

# Package ‘NanoMethViz’

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**Type** Package

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

**Version** 3.0.0

**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 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, LongRead, Visualization, DifferentialMethylation, DNAMethylation, Epigenetics, DataImport

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

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

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**Author** Shian Su [cre, aut]  
**Maintainer** Shian Su <su.s@wehi.edu.au>

## Contents

NanoMethViz-package . . . . .	3
bsseq_to_edger . . . . .	4
bsseq_to_log_methy_ratio . . . . .	4
cluster_reads . . . . .	5
cluster_regions . . . . .	6
convert_methy_format . . . . .	7
create_tabix_file . . . . .	7
exons . . . . .	8
exons_to_genes . . . . .	8
exons<- . . . . .	9
filter_methy . . . . .	9
get_example_exons_mus_musculus . . . . .	10
get_exons . . . . .	10
get_exons_homo_sapiens . . . . .	11
get_exons_mus_musculus . . . . .	11
load_example_modbamresult . . . . .	12
load_example_nanomethresult . . . . .	12
methy . . . . .	13
methy_col_names . . . . .	13
methy_to_bsseq . . . . .	14
methy_to_edger . . . . .	14
methy<- . . . . .	15
mod_code . . . . .	15
mod_code<- . . . . .	15
modbam_to_tabix . . . . .	16
ModBamFiles . . . . .	17
ModBamFiles-class . . . . .	17
ModBamResult-class . . . . .	18

NanoMethResult-class . . . . .	20
plot_agg_genes . . . . .	22
plot_agg_regions . . . . .	23
plot_gene . . . . .	24
plot_gene_heatmap . . . . .	26
plot_grange . . . . .	27
plot_grange_heatmap . . . . .	29
plot_mds . . . . .	30
plot_pca . . . . .	31
plot_region . . . . .	32
plot_region_heatmap . . . . .	35
plot_violin . . . . .	36
query_exons . . . . .	37
query_methy . . . . .	38
raw_methy_to_tabix . . . . .	39
reexports . . . . .	40
region_methy_stats . . . . .	40
samples . . . . .	41
samples<- . . . . .	41
sort_methy_file . . . . .	41
<b>Index</b>	<b>42</b>

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NanoMethViz-package     *NanoMethViz: Visualise methylation data from Oxford Nanopore sequencing*

---

## 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("UserGuide", package = "NanoMethViz")` for documentation of how to use this package.

## Author(s)

**Maintainer:** Shian Su <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	<i>Convert BSseq object to edgeR methylation matrix</i>
----------------	---

---

**Description**

Convert BSseq object to edgeR methylation matrix

**Usage**

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

**Arguments**

bsseq	the BSseq object.
regions	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	<i>Convert BSseq object to log-methylation-ratio matrix</i>
--------------------------	---

---

**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,
  drop_na = TRUE
)
```

**Arguments**

bsseq	the BSseq object.
regions	the regions to calculate log-methylation ratios over. If left NULL, ratios will be calculated per site.
prior_count	the prior count added to avoid taking log of 0.
drop_na	whether to drop rows with all NA values.

**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\_reads

*Cluster reads based on methylation*


---

**Description**

Cluster reads based on methylation

**Usage**

```
cluster_reads(x, chr, start, end, min_pts = 5)
```

**Arguments**

x	a ModBamResult object.
chr	the chromosome name where to find the region.
start	the start position of the region.
end	the end position of the region.
min_pts	the minimum number of points needed to form a cluster (default = 10).

**Value**

A tibble with information about each read's cluster assignment and read statistics.

---

cluster_regions	<i>Cluster regions by K-means</i>
-----------------	-----------------------------------

---

**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**

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)

---

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	<i>Get exon annotation</i>
-------	----------------------------

---

**Description**

Get exon annotation

**Usage**

```
exons(object)
```

---

exons_to_genes	<i>Convert exon annotation to genes</i>
----------------	---

---

**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))
```

---

exons<-	<i>Set exon annotation</i>
---------	----------------------------

---

**Description**

Set exon annotation

**Usage**

```
exons(object) <- value
```

---

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 `Brcal`, `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_grcm39()
```

```
get_exons_hg19()
```

```
get_exons_hg38()
```

**Value**

tibble (data.frame) object containing exon annotation.

**Examples**

```
mm10_exons <- get_exons_mm10()
grcm39_exons <- get_exons_grcm39()
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\_modbamresult

*Load an example ModBamResult object*

---

**Description**

Load an example ModBamResult object for demonstration of plotting functions. Run `load_example_modbamresult` without the function call to see how the object is constructed.

**Usage**

```
load_example_modbamresult()
```

**Value**

a ModBamResult object

**Examples**

```
mbr <- load_example_modbamresult()
```

---

load\_example\_nanomethresult

*Load an example NanoMethResult object*

---

**Description**

Load an example NanoMethResult object for demonstration of plotting functions. Run `load_example_nanomethresults` without the function call to see how the object is constructed.

**Usage**

```
load_example_nanomethresult()
```

**Value**

a NanoMethResults object

**Examples**

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

---

methy	<i>Get methylation data</i>
-------	-----------------------------

---

**Description**

Get methylation data

**Usage**

```
methy(object)
```

**Arguments**

object            the object.

**Value**

the path to the methylation data.

**Examples**

```
showMethods("methy")
```

---

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)
```

---

methy_to_edger	<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**

methy	the path to the methylation tabix file.
regions	the regions to calculate log-methylation ratios over. If left NULL, ratios will be calculated per site.
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 matrix compatible with the edgeR differential methylation pipeline

**Examples**

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

---

`methy<-`                      *Set methylation data*

---

**Description**

Set methylation data

**Usage**

```
methy(object) <- value
```

---

`mod_code`                      *Get mod code*

---

**Description**

Get mod code

**Usage**

```
mod_code(object)
```

---

`mod_code<-`                      *Set mod code*

---

**Description**

Set mod code

**Usage**

```
mod_code(object) <- value
```

---

modbam_to_tabix	<i>Convert BAM with modifications to tabix format</i>
-----------------	---

---

## Description

The `modbam_to_tabix` function takes a `ModBamResult` object and converts it into a tabix file format, which is efficient for indexing and querying large datasets.

## Usage

```
modbam_to_tabix(x, out_file, mod_code = NanoMethViz::mod_code(x))
```

## Arguments

<code>x</code>	the <code>ModBamResult</code> object.
<code>out_file</code>	the path of the output tabix.
<code>mod_code</code>	the modification code to use, defaults to 'm' for 5mC methylation.

## Details

The possible tags for `mod_code` can be found at <https://samtools.github.io/hts-specs/SAMtags.pdf> under the 'Base modifications' section.

## Value

invisibly returns the name of the created tabix file.

## Examples

```
out_file <- paste0(tempfile(), ".tsv.bgz")
mbr <- ModBamResult(
  methy = ModBamFiles(
    samples = "sample1",
    paths = system.file("peg3.bam", package = "NanoMethViz")
  ),
  samples = data.frame(
    sample = "sample1",
    group = "group1"
  )
)
modbam_to_tabix(mbr, out_file)
```



---

ModBamFiles	<i>Constructor for a ModBamFiles object</i>
-------------	---

---

### Description

This function creates a ModBamFiles object containing information about the samples and file paths. This constructor checks that the files are readable and have an index.

### Usage

```
ModBamFiles(samples, paths)

## S4 method for signature 'ModBamFiles'
show(object)
```

### Arguments

samples	a character vector with the names of the samples.
paths	a character vector with the file paths for the BAM files.
object	a ModBamFiles object.

### Value

A ModBamFiles object with the sample and path information.

---

ModBamFiles-class	<i>ModBamFiles class</i>
-------------------	--------------------------

---

### Description

This is a class for holding information about modbam files. It is a data.frame containing information about samples and paths to modbam files.

---

 ModBamResult-class      *Modbam methylation results*


---

### Description

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

### Usage

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

## S4 replacement method for signature 'ModBamResult,ModBamFiles'
methy(object) <- value

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

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

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

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

## S4 method for signature 'ModBamResult'
mod_code(object)

## S4 replacement method for signature 'ModBamResult,character'
mod_code(object) <- value

ModBamResult(methy, samples, exons = NULL, mod_code = "m")
```

### Arguments

object	the ModBamResult object.
value	the mod code.
methy	a ModBamFiles object.
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.

`mod_code` a character with the mod code of interest. Defaults to "m" for 5mC. See details for other options.

### Details

The possible tags for `mod_code` can be found at <https://samtools.github.io/hts-specs/SAMtags.pdf> under the 'Base modifications' section.

### Value

a NanoMethResult object to be used with plotting functions

a ModBamFiles data.frame.

the sample annotation.

the exon annotation.

the mod code.

### Functions

- `methy(ModBamResult)`: modbam information getter.
- `methy(object = ModBamResult) <- value`: modbam information setter.
- `samples(ModBamResult)`: sample annotation getter.
- `samples(object = ModBamResult) <- value`: sample annotation setter.
- `exons(ModBamResult)`: exon annotation getter.
- `exons(object = ModBamResult) <- value`: exon annotation setter.
- `mod_code(ModBamResult)`: mod code getter.
- `mod_code(object = ModBamResult) <- value`: mod code setter.
- `ModBamResult()`: Constructor

### Slots

`methy` a ModBamFiles data.frame specifying the samples and paths to bam files.

`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`.

`mod_code` the modification code of interest.

---

NanoMethResult-class *Nanopore Methylation Result*

---

## 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,ANY'
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

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	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(object = 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 setter.

### 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\_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

x	the NanoMethResult object.
regions	a table of regions containing at least columns chr, strand, start and end. Any additional columns can be used for grouping.
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.

**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")
```

---

plot\_gene

*Plot gene methylation*


---

**Description**

Plot the methylation of a gene symbol specified within the exon(x) slot.

**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 = TRUE,
  heatmap_subsample = 50,
  smoothing_window = 2000,
  gene_anno = TRUE,
  palette = ggplot2::scale_colour_brewer(palette = "Set1"),
  line_size = 1,
  mod_scale = c(0, 1),
  span = NULL
)

## S4 method for signature 'ModBamResult,character'
plot_gene(
  x,
  gene,
  window_prop = 0.3,
  anno_regions = NULL,
  binary_threshold = NULL,
  avg_method = c("mean", "median"),
  spaghetti = FALSE,
```



```

heatmap = TRUE,
heatmap_subsample = 50,
smoothing_window = 2000,
gene_anno = TRUE,
palette = ggplot2::scale_colour_brewer(palette = "Set1"),
line_size = 1,
mod_scale = c(0, 1),
span = NULL
)

```

### Arguments

x	the NanoMethResult or ModBamResult 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 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.
heatmap_subsample	how many packed rows of reads to subsample to.
smoothing_window	the window size for smoothing the trend line.
gene_anno	whether to show gene annotation.
palette	the ggplot colour palette used for groups.
line_size	the size of the lines.
mod_scale	the scale range for modification probabilities. Default c(0, 1), set to "auto" for automatic limits.
span	DEPRECATED, use smoothing_window instead. Will be removed in next version.

**Details**

This function plots the methylation data for a given gene. Since V3.0.0 NanoMethViz has changed the smoothing strategy from a loess smoothing to a weighted moving average. This is because the loess smoothing was too computationally expensive for large datasets and had a span parameter that was difficult to tune. The new smoothing strategy is controlled by the `smoothing_window` argument.

**Value**

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

**Functions**

- `plot_gene(x = ModBamResult, gene = character)`: S4 method for `ModBamResult`

**Examples**

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

---

`plot_gene_heatmap`      *Plot gene methylation heatmap*

---

**Description**

Plot the methylation heatmap of a gene symbol specified within the `exon(x)` slot.

**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
)

## S4 method for signature 'ModBamResult,character'
plot_gene_heatmap(
  x,
  gene,
  window_prop = 0.3,
  pos_style = c("to_scale", "compact"),
  subsample = 50
)
```

**Arguments**

x	the NanoMethResult or ModBamResult 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.
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_gene_heatmap(nmr, "Peg3")
```

---

plot_grange	<i>Plot GRanges</i>
-------------	---------------------

---

**Description**

Plot GRanges

**Usage**

```
plot_grange(
  x,
  grange,
  anno_regions = NULL,
  binary_threshold = NULL,
  avg_method = c("mean", "median"),
  spaghetti = FALSE,
  heatmap = TRUE,
  heatmap_subsample = 50,
  gene_anno = TRUE,
  smoothing_window = 2000,
  window_prop = 0,
  palette = ggplot2::scale_colour_brewer(palette = "Set1"),
  line_size = 1,
  span = NULL
)
```

**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.
heatmap_subsample	how many packed rows of reads to subsample to.
gene_anno	whether to show gene annotation.
smoothing_window	the window size for smoothing the trend line.
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.
span	DEPRECATED, use smoothing_window instead. Will be removed in next version.

**Value**

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,  
  legend_name = "group"  
)
```

**Arguments**

<code>x</code>	the log-methylation-ratio matrix.
<code>top</code>	the number of top genes used to calculate pairwise distances.
<code>plot_dims</code>	the numeric vector of the two dimensions to be plotted.
<code>labels</code>	the character vector of labels for data points. By default uses column names of <code>x</code> , set to <code>NULL</code> to plot points.
<code>groups</code>	the character vector of groups the data points will be coloured by. Colour palette can be adjusted using <code>scale_colour_*</code> () functions from <code>ggplot2</code> . If <code>groups</code> is numeric, the points will be coloured by a continuous colour palette. By default, <code>groups</code> is <code>NULL</code> and the points will not be coloured.
<code>legend_name</code>	the name for the legend.

**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	<i>Plot PCA</i>
----------	-----------------

---

### 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,  
  legend_name = "group"  
)
```

### Arguments

<code>x</code>	the log-methylation-ratio matrix.
<code>plot_dims</code>	the numeric vector of the two dimensions to be plotted.
<code>labels</code>	the character vector of labels for data points. By default uses column names of <code>x</code> , set to <code>NULL</code> to plot points.
<code>groups</code>	the character vector of groups the data points will be coloured by.
<code>legend_name</code>	the name for the legend.

### 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 methylation</i>
-------------	--------------------------------

---

### Description

Plot the methylation of a genomic 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 = TRUE,
  heatmap_subsample = 50,
  smoothing_window = 2000,
  gene_anno = TRUE,
  window_prop = 0,
  palette = ggplot2::scale_colour_brewer(palette = "Set1"),
  line_size = 1,
  mod_scale = c(0, 1),
  span = NULL
)

## S4 method for signature 'ModBamResult,character,numeric,numeric'
plot_region(
  x,
  chr,
  start,
  end,
  anno_regions = NULL,
  binary_threshold = NULL,
  avg_method = c("mean", "median"),
  spaghetti = FALSE,
  heatmap = TRUE,
  heatmap_subsample = 50,
  smoothing_window = 2000,
  gene_anno = TRUE,
  window_prop = 0,
```



```
palette = ggplot2::scale_colour_brewer(palette = "Set1"),
line_size = 1,
mod_scale = c(0, 1),
span = NULL
)

## 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 = TRUE,
  heatmap_subsample = 50,
  smoothing_window = 2000,
  gene_anno = TRUE,
  window_prop = 0,
  palette = ggplot2::scale_colour_brewer(palette = "Set1"),
  line_size = 1,
  mod_scale = c(0, 1),
  span = NULL
)

## S4 method for signature 'ModBamResult,factor,numeric,numeric'
plot_region(
  x,
  chr,
  start,
  end,
  anno_regions = NULL,
  binary_threshold = NULL,
  avg_method = c("mean", "median"),
  spaghetti = FALSE,
  heatmap = TRUE,
  heatmap_subsample = 50,
  smoothing_window = 2000,
  gene_anno = TRUE,
  window_prop = 0,
  palette = ggplot2::scale_colour_brewer(palette = "Set1"),
  line_size = 1,
  mod_scale = c(0, 1),
  span = NULL
)
```

**Arguments**

x	the NanoMethResult or ModBamResult 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.
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.
heatmap_subsample	how many packed rows of reads to subsample to.
smoothing_window	the window size for smoothing the trend line.
gene_anno	whether to show gene annotation.
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.
mod_scale	the scale range for modification probabilities. Default c(0, 1), set to "auto" for automatic limits.
span	DEPRECATED, use smoothing_window instead. Will be removed in next version.

**Details**

This function plots the methylation data for a given region. The region is specified by chromosome, start and end positions. The basic plot contains a smoothed line plot of the methylation of each experimental group. Since V3.0.0 NanoMethViz has changed the smoothing strategy from a loess smoothing to a weighted moving average. This is because the loess smoothing was too computationally expensive for large datasets and had a span parameter that was difficult to tune. The new smoothing strategy is controlled by the smoothing\_window argument.

**Value**

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

**Examples**

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

---

plot\_region\_heatmap *Plot region methylation heatmap*

---

**Description**

Plot the methylation heatmap of a genomic region.

**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 'ModBamResult,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
)
```

```

)

## S4 method for signature 'ModBamResult,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 or ModBamResult 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.
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)

```

---

plot\_violin

*Plot violin for regions*


---

### Description

This function plots a violin plot of the methylation proportion for each region in the regions table. The methylation proportion is calculated as the mean of the modification probability within each region and the violin represents the . The regions are then grouped and coloured by the group\_col column in the regions table or samples(x).

**Usage**

```
plot_violin(
  x,
  regions,
  binary_threshold = 0.5,
  group_col = "group",
  palette = ggplot2::scale_colour_brewer(palette = "Set1")
)
```

**Arguments**

**x** the NanoMethResult object.

**regions** a table of regions containing at least columns chr, strand, start and end. Any additional columns can be used for grouping.

**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).

**palette** the ggplot colour palette used for groups.

**Value**

a ggplot object containing the methylation violin plot.

**Examples**

```
nmr <- load_example_nanomethresult()
gene_anno <- exons_to_genes(NanoMethViz::exons(nmr))
plot_violin(nmr, gene_anno)
plot_violin(nmr, gene_anno, group_col = "sample")
```

---

query\_exons

*Query exons*


---

**Description**

Query a data.frame, NanoMethResult or ModBamResult for exon annotation.

**Usage**

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

```
query_exons_gene_id(x, gene_id)
```

```
query_exons_symbol(x, symbol)
```

**Arguments**

x	the object to query.
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

*Query methylation data*

---

**Description**

Query methylation data

**Usage**

```
query_methy(
  x,
  chr,
  start,
  end,
  simplify = TRUE,
  force = FALSE,
  truncate = TRUE,
  site_filter = getOption("NanoMethViz.site_filter", 1L)
)
```

**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.
truncate	when querying from ModBamFiles, whether or not to truncate returned results to only those within the specified region. Otherwise methylation data for entire reads overlapping the region will be returned.
site_filter	the minimum amount of coverage to report a site. This filters the queried data such that any site with less than the filter is not returned. The default is 1, which means that all sites are returned. This option can be set globally using the options(site_filter = ...) which will affect all plotting functions in NanoMethviz.

### Details

The argument `site_filter` can be set globally using the `options(site_filter = ...)` command.

### 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      *Convert methylation file to tabix format*

---

### 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	<i>Objects exported from other packages</i>
-----------	---

---

**Description**

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

**e1071** [sigmoid](#)

---

region_methy_stats	<i>Calculate region methylation statistics</i>
--------------------	--

---

**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)
```



---

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

---

**Description**

Get sample annotation

**Usage**

```
samples(object)
```

---

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

---

**Description**

Set sample annotation

**Usage**

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

---

sort_methy_file	<i>Sort methylation file</i>
-----------------	------------------------------

---

**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, 7
  - exons, 8
  - exons<-, 9
  - methy, 13
  - methy<-, 15
  - mod\_code, 15
  - mod\_code<-, 15
  - NanoMethViz-package, 3
  - raw\_methy\_to\_tabix, 39
  - reexports, 40
  - samples, 41
  - samples<-, 41
  - sort\_methy\_file, 41
- bsseq\_to\_edger, 4
- bsseq\_to\_log\_methy\_ratio, 4
- cluster\_reads, 5
- cluster\_regions, 6
- convert\_methy\_format, 7
- create\_tabix\_file, 7
- exons, 8
- exons, ModBamResult-method  
(ModBamResult-class), 18
- exons, NanoMethResult-method  
(NanoMethResult-class), 20
- exons<-, 9
- exons<-, ModBamResult, data.frame-method  
(ModBamResult-class), 18
- exons<-, NanoMethResult, data.frame-method  
(NanoMethResult-class), 20
- exons\_to\_genes, 8
- filter\_methy, 9
- get\_example\_exons\_mus\_musculus, 10
- get\_exons, 10
- get\_exons\_grcm39 (get\_exons), 10
- get\_exons\_hg19 (get\_exons), 10
- get\_exons\_hg38 (get\_exons), 10
- get\_exons\_homo\_sapiens, 11
- get\_exons\_mm10 (get\_exons), 10
- get\_exons\_mus\_musculus, 11
- load\_example\_modbamresult, 12
- load\_example\_nanomethresult, 12
- methy, 13
- methy, ModBamResult-method  
(ModBamResult-class), 18
- methy, NanoMethResult-method  
(NanoMethResult-class), 20
- methy<-, 15
- methy<-, ModBamResult, ModBamFiles-method  
(ModBamResult-class), 18
- methy<-, NanoMethResult, ANY-method  
(NanoMethResult-class), 20
- methy\_col\_names, 13
- methy\_to\_bsseq, 14
- methy\_to\_edger, 14
- mod\_code, 15
- mod\_code, ModBamResult-method  
(ModBamResult-class), 18
- mod\_code<-, 15
- mod\_code<-, ModBamResult, character-method  
(ModBamResult-class), 18
- modbam\_to\_tabix, 16
- ModBamFiles, 17
- ModBamFiles-class, 17
- ModBamResult (ModBamResult-class), 18
- ModBamResult-class, 18
- NanoMethResult (NanoMethResult-class),  
20
- NanoMethResult-class, 20
- NanoMethViz (NanoMethViz-package), 3
- NanoMethViz-package, 3
- plot\_agg\_genes, 22

plot\_agg\_regions, 23  
 plot\_gene, 24  
 plot\_gene(), 3  
 plot\_gene, ModBamResult, character-method  
     (plot\_gene), 24  
 plot\_gene, NanoMethResult, character-method  
     (plot\_gene), 24  
 plot\_gene\_heatmap, 26  
 plot\_gene\_heatmap, ModBamResult, character-method  
     (plot\_gene\_heatmap), 26  
 plot\_gene\_heatmap, NanoMethResult, character-method  
     (plot\_gene\_heatmap), 26  
 plot\_grange, 27  
 plot\_grange\_heatmap, 29  
 plot\_mds, 30  
 plot\_pca, 31  
 plot\_region, 32  
 plot\_region(), 3  
 plot\_region, ModBamResult, character, numeric, numeric-method  
     (plot\_region), 32  
 plot\_region, ModBamResult, factor, numeric, numeric-method  
     (plot\_region), 32  
 plot\_region, NanoMethResult, character, numeric, numeric-method  
     (plot\_region), 32  
 plot\_region, NanoMethResult, factor, numeric, numeric-method  
     (plot\_region), 32  
 plot\_region\_heatmap, 35  
 plot\_region\_heatmap, ModBamResult, character, numeric, numeric-method  
     (plot\_region\_heatmap), 35  
 plot\_region\_heatmap, ModBamResult, factor, numeric, numeric-method  
     (plot\_region\_heatmap), 35  
 plot\_region\_heatmap, NanoMethResult, character, numeric, numeric-method  
     (plot\_region\_heatmap), 35  
 plot\_region\_heatmap, NanoMethResult, factor, numeric, numeric-method  
     (plot\_region\_heatmap), 35  
 plot\_violin, 36  
  
 query\_exons, 37  
 query\_exons\_gene\_id(query\_exons), 37  
 query\_exons\_region(query\_exons), 37  
 query\_exons\_symbol(query\_exons), 37  
 query\_methy, 38  
  
 raw\_methy\_to\_tabix, 39  
 reexports, 40  
 region\_methy\_stats, 40  
  
 samples, 41  
 samples, ModBamResult-method  
     (ModBamResult-class), 18  
 samples, NanoMethResult-method  
     (NanoMethResult-class), 20  
 samples<-, 41  
 samples<-, ModBamResult, data.frame-method  
     (ModBamResult-class), 18  
 samples<-, NanoMethResult, data.frame-method  
     (NanoMethResult-class), 20  
 show, ModBamFiles-method (ModBamFiles),  
     17  
 sigmoid, 40  
 sigmoid(reexports), 40  
 sort\_methy\_file, 41