Package ‘bsseq’

March 28, 2017

Version 1.10.0
Title Analyze, manage and store bisulfite sequencing data
Description A collection of tools for analyzing and visualizing bisulfite sequencing data.
Depends R (>= 3.3), methods, BiocGenerics, GenomicRanges (>= 1.23.7), SummarizedExperiment (>= 0.1.1), parallel, limma
Imports IRanges (>= 2.5.17), GenomeInfoDb, scales, stats, graphics, Biobase, locfit, gtools, data.table, S4Vectors, R.utils (>= 2.0.0), matrixStats (>= 0.50.0), permute
Suggests RUnit, bsseqData, BiocStyle, knitr
License Artistic-2.0
VignetteBuilder knitr
URL https://github.com/kasperdanielhansen/bsseq
LazyData yes
LazyDataCompression xz
BugReports https://github.com/kasperdanielhansen/bsseq/issues
biocViews DNAmethylation
NeedsCompilation no
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R topics documented:

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BS.chr22

Whole-genome bisulfite sequencing for chromosome 22 from Lister et al.

Description

This dataset represents chromosome 22 from the IMR90 cell line sequenced in Lister et al. Only CpG methylation are included (there were very few non-CpG loci). The two samples are two different extractions from the same cell line (ie. technical replicates), and are pooled in the analysis in the original paper.

Usage

data(BS.chr22)

Format

An object of class BSseq.

Details

All coordinates are in hg18.

Source

Obtained from http://neomorph.salk.edu/human_methyome/data.html specifically the files mc_h1_r1.tar.gz and mc_h1_r1.tar.gz. A script which downloads these files and constructs the BS.chr22 object may be found in ‘inst/scripts/get_BS.chr22.R’, see the example.

References

BSmooth

Examples

```r
data(BS.chr22)
BS.chr22

script <- system.file("scripts", "get_BS.chr22.R", package = "bsseq")
script
readLines(script)
```

BSmooth

**BSmooth, smoothing bisulfite sequence data**

Description

This implements the BSmooth smoothing algorithm for bisulfite sequencing data.

Usage

```r
BSmooth(BSseq, ns = 70, h = 1000, maxGap = 10^8,
parallelBy = c("sample", "chromosome"), mc.preschedule = FALSE,
mc.cores = 1, keep.se = FALSE, verbose = TRUE)
```

Arguments

- **BSseq**: An object of class BSseq.
- **ns**: The minimum number of methylation loci in a smoothing window.
- **h**: The minimum smoothing window, in bases.
- **maxGap**: The maximum gap between two methylation loci, before the smoothing is broken across the gap. The default smooths each chromosome separately.
- **parallelBy**: Should the computation be parallel by chromosome or sample, see details.
- **mc.preschedule**: Passed to mclapply (should the tasks be prescheduled).
- **mc.cores**: Passed to mclapply (the number of cores used). Note that setting mc.cores to a value greater than 1 is not supported on MS Windows, see the help page for mclapply.
- **keep.se**: Should the estimated standard errors from the smoothing algorithm be kept. This will make the return object roughly 30 percent bigger and may not be used for anything.
- **verbose**: Should the function be verbose.

Details

`ns` and `h` are passed to the locfit function. The bandwidth used is the maximum (in genomic distance) of the `h` and a width big enough to contain `ns` number of methylation loci.

The function uses the parallel package to do parallel computations. In order to use this, make sure your system have enough RAM, these are typically big objects. The computation can either be split by chromosome or by sample, which is better depends on the number of samples and how many concurrent smoothings may be done.

Value

An object of class BSseq, containing smoothed values and optionally standard errors for these.
BSmooth.tstat

Compute t-statistics based on smoothed whole-genome bisulfite sequencing data.

Description

Compute t-statistics based on smoothed whole-genome bisulfite sequencing data.

Usage

```r
BSmooth.tstat(BSseq, group1, group2, 
estimate.var = c("same", "paired", "group2"), local.correct = TRUE, 
maxGap = NULL, qSd = 0.75, k = 101, mc.cores = 1, verbose = TRUE)
```

Arguments

- `BSseq` An object of class BSseq.
- `group1` A vector of sample names or indexes for the ‘treatment’ group.
- `group2` A vector of sample names or indexes for the ‘control’ group.
- `estimate.var` How is the variance estimated, see details.
- `local.correct` A logical; should local correction be used, see details.
- `maxGap` A scalar greater than 0, see details.
- `qSd` A scalar between 0 and 1, see details.
- `k` A positive scalar, see details.
- `mc.cores` The number of cores used. Note that setting mc.cores to a value greater than 1 is not supported on MS Windows, see the help page for mclapply.
- `verbose` Should the function be verbose?
BSmooth.tstat

Details

T-statistics are formed as the difference in means between group 1 and group 2 divided by an estimate of the standard deviation, assuming that the variance in the two groups are the same (same), that we have paired samples (paired) or only estimate the variance based on group 2 (group2). The standard deviation estimates are then smoothed (using a running mean with a width of k) and thresholded (using qSd which sets the minimum standard deviation to be the qSd-quantile). Optionally, the t-statistics are corrected for low-frequency patterns.

It is sometimes useful to use local.correct even if no large scale changes in methylation have been found; it makes the marginal distribution of the t-statistics more symmetric.

Additional details in the reference.

Value

An object of class BSseqTstat.

Author(s)

Kasper Daniel Hansen <khansen@jhsph.edu>

References


See Also

BSmooth for the input object and BSseq for its class. BSseqTstat describes the return class. This function is likely to be followed by the use of dmrFinder. And finally, see the package vignette(s) for more information on how to use it.

Examples

```r
if(require(bsseqData)) {
  data(keepLoci.ex)
  data(BS.cancer.ex.fit)
  BS.cancer.ex.fit <- updateObject(BS.cancer.ex.fit)
  # Remember to subset the BSseq object, see vignette for explanation
  BS.tstat <- BSmooth.tstat(BS.cancer.ex.fit[keepLoci.ex,],
                             group1 = c("C1", "C2", "C3"),
                             group2 = c("N1", "N2", "N3"),
                             estimate.var = "group2")

  BS.tstat
  # This object is also stored as BS.cancer.ex.tstat in the
  # bsseqData package
}
```
BSseq

The constructor function for BSseq objects.

Description

The constructor function for BSseq objects.

Usage

BSseq(M = NULL, Cov = NULL, coef = NULL, se.coef = NULL, trans = NULL, parameters = NULL, pData = NULL, gr = NULL, pos = NULL, chr = NULL, sampleNames = NULL, rmZeroCov = FALSE)

Arguments

M
A matrix of methylation evidence.

Cov
A matrix of coverage.

coeff
Smoothing estimates.

se.coef
Smoothing standard errors.

trans
A smoothing transformation.

parameters
A list of smoothing parameters.

data
An data.frame or DataFrame.

sampleNames
A vector of sample names.

gr
An object of type GRanges.

pos
A vector of locations.

chr
A vector of chromosomes.

rmZeroCov
Should genomic locations with zero coverage in all samples be removed.

Details

Genomic locations are specified either through gr or through chr and pos but not both. There should be the same number of genomic locations as there are rows in the M and Cov matrix.

The argument rmZeroCov may be useful in order to reduce the size of the return object be removing methylation loci with zero coverage.

In case one or more methylation loci appears multiple times, the M and Cov matrices are summed over rows linked to the same methylation loci. See the example below.

Users should never have to specify coef, se.coef, trans, and parameters, this is for internal use (they are added by BSmooth).

phenoData is a way to specify pheno data (as known from the ExpressionSet and eSet classes), at a minimum sampleNames should be given (if they are not present, the function uses col.names(M)).

Value

An object of class BSseq.

Author(s)

Kasper Daniel Hansen <khansen@jhsph.edu>
BSseq-class

See Also

BSseq

Examples

```r
M <- matrix(0:8, 3, 3)
Cov <- matrix(1:9, 3, 3)
BS1 <- BSseq(chr = c("chr1", "chr2", "chr1"), pos = c(1,2,3),
             M = M, Cov = Cov, sampleNames = c("A","B", "C"))
BS1
BS2 <- BSseq(chr = c("chr1", "chr1", "chr1"), pos = c(1,1,1),
             M = M, Cov = Cov, sampleNames = c("A","B", "C"))
BS2
```

### Description

A class for representing whole-genome or capture bisulfite sequencing data.

### Objects from the Class

An object from the class links together several pieces of information. (1) genomic locations stored as a GRanges object, a location by samples matrix of M values, a location by samples matrix of Cov (coverage) values and phenodata information. In addition, there are slots for representing smoothed data. This class is an extension of RangedSummarizedExperiment.

### Slots

- `trans`: Object of class function. This function transforms the coef slot from the scale the smoothing was done to the 0-1 methylation scale.
- `parameters`: Object of class list. A list of parameters representing for example how the data was smoothed.

### Methods

- `signature(x = "BSseq")`: Subsetting by location (using integer indices) or sample (using integers or sample names).
- `length`: Unlike for RangedSummarizedExperiment, length() is the number of methylation loci (equal to length(granges(x))).
- `sampleNames,sampleNames<-`: Sample names and its replacement function for the object. This is an alias for colnames.
- `pData,pData<-`: Obtain and replace the pData slot of the phenoData slot. This is an alias for colData.
- `show`: The show method.
- `combine`: This function combines two BSSeq objects. The genomic locations of the new object is the union of the genomic locations of the individual objects. In addition, the methylation data matrices are placed next to each other (as appropriate wrt. the new genomic locations) and zeros are entered into the matrices as needed.
Utilities

This class extends hasGRanges and therefore inherits a number of useful GRanges methods that operate on the gr slot, used for accessing and setting the genomic locations and also do subsetByOverlaps.

There are a number of almost methods-like functions for operating on objects of class BSseq, including getBSseq, collapseBSseq, and orderBSseq. They are detailed below.

collapseBSseq(BSseq, columns) is used to collapse an object of class BSseq. By collapsing we simply mean that certain columns (samples) are merge together by summing up the methylation evidence and coverage. This is a useful function if you start by reading in a dataset based on say flowcells and you (after QC) want to simply add a number of flowcells into one sample. The argument columns specify which samples are to be merged, in the following way: it is a character vector of new sample names, and the names of the column vector indicates which samples in the BSseq object are to be collapsed. If columns have the same length as the number of rows of BSseq (and has no names) it is assumed that the ordering corresponds to the sample ordering in BSseq.

orderBSseq(BSseq, seqOrder = NULL) simply orders an object of class BSseq according to (increasing) genomic locations. The seqOrder vector is a character vector of seqnames(BSseq) describing the order of the chromosomes. This is useful for ordering chr1 before chr10.

chrSelectBSseq(BSseq, seqnames = NULL, order = FALSE) subsets and optionally reorders an object of class BSseq. The seqnames vector is a character vector of seqnames(BSseq) describing which chromosomes should be retained. If order is TRUE, the chromosomes are also re-ordered using orderBSseq.

getBSseq(BSseq, type = c("Cov", "M", "gr", "coef", "se.coef", "trans", "parameters")) is a general accessor: is used to obtain a specific slot of an object of class BSseq. It is primarily intended for internal use in the package. For users we recommend granges to get the genomic locations, getCoverage to get the coverage slots and getMeth to get the smoothed values (if they exist).

hasBeenSmoothed(BSseq) This function returns a logical depending on whether or not the BSseq object has been smoothed using BSmooth.

combineList(list) This function function is a faster way of using combine on multiple objects, all containing methylation data for the exact same methylation loci. The input is a list, with each component an object of class BSseq. The (slower) alternative is to use Reduce(combine, list).

strandCollapse(BSseq, shift = TRUE) This function operates on a BSseq objects which has stranded loci (ie. loci where the strand is one of ‘+’ or ‘-’). It will collapse the methylation and coverage information across the two strands, into one position. The argument shift indicates whether the positions for the loci on the reverse strand should be shifted one (ie. the positions for these loci are the positions of the ‘G’ in the ‘CpG’; this is the case for Bismark output for example.

Coercion

Package version 1.5.2 introduced a new version of representing ‘BSseq’ objects. You can update old serialized (saved) objects by invoking x <- updateObject(x).

Assays

This class overrides the default implementation of assays to make it faster. Per default, no names are added to the returned data matrices.

Assay names can conveniently be obtained by the function assayNames(x)
**BSseqStat-class**

**Author(s)**
Kasper Daniel Hansen <khansen@jhsph.edu>

**See Also**
The package vignette. **BSseq** for the constructor function. **RangedSummarizedExperiment** for the underlying class. **getBSseq, getCoverage**, and **getMeth** for accessing the data stored in the object and finally **BSsmooth** for smoothing the bisulfite sequence data.

**Examples**

```r
M <- matrix(1:9, 3, 3)
colnames(M) <- c("A1", "A2", "A3")
BStest <- BSseq(pos = 1:3, chr = c("chr1", "chr2", "chr1"), M = M, Cov = M + 2)
chrSelectBSseq(BStest, seqnames = "chr1", order = TRUE)
collapseBSseq(BStest, columns = c("A1" = "A", "A2" = "A", "A3" = "B"))
```

---

**BSseqStat-class**

**Class BSseqStat**

**Description**
A class for representing statistics for smoothed whole-genome bisulfite sequencing data.

**Usage**

```r
BSseqStat(gr = NULL, stats = NULL, parameters = NULL)
```

**Arguments**

- **gr**: The genomic locations as an object of class GRanges.
- **stats**: The statistics, as a matrix.
- **parameters**: A list of parameters.

**Objects from the Class**
Objects can be created by calls of the form `BSseqStat(...)`. However, usually objects are returned by `BSsmooth.fstat(...)` and not constructed by the user.

**Slots**

- **stats**: This is a matrix with columns representing various statistics for methylation loci along the genome.
- **parameters**: Object of class list. A list of parameters representing how the statistics were computed.
- **gr**: Object of class GRanges giving genomic locations.

**Extends**
Class **hasGRanges**, directly.
BSseqTstat-class

Methods

The subsetting operator; one may only subset in one dimension, corresponding to methylation loci.

show The show method.

Utilities

This class extends hasGRanges and therefore inherits a number of useful GRanges methods that operate on the gr slot, used for accessing and setting the genomic locations and also do subsetByOverlaps.

Author(s)

Kasper Daniel Hansen <khansen@jhsph.edu>

See Also

hasGRanges for accessing the genomic locations. BSmooth.fstat for a function that returns objects of class BSseqStat, and smoothSds, computeStat and dmrFinder for functions that operate based on these statistics. Also see the more specialised BSseqTstat.

BSseqTstat-class

Class BSseqTstat

Description

A class for representing t-statistics for smoothed whole-genome bisulfite sequencing data.

Usage

BSseqTstat(gr = NULL, stats = NULL, parameters = NULL)

Arguments

gr The genomic locations as an object of class GRanges.
stats The statistics, as a matrix.
parameters A list of parameters.

Objects from the Class

Objects can be created by calls of the form BSseqTstat(...). However, usually objects are returned by BSmooth.tstat(...) and not constructed by the user.

Slots

stats: This is a matrix with columns representing various statistics for methylation loci along the genome.
parameters: Object of class list. A list of parameters representing how the t-statistics were computed.
gr: Object of class GRanges giving genomic locations.
data.frame2GRanges

Extends

Class hasGRanges, directly.

Methods

[] The subsetting operator; one may only subset in one dimension, corresponding to methylation loci.

show The show method.

Utilities

This class extends hasGRanges and therefore inherits a number of useful GRanges methods that operate on the gr slot, used for accessing and setting the genomic locations and also do subsetByOverlaps.

Author(s)

Kasper Daniel Hansen <khansen@jhsph.edu>

See Also

The package vignette(s). hasGRanges for accessing the genomic locations. BSmooth.tstat for a function that returns objects of class BSseqTstat, and dmrFinder for a function that computes DMRs based on the t-statistics. Also see BS.cancer.ex.tstat for an example of the class in the bsseqData package.

data.frame2GRanges # Converts a data frame to a GRanges.

Description

Converting a data.frame to a GRanges object. The data.frame needs columns like chr, start and end (strand is optional). Additional columns may be kept in the GRanges object.

Usage

data.frame2GRanges(df, keepColumns = FALSE, ignoreStrand = FALSE)

Arguments

df A data.frame with columns chr or seqnames, start, end and optionally a strand column.
keepColumns In case df has additional columns, should these columns be stored as metadata columns on the return GRanges or should they be discarded.
ignoreStrand In case df has a strand column, should this column be ignored.

Value

An object of class GRanges

Note

In case df has rownames, they will be used as names for the return object.
Author(s)

Kasper Daniel Hansen <khansen@jhsph.edu>

Examples

```r
df <- data.frame(chr = "chr1", start = 1:3, end = 2:4,
                 strand = c("+","-","+"))
data.frame2GRanges(df, ignoreStrand = TRUE)
```

---

dmrFinder

Finds differentially methylated regions for whole genome bisulfite sequencing data.

Description

Finds differentially methylated regions for whole genome bisulfite sequencing data. Essentially identifies regions of the genome where all methylation loci have an associated t-statistic that is beyond a (low, high) cutoff.

Usage

```r
dmrFinder(bstat, cutoff = NULL, qcutoff = c(0.025, 0.975),
          maxGap=300, stat = "tstat.corrected", verbose = TRUE)
```

Arguments

- `bstat`: An object of class BSseqStat or BSseqTstat.
- `cutoff`: The cutoff of the t-statistics. This should be a vector of length two giving the (low, high) cutoff. If NULL, see `qcutoff`.
- `qcutoff`: In case `cutoff` is NULL, compute the cutoff using these quantiles of the t-statistic.
- `maxGap`: If two methylation loci are separated by this distance, break a possible DMR. This guarantees that the return DMRs have CpGs that are this distance from each other.
- `stat`: Which statistic should be used?
- `verbose`: Should the function be verbose?

Details

The workhorse function is `BSsmooth.tstat` which sets up a t-statistic for a comparison between two groups.

Note that post-processing of these DMRs are likely to be necessary, filtering for example for length (or number of loci).
Value

A data.frame with columns

- `start`, `end`, `width`, `chr` - genomic locations and width.
- `n` - The number of methylation loci.
- `invdensity` - Average length per loci.
- `group1.mean` - The mean methylation level across samples and loci in 'group1'.
- `group2.mean` - The mean methylation level across samples and loci in 'group2'.
- `meanDiff` - The mean difference in methylation level; the difference between `group1.mean` and `group2.mean`.
- `idxStart`, `idxEnd`, `cluster` - Internal use.
- `areaStat` - The 'area' of the t-statistic; equal to the sum of the t-statistics for the individual methylation loci.
- `direction` - either 'hyper' or 'hypo'.
- `areaStat.corrected` - Only present if column = "tstat.corrected", contains the area of the corrected t-statistics.

Author(s)

Kasper Daniel Hansen <khansen@jhsph.edu>.

References


See Also

BSmooth.tstat for the function constructing the input object, and BSseqTstat for its class. In the example below, we use BS.cancer.ex.tstat as the actual input object. Also see the package vignette(s) for a detailed example.

Examples

```r
if(require(bsseqData)) {
  dmrs0 <- dmrFinder(BS.cancer.ex.tstat, cutoff = c(-4.6, 4.6), verbose = TRUE)
  dmrs <- subset(dmrs0, abs(meanDiff) > 0.1 & n >= 3)
}
```
fisherTests

Compute Fisher-tests for a BSseq object

Description

A function to compute Fisher-tests for an object of class BSseq.

Usage

fisherTests(BSseq, group1, group2, lookup = NULL, returnLookup = TRUE, mc.cores = 1, verbose = TRUE)

Arguments

- BSseq: An object of class BSseq.
- group1: A vector of sample names or indexes for the ‘treatment’ group.
- group2: A vector of sample names or indexes for the ‘control’ group.
- lookup: A ‘lookup’ object, see details.
- returnLookup: Should a ‘lookup’ object be returned, see details.
- mc.cores: The number of cores used. Note that setting mc.cores to a value greater than 1 is not supported on MS Windows, see the help page for mclapply.
- verbose: Should the function be verbose.

Details

This function computes row-wise Fisher’s exact tests. It uses an internal lookup table so rows which forms equivalent 2x2 tables are group together and only a single test is computed. If returnLookup is TRUE the return object contains the lookup table which may be feed to another call to the function using the lookup argument.

If group1, group2 designates more than 1 sample, the samples are added together before testing.

This function can use multiple cores on the same computer.

This test cannot model biological variability.

Value

if returnLookup is TRUE, a list with components results and lookup, otherwise just the results component. The results (component) is a matrix with the same number of rows as the BSseq argument and 2 columns p.value (the unadjusted p-values) and log2OR (log2 transformation of the odds ratio).

Author(s)

Kasper Daniel Hansen <khansen@jhsph.edu>

See Also

fisher.test for information about Fisher’s test. mclapply for the mc.cores argument.
getCoverage

Examples

```R
M <- matrix(1:9, 3,3)
colnames(M) <- c("A1", "A2", "A3")
BStest <- BSseq(pos = 1:3, chr = c("chr1", "chr2", "chr1"),
  M = M, Cov = M + 2)
results <- fisherTests(BStest, group1 = "A1", group2 = "A2",
  returnLookup = TRUE)
results
```

getCoverage  Obtain coverage for BSseq objects.

Description

Obtain coverage for BSseq objects.

Usage

```R
getCoverage(BSseq, regions = NULL, type = c("Cov", "M"),
  what = c("perBase", "perRegionAverage", "perRegionTotal"))
```

Arguments

- **BSseq** An object of class BSseq.
- **regions** An optional data.frame or GenomicRanges object specifying a number of genomic regions.
- **type** This returns either coverage or the total evidence for methylation at the loci.
- **what** The type of return object, see details.

Value

If `regions` are not specified (`regions = NULL`) a matrix (what = "perBase") or a vector (otherwise) is returned. This will either contain the per-base coverage or the genome total or average coverage.

If what = "perBase" and regions are specified, a list is returned. Each element of the list is a matrix corresponding to the genomic loci inside the region. It is conceptually the same as splitting the coverage by region.

If what = "perRegionAverage" or what = "perRegionTotal" and regions are specified the return value is a matrix. Each row of the matrix corresponds to a region and contains either the total coverage of the average coverage in the region.

Author(s)

Kasper Daniel Hansen <khansen@jhsph.edu>.

See Also

- `BSseq` for the BSseq class.
Examples

data(BS.chr22)
head(getCoverage(BS.chr22, type = "M"))
reg <- GRanges(seqnames = c("chr22", "chr22"),
  ranges = IRanges(start = c(1, 2*10^7), end = c(2*10^7 +1, 4*10^7)))
getCoverage(BS.chr22, regions = reg, what = "perRegionAverage")
cList <- getCoverage(BS.chr22, regions = reg)
length(cList)
head(cList[[1]])

getMeth

Obtain methylation estimates for BSseq objects.

Description

Obtain methylation estimates for BSseq objects, both smoothed and raw.

Usage

getMeth(BSseq, regions = NULL, type = c("smooth", "raw"),
  what = c("perBase", "perRegion"), confint = FALSE, alpha = 0.95)

Arguments

BSseq An object of class BSseq.
regions An optional data.frame or GenomicRanges object specifying a number of gen-
nomic regions.
type This returns either smoothed or raw estimates of the methylation level.
what The type of return object, see details.
confint Should a confidence interval be return for the methylation estimates (see below).
  This is only supported if what is equal to perBase.
alpha alpha value for the confidence interval.

Value

If region = NULL the what argument is ignored. This is also the only situation in which confint = TRUE
is supported. The return value is either a matrix (confint = FALSE or a list with three components
confint = TRUE (meth, upper and lower), giving the methylation estimates and (optionally) confidence intervals.
Confidence intervals for type = "smooth" is based on standard errors from the smoothing algo-
rithm (if present). Otherwise it is based on pointwise confidence intervals for binomial distributions
described in Agresti (see below), specifically the score confidence interval.
If regions are specified, what = "perBase" will make the function return a list, each element
of the list being a matrix corresponding to a genomic region (and each row of the matrix being
a loci inside the region). If what = "perRegion" the function returns a matrix, with each row
corresponding to a region and containing the average methylation level in that region.

Note

A BSseq object needs to be smoothed by the function BSmooth in order to support type = "smooth".
getStats

Author(s)
Kasper Daniel Hansen <khansen@jhsph.edu>.

References

See Also
BSseq for the BSseq class and BSmooth for smoothing such an object.

Examples
```r
data(BS.chr22)
head(getMeth(BS.chr22, type = "raw"))
reg <- GRanges(seqnames = c("chr22", "chr22"),
               ranges = IRanges(start = c(1, 2*10^7), end = c(2*10^7 +1, 4*10^7))))
head(getMeth(BS.chr22, regions = reg, type = "raw", what = "perBase"))
```

getStats Obtain statistics from a BSseqTstat object

Description
Essentially an accessor function for the statistics of a BSseqTstat object.

Usage
```r
getStats(bstat, regions = NULL, ...)
```

Arguments
- **bstat**: An object of class BSseqStat or BSseqTstat.
- **regions**: An optional data.frame or GenomicRanges object specifying a number of genomic regions.
- **...**: Additional arguments passed to the different backends based on the class of bstat; see Details.

Details
Additional argument when the bstat object is of class BSseqTstat:
- **stat**: Which statistics column should be obtained.

Value
An object of class data.frame possible restricted to the regions specified.

Author(s)
Kasper Daniel Hansen <khansen@jhsph.edu>
GoodnessOfFit

See Also

BSseqTstat for the BSseqTstat class, and getCoverage and getMeth for similar functions, operating on objects of class BSseq.

Examples

```r
if(require(bsseqData)) {
  data(BS.cancer.ex.tstat)
  head(getStats(BS.cancer.ex.tstat))
  reg <- GRanges(seqnames = c("chr22", "chr22"),
                 ranges = IRanges(start = c(1, 2*10^7),
                                 end = c(2*10^7 +1, 4*10^7)))
  head(getStats(BS.cancer.ex.tstat, regions = reg))
}
```

GoodnessOfFit

Binomial and poisson goodness of fit statistics for BSSeq objects

Description

Binomial and poisson goodness of fit statistics for BSSeq objects, including plotting capability.

Usage

```r
poissonGoodnessOfFit(BSseq, nQuantiles = 10^5)
binomialGoodnessOfFit(BSseq, method = c("MLE"), nQuantiles = 10^5)
```

Arguments

- `BSseq`: An object of class BSseq.
- `x`: A chisqGoodnessOfFit object (as produced by poissonGoodnessOfFit or binomialGoodnessOfFit).
- `nQuantiles`: The number of (evenly-spaced) quantiles stored in the return object.
- `method`: How is the parameter estimated.
- `type`: Are the chisq or the p-values being plotted.
- `plotCol`: Should the extreme quantiles be colored.
- `qqline`: Add a qqline.
- `pch`, `cex`: Plotting symbols and size.
- `...`: Additional arguments being passed to qqplot (for plot) or ignored (for print).

Details

These functions compute and plot goodness of fit statistics for BSseq objects. For each methylation loci, the Poisson goodness of fit statistic tests whether the coverage (at that loci) is independent and identically Poisson distributed across the samples. In a similar fashion, the Binomial goodness of fit statistic tests whether the number of reads supporting methylation are independent and identically binomial distributed across samples (with different size parameters given by the coverage vector). These functions do not handle NA values.
Value
The plotting method is invoked for its side effect. Both poissonGoodnessOfFit and binomialGoodnessOfFit returns an object of class chisqGoodnessOfFit which is a list with components

- `chisq` a vector of Chisq values.
- `quantiles` a vector of quantiles (of the chisq values).
- `df` degrees of freedom

Author(s)
Kasper Daniel Hansen <khansen@jhsph.edu>

See Also
For the plotting method, see qqplot.

Examples
if(require(bsseqData)) {
  data(BS.cancer.ex)
  BS.cancer.ex <- updateObject(BS.cancer.ex)
  gof <- poissonGoodnessOfFit(BS.cancer.ex)
  plot(gof)
}
seqlevels<- Getting and setting the seqlevels of the genomic locations (the gr slot).
seqnames<- Getting and setting the seqnames of the genomic locations (the gr slot).
show The show method.

findOverlaps (query = "hasGRanges", subject = "hasGRanges"): finds overlaps between the granges() of the two objects.

findOverlaps (query = "GenomicRanges", subject = "hasGRanges"): finds overlaps between query and the granges() of the subject.

findOverlaps (query = "hasGRanges", subject = "GenomicRanges"): finds overlaps between the granges() of the query and the subject.

subsetByOverlaps (query = "hasGRanges", subject = "hasGRanges"): Subset the query, keeping the genomic locations that overlaps the subject.

subsetByOverlaps (query = "hasGRanges", subject = "GenomicRanges"): Subset the query, keeping the genomic locations that overlaps the subject.

subsetByOverlaps (query = "GenomicRanges", subject = "hasGRanges"): Subset the query, keeping the genomic locations that overlaps the subject.

Note
If you extend the hasGRanges class, you should consider writing a subset method (\[]), and a show method. If the new class supports single index subsetting, the subsetByOverlaps methods show extend without problems.

Author(s)
Kasper Daniel Hansen <khansen@jhsph.edu>

Examples
showClass("hasGRanges")

plotRegion

Plotting BSmooth methylation estimates

Description
Functions for plotting BSmooth methylation estimates. Typically used to display differentially methylated regions.

Usage
plotRegion(BSseq, region = NULL, extend = 0, main = "", addRegions = NULL, annoTrack = NULL, cex.anno = 1, geneTrack = NULL, cex.gene = 1.5, col = NULL, lty = NULL, lw = NULL, BSseqStat = NULL, stat = "tstat.corrected", stat.col = "black", stat.lwd = 1, stat.lty = 1, stat.ylim = c(-8, 8), mainWithWidth = TRUE, regionCol = alpha("red", 0.1), addTicks = TRUE, addPoints = FALSE, pointsMinCov = 5, highlightMain = FALSE)

plotManyRegions(BSseq, regions = NULL, extend = 0, main = "", addRegions = NULL, annoTrack = NULL, cex.anno = 1, geneTrack = NULL, cex.gene = 1.5, col = NULL, lty = NULL, lw = NULL, BSseqStat = NULL, stat = "tstat.corrected", stat.col = "black", stat.lwd = 1, stat.lty = 1, stat.ylim = c(-8, 8), mainWithWidth = TRUE, regionCol = alpha("red", 0.1), addTicks = TRUE, addPoints = FALSE, pointsMinCov = 5, highlightMain = FALSE)
addRegions = NULL, annoTrack = NULL, cex.anno = 1, 
geneTrack = NULL, cex.gene = 1.5, col = NULL, lty = NULL, 
lwd = NULL, BSseqStat = NULL, stat = "tstat.corrected", 
stat.col = "black", stat.lwd = 1, stat.lty = 1, stat.ylim = c(-8, 8), 
mainWithWidth = TRUE, regionCol = alpha("red", 0.1), addTicks = TRUE, 
addPoints = FALSE, pointsMinCov = 5, highlightMain = FALSE, 
verbose = TRUE)

Arguments

BSseq  
An object of class BSseq.

region  
A data.frame (with start, end and chr columns) with 1 row or GRanges of 
length 1. If region is NULL the entire BSseq argument is plotted.

regions  
A data.frame (with start, end and chr columns) or GRanges.

extend  
Describes how much the plotting region should be extended in either direction. 
The total width of the plot is equal to the width of the region plus twice extend.

main  
The plot title. The default is to construct a title with information about which 
genomic region is being plotted.

addRegions  
A set of additional regions to be highlighted on the plots. As the regions argument.

annoTrack  
A named list of GRanges objects. Each component is a track and the names of 
the list are the track names. Each track will be plotted as solid bars, and we 
routinely display information such as CpG islands, exons, etc.

cex.anno  
cex argument when plotting annoTrack.

geneTrack  
**EXPERIMENTAL**: A data.frame with columns: chr, start, end, gene_ID, 
exon_number, strand, gene_name, isoforms. This interface is under active 
development and subject to change.

cex.gene  
cex argument when plotting geneTrack.

col 
The color of the methylation estimates, see details.

lty 
The line type of the methylation estimates, see details.

lwd 
The line width of the methylation estimates, see details.

BSseqStat 
An object of class BSseqStat. If present, a new panel will be shown with the 
t-statistics.

stat  
Which statistics will be plotted (only used is BSseqStat is not NULL.)

stat.col  
color for the statistics plot.

stat.lwd  
line width for the statistics plot.

stat.lty  
line type for the statistics plot.

stat.ylim  
y-limits for the statistics plot.

mainWithWidth  
Should the default title include information about width of the plot region.

regionCol  
The color used for highlighting the region.

addTicks  
Should tick marks showing the location of methylation loci, be added?

addPoints  
Should the individual unsmoothed methylation estimates be plotted. This usually 
leads to a very confusing plot, but may be useful for diagnostic purposes.

pointsMinCov  
The minimum coverage a methylation loci need in order for the raw methylation 
estimates to be plotted. Useful for filtering out low coverage loci. Only used if 
addPoints = TRUE.

highlightMain  
Should the plot region be highlighted?

verbose  
Should the function be verbose?
Details

The correct choice of aspect ratio depends on the width of the plotting region. We tend to use width = 10, height = 5.

`plotManyRegions` is used to plot many regions (hundreds or thousands), and is substantially quicker than repeated calls to `plotRegion`.

This function has grown to be rather complicated over time. For custom plotting, it is sometimes useful to use the function definition as a skeleton and directly modify the code.

Value

This function is invoked for its side effect: producing a plot.

Author(s)

Kasper Daniel Hansen <khansen@jhsph.edu>

See Also

The package vignette has an extended example.

---

**read.bismark**  
*Parsing output from the Bismark alignment suite.*

**Description**

Parsing output from the Bismark alignment suite.

**Usage**

```r
read.bismark(files,  
sampleNames,  
rmZeroCov = FALSE,  
strandCollapse = TRUE,  
fileType = c("cov", "oldBedGraph", "cytosineReport"),  
mc.cores = 1,  
verbose = TRUE)
```

**Arguments**

- `files`: Input files. Each sample is in a different file. Input files are created by running Bismark's methylation extractor; see Note for details.
- `sampleNames`: Sample names, based on the order of files.
- `rmZeroCov`: Should methylation loci that have zero coverage in all samples be removed. This will result in a much smaller object if the data originates from (targeted) capture bisulfite sequencing.
- `strandCollapse`: Should strand-symmetric methylation loci, e.g., CpGs, be collapsed across strands. This option is only available if `fileType = "cytosineReport"` since the other file types do not contain the necessary strand information.
- `fileType`: The format of the input file; see Note for details.
The number of cores used. Note that setting `mc.cores` to a value greater than 1 is not supported on MS Windows, see the help page for `mclapply`.

Make the function verbose.

An object of class `BSseq`.

Input files can either be gzipped or not.

The user must specify the relevant file format via the `fileType` argument. The format of the output of the Bismark alignment suite will depend on the version of Bismark and on various user-specified options. Please consult the Bismark documentation and the Bismark RELEASE NOTES (http://www.bioinformatics.bbsrc.ac.uk/projects/bismark/RELEASE_NOTES.txt) for the definitive list of changes between versions. When possible, it is strongly recommended that you use the most recent version of Bismark.

The "cov" and "oldBedGraph" formats both have six columns ("chromosome", "position", "strand", "methylation percentage", "count methylated", "count unmethylated"). If you are using a recent version of Bismark (v>=0.10.0) then the standard file extension for this file is ".cov". If, however, you are using an older version of Bismark (v<0.10.0) then the file extension will be ",bedGraph". Please note that the ",bedGraph" file created in recent versions of Bismark (v>=0.10.0) is not suitable for analysis with bsseq because it only contains the "methylation percentage" and not "count methylated" nor "count unmethylated".

The "cytosineReport" format has seven columns ("chromosome", "position", "strand", "count methylated", "count unmethylated", "C-context", "trinucleotide context"). There is no standard file extension for this file. The "C-context" and "trinucleotide context" columns are not currently used by bsseq.

The following is a list of some issues to be aware of when using output from Bismark’s methylation extractor:

- The program to extract methylation counts was named `methylation_extractor` in older versions of Bismark (v<0.8.0) and re-named `bismark_methylation_extractor` in recent versions of Bismark (v>=0.8.0). Furthermore, very old versions of Bismark (v<0.7.7) required that user run a separate script (called something like `genome_methylation_bismark2bedGraph`) to create the six-column "cov/oldBedGraph" file.

- The `--counts` and `--bedGraph` arguments must be supplied to `methylation_extractor/bismark_methylation_extractor` in order to use the output with `bsseq::read.bismark()`.

- The genomic co-ordinates of the Bismark output file may be zero-based or one-based depending on whether the `--zero_based` argument is used. Furthermore, the default co-ordinate system varies by version of Bismark. bsseq makes no assumptions about the basis of the genomic co-ordinates and it is left to the user to ensure that the appropriate basis is used in the analysis of their data. Since Bioconductor packages and GRanges use one-based co-ordinates, it is recommended that your Bismark files are also one-based.

Peter Hickey <peter.hickey@gmail.com>
See Also

read.bsmooth for parsing output from the BSmooth alignment suite. read.umtab for parsing legacy (old) formats from the BSmooth alignment suite. collapseBSseq for collapse (merging or summing) the data in two different directories.

Examples

```r
infile <- system.file("extdata/test_data.fastq_bismark.bismark.cov.gz", package = "Varbsseq")
bismarkBSseq <- read.bismark(files = infile, sampleNames = "test_data", rmZeroCov = FALSE, strandCollapse = FALSE, fileType = "cov", verbose = TRUE)
bismarkBSseq
```

Description

Parsing output from the BSmooth alignment suite.

Usage

```r
read.bsmooth(dirs, sampleNames = NULL, seqnames = NULL, returnRaw = FALSE, qualityCutoff = 20, rmZeroCov = FALSE, verbose = TRUE)
```

Arguments

- `dirs` Input directories. Usually each sample is in a different directory, or perhaps each (sample, lane) is a different directory.
- `sampleNames` sample names, based on the order of `dirs`. If `NULL` either set to basename(`dirs`) (if unique) or `dirs`.
- `seqnames` The default is to read all BSmooth output files in `dirs`. Using this argument, it is possible to restrict this to only files with names in `seqnames` (apart from `.cpg.tsv` and optionally `.gz`).
- `returnRaw` Should the function return the complete information in the output files?
- `qualityCutoff` Only use evidence (methylated and unmethylated evidence) for a given methylation loci, if the base in the read has a quality greater than this cutoff.
- `rmZeroCov` Should methylation loci that have zero coverage in all samples be removed. This will result in a much smaller object if the data originates from (targeted) capture bisulfite sequencing.
- `verbose` Make the function verbose.

Value

Either an object of class BSseq (if `returnRaw = FALSE`) or a list of GRanges which each component coming from a directory.
Note

Input files can either be gzipped or not. Gzipping the input files results in much greater speed of reading (and saves space), so it is recommended.

We are working on making this function faster and less memory hungry.

Author(s)

Kasper Daniel Hansen <khansen@jhsph.edu>

See Also

read.umtab for parsing legacy (old) formats from the BSmooth alignment suite. collapseBSseq for collapse (merging or summing) the data in two different directories.

read.umtab Parsing UM tab files (legacy output) containing output from the BSmooth aligner.

Description

Parsing UM tab files containing output from the bisulfite aligner Merman. This is two different legacy formats, which we keep around. These functions are likely to be deprecated in the future.

Usage


read.umtab2(dirs, sampleNames = NULL, rmZeroCov = FALSE, readCycle = FALSE, keepFilt = FALSE, pattern = NULL, keepU, keepM, verbose = TRUE)

Arguments

dirs Input directories. Usually each sample is in a different directory.

pattern An optional pattern, see list.files in the base package.

sampleNames sample names, based on the order of dirs.

rmZeroCov Should methylation loci that have zero coverage in all samples be removed. This will result in a much smaller object if the data originates from (targeted) capture bisulfite sequencing.

keepU A vector of U columns which are kept.

keepM A vector of M columns which are kept.

readCycle Should the cycle columns be returned?

keepFilt Should the filter columns be returned?

verbose Make the function verbose.
Details

`read.umtab2` is newer than `read.umtab` and both process output from older versions of the BSmooth alignment suite (versions older than 0.6.1). These functions are likely to be deprecated in the future. Newer output from the BSmooth alignment suite can be parsed using `read.bsmooth`.

A script using this function can be found in the `bsseqData` package, in the file `scripts/create_BS.cancer.R`.

Value

Both functions returns lists, the components are

- `BSdata`  An object of class `BSseq` containing the methylation evidence.
- `GC`     A vector of local GC content values.
- `Map`     A vector of local mapability values.
- `Mcy`     A matrix of the number of unique M cycles.
- `Ucy`     A matrix of the number of unique U cycles.
- `chr`     A vector of chromosome names.
- `pos`     A vector of genomic positions.
- `M`       A matrix representing methylation evidence.
- `U`       A matrix representing un-methylation evidence.
- `csums`   Description of `comp2`.

Author(s)

Kasper Daniel Hansen <khansen@jhsph.edu>

See Also

`read.bsmooth`.

Examples

```r
## Not run:
require(bsseqData)
umDir <- system.file("umtab", package = "bsseqData")
sampleNames <- list.files(numDir)
dirs <- file.path(numDir, sampleNames, "umtab")
umData <- read.umtab(dirs, sampleNames)
## End(Not run)
```
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