# Package 'DMRcaller'

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 $analyse {\tt ReadsInsideRegionsForCondition}$ 

Analyse reads inside regions for condition

### Description

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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**

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

### 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.

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#### 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**

computeDMRs

Compute DMRs

#### **Description**

This function computes the differentially methylated regions between two conditions.

### Usage

```
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)
```

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#### **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 dispay different levels of methylation in the two conditions. The "neighbourhood" method computates differentially methylated cytosines. Finally, 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 inital parameters used when calling the differentiall 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  $(K(x) = \exp(-1 \operatorname{ambda} * x^2))$ .

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".

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 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.

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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

 $filter {\tt DMRs}, {\tt mergeDMRs} Iteratively, {\tt analyseReads} Inside {\tt RegionsForCondition} \ and {\tt DMRsNoiseFilter} CG$ 

```
cores = 1)
## Not run:
# compute the DMRs in CG context with neighbourhood method
DMRsNeighbourhoodCG <- computeDMRs(methylationDataList[["WT"]],</pre>
                       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"]],</pre>
               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)
```

computeMethylationDataCoverage

Compute methylation data coverage

#### 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 specifing the different values for the thresholds when comput-

ing 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

```
\verb|plotMethylationDataCoverage|, methylationDataList|
```

### **Examples**

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 av-

eraged.

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

#### See Also

 $\verb|plotMethylationProfileFromData|, \verb|plotMethylationProfile|, methylationDataList| \\$ 

### **Examples**

computeOverlapProfile Compute Overlaps Profile

#### **Description**

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

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#### 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.

#### 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
```

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.

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.

#### Author(s)

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#### See Also

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

```
## 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"]],
                                                                       methylationDataList[["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
lowResProfileWTCG <- computeMethylationProfile(methylationDataList[["WT"]],</pre>
                                                region, windowSize = 10000, context = "CG")
lowResProfileMet13CG <- computeMethylationProfile(</pre>
                                                methylationDataList[["met1-3"]], region,
                                                windowSize = 10000, context = "CG")
lowResProfileCG <- GRangesList("WT" = lowResProfileWTCG,</pre>
                                           "met1-3" = lowResProfileMet13CG)
# compute low resolution profile in 10 Kb windows in CHG context
low Res Profile \verb|WTCHG| <- compute Methylation Profile (methylation DataList[["WT"]], the profile \verb|WTCHG| -- compute Methylation Profile (methylation DataList[["WT"]], the profile \verb|WTCHG| -- compute Methylation Profile (methylation DataList[["WT"]], the profile \verb|WTCHG| -- compute Methylation Profile (methylation DataList[["WT"]], the profile \verb|WTCHG| -- compute Methylation Profile (methylation DataList[["WT"]], the profile \verb|WTCHG| -- compute Methylation Profile (methylation DataList[["WT"]], the profile \verb|WTCHG| -- compute Methylation Profile (methylation DataList[["WT"]], the profile \verb|WTCHG| -- compute Methylation Profile (methylation DataList[["WT"]], the profile \verb|WTCHG| -- compute Methylation Profile (methylation DataList[""WT"]], the profile \verb|WTCHG| -- compute Methylation Profile (methylation DataList[""WT"]], the profile \verb|WTCHG| -- compute Methylation Profile (methylation DataList["""), the profile methylation Profil
                                                region, windowSize = 10000, context = "CHG")
lowResProfileMet13CHG <- computeMethylationProfile(</pre>
                                                methylationDataList[["met1-3"]], region,
                                                windowSize = 10000, context = "CHG")
lowResProfileCHG <- GRangesList("WT" = lowResProfileWTCHG,</pre>
                                            "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),
                                                     1ty = c(4,1)
plotMethylationProfile(lowResProfileCHG, autoscale = FALSE,
                                                     labels = LETTERS[2],
                                                     title="CHG methylation on Chromosome 3",
                                                     col=c("#0072B2", "#56B4E9"), pch = c(16,2),
```

```
1ty = c(3,2)
# 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)
# the regions where to compute the DMRs
regions <- GRanges(seqnames = Rle("Chr3"), ranges = IRanges(1,1E6))</pre>
# compute the DMRs in CG context with noise_filter method
DMRsNoiseFilterCG <- computeDMRs(methylationDataList[["WT"]],</pre>
                     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"]],</pre>
                       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"]],</pre>
               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")]</pre>
# the regions where to compute the DMRs
genes <- genes[overlapsAny(genes, regions)]</pre>
# filter genes that are differntially methylated in the two conditions
DMRsGenesCG <- filterDMRs(methylationDataList[["WT"]],</pre>
```

```
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,</pre>
                           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$type == "gene")]</pre>
# the coordinates of the area to be plotted
chr3Reg <- GRanges(seqnames = Rle("Chr3"), ranges = IRanges(510000,530000))</pre>
# load the DMRs in CG context
data(DMRsNoiseFilterCG)
DMRsCGlist <- list("noise filter"=DMRsNoiseFilterCG,</pre>
                   "neighbourhood"=DMRsNeighbourhoodCG,
                   "bins"=DMRsBinsCG,
                   "genes"=DMRsGenesCG)
# plot the CG methylation
par(mar=c(4, 4, 3, 1)+0.1)
par(mfrow=c(1,1))
plotLocalMethylationProfile(methylationDataList[["WT"]],
                           methylationDataList[["met1-3"]], chr3Reg,
                           DMRsCGlist, c("WT", "met1-3"), GEs,
                           windowSize=100, main="CG methylation")
hotspotsHypo <- computeOverlapProfile(</pre>
               DMRsNoiseFilterCG[(DMRsNoiseFilterCG$regionType == "loss")],
               region, windowSize=2000, binary=TRUE, cores=1)
hotspotsHyper <- computeOverlapProfile(</pre>
               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")
## End(Not run)
```

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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

filterDMRs

Filter DMRs

### Description

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

### Usage

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

### Arguments

methylationData1

the methylation data in condition 1 (see methylationDataList).

methylationData2

the methylation data in condition 2 (see methylationDataList).

potential DMRs a GRanges object with potential DMRs where to compute the DMRs. This can

be a a list of gene and/or transposable elements coordinates.

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

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

for Score test).

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#### 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

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

 $DMRsNoiseFilterCG, computeDMRs, analyseReadsInsideRegionsForCondition \\ \textbf{and} \\ mergeDMRsIteratively \\ DMRsNoiseFilterCG, computeDMRs, analyseReadsInsideRegionsForCondition \\ \textbf{and} \\ mergeDMRsIteratively \\ DMRsNoiseFilterCG, computeDMRs, analyseReadsInsideRegionsForCondition \\ \textbf{and} \\ mergeDMRsIteratively \\ DMRsNoiseFilterCG, computeDMRs, analyseReadsInsideRegionsForCondition \\ \textbf{and} \\ mergeDMRsIteratively \\ DMRsNoiseFilterCONDITION \\ DMRsnois$ 

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

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

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

# get all chromosomes
chromosomes <- getWholeChromosomes(methylationDataList[["WT"]])</pre>
```

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

```
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 Panjamini and Hachberr's method to control the false discovery rate.

the Benjamini and Hochberg's method to control the false discovery rate.

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

test

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

```
# 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))</pre>
# compute DMRs and remove gaps smaller than 200 bp
DMRsNoiseFilterCG200 <- computeDMRs(methylationDataList[["WT"]],</pre>
                       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)
DMRsNoiseFilterCG0 <- computeDMRs(methylationDataList[["WT"]],</pre>
                       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 = 0, minSize = 0, minReadsPerCytosine = 1,
                       cores = 1)
DMRsNoiseFilterCG0Merged200 <- mergeDMRsIteratively(DMRsNoiseFilterCG0,
                             minGap = 200, respectSigns = TRUE,
                             methylationDataList[["WT"]],
                             methylationDataList[["met1-3"]],
```

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 ${\tt methylationDataList}$ 

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**

Each element was created by by calling readBismark function on the CX report files generated by Bismark http://www.bioinformatics.babraham.ac.uk/projects/bismark/ for http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM980986 dataset in the case of Wild Type (WT) and http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM981032 dataset in the case of met1-3 mutant (met1-3).

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 intesity 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.

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 -).

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 length (DMRs)

colors. If not or if NULL, the defalut 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")]</pre>
# the coordinates of the area to be plotted
chr3Reg <- GRanges(seqnames = Rle("Chr3"), ranges = IRanges(510000,530000))</pre>
# load the DMRs in CG context
data(DMRsNoiseFilterCG)
DMRsCGlist <- list("noise filter"=DMRsNoiseFilterCG)</pre>
# 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")
```

 $\verb|plotMethylationDataCoverage| \\$ 

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 op-

tional.

breaks a numeric vector specifing the different values for the thresholds when comput-

ing 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 defalut 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 defalut symbols will be used.

1ty 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 defalut 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.

#### 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

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#### See Also

 $compute {\tt MethylationDataCoverage}, {\tt methylationDataList}$ 

#### **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)
```

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))
```

plotMethylationProfile

#### **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 autoscalled for each context

or not.

labels 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 defalut colours will be used.

pch the R symbols used to plot the data.

1ty the line types used to plot the data.

#### Value

Invisibly returns NULL

#### Author(s)

Nicolae Radu Zabet

#### See Also

plotMethylationProfileFromData, computeMethylationProfile and methylationDataList

```
# load the methylation data
data(methylationDataList)
# the region where to compute the profile
region <- GRanges(seqnames = Rle("Chr3"), ranges = IRanges(1,1E6))</pre>
# compute low resolution profile in 20 Kb windows
lowResProfileWTCG <- computeMethylationProfile(methylationDataList[["WT"]],</pre>
                     region, windowSize = 20000, context = "CG")
lowResProfilsCG <- GRangesList("WT" = lowResProfileWTCG)</pre>
#plot the low resolution profile
par(mar=c(4, 4, 3, 1)+0.1)
par(mfrow=c(1,1))
plotMethylationProfile(lowResProfilsCG, autoscale = FALSE,
                       title="CG methylation on Chromosome 3",
                       col=c("\#D55E00","\#E69F00"), pch = c(1,0),
                       1ty = c(4,1)
## Not run:
```

```
# compute low resolution profile in 10 Kb windows in CG context
lowResProfileWTCG <- computeMethylationProfile(methylationDataList[["WT"]],</pre>
                     region, windowSize = 10000, context = "CG")
lowResProfileMet13CG <- computeMethylationProfile(</pre>
                     methylationDataList[["met1-3"]], region,
                     windowSize = 10000, context = "CG")
lowResProfileCG <- GRangesList("WT" = lowResProfileWTCG,</pre>
                   "met1-3" = lowResProfileMet13CG)
# compute low resolution profile in 10 Kb windows in CHG context
lowResProfileWTCHG <- computeMethylationProfile(methylationDataList[["WT"]],</pre>
                     region, windowSize = 10000, context = "CHG")
lowResProfileMet13CHG <- computeMethylationProfile(</pre>
                     methylationDataList[["met1-3"]], region,
                     windowSize = 10000, context = "CHG")
lowResProfileCHG <- GRangesList("WT" = lowResProfileWTCHG,</pre>
                   "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)
plotMethylationProfile(lowResProfileCHG, autoscale = FALSE,
                       labels = LETTERS[2],
                       title="CHG methylation on Chromosome 3",
                       col=c("#0072B2", "#56B4E9"), pch = c(16,2),
                       1ty = c(3,2)
## End(Not run)
```

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(1, 0, 16, 2, 15, 17), lty = c(4, 1, 3, 2, 6, 5), contextPerRow = TRUE)
```

#### **Arguments**

methylationData1

the methylation data in condition 1 (see methylationDataList).

methylationData2

the methylation data in condition 2 (see methylationDataList). This is op-

tional.

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 av-

eraged.

autoscale a logical value indicating whether the values are autoscalled 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 defalut colours 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 defalut symbols will be used.

1ty 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 defalut 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

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#### **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

```
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 optinal parameter.

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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 defalut 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

```
# 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))</pre>
# compute overlaps distribution
hotspotsHypo <- computeOverlapProfile(DMRsNoiseFilterCG, largeRegion,</pre>
                 windowSize = 10000, binary = FALSE)
plotOverlapProfile(GRangesList("Chr3"=hotspotsHypo),
                   names = c("hypomethylated"), title = "CG methylation")
## Not run:
largeRegion <- GRanges(segnames = Rle("Chr3"), ranges = IRanges(1,1E6))</pre>
hotspotsHypo <- computeOverlapProfile(</pre>
               DMRsNoiseFilterCG[(DMRsNoiseFilterCG$regionType == "loss")],
               largeRegion, windowSize=2000, binary=TRUE, cores=1)
hotspotsHyper <- computeOverlapProfile(</pre>
               DMRsNoiseFilterCG[(DMRsNoiseFilterCG$regionType == "gain")],
               largeRegion, windowSize=2000, binary=TRUE, cores=1)
plotOverlapProfile(GRangesList("Chr3"=hotspotsHypo),
```

poolMethylationDatasets

Pool methylation data

### Description

This function pools together multiple methylation datasets.

#### Usage

```
poolMethylationDatasets(methylationDataList)
```

### **Arguments**

```
{\tt 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

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

# pools the two datasets together
pooledMethylationData <- poolMethylationDatasets(methylationDataList)</pre>
```

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```
poolTwoMethylationDatasets
```

Pool two methylation datasets

### **Description**

This function pools together two methylation datasets.

#### Usage

```
\verb|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

### **Examples**

readBismark

Read Bismark

### **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)
```

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#### **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 one 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"]])</pre>
```

readBismarkPool

Read Bismark pool

#### **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

```
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).

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#### Author(s)

Nicolae Radu Zabet and Jonathan Michael Foonlan Tsang

### **Examples**

saveBismark

Save Bismark

### 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).

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filename

the filename where the data will be saved.

#### Value

Invisibly returns NULL

#### Author(s)

Nicolae Radu Zabet

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

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

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