c("chr1", "chr2"), c(10000, 200)))
transformData(seq_len(10), 10:1, transformation = "log2Ratio")
```

---

trimPeaks

*Trim peaks*

---

### Description

Filter the peaks by pvalue and trim the range of peaks for an NAD or ChIP-seq experiment without biological replicates.

### Usage

```
trimPeaks(
  se,
  cutoffAdjPvalue = 0.05,
  padjust.method = "BH",
  backgroundPercentage = 0.25,
  countFilter = 1000,
  ratioAssay = "ratio",
  backgroundCorrectedAssay = "bcRatio",
  smoothedRatioAssay = "smoothedRatio",
  zscoreAssay = "zscore"
)
```

**Arguments**

|  |   |
|--|---|
| <code>se</code>  | An object of <a href="#">RangedSummarizedExperiment</a> with assays of raw counts, ratios, background corrected ratios, smoothed ratios and z-scores. It should be an element of the output of <a href="#">smoothRatiosByChromosome</a> |
| <code>cutoffAdjPvalue</code>   | numeric(1). Cutoff of adjusted p-value.   |
| <code>padjust.method</code>  | character(1). The method to use for adjusting p-values, which is passed to <code>p.adjust</code> function   |
| <code>backgroundPercentage</code>                                      | numeric(1). Cutoff value for the peaks height.  |
| <code>countFilter</code>   | numeric(1) or integer(1). Cutoff value for mean of raw reads count of the Nucleolar/ChIP samples in each window.  |
| <code>ratioAssay</code>  | character(1). The name of assay in <code>se</code> , which store the values to be smoothed.   |
| <code>backgroundCorrectedAssay, smoothedRatioAssay, zscoreAssay</code> | Assays names for background-corrected ratios, smoothed ratios and z-scores based on background corrected ratios.  |

**Value**

An object of [GRanges](#).

**Examples**

```
data(single.count)
se <- single.count
dat <- log2se(se, nucleolusCols="N18.subsampled.srt.bam", genomeCols="G18.subsampled.srt.bam",
transformation="log2CPMRatio")
## Smooth the ratios for each chromosome.
dat <- smoothRatiosByChromosome(dat, N=100, chr=c("chr18", "chr19"))
peaks <- trimPeaks(dat[["chr18"]],
backgroundPercentage=.25,
cutoffAdjPvalue=0.05, countFilter=1000)
```

---

| `triplicate.count` |
 *Counts data for chromosome 18 for an experiment with triplicates* |**Description**

Counts data for chromosome 18 for an experiment with triplicates

---

|               |                                     |
|---------------|-------------------------------------|
| zscoreOverBck | <i>Z-scores over the background</i> |
|---------------|-------------------------------------|

---

**Description**

Calculate the z-scores over the background distribution.

**Usage**

```
zscoreOverBck(ratios, backgroundPercentage = 0.25)
```

**Arguments**

|                      |   |
|----------------------|---|
| ratios               | A numeric vector containing the transformed, background corrected and smoothed ratios in each window. |
| backgroundPercentage | numeric(1). Low percentile for background distribution.   |

**Value**

A vector of numeric. Z-scores.

**Author(s)**

Jianhong Ou and Julie Zhu

**Examples**

```
r <- runif(200)
zscoreOverBck(r)
```

# Index

- \* **data**
  - single.count, 13
  - triplicate.count, 19
- backgroundCorrection, 3
- BamFileList, 14
- baseline.modpolyfit, 3
- butterFilter, 3, 14
- callPeaks, 4
- computeLibSizeChrom, 6
- cor, 8
- corrplot, 8
- coverage, 7
- cumulativePercentage, 6
- export, 7
- exportSignals, 7
- GAlignmentPairs, 14
- GAlignments, 14
- GAlignmentsList, 14
- getCorrelations, 8
- GRanges, 7, 10, 14, 19
- GRangesList, 12, 14, 15
- groupZscores, 9
- ideogramPlot, 12
- IntersectionNotStrict, 10
- loadIdeogram, 12
- log2se, 8, 10, 13
- NADfinder (NADfinder-package), 2
- NADfinder-package, 2
- normalizeBetweenArrays, 4
- peakdet, 11
- plotSig, 12
- RangedSummarizedExperiment, 4, 6–8, 11, 13–16, 19
- SimpleList, 14
- SimpleRleList, 7, 8
- single.count, 13
- smoothRatiosByChromosome, 4, 6, 7, 13, 19
- summarizeOverlaps, 10, 14, 15
- tileCount, 11, 14
- tileCount2, 15
- transformData, 17
- trimPeaks, 18
- triplicate.count, 19
- zscoreOverBck, 13, 20