# Package ‘DMRcaller’

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**Title** Differentially Methylated Regions caller  
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**Author** Nicolae Radu Zabet <n.r.zabet@gen.cam.ac.uk> and Jonathan Michael Foonlan Tsang <jmftz2@cam.ac.uk>  
**Maintainer** Nicolae Radu Zabet <n.r.zabet@gen.cam.ac.uk>  
**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.  
**License** GPL-3  
**LazyLoad** yes  
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**VignetteBuilder** knitr  
**NeedsCompilation** no

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**analyseReadsInsideRegionsForCondition**

Analyse reads inside regions for condition

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)
```

**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
computeDMRs

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")
```

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 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).
computeDMRs

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 "epanechnikov". 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".

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.
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.

Author(s)

Nicolae Radu Zabet and Jonathan Michael Foonlan Tsang

See Also

filterDMRs, mergeDMRsIteratively, analyseReadsInsideRegionsForCondition and DMRsNoiseFilterCG

Examples

# 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)
Usage

```r
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

```r
# 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)
```

---

**computeMethylationProfile**

*Compute methylation profile*

Description

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

Usage

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

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

See Also

plotMethylationProfileFromData, plotMethylationProfile, 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
lowResProfileWTCHH <- computeMethylationProfile(methylationDataList["WT"],
    region, windowSize = 20000, context = "CHH")

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

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

## End(Not run)
computeOverlapProfile  Compute Overlaps Profile

Description

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.

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

# 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.
- 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)

Nicolae Radu Zabet <n.r.zabet@gen.cam.ac.uk>, Jonathan Michael Foonlan Tsang <jmft2@cam.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"],
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"],
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
lowResProfileWTCHG <- computeMethylationProfile(methylationDataList["WT"],
region, windowSize = 10000, context = "CHG")

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

lowResProfileCHG <- GRangesList("WT" = lowResProfileWTCHG,
"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),
lty = 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))

# 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, 
cores = 1)
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")

## End(Not run)

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 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`: the methylation data in condition 1 (see `methylationDataList`).
- `methylationData2`: 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`: 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).
- `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
getWholeChromosomes

**See Also**
- `DMRsNoiseFilterCG`, `computeDMRs`, `analyseReadsInsideRegionsForCondition` and `mergeDMRsIteratively`

**Examples**

```r
# 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`

---

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**

```r
getWholeChromosomes(methylationData)
```
mergeDMRsIteratively

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

# load the methylation data
data(methylationDataList)

# get all chromosomes
cchromosomes <- getWholeChromosomes(methylationDataList["WT"])

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.
mergeDMRsIteratively

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.

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,
methylationDataList

minGap = 200, minSize = 0, minReadsPerCytosine = 1, cores = 1)

DMRsNoiseFilterCG0 <- 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 = 0, minSize = 0, minReadsPerCytosine = 1, cores = 1)

DMRsNoiseFilterCG0Merged200 <- mergeDMRsIteratively(DMRsNoiseFilterCG0, minGap = 200, respectSigns = TRUE, methylationDataList["WT"], methylationDataList["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)

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

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 -).

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").
plotMethylationDataCoverage

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 \text{4 length(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

\textit{Plot methylation data coverage}

Description
This function plots the coverage for the bisulfite sequencing data.
plotMethylationDataCoverage

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

methyla0tionData1
the methylation data in condition 1 (see methylationDataList).

methyla0tionData2
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.

c0ntext
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.

c0l
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.

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

```r
# 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

```r
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.
- `labels`: a vector of character used to add a subfigure characters to the plot. If NULL nothing is added.
- `title`: the plot title.
plotMethylationProfile

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")

lowResProfilsCG <- GRangesList("WT" = lowResProfileWTCG)

#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),
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")

lowResProfileMet13CHG <- computeMethylationProfile("
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 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.
plotMethylationProfileFromData

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.

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)
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 hy-
  pomethylated 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
poolMethylationDatasets

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

`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`).
poolTwoMethylationDatasets

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 <- poolMethylationDatasets(methylationDataList)

---

poolTwoMethylationDatasets

Pool two methylation datasets

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

Examples

# load methylation data object
data(methylationDataList)

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

```r
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**

```r
# 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"]])
```

---

**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**

```r
readBismarkPool(files)
```
**saveBismark**

**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 **methyltionDataList**).

**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)
```

**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**

```r
saveBismark(methylationData, filename)
```

**Arguments**

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

- **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")
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