Package ‘bsseq’

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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**

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

**Format**

An object of class BSSeq.

**Details**

All coordinates are in hg18.

**Source**

Obtained from [http://neomorph.salk.edu/human_methylome/data.html](http://neomorph.salk.edu/human_methylome/data.html) specifically the files `mc_imr90_r1.tar.gz` and `mc_imr90_r2.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**


**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 algorithm for estimating methylation levels from bisulfite sequencing data.

Usage
```r
BSmooth(BSseq,
    ns = 70,
    h = 1000,
    maxGap = 10^8,
    keep.se = FALSE,
    BPPARAM = bpparam(),
    chunkdim = NULL,
    level = NULL,
    verbose = getOption("verbose"))
```

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 smoothes each chromosome separately.
- **keep.se**: Should the estimated standard errors from the smoothing algorithm be kept. This will make the return object roughly 30 percent bigger and is currently not be used for anything in bsseq.
- **BPPARAM**: An optional BiocParallelParam instance determining the parallel back-end to be used during evaluation. Currently supported are SerialParam (Unix, Mac, Windows), MulticoreParam (Unix and Mac), SnowParam (Unix, Mac, and Windows, limited to single-machine clusters), and BatchtoolsParam (Unix, Mac, Windows, only with the in-memory realization backend). See sections ’Parallelization and progress monitoring’ and ’Realization backends’ for further details.
- **chunkdim**: Only applicable if BACKEND == "HDF5Array". The dimensions of the chunks to use for writing the data to disk. By default, getHDF5DumpChunkDim() using the dimensions of the returned BSseq object will be used. See ?getHDF5DumpChunkDim for more information.
- **level**: Only applicable if BACKEND == "HDF5Array". The compression level to use for writing the data to disk. By default, getHDF5DumpCompressionLevel() will be used. See ?getHDF5DumpCompressionLevel for more information.
- **verbose**: A logical(1) indicating whether progress messages should be printed (default TRUE).
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.

Value

An object of class BSseq, containing coefficients used to fit smoothed methylation values and optionally standard errors for these.

Realization backends

The BSmooth() function creates a new assay to store the coefficients used to construct the smoothed methylation estimates ((coef)). An additional assay is also created if keep.se == TRUE (se.coef).

The choice of realization backend controls whether these assay(s) are stored in-memory as an ordinary matrix or on-disk as a HDF5Array, for example.

The choice of realization backend is controlled by the BACKEND argument, which defaults to the current value of DelayedArray::getAutoRealizationBackend().

BSmooth supports the following realization backends:

• NULL (in-memory): This stores each new assay in-memory using an ordinary matrix.
• HDF5Array (on-disk): This stores each new assay on-disk in a HDF5 file using an HDF5Matrix from HDF5Array.

Please note that certain combinations of realization backend and parallelization backend are currently not supported. For example, the HDF5Array realization backend is currently only compatible when used with a single-machine parallelization backend (i.e. it is not compatible with a SnowParam that specifies an ad hoc cluster of multiple machines). BSmooth() will issue an error when given such an incompatible realization and parallelization backends. Furthermore, to avoid memory usage blow-ups, BSmooth() will issue an error if an in-memory realization backend is used when smoothing a disk-backed BSseq object.

Additional arguments related to the realization backend can be passed via the ... argument. These arguments must be named and are passed to the relevant RealizationSink constructor. For example, the ... argument can be used to specify the path to the HDF5 file to be used by BSmooth(). Please see the examples at the bottom of the page.

Parallelization and progress monitoring

BSmooth() now uses the BioCParallel package to implement parallelization. This brings some notable improvements:

• Smoothened results can now be written directly to an on-disk realization backend by the worker. This dramatically reduces memory usage compared to previous versions of bsseq that required all results be retained in-memory.
• Parallelization is now supported on Windows through the use of a SnowParam object as the value of BPPARAM.
• Detailed and extensive job logging facilities.
All parallelization options are controlled via the `BPPARAM` argument. In general, we recommend that users combine multicore (single-machine) parallelization with an on-disk realization backend (see section, ‘Realization backend’). For Unix and Mac users, this means using a `MulticoreParam`. For Windows users, this means using a single-machine `SnowParam`. Please consult the `BiocParallel` documentation to take full advantage of the more advanced features.

**Deprecated arguments:** parallelBy, mc.cores, and mc.preschedule are deprecated and will be removed in subsequent releases of `bsseq`. These arguments were necessary when `BSmooth()` used the `parallel` package to implement parallelization, but this functionality is superseded by the aforementioned use of `BiocParallel`. We recommend that users who previously relied on these arguments switch to `BPPARAM = MulticoreParam(workers = mc.cores, progressbar = TRUE)`.

**Progress monitoring:** A useful feature of `BiocParallel` are progress bars to monitor the status of long-running jobs, such as `BSmooth()`. Progress bars are controlled via the `progressbar` argument in the `BiocParallelParam` constructor. Progress bars replace the use of the deprecated `verbose` argument to print out information on the status of `BSmooth()`.

`BiocParallel` also supports extensive and detailed logging facilities. Please consult the `BiocParallel` documentation to take full advantage these advanced features.

**Author(s)**

Method and original implementation by Kasper Daniel Hansen <khansen@jhsph.edu>. Updated implementation to support disk-backed `BSseq` objects and more general parallelization by Peter Francis Hickey.

**References**


**See Also**

`locfit` in the `locfit` package, as well as `BSseq`.

**Examples**

```r
## Not run:
# Run BSmooth() on a matrix-backed BSseq object using an in-memory realization
# backend with serial evaluation.
data(BS.chr22)
# This is a matrix-backed BSseq object.
sapply(assays(BS.chr22, withDimnames = FALSE), class)
BS.fit <- BSmooth(BS.chr22, BPPARAM = SerialParam(progressbar = TRUE))
# The new 'coef' assay is an ordinary matrix.
sapply(assays(BS.fit, withDimnames = FALSE), class)
BS.fit

# Run BSmooth() on a disk-backed BSseq object using the HDF5Array realization
# backend (with data written to the file 'BSmooth_example.h5') with
# multi-core parallel evaluation.
```
BS.chr22 <- realize(BS.chr22, "HDF5Array")
# This is a disk-backed BSseq object.
sapply(assays(BS.chr22, withDimnames = FALSE), class)
BS.fit <- BSmooth(BS.chr22,
  BPPARAM = MulticoreParam(workers = 2, progressbar = TRUE),
  BACKEND = "HDF5Array",
  filepath = "BSmooth_example.h5")
# The new 'coef' assay is an HDF5Matrix.
sapply(assays(BS.fit, withDimnames = FALSE), class)
BS.fit
# The new 'coef' assay is in the HDF5 file 'BSmooth_example.h5' (in the # current working directory).
sapply(assays(BS.fit, withDimnames = FALSE), path)

## End(Not run)

BSmooth.fstat

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

Description

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

Usage

BSmooth.fstat(BSseq, design, contrasts, verbose = TRUE)

Arguments

- **BSseq**: An object of class BSseq.
- **design**: The design matrix of the bisulfite-sequencing experiment, with rows corresponding to arrays and columns to coefficients to be estimated.
- **contrasts**: Numeric matrix with rows corresponding to columns in design and columns containing contrasts. May be a vector if there is only one contrast.
- **verbose**: Should the function be verbose?

Details

TODO

Value

An object of class **BSseqStat**.

Author(s)

Kasper Daniel Hansen <khansen@jhsph.edu>
See Also

BSmooth for the input object and BSseq for its class. BSseqStat describes the return class. This function is likely to be followed by the use of smoothSds, computeStat, and dmrFinder.

Examples

```r
if(require(bsseqData)) {
  # limma required for makeContrasts()
  library(limma)
  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
  ## TODO: Kind of a forced example
  design <- model.matrix(~0 + BS.cancer.ex.fit$Type)
  colnames(design) <- gsub("BS\".cancer\".ex\".fit\".Type", "",
                         colnames(design))
  contrasts <- makeContrasts(
    cancer_vs_normal = cancer - normal,
    levels = design
  )
  BS.stat <- BSmooth.fstat(BS.cancer.ex.fit[keepLoci.ex,],
                           design,
                           contrasts)
  BS.stat

  #---------------------------------------------------------------------------
  # An example using a HDF5Array-backed BSseq object
  #
  library(HDF5Array)
  # See ?SummarizedExperiment::saveHDF5SummarizedExperiment for details
  hdf5_BS.cancer.ex.fit <- saveHDF5SummarizedExperiment(
    x = BS.cancer.ex.fit[keepLoci.ex, ],
    dir = tempfile())
  hdf5_BS.stat <- BSmooth.fstat(hdf5_BS.cancer.ex.fit,
                                design,
                                contrasts)
  hdf5_BS.stat
}
```

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.
BSmooth.tstat

Usage

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?

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

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-like object of methylation evidence (see 'Details' below).
Cov     A matrix-like object of coverage (see 'Details' below).
coef    A matrix-like object of smoothing estimates (see 'Details' below).
se.coef A matrix-like object of smoothing standard errors (see 'Details' below).
trans   A smoothing transformation.
parameters A list of smoothing parameters.
pData   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

The 'M', 'Cov', 'coef', and 'se.coef' matrix-like objects will be coerced to DelayedMatrix objects; see ?DelayedArray::DelayedMatrix for the full list of supported matrix-like objects. We recommend using matrix objects for in-memory storage of data and HDF5Matrix for on-disk storage of data.

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>

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
```

# An example using a HDF5Array-backed BSseq object

```r
library(HDF5Array)
hdf5_M <- realize(M, "HDF5Array")
hdf5_Cov <- realize(Cov, "HDF5Array")
hdf5_BS1 <- BSseq(chr = c("chr1", "chr2", "chr1"),
                  pos = c(1, 2, 3),
                  M = hdf5_M,
                  Cov = hdf5_Cov,
                  sampleNames = c("A", "B", "C"))
hdf5_BS1
hdf5_BS2 <- BSseq(chr = c("chr1", "chr1", "chr1"),
                  pos = c(1, 1, 1),
                  M = hdf5_M,
                  Cov = hdf5_Cov,
                  sampleNames = c("A", "B", "C"))
hdf5_BS2
```

---

**BSseq-class**

**Class BSseq**

**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 RangedSummarizedExperiment and therefore inherits a number of useful GRanges methods that operate on the rowRanges 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, BACKEND = NULL) This function function is a faster way of using combine on multiple BSseq objects. The input is a list, with each component an object of class BSseq. The (slower) alternative is to use Reduce(combine, list).

The BACKEND argument determines which backend should be used for the 'M' and 'Cov' matrices and, if present, the 'coef' and 'se.coef' matrices (the latter two can only be combined if all objects have the same rowRanges). The default, BACKEND = NULL, corresponds to using matrix objects. See ?DelayedArray::setAutoRealizationBackend for alternative backends.

strandCollapse(BSseq, shift = TRUE) This function operates on a BSseq objects which has stranded loci (i.e. loci where the strand is one of ‘+’ or ‘-’). It will collapse the methylation and coverage information across the two strands, unstranding the loci in the process and potentially re-ordering them.

The argument shift indicates whether the positions for the loci on the reverse strand should be shifted one (i.e. 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 versions 1.5.2 and 1.11.1 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)

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 BSmooth for smoothing the bisulfite sequence data.

Examples

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

# An example using a HDF5-backed BSseq object
#
```r
hdf5_BStest <- realize(BSseq, "HDF5Array")
chrSelectBSseq(hdf5_BStest, seqnames = "chr1", order = TRUE)
collapseBSseq(
```
BSseqStat-class

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BSseq = hdf5_BStest,
group = c("A", "A", "B"),
BACKEND = "HDF5Array",
type = "integer"

BSseqStat-class

Class BSseqStat

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

Usage
BSseqStat(gr = NULL, stats = NULL, parameters = NULL)

Arguments
gr
The genomic locations as an object of class GRanges.

stats
The statistics, as a list of matrix-like objects (see 'Details' below).

parameters
A list of parameters.

Details
The matrix-like elements of the list in the 'stats' slot will be coerced to DelayedMatrix objects; see ?DelayedArray::DelayedMatrix for the full list of supported matrix-like objects. We recommend using matrix objects for in-memory storage of data and HDF5Matrix for on-disk storage of data.

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

Slots
stats: This is a list of DelayedMatrix objects with list elements 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.
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.

Coercion

Package version 1.11.1 introduced a new version of representing ‘BSseqStat’ objects. You can update old serialized (saved) objects by invoking x <- updateObject(x).

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-like object (see ’Details’ below).
parameters A list of parameters.

Details

The ’stats’ matrix-like object will be coerced to a DelayedMatrix object; see ?DelayedArray::DelayedMatrix for the full list of supported matrix-like objects. We recommend using matrix objects for in-memory storage of data and HDF5Matrix for on-disk storage of data.
BSseqTstat-class

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 DelayedMatrix object 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.

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.

Coercion

Package version 1.11.1 introduced a new version of representing ‘BSseqTstat’ objects. You can update old serialized (saved) objects by invoking x <- updateObject(x).

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.
computeStat

Compute a test statistic based on smoothed whole-genome bisulfite sequencing data.

Description

Compute a test statistic based on smoothed whole-genome bisulfite sequencing data.

Usage

```
computeStat(BSseqStat, coef = NULL)
```

Arguments

- `BSseqStat`: An object of class `BSseqStat`, typically an object returned by `smoothSds(...)` and not constructed by the user.
- `coef`: A vector indicating for which coefficients the statistic is to be computed (coef = NULL corresponds to testing all coefficients). If the length of the coef is 1 then the corresponding t-statistic is computed, otherwise the corresponding F-statistic is computed.

Details

TODO

Value

An object of class `BSseqStat`. More specifically, the input `BSseqStat` object with the computed statistics added to the `stats` slot (accessible with `getStats`).

Author(s)

Kasper Daniel Hansen <khansen@jhsph.edu>

See Also

- `smoothSds` for the function to create the appropriate `BSseqStat` input object. `BSseqStat` also describes the return class. This function is likely to be followed by the use of `dmrFinder`.

Examples

```
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
  ## TODO: Kind of a forced example
  design <- model.matrix(~0 + BS.cancer.ex.fit$Type)
```
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,
strant = 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 `BSmooth.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.
findLoci

Find methylation loci in a genome

Description

This is a convenience function to find methylation loci, such as CpGs, in a reference genome. The result is useful as the value of the loci argument for read.bismark().

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

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)
}

findLoci
findLoci

Usage

findLoci(pattern,
subject,
include = seqlevels(subject),
strand = c("*", "+", "-"),
fixed = "subject",
resize = TRUE)

Arguments

pattern A string specifying the pattern to search for, e.g. "CG". Can contain IUPAC ambiguity codes, e.g., "CH".
subject A string containing a file path to the genome sequence, in FASTA or 2bit format, to be searched. Alternatively, a BSgenome or DNAStringSet object storing the genome sequence to be searched.
include A character vector indicating the seqlevels of subject to be used.
strand A character scaler specifying the strand of subject to be used. If strand = "+", then both the positive (strand = "+") and negative (strand = "-" strands will be searched.) It is assumed that subject contains the sequence with respect to the + strand.
fixed If "subject" (the default), IUPAC ambiguity codes in the pattern only are interpreted as wildcards, e.g., a pattern containing CH matches a subject containing CA but not vice versa. See ?Biostrings::lowlevel-matching for more information
resize A logical scalar specifying whether the ranges should be shifted to have width 1 and anchored by the start of the locus, e.g., resize a CpG dinucleotide to give the co-ordinates of the cytosine.

Details

This function provides a convenience wrapper for finding methylation loci in a genome, based on running vmatchPattern(). Users requiring finer-grained control should directly use the vmatchPattern() function and coerce the result to a GRanges object.

Value

A GRanges object storing the found loci.

Author(s)

Peter F. Hickey

See Also

- Biostrings::vmatchPattern()
- ?BSgenome::"BSgenome-utils"
**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.

Examples

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

# An example using a HDF5Array-backed BSseq object

```r
library(HDF5Array)
# See ?SummarizedExperiment::saveHDF5SummarizedExperiment for details
hdf5_BStest <- saveHDF5SummarizedExperiment(x = BSseq,
                                          dir = tempfile())
results <- fisherTests(hdf5_BStest,
                        group1 = "A1",
                        group2 = "A2",
                        returnLookup = TRUE)
results
```
Description

Classes for fixed-width IRanges and GRanges, i.e. objects where all ranges have the same width. The intention is for these classes to be added to GenomicRanges. Documented here temporarily.

Details

See description. Otherwise works like IRanges and GRanges, except there are many unimplemented methods.

This is really a private class, with private methods, but R’s NAMESPACE handling means they get unintentionally exported. Hence this documentation.

Examples

```
showClass("FWIRanges")
```

getCoverage

Obtain coverage for BSseq objects.

Description

Obtain coverage for BSseq objects.

Usage

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

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.
- **withDimnames**: A logical(1), indicating whether dimnames should be applied to extracted coverage elements. Setting withDimnames = FALSE increases the speed and memory efficiency with which coverage is extracted.
Value

NOTE: The return type of `getCoverage` varies depending on its arguments.

If regions are not specified (`regions = NULL`) a `DelayedMatrix` object (`what = "perBase"`) is returned. This will either contain the per-base coverage, the average coverage, or the genome total coverage (depending on value of `what`).

If `what = "perBase"` and regions are specified, a list is returned. Each element of the list is a `DelayedMatrix` object 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 `DelayedMatrix` object. Each row of the `DelayedMatrix` corresponds to a region and contains either the average coverage or the total 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]])

# An example using a HDF5Array-backed BSseq object
#
library(HDF5Array)
# See ?SummarizedExperiment::saveHDF5SummarizedExperiment for details
hdf5_BS.chr22 <- saveHDF5SummarizedExperiment(x = BS.chr22,
                                           dir = tempfile())
head(getCoverage(hdf5_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(hdf5_BS.chr22, regions = reg, what = "perRegionAverage")
hdf5_cList <- getCoverage(hdf5_BS.chr22, regions = reg)
length(hdf5_cList)
head(hdf5_cList[[1]])
getMeth

Obtain methylation estimates for BSseq objects.

Description

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

Usage

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

Arguments

BSseq An object of class BSseq.
regions An optional data.frame or GenomicRanges object specifying a number of geno-
mic 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.
algebra alpha value for the confidence interval.
withDimnames A logical(1), indicating whether dimnames should be applied to extracted
coverage elements. Setting withDimnames = FALSE increases the speed and
memory efficiency with which coverage is extracted.

Value

NOTE: The return type of getMeth varies depending on its arguments.
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 DelayedMatrix (confint = FALSE or a list with three DelayedMatrix 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 algorithm (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 DelayedMatrix corresponding to a genomic region (and each row of the DelayedMatrix being a loci inside the region). If what = "perRegion" the function returns a DelayedMatrix, 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".
Author(s)

Kasper Daniel Hansen <khansen@jhsph.edu>.

References


See Also

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

Examples

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

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>

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

Examples
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
poissonGoodnessOfFit(BSseq, nQuantiles = 10^5)
binomialGoodnessOfFit(BSseq, method = c("MLE"), nQuantiles = 10^5)
## S3 method for class 'chisqGoodnessOfFit'
print(x, ...)  
## S3 method for class 'chisqGoodnessOfFit'
plot(x, type = c("chisq", "pvalue"), plotCol = TRUE, qqline = TRUE, 
      pch = 16, cex = 0.75, ...)
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

```r
if(require(bsseqData)) {
  data(BS.cancer.ex)
  BS.cancer.ex <- updateObject(BS.cancer.ex)
  gof <- poissonGoodnessOfFit(BS.cancer.ex)
  plot(gof)
}
```

# An example using a HDF5Array-backed BSseq object
hasGRanges-class

### Description

A class with a GRanges slot, used as a building block for other classes. Provides basic accessor functions etc.

### Objects from the Class

Objects can be created by calls of the form `new("hasGRanges", ...)`.

### Slots

- `gr`: Object of class `GRanges`.

### Methods

- `"["` Subsets a single dimension.
- `granges` Get the GRanges object representing genomic locations.
- `start,start<-,end,end<-,width,width<-` Start, end and width for the genomic locations of the object, also replacement functions. This accessor functions operate directly on the `gr` slot.
- `strand,strand<-` Getting and setting the strand of the genomic locations (the `gr` slot).
- `seqlengths,seqlengths<-` Getting and setting the seqlengths of the genomic locations (the `gr` slot).
- `seqlevels,seqlevels<-` Getting and setting the seqlevels of the genomic locations (the `gr` slot).
- `seqnames,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 will automatically extend.

Author(s)

Kasper Daniel Hansen <khansen@jhsph.edu>

Examples

showClass("hasGRanges")

Internals

Internals

Description

The matrixOrNULL class is a class union, representing a slot which is either a matrix or NULL (representing missing information).

plotRegion

Plotting BSsmooth methylation estimates

Description

Functions for plotting BSsmooth 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, 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)

plotManyRegions(BSseq, regions = NULL, extend = 0, main = "", 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

read.bismark(files,
    loci = NULL,
    colData = NULL,
    rmZeroCov = FALSE,
    strandCollapse = TRUE,
    BPPARAM = bpparam(),
    BACKEND = NULL,
    dir = tempfile("BSseq"),
    replace = FALSE,
    chunkdim = NULL,
    level = NULL,
    nThread = 1L,
    verbose = getOption("verbose"))

Arguments

files The path to the files created by running Bismark’s methylation extractor, one sample per file. Files ending in .gz or .bz2 will be automatically decompressed to tempfile(). We strongly recommend you use the "genome wide cytosine report" output files. See section 'File formats' for further details.

loci NULL (default) or a GenomicRanges instance containing methylation loci (all with width equal to 1). If loci = NULL, then read.bismark() will perform a first pass over the Bismark file to identify candidate loci. If loci is a GenomicRanges instance, then these form the candidate loci. In either case, the candidate loci will be filtered if rmZeroCov = TRUE and collapsed if strandCollapse = TRUE to form the final set of methylation loci that form the rowRanges of the returned BSseq object. See section 'Efficient use of read.bismark()' for further details.

colData An optional DataFrame describing the samples. Row names, if present, become the column names of the BSseq object. If NULL, then a DataFrame will be created with files used as the row names.

rmZeroCov A logical(1) indicating whether methylation loci that have zero coverage in all samples be removed. For several reasons, the default rmZeroCov = FALSE is recommended even in cases where you ultimately want to remove such loci. See section 'Efficient use of read.bismark()' for further details.
strandCollapse: A logical(1) indicating whether strand-symmetric methylation loci (i.e., CpGs) should be collapsed across strands. This is only applicable for stranded methylation loci, e.g., loci extracted from 'genome wide cytosine reports' (see section 'File formats' for further details).

BPPARAM: An optional BiocParallelParam instance determining the parallel back-end to be used during evaluation. Currently supported are SerialParam (Unix, Mac, Windows), MulticoreParam (Unix and Mac), SnowParam (Unix, Mac, and Windows, limited to single-machine clusters), and BatchtoolsParam (Unix, Mac, Windows, only with the in-memory realization backend). See sections 'Parallelization and progress monitoring' and 'Realization backends' for further details.

BACKEND: NULL or a single string specifying the name of the realization backend. When the backend is set to NULL, the M and Cov assays are realized in memory as ordinary matrices, otherwise these are realized with the given BACKEND. See section 'Realization backends' for further details.

dir: Only applicable if BACKEND == "HDF5Array". The path (as a single string) to the directory where to save the HDF5-based BSseq object. The directory will be created so should not already exist, unless replace is set to TRUE.

replace: Only applicable if BACKEND == "HDF5Array". If directory dir already exists, should it be replaced with a new one? The content of the existing directory will be lost!

chunkdim: Only applicable if BACKEND == "HDF5Array". The dimensions of the chunks to use for writing the data to disk. By default, getHDF5DumpChunkDim() using the dimensions of the returned BSseq object will be used. See ?getHDF5DumpChunkDim for more information.

level: Only applicable if BACKEND == "HDF5Array". The compression level to use for writing the data to disk. By default, getHDF5DumpCompressionLevel() will be used. See ?getHDF5DumpCompressionLevel for more information.

nThread: The number of threads used by fread when reading the files. Be careful when combining a parallel backend specified with BPPARAM with nThread > 1 because each worker will use nThread.

verbose: A logical(1) indicating whether progress messages should be printed (default TRUE).

Value

A BSseq object.

File formats

The format of each file is automatically detected using the internal function bsseq:::.guessBismarkFileType(). Files ending in .gz, .bz2, .xz, or .zip will be automatically decompressed to tempdir().

Supported file formats: Bismark's 'genome wide cytosine report' (https://github.com/FelixKrueger/Bismark/tree/master/Docs#the-genome-wide-cytosine-report-optional-is-tab-delimited-in-the-following-format-1-based-coords) and 'coverage' (https://github.com/FelixKrueger/Bismark/tree/master/Docs#the-coverage-output-looks-like-this-tab-delimited-1-based-genomic-coords) formats are both supported. If setting loci = NULL, then we strongly recommend using the
'genome wide cytosine report' output format because this includes strand information for each locus. The 'coverage' output does not contain strand information and so the strand of the returned BSeq object will be set to * unless stranded loci are supplied.

Unsupported file formats: Neither the 'bedGraph' output format (https://github.com/FelixKrueger/Bismark/tree/master/Docs#the-bedgraph-output-optional-looks-like-this-tab-delimited) nor the 'bismark_methylation_extractor' output format (https://github.com/FelixKrueger/Bismark/tree/master/Docs#the-bismark_methylation_extractor-output-is-in-the-form-tab-delimited) are supported. The former does not include the required counts of methylated and unmethylated reads while the is an intermediate file containing read-level, rather than locus-level, data on methylation.

One-based vs. zero-based genomic co-ordinates: The genomic co-ordinates of the Bismark output files may be zero-based or one-based depending on whether the --zero_based argument was used when running Bismark's methylation extractor. Furthermore, the default co-ordinate counting 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 typically use one-based co-ordinates, we strongly recommend that your Bismark files are also one-based.

Efficient use of read.bismark()

We recommend the following to achieve fast and efficient importing of Bismark files:

- Specify the set of methylation loci via the loci argument.
- Use Bismark files in the 'coverage' output format.
- Leave rmZeroCov = FALSE.
- Use a BPPARAM with a moderate number of workers (cores).
- Use BACKEND = "HDF5Array".
- Use multiple threads per worker (i.e. nThread > 1).

Each point is discussed below.

Specifying loci: Specifying the set of methylation loci via the loci argument means that read.bismark() does not need to first parse all files to identify the set of candidate loci. Provided that rmZeroCov = FALSE, this means that each file is only read once. This may be a considerable saving when there are a large number of files.

If you are unsure whether the below-described shortcuts apply to your data, leave loci = NULL and let read.bismark() identify the set of candidate loci from files.

You may wish to use the findLoci() function to find all methylation loci of interest in your reference genome (e.g., all CpGs) and then pass the result via the loci argument. Alternatively, if all files are 'genome wide cytosine reports' for samples aligned to the same reference genome, then all files contain the exact same set of methylation loci. In this case, you may wish to first construct loci using the internal function bsseq:::.readBismarkAsFWGRanges() applied to a single file, e.g., loci = bsseq:::.readBismarkAsFWGRanges(files[1], rmZeroCov, strandCollapse).
Using the 'coverage' Bismark files: It will generally be faster to parse Bismark files in the 'coverage' output format than those in the 'genome wide cytosine report' format. This is because the former only includes loci with non-zero coverage and so the file size is often considerably smaller, particularly for shallowly sequenced samples (e.g., those from single-cell bisulfite sequencing).

Leaving rmZeroCov = FALSE: If you set rmZeroCov = TRUE, then read.bismark() must first parse all the files to identify which loci have zero coverage in all samples and then filter these out from the set of candidate loci. This will happen even if you supply loci with a GenomicRanges of candidate loci.

Furthermore, any coverage-based filtering of methylation loci is best left until you have constructed your final BSseq object. In our experience, the final BSseq object is often the product of combining multiple BSseq objects, each constructed with a separate call to read.bismark(). In such cases, it is premature to use rmZeroCov = TRUE when running each read.bismark(); regrettably, combining these objects will often then lead to an inefficiently stored BSseq object.

Using a BPPARAM with a moderate number of workers (cores): Each file can be processed on its own, so you can process in parallel as many files as you have workers. However, if using the HDF5Array backend, then writing to the HDF5 file cannot be performed in parallel and so becomes the bottleneck. Nonetheless, by using a moderate number of workers (2 - 10), we can ensure there is processed data available to write to disk as soon as the current write is completed.

Using BACKEND = "HDF5Array": By using the HDF5Array realization backend from HDF5Array, we reduce the amount of data that is kept in-memory at any one time. Once each file is parsed, the data are written to the HDF5 file and are no longer needed in-memory. When combined with multiple workers (cores), this means that each file will only need to read and retain in-memory 1 sample’s worth of data at a time.

Conversely, if you opt for all data to be in-memory (via BACKEND = NULL), then each worker will pass each file’s data back to the main process and memory usage will steadily accumulate to often unreasonable levels.

Using nThread > 1: read.bismark uses data.table::fread to read each file, which supports threaded-parallisation. Depending on the system, increasing nThread can achieve near-linear speed-ups in the number of threads for reading each file. Care needs to be taken when nThread > 1 is used in conjunction with a parallel backend via BPPARAM to ensure the system isn’t overloaded. For example, using BPPARAM = MulticoreParam(workers = 10) with nThread = 4 may use up to 40 workers simultaneously.

Realization backends

The read.bismark() function creates a BSseq object with two assays, M and Cov. The choice of realization backend controls whether these assays are stored in-memory as an ordinary matrix or on-disk as a HDF5Array, for example. The choice of realization backend is controlled by the BACKEND argument, which defaults to the current value of DelayedArray::getAutoRealizationBackend().

read.bismark() supports the following realization backends:

- NULL (in-memory): This stores each new assay in-memory using an ordinary matrix.
- HDF5Array (on-disk): This stores each new assay on-disk in a HDF5 file using an HDF5Matrix from HDF5Array.
Please note that certain combinations of realization backend and parallelization backend are currently not supported. For example, the HDF5Array realization backend is currently only compatible when used with a single-machine parallelization backend (i.e. it is not compatible with a SnowParam that specifies an ad hoc cluster of multiple machines).

BSmooth() will issue an error when given such incompatible realization and parallelization backends.

Additional arguments related to the realization backend can be passed via the ... argument. These arguments must be named and are passed to the relevant RealizationSink constructor. For example, the ... argument can be used to specify the path to the HDF5 file to be used by BSmooth(). Please see the examples at the bottom of the page.

Parallelization, progress monitoring, and logging

read.bismark() now uses the BiocParallel package to implement parallelization. This brings some notable improvements:

- Imported files can now be written directly to an on-disk realization backend by the worker. This dramatically reduces memory usage compared to previous versions of bsseq that required all results be retained in-memory.
- Parallelization is now supported on Windows through the use of a SnowParam object as the value of BPPARAM.
- Detailed and extensive job logging facilities.

All parallelization options are controlled via the BPPARAM argument. In general, we recommend that users combine multicore (single-machine) parallelization with an on-disk realization backend (see section, 'Realization backend'). For Unix and Mac users, this means using a MulticoreParam. For Windows users, this means using a single-machine SnowParam. Please consult the BiocParallel documentation to take full advantage of the more advanced features.

A useful feature of BiocParallel are progress bars to monitor the status of long-running jobs, such as BSmooth(). Progress bars are controlled via the progressbar argument in the BiocParallelParam constructor.

BiocParallel also supports extensive and detailed logging facilities. Please consult the BiocParallel documentation to take full advantage these advanced features.

Author(s)

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 collapsing (aggregating) data from sample’s with multiple Bismark methylation extractor files (e.g., technical replicates).
Examples

# Run read.bismark() on a single sample to construct a matrix-backed BSseq
# object.
infile <- system.file("extdata/test_data.fastq_bismark.bismark.cov.gz", 
    package = "bsseq")
bsseq <- read.bismark(files = infile, 
    colData = DataFrame(row.names = "test_data"), 
    rmZeroCov = FALSE, 
    strandCollapse = FALSE, 
    verbose = TRUE)
# This is a matrix-backed BSseq object.
sapply(assays(bsseq, withDimnames = FALSE), class)
bsseq

## Not run:
# Run read.bismark() on a single sample to construct a HDF5Array-backed BSseq
# object (with data written to 'test_dir')
test_dir <- tempfile("BSseq")
bsseq <- read.bismark(files = infile, 
    colData = DataFrame(row.names = "test_data"), 
    rmZeroCov = FALSE, 
    strandCollapse = FALSE, 
    BACKEND = "HDF5Array", 
    dir = test_dir, 
    verbose = TRUE)
# This is a HDF5Array-backed BSseq object.
sapply(assays(bsseq, withDimnames = FALSE), class)
# The 'M' and 'Cov' assays are in the HDF5 file 'assays.h5' (in 'test_dir').
sapply(assays(bsseq, withDimnames = FALSE), path)

## End(Not run)

read.bsmooth Parsing output from the BSmooth alignment suite

Description

Parsing output from the BSmooth alignment suite.

Usage

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.

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.umtab(dirs, sampleNames = NULL, rmZeroCov = FALSE,
          pattern = NULL, keepU = c("U10", "U20", "U30", "U40"),
          keepM = c("M10", "M20", "M30", "M40"), verbose = TRUE)

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’
smoothSds

Author(s)

Kasper Daniel Hansen <khansen@jhsph.edu>

See Also

read.bsmooth.

Examples

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

smoothSds

Smooth the standard deviations using a thresholded running mean based on smoothed whole-genome bisulfite sequencing data.

Description

Smooth the standard deviations using a thresholded running mean based on smoothed whole-genome bisulfite sequencing data.

Usage

smoothSds(BSseqStat, k = 101, qSd = 0.75, mc.cores = 1, maxGap = 10^8, verbose = TRUE)

Arguments

BSseqStat An object of class BSseqStat, typically an object returned by BSsmooth.fstat(...) and not constructed by the user.
k A positive scalar, see details.
qSd A scalar between 0 and 1, 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.
maxGap A scalar greater than 0, see details.
verbose Should the function be verbose?

Details

The standard deviation estimates are 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.
Value

An object of class BSseqStat. More specifically, the input BSseqStat object with the computed statistics added to the stats slot (accessible with getStats).

Author(s)

Kasper Daniel Hansen <khansen@jhsph.edu>

See Also

BS_smooth.fstat for the function to create the appropriate BSseqStat input object. BSseqStat also describes the return class. This function is likely to be followed by the use of computeStat.

Examples

```r
if(require(bsseqData)) {
    # library(limma) required for makeContrasts()
    library(limma)
    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
    ## TODO: Kind of a forced example
    design <- model.matrix(~0 + BS.cancer.ex.fit$Type)
    colnames(design) <- gsub("BS\\.cancer\\.ex\\.fit\$Type", ",
        colnames(design))
    contrasts <- makeContrasts(
        cancer_vs_normal = cancer - normal,
        levels = design
    )
    BS.stat <- BSmooth.fstat(BS.cancer.ex.fit[keepLoci.ex,],
                           design,
                           contrasts)
    BS.stat <- smoothSds(BS.stat)
    ## Comparing the raw standard deviations to the smoothed standard
    ## deviations
    summary(getStats(BS.stat, what = "rawSds"))
    summary(getStats(BS.stat, what = "smoothSds"))
}
```
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