Package 'methodical'

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Title Discovering genomic regions where methylation is strongly associated with transcriptional activity

Version 1.8.0

Description

DNA methylation is generally considered to be associated with transcriptional silencing. However, comprehensive, genome-wide investigation of this relationship requires the evaluation of potentially millions of correlation values between the methylation of individual genomic loci and expression of associated transcripts in a relatively large numbers of samples. Methodical makes this process quick and easy while keeping a low memory footprint. It also provides a novel method for identifying regions where a number of methylation sites are consistently strongly associated with transcriptional expression. In addition, Methodical enables housing DNA methylation data from diverse sources (e.g. WGBS, RRBS and methylation arrays) with a
common framework, lifting over DNA methylation data between different genome builds and creating base-resolution plots of the association between DNA methylation and transcriptional activity at transcriptional start sites.

License GPL (>= 3)

BugReports https://github.com/richardheery/methodical/issues

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`methodical-package` *methodical: A one-stop shop for dealing with big DNA methylation datasets*

Description

DNA methylation is generally considered to be associated with transcriptional silencing. However, comprehensive, genome-wide investigation of this relationship requires the evaluation of potentially millions of correlation values between the methylation of individual genomic loci and expression of associated transcripts in a relatively large numbers of samples. Methodical makes this process quick and easy while keeping a low memory footprint. It also provides a novel method for identifying regions where a number of methylation sites are consistently strongly associated with transcriptional expression. In addition, Methodical enables housing DNA methylation data from diverse sources (e.g. WGBS, RRBS and methylation arrays) with a common framework, lifting over DNA methylation data between different genome builds and creating base-resolution plots of the association between DNA methylation and transcriptional activity at transcriptional start sites.

Author(s)

Richard Heery

See Also

Useful links:

- https://github.com/richardheery/methodical
- Report bugs at https://github.com/richardheery/methodical/issues

---

`.check_input_files` *Check input files have the correct number of columns specified and that columns seem to be of correct type*

Description

Check input files have the correct number of columns specified and that columns seem to be of correct type

Usage

```
.check_input_files(input_files, meth_files_columns)
```

Arguments

`input_files`	A vector of input filepaths.
`meth_files_columns`	A list specifying the columns in meth_files.

`.chunk_regions` *Split genomic regions into balanced chunks based on the number of methylation sites that they cover*

Description

Split genomic regions into balanced chunks based on the number of methylation sites that they cover

Usage

```
.chunk_regions(
  meth_rse,
  genomic_regions,
  max_sites_per_chunk = NULL,
  ncores = 1
)
```

Arguments

meth_rse	A RangedSummarizedExperiment with methylation values.
genomic_regions	A GRanges object.
max_sites_per_chunk	The maximum number of methylation sites to load into memory at once for each chunk.
ncores	The number of cores that will be used.

Value

A GRangesList where each GRanges object overlaps approximately the number of methylation sites given by max_sites_per_chunk

---

.collapse_strands *Combine values for stranded data*

Description

Combine values for stranded data

Usage

```
.collapse_strands(meth_df, sequence_context)
```

Arguments

meth_df	A data.frame with methylation data.
sequence_context	A single character string or DNAString with the sequence context of the methylation sites e.g. CG or CHG.

.create_meth_rse_from_hdf5 *Create a RangedSummarizedExperiment for methylation values already deposited in HDF5*

Description

Create a RangedSummarizedExperiment for methylation values already deposited in HDF5

Usage

```
.create_meth_rse_from_hdf5(
  hdf5_filepath,
  hdf5_dir,
  meth_sites,
  sample_metadata
)
```

Arguments

hdf5_filepath	Path to HDF5 file
hdf5_dir	The path to the HDF5 directory.
meth_sites	A sorted GRanges object with the locations of the methylation sites of interest.
sample_metadata	A data.frame with sample metadata

Value

A RangedSummarizedExperiment with methylation values

.find_tmrs_single *Find TSS-Proximal Methylation-Controlled Regulatory Sites (TMRs)*

Description

Find TSS-Proximal Methylation-Controlled Regulatory Sites (TMRs)

Usage

```
.find_tmrs_single(
  correlation_df,
  offset_length = 10,
  p_value_threshold = 0.05,
  smoothing_factor = 0.75,
  min_gapwidth = 150,
  min_meth_sites = 5
)
```

Arguments

correlation_df	A data.frame with correlation values between methylation sites and a transcript or a path to an RDS file containing such a data.frame as returned by calculateMethSiteTranscriptCors.
offset_length	Number of methylation sites added upstream and downstream of a central methylation site to form a window, resulting in a window size of 2*offset_length + 1. Default value is 10.

p_value_threshold
: The p_value cutoff to use. Default value is 0.05.

smoothing_factor
: Smoothing factor for exponential moving average. Should be a value between 0 and 1 with higher values resulting in a greater degree of smoothing. Default is 0.75.

min_gapwidth
: Merge TMRs with the same direction separated by less than this number of base pairs. Default value is 150.

min_meth_sites
: Minimum number of methylation sites that TMRs can contain. Default value is 5.

Value

A GRanges object with the location of TMRs.

Examples

```
# Load methylation-transcript correlation results for TUBB6 gene
data("tubb6_cpg_meth_transcript_cors", package = "methodical")

# Find TMRs for
tubb6_tmrs <- methodical:::.find_tmrs_single(correlation_df = tubb6_cpg_meth_transcript_cors)
print(tubb6_tmrs)
```

.make_meth_rse_setup *Perform setup for makeMethRSEFromInputFiles or makeMethRSEFromArrayFiles*

Description

Perform setup for makeMethRSEFromInputFiles or makeMethRSEFromArrayFiles

Usage

```
.make_meth_rse_setup(
  meth_files,
  meth_sites,
  sample_metadata,
  hdf5_dir,
  overwrite,
  chunkdim,
  temporary_dir,
  ...
)
```

Arguments

meth_files	A vector of paths to files with methylation values. Automatically detects if meth_files contain a header if every field in the first line is a character.
meth_sites	A GRanges object with the locations of the methylation sites of interest. Should contain separate ranges for each stand if meth_files are stranded (i.e. separate ranges for the C and G positions of CpG sites). Any positions in meth_files that are not in meth_sites are ignored.
sample_metadata	A data.frame with sample metadata to be used as colData for the RangedSummarizedExperiment.
hdf5_dir	Directory to save HDF5 file. Is created if it doesn't exist. HDF5 file is called assays.h5.
overwrite	TRUE or FALSE indicating whether to allow overwriting if hdf5_dir already exists.
chunkdim	The dimensions of the chunks for the HDF5 file.
temporary_dir	Name to give a temporary directory to store intermediate files. A directory with this name cannot already exist.
...	Additional arguments to be passed to HDF5Array::HDF5RealizationSink.

Value

A list describing the setup to be used for makeMethRSEFromInputFiles or makeMethRSEFromArrayFiles.

.set_meth_df_columns *Process a data.frame with methylation data so that it contains the correct columns*

Description

Process a data.frame with methylation data so that it contains the correct columns

Usage

```
.set_meth_df_columns(meth_df, zero_based)
```

Arguments

meth_df	A data.frame with methylation data.
zero_based	TRUE or FALSE indicating if files are zero-based.

.split_meth_file *Split data from a single input methylation file into chunks*

Description

Split data from a single input methylation file into chunks

Usage

```
.split_meth_file(
  meth_file,
  meth_files_columns,
  grid_column,
  file_count,
  parameters
)
```

Arguments

meth_file	Path to an input methylation file.
meth_files_columns	A list specifying the columns in meth_files.
grid_column	The current grid column being processed.
file_count	The number of the current file being processed.
parameters	A list of parameters for processing meth_file.

Value

Invisibly returns NULL.

.split_meth_files_into_chunks *Split data from input methylation files into chunks*

Description

Split data from input methylation files into chunks

Usage

```
.split_meth_files_into_chunks(
  meth_files,
  meth_files_columns,
  file_grid_columns,
  meth_sites_df,
  collapse_strands,
  sequence_context,
  meth_site_groups,
  temp_chunk_dirs,
  zero_based,
  decimal_places,
  BPPARAM
)
```

Arguments

meth_files	Paths to input methylation files.
meth_files_columns	A list specifying the columns in meth_files.
file_grid_columns	The grid column number for each file.
meth_sites_df	A data.table with the positions of methylation sites.
collapse_strands	TRUE or FALSE indicating whether or not to collapse data on + and - strands.
sequence_context	A single character string or DNAString with the sequence context of the methylation sites e.g. CG or CHG.
meth_site_groups	A list with the indices of the methylation sites in each group.
temp_chunk_dirs	A vector giving the temporary directory associated with each chunk.
zero_based	TRUE or FALSE indicating if files are zero-based.
decimal_places	Integer indicating the number of decimal places to round beta values to.
BPPARAM	A BiocParallelParam object.

Value

Invisibly returns NULL.

.summarize_chunk_methylation *Summarize methylation values for regions in a chunk*

Description

Summarize methylation values for regions in a chunk

Usage

```
.summarize_chunk_methylation(
  chunk_regions,
  meth_rse,
  assay,
  col_summary_function,
  na.rm,
  ...
)
```

Arguments

chunk_regions	Chunk with genomic regions of interest.
meth_rse	A RangedSummarizedExperiment with methylation values.
assay	The assay from meth_rse to extract values from. Should be either an index or the name of an assay.
col_summary_function	A function that summarizes column values.
na.rm	TRUE or FALSE indicating whether to remove NA values when calculating summaries.
...	Additional arguments to be passed to col_summary_function.

Value

A function which returns a list with the summarized methylation values for regions in each sample.

.test_tmrs *Find TMRs where smoothed methodical scores exceed thresholds*

Description

Find TMRs where smoothed methodical scores exceed thresholds

Usage

```
.test_tmrs(
  meth_sites_gr,
  smoothed_methodical_scores,
  p_value_threshold = 0.05,
  tss_gr = NULL,
  transcript_id = NULL
)
```

Arguments

meth_sites_gr	A GRanges object with the location of methylation sites.
smoothed_methodical_scores	A numeric vector with the smoothed methodical scores associated with each methylation site.
p_value_threshold	The p_value cutoff to use. Default value is 0.05.
tss_gr	An optional GRanges object giving the location of the TSS meth_sites_gr is associated with.
transcript_id	Name of the transcript associated with the TSS.

Value

A GRanges object with the location of TMRs.

.tss_correlations *Calculate meth site-transcript correlations for given TSS*

Description

Calculate meth site-transcript correlations for given TSS

Usage

```
.tss_correlations(correlation_objects)
```

Arguments

correlation_objects	A list with a table of methylation values, expression values for transcripts, a GRangesList for the transcript and the name of the transcript.

Value

A data.frame with the correlation values

`.tss_iterator` *Create an iterator function for use with bpiterate*

Description

Create an iterator function for use with bpiterate

Usage

```
.tss_iterator(
  meth_values_chunk,
  tss_region_indices_list,
  transcript_values_list,
  tss_gr_chunk_list,
  cor_method,
  add_distance_to_region,
  min_number_complete_pairs,
  results_dir
)
```

Arguments

`meth_values_chunk`	A table with methylation values for current chunk
`tss_region_indices_list`	A list with the indices for methylation sites associated with each TSS.
`transcript_values_list`	A list with expression values for transcripts.
`tss_gr_chunk_list`	A list of GRanges with the TSS for the current chunk.
`cor_method`	Correlation method to use.
`add_distance_to_region`	TRUE or FALSE indicating whether to add distance to TSS.
`min_number_complete_pairs`	The minimum number of complete pairs required to return a p-value for a correlation.
`results_dir`	Location of results directory.

Value

An iterator function which returns a list with the parameters necessary for .tss_correlations.

.write_chunks_to_hdf5 *Write chunks of data to a HDF5 sink*

Description

Write chunks of data to a HDF5 sink

Usage

```
.write_chunks_to_hdf5(
  temp_chunk_dirs,
  files_in_chunks,
  beta_sink,
  Cov_sink,
  hdf5_grid
)
```

Arguments

temp_chunk_dirs	A vector giving the temporary directory associated with each chunk.
files_in_chunks	A list of files associated with each chunk in the order they should be placed.
beta_sink	A HDF5RealizationSink for methylation proportions.
Cov_sink	A HDF5RealizationSink for coverage.
hdf5_grid	A RegularArrayGrid.

Value

Invisibly returns TRUE.

annotatePlot *Create a plot with genomic annotation for a plot of values at methylation sites.*

Description

Works with plots returned by `plotRegionValues()`, `plotMethSiteCorCoefs()` or `plotMethodicalScores`. Can combine the meth site values plot and genomic annotation together into a single plot or return the annotation plot separately.

Usage

```
annotatePlot(
  meth_site_plot,
  annotation_grl,
  reference_tss = FALSE,
  grl_colours = NULL,
  annotation_line_size = 5,
  ylab = "Genome Annotation",
  annotation_plot_proportion = 0.5,
  keep_meth_site_plot_legend = FALSE,
  annotation_plot_only = FALSE
)
```

Arguments

meth_site_plot	A plot of methylation site values (generally methylation level or correlation of methylation with transcription) around a TSS
annotation_grl	A GRangesList object (or list coercible to a GRangesList) where each component GRanges gives the locations of different classes of regions to display. Each class of region will be given a separate colour in the plot, with regions ordered by the order of `names(annotation_grl)`.
reference_tss	TRUE or FALSE indicating whether to show distances on the X-axis relative to the TSS stored as an attribute `tss_range` of meth_site_plot. Alternatively, can provide a GRanges object with a single range for such a TSS site. In either case, will show the distance of methylation sites to the start of this region with methylation sites upstream relative to the reference_tss shown first. If FALSE (the default), the x-axis will instead show the start site coordinate of the methylation site. relative to the reference_tss shown first. If not, the x-axis will show the start site coordinate of the methylation site.
grl_colours	An optional vector of colours used to display each of the GRanges making up annotation_grl. Must have same length as annotation_grl.
annotation_line_size	Linewidth for annotation plot. Default is 5.
ylab	The title to give the Y axis in the annotation plot. Default is "Genome Annotation".
annotation_plot_proportion	A value giving the proportion of the height of the plot devoted to the annotation. Default is 0.5.
keep_meth_site_plot_legend	TRUE or FALSE indicating whether to retain the legend of meth_site_plot, if it has one. Default value is FALSE.
annotation_plot_only	TRUE or FALSE indicating whether to return only the annotation plot. Default is to combine meth_site_plot with the annotation.

Value

A ggplot object

Examples

```
# Get CpG islands from UCSC
data("hg38_cpg_islands", package = "methodical")
hg38_cpg_islands <- GRangesList(split(hg38_cpg_islands, hg38_cpg_islands$type))

# Load plot with CpG methylation correlation values for TUBB6
data("tubb6_correlation_plot", package = "methodical")

# Add positions of CpG islands to tubb6_correlation_plot
methodical::annotatePlot(tubb6_correlation_plot, annotation_grl = hg38_cpg_islands, annotation_plot_proportion =
```

---

calculateMethSiteTranscriptCors

Calculate correlation between expression of transcripts and methylation of sites surrounding their TSS

Description

Calculate correlation between expression of transcripts and methylation of sites surrounding their TSS

Usage

```
calculateMethSiteTranscriptCors(
  meth_rse,
  assay_number = 1,
  transcript_expression_table,
  samples_subset = NULL,
  tss_gr,
  tss_associated_gr,
  cor_method = "pearson",
  min_number_complete_pairs = 30,
  add_distance_to_region = TRUE,
  max_sites_per_chunk = NULL,
  BPPARAM = BiocParallel::SerialParam(),
  results_dir = NULL
)
```

Arguments

meth_rse	A RangedSummarizedExperiment for methylation sites.
assay_number	The assay from meth_rse to extract values from. Default is the first assay.
transcript_expression_table	A matrix or data.frame with the expression values for transcripts, where row names are transcript names and columns sample names. There should be a row corresponding to each transcript associated with each range in tss_gr. Names of samples must match those in meth_rse unless samples_subset provided.

samples_subset Sample names used to subset meth_rse and transcript_expression_table. Provided samples must be found in both meth_rse and transcript_expression_table. Default is to use all samples in meth_rse and transcript_expression_table.

tss_gr A GRanges object with the locations of transcription start sites. Names of regions cannot contain any duplicates and should and match those of tss_associated_gr and be present in transcript_expression table.

tss_associated_gr
A GRanges object with the locations of regions associated with each transcription start site. Names of regions cannot contain any duplicates and should and match those of tss_gr and be present in transcript_expression table.

cor_method A character string indicating which correlation coefficient is to be computed. One of either "pearson" or "spearman" or their abbreviations.

min_number_complete_pairs
The minimum number of complete pairs required to return a p-value for a correlation. Correlations with less than this number are given a p-value of NaN. Default value is 30.

add_distance_to_region
TRUE or FALSE indicating whether to add the distance of methylation sites to the TSS. Default value is TRUE. Setting to FALSE will roughly half the total running time.

max_sites_per_chunk
The approximate maximum number of methylation sites to try to load into memory at once. The actual number loaded may vary depending on the number of methylation sites overlapping each region, but so long as the size of any individual regions is not enormous (>= several MB), it should vary only very slightly. Some experimentation may be needed to choose an optimal value as low values will result in increased running time, while high values will result in a large memory footprint without much improvement in running time. Default is floor(62500000/ncol(meth_rse)), resulting in each chunk requiring approximately 500 MB of RAM.

BPPARAM A BiocParallelParam object for parallel processing. Defaults to `BiocParallel::SerialParam()`.

results_dir An optional path to a directory to save results as RDS files. There will be one RDS file for each transcript. If not provided, returns the results as a list.

Value

If results_dir is NULL, a list of data.frames with the correlation of methylation sites surrounding a specified genomic region with a given feature, p-values and adjusted q-values for the correlations. Distance of the methylation sites upstream or downstream to the center of the region is also provided. If results_dir is provided, instead returns a list with the paths to the RDS files with the results.

Examples

```
# Load TUBB6 TSS GRanges, RangedSummarizedExperiment with methylation values for CpGs around TUBB6 TSS and TUBB6 tra
data(tubb6_tss, package = "methodical")
data(tubb6_meth_rse, package = "methodical")
```

```
tubb6_meth_rse <- eval(tubb6_meth_rse)
data(tubb6_transcript_counts, package = "methodical")

# Calculate correlation values between methylation values and transcript values for TUBB6
tubb6_cpg_meth_transcript_cors <- methodical::calculateMethSiteTranscriptCors(meth_rse = tubb6_meth_rse,
  transcript_expression_table = tubb6_transcript_counts, tss_gr = tubb6_tss,
 tss_associated_gr = methodical::expand_granges(tubb6_tss, upstream = 5000, downstream = 5000))
head(tubb6_cpg_meth_transcript_cors$ENST00000591909)
```

`calculateRegionMethylationTranscriptCors` *Calculate the correlation values between the methylation of genomic regions and the expression of associated transcripts*

Description

Calculate the correlation values between the methylation of genomic regions and the expression of associated transcripts

Usage

```
calculateRegionMethylationTranscriptCors(
  meth_rse,
  assay = 1,
  transcript_expression_table,
  samples_subset = NULL,
  genomic_regions,
  genomic_region_names = NULL,
  genomic_region_transcripts = NULL,
  genomic_region_methylation = NULL,
  cor_method = "pearson",
  p_adjust_method = "BH",
  region_methylation_summary_function = colMeans,
  BPPARAM = BiocParallel::SerialParam(),
  ...
)
```

Arguments

`meth_rse`	A RangedSummarizedExperiment with methylation values for CpG sites which will be used to calculate methylation values for genomic_regions. There must be at least 3 samples in common between meth_rse and transcript_expression_table.
`assay`	The assay from meth_rse to extract values from. Should be either an index or the name of an assay. Default is the first assay.

`transcript_expression_table`
: A table with the expression values for different transcripts in different samples. Row names should give be the transcript name and column names should be the name of samples.

`samples_subset`
: Optional sample names used to subset meth_rse and transcript_expression_table. Provided samples must be found in both meth_rse and transcript_expression_table. Default is to use all samples in meth_rse and transcript_expression_table.

`genomic_regions`
: A GRanges object.

`genomic_region_names`
: A character vector of unique names to assign genomic_regions in the output table. Defaults to `names(genomic_regions)` if present or otherwise converts regions to character strings (e.g. "chr:1000-2000") to use as names.

`genomic_region_transcripts`
: Names of transcripts associated with each region in genomic_regions. If not provided, attempts to use genomic_regions$transcript_id. All transcripts must be present in transcript_expression_table.

`genomic_region_methylation`
: Optional preprovided table with methylation values for genomic_regions such as created using summarizeRegionMethylation(). Table will be created if it is not provided which will increase running time. Row names should match genomic_region_names and column names should match those of transcript_expression_table

`cor_method`
: A character string indicating which correlation coefficient is to be computed. One of either "pearson" or "spearman" or their abbreviations.

`p_adjust_method`
: Method used to adjust p-values. Same as the methods from p.adjust.methods. Default is Benjamini-Hochberg.

`region_methylation_summary_function`
: A function that summarizes column values. Default is colMeans.

`BPPARAM`
: A BiocParallelParam object for parallel processing. Defaults to `BiocParallel::SerialParam()`.

`...`
: Additional arguments to be passed to summary_function.

Value

A data.frame with the correlation values between the methylation of genomic regions and expression of transcripts associated with them

Examples

```
# Load TUBB6 TMRs, RangedSummarizedExperiment with methylation values for CpGs around TUBB6 TSS and TUBB6 transcrip
data(tubb6_tmrs, package = "methodical")
data(tubb6_meth_rse, package = "methodical")
tubb6_meth_rse <- eval(tubb6_meth_rse)
data(tubb6_transcript_counts, package = "methodical")

# Calculate correlation values between TMRs identified for TUBB6 and transcript expression
tubb6_tmrs_transcript_cors <- methodical::calculateRegionMethylationTranscriptCors(
```

```
  meth_rse = tubb6_meth_rse, transcript_expression_table = tubb6_transcript_counts,
  genomic_regions = tubb6_tmrs, genomic_region_names = tubb6_tmrs$tmr_name)
tubb6_tmrs_transcript_cors
```

`calculateSmoothedMethodicalScores` *Calculate methodical score and smooth it using a exponential weighted moving average*

Description

Calculate methodical score and smooth it using a exponential weighted moving average

Usage

```
calculateSmoothedMethodicalScores(
  correlation_df,
  offset_length = 10,
  smoothing_factor = 0.75
)
```

Arguments

`correlation_df`	A data.frame with correlation values between methylation sites and a transcript as returned by calculateMethSiteTranscriptCors.
`offset_length`	Number of methylation sites added upstream and downstream of a central methylation site to form a window, resulting in a window size of 2*offset_length + 1. Default value is 10.
`smoothing_factor`	Smoothing factor for exponential moving average. Should be a value between 0 and 1 with higher values resulting in a greater degree of smoothing. Default is 0.75.

Value

A GRanges object

Examples

```
# Load data.frame with CpG methylation-transcription correlation results for TUBB6
data("tubb6_cpg_meth_transcript_cors", package = "methodical")

# Calculate smoothed Methodical scores from correlation values
smoothed_methodical_scores <- methodical::calculateSmoothedMethodicalScores(tubb6_cpg_meth_transcript_cors)
```

chr11_subset_hg38_cpgs *chr11_subset_hg38_cpgs*

Description

All the CpG sites on both strands within the region chr11:67578812-67588812

Usage

```
chr11_subset_hg38_cpgs
```

Format

A GRanges object.

chr1_subset_hg38 *chr1_subset_hg38*

Description

A DNAStringSet for first 1,000,000 bp from chr1 from BSgenome.Hsapiens.UCSC.hg38

Usage

```
chr1_subset_hg38
```

Format

A DNAStringSet object.

chr4_subset_a_thal *chr4_subset_a_thal*

Description

A DNAStringSet for first 1,000,000 bp from chr4 from BSgenome.Athaliana.TAIR.TAIR9

Usage

```
chr4_subset_a_thal
```

Format

A DNAStringSet object.

convert_rse_to_bsseq *Convert a methylation RSE to a BSseq object*

Description

Convert a methylation RSE to a BSseq object

Usage

```
convert_rse_to_bsseq(meth_rse, proportion_assay = 1, coverage_assay = 2)
```

Arguments

meth_rse	A RangedSummarizedExperiment with methylation values.
proportion_assay	The assay of meth_rse which corresponds to the proportion of methylated reads. Can be either a numeric index or the name of the assay. Default is the first assay.
coverage_assay	The assay of meth_rse which corresponds to the coverage (the total number of reads). Can be either a numeric index or the name of the assay. Default is the second assay.

Value

A RangedSummarized experiment identical to meth_rse with two additional assays added: one for methylated reads and another for unmethylated reads.

correct_correlation_pvalues *Correct p-values for a list of methylation-transcription correlations results*

Description

Correct p-values for a list of methylation-transcription correlations results

Usage

```
correct_correlation_pvalues(correlation_list, p_adjust_method = "fdr")
```

Arguments

correlation_list	A list of data.frames with correlation values between methylation sites and a transcript as returned by calculateMethSiteTranscriptCors.
p_adjust_method	The method to use for p-value adjustment. Should be one of the methods in p.adjust.methods. Default is "fdr".

Value

A list identical to correlation_list except with p-values corrected using the indicated method.

`expand_granges` *Expand GRanges upstream and downstream*

Description

Expand ranges in a GRanges object upstream and downstream by specified numbers of bases, taking account of strand. Unstranded ranges are treated like they on the "+" strand. If any of the resulting ranges are out-of-bounds given the seqinfo of genomic_regions, they will be trimmed using trim().

Usage

```
expand_granges(genomic_regions, upstream = 0, downstream = 0)
```

Arguments

`genomic_regions`	A GRanges object
`upstream`	Number of bases to add upstream of each region in genomic_regions. Must be numeric vector of length 1 or else equal to the length of genomic_regions. Default value is 0. Negative values result in upstream end of regions being shortened, however the width of the resulting regions cannot be less than zero.
`downstream`	Number of bases to add downstream of each region in genomic_regions. Negative values result in downstream end of regions being shortened. Must be numeric vector of length 1 or else equal to the length of genomic_regions. Default value is 0. Negative values result in upstream end of regions being shortened, however the width of the resulting regions cannot be less than zero.

Value

A GRanges object

Examples

```
data(tubb6_tss, package = "methodical")
tubb6_tss
methodical::expand_granges(tubb6_tss, upstream = 5000, downstream = 5000)
```

`export_bedGraph_from_rse`

Export values for a sample in a RangedSummarizedExperiment as a bedGraph

---

Description

Export values for a sample in a RangedSummarizedExperiment as a bedGraph

Usage

```
export_bedGraph_from_rse(meth_rse, assay_number = 1, sample_name, file_name)
```

Arguments

`meth_rse`	A RangedSummarizedExperiment for methylation data.
`assay_number`	The assay from meth_rse to extract values from. Default is the first assay.
`sample_name`	The name of a single sample in meth_rse.
`file_name`	The output filename.

Value

A data.frame with the methylation site values for all sites in meth_rse which overlap genomic_ranges. Row names are the coordinates of the sites as a character vector.

`extractGRangesMethSiteValues`

Extract values for methylation sites overlapping genomic regions from a methylation RSE.

Description

Extract values for methylation sites overlapping genomic regions from a methylation RSE.

Usage

```
extractGRangesMethSiteValues(
  meth_rse,
  genomic_regions = NULL,
  samples_subset = NULL,
  assay_number = 1
)
```

Arguments

meth_rse	A RangedSummarizedExperiment for methylation data.
genomic_regions	A GRanges object. If set to NULL, returns all methylation sites in meth_rse
samples_subset	Optional sample names used to subset meth_rse.
assay_number	The assay from meth_rse to extract values from. Default is the first assay.

Value

A data.frame with the methylation site values for all sites in meth_rse which overlap genomic_ranges. Row names are the coordinates of the sites as a character vector.

Examples

```
# Load sample RangedSummarizedExperiment with CpG methylation data
data(tubb6_meth_rse, package = "methodical")
tubb6_meth_rse <- eval(tubb6_meth_rse)

# Create a sample GRanges object to use
test_region <- GRanges("chr18:12305000-12310000")

# Get methylation values for CpG sites overlapping HDAC1 gene
test_region_methylation <- methodical::extractGRangesMethSiteValues(meth_rse = tubb6_meth_rse, genomic_regions =
```

---

extractMethSitesFromGenome

Create a GRanges with methylation sites of interest from a BSgenome or DNAStringSet

---

Description

Create a GRanges with methylation sites of interest from a BSgenome or DNAStringSet

Usage

```
extractMethSitesFromGenome(
  genome,
  pattern = "CG",
  stranded = TRUE,
  standard_seqs_only = FALSE
)
```

Arguments

genome	A BSgenome object or a DNAStringSet with names indicating the sequences.
pattern	A pattern to match in genome. Default is "CG".
stranded	TRUE or FALSE indicating whether to return matches on both strands or else just the "+" strand. Strand will be set to "*" if FALSE. Default is TRUE.
standard_seqs_only	TRUE or FALSE indicating whether to only return ranges on standard sequences (those without "_" in their names). Default is FALSE.

Value

A GRanges object with genomic regions matching the pattern.

Examples

```
# Get human CpG sites for a portion of chr1 from hg38 genome build
data(chr1_subset_hg38, package = "methodical")
chr1_subset_hg38_cpgs <- methodical::extractMethSitesFromGenome(chr1_subset_hg38)
head(chr1_subset_hg38_cpgs)

# Find CHG sites in Arabidopsis thaliana
data(chr4_subset_a_thal, package = "methodical")
chr4_subset_a_thal_chg_sites <- methodical::extractMethSitesFromGenome(
genome = chr4_subset_a_thal, pattern = "CHG")
head(chr4_subset_a_thal_chg_sites)
```

---

findTMRs *Find TSS-Proximal Methylation-Controlled Regulatory Sites (TMRs)*

Description

Find TSS-Proximal Methylation-Controlled Regulatory Sites (TMRs)

Usage

```
findTMRs(
  correlation_list,
  offset_length = 10,
  p_adjust_method = "fdr",
  p_value_threshold = 0.05,
  smoothing_factor = 0.75,
  min_gapwidth = 150,
  min_meth_sites = 5,
  BPPARAM = BiocParallel::SerialParam()
)
```

Arguments

correlation_list	A list of data.frames with correlation values between methylation sites and a transcript as returned by calculateMethSiteTranscriptCors.
offset_length	Number of methylation sites added upstream and downstream of a central methylation site to form a window, resulting in a window size of 2*offset_length + 1. Default value is 10.
p_adjust_method	The method to use for p-value adjustment. Should be one of the methods in p.adjust.methods. Default is "fdr".
p_value_threshold	The p_value cutoff to use (after correcting p-values with p_adjust_method). Default value is 0.05.
smoothing_factor	Smoothing factor for exponential moving average. Should be a value between 0 and 1 with higher values resulting in a greater degree of smoothing. Default is 0.75.
min_gapwidth	Merge TMRs with the same direction separated by less than this number of base pairs. Default value is 150.
min_meth_sites	Minimum number of methylation sites that TMRs can contain. Default value is 5.
BPPARAM	A BiocParallelParam object for parallel processing. Defaults to `BiocParallel::SerialParam()`.

Value

A GRanges object with the location of TMRs.

---

hg19_chr18_cpgs *hg19_chr18_cpgs*

Description

A GRanges object with CpG sites on chr18 for hg19

Usage

```
hg19_chr18_cpgs
```

Format

A GRanges object.

hg38_cpg_islands *hg38_cpg_islands*

Description

A GRanges with CpG islands, shelves and shores for hg38

Usage

```
hg38_cpg_islands
```

Format

A GRanges object.

infinium_450k_probe_granges_hg19
infinium_450k_probe_granges_hg19

Description

The hg19 genomic coordinates for methylation sites analysed by the Infinium HumanMethylation450K array.

Usage

```
infinium_450k_probe_granges_hg19
```

Format

GRanges object with 482,421 ranges and one metadata column name giving the name of the associated probe.

Source

Derived from the manifest file downloaded from https://webdata.illumina.com/downloads/productfiles/humanmethylation450
2.csv?_gl<-1*ocsx4f* _ga*MTk1Nzc4MDkwMy4xNjg3ODcxNjg0*_ga_VVVPY8BDYL*MTY4Nzg3MTY4My4xLjEuMTY4Nz

`liftoverMethRSE` *Liftover rowRanges of a RangedSummarizedExperiment for methylation data from one genome build to another*

Description

Removes methylation sites which cannot be mapped to the target genome build and those which result in many-to-one mappings. Also removes one-to-many mappings by default and can remove sites which do not map to allowed regions in the target genome e.g. CpG sites.

Usage

```
liftoverMethRSE(
  meth_rse,
  chain,
  remove_one_to_many_mapping = TRUE,
  permitted_target_regions = NULL,
  seqlevels = NULL
)
```

Arguments

`meth_rse`	A RangedSummarizedExperiment for methylation data
`chain`	A "Chain" object to be used with rtracklayer::liftOver
`remove_one_to_many_mapping`	TRUE or FALSE indicating whether to remove regions in the source genome which map to multiple regions in the target genome. Default is TRUE.
`permitted_target_regions`	An optional GRanges object used to filter the rowRanges by overlaps after liftover, for example CpG sites from the target genome. Any regions which do not overlap permitted_target_regions will be removed. GRangesList to GRanges if all remaining source regions can be uniquely mapped to the target genome.
`seqlevels`	An optional character vector giving the order to use for seqlevels of the rowRanges of the returned RangedSummarizedExperiment.

Value

A RangedSummarizedExperiment with rowRanges lifted over to the genome build indicated by chain.

Examples

```
# Load sample RangedSummarizedExperiment with CpG methylation data
data(tubb6_meth_rse, package = "methodical")
tubb6_meth_rse <- eval(tubb6_meth_rse)

# Get CpG sites for hg19
```

```
data(hg19_chr18_cpgs, package = "methodical")

# Get liftover chain for mapping hg38 to hg19
library(AnnotationHub)
ah <- AnnotationHub()
chain <- ah[["AH14108"]]

# Liftover tubb6_meth_rse from hg38 to hg19, keeping only sites that were mapped to CpG sites in hg19
tubb6_meth_rse_hg19 <- methodical::liftoverMethRSE(tubb6_meth_rse, chain = chain,
  permitted_target_regions = hg19_chr18_cpgs)
```

`makeMethRSEFromInputFiles` *Create a HDF5-backed RangedSummarizedExperiment for methylation values in meth_files*

Description

Create a HDF5-backed RangedSummarizedExperiment for methylation values in meth_files

Usage

```
makeMethRSEFromInputFiles(
  meth_files,
  seqnames_col,
  start_col,
  total_reads_col = NULL,
  meth_reads_col = NULL,
  unmeth_reads_col = NULL,
  meth_fraction_col = NULL,
  zero_based,
  meth_sites,
  sequence_context = "CG",
  collapse_strands = TRUE,
  decimal_places = NA,
  sample_metadata = NULL,
  hdf5_dir,
  overwrite = FALSE,
  chunkdim = NULL,
  temporary_dir = NULL,
  BPPARAM = BiocParallel::SerialParam(),
  ...
)
```

Arguments

`meth_files`	A vector of paths to input methylation files. All sites in each file are assumed to be for the same sequence context e.g. CG or CHG. Automatically detects if meth_files contain a header if every field in the first line is a character.

`seqnames_col` The column number in meth_files which corresponds to the sequence names.

`start_col` The column number in meth_files which corresponds to the genomic start coordinate.

`total_reads_col` The column number in meth_files which corresponds to the total number of reads for the position.

`meth_reads_col` The column number in meth_files which corresponds to the number of methylated reads for the position.

`unmeth_reads_col` The column number in meth_files which corresponds to the number of unmethylated reads for the position.

`meth_fraction_col` The column number in meth_files which corresponds to the fraction of reads that support methylation at the position. Will be converted to a proportion if it appears to be a fraction.

`zero_based` TRUE or FALSE indicating if files are zero-based.

`meth_sites` A GRanges object with non-overlapping locations of methylation sites of interest e.g. CpG sites. Any methylation sites in meth_files that are not in meth_sites are ignored.

`sequence_context` A single character string or DNAString with the sequence context of the methylation sites e.g. CG or CHG. If a character, must be coercible to a DNAString. Default is "CG".

`collapse_strands` TRUE or FALSE indicating whether or not to collapse data on + and - strands. Only makes sense for symmetrically methylated contexts e.g. CG or CHG and meth_sites should include ranges on both the + and - strands if TRUE.

`decimal_places` Optional integer indicating the number of decimal places to round beta values to. Default is not to round.

`sample_metadata` Sample metadata to be used as colData for the RangedSummarizedExperiment.

`hdf5_dir` Directory to save HDF5 file. Is created if it doesn't exist. HDF5 file is called assays.h5.

`overwrite` TRUE or FALSE indicating whether to allow overwriting if hdf5_dir already exists. Default is FALSE.

`chunkdim` The dimensions of the chunks for the HDF5 file. Should be a vector of length 2 giving the number of rows and then the number of columns in each chunk. Uses HDF5Array::getHDF5DumpChunkDim(length(meth_sites), length(meth_files))) by default.

`temporary_dir` Name to give temporary directory created to store intermediate files. A directory with this name cannot already exist. Default is to create a subdirectory named "temporary_meth_chunks_" inside the directory given by `tempdir()`. Will be deleted after completion.

`BPPARAM` A BiocParallelParam object for parallel processing. Defaults to `BiocParallel::SerialParam()`.

`...` Additional arguments to be passed to HDF5Array::HDF5RealizationSink() for controlling the physical properties of the created HDF5 file, such as compression level. Uses the defaults for any properties that are not specified.

Value

A RangedSummarizedExperiment with two assays for all methylation sites in meth_sites, beta with the proportion of methylated reads and Cov with the total number of reads for each site. methylation sites will be in the same order as sort(meth_sites).

Examples

```
# Load CpGs from subset of chromosome 11 as a GRanges object
data("chr11_subset_hg38_cpgs", package = "methodical")

# Get paths to meth_files
meth_files <- list.files(path = system.file('extdata', package = 'methodical'),
  pattern = ".CX_report.txt.gz", full.names = TRUE)

# Create sample metadata
sample_metadata <- data.frame(
  sample_type = ifelse(grepl("N", basename(meth_files)), "Normal", "Tumour"),
  row.names = gsub("_.*", "", basename(meth_files))
)

# Create a HDF5-backed RangedSummarizedExperiment from meth_files
meth_rse <- makeMethRSEFromInputFiles(meth_files = meth_files,
  seqnames_col = 1, start_col = 2, meth_reads_col = 4, unmeth_reads_col = 5,
 zero_based = FALSE, meth_sites = chr11_subset_hg38_cpgs, sample_metadata = sample_metadata,
  hdf5_dir = paste0(tempdir(), "/test_hdf5_1"))

# Show beta values and coverage
assay(meth_rse, "beta")
assay(meth_rse, "Cov")
```

---

maskRangesInRSE *Mask regions in a ranged summarized experiment*

Description

Mask regions in a ranged summarized experiment

Usage

```
maskRangesInRSE(rse, mask_ranges, assay_number = 1)
```

Arguments

rse	A RangedSummarizedExperiment.
mask_ranges	Either a GRanges with regions to be masked in all samples (e.g. repetitive sequences) or a GRangesList object with different regions to mask in each sample (e.g. mutations). If using a GRangesList object, names of the list elements should be the names of samples in rse.
assay_number	Assay to perform masking. Default is first assay

Value

A RangedSummarizedExperiment with the regions present in mask_ranges masked

Examples

```
# Load sample RangedSummarizedExperiment with CpG methylation data
data(tubb6_meth_rse, package = "methodical")
tubb6_meth_rse <- eval(tubb6_meth_rse)

# Create a sample GRanges object to use to mask tubb6_meth_rse
mask_ranges <- GRanges("chr18:12305000-12310000")

# Mask regions in tubb6_meth_rse
tubb6_meth_rse_masked <- methodical::maskRangesInRSE(tubb6_meth_rse, mask_ranges)

# Count the number of NA values before and after masking
sum(is.na(assay(tubb6_meth_rse)))
sum(is.na(assay(tubb6_meth_rse_masked)))
```

---

methrixToRSE *Convert a Methrix object into a RangedSummarizedExperiment*

Description

Convert a Methrix object into a RangedSummarizedExperiment

Usage

```
methrixToRSE(methrix, assays = c("beta", "cov"))
```

Arguments

methrix	A methrix object
assays	A vector indicating the names of assays in methrix used to create a RangedSummarizedExperiment. Can be one or both of "beta" and "cov". Default is both "beta" and "cov" assays.

Value

A RangedSummarizedExperiment

Examples

```
# Load a sample methrix object
data("methrix_data", package = "methrix")

# Convert methrix to a RangedSummarizedExperiment with one assay for the methylation beta values
meth_rse <- methodical::methrixToRSE(methrix_data, assays = "beta")
print(meth_rse)
```

`plotMethodicalScores` *Create plot of Methodical score values for methylation sites around a TSS*

Description

Create plot of Methodical score values for methylation sites around a TSS

Usage

```
plotMethodicalScores(
  genomic_region_values,
  reference_tss = NULL,
  p_value_threshold = 0.005,
  smooth_scores = TRUE,
  offset_length = 10,
  smoothing_factor = 0.75,
  smoothed_curve_colour = "black",
  linewidth = 1,
  curve_alpha = 0.75,
  title = NULL,
  xlabel = "Genomic Position",
  low_colour = "#7B5C90",
  high_colour = "#BFAB25"
)
```

Arguments

`genomic_region_values`
: A data.frame with correlation values for methylation sites. There should be one column called "cor". and another called "p_val" which are used to calculate the Methodical score. row.names should be the names of methylation sites and all methylation sites must be located on the same sequence.

`reference_tss`
: An optional GRanges object with a single range. If provided, the x-axis will show the distance of methylation sites to the start of this region with methylation sites upstream. relative to the reference_tss shown first. If not, the x-axis will show the start site coordinate of the methylation site.

`p_value_threshold`
: The p-value threshold used to identify TMRs. Default value is 0.005. Set to NULL to turn off significance thresholds.

`smooth_scores`
: TRUE or FALSE indicating whether to display a curve of smoothed Methodical scores on top of the plot. Default is TRUE.

`offset_length`
: Offset length to be supplied to calculateSmoothedMethodicalScores. Default is 10.

`smoothing_factor`
: Smoothing factor to be provided to calculateSmoothedMethodicalScores. Default is 0.75.

smoothed_curve_colour
: Colour of the smoothed curve. Default is "black".

linewidth
: Line width of the smoothed curve. Default value is 1.

curve_alpha
: Alpha value for the curve. Default value is 0.75.

title
: Title of the plot. Default is no title.

xlabel
: Label for the X axis in the plot. Default is "Genomic Position".

low_colour
: Colour to use for low values. Default value is "#7B5C90".

high_colour
: Colour to use for high values. Default value is "#BFAB25".

Value

A ggplot object

Examples

```
# Load methylation-transcript correlation results for TUBB6 gene
data("tubb6_cpg_meth_transcript_cors", package = "methodical")

# Calculate and plot Methodical scores from correlation values
methodical::plotMethodicalScores(tubb6_cpg_meth_transcript_cors, reference_tss = attributes(tubb6_cpg_meth_tran
```

---

plotMethSiteCorCoefs *Plot the correlation coefficients for methylation sites within a region and an associated feature of interest*

Description

Plot the correlation coefficients for methylation sites within a region and an associated feature of interest

Usage

```
plotMethSiteCorCoefs(
  meth_site_cor_values,
  reference_tss = FALSE,
  title = NULL,
  xlabel = NULL,
  ylabel = "Correlation Coefficient",
  value_colours = c("#7B5C90", "#bfab25"),
  reverse_x_axis = FALSE
)
```

Arguments

meth_site_cor_values	A data.frame with correlation values associated with methylation sites, such as returned by calculateMethSiteTranscriptCors. There should be one column called meth_site giving the coordinates of methylation sites in character format and another column called cor giving the correlation between the methylation values of the methylation sites and a feature of interest. All methylation sites must be located on the same sequence.
reference_tss	TRUE or FALSE indicating whether to show distances on the X-axis relative to the TSS stored as an attribute tss_range of meth_site_cor_values. Alternatively, can provide a GRanges object with a single range for such a TSS site. In either case, will show the distance of methylation sites to the start of this region with methylation sites upstream relative to the reference_tss shown first. If FALSE (the default), the x-axis will instead show the start site coordinate of the methylation site.
title	Title of the plot. Default is no title.
xlabel	Label for the X axis in the plot. Defaults to "Distance to TSS" if reference_tss is used or "seqname position" where seqname is the name of the relevant sequence.
ylabel	Label for the Y axis in the plot. Default is "Correlation Coefficient".
value_colours	A vector with two colours to use, the first for low values and the second for high values. Defaults are c("#7B5C90", "#bfab25").
reverse_x_axis	TRUE or FALSE indicating whether x-axis should be reversed, for example if plotting a region on the reverse strand so that left side of plot corresponds to upstream.

Value

A ggplot object

Examples

```
# Load methylation-transcript correlation results for TUBB6 gene
data("tubb6_cpg_meth_transcript_cors", package = "methodical")

# Plot methylation-transcript correlation values around TUBB6 TSS
methodical::plotMethSiteCorCoefs(tubb6_cpg_meth_transcript_cors, ylabel = "Spearman Correlation")

# Create same plot but showing the distance to the TUBB6 TSS on the x-axis
methodical::plotMethSiteCorCoefs(tubb6_cpg_meth_transcript_cors,
 ylabel = "Spearman Correlation", reference_tss = attributes(tubb6_cpg_meth_transcript_cors)$tss_range)
```

plotRegionValues *Create a scatter plot with smoothed curve for values along adjacent loci in a genomic region*

Description

Create a scatter plot with smoothed curve for values along adjacent loci in a genomic region

Usage

```
plotRegionValues(
  genomic_region_values,
  sample_name = NULL,
  reference_tss = FALSE,
  geom_point_params = list(),
  geom_smooth_params = list(),
  title = NULL,
  xlabel = NULL,
  ylabel = "Genomic Region Value",
  value_colours = c("#53868B", "#CD2626"),
  reverse_x_axis = FALSE
)
```

Arguments

`genomic_region_values`
: A data.frame with values associated with genomic regions. Row names must be the coordinates of genomic regions in character format (e.g chr1:1000-2000) and all regions must be located on the same sequence. The position of the first base in each region is used as the x-axis coordinate for the plot.

`sample_name`
: Name of column in genomic_region_values to plot. Defaults to first column if none provided.

`reference_tss`
: TRUE or FALSE indicating whether to show distances on the X-axis relative to the TSS stored as an attribute `tss_range` of genomic_region_values. Alternatively, can provide a GRanges object with a single range for such a TSS site. In either case, will show the distance of genomic regions to the start of this region with genomic regions upstream relative to the reference_tss shown first. If FALSE (the default), the x-axis will instead show the start site coordinate of the genomic region.

`geom_point_params`
: An optional list to explicitly set values of parameters to use with geom_point(). Use list(alpha = 0) to make points invisible.

`geom_smooth_params`
: An optional list to explicitly set values of parameters to use with geom_smooth(). Use list(alpha = 0) to make line invisible.

`title`
: Title of the plot. Default is no title.

xlabel	Label for the X axis in the plot. Defaults to "Distance to TSS" if reference_tss is used or "seqname position" where seqname is the name of the relevant sequence.
ylabel	Label for the Y axis in the plot. Default is "Genomic Region Value".
value_colours	A vector with two colours to use, the first for low values and the second for high values. Defaults are c("#53868B", "#CD2626").
reverse_x_axis	TRUE or FALSE indicating whether x-axis should be reversed, for example if plotting a region on the reverse strand so that left side of plot corresponds to upstream.

Value

A ggplot object

Examples

```
# Load methylation-values around the TUBB6 TSS
data("tubb6_meth_rse", package = "methodical")
tubb6_meth_rse <- eval(tubb6_meth_rse)

# Extract methylation values from tubb6_meth_rse
tubb6_methylation_values = methodical::extractGRangesMethSiteValues(meth_rse = tubb6_meth_rse)

# Plot methylation values around TUBB6 TSS
methodical::plotRegionValues(tubb6_methylation_values, sample_name = "N1", ylabel = "Methylation Value")

# Create same plot but showing the distance to the TUBB6 TSS on the x-axis
data("tubb6_tss", package = "methodical")
methodical::plotRegionValues(tubb6_methylation_values, sample_name = "N1",
  reference_tss = tubb6_tss, ylabel = "Methylation Value")
```

---

plotTMRs *Add TMRs to a methylation site value plot*

Description

Add TMRs to a methylation site value plot

Usage

```
plotTMRs(
  meth_site_plot,
  tmrs_gr,
  reference_tss = NULL,
  transcript_id = NULL,
  tmr_colours = c("#A28CB1", "#D2C465"),
  linewidth = 5
)
```

Arguments

meth_site_plot	A plot of Value around a TSS.
tmrs_gr	A GRanges object giving the position of TMRs.
reference_tss	An optional GRanges object with a single range. If provided, the x-axis will show the distance of methylation sites to the start of this region with methylation sites upstream relative to the reference_tss shown first. If not, the x-axis will show the start site coordinate of the methylation site.
transcript_id	An optional transcript ID. If provided, will attempt to filter tmrs_gr and reference_tss using a metadata column called transcript_id with a value identical to the provided one.
tmr_colours	A vector with colours to use for negative and positive TMRs. Defaults to "#7B5C90" for negative and "#BFAB25" for positive TMRs.
linewidth	A numeric value to be provided as linewidth for geom_segment().

Value

A ggplot object

Examples

```
# Load methylation-transcript correlation results for TUBB6 gene
data("tubb6_cpg_meth_transcript_cors", package = "methodical")

# Plot methylation-transcript correlation values around TUBB6 TSS
tubb6_correlation_plot <- methodical::plotMethSiteCorCoefs(tubb6_cpg_meth_transcript_cors, ylabel = "Spearman Co

# Find TMRs for TUBB6
tubb6_tmrs <- findTMRs(correlation_list = list(ENST00000591909 = tubb6_cpg_meth_transcript_cors))

# Plot TMRs on top of tubb6_correlation_plot
methodical::plotTMRs(tubb6_correlation_plot, tmrs_gr = tubb6_tmrs)
```

---

rangesRelativeToTSS *Find locations of genomic regions relative to transcription start sites.*

Description

Find locations of genomic regions relative to transcription start sites.

Usage

```
rangesRelativeToTSS(genomic_regions, tss_gr)
```

Arguments

genomic_regions	A GRanges object.
tss_gr	A GRanges object with transcription start sites. Each range should have width 1. Upstream and downstream are relative to strand of tss_gr.

Value

A GRanges object where all regions have "relative" as the sequence names and ranges are the location of TMRs relative to the TSS.

Examples

```
# Create query and subject GRanges
genomic_regions <- GenomicRanges::GRanges(c("chr1:100-1000:+", "chr1:2000-3000:-"))
tss_gr <- GenomicRanges::GRanges(c("chr1:1500:+", "chr1:4000:-"))

# Calculate distances between query and subject
methodical::rangesRelativeToTSS(genomic_regions, tss_gr)
```

---

rapidCorTest — *Rapidly calculate the correlation and the significance of pairs of columns from two data.frames*

Description

Rapidly calculate the correlation and the significance of pairs of columns from two data.frames

Usage

```
rapidCorTest(
  table1,
  table2,
  cor_method = "pearson",
  table1_name = "table1",
  table2_name = "table2",
  p_adjust_method = "BH",
  n_covariates = 0,
  min_number_complete_pairs = 30
)
```

Arguments

table1	A data.frame
table2	A data.frame
cor_method	A character string indicating which correlation coefficient is to be computed. One of either "pearson" or "spearman" or their abbreviations.

table1_name Name to give the column giving the name of features in table1. Default is "table1".

table2_name Name to give the column giving the name of features in table2. Default is "table2".

p_adjust_method Method used to adjust p-values. Same as the methods from p.adjust.methods. Default is Benjamini-Hochberg. Setting to "none" will result in no adjusted p-values being calculated.

n_covariates Number of covariates if calculating partial correlations. Defaults to 0.

min_number_complete_pairs The minimum number of complete pairs required to return a p-value for a correlation. Correlations with less than this number are given a p-value of NaN. Default value is 30.

Value

A data.frame with the correlation and its significance for all pairs consisting of a variable from table1 and a variable from table2.

Examples

```
# Divide mtcars into two tables
table1 <- mtcars[, 1:5]
table2 <- mtcars[, 6:11]

# Calculate correlation between table1 and table2
cor_results <- methodical::rapidCorTest(table1, table2, cor_method = "spearman",
  table1_name = "feature1", table2_name = "feature2")
head(cor_results)
```

---

sampleMethSites *Randomly sample sites from a methylation RSE.*

Description

Randomly sample sites from a methylation RSE.

Usage

```
sampleMethSites(
  meth_rse,
  n_sites = 1000,
  seqnames_filter = NULL,
  genomic_ranges_filter = NULL,
  invert_granges_filter = FALSE,
  samples_subset = NULL
)
```

Arguments

meth_rse	A RangedSummarizedExperiment for methylation data.
n_sites	Number of sites to randomly sample. Default is 1000. Will give an error if there are less than this number of sites available to sample after applying any of the optional filters.
seqnames_filter	An optional character vector giving the names of sequences to filter meth_rse for.
genomic_ranges_filter	An optional GRanges object used to first subset meth_rse. Sites will then be chosen randomly from those overlapping these ranges.
invert_granges_filter	TRUE or FALSE indicating whether to invert the genomic_ranges_filter so as to exclude sites overlapping these regions. Default value is FALSE.
samples_subset	Optional sample names used to subset meth_rse.

Value

A RangedSummarizedExperiment with the specified number of randomly sampled sites after applying the different filters.

Examples

```
# Load sample RangedSummarizedExperiment with CpG methylation data
data(tubb6_meth_rse, package = "methodical")
tubb6_meth_rse <- eval(tubb6_meth_rse)

# Create a sample GRanges object to use to mask tubb6_meth_rse
mask_ranges <- GRanges("chr18:12305000-12310000")

# Get 20 random CpG sites outside mask_ranges
random_cpgs <- methodical::sampleMethSites(tubb6_meth_rse, n_sites = 20, genomic_ranges_filter = mask_ranges,
  invert_granges_filter = TRUE)

# Check that no CpGs overlap repeats
intersect(rowRanges(random_cpgs), mask_ranges)
```

---

strandedDistance *Calculate distances of query GRanges upstream or downstream of subject GRanges*

Description

Upstream ranges are assigned negative distances and downstream regions positive distances and are relative to the strand of subject_gr. Unstranded ranges are treated the same as regions on the "+" strand. If subject_gr has a length of 1, then distances are calculated between each range in query_gr and this range, otherwise distances are calculated in a pairwise manner between ranges in query_gr and subject_gr.

Usage

```
strandedDistance(query_gr, subject_gr)
```

Arguments

query_gr	A GRanges object.
subject_gr	A GRanges object.

Value

A numeric vector of distances.

Examples

```
# Create query and subject GRanges
query_gr <- GenomicRanges::GRanges(c("chr1:100-1000:+", "chr1:2000-3000:-"))
subject_gr <- GenomicRanges::GRanges(c("chr1:1500-1600:+", "chr1:4000-4500:-"))

# Calculate distances between query and subject
methodical::strandedDistance(query_gr, subject_gr)
```

---

summarizeRegionMethylation

Summarize methylation of genomic regions within samples

Description

Summarize methylation of genomic regions within samples

Usage

```
summarizeRegionMethylation(
  meth_rse,
  assay = 1,
  genomic_regions,
  genomic_region_names = NULL,
  col_summary_function = "colMeans2",
  keep_metadata_cols = FALSE,
  max_sites_per_chunk = floor(62500000/ncol(meth_rse)),
  na.rm = TRUE,
  BPPARAM = BiocParallel::SerialParam(),
  ...
)
```

Arguments

meth_rse	A RangedSummarizedExperiment with methylation values.
assay	The assay from meth_rse to extract values from. Should be either an index or the name of an assay. Default is the first assay.
genomic_regions	GRanges object with regions to summarize methylation values for.
genomic_region_names	A character vector of unique names to assign genomic_regions in the output table. Defaults to `names(genomic_regions)` if present or otherwise converts regions to character strings (e.g. "chr:1000-2000") to use as names.
col_summary_function	A function that summarizes column values. Should be the name of one of the column summary functions from MatrixGenerics. Default is "colMeans2".
keep_metadata_cols	TRUE or FALSE indicating whether to add the metadata columns of genomic_regions to the output. Default is FALSE.
max_sites_per_chunk	The approximate maximum number of methylation sites to try to load into memory at once. The actual number loaded may vary depending on the number of methylation sites overlapping each region, but so long as the size of any individual regions is not enormous (>= several MB), it should vary only very slightly. Some experimentation may be needed to choose an optimal value as low values will result in increased running time, while high values will result in a large memory footprint without much improvement in running time. Default is floor(62500000/ncol(meth_rse)), resulting in each chunk requiring approximately 500 MB of RAM.
na.rm	TRUE or FALSE indicating whether to remove NA values when calculating summaries. Default value is TRUE.
BPPARAM	A BiocParallelParam object. Defaults to `BiocParallel::SerialParam()`.
...	Additional arguments to be passed to col_summary_function.

Value

A data.table with the summary of methylation of each region in genomic_regions for each sample.

Examples

```
# Load sample RangedSummarizedExperiment with CpG methylation data
data(tubb6_meth_rse, package = "methodical")
tubb6_meth_rse <- eval(tubb6_meth_rse)

# Create a sample GRanges
test_gr <- GRanges(c("chr18:12303400-12303500", "chr18:12303600-12303750", "chr18:12304000-12306000"))
names(test_gr) <- paste("region", 1:3, sep = "_")

# Calculate mean methylation values for regions in test_gr
test_gr_methylation <- methodical::summarizeRegionMethylation(tubb6_meth_rse, genomic_regions = test_gr,
```

```
    genomic_region_names = names(test_gr))
```

`tubb6_correlation_plot`
tubb6_correlation_plot

Description

A plot of the correlation values between methylation-transcription correlations for CpG sites around the TUBB6 TSS.

Usage

```
tubb6_correlation_plot
```

Format

A ggplot object.

`tubb6_cpg_meth_transcript_cors`
tubb6_cpg_meth_transcript_cors

Description

A data.frame with the methylation-transcription correlation results for CpGs around the TUBB6 TSS.

A data.frame with the correlation results for CpG sites within +/- 5 KB of the TUBB6 (ENST00000591909) TSS.

Usage

```
tubb6_cpg_meth_transcript_cors

tubb6_cpg_meth_transcript_cors
```

Format

A ggplot object.

A data.frame with 5 columns giving the name of the CpG site (meth_site), name of the transcript associated with the TSS, Spearman correlation value between the methylation of the CpG site and expression of the transcript, p-value associated with the correlations and distance from the CpG site to the TSS.

tubb6_meth_rse *tubb6_meth_rse*

Description

A RangedSummarizedExperiment with methyletion data for TUBB6.

Usage

```
tubb6_meth_rse
```

Format

A call to create a RangedSummarizedExperiment with methylation data for 355 CpG sites within +/- 5,000 base pairs of the TUBB6 TSS in 126 normal prostate samples. Should be evaluated after loading using `tubb6_meth_rse <- tubb6_meth_rse <- eval(tubb6_meth_rse)` to restore the RangedSummarizedExperiment.

Source

WGBS data from 'Li, Jing, et al. "A genomic and epigenomic atlas of prostate cancer in Asian populations." Nature 580.7801 (2020): 93-99.'

tubb6_tmrs *tubb6_tmrs*

Description

TMRs identified for TUBB6

Usage

```
tubb6_tmrs
```

Format

A GRanges object with two ranges.

tubb6_transcript_counts *tubb6_transcript_counts*

Description

Transcript counts for TUBB6 in normal prostate samples.

Usage

```
tubb6_transcript_counts
```

Format

A data.frame with normalized transcript counts for TUBB6 in 126 normal prostate samples.

Source

RNA-seq data from 'Li, Jing, et al. "A genomic and epigenomic atlas of prostate cancer in Asian populations." Nature 580.7801 (2020): 93-99.'

tubb6_tss *tubb6_tss*

Description

The location of the TSS for TUBB6.

Usage

```
tubb6_tss
```

Format

GRanges object with 1 ranges for the TUBB6 TSS.

Source

The TSS of the ENST00000591909 transcript.

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