Package ‘DMRcaller’

May 3, 2024

Type Package

Title Differentially Methylated Regions caller

Version 1.36.0

Date 2021-11-17

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Description Uses Bisulfite sequencing data in two conditions and identifies differentially methylated regions between the conditions in CG and non-CG context. The input is the CX report files produced by Bismark and the output is a list of DMRs stored as GRanges objects.

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LazyLoad yes

Imports parallel, Rcpp, RcppRoll, betareg, grDevices, graphics, methods, stats, utils

Depends R (>= 3.5), GenomicRanges, IRanges, S4Vectors (>= 0.23.10)

Suggests knitr, RUnit, BiocGenerics

biocViews DifferentialMethylation, DNAMethylation, Software, Sequencing, Coverage

VignetteBuilder knitr

NeedsCompilation no

git_url https://git.bioconductor.org/packages/DMRcaller

git_branch RELEASE_3_19

git_last_commit 0b1facf

git_last_commit_date 2024-04-30

Repository Bioconductor 3.19

Date/Publication 2024-05-03
analyseReadsInsideRegionsForCondition

Analyse reads inside regions for condition

Description

This function extracts from the methylation data the total number of reads, the number of methylated reads and the number of cytosines in the specific context from a region (e.g. DMRs)

Usage

```r
analyseReadsInsideRegionsForCondition(regions, methylationData, context, label = "", cores = 1)
```
analyseReadsInsideRegionsForCondition

Arguments

- `regions` a `GRanges` object with a list of regions on the genome; e.g. could be a list of DMRs
- `methylationData` the methylation data in one condition (see `methylationDataList`).
- `context` the context in which to extract the reads ("CG", "CHG" or "CHH").
- `label` a string to be added to the columns to identify the condition
- `cores` the number of cores used to compute the DMRs.

Value

A `GRanges` object with additional four metadata columns

- `sumReadsM` the number of methylated reads
- `sumReadsN` the total number of reads
- `proportion` the proportion methylated reads
- `cytosinesCount` the number of cytosines in the regions

Author(s)

Nicolae Radu Zabet

See Also

`filterDMRs`, `computeDMRs`, `DMRsNoiseFilterCG`, and `mergeDMRsIteratively`

Examples

```r
# load the methylation data
data(methylationDataList)

# load the DMRs in CG context. These DMRs were computed with minGap = 200.
data(DMRsNoiseFilterCG)

# retrieve the number of reads in CHH context in WT
DMRsNoiseFilterCGreadsCHH <- analyseReadsInsideRegionsForCondition(
  DMRsNoiseFilterCG[1:10],
  methylationDataList["WT"], context = "CHH",
  label = "WT")
```
computeDMRs

**Description**

This function computes the differentially methylated regions between two conditions.

**Usage**

```r
computeDMRs(methylationData1, methylationData2, regions = NULL,
context = "CG", method = "noise_filter", windowSize = 100,
kernelFunction = "triangular", lambda = 0.5, binSize = 100,
test = "fisher", pValueThreshold = 0.01, minCytosinesCount = 4,
minProportionDifference = 0.4, minGap = 200, minSize = 50,
minReadsPerCytosine = 4, cores = 1)
```

**Arguments**

- `methylationData1`
  the methylation data in condition 1 (see `methylationDataList`).
- `methylationData2`
  the methylation data in condition 2 (see `methylationDataList`).
- `regions`
  a `GRanges` object with the regions where to compute the DMRs. If NULL, the DMRs are computed genome-wide.
- `context`
  the context in which the DMRs are computed ("CG", "CHG" or "CHH").
- `method`
  the method used to compute the DMRs ("noise_filter", "neighbourhood" or "bins"). The "noise_filter" method uses a triangular kernel to smooth the number of reads and then performs a statistical test to determine which regions display different levels of methylation in the two conditions. The "neighbourhood" method computes differentially methylated cytosines. Finally, the "bins" method partitions the genome into equal sized tiling bins and performs the statistical test between the two conditions in each bin. For all three methods, the cytosines or bins are then merged into DMRs without affecting the initial parameters used when calling the differentially methylated cytosines/bins (p-value, difference in methylation levels, minimum number of reads per cytosine).
- `windowSize`
  the size of the triangle base measured in nucleotides. This parameter is required only if the selected method is "noise_filter".
- `kernelFunction`
  a character indicating which kernel function to be used. Can be one of "uniform", "triangular", "gaussian" or "epanechnicov". This is required only if the selected method is "noise_filter".
- `lambda`
  numeric value required for the Gaussian filter \( K(x) = \exp(-\lambda x^2) \). This is required only if the selected method is "noise_filter" and the selected kernel function is "gaussian".
- `binSize`
  the size of the tiling bins in nucleotides. This parameter is required only if the selected method is "bins".
computeDMRs

test  the statistical test used to call DMRs ("fisher" for Fisher’s exact test or "score" for Score test).
pValueThreshold  DMRs with p-values (when performing the statistical test; see test) higher or equal than pValueThreshold are discarded. Note that we adjust the p-values using the Benjamini and Hochberg’s method to control the false discovery rate.
minCytosinesCount  DMRs with less cytosines in the specified context than minCytosinesCount will be discarded.
minProportionDifference  DMRs where the difference in methylation proportion between the two conditions is lower than minProportionDifference are discarded.
minGap  DMRs separated by a gap of at least minGap are not merged. Note that only DMRs where the change in methylation is in the same direction are joined.
minSize  DMRs with a size smaller than minSize are discarded.
minReadsPerCytosine  DMRs with the average number of reads lower than minReadsPerCytosine are discarded.
cores  the number of cores used to compute the DMRs.

Value

the DMRs stored as a GRanges object with the following metadata columns:

direction  a number indicating whether the region lost (-1) or gain (+1) methylation in condition 2 compared to condition 1.
context  the context in which the DMRs was computed ("CG", "CHG" or "CHH").
sumReadsM1  the number of methylated reads in condition 1.
sumReadsN1  the total number of reads in condition 1.
proportion1  the proportion methylated reads in condition 1.
sumReadsM2  the number of methylated reads in condition 2.
sumReadsN2  the total number reads in condition 2.
proportion2  the proportion methylated reads in condition 2.
cytosinesCount  the number of cytosines in the DMR.
regionType  a string indicating whether the region lost ("loss") or gained ("gain") methylation in condition 2 compared to condition 1.
pValue  the p-value (adjusted to control the false discovery rate with the Benjamini and Hochberg’s method) of the statistical test when the DMR was called.

Author(s)

Nicolae Radu Zabet and Jonathan Michael Foonlan Tsang

See Also

filterDMRs, mergeDMRsIteratively, analyseReadsInsideRegionsForCondition and DMRsNoiseFilterCG
Examples

```r
# load the methylation data
data(methylationDataList)

# the regions where to compute the DMRs
regions <- GRanges(seqnames = Rle("Chr3"), ranges = IRanges(1,1E5))

# compute the DMRs in CG context with noise_filter method
DMRsNoiseFilterCG <- computeDMRs(methylationDataList["WT"],
                                   methylationDataList["met1-3"],
                                   regions = regions,
                                   context = "CG", method = "noise_filter",
                                   windowSize = 100, kernelFunction = "triangular",
                                   test = "score", pValueThreshold = 0.01,
                                   minCytosinesCount = 4, minProportionDifference = 0.4,
                                   minGap = 200, minSize = 50, minReadsPerCytosine = 4,
                                   cores = 1)

## Not run:
## compute the DMRs in CG context with neighbourhood method
DMRsNeighbourhoodCG <- computeDMRs(methylationDataList["WT"],
                                      methylationDataList["met1-3"],
                                      regions = regions,
                                      context = "CG", method = "neighbourhood",
                                      test = "score", pValueThreshold = 0.01,
                                      minCytosinesCount = 4, minProportionDifference = 0.4,
                                      minGap = 200, minSize = 50, minReadsPerCytosine = 4,
                                      cores = 1)

# compute the DMRs in CG context with bins method
DMRsBinsCG <- computeDMRs(methylationDataList["WT"],
                           methylationDataList["met1-3"],
                           regions = regions,
                           context = "CG", method = "bins",
                           binSize = 100,
                           test = "score", pValueThreshold = 0.01,
                           minCytosinesCount = 4, minProportionDifference = 0.4,
                           minGap = 200, minSize = 50, minReadsPerCytosine = 4,
                           cores = 1)

## End(Not run)
```

Description

This function computes the differentially methylated regions between replicates with two conditions.

Usage

```r
computeDMRsReplicates(methylationData, condition = NULL, regions = NULL,
                       context = NULL, method = NULL, test = NULL, pValueThreshold = NULL,
                       minCytosinesCount = NULL, minProportionDifference = NULL,
                       minGap = NULL, minSize = NULL, minReadsPerCytosine = NULL,
                       cores = NULL)
```
context = "CG", method = "neighbourhood", binSize = 100,
test = "betareg", pseudocountM = 1, pseudocountN = 2,
pValueThreshold = 0.01, minCytosinesCount = 4,
minProportionDifference = 0.4, minGap = 200, minSize = 50,
minReadsPerCytosine = 4, cores = 1)

Arguments

methylationData
the methylation data containing all the conditions for all the replicates.

condition
a vector of strings indicating the conditions for each sample in methylationData.
Two different values are allowed (for the two conditions).

regions
a GRanges object with the regions where to compute the DMRs. If NULL, the
DMRs are computed genome-wide.

context
the context in which the DMRs are computed ("CG", "CHG" or "CHH").

method
the method used to compute the DMRs "neighbourhood" or "bins". The
"neighbourhood" method computates differentially methylated cytosines. Fi-
nally, the "bins" method partiones the genome into equal sized tilling bins and
performs the statistical test between the two conditions in each bin. For all three
methods, the cytosines or bins are then merged into DMRs without affecting the
initial parameters used when calling the differential methylated cytosines/bins
(p-value, difference in methylation levels, minimum number of reads per cyto-
sine).

binSize
the size of the tiling bins in nucleotides. This parameter is required only if the
selected method is "bins".

test
the statistical test used to call DMRs ("betareg" for Beta regression).
pseudocountM
numerical value to be added to the methylated reads before modelling beta re-
gression.
pseudocountN
numerical value to be added to the total reads before modelling beta regression.
pValueThreshold
DMRs with p-values (when performing the statistical test; see test) higher or
equal than pValueThreshold are discarded. Note that we adjust the p-values
using the Benjamini and Hochberg’s method to control the false discovery rate.

minCytosinesCount
DMRs with less cytosines in the specified context than minCytosinesCount
will be discarded.

minProportionDifference
DMRs where the difference in methylation proportion between the two condi-
tions is lower than minProportionDifference are discarded.

minGap
DMRs separated by a gap of at least minGap are not merged. Note that only
DMRs where the change in methylation is in the same direction are joined.

minSize
DMRs with a size smaller than minSize are discarded.

minReadsPerCytosine
DMRs with the average number of reads lower than minReadsPerCytosine are
discarded.

cores
the number of cores used to compute the DMRs.
computeDMRsReplicates

Value

the DMRs stored as a GRanges object with the following metadata columns:

direction a number indicating whether the region lost (-1) or gain (+1) methylation in condition 2 compared to condition 1.
context the context in which the DMRs was computed ("CG", "CHG", or "CHH").
sumReadsM1 the number of methylated reads in condition 1.
sumReadsN1 the total number of reads in condition 1.
proportion1 the proportion methylated reads in condition 1.
sumReadsM2 the number of methylated reads in condition 2.
sumReadsN2 the total number reads in condition 2.
proportion2 the proportion methylated reads in condition 2.
cytosinesCount the number of cytosines in the DMR.
regionType a string indicating whether the region lost ("loss") or gained ("gain") methylation in condition 2 compared to condition 1.
pValue the p-value (adjusted to control the false discovery rate with the Benjamini and Hochberg’s method) of the statistical test when the DMR was called.

Author(s)

Alessandro Pio Greco and Nicolae Radu Zabet

Examples

```r
## Not run:
# starting with data joined using joinReplicates
data("syntheticDataReplicates")

# compute the DMRs in CG context with neighbourhood method

# creating condition vector
c <- c("a", "a", "b", "b")

# computing DMRs using the neighbourhood method
DMRsReplicatesNeighbourhood <- computeDMRsReplicates(methylationData = methylationData, condition = c, regions = NULL, context = "CHH", method = "neighbourhood", test = "betareg", pseudocountM = 1, pseudocountN = 2, pValueThreshold = 0.01, minCytosinesCount = 4, minProportionDifference = 0.4, minGap = 200, minSize = 50, minReadsPerCytosine = 4,
```

```
### Description
This function computes the coverage for bisulfite sequencing data. It returns a vector with the proportion (or raw count) of cytosines that have the number of reads higher or equal than a vector of specified thresholds.

### Usage
```
computeMethylationDataCoverage(methylationData, regions = NULL, context = "CG", breaks = NULL, proportion = TRUE)
```

### Arguments
- `methylationData` the methylation data stored as a `GRanges` object with four metadata columns (see `methylationDataList`).
- `regions` a `GRanges` object with the regions where to compute the coverage. If NULL, the coverage is computed genome-wide.
- `context` the context in which the DMRs are computed ("CG", "CHG" or "CHH").
- `breaks` a numeric vector specifying the different values for the thresholds when computing the coverage.
- `proportion` a logical value indicating whether to compute the proportion (TRUE) or raw counts (FALSE).

### Value
a vector with the proportion (or raw count) of cytosines that have the number of reads higher or equal than the threshold values specified in the breaks vector.

### Author(s)
Nicolae Radu Zabet and Jonathan Michael Foonlan Tsang

### See Also
- `plotMethylationDataCoverage`
- `methylationDataList`
Examples

# load the methylation data
data(methylationDataList)

# compute coverage in CG context
breaks <- c(1,5,10,15)
coverage_CG_wt <- computeMethylationDataCoverage(methylationDataList["WT"],
context="CG", breaks=breaks)

computeMethylationDataSpatialCorrelation

Compute methylation data spatial correlation

Description

This function computes the correlation of the methylation levels as a function of the distances between the Cytosines. The function returns a vector with the correlation of methylation levels at distance equal to a vector of specified thresholds.

Usage

computeMethylationDataSpatialCorrelation(methylationData, regions = NULL,
context = "CG", distances = NULL)

Arguments

methylationData
the methylation data stored as a GRanges object with four metadata columns (see methylationDataList).

regions
a GRanges object with the regions where to compute the correlation. If NULL, the correlation is computed genome-wide.

context
the context in which the correlation is computed ("CG", "CHG" or "CHH").

distances
a numeric vector specifying the different values for the distances when computing the correlation.

Value

a vector with the correlation of the methylation levels for Cytosines located at distances specified in the distances vector.

Author(s)

Nicolae Radu Zabet

See Also

plotMethylationDataSpatialCorrelation, methylationDataList
computeMethylationProfile

Compute methylation profile

Description
This function computes the low resolution profiles for the bisulfite sequencing data.

Usage
computeMethylationProfile(methylationData, region, windowSize = floor(width(region)/500), context = "CG")

Arguments
- methylationData: the methylation data stored as a GRanges object with four metadata columns (see methylationDataList).
- region: a GRanges object with the regions where to compute the DMRs.
- windowSize: a numeric value indicating the size of the window in which methylation is averaged.
- context: the context in which the DMRs are computed ("CG", "CHG" or "CHH").

Value
- a GRanges object with equal sized tiles of the region. The object consists of the following metadata:
  - sumReadsM: the number of methylated reads.
  - sumReadsN: the total number of reads.
  - Proportion: the proportion of methylated reads.
  - context: the context ("CG", "CHG" or "CHH").

Author(s)
Nicolae Radu Zabet and Jonathan Michael Foonlan Tsang
computeOverlapProfile

Compute Overlaps Profile

This function computes the distribution of a subset of regions (GRanges object) over a large region (GRanges object).

Usage

computeOverlapProfile(subRegions, largeRegion, windowSize = floor(width(largeRegion)/500), binary = TRUE, cores = 1)

Arguments

subRegions a GRanges object with the sub regions; e.g. can be the DMRs.
largeRegion a GRanges object with the region where to compute the overlaps; e.g. a chromosome
windowSize The largeRegion is partitioned into equal sized tiles of width windowSize.
binary a value indicating whether to count 1 for each overlap or to compute the width of the overlap
cores the number of cores used to compute the DMRs.
DMRcaller

Value

a `GRanges` object with equal sized tiles of the regions. The object has one metadata file `score` which represents: the number of subRegions overlapping with the tile, in the case of `binary = TRUE`, and the width of the subRegions overlapping with the tile, in the case of `binary = FALSE`.

Author(s)

Nicolae Radu Zabet

See Also

`plotOverlapProfile`, `filterDMRs`, `computeDMRs`, and `mergeDMRsIteratively`

Examples

```r
# load the methylation data
data(methylationDataList)

# load the DMRs in CG context
data(DMRsNoiseFilterCG)

# the coordinates of the area to be plotted
largeRegion <- GRanges(seqnames = Rle("Chr3"), ranges = IRanges(1,1E5))

# compute overlaps distribution
hotspots <- computeOverlapProfile(DMRsNoiseFilterCG, largeRegion,
                                   windowSize = 10000, binary = FALSE)
```

---

DMRcaller  

*Call Differentially Methylated Regions (DMRs) between two samples*

Description

Uses bisulfite sequencing data in two conditions and identifies differentially methylated regions between the conditions in CG and non-CG context. The input is the CX report files produced by Bismark and the output is a list of DMRs stored as GRanges objects.

Details

The most important functions in the **DMRcaller** are:

- `readBismark` reads the Bismark CX report files in a `GRanges` object.
- `readBismarkPool` Reads multiple CX report files and pools them together.
- `saveBismark` saves the methylation data stored in a `GRanges` object into a Bismark CX report file.
- `poolMethylationDatasets` pools together multiple methylation datasets.
- `poolTwoMethylationDatasets` pools together two methylation datasets.
- `computeMethylationDataCoverage` Computes the coverage for the bisulfite sequencing data.
plotMethylationDataCoverage  Plots the coverage for the bisulfite sequencing data.
computeMethylationDataSpatialCorrelation  Computes the correlation between methylation levels as a function of the distances between the Cytosines.
plotMethylationDataSpatialCorrelation  Plots the correlation of methylation levels for Cytosines located at a certain distance apart.
computeMethylationProfile  Computes the low resolution profiles for the bisulfite sequencing data at certain locations.
plotMethylationProfile  Plots the low resolution profiles for the bisulfite sequencing data at certain locations.
plotMethylationProfileFromData  Plots the low resolution profiles for the loaded bisulfite sequencing data.
computeDMRs  Computes the differentially methylated regions between two conditions.
filterDMRs  Filters a list of (potential) differentially methylated regions.
mergeDMRsIteratively  Merge DMRs iteratively.
analyseReadsInsideRegionsForCondition  Analyse reads inside regions for condition.
plotLocalMethylationProfile  Plots the methylation profile at one locus for the bisulfite sequencing data.
computeOverlapProfile  Computes the distribution of a set of subregions on a large region.
plotOverlapProfile  Plots the distribution of a set of subregions on a large region.
getWholeChromosomes  Computes the GRanges objects with each chromosome as an element from the methylationData.
joinReplicates  Merges two GRanges objects with single reads columns. It is necessary to start the analysis of DMRs with biological replicates.
computeDMRsReplicates  Computes the differentially methylated regions between two conditions with multiple biological replicates.

Author(s)
Nicolae Radu Zabet <n.r.zabet@gen.cam.ac.uk>, Jonathan Michael Foonlan Tsang <jmft2@cam.ac.uk>
Alessandro Pio Greco <apgrec@essex.ac.uk>
Maintainer: Nicolae Radu Zabet <n.r.zabet@gen.cam.ac.uk>

See Also
See vignette("rd", package = "DMRcaller") for an overview of the package.

Examples
```r
## Not run:
# load the methylation data
data(methylationDataList)

#plot the low resolution profile at 5 Kb resolution
par(mar=c(4, 4, 3, 1)+0.1)
plotMethylationProfileFromData(methylationDataList[["WT"]],
```
methylDataList[c("met1-3"),
  conditionsNames=c("WT", "met1-3"),
  windowSize = 5000, autoscale = TRUE,
  context = c("CG", "CHG", "CHH"),
  labels = LETTERS)

# compute low resolution profile in 10 Kb windows in CG context
lowResProfileWT <- computeMethylationProfile(methylDataList[c("WT")],
  region, windowSize = 10000, context = "CG")

lowResProfileMet13 <- computeMethylationProfile(
  methylDataList[c("met1-3")], region,
  windowSize = 10000, context = "CG")

lowResProfile <- GRangesList("WT" = lowResProfileWT,
  "met1-3" = lowResProfileMet13)

# compute low resolution profile in 10 Kb windows in CHG context
lowResProfileWT <- computeMethylationProfile(methylDataList[c("WT")],
  region, windowSize = 10000, context = "CHG")

lowResProfileMet13 <- computeMethylationProfile(
  methylDataList[c("met1-3")], region,
  windowSize = 10000, context = "CHG")

lowResProfileCHG <- GRangesList("WT" = lowResProfileWT,
  "met1-3" = lowResProfileMet13)

# plot the low resolution profile
par(mar=c(4, 4, 3, 1)+0.1)
par(mfrow=c(2,1))
plotMethylationProfile(lowResProfile, autoscale = FALSE,
  labels = LETTERS[1],
  title="CG methylation on Chromosome 3",
  col=c("#D55E00","#E69F00"), pch = c(1,0),
  lty = c(4,1))

plotMethylationProfile(lowResProfileCHG, autoscale = FALSE,
  labels = LETTERS[2],
  title="CHG methylation on Chromosome 3",
  col=c("#0072B2","#56B4E9"), pch = c(16,2),
  lty = c(3,2))

# plot the coverage in all three contexts
plotMethylationDataCoverage(methylDataList[c("WT")],
  methylDataList[c("met1-3")],
  breaks = 1:15, regions = NULL,
  conditionsNames = c("WT","met1-3"),
  context = c("CG", "CHG", "CHH"),
  proportion = TRUE, labels = LETTERS, col = NULL,
  pch = c(1,0,16,2,15,17), lty = c(4,1,3,2,6,5),
  contextPerRow = FALSE)

# plot the correlation of methylation levels as a function of distance
plotMethylationDataSpatialCorrelation(methylationDataList[["WT"]],
distances = c(1,5,10,15), regions = NULL,
conditionsNames = c("WT","met1-3"),
context = c("CG"),
labels = LETTERS, col = NULL,
pch = c(1,0,16,2,15,17), lty = c(4,1,3,2,6,5),
contextPerRow = FALSE)

# the regions where to compute the DMRs
regions <- GRanges(seqnames = Rle("Chr3"), ranges = IRanges(1,1E6))

# compute the DMRs in CG context with noise_filter method
DMRsNoiseFilterCG <- computeDMRs(methylationDataList[["WT"]],
methylationDataList[["met1-3"]], regions = regions,
context = "CG", method = "noise_filter",
windowSize = 100, kernelFunction = "triangular",
test = "score", pValueThreshold = 0.01,
minCytosinesCount = 4, minProportionDifference = 0.4,
minGap = 200, minSize = 50, minReadsPerCytosine = 4,
cores = 1)

# compute the DMRs in CG context with neighbourhood method
DMRsNeighbourhoodCG <- computeDMRs(methylationDataList[["WT"]],
methylationDataList[["met1-3"]], regions = regions,
context = "CG", method = "neighbourhood",
test = "score", pValueThreshold = 0.01,
minCytosinesCount = 4, minProportionDifference = 0.4,
minGap = 200, minSize = 50, minReadsPerCytosine = 4,
cores = 1)

# compute the DMRs in CG context with bins method
DMRsBinsCG <- computeDMRs(methylationDataList[["WT"]],
methylationDataList[["met1-3"]], regions = regions,
context = "CG", method = "bins", binSize = 100,
test = "score", pValueThreshold = 0.01,
minCytosinesCount = 4, minProportionDifference = 0.4,
minGap = 200, minSize = 50, minReadsPerCytosine = 4,
cores = 1)

# load the gene annotation data
data(GEs)

# select the genes
genes <- GEs[which(GEs$type == "gene")]

# the regions where to compute the DMRs
genes <- genes[overlapsAny(genes, regions)]

# filter genes that are differentially methylated in the two conditions
DMRsGenesCG <- filterDMRs(methylationDataList[["WT"]],
methylationDataList[["met1-3"]], potentialDMRs = genes,
context = "CG", test = "score", pValueThreshold = 0.01,
minCytosinesCount = 4, minProportionDifference = 0.4,
minReadsPerCytosine = 3, cores = 1)
#merge the DMRs
DMRsNoiseFilterCGLarger <- mergeDMRsIteratively(DMRsNoiseFilterCG, minGap = 500, respectSigns = TRUE, methylationDataList[['WT']], methylationDataList[['met1-3']], context = "CG", minProportionDifference=0.4, minReadsPerCytosine = 1, pValueThreshold=0.01, test="score",alternative = "two.sided")

#select the genes
genes <- GEs[which(GEs$type == "gene")]

# the coordinates of the area to be plotted
chr3Reg <- GRanges(seqnames = Rle("Chr3"), ranges = IRanges(510000,530000))

# load the DMRs in CG context
data(DMRsNoiseFilterCG)

DMRsCGlist <- list("noise filter"=DMRsNoiseFilterCG, "neighbourhood"=DMRsNeighbourhoodCG, "bins"=DMRsBinsCG, "genes"=DMRsGenesCG)

# plot the CG methylation
par(mar=c(4, 4, 3, 1)+0.1)
par(mfrow=c(1,1))
plotLocalMethylationProfile(methylationDataList[['WT']], methylationDataList[['met1-3']], chr3Reg, DMRsCGlist, c("WT", "met1-3"), GEs, windowSize=100, main="CG methylation")

hotspotsHypo <- computeOverlapProfile(DMRsNoiseFilterCG[DMRsNoiseFilterCG$regionType == "loss"], region, windowSize=2000, binary=TRUE, cores=1)

hotspotsHyper <- computeOverlapProfile(DMRsNoiseFilterCG[DMRsNoiseFilterCG$regionType == "gain"], region, windowSize=2000, binary=TRUE, cores=1)

plotOverlapProfile(GRangesList("Chr3"=hotspotsHypo), GRangesList("Chr3"=hotspotsHyper), names=c("loss", "gain"), title="CG methylation")

# loading synthetic data
data("syntheticDataReplicates")

# creating condition vector
condition <- c("a", "a", "b", "b")

# computing DMRs using the neighbourhood method
DMRsReplicatesNeighbourhood <- computeDMRsReplicates(methylationData = methylationData,
DMRsNoiseFilterCG  

The DMRs between WT and met1-3 in CG context

Description

A GRangesList object containing the DMRs between Wild Type (WT) and met1-3 mutant (met1-3) in Arabidopsis thaliana (see methylationDataList). The DMRs were computed on the first 1 Mbp from Chromosome 3 with noise filter method using a triangular kernel and a windowSize of 100 bp

Format

The GRanges element contain 11 metadata columns; see computeDMRs

See Also

filterDMRs, computeDMRs, analyseReadsInsideRegionsForCondition and mergeDMRsIteratively

extractGC  

Extract GC

Description

This function extracts GC sites in the genome

Usage

extractGC(methylationData, genome, contexts = c("ALL", "CG", "CHG", "CHH"))
## Filter DMRs

This function verifies whether a set of potential DMRs (e.g. genes, transposons, CpG islands) are differentially methylated or not.

### Usage

```r
filterDMRs(methylationData1, methylationData2, potentialDMRs, context = "CG", test = "fisher", pValueThreshold = 0.01, minCytosinesCount = 4, minProportionDifference = 0.4, minReadsPerCytosine = 3, cores = 1)
```
Arguments

methylationData1
de the methylation data in condition 1 (see methylationDataList).

methylationData2
de the methylation data in condition 2 (see methylationDataList).

potentialDMRs a GRanges object with potential DMRs where to compute the DMRs. This can be a list of gene and/or transposable elements coordinates.

context
de the context in which the DMRs are computed ("CG", "CHG" or "CHH").

test
de the statistical test used to call DMRs ("fisher" for Fisher’s exact test or "score" for Score test).

pValueThreshold
DMRs with p-values (when performing the statistical test; see test) higher or equal than pValueThreshold are discarded. Note that we adjust the p-values using the Benjamini and Hochberg’s method to control the false discovery rate.

minCytosinesCount
DMRs with less cytosines in the specified context than minCytosinesCount will be discarded.

minProportionDifference
DMRs where the difference in methylation proportion between the two conditions is lower than minProportionDifference are discarded.

minReadsPerCytosine
DMRs with the average number of reads lower than minReadsPerCytosine are discarded.

cores
de the number of cores used to compute the DMRs.

Value
a GRanges object with 11 metadata columns that contain the DMRs; see computeDMRs.

Author(s)
Nicolae Radu Zabet

See Also
DMRsNoiseFilterCG, computeDMRs, analyseReadsInsideRegionsForCondition and mergeDMRsIteratively

Examples

# load the methylation data
data(methylationDataList)
# load the gene annotation data
data(GEs)

#select the genes
genes <- GEs[which(GEs$type == "gene")]

# the regions where to compute the DMRs
regions <- GRanges(seqnames = Rle("Chr3"), ranges = IRanges(1, 1E5))
genes <- genes[overlapsAny(genes, regions)]

# filter genes that are differentially methylated in the two conditions
DMRsGenesCG <- filterDMRs(methylationDataList["WT"], methylationDataList["met1-3"], potentialDMRs = genes, context = "CG", test = "score", pValueThreshold = 0.01, minCytosinesCount = 4, minProportionDifference = 0.4, minReadsPerCytosine = 3, cores = 1)

---

**GEs**  
*The genetic elements data*

**Description**

A GRanges object containing the annotation of the Arabidopsis thaliana

**Format**

A GRanges object

**Source**

The object was created by calling `import.gff3` function from `rtracklayer` package for [ftp://ftp.arabidopsis.org/Maps/gbrowse_data/TAIR10/TAIR10_GFF3_genes_transposons.gff](ftp://ftp.arabidopsis.org/Maps/gbrowse_data/TAIR10/TAIR10_GFF3_genes_transposons.gff)

---

**getWholeChromosomes**  
*Get whole chromosomes from methylation data*

**Description**

Returns a GRanges object spanning from the first cytocine until the last one on each chromosome

**Usage**

getWholeChromosomes(methylationData)

**Arguments**

methylationData

the methylation data stored as a GRanges object with four metadata columns (see `methylationDataList`).

**Value**

a GRanges object will all chromosomes.
Author(s)
Nicolae Radu Zabet

Examples

```r
data(methylationDataList)

chromosomes <- getWholeChromosomes(methylationDataList[['WT']])
```

joinReplicates Joins together two GRanges objects in a single containing all the replicates

Description
This function joins together data that come from biological replicates to perform analysis.

Usage

```r
joinReplicates(methylationData1, methylationData2, usecomplete = FALSE)
```

Arguments

methylationData1
the methylation data stored as a GRanges object with four metadata columns (see methylationDataList).

methylationData2
the methylation data stored as a GRanges object with four metadata columns (see methylationDataList).

usecomplete
Boolean, determine whether, when the two dataset differ for number of cytosines, if the smaller dataset should be added with zero reads to match the bigger dataset.

Value

returns a GRanges object containing multiple metadata columns with the reads from each object passed as parameter.

Author(s)
Alessandro Pio Greco and Nicolae Radu Zabet
mergeDMRsIteratively

Examples

```r
## Not run:
# load the methylation data
data(methylationDataList)

# Joins the wildtype and the mutant in a single object
joined_data <- joinReplicates(methylationDataList["WT"],
                             methylationDataList["met1-3"], FALSE)

## End(Not run)
```

mergeDMRsIteratively  Merge DMRs iteratively

Description

This function takes a list of DMRs and attempts to merge DMRs while keeping the new DMRs statistically significant.

Usage

```r
mergeDMRsIteratively(DMRs, minGap, respectSigns = TRUE, methylationData1,
                      methylationData2, context = "CG", minProportionDifference = 0.4,
                      minReadsPerCytosine = 4, pValueThreshold = 0.01, test = "fisher",
                      alternative = "two.sided", cores = 1)
```

Arguments

- **DMRs**  the list of DMRs as a `GRanges` object; e.g. see `computeDMRs`
- **minGap**  DMRs separated by a gap of at least `minGap` are not merged.
- **respectSigns**  logical value indicating whether to respect the sign when joining DMRs.
- **methylationData1**  the methylation data in condition 1 (see `methylationDataList`).
- **methylationData2**  the methylation data in condition 2 (see `methylationDataList`).
- **context**  the context in which the DMRs are computed ("CG", "CHG" or "CHH").
- **minProportionDifference**  two adjacent DMRs are merged only if the difference in methylation proportion of the new DMR is higher than `minProportionDifference`.
- **minReadsPerCytosine**  two adjacent DMRs are merged only if the number of reads per cytosine of the new DMR is higher than `minReadsPerCytosine`.
- **pValueThreshold**  two adjacent DMRs are merged only if the p-value of the new DMR (see `test` below) is lower than `pValueThreshold`. Note that we adjust the p-values using the Benjamini and Hochberg’s method to control the false discovery rate.
mergeDMRsIteratively

test the statistical test used to call DMRs ("fisher" for Fisher's exact test or "score" for Score test).

alternative indicates the alternative hypothesis and must be one of "two.sided", "greater" or "less".

cores the number of cores used to compute the DMRs.

Value

the reduced list of DMRs as a GRanges object; e.g. see computeDMRs

Author(s)

Nicolae Radu Zabet

See Also

filterDMRs, computeDMRs, analyseReadsInsideRegionsForCondition and DMRsNoiseFilterCG

Examples

# load the methylation data
data(methylationDataList)

# load the DMRs in CG context they were computed with minGap = 200
data(DMRsNoiseFilterCG)

# merge the DMRs
DMRsNoiseFilterCGLarger <- mergeDMRsIteratively(DMRsNoiseFilterCG[1:100],
  minGap = 500, respectSigns = TRUE,
  methylationDataList[["WT"]],
  methylationDataList[["met1-3"]],
  context = "CG", minProportionDifference = 0.4,
  minReadsPerCytosine = 1, pValueThreshold = 0.01,
  test = "score", alternative = "two.sided")

## Not run:
# set genomic coordinates where to compute DMRs
regions <- GRanges(seqnames = Rle("Chr3"), ranges = IRanges(1,1E5))

# compute DMRs and remove gaps smaller than 200 bp
DMRsNoiseFilterCG200 <- computeDMRs(methylationDataList[["WT"]],
  methylationDataList[["met1-3"]], regions = regions,
  context = "CG", method = "noise_filter",
  windowSize = 100, kernelFunction = "triangular",
  test = "score", pValueThreshold = 0.01,
  minCytosinesCount = 1, minProportionDifference = 0.4,
  minGap = 200, minSize = 0, minReadsPerCytosine = 1,
  cores = 1)
methylDataList <- computeDMRs(methylDataList["WT"],
methylDataList["met1-3"], regions = regions, 
context = "CG", method = "noise_filter", 
windowSize = 100, kernelFunction = "triangular", 
test = "score", pValueThreshold = 0.01,
minCytosinesCount = 1, minProportionDifference = 0.4,
minGap = 0, minSize = 0, minReadsPerCytosine = 1, 
cores = 1)

DMRsNoiseFilterCG0Merged200 <- mergeDMRsIteratively(DMRsNoiseFilterCG0,
minGap = 200, respectSigns = TRUE,
methylDataList["WT"],
methylDataList["met1-3"],
context = "CG", minProportionDifference=0.4,
minReadsPerCytosine = 1, pValueThreshold=0.01,
test="score",alternative = "two.sided")

#check that all newley computed DMRs are identical
print(all(DMRsNoiseFilterCG200 == DMRsNoiseFilterCG0Merged200))

## End(Not run)

methylDataList  The methylation data list

Description

A GRangesList object containing the methylation data at each cytosine location in the genome in Wild Type (WT) and met1-3 mutant (met1-3) in Arabidopsis thaliana. The data only contains the first 1 Mbp from Chromosome 3.

Format

The GRanges elements contain four metadata columns

context  the context in which the DMRs are computed ("CG", "CHG" or "CHH").
readsM  the number of methylated reads.
readsN  the total number of reads.
trinucleotide_context  the specific context of the cytosine (H is replaced by the actual nucleotide).

Source

plotLocalMethylationProfile

Plot local methylation profile

Description

This function plots the methylation profile at one locus for the bisulfite sequencing data. The points on the graph represent methylation proportion of individual cytosines, their colour which sample they belong to and the intensity of the the colour how many reads that particular cytosine had. This means that darker colors indicate stronger evidence that the corresponding cytosine has the corresponding methylation proportion, while lighter colors indicate a weaker evidence. The solid lines represent the smoothed profiles and the intensity of the line the coverage at the corresponding position (darker colors indicate more reads while lighter ones less reads). The boxes on top represent the DMRs, where a filled box will represent a DMR which gained methylation while a box with a pattern represent a DMR that lost methylation. The DMRs need to have a metadafield "regionType" which can be either "gain" (where there is more methylation in condition 2 compared to condition 1) or "loss" (where there is less methylation in condition 2 compared to condition 1). In case this metadafield is missing all DMRs are drawn using a filled box. Finally, we also allow annotation of the DNA sequence. We represent by a black boxes all the exons, which are joined by a horizontal black line, thus, marking the full body of the gene. With grey boxes we mark the transposable elements. Both for genes and transposable elements we plot them over a mid line if they are on the positive strand and under the mid line if they are on the negative strand.

Usage

plotLocalMethylationProfile(methylationData1, methylationData2, region,
   DMRs = NULL, conditionsNames = NULL, gff = NULL, windowSize = 150,
   context = "CG", labels = NULL, col = NULL, main = "",
   plotMeanLines = TRUE, plotPoints = TRUE)

Arguments

methylationData1
   the methylation data in condition 1 (see methylationDataList).
methylationData2
   the methylation data in condition 2 (see methylationDataList).
region
   a GRanges object with the region where to plot the high resolution profile.
DMRs
   a GRangesList object or a list with the list of DMRs (see computeDMRs or filterDMRs).
conditionsNames
   the names of the two conditions. This will be used to plot the legend.
gff
   a GRanges object with all elements usually imported from a GFF3 file. The gff file needs to have an metafield "type". Only the elements of type "gene", "exon" and "transposable_element" are plotted. Genes are represented as horizontal black lines, exons as a black rectangle and transposable elements as a grey rectangle. The elements are plotted on the corresponding strand (+ or -).
```r
plotLocalMethylationProfile

windowSize     the size of the triangle base used to smooth the average methylation profile.
context        the context in which the DMRs are computed ("CG", "CHG" or "CHH").
labels         a vector of character used to add a subfigure characters to the plot. If NULL nothing is added.
col            a character vector with the colors. It needs to contain a minimum of 4 \text{length}(\text{DMRs}) colors. If not or if NULL, the default colors will be used.
main           a character with the title of the plot
plotMeanLines  a logical value indicating whether to plot the mean lines or not.
plotPoints     a logical value indicating whether to plot the points or not.

Value

Invisibly returns NULL

Author(s)

Nicolae Radu Zabet

Examples

# load the methylation data
data(methylationDataList)
# load the gene annotation data
data(GEs)

#select the genes
genes <- GEs[which(GEs$type == "gene")]

# the coordinates of the area to be plotted
chr3Reg <- GRanges(seqnames = Rle("Chr3"), ranges = IRanges(510000,530000))

# load the DMRs in CG context
data(DMRsNoiseFilterCG)

DMRsCGlist <- list("noise filter"=DMRsNoiseFilterCG)

# plot the CG methylation
par(mar=c(4, 4, 3, 1)+0.1)
par(mfrow=c(1,1))
plotLocalMethylationProfile(methylationDataList[["WT"]],
                          methylationDataList[["met1-3"]], chr3Reg,
                          DMRsCGlist, c("WT", "met1-3"), GEs,
                          windowSize=100, main="CG methylation")
```
plotMethylationDataCoverage

Plot methylation data coverage

Description

This function plots the coverage for the bisulfite sequencing data.

Usage

plotMethylationDataCoverage(methylationData1, methylationData2 = NULL, breaks, regions = NULL, conditionsNames = NULL, context = "CG", proportion = TRUE, labels = NULL, col = NULL, pch = c(1, 0, 16, 2, 15, 17), lty = c(4, 1, 3, 2, 6, 5), contextPerRow = FALSE)

Arguments

methylationData1
the methylation data in condition 1 (see methylationDataList).

methylationData2
the methylation data in condition 2 (see methylationDataList). This is optional.

breaks
a numeric vector specifying the different values for the thresholds when computing the coverage.

regions
a GRanges object with the regions where to compute the coverage. If NULL, the coverage is computed genome-wide.

conditionsNames
a vector of character with the names of the conditions for methylationData1 and methylationData2.

context
the context in which the DMRs are computed ("CG", "CHG" or "CHH").

proportion
a logical value indicating whether proportion or counts will be plotted.

labels
a vector of character used to add a subfigure character to the plot. If NULL nothing is added.

col
a character vector with the colors. It needs to contain a minimum of 2 colors per condition. If not or if NULL, the default colors will be used.

pch
the R symbols used to plot the data. It needs to contain a minimum of 2 symbols per condition. If not or if NULL, the default symbols will be used.

lty
the line types used to plot the data. It needs to contain a minimum of 2 line types per condition. If not or if NULL, the default line types will be used.

contextPerRow
a logical value indicating if the each row represents an individual context. If FALSE, each column will represent an individual context.
plotMethylationDataCoverage

Details

This function plots the proportion of cytosines in a specific context that have at least a certain number of reads (x-axis)

Value

Invisibly returns NULL

Author(s)

Nicolae Radu Zabet

See Also

computeMethylationDataCoverage, methylationDataList

Examples

# load the methylation data
data(methylationDataList)

# plot the coverage in CG context
par(mar=c(4, 4, 3, 1)+0.1)
plotMethylationDataCoverage(methylationDataList["WT"], methylationDataList["met1-3"], breaks = c(1,5,10,15), regions = NULL, conditionsNames = c("WT","met1-3"), context = c("CG"), proportion = TRUE, labels = LETTERS, col = NULL, pch = c(1,0,16,2,15,17), lty = c(4,1,3,2,6,5), contextPerRow = FALSE)

## Not run:
# plot the coverage in all three contexts
plotMethylationDataCoverage(methylationDataList["WT"], methylationDataList["met1-3"], breaks = 1:15, regions = NULL, conditionsNames = c("WT","met1-3"), context = c("CG", "CHG", "CHH"), proportion = TRUE, labels = LETTERS, col = NULL, pch = c(1,0,16,2,15,17), lty = c(4,1,3,2,6,5), contextPerRow = FALSE)

## End(Not run)
### plotMethylationDataSpatialCorrelation

**Plot methylation data spatial correlation**

#### Description

This function plots the correlation of methylation levels for Cytosines located at a certain distance apart.

#### Usage

```r
plotMethylationDataSpatialCorrelation(methylationData1, methylationData2 = NULL, distances, regions = NULL, conditionsNames = NULL, context = "CG", labels = NULL, col = NULL, pch = c(1, 0, 16, 2, 15, 17), lty = c(4, 1, 3, 2, 6, 5), contextPerRow = FALSE, log = "")
```

#### Arguments

- `methylationData1`: the methylation data in condition 1 (see `methylationDataList`).
- `methylationData2`: the methylation data in condition 2 (see `methylationDataList`). This is optional.
- `distances`: a numeric vector specifying the different values for the distances when computing the correlation.
- `regions`: a `GRanges` object with the regions where to compute the correlation. If `NULL`, the coverage is computed genome-wide.
- `conditionsNames`: a vector of character with the names of the conditions for `methylationData1` and `methylationData2`.
- `context`: the context in which the DMRs are computed ("CG", "CHG" or "CHH").
- `labels`: a vector of character used to add a subfigure character to the plot. If `NULL`, nothing is added.
- `col`: a character vector with the colors. It needs to contain a minimum of 2 colors per condition. If not or if `NULL`, the default colors will be used.
- `pch`: the R symbols used to plot the data. It needs to contain a minimum of 2 symbols per condition. If not or if `NULL`, the default symbols will be used.
- `lty`: the line types used to plot the data. It needs to contain a minimum of 2 line types per condition. If not or if `NULL`, the default line types will be used.
- `contextPerRow`: a logical value indicating if the each row represents an individual context. If `FALSE`, each column will represent an individual context.
- `log`: a character indicating if any of the axes will be displayed on log scale. This argument will be passed to `plot` function.
plotMethylationDataSpatialCorrelation

Details

This function plots the proportion of cytosines in a specific context that have at least a certain number of reads (x-axis)

Value

Invisibly returns NULL

Author(s)

Nicolae Radu Zabet

See Also

computeMethylationDataSpatialCorrelation, methylationDataList

Examples

## Not run:
# load the methylation data
data(methylationDataList)

# plot the spatial correlation in CG context
par(mar=c(4, 4, 3, 1)+0.1)
plotMethylationDataSpatialCorrelation(methylationDataList[["WT"]],
    distances = c(1,5,10,15), regions = NULL,
    conditionsNames = c("WT","met1-3"),
    context = c("CG"),
    labels = LETTERS, col = NULL,
    pch = c(1,0,16,2,15,17), lty = c(4,1,3,2,6,5),
    contextPerRow = FALSE)

# plot the spatial correlation in all three contexts
plotMethylationDataSpatialCorrelation(methylationDataList[["WT"]],
    methylationDataList[["met1-3"]],
    distances = c(1,5,10,15,20,50,100,150,200,500,1000),
    regions = NULL, conditionsNames = c("WT","met1-3"),
    context = c("CG", "CHG", "CHH"),
    labels = LETTERS, col = NULL,
    pch = c(1,0,16,2,15,17), lty = c(4,1,3,2,6,5),
    contextPerRow = FALSE, log="x")

## End(Not run)
plotMethylationProfile

Plot Methylation Profile

Description

This function plots the low resolution profiles for the bisulfite sequencing data.

Usage

plotMethylationProfile(methylationProfiles, autoscale = FALSE,
labels = NULL, title = "", col = NULL, pch = c(1, 0, 16, 2, 15, 17),
lty = c(4, 1, 3, 2, 6, 5))

Arguments

methylationProfiles
  a GRangesList object. Each GRanges object in the list is generated by calling
  the function computeMethylationProfile.
autoscale
  a logical value indicating whether the values are autoscaled for each context
  or not.
lables
  a vector of character used to add a subfigure characters to the plot. If NULL
  nothing is added.
title
the plot title.
col
  a character vector with the colours. It needs to contain a minimum of 2 colours
  per context. If not or if NULL, the default colours will be used.
pch
  the R symbols used to plot the data.
lty
  the line types used to plot the data.

Value

Invisibly returns NULL

Author(s)

Nicolae Radu Zabet

See Also

plotMethylationProfileFromData, computeMethylationProfile and methylationDataList
Examples

# load the methylation data
data(methylationDataList)

# the region where to compute the profile
region <- GRanges(seqnames = Rle("Chr3"), ranges = IRanges(1,1E6))

# compute low resolution profile in 20 Kb windows
lowResProfileWTCG <- computeMethylationProfile(methylationDataList["WT"],
region, windowSize = 20000, context = "CG")

lowResProfileCG <- GRangesList("WT" = lowResProfileWTCG)

#plot the low resolution profile
par(mar=c(4, 4, 3, 1)+0.1)
par(mfrow=c(1,1))
plotMethylationProfile(lowResProfileCG, autoscale = FALSE,
title="CG methylation on Chromosome 3",
col=c("#D55E00","#E69F00"), pch = c(1,0),
lty = c(4,1))

## Not run:
# compute low resolution profile in 10 Kb windows in CG context
lowResProfileWTCG <- computeMethylationProfile(methylationDataList["WT"],
region, windowSize = 10000, context = "CG")

lowResProfileMet13CG <- computeMethylationProfile(
methylationDataList["met1-3"], region,
windowSize = 10000, context = "CG")

lowResProfileCG <- GRangesList("WT" = lowResProfileWTCG,
"met1-3" = lowResProfileMet13CG)

# compute low resolution profile in 10 Kb windows in CHG context
lowResProfileWTCG <- computeMethylationProfile(methylationDataList["WT"],
region, windowSize = 10000, context = "CHG")

lowResProfileMet13CHG <- computeMethylationProfile(
methylationDataList["met1-3"], region,
windowSize = 10000, context = "CHG")

lowResProfileCHG <- GRangesList("WT" = lowResProfileWTCG,
"met1-3" = lowResProfileMet13CHG)

# plot the low resolution profile
par(mar=c(4, 4, 3, 1)+0.1)
par(mfrow=c(2,1))
plotMethylationProfile(lowResProfileCG, autoscale = FALSE,
labels = LETTERS[1],
title="CG methylation on Chromosome 3",
col=c("#D55E00","#E69F00"), pch = c(1,0),
lty = c(4,1))
plotMethylationProfileFromData

Plot methylation profile from data

Description

This function plots the low resolution profiles for all bisulfite sequencing data.

Usage

plotMethylationProfileFromData(methylationData1, methylationData2 = NULL, regions = NULL, conditionsNames = NULL, context = "CG", windowSize = NULL, autoscale = FALSE, labels = NULL, col = NULL, pch = c(16, 2), lty = c(3, 2), contextPerRow = TRUE)

Arguments

methylationData1
the methylation data in condition 1 (see methylationDataList).

methylationData2
the methylation data in condition 2 (see methylationDataList). This is optional.

regions a GRanges object with the regions where to plot the profiles.

conditionsNames the names of the two conditions. This will be used to plot the legend.

context a vector with all contexts in which the DMRs are computed ("CG", "CHG" or "CHH").

windowSize a numeric value indicating the size of the window in which methylation is averaged.

autoscale a logical value indicating whether the values are autoscaled for each context or not.

labels a vector of character used to add a subfigure character to the plot. If NULL nothing is added.

col a character vector with the colours. It needs to contain a minimum of 2 colours per condition. If not or if NULL, the default colours will be used.
plotMethylationProfileFromData

pch the R symbols used to plot the data. It needs to contain a minimum of 2 symbols per condition. If not or if NULL, the default symbols will be used.

lty the line types used to plot the data. It needs to contain a minimum of 2 line types per condition. If not or if NULL, the default line types will be used.

contextPerRow a logical value indicating if the each row represents an individual context. If FALSE, each column will represent an individual context.

Value

Invisibly returns NULL

Author(s)

Nicolae Radu Zabet

See Also

plotMethylationProfile, computeMethylationProfile and methylationDataList

Examples

# load the methylation data
data(methylationDataList)

# plot the low resolution profile at 10 Kb resolution
par(mar=c(4, 4, 3, 1)+0.1)
plotMethylationProfileFromData(methylationDataList[["WT"]],
methylationDataList[["met1-3"]],
conditionsNames=c("WT", "met1-3"),
windowSize = 20000, autoscale = TRUE,
context = c("CHG"))

## Not run:
# plot the low resolution profile at 5 Kb resolution
par(mar=c(4, 4, 3, 1)+0.1)
plotMethylationProfileFromData(methylationDataList[["WT"]],
methylationDataList[["met1-3"]],
conditionsNames=c("WT", "met1-3"),
windowSize = 5000, autoscale = TRUE,
context = c("CG", "CHG", "CHH"),
labels = LETTERS)

## End(Not run)
plotOverlapProfile  
Plot overlap profile

Description
This function plots the distribution of a set of subregions on a large region.

Usage
```r
plotOverlapProfile(overlapsProfiles1, overlapsProfiles2 = NULL,
                   names = NULL, labels = NULL, col = NULL, title = "",
                   logscale = FALSE, maxValue = NULL)
```

Arguments
- `overlapsProfiles1`  
a `GRanges` object with the overlaps profile; see `computeOverlapProfile`.
- `overlapsProfiles2`  
a `GRanges` object with the overlaps profile; see `computeOverlapProfile`. This is optional. For example, one can be use `overlapsProfiles1` to display hypomethylated regions and `overlapsProfiles2` the hypermethylated regions.
- `names`  
a vector of character to add labels for the two `overlapsProfiles`. This is an optional parameter.
- `labels`  
a vector of character used to add a subfigure character to the plot. If NULL nothing is added.
- `col`  
a character vector with the colours. It needs to contain 2 colours. If not or if NULL, the default colours will be used.
- `title`  
the title of the plot.
- `logscale`  
a logical value indicating if the colours are on logscale or not.
- `maxValue`  
a maximum value in a region. Used for the colour scheme.

Value
Invisibly returns NULL.

Author(s)
Nicolae Radu Zabet

See Also
`computeOverlapProfile`, `filterDMRs`, `computeDMRs` and `mergeDMRsIteratively`
Examples

```r
# load the methylation data
data(methylationDataList)

# load the DMRs in CG context
data(DMRsNoiseFilterCG)

# the coordinates of the area to be plotted
largeRegion <- GRanges(seqnames = Rle("Chr3"), ranges = IRanges(1,1E5))

# compute overlaps distribution
hotspotsHypo <- computeOverlapProfile(DMRsNoiseFilterCG, largeRegion, windowSize = 10000, binary = FALSE)
plotOverlapProfile(GRangesList("Chr3"=hotspotsHypo), names = c("hypomethylated"), title = "CG methylation")

## Not run:
largeRegion <- GRanges(seqnames = Rle("Chr3"), ranges = IRanges(1,1E6))
hotspotsHypo <- computeOverlapProfile(DMRsNoiseFilterCG[(DMRsNoiseFilterCG$regionType == "loss")], largeRegion, windowSize=2000, binary=TRUE, cores=1)
hotspotsHyper <- computeOverlapProfile(DMRsNoiseFilterCG[(DMRsNoiseFilterCG$regionType == "gain")], largeRegion, windowSize=2000, binary=TRUE, cores=1)
plotOverlapProfile(GRangesList("Chr3"=hotspotsHypo), GRangesList("Chr3"=hotspotsHyper), names=c("loss", "gain"), title="CG methylation")

## End(Not run)
```

---

poolMethylationDatasets

*Pool methylation data*

Description

This function pools together multiple methylation datasets.

Usage

```r
poolMethylationDatasets(methylationDataList)
```
Arguments

methylationDataList

a GRangesList object where each element of the list is a GRanges object with the methylation data in the corresponding condition (see methylationDataList).

Value

the methylation data stored as a GRanges object with four metadata columns (see methylationDataList).

Author(s)

Nicolae Radu Zabet

Examples

# load methylation data object
data(methylationDataList)

# pools the two datasets together
pooledMethylationData <- poolTwoMethylationDatasets(methylationDataList)

Description

This function pools together two methylation datasets.

Usage

poolTwoMethylationDatasets(methylationData1, methylationData2)

Arguments

methylationData1

a GRanges object with the methylation data (see methylationDataList).

methylationData2

a GRanges object with the methylation data (see methylationDataList).

Value

the methylation data stored as a GRanges object with four metadata columns (see methylationDataList).

Author(s)

Nicolae Radu Zabet
readBismark

Examples

# load methylation data object
data(methylationDataList)

# save the two datasets together
pooledMethylationData <- poolTwoMethylationDatasets(methylationDataList[[1]],
methylationDataList[[2]])

Description

This function takes as input a CX report file produced by Bismark and returns a GRanges object with four metadata columns. The file represents the bisulfite sequencing methylation data.

Usage

readBismark(file)

Arguments

file The filename (including path) of the methylation (CX report generated by Bismark) to be read.

Value

the methylation data stored as a GRanges object with four metadata columns (see methylationDataList).

Author(s)

Nicolae Radu Zabet and Jonathan Michael Foonlan Tsang

Examples

# load methylation data object
data(methylationDataList)

# save the two datasets into a file
saveBismark(methylationDataList[["WT"]], "chr3test_a_thaliana_wt.CX_report")

# load the data
methylationDataWT <- readBismark("chr3test_a_thaliana_wt.CX_report")

# check that the loading worked
all(methylationDataWT == methylationDataList[["WT"]])
Description

This function takes as input a vector of CX report file produced by Bismark and returns a `GRanges` object with four metadata columns (see `methylationDataList`). The file represents the pooled bisulfite sequencing data.

Usage

```r
readBismarkPool(files)
```

Arguments

- `files`: The filenames (including path) of the methylation (CX report generated with Bismark) to be read

Value

the methylation data stored as a `GRanges` object with four metadata columns (see `methylationDataList`).

Author(s)

Nicolae Radu Zabet and Jonathan Michael Foonlan Tsang

Examples

```r
# load methylation data object
data(methylationDataList)

# save the two datasets
saveBismark(methylationDataList[["WT"]],
            "chr3test_a_thaliana_wt.CX_report")
saveBismark(methylationDataList[["met1-3"]],
            "chr3test_a_thaliana_met13.CX_report")

# reload the two datasets and pool them
filenames <- c("chr3test_a_thaliana_wt.CX_report",
               "chr3test_a_thaliana_met13.CX_report")
methylationDataPool <- readBismarkPool(filenames)
```
saveBismark

Description

This function takes as input a GRanges object generated with readBismark and saves the output to a file using Bismark CX report format.

Usage

saveBismark(methylationData, filename)

Arguments

methylationData

the methylation data stored as a GRanges object with four metadata columns (see methylationDataList).

filename

the filename where the data will be saved.

Value

Invisibly returns NULL

Author(s)

Nicolae Radu Zabet

Examples

# load methylation data object
data(methylationDataList)

# save one dataset to a file
saveBismark(methylationDataList[["WT"]], "chr3test_a_thaliana_wt.CX_report")
Format

A GRanges object containing multiple metadata columns with the reads from each object passed as parameter.

Source

The object was created by calling joinReplicates function.
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