

Package ‘methylSig’

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Title MethylSig: Differential Methylation Testing for WGBS and RRBS Data

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Description MethylSig is a package for testing for differentially methylated cytosines (DMCs) or regions (DMRs) in whole-genome bisulfite sequencing (WGBS) or reduced representation bisulfite sequencing (RRBS) experiments. MethylSig uses a beta binomial model to test for significant differences between groups of samples. Several options exist for either site-specific or sliding window tests, and variance estimation.

Depends R (>= 3.6)

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Suggests BiocStyle, bsseqData, knitr, rmarkdown, testthat (>= 2.1.0), covr

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BugReports <https://github.com/sartorlab/methylSig/issues>

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bsseq_destranded	<i>BSseq object read from destranded coverage files</i>
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Description

Data contains 6 methylation loci and 2 samples

Usage

`bsseq_destranded`

Format

A BSseq object

Source

`data/02-create_bsseq_rda.R`

Examples

```
data(bsseq_destranded, package = 'methylSig')
```

bsseq_multichrom *BSseq object with loci on multiple chromosomes*

Description

Data contains 4 methylation loci for 2 samples on 2 chromosomes

Usage

`bsseq_multichrom`

Format

A BSseq object

Source

`data-raw/02-create_bsseq_rda.R`

Examples

`data(bsseq_multichrom, package = 'methylSig')`

bsseq_stranded *BSseq object read from stranded coverage files*

Description

Data contains 11 methylation loci and 2 samples

Usage

`bsseq_stranded`

Format

A BSseq object

Source

`data-raw/02-create_bsseq_rda.R`

Examples

`data(bsseq_stranded, package = 'methylSig')`

diff_binomial	<i>Differential methylation analysis using binomial model</i>
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Description

This function calculates differential methylation statistics using a binomial-based approach. See ‘Warning’ message below.

Usage

```
diff_binomial(bs, group_column, comparison_groups)
```

Arguments

bs	A BSseq-class object to calculate differential methylation statistics. See <code>methylSigReadData</code> for how to read in methylation data.
group_column	a character string indicating the column of <code>pData(bs)</code> to use for determining group membership.
comparison_groups	a named character vector indicating the case and control factors of <code>group_column</code> for the comparison.

Details

This function uses a binomial-based model to calculate differential methylation statistics. It is nearly identical to the `methylKit::calculateDiffMeth` function in the `methylKit` R package except that only the likelihood ratio test and `p.adjust(..., method='BH')` are used to calculate significance levels. It is significantly faster than `methylKit::calculateDiffMeth` function.

Value

A GRanges object containing the following mcols:

- meth_case:** Methylation estimate for case.
- meth_control:** Methylation estimate for control.
- meth_diff:** The difference `meth_case - meth_control`.
- direction:** The group for which the locus is hyper-methylated. Note, this is not subject to significance thresholds.
- pvalue:** The p-value from the t-test (`t_approx = TRUE`) or the Chi-Square test (`t_approx = FALSE`).
- fdr:** The Benjamini-Hochberg adjusted p-values using `p.adjust(method = 'BH')`.
- log_likelihood_ratio:** The log likelihood ratio.

Warning

This function does not take into account the variability among samples in each group being compared.

Examples

```
data(BS.cancer.ex, package = 'bsseqData')

bs = filter_loci_by_group_coverage(
  bs = BS.cancer.ex,
  group_column = 'Type',
  c('cancer' = 2, 'normal' = 2))

small_test = bs[1:50]

diff_gr = diff_binomial(
  bs = small_test,
  group_column = 'Type',
  comparison_groups = c('case' = 'cancer', 'control' = 'normal'))
```

diff_dss_fit

Performs model fit for general experimental design

Description

This function is a wrapper for DSS::DMLfit.multiFactor.

Usage

```
diff_dss_fit(bs, design, formula)
```

Arguments

bs	a BSseq object to calculate differential methylation statistics.
design	a data.frame or DataFrame for experimental design. Should contain as many rows as there are columns (samples) in bs, and the order of the rows should match the columns of bs. If omitted, will default to pData(bs).
formula	a formula for the linear model. It should refer to column names from design. NOTE: The intercept is included by default if omitted. One can omit the intercept with a formula such as ' $\sim 0 + \text{group}$ '. For clarity, it helps to include the intercept explicitly as in ' $\sim 1 + \text{group}$ '.

Value

A list object with:

gr: a GRanges object with loci fit.

design: the data.frame input as the experimental design.

formula: the formula representing the model. Can be character or formula.

X: the design matrix used in regression based on the design and formula. This should be consulted to determine the appropriate contrast to use in dss_fit_test().

fit: a list with model fitting results. It has components beta, the estimated coefficients, and var.beta the estimated variance/covariance matrix for beta.

Examples

```
data(BS.cancer.ex, package = 'bsseqData')

bs = filter_loci_by_group_coverage(
  bs = BS.cancer.ex,
  group_column = 'Type',
  c('cancer' = 2, 'normal' = 2))

small_test = bs[1:50]

diff_fit = diff_dss_fit(
  bs = small_test,
  design = bsseq::pData(bs),
  formula = '~ Type')
```

diff_dss_test

Calculates differential methylation statistics under general experimental design

Description

This function is a wrapper for DSS::DMLtest.multiFactor with the added feature of reporting methylation rates alongside the test results via the `methylation_group_column` and `methylation_groups` parameters. See documentation below.

Usage

```
diff_dss_test(
  bs,
  diff_fit,
  contrast,
  methylation_group_column = NA,
  methylation_groups = NA
)
```

Arguments

<code>bs</code>	a BSseq, the same used used to create <code>diff_fit</code> .
<code>diff_fit</code>	a list object output by <code>diff_dss_fit()</code> .
<code>contrast</code>	a contrast matrix for hypothesis testing. The number of rows should match the number of columns <code>design</code> . Consult <code>diff_fit\$X</code> to ensure the contrast corresponds to the intended test.
<code>methylation_group_column</code>	Optionally, a column from <code>diff_fit\$design</code> by which to group samples and capture methylation rates. This column can be a <code>character</code> , <code>factor</code> , or <code>numeric</code> . In the case of <code>numeric</code> the samples are grouped according to the top and bottom

25 percentiles of the covariate, and the mean methylation for each group is calculated. If not a numeric, use the `methylation_groups` parameter to specify case and control.

`methylation_groups`

Optionally, a named character vector indicating the case and control factors of `methylation_group_column` by which to group samples and capture methylation rates. If specified, must also specify `methylation_group_column`.

Value

A GRanges object containing the following mcols:

stat: The test statistic.

pvalue: The p-value.

fdr: The Benjamini-Hochberg adjusted p-values using `p.adjust(method = 'BH')`.

If `methylation_group_column` is specified, also the following mcols:

meth_case: Methylation estimate for case.

meth_control: Methylation estimate for control.

meth_diff: The difference `meth_case - meth_control`.

direction: The group for which the locus is hyper-methylated. Note, this is not subject to significance thresholds.

Examples

```
data(BS.cancer.ex, package = 'bsseqData')

bs = filter_loci_by_group_coverage(
  bs = BS.cancer.ex,
  group_column = 'Type',
  c('cancer' = 2, 'normal' = 2))

small_test = bs[1:50]

diff_fit = diff_dss_fit(
  bs = small_test,
  design = bsseq::pData(bs),
  formula = ' ~ Type')

result = diff_dss_test(
  bs = small_test,
  diff_fit = diff_fit,
  contrast = matrix(c(0,1), ncol = 1)
)

result_with_meth = diff_dss_test(
  bs = small_test,
  diff_fit = diff_fit,
  contrast = matrix(c(0,1), ncol = 1),
  methylation_group_column = 'Type',
```

```

methylation_groups = c('case' = 'cancer', 'control' = 'normal')
)

```

diff_methylsig	<i>Calculates differential methylation statistics using a Beta-binomial approach</i>
----------------	--

Description

The function calculates differential methylation statistics between two groups of samples using a beta-binomial approach to calculate differential methylation statistics, accounting for variation among samples within each group. The function can be applied to a BSseq object subjected to `filter_loci_by_coverage()`, `filter_loci_by_snps()`, `filter_loci_by_group_coverage()` or any combination thereof. Moreover, the function can be applied to a BSseq object which has been tiled with `tile_by_regions()` or `tile_by_windows()`.

Usage

```

diff_methylsig(
  bs,
  group_column,
  comparison_groups,
  disp_groups,
  local_window_size = 0,
  local_weight_function,
  t_approx = TRUE,
  n_cores = 1
)

```

Arguments

<code>bs</code>	a BSseq object.
<code>group_column</code>	a character string indicating the column of <code>pData(bs)</code> to use for determining group membership.
<code>comparison_groups</code>	a named character vector indicating the case and control factors of <code>group_column</code> for the comparison.
<code>disp_groups</code>	a named logical vector indicating the whether to use <code>case</code> , <code>control</code> , or both to estimate the dispersion.
<code>local_window_size</code>	an integer indicating the size of the window for use in determining local information to improve mean and dispersion parameter estimations. In addition to a the distance constraint, a maximum of 5 loci upstream and downstream of the locus are used. The default is 0, indicating no local information is used.

local_weight_function	a weight kernel function. The default is the tri-weight kernel function defined as function(u) = (1-u^2)^3. The domain of any given weight function should be [-1,1], and the range should be [0,1].
t_approx	a logical value indicating whether to use squared t approximation for the likelihood ratio statistics. Chi-square approximation (t_approx = FALSE) is recommended when the sample size is large. Default is TRUE.
n_cores	an integer denoting how many cores should be used for differential methylation calculations.

Value

A GRanges object containing the following ncols:

- meth_case:** Methylation estimate for case.
- meth_control:** Methylation estimate for control.
- meth_diff:** The difference meth_case - meth_control.
- direction:** The group for which the locus is hyper-methylated. Note, this is not subject to significance thresholds.
- pvalue:** The p-value from the t-test (t_approx = TRUE) or the Chi-Square test (t_approx = FALSE).
- fdr:** The Benjamini-Hochberg adjusted p-values using p.adjust(method = 'BH').
- disp_est:** The dispersion estimate.
- log_likelihood_ratio:** The log likelihood ratio.
- df:** Degrees of freedom used when t_approx = TRUE.

Examples

```
data(BS.cancer.ex, package = 'bsseqData')

bs = filter_loci_by_group_coverage(
  bs = BS.cancer.ex,
  group_column = 'Type',
  c('cancer' = 2, 'normal' = 2))

small_test = bs[seq(50)]

diff_gr = diff_methylsig(
  bs = small_test,
  group_column = 'Type',
  comparison_groups = c('case' = 'cancer', 'control' = 'normal'),
  disp_groups = c('case' = TRUE, 'control' = TRUE),
  local_window_size = 0,
  t_approx = TRUE,
  n_cores = 1)
```

filter_loci_by_coverage*Filter BSseq object by coverage*

Description

Used after `bsseq::read.bismark` to mark loci in samples below `min_count` or above `max_count` to 0. These loci will then be removed prior to differential analysis by `filter_loci_by_group_coverage()` if there are not a sufficient number of samples with appropriate coverage.

Usage

```
filter_loci_by_coverage(bs, min_count = 5, max_count = 500)
```

Arguments

<code>bs</code>	a BSseq object resulting from <code>bsseq::read.bismark</code> or constructed manually by the user.
<code>min_count</code>	an integer giving the minimum coverage required at a locus.
<code>max_count</code>	an integer giving the maximum coverage allowed at a locus.

Value

A BSseq object with samples/loci in the coverage and methylation matrix set to 0 where the coverage was less than `min_count` or greater than `max_count`. The number of samples and loci are conserved.

Examples

```
bis_cov_file1 = system.file('extdata', 'bis_cov1 cov', package = 'methylSig')
bis_cov_file2 = system.file('extdata', 'bis_cov2 cov', package = 'methylSig')
test = bsseq::read.bismark(
  files = c(bis_cov_file1, bis_cov_file2),
  colData = data.frame(row.names = c('test1', 'test2')),
  rmZeroCov = FALSE,
  strandCollapse = FALSE
)
test = filter_loci_by_coverage(bs = test, min_count = 10, max_count = 500)
```

filter_loci_by_group_coverage

Filter loci based on coverage threshold per sample per group

Description

An optional function to remove loci not satisfying coverage thresholds from filter_loci_by_coverage in a minimum number of samples per group.

Usage

```
filter_loci_by_group_coverage(bs, group_column, min_samples_per_group)
```

Arguments

<code>bs</code>	a BSseq object.
<code>group_column</code>	a character string indicating the column of pData(bs) to use for determining group membership.
<code>min_samples_per_group</code>	a named integer vector indicating the minimum number of samples with non-zero coverage required for maintaining a locus.

Details

The filter_loci_by_coverage function marked locus/sample pairs in the coverage matrix as 0 if said pair had coverage less than `minCount` or more than `maxCount`. This function enforces a threshold on the minimum number of samples per group required for a locus to be tested in downstream testing functions.

Value

A BSseq object with only those loci having `min_samples_per_group`.

Examples

```
data(BS.cancer.ex, package = 'bsseqData')

filter_loci_by_group_coverage(
  bs = BS.cancer.ex,
  group_column = 'Type',
  min_samples_per_group = c('cancer' = 3, 'normal' = 3)
)
```

filter_loci_by_location

Remove loci by overlap with a GRanges object

Description

A function to remove loci from a BSseq object based on intersection with loci in a GRanges object.

Usage

```
filter_loci_by_location(bs, gr)
```

Arguments

bs	a BSseq object.
gr	a GRanges object.

Value

A BSseq object with loci intersecting gr removed.

Examples

```
data(bsseq_stranded, package = 'methylSig')
regions = GenomicRanges::GRanges(
  seqnames = c('chr1','chr1','chr1','chr1'),
  ranges = IRanges::IRanges(
    start = c(5,25,45,70),
    end = c(15,40,55,80)
  )
)
filtered = filter_loci_by_location(bs = bsseq_stranded, gr = regions)
```

Description

MethylSig is a package for testing for differentially methylated cytosines (DMCs) or regions (DMRs) in whole-genome bisulfite sequencing (WGBS) or reduced representation bisulfite sequencing (RRBS) experiments. MethylSig uses a beta binomial model to test for significant differences between groups of samples. Several options exist for either site-specific or sliding window tests, and variance estimation.

methylSig functions

filter_loci_by_coverage() filter_loci_by_snps() tile_by_regions() tile_by_windows() filter_loci_by_group_coverage()
diff_binomial() diff_methylsig() diff_methylsig_dss() annotate_diff() visualize_diff() region_enrichment_diff()

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See Also

Useful links:

- Report bugs at <https://github.com/sartorlab/methylSig/issues>

promoters_gr

GRanges object with collapsed promoters on chr21 and chr22

Description

Data contains 1466 promoters for use in the vignette

Usage

`promoters_gr`

Format

A GRanges object

Source

`data/02-create_bsseq_rda.R`

Examples

```
data(promoters_gr, package = 'methylSig')
```

tile_by_regions	<i>Group cytosine / CpG level data into regions based on genomic regions</i>
-----------------	--

Description

An optional function to aggregate cytosine / CpG level data into regions based on a GRanges set of genomic regions.

Usage

```
tile_by_regions(bs, gr)
```

Arguments

bs	a BSseq object.
gr	a GRanges object.

Value

A BSseq object with loci of regions matching gr. Coverage and methylation read count matrices are aggregated by the sums of the cytosines / CpGs in the regions per sample.

Examples

```
data(bsseq_stranded, package = 'methylSig')
regions = GenomicRanges::GRanges(
  seqnames = c('chr1', 'chr1', 'chr1'),
  ranges = IRanges::IRanges(
    start = c(5, 35, 75),
    end = c(30, 70, 80)
  )
)
tiled = tile_by_regions(bs = bsseq_stranded, gr = regions)
```

tile_by_windows	<i>Group cytosine / CpG level data into regions based on genomic windows</i>
-----------------	--

Description

An optional function to aggregate cytosine / CpG level data into regions based on a tiling of the genome by win_size.

Usage

```
tile_by_windows(bs, win_size = 200)
```

Arguments

<code>bs</code>	a BSseq object.
<code>win_size</code>	an integer indicating the size of the tiles. Default is 200bp.

Value

A BSseq object with loci consisting of a tiling of the genome by `win_size` bp tiles. Coverage and methylation read count matrices are aggregated by the sums of the cytosines / CpGs in the regions per sample.

Examples

```
data(bsseq_stranded, package = 'methylSig')  
  
tiled = tile_by_windows(bs = bsseq_stranded, win_size = 50)
```

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