

# Package ‘NanoMethViz’

November 17, 2022

**Type** Package

**Title** Visualise methylation data from Oxford Nanopore sequencing

**Version** 2.5.0

**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 nanoprocess, f5c and megalodon. The plots in this package allow the visualisation of methylation profiles aggregated over experimental groups and across classes of genomic features.

**bioViews** 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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## R topics documented:

NanoMethViz-package . . . . .	3
bsseq_to_edger . . . . .	4
bsseq_to_log_methyl_ratio . . . . .	4
cluster_regions . . . . .	5
convert_methyl_format . . . . .	6
create_tabix_file . . . . .	6
exons . . . . .	7
exons<- . . . . .	8
exons_to_genes . . . . .	8
filter_methyl . . . . .	9
get_example_exons_mus_musculus . . . . .	9
get_exons . . . . .	10
get_exons_homo_sapiens . . . . .	10
get_exons_mus_musculus . . . . .	11
load_example_nanomethresult . . . . .	11
methyl . . . . .	12
methyl<- . . . . .	12
methyl_col_names . . . . .	13
methyl_to_bsseq . . . . .	13
methyl_to_edger . . . . .	14
NanoMethResult-class . . . . .	14
plot_agg_genes . . . . .	16
plot_agg_regions . . . . .	17
plot_gene . . . . .	18
plot_gene_heatmap . . . . .	20
plot_grange . . . . .	21
plot_grange_heatmap . . . . .	22
plot_mds . . . . .	23
plot_pca . . . . .	24
plot_region . . . . .	25
plot_region_heatmap . . . . .	26
query_exons . . . . .	28

<i>NanoMethViz-package</i>	3
----------------------------	---

query_methy . . . . .	29
raw_methy_to_tabix . . . . .	29
reexports . . . . .	30
region_methy_stats . . . . .	30
samples . . . . .	31
samples<- . . . . .	31
sort_methy_file . . . . .	32

<b>Index</b>	33
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**NanoMethViz-package** *NanoMethViz: 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.

## Details

The main plotting functions in this package are `plot_gene()` and `plot_region()`.

- See `vignette("ImportingData", package = "NanoMethViz")` for how to import data from Nanopolish and f5c.
- See `vignette("Introduction", package = "NanoMethViz")` for how to create visualisations using this package.

## Author(s)

**Maintainer:** Shian Su <[su.s@wehi.edu.au](mailto:su.s@wehi.edu.au)>

## See Also

Useful links:

- <https://github.com/shians/NanoMethViz>
- Report bugs at <https://github.com/Shians/NanoMethViz/issues>

**bsseq\_to\_edger**      *Convert BSseq object to edgeR methylation matrix*

### Description

Convert BSseq object to edgeR methylation matrix

### Usage

```
bsseq_to_edger(bsseq, regions = NULL)
```

### Arguments

<code>bsseq</code>	the BSseq object.
<code>regions</code>	the regions to calculate log-methylation ratios over. If left NULL, ratios will be calculated per site.

### 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, regions = NULL, prior_count = 2)
```

### Arguments

<code>bsseq</code>	the BSseq object.
<code>regions</code>	the regions to calculate log-methylation ratios over. If left NULL, ratios will be calculated per site.
<code>prior_count</code>	the prior count added to avoid taking log of 0.

**Value**

a matrix containing log-methylation-ratios.

**Examples**

```
nmr <- load_example_nanomethresult()
bsseq <- methy_to_bsseq(nmr)
regions <- exons_to_genes(NanoMethViz::exons(nmr))
log_m_ratio <- bsseq_to_log_methy_ratio(bsseq, regions)
```

---

cluster\_regions

*Cluster regions by K-means*

---

**Description**

Cluster regions by k-means based on their methylation profiles. In order to cluster using k-means the methylation profile of each region is interpolated and sampled at fixed points. The first 10 principal components are used for the k-means clustering. The clustering is best behaved in regions of similar width and CpG density.

**Usage**

```
cluster_regions(x, regions, centers = 2, grid_method = c("density", "uniform"))
```

**Arguments**

- |             |  |
|-------------|--|
| x           | the NanoMethResult object.   |
| regions     | a table of regions containing at least columns chr, strand, start and end.   |
| centers     | number of centers for k-means, identical to the number of output clusters.   |
| grid_method | the method for generating the sampling grid. The default option "density" attempts to create a grid with similar density as the data, "uniform" creates a grid of uniform density. |

**Value**

the table of regions given by the 'regions' argument with the column 'cluster' added.

**Examples**

```
nmr <- load_example_nanomethresult()
gene_anno <- exons_to_genes(NanoMethViz::exons(nmr))
# uniform grid due to low number of input features
gene_anno_clustered <- cluster_regions(nmr, gene_anno, centers = 2, grid_method = "uniform")
plot_agg_regions(nmr, gene_anno_clustered, group_col = "cluster")
```

`convert_methy_format`    *Convert methylation calls to NanoMethViz format*

### Description

Convert methylation calls to NanoMethViz format

### Usage

```
convert_methy_format(
  input_files,
  output_file,
  samples = fs::path_ext_remove(fs::path_file(input_files)),
  verbose = TRUE
)
```

### Arguments

<code>input_files</code>	the files to convert
<code>output_file</code>	the output file to write results to (must end in .bgz)
<code>samples</code>	the names of samples corresponding to each file
<code>verbose</code>	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)

`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**

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

create_tabix_file(methyl_calls, temp_file)
```

---

exons

*Get exon annotation*

---

**Description**

Get exon annotation

**Usage**

```
exons(object)
```

**Arguments**

object	the object.
--------	-------------

**Value**

the exon annotation.

**Examples**

```
showMethods("exons")
```

exons&lt;-

*Set exon annotation***Description**

Set exon annotation

**Usage**

exons(object) &lt;- value

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))
```

---

filter_methy	<i>Create filtered methylation file</i>
--------------	---

---

## Description

Create a filtered methylation file from an existing one.

## Usage

```
filter_methy(x, output_file, ...)
```

## Arguments

- |             |  |
|-------------|--|
| x           | the path to the methylation file or a NanoMethResult object.                                   |
| output_file | the output file to write results to (must end in .bgz).  |
| ...         | filtering criteria given in dplyr syntax. Use methy_col_names() to get available column names. |

## Value

invisibly returns 'output\_file' if x is a file path, otherwise returns NanoMethResult object with methy(x) replaced with filtered value.

## Examples

```
nmr <- load_example_nanomethresult()
output_file <- paste0(tempfile(), ".tsv.bgz")
filter_methy(nmr, output_file = output_file, chr == "chrX")
filter_methy(methy(nmr), output_file = output_file, chr == "chrX")
```

---

get\_example\_exons\_mus\_musculus

*Get example exon annotations for mus musculus (mm10)*

---

## 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`*Get exon annotations***Description**

Helper functions are provided for obtaining exon annotations from relevant TxDb packages on Bioconductor for the construction of NanoMethResults objects.

**Usage**

```
get_exons_mm10()
get_exons_hg19()
get_exons_hg38()
```

**Value**

`data.frame` containing exons

**Examples**

```
mm10_exons <- get_exons_mm10()
hg19_exons <- get_exons_hg19()
hg38_exons <- get_exons_hg38()
```

`get_exons_homo_sapiens`*Get exon annotations for Homo sapiens (hg19)***Description**

Get exon annotations for Homo sapiens (hg19)

**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 (mm10)*

---

**Description**

Get exon annotations for Mus musculus (mm10)

**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

---

*Get methylation data*

---

### Description

Get methylation data

### Usage

`methy(object)`

### Arguments

`object` the object.

### Value

the path to the methylation data.

### Examples

`showMethods("methy")`

---

---

methy<-

---

*Set methylation data*

---

### Description

Set methylation data

### Usage

`methy(object) <- value`

---

methy_col_names	<i>Column names for methylation data</i>
-----------------	--

---

**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**

```
nmr <- load_example_nanomethresult()
bsseq <- methy_to_bsseq(nmr)
```

<code>methy_to_edger</code>	<i>Convert NanoMethResult object to edgeR methylation matrix</i>
-----------------------------	--

## Description

Convert NanoMethResult object to edgeR methylation matrix

## Usage

```
methy_to_edger(methy, regions = NULL, out_folder = tempdir(), verbose = TRUE)
```

## Arguments

<code>methy</code>	the path to the methylation tabix file.
<code>regions</code>	the regions to calculate log-methylation ratios over. If left NULL, ratios will be calculated per site.
<code>out_folder</code>	the folder to store intermediate files. One file is created for each sample and contains columns "chr", "pos", "total" and "methylated".
<code>verbose</code>	TRUE if progress messages are to be printed

## Value

a matrix compatible with the edgeR differential methylation pipeline

## Examples

```
nmr <- load_example_nanomethresult()
edger_mat <- methy_to_edger(nmr)
```

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

## 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 replacement method for signature 'NanoMethResult'
methy(object) <- value

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

## S4 replacement method for signature 'NanoMethResult,data.frame'
samples(object) <- value

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

## S4 replacement method for signature 'NanoMethResult,data.frame'
exons(object) <- value
```

**Arguments**

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

**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)`: methylation data path getter.
- `methy(NanoMethResult) <- value`: methylation data path setter.
- `samples(NanoMethResult)`: sample annotation getter.
- `samples(object = NanoMethResult) <- value`: sample annotation setter.
- `exons(NanoMethResult)`: exon annotation getter.
- `exons(object = NanoMethResult) <- value`: exon annotation getter.

**Slots**

**methyl** 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**

```

methyl <- 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(methyl, sample_anno, exon_tibble)

x <- load_example_nanomethresult()
methyl(x)

```

**plot\_agg\_genes** *Plot gene aggregate plot*

**Description**

Plot gene aggregate plot

**Usage**

```

plot_agg_genes(
  x,
  genes = NULL,
  binary_threshold = 0.5,
  group_col = NULL,
  flank = 2000,

```

```

stranded = TRUE,
span = 0.05,
palette = ggplot2::scale_colour_brewer(palette = "Set1")
)

```

## Arguments

x	the NanoMethResult object.
genes	a character vector of genes to include in aggregate plot, if NULL then all genes are used.
binary_threshold	the modification probability such that calls with modification probability above the threshold are considered methylated, and those with probability equal or below are considered unmethylated.
group_col	the column to group aggregated trends by. This column can be in from the regions table or samples(x).
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 ggplot colour palette used for groups.

## Value

a ggplot object containing the aggregate methylation trend of genes.

## Examples

```

nmr <- load_example_nanomethresult()
plot_agg_genes(nmr)

```

plot\_agg\_regions      *Plot aggregate regions*

## Description

Plot aggregate regions

## Usage

```

plot_agg_regions(
  x,
  regions,
  binary_threshold = 0.5,
  group_col = NULL,

```

```

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

```

### Arguments

<code>x</code>	the NanoMethResult object.
<code>regions</code>	a table of regions containing at least columns chr, strand, start and end. Any additional columns can be used for grouping.
<code>binary_threshold</code>	the modification probability such that calls with modification probability above the threshold are considered methylated, and those with probability equal or below are considered unmethylated.
<code>group_col</code>	the column to group aggregated trends by. This column can be in from the regions table or samples(x).
<code>flank</code>	the number of flanking bases to add to each side of each region.
<code>stranded</code>	TRUE if negative strand features should have coordinates flipped to reflect features like transcription start sites.
<code>span</code>	the span for loess smoothing.
<code>palette</code>	the ggplot 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(nmr, gene_anno, group_col = "sample")
plot_agg_regions(nmr, gene_anno, group_col = "group")

```

### 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,
  binary_threshold = NULL,
  avg_method = c("mean", "median"),
  spaghetti = FALSE,
  heatmap = FALSE,
  span = NULL,
  gene_anno = TRUE,
  palette = ggplot2::scale_colour_brewer(palette = "Set1"),
  line_size = 2
)
```

**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.
binary_threshold	the modification probability such that calls with modification probability above the threshold are set to 1 and probabilities equal to or below the threshold are set to 0.
avg_method	the average method for pre-smoothing at each genomic position. Data is pre-smoothed at each genomic position before the smoothed aggregate line is generated for performance reasons. The default is "mean" which corresponds to the average methylation fraction. The alternative "median" option is closer to an average within the more common methylation state.
spaghetti	whether or not individual reads should be shown.
heatmap	whether or not read-methylation heatmap should be shown.
span	the span for loess smoothing.
gene_anno	whether or not gene annotation tracks are plotted.
palette	the ggplot colour palette used for groups.
line_size	the size of the lines.

**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"),
  subsample = 50
)
```

**Arguments**

<code>x</code>	the NanoMethResult object.
<code>gene</code>	the gene symbol for the gene to plot.
<code>...</code>	additional arguments
<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.
<code>pos_style</code>	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.
<code>subsample</code>	the number of read of packed read rows to subsample to.

**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,  
  binary_threshold = NULL,  
  avg_method = c("mean", "median"),  
  spaghetti = FALSE,  
  heatmap = FALSE,  
  span = NULL,  
  window_prop = 0,  
  palette = ggplot2::scale_colour_brewer(palette = "Set1"),  
  line_size = 2  
)
```

**Arguments**

x	the NanoMethResult object.
grange	the GRanges object with one entry.
anno_regions	the data.frame of regions to be annotated.
binary_threshold	the modification probability such that calls with modification probability above the threshold are set to 1 and probabilities equal to or below the threshold are set to 0.

avg_method	the average method for pre-smoothing at each genomic position. Data is pre-smoothed at each genomic position before the smoothed aggregate line is generated for performance reasons. The default is "mean" which corresponds to the average methylation fraction. The alternative "median" option is closer to an average within the more common methylation state.
spaghetti	whether or not individual reads should be shown.
heatmap	whether or not read-methylation heatmap 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.
palette	the ggplot colour palette used for groups.
line_size	the size of the lines.

### 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, GenomicRanges::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,
  subsample = 50
)
```

**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.
subsample	the number of read of packed read rows to subsample to.

**Value**

a ggplot plot containing the heatmap.

**Examples**

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

plot\_mds

*Plot MDS***Description**

Plot multi-dimensional scaling plot using algorithm of limma::plotMDS(). It is recommended this be done with the log-methylation-ratio matrix generated by bsseq\_to\_log\_methy\_ratio().

**Usage**

```
plot_mds(
  x,
  top = 500,
  plot_dims = c(1, 2),
  labels = colnames(x),
  groups = NULL
)
```

**Arguments**

x	the log-methylation-ratio matrix.
top	the number of top genes used to calculate pairwise distances.
plot_dims	the numeric vector of the two dimensions to be plotted.
labels	the character vector of labels for data points. By default uses column names of x, set to NULL to plot points.
groups	the character vector of groups the data points will be coloured by. Colour palette can be adjusted using scale_colour_*( ) functions from ggplot2.

**Value**

ggplot object of the MDS plot.

**Examples**

```
nmr <- load_example_nanomethresult()
bss <- methy_to_bsseq(nmr)
lmr <- bsseq_to_log_methy_ratio(bss)
plot_mds(lmr)
```

---

**plot\_pca**

*Plot PCA*

---

**Description**

Plot multi-dimensional scaling plot using algorithm of BiocSingular::runPCA(). It is recommended this be done with the log-methylation-ratio matrix generated by bsseq\_to\_log\_methy\_ratio().

**Usage**

```
plot_pca(x, plot_dims = c(1, 2), labels = colnames(x), groups = NULL)
```

**Arguments**

- x** the log-methylation-ratio matrix.
- plot\_dims** the numeric vector of the two dimensions to be plotted.
- labels** the character vector of labels for data points. By default uses column names of x, set to NULL to plot points.
- groups** the character vector of groups the data points will be coloured by.

**Value**

ggplot object of the MDS plot.

**Examples**

```
nmr <- load_example_nanomethresult()
bss <- methy_to_bsseq(nmr)
lmr <- bsseq_to_log_methy_ratio(bss)
plot_pca(lmr)
```

---

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,
  binary_threshold = NULL,
  avg_method = c("mean", "median"),
  spaghetti = FALSE,
  heatmap = FALSE,
  span = NULL,
  window_prop = 0,
  palette = ggplot2::scale_colour_brewer(palette = "Set1"),
  line_size = 2
)

## S4 method for signature 'NanoMethResult,factor,numeric,numeric'
plot_region(
  x,
  chr,
  start,
  end,
  anno_regions = NULL,
  binary_threshold = NULL,
  avg_method = c("mean", "median"),
  spaghetti = FALSE,
  heatmap = FALSE,
  span = NULL,
  window_prop = 0,
  palette = ggplot2::scale_colour_brewer(palette = "Set1"),
  line_size = 2
)
```

## Arguments

x the NanoMethResult object.

<code>chr</code>	the chromosome to plot.
<code>start</code>	the start of the plotting region.
<code>end</code>	the end of the plotting region.
<code>...</code>	additional arguments.
<code>anno_regions</code>	the data.frame of regions to be annotated.
<code>binary_threshold</code>	the modification probability such that calls with modification probability above the threshold are set to 1 and probabilities equal to or below the threshold are set to 0.
<code>avg_method</code>	the average method for pre-smoothing at each genomic position. Data is pre-smoothed at each genomic position before the smoothed aggregate line is generated for performance reasons. The default is "mean" which corresponds to the average methylation fraction. The alternative "median" option is closer to an average within the more common methylation state.
<code>spaghetti</code>	whether or not individual reads should be shown.
<code>heatmap</code>	whether or not read-methylation heatmap 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.
<code>palette</code>	the ggplot colour palette used for groups.
<code>line_size</code>	the size of the lines.

### 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,
  subsample = 50
)

## S4 method for signature 'NanoMethResult,factor,numeric,numeric'
plot_region_heatmap(
  x,
  chr,
  start,
  end,
  pos_style = c("to_scale", "compact"),
  window_prop = 0,
  subsample = 50
)
```

**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" option 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.
subsample	the number of read of packed read rows to subsample to.

**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

<code>exons</code>	the data.frame of exons.
<code>chr</code>	the chromosome to query.
<code>start</code>	the start of the query region.
<code>end</code>	the end of the query region.
<code>gene_id</code>	the gene_id to query.
<code>symbol</code>	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, force = FALSE)
```

### Arguments

x	the NanoMethResults object or a 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
force	whether to force empty output when query region 'chr' does not appear in data. Without 'force', an empty result indicates that the requested 'chr' appears in the data but no data overlaps with requested region, and an invalid 'chr' will cause an error.

### 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)
```

---

raw_methy_to_tabix	<i>Convert methylation file to tabix format</i>
--------------------	---

---

### Description

Convert methylation file to tabix format

### Usage

```
raw_methy_to_tabix(x)
```

**Arguments**

- x the path to the sorted methylation file

**Value**

invisibly returns the path to the tabix file

reexports

*Objects exported from other packages***Description**

These objects are imported from other packages. Follow the links below to see their documentation.

[e1071 sigmoid](#)

region\_methy\_stats

*Calculate region methylation statistics***Description**

Calculate the average methylation probability and prevalence based on specified probability threshold.

**Usage**

```
region_methy_stats(nmr, regions, threshold = 0.5)
```

**Arguments**

- |           |   |
|-----------|---|
| nmr       | the NanoMethResult object.  |
| regions   | the table of regions to query statistics for.   |
| threshold | the threshold to use for determining methylation calls for the calculation of prevalence. |

**Value**

table of regions with additional columns of methylation summary statistics.

**Examples**

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

---

<i>samples</i>	<i>Get sample annotation</i>
----------------	------------------------------

---

## Description

Get sample annotation

## Usage

```
samples(object)
```

## Arguments

object            the object.

## Value

the sample annotation.

## Examples

```
showMethods("samples")
```

---

<i>samples&lt;-</i>	<i>Set sample annotation</i>
---------------------	------------------------------

---

## Description

Set sample annotation

## Usage

```
samples(object) <- value
```

---

sort\_methy\_file      *Sort methylation file*

---

**Description**

Sort methylation file

**Usage**

`sort_methy_file(x)`

**Arguments**

`x`                  the path to the methylation file to sort

**Value**

invisibly returns path of sorted file

# Index

\* **internal**

- convert\_methy\_format, 6
- exons, 7
- exons<-, 8
- methy, 12
- methy<-, 12
- NanoMethViz-package, 3
- raw\_methy\_to\_tabix, 29
- reexports, 30
- samples, 31
- samples<-, 31
- sort\_methy\_file, 32

bsseq\_to\_edger, 4

bsseq\_to\_log\_methy\_ratio, 4

cluster\_regions, 5

convert\_methy\_format, 6

create\_tabix\_file, 6

exons, 7

exons, NanoMethResult-method

- (NanoMethResult-class), 14

exons<-, 8

exons<-, NanoMethResult, data.frame-method

- (NanoMethResult-class), 14

exons\_to\_genes, 8

filter\_methy, 9

get\_example\_exons\_mus\_musculus, 9

get\_exons, 10

get\_exons\_hg19 (get\_exons), 10

get\_exons\_hg38 (get\_exons), 10

get\_exons\_homo\_sapiens, 10

get\_exons\_mm10 (get\_exons), 10

get\_exons\_mus\_musculus, 11

load\_example\_nanomethresult, 11

methy, 12

methy, NanoMethResult-method

- (NanoMethResult-class), 14

methy<-, 12

methy<-, NanoMethResult-method

- (NanoMethResult-class), 14

methy\_col\_names, 13

methy\_to\_bsseq, 13

methy\_to\_edger, 14

NanoMethResult (NanoMethResult-class), 14

NanoMethResult-class, 14

NanoMethViz (NanoMethViz-package), 3

NanoMethViz-package, 3

plot\_agg\_genes, 16

plot\_agg\_regions, 17

plot\_gene, 18

plot\_gene(), 3

plot\_gene, NanoMethResult, character-method

- (plot\_gene), 18

plot\_gene\_heatmap, 20

plot\_gene\_heatmap, NanoMethResult, character-method

- (plot\_gene\_heatmap), 20

plot\_grange, 21

plot\_grange\_heatmap, 22

plot\_mds, 23

plot\_pca, 24

plot\_region, 25

plot\_region(), 3

plot\_region, NanoMethResult, character, numeric, numeric-method

- (plot\_region), 25

plot\_region, NanoMethResult, factor, numeric, numeric-method

- (plot\_region), 25

plot\_region\_heatmap, 26

plot\_region\_heatmap, NanoMethResult, character, numeric, numeric-method

- (plot\_region\_heatmap), 26

plot\_region\_heatmap, NanoMethResult, factor, numeric, numeric-method

- (plot\_region\_heatmap), 26

query\_exons, 28

query\_exons\_gene\_id (query\_exons), 28  
query\_exons\_region (query\_exons), 28  
query\_exons\_symbol (query\_exons), 28  
query\_methy, 29  
  
raw\_methy\_to\_tabix, 29  
reexports, 30  
region\_methy\_stats, 30  
  
samples, 31  
samples, NanoMethResult-method  
    (NanoMethResult-class), 14  
samples<-, 31  
samples<-, NanoMethResult, data.frame-method  
    (NanoMethResult-class), 14  
sigmoid, 30  
sigmoid (reexports), 30  
sort\_methy\_file, 32