Package ‘methylKit’

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**Type**  Package  
**Title**  DNA methylation analysis from high-throughput bisulfite sequencing results  
**Version**  1.30.0  
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**Description**  methylKit is an R package for DNA methylation analysis and annotation from high-throughput bisulfite sequencing. The package is designed to deal with sequencing data from RRBS and its variants, but also target-capture methods and whole genome bisulfite sequencing. It also has functions to analyze base-pair resolution 5hmC data from experimental protocols such as oxBs-Seq and TAB-Seq. Methylation calling can be performed directly from Bismark aligned BAM files.  
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Collate 'methylKit.R' 'backbone.R' 'diffMeth.R' 'clusterSamples.R'
   'regionalize.R' 'processBismarkAln.R' 'RcppExports.R'
   'document_data.R' 'bedgraph.R' 'reorganize.R'
   'percMethylation.R' 'normalizeCoverage.R' 'pool.R'
   'adjustMethylC.R' 'updateMethObject.R' 'batchControl.R'
   'dataSim.R' 'methylDBClasses.R' 'methylDBFunctions.R'
   'tabix.functions.R' 'methSeg.R' 'diffMethDSS.R'
   'deprecated_defunct.R' 'onUnload.R'

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**adjustMethylC**

.adjustMethylC

**Adjust measured 5mC levels using 5hmC levels**

**Description**

Measured 5mC levels via bisulfite sequencing might be a combination of 5hmC and 5mC levels since bisulfite sequencing cannot distinguish between the two. This function can adjust 5mC levels of a bisulfite sequencing experiment if the user supplies corresponding 5hmC levels from the same sample.

**Usage**

```r
adjustMethylC(mc, hmc, save.db = FALSE, ..., chunk.size)
```

## S4 method for signature 'methylRaw,methylRaw'

```r
adjustMethylC(mc, hmc, save.db = FALSE, ..., chunk.size = 1e+06)
```

## S4 method for signature 'methylRawList,methylRawList'

```r
```
adjustMethylC(mc, hmc, save.db = FALSE, ..., chunk.size = 1e+06)

## S4 method for signature 'methylRawDB,methylRawDB'
adjustMethylC(mc, hmc, save.db = TRUE, ..., chunk.size = 1e+06)

## S4 method for signature 'methylRawListDB,methylRawListDB'
adjustMethylC(mc, hmc, save.db = TRUE, ..., chunk.size = 1e+06)

Arguments

  mc            a methylRawList, methylRaw, methylRawDB or methylRawListDB containing
                5mC levels of a sample or set of samples
  hmc          a methylRawList, methylRaw, methylRawDB or methylRawListDB containing
                5hmC levels of a sample or set of samples. If a methylRawList or methylRawListDB
                given the sample order should be same as "mc" methylRawList or methylRawListDB
                object.
  save.db      A Logical to decide whether the resulting object should be saved as flat file
                database or not, default: explained in Details sections
  ...          optional Arguments used when save.db is TRUE
  suffix       A character string to append to the name of the output flat file database,
                only used if save.db is true, default actions: append "_filtered" to current file-
                name if database already exists or generate new file with filename “sampleID_filtered”
  dbdir        The directory where flat file database(s) should be stored, defaults to
                getwd(), working directory for newly stored databases and to same directory for
                already existing database
  dbtype       The type of the flat file database, currently only option is "tabix" (only
                used for newly stored databases)
  chunk.size   Number of rows to be taken as a chunk for processing the methylRawDB or
                methylRawListDB objects (default: 1e6)

Value

returns adjusted 5-methyl cytosine levels in the form of methylRawList, methylRaw, methylRawDB
or methylRawListDB object depending on the input object

Details

The parameter chunk.size is only used when working with methylRawDB or methylRawListDB
objects, as they are read in chunk by chunk to enable processing large-sized objects which are
stored as flat file database. Per default the chunk.size is set to 1M rows, which should work for most
systems. If you encounter memory problems or have a high amount of memory available feel free
to adjust the chunk.size.

The parameter save.db is per default TRUE for methylDB objects as methylRawDB and methylRawListDB,
while being per default FALSE for methylRaw and methylRawList. If you wish to save the result
of an in-memory-calculation as flat file database or if the size of the database allows the calculation
in-memory, then you might change the value of this parameter.
assocComp

References


Examples

# read 5hmC and 5mC files
hmc.file = system.file("extdata", "test1.myCpG.txt", package = "methylKit")
mc.file = system.file("extdata", "test2.myCpG.txt", package = "methylKit")

my5hmC = methRead( hmc.file, sample.id="hmc", assembly="hg18")
my5mC = methRead( mc.file, sample.id="mc", assembly="hg18")

# adjusting the 5mC levels using 5hmC levels
adjusted.5mC = adjustMethylC(my5mC, my5hmC)

assocComp

Associate principal components with sample annotations

Description

This function associates principal components with sample annotations such as age, gender, batch_id. Can be used to detect which batch effects are associated with the variation in the methylation values.

Usage

assocComp(mBase, sampleAnnotation)

Arguments

mBase methylBase or methylBaseDB object with no NA values in the data part.
sampleAnnotation a data frame where columns are different annotations and rows are the samples, in the same order as in the methylBase object.

Value

a named list of principal component matrix (named 'pcs'), by principal components (named 'vars') and a p-value matrix showing association p-values between sample annotations and principal components (named 'association').

Author(s)

Altuna Akalin
Examples

```r
data(methylKit)
sampleAnnotation=data.frame(batch_id=c("a","a","b","b"),age=c(19,34,23,40))
as=assocComp(mBase=methylBase.obj,sampleAnnotation)
```

---

**bedgraph**

Get bedgraph from methylRaw, methylRawList and methylDiff objects

---

**Description**

The function converts `methylRaw`, `methylRawDB`, `methylRawList`, `methylRawListDB`, `methylDiff` or `methylDiffDB` object into a bedgraph format. It either writes as a file or returns a `data.frame`

**Usage**

```r
bedgraph(
    methylObj,
    file.name = NULL,
    col.name,
    unmeth = FALSE,
    log.transform = FALSE,
    negative = FALSE,
    add.on = "",
    chunk.size = 1e+06
)
```

```r
## S4 method for signature 'methylDiff'
bedgraph(
    methylObj,
    file.name,
    col.name,
    unmeth,
    log.transform,
    negative,
    add.on
)
```

```r
## S4 method for signature 'methylRaw'
bedgraph(
    methylObj,
    file.name,
    col.name,
    unmeth,
    log.transform,
```
negative,
add.on
)

## S4 method for signature 'methylRawList'
bedgraph(
methylObj,
file.name,
col.name,
unmeth,
log.transform,
negative,
add.on
)

## S4 method for signature 'methylRawDB'
bedgraph(
methylObj,
file.name = NULL,
col.name,
unmeth = FALSE,
log.transform = FALSE,
negative = FALSE,
add.on = "",
chunk.size = 1e+06
)

## S4 method for signature 'methylRawListDB'
bedgraph(
methylObj,
file.name = NULL,
col.name,
unmeth = FALSE,
log.transform = FALSE,
negative = FALSE,
add.on = "",
chunk.size = 1e+06
)

## S4 method for signature 'methylDiffDB'
bedgraph(
methylObj,
file.name,
col.name,
log.transform,
negative,
add.on,
chunk.size
Arguments

methylObj a methylRaw, methylRawDB, methylRawList, methylRawListDB, methylDiff or methylDiffDB object

file.name Default: NULL. if a string is given a bedgraph file will be written, if NULL a data.frame or a list of data frames will be returned

col.name name of the column in methylRaw, methylRawDB, methylRawList, methylRawListDB, methylDiff or methylDiffDB objects to be used as a score for the bedgraph. For methylDiff or methylDiffDB, col.name must be one of the following 'pvalue','qvalue', 'meth.diff'. For methylRaw, methylRawDB, methylRawList and methylRawListDB it must be one of the following 'coverage', 'numCs', 'numTs', 'perc.meth'

unmeth when working with methylRaw, methylRawDB, methylRawList and methylRawListDB objects should you output unmethylated C percentage this makes it easier to see the unmethylated bases because their methylation percentage values will be zero. Only invoked when file.name is not NULL.

log.transform Default FALSE, If TRUE the score column of the bedgraph will be in log10 scale. Ignored when col.name='perc.meth'

negative Default FALSE, If TRUE, the score column of the bedgraph will be multiplied by -1. Ignored when col.name='perc.meth'

add.on additional string to be add on the track line of bedgraph. can be viewlimits,priority etc. Check bedgraph track line options at UCSC browser

chunk.size Number of rows to be taken as a chunk for processing the methylRawDB, methylRawListDB or methylDiffDB objects, default: 1e6

Value

Returns a data.frame or list of data.frames if file.name=NULL, if a file.name given appropriate bed file will be written to that file

Details

The parameter chunk.size is only used when working with methylRawDB, methylRawListDB or methylDiffDB objects, as they are read in chunk by chunk to enable processing large-sized objects which are stored as flat file database. Per default the chunk.size is set to 1M rows, which should work for most systems. If you encounter memory problems or have a high amount of memory available feel free to adjust the chunk.size.

Examples

data(methylKit)

# getting a bedgraph file from a methylDiff object containing differential
# methylation percentages
bedgraph(methylDiff.obj, file.name="test.bed", col.name="meth.diff",
unmeth=FALSE,log.transform=FALSE,negative=FALSE,add.on="")
calculateDiffMeth

# remove the file
unlink("test.bed")

# getting a bedgraph file from a methylBase object containing percent methylation values
bedgraph(methylRawList.obj[[1]], file.name="test2.bed", col.name="perc.meth", unmeth=FALSE, log.transform=FALSE, negative=FALSE, add.on="")

unlink("test2.bed") # remove the file

calculateDiffMeth  Calculate differential methylation statistics

Description

The function calculates differential methylation statistics between two groups of samples. The function uses either logistic regression test or Fisher’s Exact test to calculate differential methylation. See the rest of the help page and references for detailed explanation on statistics.

Usage

calculateDiffMeth(
  .Object,
  covariates = NULL,
  overdispersion = c("none", "MN", "shrinkMN"),
  adjust = c("SLIM", "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none", "qvalue"),
  effect = c("wmean", "mean", "predicted"),
  parShrinkMN = list(),
  test = c("F", "Chisq", "fast.fisher", "midPval"),
  mc.cores = 1,
  slim = TRUE,
  weighted.mean = TRUE,
  chunk.size = 1e+06,
  save.db = FALSE,
  ...
)

## S4 method for signature 'methylBaseDB'
calculateDiffMeth(
  .Object,
  covariates = NULL,
  overdispersion = c("none", "MN", "shrinkMN"),
  adjust = c("SLIM", "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none", "qvalue"),
  effect = c("wmean", "mean", "predicted"),
  ...)
parShrinkMN = list(),
test = c("F", "Chisq", "fast.fisher", "midPval"),
mc.cores = 1,
slim = TRUE,
weighted.mean = TRUE,
chunk.size = 1e+06,
save.db = TRUE,
...)

Arguments

.Object a methylBase or methylBaseDB object to calculate differential methylation

covariates a data.frame containing covariates, which should be included in the test.

overdispersion If set to "none"(default), no overdispersion correction will be attempted. If set to "MN", basic overdispersion correction, proposed by McCullagh and Nelder (1989) will be applied. This correction applies a scaling parameter to variance estimated by the model. EXPERIMENTAL: If set to "shrinkMN", scaling parameter will be shrunk towards a common value (not thoroughly tested as of yet).

adjust different methods to correct the p-values for multiple testing. Default is "SLIM" from methylKit. For "qvalue" please see qvalue and for all other methods see p.adjust.

effect method to calculate the mean methylation different between groups using read coverage as weights (default). When set to "mean", the generic mean is applied and when set to "predicted", predicted means from the logistic regression model is used for calculating the effect.

parShrinkMN a list for squeezeVar(). (NOT IMPLEMENTED)

test the statistical test used to determine the methylation differences. The Chisq-test is used by default for more than two groups, while the F-test can be chosen if overdispersion control is applied. If there is one sample per group the Fisher’s exact test will be applied using "fast.fisher", while "midPval" can be choosen to boost calculation speed. See details section for more information.

mc.cores integer denoting how many cores should be used for parallel differential methylation calculations (can only be used in machines with multiple cores).

slim If set to FALSE, adjust will be set to "BH" (default behaviour of earlier versions)

weighted.mean If set to FALSE, effect will be set to "mean" (default behaviour of earlier versions)

chunk.size Number of rows to be taken as a chunk for processing the methylBaseDB objects (default: 1e6)

save.db A Logical to decide whether the resulting object should be saved as flat file database or not, default: explained in Details section.

... optional Arguments used when save.db is TRUE
suffix A character string to append to the name of the output flat file database, only used if save.db is true. Default actions: The default suffix is a 13-character random string appended to the fixed prefix "methylDiff", e.g., "methylDiff_16d3047c1a254.txt.bgz". 
dbdir The directory where flat file database(s) should be stored, defaults to getwd(), working directory for newly stored databases and to same directory for already existing database 
dbtype The type of the flat file database, currently only option is "tabix" (only used for newly stored databases)

Value

A methylDiff object containing the differential methylation statistics and locations for regions or bases

Details

Covariates can be included in the analysis. The function will then try to separate the influence of the covariates from the treatment effect via a linear model. The Chisq-test is used per default only when no overdispersion correction is applied. If overdispersion correction is applied, the function automatically switches to the F-test. The Chisq-test can be manually chosen in this case as well, but the F-test only works with overdispersion correction switched on.

If there is one sample in each group, e.g., after applying the pooling samples, the Fisher's exact test will be applied for differential methylation. methyKit offers two implementations to perform this test, which yield slightly different results but differ much in computation time. "fast.fisher" is a cut down version of 'fisher.test()' that should produce the exact same results as the base implementation, while "midPval" will produce marginaly different p-values, but offers a large boost in calculation speed.

The parameter chunk.size is only used when working with methylBaseDB objects, as they are read in chunk by chunk to enable processing large-sized objects which are stored as flat file database. Per default the chunk.size is set to 1M rows, which should work for most systems. If you encounter memory problems or have a high amount of memory available feel free to adjust the chunk.size.

The parameter save.db is per default TRUE for methylDB objects as methylBaseDB, while being per default FALSE for methyl1Base. If you wish to save the result of an in-memory-calculation as flat file database or if the size of the database allows the calculation in-memory, then you might want to change the value of this parameter.

References


See Also

calculateDiffMethDSS

dataSim, pool, reorganize

Examples

data(methylKit)

# The Chisq-test will be applied when no overdispersion control is chosen.
my.diffMeth=calculateDiffMeth(methylBase.obj, covariates=NULL,
    overdispersion="none",
    adjust="SLIM")

# pool samples in each group
pooled.methylBase=pool(methylBase.obj, sample.ids=c("test", "control")

# After applying the pool() function, there is one sample in each group.
# The Fisher’s exact test will be applied for differential methylation.
my.diffMeth2=calculateDiffMeth(pooled.methylBase, covariates=NULL,
    overdispersion="none",
    adjust="SLIM")

# Covariates and overdispersion control:
# generate a methylBase object with age as a covariate
covariates=data.frame(age=c(30,80,30,80))
sim.methylBase=dataSim(replicates=4, sites=1000, treatment=c(1,1,0,0),
    covariates=covariates,
    sample.ids=c("test1", "test2", "ctrl1", "ctrl2")

# Apply overdispersion correction and include covariates
# in differential methylation calculations.
my.diffMeth3=calculateDiffMeth(sim.methylBase,
    covariates=covariates,
    overdispersion="MN", test="Chisq", mc.cores=1)
## methylBase

```r
## S4 method for signature 'methylBase'
calculateDiffMethDSS(
  meth,
  adjust = c("SLIM", "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none", "qvalue"),
  mc.cores = 1
)
```

### Arguments

- **meth**: a methylBase object
- **adjust**: different methods to correct the p-values for multiple testing. Default is "SLIM" from methylKit. For "qvalue" please see `qvalue` and for all other methods see `p.adjust`
- **mc.cores**: integer denoting how many cores should be used for parallel differential methylation calculations (can only be used in machines with multiple cores).

### Value

- a methylDiff object

### References

Feng H, Conneely K and Wu H (2014). A bayesian hierarchical model to detect differentially methylated loci from single nucleotide resolution sequencing data. Nucleic acids research

### Examples

```r
data(methylKit)
dssDiffay <- calculateDiffMethDSS(methylBase.obj, adjust="SLIM", mc.cores=1)
```

---

### clusterSamples

Hierarchical Clustering using methylation data

The function clusters samples using `hclust` function and various distance metrics derived from percent methylation per base or per region for each sample.

### Description

Hierarchical Clustering using methylation data

The function clusters samples using `hclust` function and various distance metrics derived from percent methylation per base or per region for each sample.
Usage

clusterSamples(.Object, dist="correlation", method="ward",
               sd.filter=TRUE, sd.threshold=0.5,
               filterByQuantile=TRUE, plot=TRUE, chunk.size)

## S4 method for signature 'methylBase'
clusterSamples(
  .Object,
  dist,
  method,
  sd.filter,
  sd.threshold,
  filterByQuantile,
  plot
)

## S4 method for signature 'methylBaseDB'
clusterSamples(
  .Object,
  dist = "correlation",
  method = "ward",
  sd.filter = TRUE,
  sd.threshold = 0.5,
  filterByQuantile = TRUE,
  plot = TRUE,
  chunk.size = 1e+06
)

Arguments

.Object a methylBase or methylBaseDB object
dist the distance measure to be used. This must be one of "correlation", "euclidean", "maximum", "manhattan", "canberra", "binary" or "minkowski". Any unambiguous abbreviation can be given. (default: "correlation")
method the agglomeration method to be used. This should be (an unambiguous abbreviation of) one of "ward", "ward.D", "ward.D2", "single", "complete", "average", "mcquitty", "median" or "centroid". (default: "ward")
sd.filter If TRUE, the bases/regions with low variation will be discarded prior to clustering (default: TRUE)
sd.threshold A numeric value. If filterByQuantile is TRUE, features whose standard deviations is less than the quantile denoted by sd.threshold will be removed. If filterByQuantile is FALSE, then features whose standard deviations is less than the value of sd.threshold will be removed. (default: 0.5)
filterByQuantile A logical determining if sd.threshold is to be interpreted as a quantile of all Standard Deviation values from bases/regions (the default), or as an absolute value
dataSim

plot: a logical value indicating whether to plot hierarchical clustering. (default: TRUE)
chunk.size: Number of rows to be taken as a chunk for processing the methylBaseDB objects, default: 1e6

Value

dataSim

Simulate DNA methylation data

Description

The function simulates DNA methylation data from multiple samples. See references for detailed explanation on statistics.

Usage

dataSim(
  replicates,
  sites,
  treatment,
  percentage = 10,
  effect = 25,
  alpha = 0.4,
  beta = 0.5,
  theta = 10,
  covariates = NULL,
  sample.ids = NULL,
  assembly = "hg18",
  context = "CpG",
)
dataSim

add.info = FALSE

Arguments

replicates the number of samples that should be simulated.
sites the number of CpG sites per sample.
treatment a vector containing treatment information.
percentage the proportion of sites which should be affected by the treatment.
effect a number between 0 and 100 specifying the effect size of the treatment. This is essentially describing the average percent methylation difference between differentially methylated bases. See 'Examples' and 'Details'.
alpha shape1 parameter for beta distribution (used for initial sampling of methylation proportions)
beta shape2 parameter for beta distribution (used for initial sampling of methylation proportions)
theta dispersion parameter for beta distribution (initial sampling of methylation proportions)
covariates a data.frame containing covariates (optional)
sample.ids will be generated automatically from treatment, but can be overwritten by a character vector containing sample names.
assembly the assembly description (e.g. "hg18"). Only needed for book keeping.
context the experimental context of the data (e.g. "CpG"). Only needed for book keeping.
add.info if set to TRUE, the output will be a list with the first element being the methylbase object and a vector of indices that indicate which CpGs should be differentially methylated. This vector can be used to subset simulated methylBase or methylDiff object with differentially methylated bases.

Value

a methylBase object containing simulated methylation data, or if add.info=TRUE a list containing the methylbase object and the indices of all treated sites (differentially methylated bases or regions) as the second element.

Details

The function uses a Beta distribution to simulate the methylation proportion background across all samples. The parameters alpha, beta used in a beta distribution to draw methylation proportions, mu, from a typical bimodal distribution. For each initial methylation proportion drawn using the parameters above, a range of methylation proportions is distributed around the original mu with overdispersion parameter theta, this is using an alternative parameterization of Beta distribution: Beta(mu, theta). The parameters percentage and effect determine the proportion of sites that are affected by the treatment (meaning differential sites) and the strength of this influence, respectively. effect is added on top of mu for the CpGs that are affected by the treatment. The affected group of samples
for that particular CpG will now be distributed by $Beta(\mu + effect, \theta)$. The coverage is modeled with a negative binomial distribution, using `rnbinom` function with `size=1` and `prob=0.01`. The additional information needed for a valid methylBase object, such as CpG start, end and strand, is generated as "dummy values", but can be overwritten as needed.

Examples

```r
data(methylKit)

# Simulate data for 4 samples with 20000 sites each.
# The methylation in 10% of the sites are elevated by 25%.
my.methylBase=dataSim(replicates=4, sites=2000, treatment=c(1,1,0,0),
percentage=10, effect=25)
```

---

**diffMethPerChr**

Get and plot the number of hyper/hypo methylated regions/bases per chromosome

Description

This function gets number of hyper/hypo methylated regions/bases from `methylDiff` object. It can also plot percentages of differentially methylated bases per chromosome.

Usage

```r
diffMethPerChr(
  x,
  plot = TRUE,
  qvalue.cutoff = 0.01,
  meth.cutoff = 25,
  exclude = NULL,
  keep.empty.chrom = FALSE,
  ...
)
```

```r
## S4 method for signature 'methylDiff'
diffMethPerChr(
  x,
  plot = TRUE,
  qvalue.cutoff = 0.01,
  meth.cutoff = 25,
  exclude = NULL,
  keep.empty.chrom = FALSE,
  ...
)
```
S4 method for signature 'methylDiffDB'

diffMethPerChr(
  x,
  plot = TRUE,
  qvalue.cutoff = 0.01,
  meth.cutoff = 25,
  exclude = NULL,
  keep.empty.chrom = FALSE,
  ...
)

Arguments

- x: a methylDiff object
- plot: TRUE|FALSE. If TRUE horizontal barplots for proportion of hypo/hyper methylated bases/regions
- qvalue.cutoff: cutoff for q-value
- meth.cutoff: cutoff for percent methylation difference
- exclude: names of chromosomes to be excluded from plot
- keep.empty.chrom: keep chromosome in list / plot, even if it contains no hyper/hypo sites
  ...
- extra graphical parameters to be passed to barplot function

Value

plots a piechart or a barplot for percentage of the target features overlapping with annotation

Examples

data(methylKit)

# get a list of differentially methylated bases/regions per chromosome and overall
diffMethPerChr(methylDiff.obj, plot=FALSE,qvalue.cutoff=0.01,
  meth.cutoff=25,exclude=NULL)

Description

The function extracts part of the data and returns a new object.
## Usage

```r
## S4 method for signature 'methylRaw,ANY,ANY,ANY'
x[i, j]
```

```r
## S4 method for signature 'methylBase,ANY,ANY,ANY'
x[i, j]
```

```r
## S4 method for signature 'methylDiff,ANY,ANY,ANY'
x[i, j]
```

```r
## S4 method for signature 'methylRawDB,ANY,ANY,ANY'
x[i, j]
```

```r
## S4 method for signature 'methylBaseDB,ANY,ANY,ANY'
x[i, j]
```

```r
## S4 method for signature 'methylDiffDB,ANY,ANY,ANY'
x[i, j]
```

## Arguments

- `x`: an `methylBase`, `methylBaseDB`, `methylRaw`, `methylRawDB` or `methylDiff` object.
- `i`: a numeric or logical vector. This vector corresponds to bases or regions contained in `methylKit` objects. The vector is used to subset the data.
- `j`: This argument can not be used for the extraction of columns. As unintentional extraction of the columns will cause an error in the downstream analysis. Using this argument will cause an error. Use `getData` to access the data part of the objects.

## Examples

```r
data(methylKit)

# selects first hundred rows, returns a methylRaw object
subset1=methylRawList.obj[[1]][1:100]

# selects first hundred rows, returns a methylBase object
subset2=methylBase.obj[1:100,]

# selects first hundred rows, returns a methylDiff object
subset3=methylDiff.obj[1:100,]

# This will get chromosomes, will return a factor
# That means the resulting object will ceases to be a methylKit object
chrs=methylDiff.obj[[2]]
```
filterByCoverage  

Filter methylRaw, methylRawDB, methylRawList and methylRawListDB object based on read coverage

Description

This function filters methylRaw, methylRawDB, methylRawList and methylRawListDB objects. You can filter based on lower read cutoff or high read cutoff. Higher read cutoff is useful to eliminate PCR effects. Lower read cutoff is useful for doing better statistical tests.

Usage

```r
filterByCoverage(
  methylObj,
  lo.count = NULL,
  lo.perc = NULL,
  hi.count = NULL,
  hi.perc = NULL,
  chunk.size = 1e+06,
  save.db = FALSE,
  ...
)
```

## S4 method for signature 'methylRaw'

```r
filterByCoverage(
  methylObj,
  lo.count = NULL,
  lo.perc = NULL,
  hi.count = NULL,
  hi.perc = NULL,
  chunk.size = 1e+06,
  save.db = FALSE,
  ...
)
```

## S4 method for signature 'methylRawList'

```r
filterByCoverage(
  methylObj,
  lo.count = NULL,
  lo.perc = NULL,
  hi.count = NULL,
  hi.perc = NULL,
  chunk.size = 1e+06,
  save.db = FALSE,
  ...
)
```
filterByCoverage

## S4 method for signature 'methylRawDB'
filterByCoverage(
  methylObj,
  lo.count = NULL,
  lo.perc = NULL,
  hi.count = NULL,
  hi.perc = NULL,
  chunk.size = 1e+06,
  save.db = TRUE,
  ...
)

## S4 method for signature 'methylRawListDB'
filterByCoverage(
  methylObj,
  lo.count = NULL,
  lo.perc = NULL,
  hi.count = NULL,
  hi.perc = NULL,
  chunk.size = 1e+06,
  save.db = TRUE,
  ...
)

Arguments

methylObj  a methylRaw, methylRawDB, methylRawList or methylRawListDB object
lo.count  An integer for read counts. Bases/regions having lower coverage than this count is discarded
lo.perc  A double [0-100] for percentile of read counts. Bases/regions having lower coverage than this percentile is discarded
hi.count  An integer for read counts. Bases/regions having higher coverage than this is count discarded
hi.perc  A double [0-100] for percentile of read counts. Bases/regions having higher coverage than this percentile is discarded
chunk.size  Number of rows to be taken as a chunk for processing the methylRawDB or methylRawListDB objects, default: 1e6
save.db  A Logical to decide whether the resulting object should be saved as flat file database or not, default: explained in Details sections
...

suffix  A character string to append to the name of the output flat file database, only used if save.db is true, default actions: append "_filtered" to current file-name if database already exists or generate new file with filename "sampleID_filtered"
dbdir  The directory where flat file database(s) should be stored, defaults to getwd(), working directory for newly stored databases and to original directory for already existing database
getAssembly

dbtype The type of the flat file database, currently only option is "tabix" (only used for newly stored databases)

Value

methylRaw, methylRawDB, methylRawList or methylRawListDB object depending on input object

Details

The parameter chunk.size is only used when working with methylRawDB or methylRawListDB objects, as they are read in chunk by chunk to enable processing large-sized objects which are stored as flat file database. Per default the chunk.size is set to 1M rows, which should work for most systems. If you encounter memory problems or have a high amount of memory available feel free to adjust the chunk.size.

The parameter save.db is per default TRUE for methylDB objects as methylRawDB and methylRawListDB, while being per default FALSE for methylRaw and methylRawList. If you wish to save the result of an in-memory-calculation as flat file database or if the size of the database allows the calculation in-memory, then you might change the value of this parameter.

Examples

data(methylKit)

# filter out bases with coverage above 500 reads
filtered1=filterByCoverage(methylRawList.obj, lo.count=NULL, lo.perc=NULL, hi.count=500, hi.perc=NULL)

# filter out bases with coverage above 99.9th percentile of coverage
# distribution
filtered2=filterByCoverage(methylRawList.obj, lo.count=NULL, lo.perc=NULL, hi.count=NULL, hi.perc=99.9)

# filter out bases with coverage above 500 reads and save to database
# "test1_max500.txt.bgz"
# in directory "methylDB", filtered3 now becomes a \code{methylRawDB} object
filtered3=filterByCoverage(methylRawList.obj[[1]], lo.count=NULL, lo.perc=NULL, hi.count=500, hi.perc=NULL, save.db=TRUE, suffix="max500", dbdir="methylDB")

# tidy up
rm(filtered3)
unlink("methylDB", recursive=TRUE)

getAssembly

get assembly of the genome
**getAssembly**

**Description**

The function returns the genome assembly stored in any of the `methylBase, methylBaseDB, methylRaw, methylRawDB, methylDiff` objects.

**Usage**

```r
getAssembly(x)
```

```r
## S4 method for signature 'methylBase'
getAssembly(x)
```

```r
## S4 method for signature 'methylRaw'
getAssembly(x)
```

```r
## S4 method for signature 'methylDiff'
getAssembly(x)
```

```r
## S4 method for signature 'methylRawDB'
getAssembly(x)
```

```r
## S4 method for signature 'methylBaseDB'
getAssembly(x)
```

```r
## S4 method for signature 'methylDiffDB'
getAssembly(x)
```

**Arguments**

- `x`: an `methylBase, methylBaseDB, methylRaw, methylRawDB` or `methylDiff` object.

**Value**

the assembly string for the object.

**Examples**

```r
data(methylKit)
getAssembly(methylBase.obj)
getAssembly(methylDiff.obj)
getAssembly(methylRawList.obj[[1]])
```
getContext  

get the context of methylation

Description

The function returns the context of methylation. For example: "CpG","CHH" or "CHG"

Usage

getContext(x)

## S4 method for signature 'methylBase'
getContext(x)

## S4 method for signature 'methylRaw'
getContext(x)

## S4 method for signature 'methylDiff'
getContext(x)

## S4 method for signature 'methylRawDB'
getContext(x)

## S4 method for signature 'methylBaseDB'
getContext(x)

## S4 method for signature 'methylDiffDB'
getContext(x)

Arguments

x an methylBase,methylBaseDB, methylRaw,methylRawDB or an methylDiff object

Value

a string for the context methylation

Examples

data(methylKit)

getContext(methylBase.obj)
getContext(methylDiff.obj)
getContext(methylRawList.obj[[1]])
getCorrelation

get correlation between samples in methylBase or methylBaseDB object

Description

The functions returns a matrix of correlation coefficients and/or a set of scatterplots showing the relationship between samples. The scatterplots will contain also fitted lines using lm() for linear regression and lowess for polynomial regression.

Usage

getCorrelation(object, method="pearson", plot=FALSE, nrow)

## S4 method for signature 'methylBase'
getCorrelation(object, method = c("pearson", "kendall", "spearman"), plot)

## S4 method for signature 'methylBaseDB'
getCorrelation(object, method = "pearson", plot = FALSE, nrow = 2e+06)

Arguments

object a methylBase or methylBaseDB object

method a character string indicating which correlation coefficient (or covariance) is to be computed (default: "pearson", other options are "kendall" and "spearman")

plot scatterPlot if TRUE (default: FALSE)

nrow a numeric giving the number of lines to read in of methylBaseDB object, defaults to 2e6

Value

a correlation matrix object and plot scatterPlot

Details

The argument 'nrow' is only evaluated if the input is a methylBaseDB object. If 'nrow' is not specified getCorrelation will read the first 2M records of the given object, but if you want to read all records 'nrow' has to be NULL. You should change 'nrow' if using getCorrelation with all records of the methylBaseDB object would take too long.

If the scatter plot is plotted, the red line in the plot is from linear regression fit and the green line is from polynomial regression fit with stats::lowess.
getCoverageStats

get coverage stats from methylRaw object

Description

The function returns basic statistics about read coverage per base. It can also plot a histogram of read coverage values.

Usage

getCoverageStats(
  object,
  plot = FALSE,
  both.strands = FALSE,
  labels = TRUE,
  ...
)

## S4 method for signature 'methylRaw'
getCoverageStats(
  object,
  plot = FALSE,
  both.strands = FALSE,
  labels = TRUE,
  ...
)

## S4 method for signature 'methylRawDB'

Examples

data(methylKit)

getCorrelation(methylBase.obj,method="pearson",plot=FALSE)

# create methylBaseDB
methylBaseDB.obj <- unite(methylRawList.obj,save.db=TRUE,dbdir="methylDB")

getCorrelation(methylBaseDB.obj,method="pearson",plot=FALSE,nrow=10000)

# remove Database again
rm(methylBaseDB.obj)
unlink("methylDB",recursive=TRUE)
getCoverageStats

getCoverageStats(
  object,
  plot = FALSE,
  both.strands = FALSE,
  labels = TRUE,
  ...,
  chunk.size = 1e+06
)

Arguments

  object  a methylRaw or methylRawDB object
  plot    plot a histogram of coverage if TRUE (default:FALSE)
  both.strands  do stats and plot for both strands if TRUE (default:FALSE)
  labels  should the bars of the histogram have labels showing the percentage of values
           in each bin (default:TRUE)
  ...     options to be passed to hist function
  chunk.size  Number of rows to be taken as a chunk for processing the methylRawDB objects
              (default: 1e6)

Value

  a summary of coverage statistics or plot a histogram of coverage

Details

  The parameter chunk.size is only used when working with methylRawDB or methylRawListDB
  objects, as they are read in chunk by chunk to enable processing large-sized objects which are
  stored as flat file database. Per default the chunk.size is set to 1M rows, which should work for most
  systems. If you encounter memory problems or have a high amount of memory available feel free
  to adjust the chunk.size.

Examples

data(methylKit)

  # gets coverage stats for the first sample in methylRawList.obj object
  getCoverageStats(methylRawList.obj[[1]],plot=TRUE,both.strands=FALSE,labels=TRUE)
**Description**

The functions retrieves the table containing methylation information from methylKit Objects. The data retrieved from this function is of a `data.frame`. This is basically containing all relevant methylation information per genomic region or base.

**Usage**

```r
getData(x)
```

## S4 method for signature 'methylBase'
```
getData(x)
```

## S4 method for signature 'methylRaw'
```
getData(x)
```

## S4 method for signature 'methylDiff'
```
getData(x)
```

## S4 method for signature 'methylRawDB'
```
getData(x)
```

## S4 method for signature 'methylBaseDB'
```
getData(x)
```

## S4 method for signature 'methylDiffDB'
```
getData(x)
```

**Arguments**

- `x` an `methylBase`, `methylBaseDB`, `methylRaw`, `methylRawDB` or `methylDiff` object

**Value**

data frame for methylation events

**Examples**

data(methylKit)

# following commands show first lines of returned data.frames from getData() function
head(
  getData(methylBase.obj))
getDBPath

getDBPath

getDBPath = Get path to database of the methylDB objects

Description

The function returns the path to the flat file database that stores the data of the methylRawDB, methylRawListDB, methylBaseDB or methylDiffDB objects.

Usage

getDBPath(x)

## S4 method for signature 'methylRawListDB'
getDBPath(x)

## S4 method for signature 'methylBaseDB'
getDBPath(x)

## S4 method for signature 'methylRawDB'
getDBPath(x)

## S4 method for signature 'methylDiffDB'
getDBPath(x)

Arguments

x an methylBaseDB,methylRawDB,methylRawListDB or methylDiffDB object

Examples

data(methylKit)

methylBaseDB.obj <- unite(methylRawList.obj,save.db=TRUE,dbdir="methylDB")

#The path to the database is returned
getDBPath(methylBaseDB.obj)

# remove Database again
rm(methylBaseDB.obj)
getMethylationStats  get Methylation stats from methylRaw or methylRawDB object

Description

The function returns basic statistics about It can also plot a histogram of

Usage

getMethylationStats(
  object,
  plot = FALSE,
  both.strands = FALSE,
  labels = TRUE,
  ...,  
  chunk.size = 1e+06
)

## S4 method for signature 'methylRaw'
getMethylationStats(
  object,
  plot = FALSE,
  both.strands = FALSE,
  labels = TRUE,
  ...,  
  chunk.size = 1e+06
)

## S4 method for signature 'methylRawDB'
getMethylationStats(
  object,
  plot = FALSE,
  both.strands = FALSE,
  labels = TRUE,
  ...,  
  chunk.size = 1e+06
)

Arguments

object a methylRaw or methylRawDB object
plot plot a histogram of Methylation if TRUE (default:FALSE)
both.strands do plots and stats for both strands seperately if TRUE (default:FALSE)
**getMethylDiff**

labels should the bars of the histogram have labels showing the percentage of values in each bin (default: TRUE)

chunk.size Number of rows to be taken as a chunk for processing the methylRawDB objects (default: 1e6)

Value

a summary of Methylation statistics or plot a histogram of coverage

Details

The parameter chunk.size is only used when working with methylRawDB or methylRawListDB objects, as they are read in chunk by chunk to enable processing large-sized objects which are stored as flat file database. Per default the chunk.size is set to 1M rows, which should work for most systems. If you encounter memory problems or have a high amount of memory available feel free to adjust the chunk.size.

Examples

data(methylKit)

# gets Methylation stats for the first sample in methylRawList.obj object
getMethylationStats(methylRawList.obj[[1]],plot=TRUE,
both.strands=FALSE,labels=TRUE)

---

**getMethylDiff**

`getMethylDiff` gets differentially methylated regions/bases based on cutoffs

Description

The function subsets a `methylDiff` or `methylDiffDB` object in order to get differentially methylated bases/regions satisfying thresholds.

Usage

```r
getMethylDiff(
  .Object,
  difference = 25,
  qvalue = 0.01,
  type = "all",
  chunk.size = 1e+06,
  save.db = FALSE,
  ...)
```

getMethylDiff

## S4 method for signature 'methylDiff'
getMethylDiff(
  .Object,
  difference = 25,
  qvalue = 0.01,
  type = "all",
  chunk.size = 1e+06,
  save.db = FALSE,
  ...
)

## S4 method for signature 'methylDiffDB'
getMethylDiff(
  .Object,
  difference = 25,
  qvalue = 0.01,
  type = "all",
  chunk.size = 1e+06,
  save.db = TRUE,
  ...
)

Arguments

**.Object**
- a `methylDiff` or `methylDiffDB` object

**difference**
- cutoff for absolute value of methylation percentage change between test and control (default: 25)

**qvalue**
- cutoff for qvalue of differential methylation statistic (default: 0.01)

**type**
- one of the "hyper", "hypo" or "all" strings. Specifies what type of differentially methylated bases/regions should be returned. For retrieving Hyper-methylated regions/bases type="hyper", for hypo-methylated type="hypo" (default: "all")

**chunk.size**
- Number of rows to be taken as a chunk for processing the `methylDiffDB` objects (default: 1e6)

**save.db**
- A Logical to decide whether the resulting object should be saved as flat file database or not, default: see Details

**...**
- optional Arguments used when `save.db` is TRUE

**suffix**
- A character string to append to the name of the output flat file database, only used if `save.db` is true, default actions: append "_type" to current filename if database already exists or generate new file with filename "methylDiff_type"

**dbdir**
- The directory where flat file database(s) should be stored, defaults to `getwd()`, working directory for newly stored databases and to same directory for already existing database

**dbtype**
- The type of the flat file database, currently only option is "tabix" (only used for newly stored databases)
getSampleID

Value

a methylDiff or methylDiffDB object containing the differential methylated locations satisfying the criteria

Details

The parameter chunk.size is only used when working with methylDiffDB objects, as they are read in chunk by chunk to enable processing large-sized objects which are stored as flat file database. Per default the chunk.size is set to 1M rows, which should work for most systems. If you encounter memory problems or have a high amount of memory available feel free to adjust the chunk.size.

The parameter save.db is per default TRUE for methylDB objects as methylDiffDB, while being per default FALSE for methylDiff. If you wish to save the result of an in-memory-calculation as flat file database or if the size of the database allows the calculation in-memory, then you might want to change the value of this parameter.

Examples

data(methylKit)

# get differentially methylated bases/regions with specific cutoffs
all.diff=getMethylDiff(methylDiff.obj,difference=25,qvalue=0.01,type="all")

# get hyper-methylated
hyper=getMethylDiff(methylDiff.obj,difference=25,qvalue=0.01,type="hyper")

# get hypo-methylated
hypo=getMethylDiff(methylDiff.obj,difference=25,qvalue=0.01,type="hypo")

getSampleID

Get or Set Sample-IDs of the methylKit objects

Description

The function returns or replaces the sample-ids stored in any of the following methylKit objects:
methylRaw, methylRawDB, methylBase, methylBaseDB, methylRawList, methylRawListDB, methylDiff, methylDiffDB.

Usage

g.getSampleID(x) <- value

g.getSampleID(x) <- value

## S4 method for signature 'methylRawList'
g.getSampleID(x)
## S4 replacement method for signature 'methylRawList'
getSampleID(x) <- value

## S4 method for signature 'methylBase'
getSampleID(x)

## S4 replacement method for signature 'methylBase'
getSampleID(x) <- value

## S4 method for signature 'methylRaw'
getSampleID(x)

## S4 replacement method for signature 'methylRaw'
getSampleID(x) <- value

## S4 method for signature 'methylDiff'
getSampleID(x)

## S4 replacement method for signature 'methylDiff'
getSampleID(x) <- value

## S4 method for signature 'methylRawListDB'
getSampleID(x)

## S4 replacement method for signature 'methylRawListDB'
getSampleID(x) <- value

## S4 method for signature 'methylBaseDB'
getSampleID(x)

## S4 replacement method for signature 'methylBaseDB'
getSampleID(x) <- value

## S4 method for signature 'methylRawDB'
getSampleID(x)

## S4 replacement method for signature 'methylRawDB'
getSampleID(x) <- value

## S4 method for signature 'methylDiffDB'
getSampleID(x)

## S4 replacement method for signature 'methylDiffDB'
getSampleID(x) <- value

**Arguments**

- `x` an `methylBaseDB`, `methylRawListDB` or `methylDiffDB` object
getTreatment

value a valid replacement vector for the sample-ids of the object

Examples

data(methylKit)

# The Sample-Ids can be printed ..
getSampleID(methylBase.obj)

# .. or replaced.
newObj <- methylBase.obj
getSampleID(newObj) <- c("sample1","sample2","sample3","sample4")
getSampleID(newObj)

getTreatment

Get or Set treatment vector of methylKit object

Description

The function returns or replaces the treatment vector stored in any of the following methylKit objects: methylBase, methylRawList, methylBaseDB, methylRawListDB, methylDiff, methylDiffDB.

Usage

getTreatment(x)
getTreatment(x) <- value

getTreatment(x) <- value

## S4 method for signature 'methylRawList'
getTreatment(x)

## S4 replacement method for signature 'methylRawList'
getTreatment(x) <- value

## S4 method for signature 'methylBase'
getTreatment(x)

## S4 replacement method for signature 'methylBase'
getTreatment(x) <- value

## S4 method for signature 'methylDiff'
getTreatment(x)

## S4 replacement method for signature 'methylDiff'
getTreatment(x) <- value
## S4 method for signature 'methylRawListDB'
getTreatment(x)

## S4 replacement method for signature 'methylRawListDB'
getTreatment(x) <- value

## S4 method for signature 'methylBaseDB'
getTreatment(x)

## S4 replacement method for signature 'methylBaseDB'
getTreatment(x) <- value

## S4 method for signature 'methylDiffDB'
getTreatment(x)

## S4 replacement method for signature 'methylDiffDB'
getTreatment(x) <- value

### Arguments

- **x**
  - A `methylKit` object

- **value**
  - A valid replacement for the treatment vector of the object

### Examples

```r
data(methylKit)

# The treatment vector can be printed ..
getTreatment(methylBase.obj)

# .. or replaced with a new one
newObj <- methylBase.obj
getTreatment(newObj) <- c(1,2,3,4)
getTreatment(newObj)
```

---

**joinSegmentNeighbours**  
*Join directly neighbouring segments produced by methSeg*

### Description

Segmentation and clustering are two distinct steps in `methSeg()`, leading to adjacent segments of the same class. This leads to a bias segment length distributions, which is removed by joining those neighbours.
Usage

joinSegmentNeighbours(res)

Arguments

res A GRanges object with segment classification and information pruduced by methSeg

Value

A GRanges object with segment classification and information.

See Also

methSeg

Description

The function converts in-memory methylKit objects to methylDB objects

Usage

makeMethylDB(obj, dbdir = getwd())

## S4 method for signature 'methylBase'
makeMethylDB(obj, dbdir = getwd())

## S4 method for signature 'methylRaw'
makeMethylDB(obj, dbdir = getwd())

## S4 method for signature 'methylDiff'
makeMethylDB(obj, dbdir = getwd())

## S4 method for signature 'methylRawList'
makeMethylDB(obj, dbdir = getwd())

Arguments

obj an methylBase, methylRaw, methylRawList or an methylDiff object
dbdir directory where flat file database(s) should be stored, defaults to getwd(), working directory.

Value

an methylBaseDB, methylRawDB, methylRawListDB or an methylDiffDB object
Examples

## Not run:
data(methylKit)

makeMethylDB(methylBase.obj,"my/path")

## End(Not run)

---

methRead  
read file(s) to methylRaw or methylRawList objects

Description

The function reads a list of files or single files with methylation information for bases/region in the genome and creates a methylrawList or methylraw object. The information can be stored as flat file database by creating a methylrawlistDB or methylrawDB object.

Usage

methRead(
  location,
  sample.id,
  assembly,
  dbtype = NA,
  pipeline = "amp",
  header = TRUE,
  skip = 0,
  sep = "\t",
  context = "CpG",
  resolution = "base",
  treatment = NA,
  dbdir = getwd(),
  mincov = 10
)

## S4 method for signature 'character,character,character'
methRead(
  location,
  sample.id,
  assembly,
  dbtype,
  pipeline,
  header,
  skip,
  sep,
methRead

## S4 method for signature 'character,ANY,ANY'
methRead(
  location,
  sample.id, 
  assembly,
  dbtype, 
  pipeline, 
  header, 
  skip, 
  sep, 
  context, 
  resolution, 
  dbdir, 
  mincov
)

## S4 method for signature 'list,list,character'
methRead(
  location,
  sample.id, 
  assembly,
  dbtype = NA, 
  pipeline = "amp", 
  header = TRUE, 
  skip = 0, 
  sep = "\t", 
  context = "CpG", 
  resolution = "base", 
  treatment = NA, 
  dbdir = getwd(), 
  mincov = 10
)

## S4 method for signature 'list,ANY,ANY'
methRead(
  location,
  sample.id, 
  assembly,
  dbtype = NA, 
  pipeline = "amp", 
  header = TRUE, 
  skip = 0,
sep = "\t",
context = "CpG",
resolution = "base",
treatment = NA,
dbdir = getwd(),
mincov = 10
)

Arguments

location file location(s), either a list of locations (each a character string) or one location string
sample.id sample.id(s)
assembly a string that defines the genome assembly such as hg18, mm9. this is just a string for book keeping. It can be any string. Although, when using multiple files from the same assembly, this string should be consistent in each object.
dbtype type of the flat file database, currently only option other than NA is "tabix". When "tabix" is given the objects are stored in tabix files, which are compressed and indexed. The default value is NA, in which case the objects are stored in memory.
pipeline name of the alignment pipeline, it can be either "amp", "bismark","bismarkCoverage", "bismarkCytosineReport" or a list (default:'amp'). The methylation text files generated from other pipelines can be read as generic methylation text files by supplying a named list argument as "pipeline" argument. The named list should contain column numbers which denotes which column of the text file corresponds to values and genomic location of the methylation events. See Details for more on possible values for this argument.
header if the input file has a header or not (default: TRUE)
skip number of lines to skip when reading. Can be set to 1 for bed files with track line (default: 0)
sep seperator between fields, same as read.table argument (default: "\t")
context methylation context string, ex: CpG,CHG,CHH, etc. (default:CpG)
resolution designates whether methylation information is base-pair resolution or regional resolution. allowed values 'base' or 'region'. Default 'base'
treatment a vector contatining 0 and 1 denoting which samples are control which samples are test
dbdir directory where flat file database(s) should be stored, defaults to getwd(), working directory.
mincov minimum read coverage to be included in the methylKit objects. defaults to 10. Any methylated base/region in the text files below the mincov value will be ignored.

Value

returns methylRaw, methylRawList, methylRawDB, methylRawListDB object
methRead

Details

The output of methRead is determined by specific input arguments, as there are location, sample.id, assembly and dbtype. The first three are obligatory, while if the last argument is given database features are enabled. If then location refers to an uncompressed file the function will create a flat file database and the associated methylRawDB object will link to this database. If then location refers to an earlier created database file then the object will directly link to this database, skipping the preprocessing steps.

When pipeline argument is a list, it is expected to provide a named list with following names. 'fraction' is a logical value, denoting if the column frequency of Cs has a range from [0-1] or [0-100]. If true it assumes range is [0-1]. 'chr.col' is the number of the column that has chromosome string. 'start.col' is the number of the column that has start coordinate of the base/region of the methylation event. 'end.col' is the number of the column that has end coordinate of the base/region of the methylation event. 'coverage.col' is the number of the column that has read coverage values. 'strand.col' is the number of the column that has strand information, the strand information in the file has to be in the form of '+' or '-'. 'freqC.col' is the number of the column that has the frequency of Cs. See examples to see how to read a generic methylation text file.

Other possible values for pipeline argument are 'amp', 'bismark', 'bismarkCoverage' and 'bismarkCytosineReport'. For 'amp' and 'bismark' the function expects a tabular format shown in the webpage (http://github.com/al2na/methylKit). "amp" and "bismark" expect identical input and are kept for historical reasons. 'amp' was a pipeline used in Akalin et al. 2012 Plos Genetics paper, publicly available in googlecode.

Bismark aligner can output methylation information per base in multiple formats. With pipeline='bismarkCoverage', the function reads bismark coverage files, which have chr,start,end, number of cytosines (methylated bases) and number of thymines (unmethylated bases) format. If bismark coverage files are used the function will not have the strand information, so beware of that fact. With pipeline='bismarkCytosineReport', the function expects cytosine report files from Bismark, which have chr,start, strand, number of cytosines (methylated bases), number of thymines (unmethylated bases), context and trinucleotide context format.

The function can also read gzipped files. On unix systems, this is achieved by using zcat filename and feeding that into data.table::fread. On Windows, the file is first uncompressed then read into R using data.table::fread.

Examples

# this is a list of example files, ships with the package
# for your own analysis you will just need to provide set of paths to files
# you will not need the "system.file(..." part
file.list=list(
    system.file("extdata", "test1.myCpG.txt", package = "methylKit"),
    system.file("extdata", "test2.myCpG.txt", package = "methylKit"),
    system.file("extdata", "control1.myCpG.txt", package = "methylKit"),
    system.file("extdata", "control2.myCpG.txt", package = "methylKit"))

# read the files to a methylRawList object: myobj
myobj=methRead(file.list,
    sample.id=list("test1","test2","ctrl1","ctrl2"),
    assembly="hg18",treatment=c(1,1,0,0))
# read one file as methylRaw object
myobj=methRead( file.list[[1]],
               sample.id="test1", assembly="hg18")

# read a generic text file containing CpG methylation values
# let's first look at the content of the file
generic.file=system.file("extdata", "generic1.CpG.txt", package = "methylKit")
read.table(generic.file, header=TRUE)

# And this is how you can read that generic file as a methylKit object
myobj=methRead( generic.file,
               pipeline=list(fraction=FALSE,chr.col=1,start.col=2,end.col=2,
                             coverage.col=4,strand.col=3,freqC.col=5),
               sample.id="test1", assembly="hg18")

# This creates tabix files that save methylation data
# Without specified dbdir first creates a folder named the following
# in working directory:
# paste("methylDB",Sys.Date(),paste(sample(c(0:9, letters, LETTERS),3,
# replace=TRUE),collapse=""))
#
# Then, saves tabix files from methylKit objects there
myobj=methRead( file.list,
               sample.id=list("test1","test2","ctrl1","ctrl2"),
               assembly="hg18", treatment=c(1,1,0,0),
               dbtype="tabix")

# This creates a single tabix files that saves methylation data
# first creates a "methylDB_objects" directory
# Then, saves tabix file from methylKit objects there
myobj=methRead(file.list[[1]],
               sample.id="test1",
               assembly="hg18",
               dbtype="tabix", dbdir="methylDB_objects")

# tidy up
rm(myobj)
unlink(list.files(pattern = "methylDB", full.names = TRUE), recursive = TRUE)

---

**methSeg**

**Segment methylation or differential methylation profile**

**Description**

The function uses a segmentation algorithm (**fastseg**) to segment the methylation profiles. Following that, it uses gaussian mixture modelling to cluster the segments into k components. This
methSeg

process uses mean methylation value of each segment in the modeling phase. Each component ideally indicates quantitative classification of segments, such as high or low methylated regions.

Usage

methSeg(
    obj,
    diagnostic.plot = TRUE,
    join.neighbours = FALSE,
    initialize.on.subset = 1,
    ...
)

Arguments

obj GRanges, methylDiff, methylDiffDB, methylRaw or methylRawDB. If the object is a GRanges it should have one meta column with methylation scores and has to be sorted by position, i.e. ignoring the strand information.

diagnostic.plot
if TRUE a diagnostic plot is plotted. The plot shows methylation and length statistics per segment group. In addition, it shows diagnostics from mixture modeling: the density function estimated and BIC criterion used to decide the optimum number of components in mixture modeling.

join.neighbours
if TRUE neighbouring segments that cluster to the same seg.group are joined by extending the ranges, summing up num.marks and averaging over seg.means.

initialize.on.subset
a numeric value indicating either percentage or absolute value of regions to subsample from segments before performing the mixture modeling. The value can be either between 0 and 1, e.g. 0.1 means that 10 integer higher than 1 to define the number of regions to sample. By default uses the whole dataset, which can be time consuming on large datasets. (Default: 1)

... arguments to fastseg function in fastseg package, or to densityMclust in Mclust package, could be used to fine tune the segmentation algorithm. E.g. Increasing "alpha" will give more segments. Increasing "cyberWeight" will give also more segments."maxInt" controls the segment extension around a breakpoint. "minSeg" controls the minimum segment length. "G" argument denotes number of components used in BIC selection in mixture modeling. For more details see fastseg and Mclust documentation.

Details

To be sure that the algorithm will work on your data, the object should have at least 5000 records After initial segmentation with fastseg(), segments are clustered into self-similar groups based on their mean methylation profile using mixture modeling. Mixture modeling estimates the initial parameters of the distributions by using the whole dataset. If "initialize.on.subset" argument set as described, the function will use a subset of the data to initialize the parameters of the mixture modeling prior to the Expectation-maximization (EM) algorithm used by Mclust package.
Value

A `GRanges` object with segment classification and information. `seg.mean` column shows the mean methylation per segment. `seg.group` column shows the segment groups obtained by mixture modeling.

Author(s)

Altuna Akalin, contributions by Arsene Wabo and Katarzyna Wreczycka

See Also

`methSeg2bed`, `joinSegmentNeighbours`

Examples

download.file(
  destfile="H1.chr21.chr22.rds",method="curl")

mbw=readRDS("H1.chr21.chr22.rds")

# it finds the optimal number of componets as 6
res=methSeg(mbw,diagnostic.plot=TRUE,maxInt=100,minSeg=10)

# however the BIC stabilizes after 4, we can also try 4 componets
res=methSeg(mbw,diagnostic.plot=TRUE,maxInt=100,minSeg=10,G=1:4)

# get segments to BED file
methSeg2bed(res,filename="H1.chr21.chr22.trial.seg.bed")

unlink(list.files(pattern="H1.chr21.chr22",full.names=TRUE))

methSeg2bed

Export segments to BED files

Description

The segments are color coded based on their score (methylation or differential methylation value). They are named by segment group (components in mixture modeling) and the score in the BED file is obtained from `seg.mean` column of segments object.
methylBase-class

Usage

methSeg2bed(
  segments,
  filename,
  trackLine = "track name='meth segments' description='meth segments' itemRgb=On",
  colramp = colorRamp(c("gray", "green", "darkgreen"))
)

Arguments

segments
  GRanges object with segment classification and information. This should be the result of methSeg function
filename
  name of the output data
trackLine
  UCSC browser trackline
colramp
  color scale to be used in the BED display defaults to gray, green, darkgreen scale.

Value

A BED files with the segmented data which can be visualized in the UCSC browser

See Also

methSeg

methylBase-class

An S4 class for methylation events sampled in multiple experiments

Description

This class is designed to contain methylation information such as coverage, number of methylated bases, etc.. The methylation events contained in the class must be sampled in multiple experiments (ex: only CpG bases covered in multiple experiments are stored in the object of this class). The class extends data.frame and creates an object that holds methylation information and genomic location. The object belonging to this class is produced by unite function.

Slots

sample.ids: character vector for ids of samples in the object
assembly: name of the genome assembly
context: context of methylation. Ex: CpG,CpH,CHH, etc
treatment: treatment vector denoting which samples are test and control
coverage.index: vector denoting which columns in the data correspons to coverage values
numCs.index: vector denoting which columns in the data correspons to number of methylatedCs values
numTs.index: vector denoting which columns in the data corresponds to number of unmethylated Cs values
destranded: logical value. If TRUE object is destranded, if FALSE it is not.
resolution: resolution of methylation information, allowed values: 'base' or 'region'

Details

methylBase class extends data.frame class therefore providing novice and experienced R users with a data structure that is well known and ubiquitous in many R packages.

Subsetting

In the following code snippets, x is a methylBase. Subsetting by x[i,] will produce a new object if subsetting is done on rows. Column subsetting is not directly allowed to prevent errors in the downstream analysis. see ?methylKit

Accessors

The following functions provides access to data slots of methylDiffDB: - getData: get the data slot from the methylKit objects, - getAssembly: get assembly of the genome, - getContext: get the context of methylation

Coercion

methylBase object can be coerced to GRanges object via as function.

Examples

data(methylKit)
library(GenomicRanges)
my.gr=as(methylBase.obj,"GRanges")

---

methylBase.obj  Example methylBase object.

Description

methylBase, methylDiff and methylRawList. You can load the data using data(methylKit)

Format

methylBase.obj object stores the location and methylation information for bases that are covered in all samples. methylBase partially extends data.frame S3 class.
methylBaseDB-class

An S4 class for storing methylation events sampled in multiple experiments as flat file database

Description

This class is designed to contain methylation information such as coverage, number of methylated bases, etc... The class creates an object that holds methylation information and genomic location as flat file database. The object belonging to this class is produced by `unite` function.

Slots

dbpath: path to flat file database(s)
num.records: number of records (lines) in the object
sample.ids: character vector for ids of samples in the object
assembly: name of the genome assembly
context: context of methylation. Ex: CpG, CpH, CHH, etc
treatment: treatment vector denoting which samples are test and control
coverage.index: vector denoting which columns in the data correspond to coverage values
numC.s.index: vector denoting which columns in the data correspond to number of methylated Cs values
numT.s.index: vector denoting which columns in the data correspond to number of unmethylated Cs values
destranded: logical value. If TRUE object is destranded, if FALSE it is not.
resolution: resolution of methylation information, allowed values: 'base' or 'region'
dbtype: string for type of the flat file database, ex: tabix

Details

methylBaseDB class has the same functionality as methylBase class, but the data is saved in a flat database file and therefore allocates less space in memory.

Subsetting

In the following code snippets, x is a methylBaseDB. Subsetting by x[i,] will produce a new methylBase object if subsetting is done on rows. Column subsetting is not directly allowed to prevent errors in the downstream analysis. see ?methylKit.

Accessors

The following functions provides access to data slots of methylDiffDB: - `getData`: get the data slot from the methylKit objects, - `getAssembly`: get assembly of the genome, - `getContext`: get the context of methylation
methylDiff-class

Coercion

methylBaseDB object can be coerced to: GRanges object via as function. methylBase object via as function.

Examples

data(methylKit)
methylBaseDB.obj <- unite(methylRawList.obj,save.db=TRUE,dbdir="methylDB")
library(GenomicRanges)
my.gr=as(methylBaseDB.obj,"GRanges")

# remove Database again
rm(methylBaseDB.obj)
unlink("methylDB",recursive=TRUE)

methylDiff-class

An S4 class that holds differential methylation information

Description

This class is designed to hold statistics and locations for differentially methylated regions/bases. It extends data.frame class. calculateDiffMeth function returns an object with methylDiff class.

Slots

sample.ids ids/names of samples in a vector
assembly a name of genome assembly, such as :hg18,mm9, etc
context numeric vector identifying which samples are which group
treatment numeric vector identifying which samples are which group
destranded logical denoting if methylation information is destranded or not
resolution string either 'base' or 'region' defining the resolution of methylation information
.Data data.frame holding the locations and statistics

Details

methylDiff class extends data.frame class therefore providing novice and experienced R users with a data structure that is well known and ubiquitous in many R packages.

Subsetting

In the following code snippets, x is a methylDiff object. Subsetting by x[i,] will produce a new object if subsetting is done on rows. Column subsetting is not directly allowed to prevent errors in the downstream analysis. see ?methylKit.
Coercion

The `methylDiff` object can be coerced to `GRanges` object via `as` function.

Accessors

The following functions provides access to data slots of `methylDiffDB`: - `getData`: get the data slot from the `methylKit` objects, - `getAssembly`: get assembly of the genome, - `getContext`: get the context of methylation

Examples

data(methylKit)
library(GenomicRanges)
my.gr=as(methylDiff.obj,"GRanges")

Description

`methylBase`, `methylDiff` and `methylRawList`. You can load the data using `data(methylKit)`

Format

The Differential methylation information is stored in `methylDiff.obj` object. `methylBase` partially extends `data.frame` S3 class.

Description

This class is designed to hold statistics and locations for differentially methylated regions/bases as flat file database. `calculateDiffMeth` function returns an object with `methylDiffDB` class.

Slots

dbpath: path to flat file database(s)
um.records: number of records (lines) in the object
sample.ids: ids/names of samples in a vector
assembly: a name of genome assembly, such as :hg18,mm9, etc
context: numeric vector identifying which samples are which group
treatment numeric vector identifying which samples are which group
destranded logical denoting if methylation information is destranded or not
resolution string either 'base' or 'region' defining the resolution of methylation information
dbtype: string for type of the flat file database, ex: tabix

Details

methylDiffDB class has the same functionality as methylDiff class, but the data is saved in a flat database file and therefore allocates less space in memory.

Subsetting

In the following code snippets, x is a methylDiffDB. Subsetting by x[i,] will produce a new object if subsetting is done on rows. Column subsetting is not directly allowed to prevent errors in the downstream analysis. see ?methylKit.

Coercion

methylDiffDB object can be coerced to: GRanges object via as function. methylDiff object via as function.

Accessors

The following functions provides access to data slots of methylDiffDB: - getData: get the data slot from the methylKit objects, - getAssembly: get assembly of the genome, - getContext: get the context of methylation

Examples

data(methylKit)
methylDiffDB.obj <- calculateDiffMeth(methylBase.obj,save.db=TRUE,dbdir="methylDB")

library(GenomicRanges)
my.gr=as(methylDiffDB.obj,"GRanges")

# remove Database again
rm(methylDiffDB.obj)
unlink("methylDB",recursive=TRUE)
Deprecation/Defunct Functions

Description

These are deprecated or defunct functions. Most of them are replaced by genomanation functions. See the vignette for examples on how to use genomanation functions for annotation purposes.

Usage

annotate.WithFeature()
annotate.WithFeature.Flank()
annotate.WithGenicParts()
read.bed()
read.feature.flank()
read.transcript.features()
getFeatsWithTargetsStats()
getFlanks()
getMembers()
getTargetAnnotationStats()
plotTargetAnnotation()
read()
read.bismark()
adjust.methylC()
get.methylDiff()

methylRaw-class

An S4 class for holding raw methylation data from an alignment pipeline.
methylRaw-class

Description
This object stores the raw methylation data that is read in through read function and extends data.frame. The raw methylation data is basically percent methylation values and read coverage values per genomic base/region.

Slots
sample.id: string for an identifier of the sample
assembly: string for genome assembly, ex: hg18, hg19, mm9
context: methylation context string, ex: CpG, CpH, CHH, etc.
resolution: resolution of methylation information, 'base' or 'region'

Details
methylRaw class extends data.frame class therefore providing novice and experienced R users with a data structure that is well known and ubiquitous in many R packages.

Subsetting
In the following code snippets, x is a methylRaw. Subsetting by x[i,j] will produce a new object if subsetting is done on rows. Column subsetting is not directly allowed to prevent errors in the downstream analysis. see ?methylKit.

Accessors
The following functions provides access to data slots of methylDiffDB: - getData: get the data slot from the methylKit objects, - getAssembly: get assembly of the genome, - getContext: get the context of methylation

Coercion
methylRaw object can be coerced to GRanges object via as function.

Examples
# example of a raw methylation data as a text file
read.table(system.file("extdata", "control1.myCpG.txt", 
package = "methylKit"), 
header=TRUE,nrows=5)
data(methylKit)

# example of a methylRaw object
head(methylRawList.obj[[1]])
str(head(methylRawList.obj[[1]]))

library(GenomicRanges)

#coercing methylRaw object to GRanges object
my.gr=as(methylRawList.obj[[1]],"GRanges")
methylRawDB-class

An S4 class for storing raw methylation data as flat file database.

Description

This object stores the raw methylation data that is read in through read function as flat file database. The raw methylation data is basically percent methylation values and read coverage values per genomic base/region.

Slots

- `dbpath`: path to flat file database
- `num.records`: number of records (lines) in the object
- `sample.id`: string for an identifier of the sample
- `assembly`: string for genome assembly, ex: hg18,hg19,mm9
- `context`: methylation context string, ex: CpG,CpH,CHH, etc.
- `resolution`: resolution of methylation information, 'base' or 'region'
- `dbtype`: string for type of the flat file database, ex: tabix

Details

methylRawDB is created via `read` function and has the same functionality as `methylRaw` class, but the data is saved in a flat database file and therefore allocates less space in memory.

Subsetting

In the following code snippets, x is a `methylRawDB`. Subsetting by `x[i,]` will produce a new `methylRaw` object if subsetting is done on rows. Column subsetting is not directly allowed to prevent errors in the downstream analysis. see `?methylKit`. `x[]` will return the `methylRawDB` object as new `methylRaw` object

Accessors

The following functions provides access to data slots of methylDiffDB: - `getData`: get the data slot from the methylKit objects, - `getAssembly`: get assembly of the genome, - `getContext`: get the context of methylation

Coercion

`methylRawDB` object can be coerced to: `GRanges` object via `as` function. `methylRaw` object via `as` function.
Examples

# example of a raw methylation data contained as a text file
read.table(system.file("extdata", "control1.myCpG.txt", package = "methylKit"),
header=TRUE,nrows=5)

methylRawDB.obj <- methRead(
  system.file("extdata", "control1.myCpG.txt", package = "methylKit"),
  sample.id = "ctrl1", assembly = "hg18",
  dbtype = "tabix", dbdir = "methylDB")

# example of a methylRawDB object
methylRawDB.obj
str(methylRawDB.obj)

library(GenomicRanges)

#coercing methylRawDB object to GRanges object
my.gr=as(methylRawDB.obj,"GRanges")

#coercing methylRawDB object to methylRaw object
myRaw=as(methylRawDB.obj,"methylRaw")

# remove Database again
rm(methylRawDB.obj)
unlink("methylDB",recursive=TRUE)

methylRawList-class  An S4 class for holding a list of methylRaw objects.

Description

This class stores the list of methylRaw objects. Functions such as lapply can be used on this list. It extends list class. This object is primarily produced by methRead function.

Usage

methylRawList(..., treatment)

Arguments

... vector of methylRaw objects
treatment vector of treatment values

Slots

treatment numeric vector denoting control and test samples
.Data a list of methylRaw objects
methylRawList.obj

**Constructor**

methylRawList(...) combine multiple methylRaw objects supplied in ... into a methylRawList object.

**Examples**

data(methylKit)

#applying functions designed for methylRaw on methylRawList object
lapply(methylRawList.obj,"getAssembly")

---

**methylRawList.obj**  Example methylRawList object.

---

**Description**

methylBase, methylDiff and methylRawList. You can load the data using data(methylKit)

**Format**

Methylation data from multiple the samples regardless of common coverage are stored in methylRawList.obj object. methylRawList extends list S3 class

---

**methylRawListDB-class**  An S4 class for holding a list of methylRawDB objects.

---

**Description**

This class stores the list of methylRawDB objects. Functions such as lapply can be used on this list. It extends list class. This object is primarily produced by methRead function.

**Usage**

methylRawListDB(..., treatment)

**Arguments**

... vector of methylRawDB files
treatment vector of treatment values

**Slots**

treatment numeric vector denoting control and test samples
.Data a list of methylRawDB objects
normalizeCoverage

Constructor
methylRawListDB(...) combine multiple methylRawDB objects supplied in ... into a methyl-
RawListDB object.

Examples
file.list=list( system.file("extdata", "test1.myCpG.txt", package = "methylKit"),
    system.file("extdata", "test2.myCpG.txt", package = "methylKit"),
    system.file("extdata", "control1.myCpG.txt", package = "methylKit"),
    system.file("extdata", "control2.myCpG.txt", package = "methylKit") )
methylRawListDB.obj <- methRead(file.list,
    sample.id = list("test1","test2","ctrl1","ctrl2"),
    assembly = "hg18",treatment = c(1,1,0,0),
    dbtype = "tabix",dbdir = "methylDB")

#applying functions designed for methylRawDB on methylRawListDB object
lapply(methylRawListDB.obj,"getAssembly")

# remove Database again
rm(methylRawListDB.obj)
unlink("methylDB",recursive=TRUE)

normalizeCoverage normalize read coverage between samples

Description
The function normalizes coverage values between samples using a scaling factor derived from dif-
fferences between mean or median of coverage distributions

Usage
normalizeCoverage(obj,method="median",chunk.size,save.db,...)

## S4 method for signature 'methylRawList'
normalizeCoverage(  
    obj,
    method = "median",
    chunk.size = 1e+06,
    save.db = FALSE,
    ...  
)
## S4 method for signature 'methylRawListDB'
normalizeCoverage(
  obj,
  method = "median",
  chunk.size = 1e+06,
  save.db = TRUE,
  ...
)

### Arguments

- **obj**: methylRawList or methylRawListDB object
- **method**: a string "mean" or "median" which denotes median or mean should be used to calculate scaling factor. (Default: median)
- **chunk.size**: Number of rows to be taken as a chunk for processing the methylRawListDB objects. (Default: 1e6)
- **save.db**: A Logical to decide whether the resulting object should be saved as flat file database or not, default: explained in Details sections
- **...**: optional Arguments used when save.db is TRUE
  - **suffix**: A character string to append to the name of the output flat file database, only used if save.db is true, default actions: append "_filtered" to current filename if database already exists or generate new file with filename "sampleID_filtered"
  - **dbdir**: The directory where flat file database(s) should be stored, defaults to getwd(), working directory for newly stored databases and to same directory for already existing database

### Value

a methylRawList or methylRawList object depending on class of input object

### Details

The parameter chunk.size is only used when working with methylRawListDB objects, as they are read in chunk by chunk to enable processing large-sized objects which are stored as flat file database. Per default the chunk.size is set to 1M rows, which should work for most systems. If you encounter memory problems or have a high amount of memory available feel free to adjust the chunk.size.

The parameter save.db is per default TRUE for methylDB objects as methylRawListDB, while being per default FALSE for methylRawList. If you wish to save the result of an in-memory-calculation as flat file database or if the size of the database allows the calculation in-memory, then you might want to change the value of this parameter.

### Author(s)

Altuna Akalin
Examples

data(methylKit)
# normalize by the median coverage
newObj = normalizeCoverage(methylRawList.obj, method="median")

# normalize by mean coverage and save to database in folder methylDB
newDBObj = normalizeCoverage(methylRawList.obj, method="mean",
save.db=TRUE, dbdir="methylDB")

---

PCASamples  Principal Components Analysis of Methylation data

Description

The function does a PCA analysis using \texttt{prcomp} function using percent methylation matrix as an input.

Usage

PCASamples(.Object, screeplot=FALSE, adj.lim=c(0.0004,0.1), scale=TRUE,
center=TRUE, comp=c(1,2), transpose=TRUE, sd.filter=TRUE,
sd.threshold=0.5, filterByQuantile=TRUE, obj.return=FALSE, chunk.size)

## S4 method for signature 'methylBase'
PCASamples( 
  .Object, 
  screeplot, 
  adj.lim, 
  scale, 
  center, 
  comp, 
  transpose, 
  sd.filter, 
  sd.threshold, 
  filterByQuantile, 
  obj.return
)

## S4 method for signature 'methylBaseDB'
PCASamples( 
  .Object, 
  screeplot = FALSE, 
  adj.lim = c(4e-04, 0.1), 
  scale = TRUE, 
  center = TRUE, 
  comp = c(1, 2), 
  ...)
transpose = TRUE,
sd.filter = TRUE,
sd.threshold = 0.5,
filterByQuantile = TRUE,
obj.return = FALSE,
chunk.size = 1e+06
)

Arguments

- **Object**: a methylBase or methylBaseDB object
- **screeplot**: a logical value indicating whether to plot the variances against the number of the principal component. (default: FALSE)
- **adj.lim**: a vector indicating the proportional adjustment of xlim (adj.lim[1]) and ylim (adj.lim[2]). This is primarily used for adjusting the visibility of sample labels on the on the PCA plot. (default: c(0.0004,0.1))
- **scale**: logical indicating if prcomp should scale the data to have unit variance or not (default: TRUE)
- **center**: logical indicating if prcomp should center the data or not (default: TRUE)
- **comp**: vector of integers with 2 elements specifying which components to be plotted.
- **transpose**: if TRUE (default) percent methylation matrix will be transposed, this is equivalent to doing PCA on variables that are regions/bases. The resulting plot will location of samples in the new coordinate system if FALSE the variables for the matrix will be samples and the resulting plot will show how each sample (variable) contributes to the principle component. The samples that are highly correlated should have similar contributions to the principal components.
- **sd.filter**: If TRUE, the bases/regions with low variation will be discarded prior to PCA (default: TRUE)
- **sd.threshold**: A numeric value. If filterByQuantile is TRUE, the value should be between 0 and 1 and the features whose standard deviations is less than the quantile denoted by sd.threshold will be removed. If filterByQuantile is FALSE, then features whose standard deviations is less than the value of sd.threshold will be removed. (default: 0.5)
- **filterByQuantile**: A logical determining if sd.threshold is to be interpreted as a quantile of all standard deviation values from bases/regions (the default), or as an absolute value
- **obj.return**: if the result of prcomp function should be returned or not. (Default: FALSE)
- **chunk.size**: Number of rows to be taken as a chunk for processing the methylRawListDB objects, default: 1e6

Value

The form of the value returned by PCASamples is the summary of principal component analysis by prcomp.
Details

The parameter chunk.size is only used when working with methylBaseDB objects, as they are read in chunk by chunk to enable processing large-sized objects which are stored as flat file database. Per default the chunk.size is set to 1M rows, which should work for most systems. If you encounter memory problems or have a high amount of memory available feel free to adjust the chunk.size.

Note

cor option is not in use anymore, since prcomp is used for PCA analysis instead of princomp

Examples

data(methylKit)

# do PCA with filtering rows with low variation, filter rows with standard deviation lower than the 50th percentile of Standard deviation distribution
PCASamples(methylBase.obj,screeplot=FALSE, adj.lim=c(0.0004,0.1),
   scale=TRUE,center=TRUE,comp=c(1,2),transpose=TRUE,sd.filter=TRUE,
   sd.threshold=0.5,filterByQuantile=TRUE,obj.return=FALSE)

percMethylation

describe get percent methylation scores from methylBase or methylBaseDB object

Usage

percMethylation(
   methylBase.obj,
   rowids = FALSE,
   save.txt = FALSE,
   chunk.size = 1e+06
)

## S4 method for signature 'methylBase'
percMethylation(methylBase.obj, rowids = FALSE)

## S4 method for signature 'methylBaseDB'
percMethylation(
   methylBase.obj,
   rowids = FALSE,
   save.txt = FALSE,
   chunk.size = 1e+06
)
pool

Arguments

methylBase.obj  a methylBase or methylBaseDB object
rowids          if TRUE, matrix rownames have identifiers as base/region location (default: FALSE)
save.txt       if TRUE, the matrix will be written to a text file, but only for methylBaseDB objects (default: FALSE)
chunk.size     Number of rows to be taken as a chunk for processing the methylBaseDB objects (default: 1e6)

Value

matrix with percent methylation values per base/region across all samples, row names would be base/region identifiers

Details

The parameter chunk.size is only used when working with methylBaseDB objects, as they are read in chunk by chunk to enable processing large-sized objects which are stored as flat file database. Per default the chunk.size is set to 1M rows, which should work for most systems. If you encounter memory problems or have a high amount of memory available feel free to adjust the chunk.size.

Examples

data(methylKit)
mat=percMethylation(methylBase.obj)
head(mat)

Description

The function sums up coverage, numCs and numTs values within each group so one representative sample for each group will be created in a new methylBase object

Usage

pool(obj, sample.ids, chunk.size = 1e+06, save.db = FALSE, ...)

## S4 method for signature 'methylBase'
pool(obj, sample.ids, chunk.size = 1e+06, save.db = FALSE, ...)

## S4 method for signature 'methylBaseDB'
pool(obj, sample.ids, chunk.size = 1e+06, save.db = TRUE, ...)
Arguments

obj: methylBase or methylBaseDB object with two groups or more and each group should have multiple samples

sample.ids: a character vector of new sample.ids ex: c("test","control"), should follow the same order as unique treatment vector, and should be equal to the length of the unique treatment vector

chunk.size: Number of rows to be taken as a chunk for processing the methylRawListDB objects, default: 1e6

save.db: A Logical to decide whether the resulting object should be saved as flat file database or not, default: explained in Details sections

... optional Arguments used when save.db is TRUE

suffix: A character string to append to the name of the output flat file database, only used if save.db is true, default actions: The default suffix is a 13-character random string appended to the fixed prefix "methylBase", e.g. "methylBase_16d3047c1a254.txt.bgz".

dbdir: The directory where flat file database(s) should be stored, defaults to getwd(), working directory for newly stored databases and to same directory for already existing database

dbtype: The type of the flat file database, currently only option is "tabix" (only used for newly stored databases)

Value

a methylBase or methylBaseDB object depending on class of input object

Details

The parameter chunk.size is only used when working with methylBaseDB objects, as they are read in chunk by chunk to enable processing large-sized objects which are stored as flat file database. Per default the chunk.size is set to 1M rows, which should work for most systems. If you encounter memory problems or have a high amount of memory available feel free to adjust the chunk.size.

The parameter save.db is per default TRUE for methylDB objects as methylBaseDB, while being per default FALSE for methylBase. If you wish to save the result of an in-memory-calculation as flat file database or if the size of the database allows the calculation in-memory, then you might want to change the value of this parameter.

Author(s)

Altuna Akalin

Examples

data(methylKit)

# methylBase.obj has two groups, each group has two samples,
# the following function will pool the samples in each group
# so that each group will be represented by one pooled sample
pooled.methylBase=pool(methylBase.obj,sample.ids=c("test","control"))
processBismarkAln

Get methylation percentage from sorted Bismark alignments

Description

The function calls methylation percentage per base from sorted Bismark SAM or BAM files and reads methylation information as methylKit objects. Bismark is a popular aligner for high-throughput bisulfite sequencing experiments and it outputs its results in SAM format by default, and can be converted to BAM. Bismark SAM/BAM format contains aligner specific tags which are absolutely necessary for methylation percentage calling using processBismarkAln. SAM/BAM files from other aligners will not work with this function.

Usage

processBismarkAln(
  location,  
  sample.id,  
  assembly,  
  save.folder = NULL,  
  save.context = c("CpG"),  
  read.context = "CpG",  
  nolap = FALSE,  
  mincov = 10,  
  minqual = 20,  
  phred64 = FALSE,  
  treatment = NULL,  
  save.db = FALSE,  
  verbose = 1
)

## S4 method for signature 'character,character,character'
processBismarkAln(
  location,  
  sample.id,  
  assembly,  
  save.folder = NULL,  
  save.context = c("CpG"),  
  read.context = "CpG",  
  nolap = FALSE,  
  mincov = 10,  
  minqual = 20,  
  phred64 = FALSE,  
  treatment = NULL,  
  save.db = FALSE,  
  verbose = 1
)
## S4 method for signature 'list, list, character'
processBismarkAln(
  location,
  sample.id,
  assembly,
  save.folder = NULL,
  save.context = c("CpG"),
  read.context = "CpG",
  nolap = FALSE,
  mincov = 10,
  minqual = 20,
  phred64 = FALSE,
  treatment = NULL,
  save.db = FALSE,
  verbose = 1
)

### Arguments

- **location**: location of sam or bam file(s). If multiple files are given this argument must be a list.
- **sample.id**: the id(s) of samples in the same order as file. If multiple sam files are given this argument must be a list.
- **assembly**: string that determines the genome assembly. Ex: mm9, hg18 etc. This is just a string for book keeping. It can be any string. Although, when using multiple files from the same assembly, this string should be consistent in each object.
- **save.folder**: The folder which will be used to save methylation call files, if set to NULL no methylation call file will be saved as a text file. The files saved can be read into R in less time using `methRead` function in `methylKit`
- **save.context**: A character vector consisting following strings: "CpG", "CHG", "CHH". The methylation percentages for these methylation contexts will be saved to save.folder
- **read.context**: One of the 'CpG', 'CHG', 'CHH' or 'none' strings. Determines what type of methylation context will be read-in to the memory which can be immediately used for analysis. If given as 'none', processBismarkAln will not return any object, but if a save.folder argument given it will save the methylation percentage call files.
- **nolap**: if set to TRUE and the SAM/BAM file has paired-end reads, the one read of the overlapping paired-end read pair will be ignored for methylation calling.
- **mincov**: minimum read coverage to call a methylation status for a base.
- **minqual**: minimum phred quality score to call a methylation status for a base.
- **phred64**: logical (default: FALSE) you will not need to set this TRUE, Currently bismark gives only phred33 scale
- **treatment**: treatment vector only to be used when location and sample.id parameters are lists and you are trying to read-in multiple samples that are related to each other in down-stream analysis.
**save.db**
A Logical to decide whether the resulting object should be saved as flat file database or not (default: FALSE). With the default value, a text file containing methylation values will be saved. If TRUE, database will either be saved to location `save.folder` or if this is NULL, to a new folder in the current working directory named after this scheme: "methylDB <Date> <3randomlettersornumbers>"

**verbose**
logical set verbosity of methCall (default: TRUE)

**Value**
methylRaw or methylRawList object

**Note**
SAM files should be sorted with samtools sort or unix sort. Other sorting methods can alter the order of fields(columns) in the SAM file and that will result in an error when using `processBismarkAln()`.

**Examples**

```r
# reading one bismark file:
my.file=system.file("extdata", "test.fastq_bismark.sorted.min.sam",
                   package = "methylKit")
obj=processBismarkAln(my.file,"test",assembly="hg18",save.folder=NULL,
                   save.context="CpG",read.context="CpG")

# reading multiple files
file.list2=list(system.file("extdata", "test.fastq_bismark.sorted.min.sam",
                            package = "methylKit"),
                   system.file("extdata", "test.fastq_bismark.sorted.min.sam",
                                package = "methylKit"),
                   system.file("extdata", "test.fastq_bismark.sorted.min.sam",
                                package = "methylKit"),
                   system.file("extdata", "test.fastq_bismark.sorted.min.sam",
                                package = "methylKit"))
objs=processBismarkAln(location=file.list2
                      ,sample.id=list("test1","test2","ctrl1","ctrl1"),assembly="hg18",
                      save.folder=NULL,save.context=NULL,read.context="CpG",
                      nolap=FALSE,mincov=10,minqual=20,phred64=FALSE,
                      treatment=c(1,1,0,0))
```

**Description**
The function reads the header from a given tabix file and loads it into corresponding methylDB object.
Usage
readMethylDB(dbpath)

Arguments
dbpath path to a tabix file with header

Value
an \texttt{methylBaseDB}, \texttt{methylRawDB}, \texttt{methylRawListDB} or an \texttt{methylDiffDB} object

Examples
## Not run:
data(methylKit)
baseDB.obj <- makeMethylDB(methylBase.obj,"my/path")
mydbpath <- getDBPath(baseDB.obj)
rm(baseDB.obj)
readMethylDB(mydbpath)

## End(Not run)

---

**reconstruct**

*Reconstruct methylBase or methylBaseDB object based on a new methylation percentage matrix*

Description

The function reconstructs a new methylBase object from an input methylBase object and percent methylation matrix. Basically, it uses the read coverages in the input methylBase object and deduces new number of methylated Cs and unmethylated Cs based on the input percent methylation matrix. It is ideally to be used to reconstruct methylBase objects after batch correction on percent methylation values. The percent methylation matrix rows must match methylBase object rows in order, and in addition column order (the order of samples) in input methylBase must match the order in percent methylation matrix.

Usage
reconstruct(methMat, mBase, chunk.size = 1e+06, save.db = FALSE, ...)

## S4 method for signature 'ANY,methylBase'
reconstruct(methMat, mBase, chunk.size = 1e+06, save.db = FALSE, ...)

## S4 method for signature 'ANY,methylBaseDB'
reconstruct(methMat, mBase, chunk.size = 1e+06, save.db = TRUE, ...)
reconstruct

Arguments

methMat  percent methylation matrix, row order and order of the samples same as the methylBase object

mBase  methylBase or methylBaseDB object to be reconstructed

chunk.size  Number of rows to be taken as a chunk for processing the methylBaseDB objects (default: 1e6)

save.db  A Logical to decide whether the resulting object should be saved as flat file database or not, default: explained in Details sections

...  optional Arguments used when save.db is TRUE

suffix  A character string to append to the name of the output flat file database, only used if save.db is true, default actions: append "_reconstructed" to current filename if database already exists or generate new file with filename “methylBase_reconstructed”

dbdir  The directory where flat file database(s) should be stored, defaults to getwd(), working directory for newly stored databases and to same directory for already existing database

dbtype  The type of the flat file database, currently only option is "tabix" (only used for newly stored databases)

Value

new methylBase or methylBase object where methylation percentage matches input methMat and coverages matches input mBase

Details

The parameter chunk.size is only used when working with methylBaseDB objects, as they are read in chunk by chunk to enable processing large-sized objects which are stored as flat file database. Per default the chunk.size is set to 1M rows, which should work for most systems. If you encounter memory problems or have a high amount of memory available feel free to adjust the chunk.size. The parameter save.db is per default TRUE for methylDB objects as methylBase0B, while being per default FALSE for methylBase. If you wish to save the result of an in-memory-calculation as flat file database or if the size of the database allows the calculation in-memory, then you might want to change the value of this parameter.

Note

Batch effect correction (if any batch effect exists) is a tricky issue. We provide some simple ways to deal with it (see assocComp and removeComp), But if you can find other ways to correct for batch effects and want to create a methylBase object with the corrected percent methylation values, you can use this function.

Author(s)

Altuna Akalin
regionCounts

Examples

data(methylKit)

# get percent methylation
mat=percMethylation(methylBase.obj)

# do some changes in the matrix
# this is just a toy example
# ideally you want to correct the matrix
# for batch effects
mat[mat==100]=80

# reconstruct the methylBase from the corrected matrix
newobj=reconstruct(mat,methylBase.obj)

regionCounts

Get regional counts for given GRanges or GRangesList object

Description

Convert methylRaw, methylRawDB, methylRawList, methylRawListDB, methylBase or methylBaseDB object into regional counts for a given GRanges or GRangesList object.

Usage

regionCounts(object, regions, cov.bases=0, strand.aware=FALSE, chunk.size, save.db, ...)

## S4 method for signature 'methylRaw,GRanges'
regionCounts(
    object, regions, cov.bases = 0, strand.aware = FALSE, chunk.size = 1e+06, save.db = FALSE, ...
)

## S4 method for signature 'methylBase,GRanges'
regionCounts(
    object, regions, cov.bases = 0, strand.aware = FALSE, chunk.size = 1e+06, save.db = FALSE, ...
)
regionCounts

## S4 method for signature 'methylRaw,GRangesList'
regionCounts(
  object,
  regions,
  cov.bases = 0,
  strand.aware = FALSE,
  chunk.size = 1e+06,
  save.db = FALSE,
  ...
)

## S4 method for signature 'methylBase,GRangesList'
regionCounts(
  object,
  regions,
  cov.bases = 0,
  strand.aware = FALSE,
  chunk.size = 1e+06,
  save.db = FALSE,
  ...
)

## S4 method for signature 'methylRawList,GRanges'
regionCounts(
  object,
  regions,
  cov.bases = 0,
  strand.aware = FALSE,
  chunk.size = 1e+06,
  save.db = FALSE,
  ...
)

## S4 method for signature 'methylRawList,GRangesList'
regionCounts(
  object,
  regions,
  cov.bases = 0,
  strand.aware = FALSE,
  chunk.size = 1e+06,
  save.db = FALSE,
  ...
)

## S4 method for signature 'methylRawDB,GRanges'
regionCounts(
regionCounts

object,
regions,
cov.bases = 0,
strand.aware = FALSE,
chunk.size = 1e+06,
save.db = TRUE,
...

## S4 method for signature 'methylRawDB,GRangesList'
regionCounts(
  object,
  regions,
  cov.bases = 0,
  strand.aware = FALSE,
  chunk.size = 1e+06,
  save.db = TRUE,
  ...
)

## S4 method for signature 'methylRawListDB,GRanges'
regionCounts(
  object,
  regions,
  cov.bases = 0,
  strand.aware = FALSE,
  chunk.size = 1e+06,
  save.db = TRUE,
  ...
)

## S4 method for signature 'methylRawListDB,GRangesList'
regionCounts(
  object,
  regions,
  cov.bases = 0,
  strand.aware = FALSE,
  chunk.size = 1e+06,
  save.db = TRUE,
  ...
)

## S4 method for signature 'methylBaseDB,GRanges'
regionCounts(
  object,
  regions,
  cov.bases = 0,
  strand.aware = FALSE,
regionCounts

chunk.size = 1e+06,
save.db = TRUE,
...
)

## S4 method for signature 'methylBaseDB,GRangesList'
regionCounts(
  object,
  regions,
  cov.bases = 0,
  strand.aware = FALSE,
  chunk.size = 1e+06,
  save.db = TRUE,
  ...
)

Arguments

object a methylRaw, methylRawDB, methylRawList, methylRawListDB, methylBase or methylBaseDB object

NOTE: The given regions (Granges/GrangesList object) will be ordered based on chromosome and position before searching for overlaps, so the resulting methylKit object might have a different ordering than expected. See details section for the reasoning of this choice and ways to still get custom ordering of regions.

regions a GRanges or GRangesList object. Make sure that the GRanges objects are unique in chr,start,end and strand columns. You can make them unique by using unique() function.

cov.bases number minimum bases covered per region (Default: 0). Only regions with base coverage above this threshold are returned.

strand.aware if set to TRUE only CpGs that match the strand of the region will be summarized. (default: FALSE)

chunk.size Number of rows to be taken as a chunk for processing the methylDB objects (default: 1e6)

save.db A Logical to decide whether the resulting object should be saved as flat file database or not, default: explained in Details sections

... optional Arguments used when save.db is TRUE

suffix A character string to append to the name of the output flat file database, only used if save.db is true, default actions: append “_regions” to current filename if database already exists or generate new file with filename “sampleID_regions” or “methylBase_filtered” dependent on input object

dbdir The directory where flat file database(s) should be stored, defaults to getwd(), working directory for newly stored databases and to same directory for already existing database

dbtype The type of the flat file database, currently only option is "tabix" (only used for newly stored databases)


Value

a new methylRaw, methylBase or methylRawList object. If strand.aware is set to FALSE (default). Even though the resulting object will have the strand information of regions it will still contain methylation information from both strands.

Details

The given regions (Granges/GrangesList object) will be ordered based on chromosome and position before searching for overlaps, so the resulting methylKit object might have a different order than expected. We are doing this is to ensure that resulting output is consistent for in-memory and database based objects, as database based objects always have to be sorted to enable tabix indexing and providing fast random access.

If you still want get a custom ordering of the output regions you can access the single regions in any object providing your indices to the select or extract functions.

The parameter chunk.size is only used when working with methylRawDB, methylBaseDB or methylRawListDB objects, as they are read in chunk by chunk to enable processing large-sized objects which are stored as flat file database. Per default the chunk.size is set to 1M rows, which should work for most systems. If you encounter memory problems or have a high amount of memory available feel free to adjust the chunk.size.

The parameter save.db is per default TRUE for methylDB objects as methylRawDB, methylBaseDB or methylRawListDB, while being per default FALSE for methylRaw, methylBase or methylRawList. If you wish to save the result of an in-memory-calculation as flat file database or if the size of the database allows the calculation in-memory, then you might want to change the value of this parameter.

Examples

data(methylKit)

# get the windows of interest as a GRanges object, this can be any set # of genomic locations
library(GenomicRanges)
my.win=GRanges(seqnames="chr21",
ranges=IRanges(start=seq(from=9764513,by=10000,length.out=20),width=5000) )

# getting counts per region
regional.methylRaw=regionCounts(object=methylRawList.obj, regions=my.win, cov.bases=0,strand.aware=FALSE)

removeComp

Remove principal components from a methylBase object
removeComp

Description

This function can remove a given principal component from a given methylBase object. First, it calculates principal components from percent methylation matrix and removes the given component(s), reconstructs the methylation matrix then reconstructs number of methylated and unmethylated Cs per position based on the reconstructed percent methylation matrix, and finally returns a new methylBase object.

Usage

removeComp(mBase, comp = NULL, chunk.size = 1e+06, save.db = FALSE, ...)

## S4 method for signature 'methylBase'
removeComp(mBase, comp = NULL, chunk.size = 1e+06, save.db = FALSE, ...)

## S4 method for signature 'methylBaseDB'
removeComp(mBase, comp = NULL, chunk.size = 1e+06, save.db = TRUE, ...)

Arguments

mBase methylBase or methylBaseDB object with no NA values, that means all bases should be covered in all samples.
comp vector of component numbers to be removed
chunk.size Number of rows to be taken as a chunk for processing the methylBaseDB objects (default: 1e6)
save.db A Logical to decide whether the resulting object should be saved as flat file database or not, default: explained in Details sections
...

optional Arguments used when save.db is TRUE

suffix A character string to append to the name of the output flat file database, only used if save.db is true, default actions: append "\_reconstructed" to current filename if database already exists or generate new file with filename "methylBase\_reconstructed"
dbdire The directory where flat file database(s) should be stored, defaults to getwd(), working directory for newly stored databases and to same directory for already existing database
dbtype The type of the flat file database, currently only option "tabix"

Value

new methylBase or methylBaseDB object

Details

The parameter chunk.size is only used when working with methylBaseDB objects, as they are read in chunk by chunk to enable processing large-sized objects which are stored as flat file database. Per default the chunk.size is set to 1M rows, which should work for most systems. If you encounter memory problems or have a high amount of memory available feel free to adjust the chunk.size.
reorganize

The parameter `save.db` is per default `TRUE` for `methylDB` objects as `methylBaseDB`, while being per default `FALSE` for `methylBase`. If you wish to save the result of an in-memory-calculation as flat file database or if the size of the database allows the calculation in-memory, then you might want to change the value of this parameter.

Examples

data(methylKit)

# remove 1st principal component
newObj=removeComp(methylBase.obj,comp=1)

# remove 3rd and 4th principal components
newObj=removeComp(methylBase.obj,comp=c(3,4))

---

reorganize  
*Reorganize methylKit objects by creating new objects from subset of samples*

Description

The function creates a new `methylRawList`, `methylRawListDB`, `methylBase` or `methylBaseDB` object by selecting a subset of samples from the input object, which is a `methylRawList` or `methylBase` object. You can use the function to partition a large `methylRawList` or `methylBase` object to smaller object based on sample ids or when you want to reorder samples and/or give a new treatment vector.

Usage

```r
reorganize(
  methylObj,  
  sample.ids,  
  treatment,  
  chunk.size = 1e+06,  
  save.db = FALSE,  
  ...  
)
```

```r
## S4 method for signature 'methylBase'
reorganize(
  methylObj,  
  sample.ids,  
  treatment,  
  chunk.size = 1e+06,  
  save.db = FALSE,  
  ...  
)
```
## S4 method for signature 'methylRawList'
reorganize(
  methylObj,
  sample.ids,
  treatment,
  chunk.size = 1e+06,
  save.db = FALSE,
  ...
)

## S4 method for signature 'methylRawListDB'
reorganize(
  methylObj,
  sample.ids,
  treatment,
  chunk.size = 1e+06,
  save.db = TRUE,
  ...
)

## S4 method for signature 'methylBaseDB'
reorganize(
  methylObj,
  sample.ids,
  treatment,
  chunk.size = 1e+06,
  save.db = TRUE,
  ...
)

### Arguments

- **methylObj**: a methylRawList, methylRawListDB, methylBase or methylBaseDB object
- **sample.ids**: a vector for sample.ids to be subset. Order is important and the order should be similar to treatment. sample.ids should be a subset or reordered version of sample ids in the input object.
- **treatment**: treatment vector, should be same length as sample.ids vector
- **chunk.size**: Number of rows to be taken as a chunk for processing the methylBaseDB or methylRawListDB objects, default: 1e6
- **save.db**: A Logical to decide whether the resulting object should be saved as flat file database or not, default: explained in Details sections
- **...**: optional Arguments used when save.db is TRUE
- **suffix**: A character string to append to the name of the output flat file database, only used if save.db is true, default actions: For “methylBase”: The default suffix is a 13-character random string appended to the fixed prefix “methylBase”, e.g. “methylBase_16d3047c1a254.txt.bgz”. For “methylRawList”: ignored.
dbdir: The directory where flat file database(s) should be stored, defaults to getwd(), working directory for newly stored databases and to same directory for already existing database.

dbtype: The type of the flat file database, currently only option "tabix"

Value

returns a methylRawList, methylRawListDB, methylBase or methylBaseDB object depending on the input object.

Details

The parameter chunk.size is only used when working with methylBaseDB or methylRawListDB objects, as they are read in chunk by chunk to enable processing large-sized objects which are stored as flat file database. Per default the chunk.size is set to 1M rows, which should work for most systems. If you encounter memory problems or have a high amount of memory available feel free to adjust the chunk.size.

The parameter save.db is per default TRUE for methylDB objects as methylBaseDB and methylRawListDB, while being per default FALSE for methylBase and methylRawList. If you wish to save the result of an in-memory-calculation as flat file database or if the size of the database allows the calculation in-memory, then you might want to change the value of this parameter.

Examples

# this is a list of example files, ships with the package
file.list=list( system.file("extdata", "test1.myCpG.txt", package = "methylKit"),
    system.file("extdata", "test2.myCpG.txt", package = "methylKit"),
    system.file("extdata", "control1.myCpG.txt", package = "methylKit"),
    system.file("extdata", "control2.myCpG.txt", package = "methylKit") )

# read the files to a methylRawList object: myobj
myobj=methRead( file.list,
    sample.id=list("test1","test2","ctrl1","ctrl2"),
    assembly="hg18",pipeline="amp",treatment=c(1,1,0,0))
meth=unite(myobj,destrand=TRUE)

# get samples named "test1" and "ctrl2" from myobj and create a new methylRawList object
myobj2=reorganize(myobj,sample.ids=c("test1","ctrl2"),treatment=c(1,0) )

# get samples named "test1" and "ctrl2" from meth and create a new methylBase object
meth2 =reorganize(meth,sample.ids=c("test1","ctrl2"),treatment=c(1,0) )
select  

selects rows from of methylKit objects

Description

The function returns a subset of data contained in the methylKit objects.

Usage

select(x, i)

## S4 method for signature 'methylBase'
select(x, i)

## S4 method for signature 'methylRaw'
select(x, i)

## S4 method for signature 'methylDiff'
select(x, i)

## S4 method for signature 'methylRawDB'
select(x, i)

## S4 method for signature 'methylBaseDB'
select(x, i)

## S4 method for signature 'methylDiffDB'
select(x, i)

Arguments

x  
an methylBase, methylBaseDB, methylRaw, methylRawDB or methylDiff object

i  
a numeric or logical vector. This vector corresponds to bases or regions contained in methylKit objects. The vector is used to subset the data.

Value

a methylBase, methylRaw or methylDiff object depending on the input object.

Examples

data(methylKit)

methylRawDB.obj=methRead( system.file("extdata","test1.txt.bgz",package="methylKit"),
  sample.id="test1", assembly="hg18",
  dbtype = "tabix", dbdir = "methylDB")
selectByOverlap

selectByOverlap selects records of methylDB objects lying inside a GRanges range.

Description
The function selects records from any methylKit object that lie inside the regions given by ranges of class GRanges and returns an in-memory equivalent of this object.

Usage

\[
\text{selectByOverlap}(\text{object}, \text{ranges})
\]

## S4 method for signature 'methylRaw,GRanges'
selectByOverlap(object, ranges)

## S4 method for signature 'methylRawList,GRanges'
selectByOverlap(object, ranges)

## S4 method for signature 'methylBase,GRanges'
selectByOverlap(object, ranges)

## S4 method for signature 'methylDiff,GRanges'
selectByOverlap(object, ranges)

## S4 method for signature 'methylRawDB,GRanges'
selectByOverlap(object, ranges)

## S4 method for signature 'methylRawListDB,GRanges'
selectByOverlap(object, ranges)

## S4 method for signature 'methylBaseDB,GRanges'
selectByOverlap(object, ranges)

## S4 method for signature 'methylDiffDB,GRanges'
selectByOverlap(object, ranges)

### Arguments

- **object**: an `methylRaw`, `methylRawDB`, `methylRawList`, `methylRawListDB`, `methylBase`, `methylBaseDB`, `methylDiff` or `methylDiffDB` object
- **ranges**: a `GRanges` object specifying the regions of interest

### Value

A `methylBase`, `methylRaw`, `methylRawList` or `methylDiff` object depending on the input object.

### Author(s)

Alexander Gosdschan

### Examples

```r
data(methylKit)

file.list = list(
  system.file("extdata", "test1.myCpG.txt", package = "methylKit"),
  system.file("extdata", "test2.myCpG.txt", package = "methylKit"),
  system.file("extdata", "control1.myCpG.txt", package = "methylKit"),
  system.file("extdata", "control2.myCpG.txt", package = "methylKit")
)

methylRawListDB.obj = methRead(file.list,
  sample.id = list("test1", "test2", "ctrl1", "ctrl2"),
  assembly = "hg18", treatment = c(1, 1, 0, 0),
  dbtype = "tabix", dbdir = "methylDB")

methylBaseDB.obj = unite(methylRawListDB.obj)

methylDiffDB.obj = calculateDiffMeth(methylBaseDB.obj)

# define the windows of interest as a GRanges object, this can be any set
# of genomic locations
library(GenomicRanges)
my.win = GRanges(seqnames = "chr21",
  ranges = IRanges(start = seq(from = 9764513, by = 10000, length.out = 20),
    width = 5000))

# selects the records that lie inside the regions
myRaw <- selectByOverlap(methylRawListDB.obj[[1]], my.win)
```
# selects the records that lie inside the regions
myBase <- selectByOverlap(methylBaseDB.obj, my.win)

# selects the records that lie inside the regions
myDiff <- selectByOverlap(methylDiffDB.obj, my.win)

# selects the records that lie inside the regions
myRaw2 <- selectByOverlap(methylRawList.obj[[1]], my.win)

# selects the records that lie inside the regions
myRawList2 <- selectByOverlap(methylRawList.obj, my.win)

# selects the records that lie inside the regions
myBase2 <- selectByOverlap(methylBase.obj, my.win)

# selects the records that lie inside the regions
myDiff2 <- selectByOverlap(methylDiff.obj, my.win)

rm(methylRawListDB.obj)
rm(methylBaseDB.obj)
rm(methylDiffDB.obj)
unlink("methylDB", recursive=TRUE)

---

show, methylBase-method

show method for methylKit classes

Description

The show method works for methylRaw, methylRawDB, methylRawList, methylRawListDB, methylBase, methylBaseDB and methylDiff objects

Usage

```r
## S4 method for signature 'methylBase'
show(object)

## S4 method for signature 'methylRaw'
show(object)

## S4 method for signature 'methylRawList'
show(object)

## S4 method for signature 'methylDiff'
show(object)
```
tileMethylCounts

## S4 method for signature 'methylRawDB'
show(object)

## S4 method for signature 'methylRawListDB'
show(object)

## S4 method for signature 'methylBaseDB'
show(object)

## S4 method for signature 'methylDiffDB'
show(object)

Arguments

object any methylKit object

Examples

data(methylKit)
methylDiff.obj
show(methylDiff.obj)

tileMethylCounts

---

### tileMethylCounts

*Get methylated/unmethylated base counts for tilling windows*

Description

The function summarizes methylated/unmethylated base counts over tilling windows across genome. This function can be used when differential methylation analysis is preferable to tilling windows instead of base pairs.

Usage

tileMethylCounts(object, win.size = 1000, step.size = 1000, cov.bases = 0, mc.cores = 1, save.db, ...)

## S4 method for signature 'methylRaw'
tileMethylCounts(
  object,
  win.size = 1000,
  step.size = 1000,
  cov.bases = 0,
  mc.cores = 1,
  save.db = FALSE,
  ...
)
## S4 method for signature 'methylRawList'
tileMethylCounts(
  object,
  win.size = 1000,
  step.size = 1000,
  cov.bases = 0,
  mc.cores = 1,
  save.db = FALSE,
  ...
)

## S4 method for signature 'methylBase'
tileMethylCounts(
  object,
  win.size = 1000,
  step.size = 1000,
  cov.bases = 0,
  mc.cores = 1,
  save.db = FALSE,
  ...
)

## S4 method for signature 'methylRawDB'
tileMethylCounts(
  object,
  win.size = 1000,
  step.size = 1000,
  cov.bases = 0,
  mc.cores = 1,
  save.db = TRUE,
  ...
)

## S4 method for signature 'methylRawListDB'
tileMethylCounts(
  object,
  win.size = 1000,
  step.size = 1000,
  cov.bases = 0,
  mc.cores = 1,
  save.db = TRUE,
  ...
)

## S4 method for signature 'methylBaseDB'
tileMethylCounts(
  object,
Arguments

- **object**: methylRaw, methylRawDB, methylRawList, methylRawListDB, methylBase or methylBaseDB object containing base pair resolution methylation information
- **win.size**: an integer for the size of the tiling windows
- **step.size**: an integer for the step size of tiling windows
- **cov.bases**: minimum number of bases to be covered in a given window
- **mc.cores**: number of cores to use when processing methylDB objects, default: 1, but always 1 for Windows
- **save.db**: A Logical to decide whether the resulting object should be saved as flat file database or not, default: explained in Details sections
- **suffix**: A character string to append to the name of the output flat file database, only used if save.db is true, default actions: append “_tiled” to current filename if database already exists or generate new file with filename “sampleID_tiled” or “methylBase_tiled” dependent on input object
- **dbdir**: The directory where flat file database(s) should be stored, defaults to getwd(), working directory for newly stored databases and to same directory for already existing database

Value

methylRaw, methylBase or methylRawList object

Details

The parameter chunk.size is only used when working with methylRawDB, methylBaseDB or methylRawListDB objects, as they are read in chunk by chunk to enable processing large-sized objects which are stored as flat file database. Per default the chunk.size is set to 1M rows, which should work for most systems. If you encounter memory problems or have a high amount of memory available feel free to adjust the chunk.size.

The parameter save.db is per default TRUE for methylDB objects as methylRawDB, methylBaseDB or methylRawListDB, while being per default FALSE for methylRaw, methylBase or methylRawList. If you wish to save the result of an in-memory-calculation as flat file database or if the size of the database allows the calculation in-memory, then you might want to change the value of this parameter.
Examples

data(methylKit)

tiled.methylRaw=tileMethylCounts(object=methylRawList.obj,win.size=1000,
    step.size=1000,cov.bases=0)

unite methylRawList to a single table

Description

This functions unites methylRawList and methylRawListDB objects that only bases with coverage from all samples are retained. The resulting object is either of class methylBase or methylBaseDB depending on input.

Usage

unite(
    object,
    destrand = FALSE,
    min.per.group = NULL,
    chunk.size = 1e+06,
    mc.cores = 1,
    save.db = FALSE,
    ...
)

## S4 method for signature 'methylRawList'
unite(
    object,
    destrand = FALSE,
    min.per.group = NULL,
    chunk.size = 1e+06,
    mc.cores = 1,
    save.db = FALSE,
    ...
)

## S4 method for signature 'methylRawListDB'
unite(
    object,
    destrand = FALSE,
    min.per.group = NULL,
    chunk.size = 1e+06,
    mc.cores = 1,
save.db = TRUE,
...
)

Arguments

object a methylRawList or methylRawListDB object to be merged by common locations covered by reads
destrand if TRUE, reads covering both strands of a CpG dinucleotide will be merged, do not set to TRUE if not only interested in CpGs (default: FALSE). If the methylRawList object contains regions rather than bases setting destrand to TRUE will have no effect.

min.per.group an integer denoting minimum number of samples per replicate needed to cover a region/base. By default only regions/bases that are covered in all samples are united as methylBase object, however by supplying an integer for this argument users can control how many samples needed to cover region/base to be united as methylBase object. For example, if min.per.group set to 2 and there are 3 replicates per condition, the bases/regions that are covered in at least 2 replicates will be united and missing data for uncovered bases/regions will appear as NAs.

chunk.size Number of rows to be taken as a chunk for processing the methylRawListDB objects, default: 1e6
mc.cores number of cores to use when processing methylRawListDB objects, default: 1, but always 1 for Windows)
save.db A Logical to decide whether the resulting object should be saved as flat file database or not, default: explained in Details sections

... optional Arguments used when save.db is TRUE

suffix A character string to append to the name of the output flat file database, only used if save.db is true, default actions: The default suffix is a 13-character random string appended to the fixed prefix “methylBase”, e.g. “methylBase_16d3047c1a254.txt.bgz”.
dbdir The directory where flat file database(s) should be stored, defaults to getwd(), working directory for newly stored databases and to same directory for already existing database
dbtype The type of the flat file database, currently only option is "tabix" (only used for newly stored databases)

Value

a methylBase or methylBaseDB object depending on input

Details

The parameter chunk.size is only used when working with methylRawDB or methylRawListDB objects, as they are read in chunk by chunk to enable processing large-sized objects which are stored as flat file database. Per default the chunk.size is set to 1M rows, which should work for most systems. If you encounter memory problems or have a high amount of memory available feel free to adjust the chunk.size.
The parameter `save.db` is per default TRUE for methylDB objects as `methylRawListDB`, while being per default FALSE for `methylRawList`. If you wish to save the result of an in-memory-calculation as flat file database or if the size of the database allows the calculation in-memory, then you might change the value of this parameter.

**Examples**

```r
data(methylKit)
## Following
my.methylBase=unite(methylRawList.obj)
my.methylBase=unite(methylRawList.obj,destrand=TRUE)
```

**updateMethObject**

*update methylKit objects The method updates object from earlier versions (<v0.9.1) to latest object.*

**Description**

update methylKit objects

The method updates object from earlier versions (<v0.9.1) to latest object.

**Usage**

```r
updateMethObject(object)
```

**Arguments**

- **object**
  - a methylKit object: methylRaw, methylRawList, methylBase or methylDiff

**Value**

- `methylRaw,methylDiff,methylBase` or `methylRawList` object
- @export @docType methods @rdname updateMethObject
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