

Package ‘NanoMethViz’

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Type Package

Title Visualise methylation data from Oxford Nanopore sequencing

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Description NanoMethViz is a toolkit for visualising methylation data from Oxford Nanopore sequencing. It can be used to explore methylation patterns from reads derived from Oxford Nanopore direct DNA sequencing with methylation called by callers including nanopolish, f5c and megalodon. The plots in this package allow the visualisation of methylation profiles aggregated over experimental groups and across classes of genomic features.

biocViews Software, Visualization, DifferentialMethylation

URL <https://github.com/shians/NanoMethViz>

BugReports <https://github.com/Shians/NanoMethViz/issues>

Depends R (>= 4.0.0), methods, ggplot2

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bsseq_to_edger	<i>Convert BSseq object to edgeR methylation matrix</i>
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Description

Convert BSseq object to edgeR methylation matrix

Usage

```
bsseq_to_edger(bsseq)
```

Arguments

bsseq the BSseq object.

Value

a matrix compatible with the edgeR differential methylation pipeline

Examples

```
methy <- system.file("methy_subset.tsv.bgz", package = "NanoMethViz")
bsseq <- methy_to_bsseq(methy)
edger_mat <- bsseq_to_edger(bsseq)
```

bsseq_to_log_methy_ratio

Convert BSseq object to log-methylation-ratio matrix

Description

Creates a log-methylation-ratio matrix from a BSseq object that is useful for dimensionality reduction plots.

Usage

```
bsseq_to_log_methy_ratio(bsseq, prior_count = 2)
```

Arguments

bsseq the BSseq object.

prior_count the prior count added to avoid taking log of 0.

Value

a matrix containing log-methylation-ratios.

Examples

```
methy <- system.file("methy_subset.tsv.bgz", package = "NanoMethViz")
bsseq <- methy_to_bsseq(methy)
log_m_ratio <- bsseq_to_log_methy_ratio(bsseq)
```

create_tabix_file *Create a tabix file using methylation calls*

Description

Create a tabix file using methylation calls

Usage

```
create_tabix_file(  
  input_files,  
  output_file,  
  samples = extract_file_names(input_files),  
  verbose = TRUE  
)
```

Arguments

input_files	the files to convert
output_file	the output file to write results to (must end in .bgz)
samples	the names of samples corresponding to each file
verbose	TRUE if progress messages are to be printed

Value

invisibly returns the output file path, creates a tabix file (.bgz) and its index (.bgz.tbi)

Examples

```
methy_calls <- system.file(package = "NanoMethViz",  
  c("sample1_nanopolish.tsv.gz", "sample2_nanopolish.tsv.gz"))  
temp_file <- paste0(tempfile(), ".tsv.bgz")  
  
create_tabix_file(methy_calls, temp_file)
```

exons_to_genes *Convert exon annotation to genes*

Description

Convert exon annotation to genes

Usage

```
exons_to_genes(x)
```

Arguments

x the exon level annotation containing columns "gene_id", "chr", "strand" and "symbol".

Value

the gene level annotation where each gene is taken to span the earliest start position and latest end position of its exons.

Examples

```
nmr <- load_example_nanomethresult()
exons_to_genes(NanoMethViz::exons(nmr))
```

`get_example_exons_mus_musculus`

Get example exon annotations for mus musculus

Description

This is a small subset of the exons returned by `get_exons_mus_musculus()` for demonstrative purposes. It contains the exons for the genes `Brca1`, `Brca2`, `Impact`, `Meg3`, `Peg3` and `Xist`.

Usage

```
get_example_exons_mus_musculus()
```

Value

data.frame containing exons

Examples

```
example_exons <- get_example_exons_mus_musculus()
```

```
get_exons_homo_sapiens
```

Get exon annotations for homo sapiens

Description

Get exon annotations for homo sapiens

Usage

```
get_exons_homo_sapiens()
```

Value

data.frame containing exons

Examples

```
h_sapiens_exons <- get_exons_homo_sapiens()
```

```
get_exons_mus_musculus
```

Get exon annotations for mus musculus

Description

Get exon annotations for mus musculus

Usage

```
get_exons_mus_musculus()
```

Value

data.frame containing exons

Examples

```
m_musculus_exons <- get_exons_mus_musculus()
```

load_example_nanomethresult
Load an example NanoMethResult object

Description

Load an example NanoMethResult object

Usage

load_example_nanomethresult()

Value

a NanoMethResults object

Examples

```
nmr <- load_example_nanomethresult()
```

methy_col_names *Column names for methylation data*

Description

Column names for methylation data

Usage

methy_col_names()

Value

column names for methylation data

Examples

```
methy_col_names()
```

methy_to_bsseq	<i>Create BSSeq object from methylation tabix file</i>
----------------	--

Description

Create BSSeq object from methylation tabix file

Usage

```
methy_to_bsseq(methy, out_folder = tempdir(), verbose = TRUE)
```

Arguments

methy	the path to the methylation tabix file.
out_folder	the folder to store intermediate files. One file is created for each sample and contains columns "chr", "pos", "total" and "methylated".
verbose	TRUE if progress messages are to be printed

Value

a BSSeq object.

Examples

```
methy <- system.file("methy_subset.tsv.bgz", package = "NanoMethViz")
bsseq <- methy_to_bsseq(methy)
```

NanoMethResult-class	<i>Nanopore Methylation Result</i>
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Description

A NanoMethResult object stores data used for NanoMethViz visualisation. It contains stores a path to the methylation data, sample information and optional exon information. The object is constructed using the NanoMethResult() constructor function described in "Usage".

Usage

```
NanoMethResult(methy, samples, exons = NULL)
```

```
## S4 method for signature 'NanoMethResult'
methy(object)
```

```
## S4 method for signature 'NanoMethResult'
samples(object)
```

```
## S4 method for signature 'NanoMethResult'
exons(object)
```

Arguments

methy	the path to the methylation tabix file.
samples	the data.frame of sample annotation containing at least columns sample and group.
exons	(optional) the data.frame of exon information containing at least columns gene_id, chr, strand, start, end, transcript_id and symbol.
object	the NanoMethResult object.

Value

a NanoMethResult object to be used with plotting functions
 the path to the methylation data.
 the sample annotation.
 the exon annotation.

Functions

- NanoMethResult: Constructor
- methy, NanoMethResult-method: methylation data path getter.
- samples, NanoMethResult-method: sample annotation getter.
- exons, NanoMethResult-method: exon annotation getter.

Slots

methy the path to the methylation tabix file.
 samples the data.frame of sample annotation containing at least columns sample and group.
 exons the data.frame of exon information containing at least columns gene_id, chr, strand, start, end, transcript_id and symbol.

Examples

```
methy <- system.file(package = "NanoMethViz", "methy_subset.tsv.bgz")
sample <- c(
  "B6Cast_Prom_1_b16",
  "B6Cast_Prom_1_cast",
  "B6Cast_Prom_2_b16",
  "B6Cast_Prom_2_cast",
  "B6Cast_Prom_3_b16",
  "B6Cast_Prom_3_cast"
)
group <- c(
  "b16",
  "cast",
  "b16",
  "cast",
  "b16",
  "cast"
)
```

```

sample_anno <- data.frame(sample, group, stringsAsFactors = FALSE)
exon_tibble <- get_example_exons_mus_musculus()
NanoMethResult(methy, sample_anno, exon_tibble)

x <- load_example_nanomethresult()
methy(x)

```

plot_agg_regions *Plot aggregate regions*

Description

Plot aggregate regions

Usage

```

plot_agg_regions(
  x,
  regions,
  features_group = NULL,
  flank = 2000,
  stranded = TRUE,
  span = 0.05,
  palette = ggplot2::scale_colour_brewer(palette = "Set1")
)

```

Arguments

x	the NanoMethResult object.
regions	a table of regions or GRanges, or a list of such objects. The table of regions must contain chr, strand, start and end columns.
features_group	if 'features' is a list, a vector of characters of the same length as the list containing names for each member.
flank	the number of flanking bases to add to each side of each region.
stranded	TRUE if negative strand features should have coordinates flipped to reflect features like transcription start sites.
span	the span for loess smoothing.
palette	the colour palette used for groups.

Value

a ggplot object containing the aggregate methylation trend.

Examples

```
nmr <- load_example_nanomethresult()
gene_anno <- exons_to_genes(NanoMethViz::exons(nmr))
plot_agg_regions(nmr, gene_anno)
```

plot_agg_regions_sample_grouped

Plot aggregate regions with grouped samples

Description

Plot aggregate regions with grouped samples

Usage

```
plot_agg_regions_sample_grouped(
  x,
  regions,
  flank = 2000,
  stranded = TRUE,
  span = 0.05,
  palette = ggplot2::scale_colour_brewer(palette = "Set1")
)
```

Arguments

x	the NanoMethResult object.
regions	a table of regions or GRanges, or a list of such objects. The table of regions must contain chr, strand, start and end columns.
flank	the number of flanking bases to add to each side of each region.
stranded	TRUE if negative strand features should have coordinates flipped to reflect features like transcription start sites.
span	the span for loess smoothing.
palette	the colour palette used for samples.

Value

a ggplot object containing the aggregate methylation trend.

Examples

```
nmr <- load_example_nanomethresult()
gene_anno <- exons_to_genes(NanoMethViz::exons(nmr))
plot_agg_regions_sample_grouped(nmr, gene_anno)
```

 plot_gene

Plot gene

Description

Plot gene

Usage

```
plot_gene(x, gene, ...)
```

```
## S4 method for signature 'NanoMethResult,character'
plot_gene(
  x,
  gene,
  window_prop = 0.3,
  anno_regions = NULL,
  spaghetti = FALSE,
  span = NULL,
  gene_anno = TRUE
)
```

Arguments

x	the NanoMethResult object.
gene	the gene symbol for the gene to plot.
...	additional arguments
window_prop	the size of flanking region to plot. Can be a vector of two values for left and right window size. Values indicate proportion of gene length.
anno_regions	the data.frame of regions to annotate.
spaghetti	whether or not individual reads should be shown.
span	the span for loess smoothing.
gene_anno	whether or not gene annotation tracks are plotted.

Value

a patchwork plot containing the methylation profile in the specified region.

a patchwork plot containing the methylation profile in the specified region.

Examples

```
nmr <- load_example_nanomethresult()
plot_gene(nmr, "Peg3")
```

```
nmr <- load_example_nanomethresult()
plot_gene(nmr, "Peg3")
```

plot_gene_heatmap *Plot gene methylation heatmap*

Description

Plot gene methylation heatmap

Usage

```
plot_gene_heatmap(x, gene, ...)  
  
## S4 method for signature 'NanoMethResult,character'  
plot_gene_heatmap(  
  x,  
  gene,  
  window_prop = 0.3,  
  pos_style = c("to_scale", "compact")  
)
```

Arguments

x	the NanoMethResult object.
gene	the gene symbol for the gene to plot.
...	additional arguments
window_prop	the size of flanking region to plot. Can be a vector of two values for left and right window size. Values indicate proportion of gene length.
pos_style	the style for plotting the base positions along the x-axis. Defaults to "to_scale", plotting (potentially) overlapping squares along the genomic position to scale. The "compact" options plots only the positions with measured modification.

Value

a ggplot object of the heatmap
a ggplot plot containing the heatmap.

Examples

```
nmr <- load_example_nanomethresult()  
plot_gene_heatmap(nmr, "Peg3")  
  
nmr <- load_example_nanomethresult()  
plot_gene_heatmap(nmr, "Peg3")
```

`plot_grange`*Plot GRanges*

Description

Plot GRanges

Usage

```
plot_grange(  
  x,  
  grange,  
  anno_regions = NULL,  
  spaghetti = FALSE,  
  span = NULL,  
  window_prop = 0.3  
)
```

Arguments

<code>x</code>	the NanoMethResult object.
<code>grange</code>	the GRanges object with one entry.
<code>anno_regions</code>	the data.frame of regions to be annotated.
<code>spaghetti</code>	whether or not individual reads should be shown.
<code>span</code>	the span for loess smoothing.
<code>window_prop</code>	the size of flanking region to plot. Can be a vector of two values for left and right window size. Values indicate proportion of gene length.

Value

a patchwork plot containing the methylation profile in the specified region.

a patchwork plot containing the methylation profile in the specified region.

Examples

```
nmr <- load_example_nanomethresult()  
plot_grange(nmr, GRanges("chr7:6703892-6730431"))
```

plot_grange_heatmap *Plot GRanges heatmap*

Description

Plot GRanges heatmap

Usage

```
plot_grange_heatmap(  
  x,  
  grange,  
  pos_style = c("to_scale", "compact"),  
  window_prop = 0.3  
)
```

Arguments

x	the NanoMethResult object.
grange	the GRanges object with one entry.
pos_style	the style for plotting the base positions along the x-axis. Defaults to "to_scale", plotting (potentially) overlapping squares along the genomic position to scale. The "compact" options plots only the positions with measured modification.
window_prop	the size of flanking region to plot. Can be a vector of two values for left and right window size. Values indicate proportion of gene length.

Value

a ggplot plot containing the heatmap.

Examples

```
nmr <- load_example_nanomethresult()  
gr <- GenomicRanges::GRanges(data.frame(chr = "chr7", start = 6703892, end = 6730431))  
plot_grange_heatmap(nmr, gr[1, ])
```

plot_region	<i>Plot region</i>
-------------	--------------------

Description

Plot region

Usage

```
plot_region(x, chr, start, end, ...)
```

```
## S4 method for signature 'NanoMethResult,character,numeric,numeric'
```

```
plot_region(
  x,
  chr,
  start,
  end,
  anno_regions = NULL,
  spaghetti = FALSE,
  span = NULL,
  window_prop = 0.3
)
```

```
## S4 method for signature 'NanoMethResult,factor,numeric,numeric'
```

```
plot_region(
  x,
  chr,
  start,
  end,
  anno_regions = NULL,
  spaghetti = FALSE,
  span = NULL,
  window_prop = 0.3
)
```

Arguments

x	the NanoMethResult object.
chr	the chromosome to plot.
start	the start of the plotting region.
end	the end of the plotting region.
...	additional arguments.
anno_regions	the data.frame of regions to be annotated.
spaghetti	whether or not individual reads should be shown.
span	the span for loess smoothing.

window_prop the size of flanking region to plot. Can be a vector of two values for left and right window size. Values indicate proportion of gene length.

Value

a patchwork plot containing the methylation profile in the specified region.

a patchwork plot containing the methylation profile in the specified region.

Examples

```
nmr <- load_example_nanomethresult()
plot_region(nmr, "chr7", 6703892, 6730431)
```

```
nmr <- load_example_nanomethresult()
plot_region(nmr, "chr7", 6703892, 6730431)
```

plot_region_heatmap *Plot region methylation heatmap*

Description

Plot region methylation heatmap

Usage

```
plot_region_heatmap(x, chr, start, end, ...)
```

```
## S4 method for signature 'NanoMethResult,character,numeric,numeric'
```

```
plot_region_heatmap(
  x,
  chr,
  start,
  end,
  pos_style = c("to_scale", "compact"),
  window_prop = 0.3
)
```

```
## S4 method for signature 'NanoMethResult,factor,numeric,numeric'
```

```
plot_region_heatmap(
  x,
  chr,
  start,
  end,
  pos_style = c("to_scale", "compact"),
  window_prop = 0.3
)
```

Arguments

x	the NanoMethResult object.
chr	the chromosome to plot.
start	the start of the plotting region.
end	the end of the plotting region.
...	additional arguments.
pos_style	the style for plotting the base positions along the x-axis. Defaults to "to_scale", plotting (potentially) overlapping squares along the genomic position to scale. The "compact" options plots only the positions with measured modification.
window_prop	the size of flanking region to plot. Can be a vector of two values for left and right window size. Values indicate proportion of gene length.

Value

- a ggplot object of the heatmap.
- a ggplot plot containing the heatmap.

Examples

```
nmr <- load_example_nanomethresult()
plot_region_heatmap(nmr, "chr7", 6703892, 6730431)

nmr <- load_example_nanomethresult()
plot_region_heatmap(nmr, "chr7", 6703892, 6730431)
```

query_exons

Query exons

Description

Query a data.frame of exons for a subset.

Usage

```
query_exons_region(exons, chr, start, end)

query_exons_gene_id(exons, gene_id)

query_exons_symbol(exons, symbol)
```

Arguments

exons	the data.frame of exons.
chr	the chromosome to query.
start	the start of the query region.
end	the end of the query region.
gene_id	the gene_id to query.
symbol	the gene_id to query.

Value

data.frame of queried exons.

Functions

- query_exons_region: Query region.
- query_exons_gene_id: Query gene ID.
- query_exons_symbol: Query gene symbol.

query_methy	<i>Query methylation data</i>
-------------	-------------------------------

Description

Query methylation data

Usage

```
query_methy(x, chr, start, end, simplify = TRUE)
```

Arguments

x	the path to the methylation data (tabix-bgzipped)
chr	the vector of chromosomes
start	the vector of start positions
end	the vector of end positions
simplify	whether returned results should be row-concatenated

Value

A table containing the data within the queried regions. If simplify is TRUE (default) then all data is contained within one table, otherwise it is a list of tables where each element is the data from one region.

Examples

```
nmr <- load_example_nanomethresult()
query_methy(methy(nmr), "chr7", 6703892, 6730431)
```

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