# Package 'DMRcaller'

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**Author** Nicolae Radu Zabet <r.zabet@qmul.ac.uk>, Jonathan Michael Foonlan Tsang <jmft2@cam.ac.uk>, Alessandro Pio Greco <apgrec@essex.ac.uk>, Ryan Merritt <rmerri@essex.ac.uk> and Young Jun Kim <qc25039@qmul.ac.uk>

**Maintainer** Nicolae Radu Zabet <r.zabet@qmul.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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---

`analyseReadsInsideRegionsForCondition`
*Analyse reads inside regions for condition*

---

## Description

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

## Usage

```
analyseReadsInsideRegionsForCondition(
  regions,
  methylationData,
  context,
  label = "",
  parallel = FALSE,
  BPPARAM = NULL,
  cores = NULL
)
```

## 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 |
| `parallel` | Logical; run in parallel if `TRUE`. |
| `BPPARAM` | A `BiocParallelParam` object controlling parallel execution. This value will automatically set when parallel is `TRUE`, also able to set as manually. |
| `cores` | Integer number of workers (must not exceed BPPARAM$workers). This value will automatically set as the maximum number of system workers, also able to set as manually. |

## 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

readONTbam, filterDMRs, computeDMRs, DMRsNoiseFilterCG, and mergeDMRsIteratively

## Examples

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

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

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

---

# analyseReadsInsideRegionsForConditionPMD

*Analyse reads inside regions for condition*

---

## Description

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

## Usage

```
analyseReadsInsideRegionsForConditionPMD(
  regions,
  methylationData,
  context,
  label = "",
  parallel = FALSE,
  BPPARAM = NULL,
  cores = NULL
)
```

## Arguments

| | |
|---|---|
| regions | a GRanges object with a list of regions on the genome; e.g. could be a list of PMDs |
| methylationData | the methylation data in one condition (see ontSampleGRangesList). |
| 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 |
| parallel | Logical; run in parallel if TRUE. |
| BPPARAM | A BiocParallelParam object controlling parallel execution. This value will automatically set when parallel is TRUE, also able to set as manually. |
| cores | Integer number of workers (must not exceed BPPARAM$workers). This value will automatically set as the maximum number of system workers, also able to set as manually. |

## 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 and Young Jun Kim

## See Also

filterPMDs, computePMDs, PMDsNoiseFilterCG, and mergePMDsIteratively

## Examples

```
# load the ONT methylation data
data(ontSampleGRangesList)

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

#retrive the number of reads in CG context in GM18501
PMDsNoiseFilterCGreadsCG <- analyseReadsInsideRegionsForConditionPMD(
                            PMDsNoiseFilterCG[1:10],
                            ontSampleGRangesList[["GM18501"]], context = "CG",
                            label = "GM18501")
```

---

`computeCoMethylatedPositions` *Compute pairwise co-methylation statistics for cytosine sites within regions*

---

## Description

`computeCoMethylatedPositions()` calculates pairwise co-methylation between all cytosine sites within each given region, using ONT methylation calls annotated to each site. For each pair of cytosines within the same strand and PMD, it builds a 2x2 contingency table reflecting the overlap state of reads (both methylated, only one methylated, or neither), performs a statistical test (Fisher's exact by default), and reports FDR-adjusted p-values.

## Usage

```
computeCoMethylatedPositions(
  methylationData,
  regions,
  minDistance = 150,
  maxDistance = 1000,
  minCoverage = 4,
  pValueThreshold = 0.01,
  alternative = "two.sided",
  test = "fisher",
  parallel = FALSE,
  BPPARAM = NULL,
  cores = NULL
)
```

## Arguments

`methylationData`
: A `GRanges` object containing cytosine sites, annotated with per-site ONT methylation calls (columns `ONT_Cm`, `ONT_C`, `readsN`, etc).

| | |
|---|---|
| regions | A GRanges object with list including genomic context such as gene and/or transposable elements coordinates which possibly have DMRs, VMRs or PMDs. |
| minDistance | Minimum distance (in bp) between two cytosines to consider for co-methylation (default: 150). |
| maxDistance | Maximum distance (in bp) between two cytosines to consider (default: 1000). |
| minCoverage | Minimum read coverage required for both cytosines in a pair (default: 4). |
| pValueThreshold | FDR-adjusted p-value threshold for reporting significant co-methylation (default: 0.01). |
| alternative | indicates the alternative hypothesis and must be one of "two.sided", "greater" or "less". You can specify just the initial letter. Only used in the 2 by 2 case. This is used only for Fisher's test. |
| test | Statistical test to use for co-methylation ("fisher" for Fisher's exact [default], or "permutation" for chi-squared). NOTE: highly recommended to do parallel when use permutation test. |
| parallel | Logical; run in parallel if TRUE. |
| BPPARAM | A BiocParallelParam object controlling parallel execution. This value will automatically set when parallel is TRUE, also able to set as manually. |
| cores | Integer number of workers (must not exceed BPPARAM$workers). This value will automatically set as the maximum number of system workers, also able to set as manually. |

## Details

Compute Co-Methylation Positions within Regions (CMPs)

Pairwise tests are performed separately for each strand (+ and -) within each region. FDR correction is performed for all pairs within each region and strand.

## Value

A list of length equal to regions, where each entry is a GInteractions object of significant cytosine pairs (by strand), annotated with:

**C1_C2** number of reads methylated at both cytosines

**C1_only** number methylated at only first cytosine

**C2_only** number methylated at only second cytosine

**neither** number methylated at neither cytosines

**strand** The DNA strand ("+" or "-") on which the two CpGs reside.

**genomic_position** The original region (from regions) containing this cytosines pair, formatted in UCSC or IGV style, e.g. "chr1:1522971-1523970".

**p.value** FDR-adjusted p-value for co-methylation association

## Author(s)

Nicolae Radu Zabet and Young Jun Kim

## See Also

readONTbam, computePMDs, ontSampleGRangesList

## Examples

```
## Not run:
# load the ONT methylation data and PMD data
data(ont_gr_GM18870_chr1_PMD_bins_1k)
data(ont_gr_GM18870_chr1_sorted_bins_1k)

# compute the co-methylations with Fisher's exact test
coMetylationFisher <- computeCoMethylatedPositions(
  ont_gr_GM18870_chr1_sorted_bins_1k,
  regions = ont_gr_GM18870_chr1_PMD_bins_1k[1:4],
  minDistance = 150,
  maxDistance = 1000,
  minCoverage = 4,
  pValueThreshold = 0.01,
  test = "fisher",
  parallel = FALSE)

# compute the co-methylations with Permuation test
coMetylationPermutation <- computeCoMethylatedPositions(
  ont_gr_GM18870_chr1_sorted_bins_1k,
  regions = ont_gr_GM18870_chr1_PMD_bins_1k[1:4],
  minDistance = 150,
  maxDistance = 1000,
  minCoverage = 4,
  pValueThreshold = 0.01,
  test = "permutation",
  parallel = FALSE) # highly recommended to set as TRUE

## End(Not run)
```

---

`computeCoMethylatedRegions` *Compute pairwise co-methylation statistics between regions*

---

## Description

`computeCoMethylatedRegions()` calculates pairwise correlation statistics for methylation levels across defined genomic regions (e.g., PMDs, Enhancer binding sites). For each region pair within the specified distance range, the function computes per-read methylation proportions and performs correlation testing (Pearson, Spearman, or Kendall). Pairs with strong correlations (beyond user-defined thresholds) and significant p-values (FDR-adjusted) are returned.

## Usage

```
computeCoMethylatedRegions(
  methylationData,
  regions,
  minDistance = 500,
  maxDistance = 50000,
  minCoverage = 4,
  pValueThreshold = 0.05,
  correlation_test = "pearson",
  minCorrelation = -0.5,
  maxCorrelation = 0.5,
  parallel = FALSE,
  BPPARAM = NULL,
  cores = NULL
)
```

## Arguments

| | |
|---|---|
| methylationData | A GRanges object containing cytosine sites, annotated with ONT methylation metadata (columns ONT_Cm, ONT_C, etc.). |
| regions | A GRanges object defining genomic regions (e.g., PMDs, Enhancer binding sites) to evaluate for CMRs. |
| minDistance | Minimum genomic distance (in base pairs) between two regions to be considered (default: 500). |
| maxDistance | Maximum genomic distance (in base pairs) between two regions (default: 50,000). |
| minCoverage | Minimum number of shared reads (per region pair) required to compute correlation (default: 4). |
| pValueThreshold | Significance threshold for FDR-adjusted p-values (default: 0.05). |
| correlation_test | Statistical method to compute correlation; must be one of "pearson", "spearman", or "kendall" (default: "pearson"). |
| minCorrelation | Minimum allowed correlation value for a significant result (must be in between -1 and 0; default: -0.5). |
| maxCorrelation | Maximum allowed correlation value for a significant result (must be in between 0 and 1; default: 0.5). |
| parallel | Logical; run in parallel if TRUE. |
| BPPARAM | A BiocParallelParam object controlling parallel execution. This value will automatically set when parallel is TRUE, also able to set as manually. |
| cores | Integer number of workers (must not exceed BPPARAM$workers). This value will automatically set as the maximum number of system workers, also able to set as manually. |

## Details

Compute Co-Methylated Regions (CMRs)

The function first identifies all region pairs within the user-defined distance range. For each pair, it calculates methylation proportions per read across both regions, extracts common reads, and tests correlation using the selected method. FDR correction is applied globally across all region pairs.

## Value

A `GInteractions` object, where each row represents a significantly correlated pair of genomic regions from the input `regions`. The anchors of each interaction correspond to original regions, and their genomic coordinates are retained in the `anchor1` and `anchor2` slots.

Additionally, a `genomic_position` meta-column is included to indicate the original coordinate ranges (in UCSC/IGV format) for each interaction, aiding downstream interpretation or visualisation.

Each interaction is annotated with:

**correlation** Correlation coefficient between the two regions

**coverage** Number of shared reads used for correlation

**p.value** FDR-adjusted p-value for the correlation test

## Author(s)

Nicolae Radu Zabet and Young Jun Kim

## See Also

`readONTbam`, `computePMDs`, `ontSampleGRangesList`

## Examples

```
# Load methylation data and PMD regions
data("ont_gr_GM18870_chr1_sorted_bins_1k")
data("ont_gr_GM18870_chr1_PMD_bins_1k")

# Compute highly correlated regions (Pearson)
coMethylationRegion_pearson <- computeCoMethylatedRegions(ont_gr_GM18870_chr1_sorted_bins_1k,
                                                          ont_gr_GM18870_chr1_PMD_bins_1k[1:5],
                                                                 minDistance = 500,
                                                                 maxDistance = 50000,
                                                                 minCoverage = 4,
                                                                 pValueThreshold = 0.05,
                                                                 correlation_test = "pearson",
                                                                 minCorrelation = -0.5,
                                                                 maxCorrelation = 0.5,
                                                                 parallel = FALSE,
                                                                 BPPARAM  = NULL)
```

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,
  parallel = FALSE,
  BPPARAM = NULL,
  cores = NULL
)
```

## 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(-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.

`parallel` Logical; run in parallel if `TRUE`.

`BPPARAM` A `BiocParallelParam` object controlling parallel execution. This value will automatically set when parallel is `TRUE`, also able to set as manually.

`cores` Integer number of workers (must not exceed BPPARAM$workers). This value will automatically set as the maximum number of system workers, also able to set as manually.

## Value

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

**direction** a number indicating whether the region lost (-1) or gain (+1) methylation in condition 2 compared to condition 1.

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

**sumReadsM1** the number of methylated reads in condition 1.

**sumReadsN1** the total number of reads in condition 1.

**proportion1** the proportion methylated reads in condition 1.

**sumReadsM2** the number of methylated reads in condition 2.

**sumReadsN2** the total number reads in condition 2.

**proportion2** the proportion methylated reads in condition 2.

**cytosinesCount** the number of cytosines in the DMR.

**regionType** a string indicating whether the region lost ("loss") or gained ("gain") methylation in condition 2 compared to condition 1.

**pValue** the p-value (adjusted to control the false discovery rate with the Benjamini and Hochberg's method) of the statistical test when the DMR was called.

## Author(s)

Nicolae Radu Zabet and Jonathan Michael Foonlan Tsang

## See Also

filterDMRs, mergeDMRsIteratively, analyseReadsInsideRegionsForCondition and DMRsNoiseFilterCG

## Examples

```
# 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)
```

---

`computeDMRsReplicates` *Compute DMRs*

---

## Description

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

## Usage

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

## Arguments

| | |
|---|---|
| `methylationData` | the methylation data containing all the conditions for all the replicates. |
| `condition` | a vector of strings indicating the conditions for each sample in `methylationData`. Two different values are allowed (for the two conditions). |

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

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

method — the method used to compute the DMRs "neighbourhood" or "bins"). The "neighbourhood" method computates differentially methylated cytosines. 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).

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

test — the statistical test used to call DMRs ("betareg" for Beta regression).

pseudocountM — numerical value to be added to the methylated reads before modelling beta regression.

pseudocountN — numerical value to be added to the total reads before modelling beta regression.

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

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

minProportionDifference — DMRs where the difference in methylation proportion between the two 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.

parallel — Logical; run in parallel if TRUE.

BPPARAM — A BiocParallelParam object controlling parallel execution. This value will automatically set when parallel is TRUE, also able to set as manually.

cores — Integer number of workers (must not exceed BPPARAM$workers). This value will automatically set as the maximum number of system workers, also able to set as manually.

## Value

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

**direction** a number indicating whether the region lost (-1) or gain (+1) methylation in condition 2 compared to condition 1.

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

**sumReadsM1** the number of methylated reads in condition 1.

**sumReadsN1** the total number of reads in condition 1.

**proportion1** the proportion methylated reads in condition 1.

**sumReadsM2** the number of methylated reads in condition 2.

**sumReadsN2** the total number reads in condition 2.

**proportion2** the proportion methylated reads in condition 2.

**cytosinesCount** the number of cytosines in the DMR.

**regionType** a string indicating whether the region lost ("loss") or gained ("gain") methylation in condition 2 compared to condition 1.

**pValue** the p-value (adjusted to control the false discovery rate with the Benjamini and Hochberg's method) of the statistical test when the DMR was called.

## Author(s)

Alessandro Pio Greco and Nicolae Radu Zabet

## Examples

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

# compute the DMRs in CG context with neighbourhood method

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

# computing DMRs using the neighbourhood method
DMRsReplicatesNeighbourhood <- computeDMRsReplicates(methylationData = syntheticDataReplicates,
                                                     condition = condition,
                                                     regions = NULL,
                                                     context = "CHH",
                                                     method = "neighbourhood",
                                                     test = "betareg",
                                                     pseudocountM = 1,
                                                     pseudocountN = 2,
                                                     pValueThreshold = 0.01,
                                                     minCytosinesCount = 4,
                                                     minProportionDifference = 0.4,
                                                     minGap = 200,
                                                     minSize = 50,
                                                     minReadsPerCytosine = 4,
                                                     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 computing the coverage. |
| `proportion` | a `logical` value indicating whether to compute the proportion (`TRUE`) or raw counts (`FALSE`). |

## Value

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

## Author(s)

Nicolae Radu Zabet and Jonathan Michael Foonlan Tsang

## See Also

`plotMethylationDataCoverage`, `methylationDataList`

## Examples

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

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

---

`computeMethylationDataSpatialCorrelation`
*Compute methylation data spatial correlation*

---

## Description

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

## Usage

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

## Arguments

| | |
|---|---|
| `methylationData` | the methylation data stored as a `GRanges` object with four metadata columns (see `methylationDataList`). |
| `regions` | a `GRanges` object with the regions where to compute the correlation. If `NULL`, the correlation is computed genome-wide. |
| `context` | the context in which the correlation is computed (`"CG"`, `"CHG"` or `"CHH"`). |
| `distances` | a `numeric` vector specifing the different values for the distances when computing the correlation. |

## Value

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

### Author(s)

Nicolae Radu Zabet

### See Also

plotMethylationDataSpatialCorrelation, methylationDataList

### Examples

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

# compute spatial correlation in CG context
distances <- c(1,5,10,15)
correlation_CG_wt <- computeMethylationDataSpatialCorrelation(methylationDataList[["WT"]],
                 context="CG", distances=distances)
```

---

## computeMethylationProfile

*Compute methylation profile*

---

### Description

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

### Usage

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

### Arguments

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

## Value

a GRanges object with equal sized tiles of the region. The object consists of the following metadata

**sumReadsM** the number of methylated reads.

**sumReadsN** the total number of reads.

**Proportion** the proportion of methylated reads.

**cytosinesCount** the number of cytosines in the regions

**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)
```

computePMDs *Compute PMDs*

## Description

This function computes the partially methylated domains between pre-set min and max proportion values.

## Usage

```
computePMDs(
  methylationData,
  regions = NULL,
  context = "CG",
  method = "noise_filter",
  windowSize = 100,
  kernelFunction = "triangular",
  lambda = 0.5,
  binSize = 100,
  minCytosinesCount = 4,
  minMethylation = 0.4,
  maxMethylation = 0.6,
  minGap = 200,
  minSize = 50,
  minReadsPerCytosine = 4,
  parallel = FALSE,
  BPPARAM = NULL,
  cores = NULL
)
```

## Arguments

| | |
|---|---|
| methylationData | the methylation data in condition (see ontSampleGRangesList). |
| regions | a GRanges object with the regions where to compute the PMDs. If NULL, the PMDs are computed genome-wide. |
| context | the context in which the PMDs are computed ("CG", "CHG" or "CHH"). |
| method | Character string specifying the algorithm for PMD detection. If "noise_filter", a sliding window of size windowSize is applied with the specified kernelFunction (and lambda for a Gaussian kernel) to smooth methylation counts before calling and merging PMDs. If "neighbourhood", individual partially methylated cytosines are identified first and then merged into PMDs. If "bins", the genome is partitioned into fixed bins of size binSize, partially methylation is sorted per bin, and significant bins are merged. |
| windowSize | the size of the triangle base measured in nucleotides. This parameter is required only if the selected method is "noise_filter". |

kernelFunction a character indicating which kernel function to be used. Can be one of "uniform", "triangular", "gaussian" or "epanechnicov". This is required only if the selected method is "noise_filter".

lambda numeric value required for the Gaussian filter (K(x) = exp(-lambda*x^2)). This is required only if the selected method is "noise_filter" and the selected kernel function is "gaussian".

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

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

minMethylation Numeric [0,1]; minimum mean methylation proportion.

maxMethylation Numeric [0,1]; maximum mean methylation proportion.

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

minSize PMDs with a size smaller than minSize are discarded.

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

parallel Logical; run in parallel if TRUE.

BPPARAM A BiocParallelParam object controlling parallel execution. This value will automatically set when parallel is TRUE, also able to set as manually.

cores Integer number of workers (must not exceed BPPARAM$workers). This value will automatically set as the maximum number of system workers, also able to set as manually.

## Value

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

**context** the context in which the PMDs was computed ("CG", "CHG" or "CHH").

**sumReadsM** the number of methylated reads.

**sumReadsN** the total number of reads.

**proportion** the proportion methylated reads filtered between minMethylation and maxMethylation.

**cytosinesCount** the number of cytosines in the PMDs.

## Author(s)

Nicolae Radu Zabet, Jonathan Michael Foonlan Tsang and Young Jun Kim

## See Also

readONTbam, filterPMDs, mergePMDsIteratively, analyseReadsInsideRegionsForConditionPMD and PMDsNoiseFilterCG

## Examples

```
# load the ONT methylation data
data(ontSampleGRangesList)

# the regions where to compute the PMDs
chr1_ranges <- GRanges(seqnames = Rle("chr1"), ranges = IRanges(1E6+5E5,2E6))

# compute the PMDs in CG context with noise_filter method
PMDsNoiseFilterCG <- computePMDs(ontSampleGRangesList[["GM18501"]],
                                 regions = chr1_ranges,
                                 context = "CG",
                                 windowSize = 100,
                                 method = "noise_filter",
                                 kernelFunction = "triangular",
                                 lambda = 0.5,
                                 minCytosinesCount = 4,
                                 minMethylation = 0.4,
                                 maxMethylation = 0.6,
                                 minGap = 200,
                                 minSize = 50,
                                 minReadsPerCytosine = 4,
                                 cores = 1,
                                 parallel = FALSE)

## Not run:
# compute the PMDs in CG context with neighbourhood method
PMDsNeighbourhoodCG <- computePMDs(ontSampleGRangesList[["GM18501"]],
                                   regions = chr1_ranges,
                                   context = "CG",
                                   method = "neighbourhood"
                                   minCytosinesCount = 4,
                                   minMethylation = 0.4,
                                   maxMethylation = 0.6,
                                   minGap = 200,
                                   minSize = 50,
                                   minReadsPerCytosine = 4,
                                   cores = 1,
                                   parallel = FALSE)

# compute the PMDs in CG context with bins method
PMDsBinsCG <- computePMDs(ontSampleGRangesList[["GM18501"]],
                          regions = chr1_ranges,
                          context = "CG",
                          method = "bins",
                          binSize = 100,
                          minCytosinesCount = 4,
                          minMethylation = 0.4,
                          maxMethylation = 0.6,
                          minGap = 200,
                          minSize = 50,
                          minReadsPerCytosine = 4,
                          cores = 1,
```

```
                          parallel = FALSE)

## End(Not run)
```

---

computeVMDs *Compute VMDs*

---

## Description

This function computes the variance methylated domains between pre-set min and max proportion values.

## Usage

```
computeVMDs(
  methylationData,
  regions = NULL,
  context = "CG",
  binSize = 100,
  minCytosinesCount = 4,
  sdCutoffMethod = "per.high",
  percentage = 0.05,
  minGap = 200,
  minSize = 50,
  minReadsPerCytosine = 4,
  parallel = FALSE,
  BPPARAM = NULL,
  cores = NULL
)
```

## Arguments

| | |
|---|---|
| methylationData | the methylation data in condition (see ontSampleGRangesList). |
| regions | a GRanges object with the regions where to compute the VMDs. If NULL, the VMDs are computed genome-wide. |
| context | the context in which the VMDs are computed ("CG", "CHG" or "CHH"). |
| binSize | the size of the tiling bins in nucleotides. This parameter is required only if the selected method is "bins". |
| minCytosinesCount | VMDs with less cytosines in the specified context than minCytosinesCount will be discarded. |
| sdCutoffMethod | Character string specifying how to determine the cutoff for filtering VMDs based on their methylation variance (standard deviation). Available options are: |
| | "per.high" Selects the top percentage of regions with the highest variance (standard deviation). |

"per.low" Selects the bottom percentage of regions with the lowest variance.

"EDE.high" Uses the elbow point (inflection/knee) from the descendingly sorted variance values to determine a data-driven high-variance cutoff. Retains regions with SD above this elbow point.

"EDE.low" Uses the elbow point from the ascendingly sorted variance values to define a low-variance cutoff. Retains regions with SD below this point.

This allows either quantile-based filtering or automatic detection of variance thresholds based on distribution shape.

percentage Numeric cutoff used when sdCutoffMethod is set to "per.high" or "per.low". Represents the quantile threshold: for example, percentage = 0.05 keeps the top 5% or bottom 5% of bins based on standard deviation, depending on the selected method.

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

minSize VMDs with a size smaller than minSize are discarded.

minReadsPerCytosine

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

parallel Logical; run in parallel if TRUE.

BPPARAM A BiocParallelParam object controlling parallel execution. This value will automatically set when parallel is TRUE, also able to set as manually.

cores Integer number of workers (must not exceed BPPARAM$workers). This value will automatically set as the maximum number of system workers, also able to set as manually.

## Value

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

**context** the context in which the VMDs was computed ("CG", "CHG" or "CHH").

**sumReadsM** the number of methylated reads.

**sumReadsN** the total number of reads.

**proportion** the proportion from total methylated reads.

**cytosinesCount** the number of cytosines in the VMDs.

**mean** mean value comparing per-read proportions

**sd** standard deviation comparing per-read proportions

**w_mean** weighted mean value comparing per-read proportions

**w_sd** weighted standard deviation comparing per-read proportions

## Author(s)

Nicolae Radu Zabet, Jonathan Michael Foonlan Tsang and Young Jun Kim

## See Also

readONTbam, filterVMDs and analyseReadsInsideRegionsForCondition

## Examples

```
## Not run:
# load the ONT methylation data
data(ontSampleGRangesList)

# the regions where to compute the VMDs
chr1_ranges <- GRanges(seqnames = Rle("chr1"), ranges = IRanges(1E6+5E5,1E6+6E5))

# compute the VMDs in CG context with bins method
VMDsBinsCG <- computeVMDs(ontSampleGRangesList[["GM18501"]],
                          regions = NULL,
                          context = "CG",
                          binSize = 100,
                          minCytosinesCount = 4,
                          sdCutoffMethod = "EDE.high",
                          percentage = 0.05,
                          minGap = 200,
                          minSize = 50,
                          minReadsPerCytosine = 4,
                          parallel = FALSE,
                          BPPARAM = NULL,
                          cores = 1)

## End(Not run)
```

---

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

---

## Description

Supports both Bisulfite Sequencing (Bismark CX reports) and Oxford Nanopore Sequencing (MM/ML tags) for per-site methylation calling. Identifies differentially methylated regions between two samples in CG and non-CG contexts.

## Details

For bisulfite data, the input is Bismark CX report files and the output is a list of DMRs stored as a `GRanges` object.

- `readsM` — count of modified reads per site
- `readsN` — total same-strand coverage per site

For Nanopore data, the input is an indexed ONT BAM with calling `readONTbam` function in this package and the output is a `GRanges` augmented with metadata columns:

- `ONT_Cm` — comma-delimited read-indices called "modified"
- `ONT_C` — comma-delimited read-indices covering but not modified
- `readsM` — count of modified reads per site

- readsN — total same-strand coverage per site

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.

selectCytosine Enumerates cytosine positions in a BSgenome reference, optionally filtering by methylation context (CG/CHG/CHH), chromosome and genomic region.

readONTbam Loads an Oxford Nanopore BAM (with MM/ML tags), decodes per-C modification probabilities and counts modified vs. unmodified reads per site.

poolMethylationDatasets pools together multiple methylation datasets.

poolTwoMethylationDatasets pools together two methylation datasets.

computeMethylationDataCoverage Computes the coverage for the bisulfite sequencing data.

plotMethylationDataCoverage Plots the coverage for the bisulfite sequencing data.

computeMethylationDataSpatialCorrelation Computes the correlation between methylation levels as a function of the distances between the Cytosines.

plotMethylationDataSpatialCorrelation Plots the correlation of methylation levels for Cytosines located at a certain distance apart.

computeMethylationProfile Computes the low resolution profiles for the bisulfite sequencing data at certain locations.

plotMethylationProfile Plots the low resolution profiles for the bisulfite sequencing data at certain locations.

plotMethylationProfileFromData Plots the low resolution profiles for the loaded bisulfite sequencing data.

computeDMRs Computes the differentially methylated regions between two conditions.

filterDMRs Filters a list of (potential) differentially methylated regions.

mergeDMRsIteratively Merge DMRs iteratively.

analyseReadsInsideRegionsForCondition Analyse reads inside regions for condition.

plotLocalMethylationProfile Plots the methylation profile at one locus for the bisulfite sequencing data.

computeOverlapProfile Computes the distribution of a set of subregions on a large region.

plotOverlapProfile Plots the distribution of a set of subregions on a large region.

getWholeChromosomes Computes the GRanges objects with each chromosome as an element from the methylationData.

joinReplicates Merges two GRanges objects with single reads columns. It is necessary to start the analysis of DMRs with biological replicates.

computeDMRsReplicates Computes the differentially methylated regions between two conditions with multiple biological replicates.

selectCytosine Enumerates cytosine positions in a BSgenome reference.

readONTbam Loads an ONT BAM (MM/ML tags), decodes per-C modification probabilities, and counts modified vs. unmodified reads per site.

computePMDs Partitions the genome into PMDs via three methods ("noise_filter", "neighbourhood", "bins").

filterPMDs Filters a set of PMDs by methylation level and read depth.

mergePMDsIteratively Merge PMDs while preserving statistical significance.

analyseReadsInsideRegionsForConditionPMD Counts reads in each PMD for one condition.

computeVMDs Computes the variance methylated domains between pre-set min and max proportion values

filterVMRsONT Filters VMRs with ONT-specific variance tests and CI filters

computeCoMethylatedPositions Computes pairwise co-methylation between Cytosine sites within regions.

computeCoMethylatedRegions Computes pairwise co-methylation statistics between regions.

## Author(s)

Nicolae Radu Zabet <r.zabet@qmul.ac.uk>, Jonathan Michael Foonlan Tsang <jmft2@cam.ac.uk>, Alessandro Pio Greco <apgrec@essex.ac.uk>, Young Jun Kim <qc25039@qmul.ac.uk>

Maintainer: Nicolae Radu Zabet <r.zabet@qmul.ac.uk>

## See Also

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

## Examples

```
## Not run:
# load the methylation data
data(methylationDataList)
library(BSgenome.Hsapiens.UCSC.hg38)

# All cytosines in hg38:
gr_all <- selectCytosine()

# Only CpG sites on chr1 and chr2:
gr_chr1_2 <- selectCytosine(context="CG", chr=c("chr1","chr2"))

# CHH sites in a specific region on chr3:
my_region <- GRanges("chr3", IRanges(1e6, 1e6 + 1e5))
gr_region <- selectCytosine(context="CHH", chr="chr3", region=my_region)

# set the bam file directory
bam_path <- system.file("extdata", "scanBamChr1Random5.bam", package="DMRcaller")

# read ONTbam file (chromosome 1 only) in CG context with BSgenome.Hsapiens.UCSC.hg38
ONTSampleGRanges <- readONTbam(bamfile = bam_path, ref_gr = NULL, modif = "C+m?",
                          prob_thresh = 0.50,genome = BSgenome.Hsapiens.UCSC.hg38,
                          context = "CG", chr = "chr1", region = NULL,
                          synonymous = FALSE, parallel = FALSE, BPPARAM = NULL)

# 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)
```

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

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

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

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

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

# load the gene annotation data
data(GEs)

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

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

# filter genes that are differntially methylated in the two conditions
DMRsGenesCG <- filterDMRs(methylationDataList[["WT"]],
                methylationDataList[["met1-3"]], potentialDMRs = genes,
                context = "CG", test = "score", pValueThreshold = 0.01,
```

```
                minCytosinesCount = 4, minProportionDifference = 0.4,
                minReadsPerCytosine = 3, cores = 1)

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

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

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

# load the DMRs in CG context
data(DMRsNoiseFilterCG)

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


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


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

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

plotOverlapProfile(GRangesList("Chr3"=hotspotsHypo),
                   GRangesList("Chr3"=hotspotsHyper),
                   names=c("loss", "gain"), title="CG methylation")
# loading synthetic data
data("syntheticDataReplicates")

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

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

# the regions where to compute the PMDs
chr1_ranges <- GRanges(seqnames = Rle("chr1"), ranges = IRanges(1E6+5E5,2E6))

# compute the PMDs in CG context with noise_filter method
PMDsNoiseFilterCG <- computePMDs(ontSampleGRangesList[["GM18501"]],
                                 regions = chr1_ranges,
                                 context = "CG",
                                 windowSize = 100,
                                 method = "noise_filter",
                                 kernelFunction = "triangular",
                                 lambda = 0.5,
                                 minCytosinesCount = 4,
                                 minMethylation = 0.4,
                                 maxMethylation = 0.6,
                                 minGap = 200,
                                 minSize = 50,
                                 minReadsPerCytosine = 4,
                                 cores = 1,
                                 parallel = FALSE)

# compute the PMDs in CG context with neighbourhood method
PMDsNeighbourhoodCG <- computePMDs(ontSampleGRangesList[["GM18501"]],
                                   regions = chr1_ranges,
                                   context = "CG",
                                   method = "neighbourhood"
                                   minCytosinesCount = 4,
                                   minMethylation = 0.4,
                                   maxMethylation = 0.6,
                                   minGap = 200,
                                   minSize = 50,
                                   minReadsPerCytosine = 4,
                                   cores = 1,
                                   parallel = FALSE)
```

```
# compute the PMDs in CG context with bins method
PMDsBinsCG <- computePMDs(ontSampleGRangesList[["GM18501"]],
                          regions = chr1_ranges,
                          context = "CG",
                          method = "bins",
                          binSize = 100,
                          minCytosinesCount = 4,
                          minMethylation = 0.4,
                          maxMethylation = 0.6,
                          minGap = 200,
                          minSize = 50,
                          minReadsPerCytosine = 4,
                          cores = 1,
                          parallel = FALSE)

# load the gene annotation data
data(GEs_hg38)

# select the transcript
transcript <- GEs_hg38[which(GEs_hg38$type == "transcript")]

# the regions where to compute the PMDs
regions <- GRanges(seqnames = Rle("chr1"), ranges = IRanges(1E6+5E5,2E6))
transcript <- transcript[overlapsAny(transcript, regions)]

# filter genes that are partially methylated in the two conditions
PMDsGenesCG <- filterPMDs(ontSampleGRangesList[["GM18501"]],
               potentialPMDs = transcript,
               context = "CG", minMethylation = 0.4, maxMethylation = 0.6,
               minCytosinesCount = 4, minReadsPerCytosine = 3, cores = 1)

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


# merge the PMDs
PMDsNoiseFilterCGLarger <- mergePMDsIteratively(PMDsNoiseFilterCG[1:100],
                           minGap = 500, respectSigns = TRUE,
                           ontSampleGRangesList[["GM18501"]], context = "CG",
                           minReadsPerCytosine = 4, minMethylation = 0.4,
                           maxMethylation = 0.6, cores = 1)

# set genomic coordinates where to compute PMDs
chr1_ranges <- GRanges(seqnames = Rle("chr1"), ranges = IRanges(1E6+5E5,2E6))

# compute PMDs and remove gaps smaller than 200 bp
PMDsNoiseFilterCG200 <- computePMDs(ontSampleGRangesList[["GM18501"]],
                        regions = chr1_ranges, context = "CG", method = "noise_filter",
                        windowSize = 100, kernelFunction = "triangular",
                        minCytosinesCount = 1, minMethylation = 0.4,
                        maxMethylation = 0.6, minGap = 0, minSize = 200,
                        minReadsPerCytosine = 1, cores = 1)
PMDsNoiseFilterCG0 <- computePMDs(ontSampleGRangesList[["GM18501"]],
```

```
                        regions = chr1_ranges, context = "CG", method = "noise_filter",
                        windowSize = 100, kernelFunction = "triangular",
                        minCytosinesCount = 1, minMethylation = 0.4,
                        maxMethylation = 0.6, minGap = 0, minSize = 0,
                        minReadsPerCytosine = 1, cores = 1)
PMDsNoiseFilterCG0Merged200 <- mergePMDsIteratively(PMDsNoiseFilterCG0,
                             minGap = 200, respectSigns = TRUE,
                             ontSampleGRangesList[["GM18501"]], context = "CG",
                             minReadsPerCytosine = 4, minMethylation = 0.4,
                             maxMethylation = 0.6, cores = 1)

#check that all newley computed PMDs are identical
print(all(PMDsNoiseFilterCG200 == PMDsNoiseFilterCG0Merged200))

#retrive the number of reads in CG context in GM18501
PMDsNoiseFilterCGreadsCG <- analyseReadsInsideRegionsForConditionPMD(
                             PMDsNoiseFilterCG[1:10],
                             ontSampleGRangesList[["GM18501"]], context = "CG",
                             label = "GM18501")

# load the PMD data
data(PMDsBinsCG)

# compute the co-methylations with Fisher's exact test
coMetylationFisher <- computeCoMethylatedPositions(
  ontSampleGRangesList[[1]],
  regions = PMDsBinsCG,
  minDistance = 150,
  maxDistance = 1000,
  minCoverage = 4,
  pValueThreshold = 0.01,
  test = "fisher",
  parallel = FALSE)

# compute the co-methylations with Permuation test
coMetylationPermutation <- computeCoMethylatedPositions(
  ontSampleGRangesList[[1]],
  regions = PMDsBinsCG,
  minDistance = 150,
  maxDistance = 1000,
  minCoverage = 4,
  pValueThreshold = 0.01,
  test = "permutation",
  parallel = FALSE) # highly recommended to set as TRUE

# select the transcript
transcript <- GEs_hg38[which(GEs_hg38$type == "transcript")]

# the regions where to compute the PMDs
regions <- GRanges(seqnames = Rle("chr1"), ranges = IRanges(1E6+5E5,2E6))
transcript <- transcript[overlapsAny(transcript, regions)]

# filter genes that are differntially methylated in the two conditions
```

```
VMRsGenesCG <- filterVMRsONT(ontSampleGRangesList[["GM18501"]],
               ontSampleGRangesList[["GM18876"]], potentialVMRs = transcript,
               context = "CG", pValueThreshold = 0.01,
               minCytosinesCount = 4, minProportionDifference = 0.01,
               minReadsPerCytosine = 3, ciExcludesOne = TRUE,
               varRatioFc = NULL, parallel = TRUE) # parallel recommended


## 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

---

## extractGC *Extract GC*

---

### Description

This function extracts GC sites in the genome

### Usage

```
extractGC(methylationData, genome, contexts = c("ALL", "CG", "CHG", "CHH"))
```

### Arguments

| | |
|---|---|
| methylationData | the methylation data stored as a GRanges object with four metadata columns (see methylationDataList). |
| genome | a BSgenome with the DNA sequence of the organism |
| contexts | the context in which the DMRs are computed ("ALL", "CG", "CHG" or "CHH"). |

## Value

the a subset of `methylationData` consisting of all GC sites.

## Author(s)

Ryan Merritt

## Examples

```
## Not run:
# load the genome sequence
if(!require("BSgenome.Athaliana.TAIR.TAIR9", character.only = TRUE)){
  if (!requireNamespace("BiocManager", quietly=TRUE))
  install.packages("BiocManager")
  BiocManager::install("BSgenome.Athaliana.TAIR.TAIR9")
}
library(BSgenome.Athaliana.TAIR.TAIR9)

# load the methylation data
data(methylationDataList)

methylationDataWTGpCpG <- extractGC(methylationDataList[["WT"]],
                                    BSgenome.Athaliana.TAIR.TAIR9,
                                    "CG")


## End(Not run)
```

---

`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,
  parallel = FALSE,
  BPPARAM = NULL,
  cores = NULL
)
```

## 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 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. |
| parallel | Logical; run in parallel if TRUE. |
| BPPARAM | A BiocParallelParam object controlling parallel execution. This value will automatically set when parallel is TRUE, also able to set as manually. |
| cores | Integer number of workers (must not exceed BPPARAM$workers). This value will automatically set as the maximum number of system workers, also able to set as manually. |

## Value

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

## Author(s)

Nicolae Radu Zabet

## See Also

DMRsNoiseFilterCG, computeDMRs, analyseReadsInsideRegionsForCondition and mergeDMRsIteratively

## Examples

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

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

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

# filter genes that are differntially 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)
```

---

`filterPMDs` *Filter PMDs*

---

## Description

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

## Usage

```
filterPMDs(
  methylationData,
  potentialPMDs,
  context = "CG",
  minCytosinesCount = 4,
  minMethylation = 0.4,
  maxMethylation = 0.6,
  minReadsPerCytosine = 3,
  parallel = FALSE,
  BPPARAM = NULL,
  cores = NULL
)
```

## Arguments

`methylationData`
: the methylation data in condition (see `ontSampleGRangesList`).

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

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

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

minMethylation — Numeric [0,1]; minimum mean methylation proportion.

maxMethylation — Numeric [0,1]; maximum mean methylation proportion.

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

parallel — Logical; run in parallel if TRUE.

BPPARAM — A BiocParallelParam object controlling parallel execution. This value will automatically set when parallel is TRUE, also able to set as manually.

cores — Integer number of workers (must not exceed BPPARAM$workers). This value will automatically set as the maximum number of system workers, also able to set as manually.

## Value

a GRanges object with 5 metadata columns that contain the PMDs; see computePMDs.

## Author(s)

Nicolae Radu Zabet and Young Jun Kim

## See Also

PMDsNoiseFilterCG, computePMDs, analyseReadsInsideRegionsForCondition and mergePMDsIteratively

## Examples

```
# load the ONT methylation data
data(ontSampleGRangesList)
# load the gene annotation data
data(GEs_hg38)

# select the transcript
transcript <- GEs_hg38[which(GEs_hg38$type == "transcript")]

# the regions where to compute the PMDs
regions <- GRanges(seqnames = Rle("chr1"), ranges = IRanges(1E6+5E5,2E6))
transcript <- transcript[overlapsAny(transcript, regions)]

# filter genes that are partially methylated in the two conditions
PMDsGenesCG <- filterPMDs(ontSampleGRangesList[["GM18501"]],
               potentialPMDs = transcript,
               context = "CG", minMethylation = 0.4, maxMethylation = 0.6,
               minCytosinesCount = 4, minReadsPerCytosine = 3, cores = 1)
```

`filterVMDs` *Filter VMDs*

## Description

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

## Usage

```
filterVMDs(
  methylationData,
  potentialVMDs,
  context = "CG",
  minCytosinesCount = 4,
  minReadsPerCytosine = 3,
  sdCutoffMethod = "per.high",
  percentage = 0.05,
  parallel = FALSE,
  BPPARAM = NULL,
  cores = NULL
)
```

## Arguments

`methylationData`
: the methylation data in condition (see `ontSampleGRangesList`).

`potentialVMDs`
: a `GRanges` object with potential VMDs where to compute the VMDs. This can be a a list of gene and/or transposable elements coordinates.

`context`
: the context in which the VMDs are computed (`"CG"`, `"CHG"` or `"CHH"`).

`minCytosinesCount`
: VMDs with less cytosines in the specified context than `minCytosinesCount` will be discarded.

`minReadsPerCytosine`
: VMDs with the average number of reads lower than `minReadsPerCytosine` are discarded.

`sdCutoffMethod`
: Character string specifying how to determine the cutoff for filtering VMDs based on their methylation variance (weighted standard deviation). Available options are:

  `"per.high"` Selects the top `percentage` of regions with the highest variance (standard deviation).

  `"per.low"` Selects the bottom `percentage` of regions with the lowest variance.

  `"EDE.high"` Uses the elbow point (inflection/knee) from the descendingly sorted variance values to determine a data-driven high-variance cutoff. Retains regions with SD above this elbow point.

"EDE.low" Uses the elbow point from the ascendingly sorted variance values to define a low-variance cutoff. Retains regions with SD below this point.

This allows either quantile-based filtering or automatic detection of variance thresholds based on distribution shape.

percentage — Numeric cutoff used when sdCutoffMethod is set to "per.high" or "per.low". Represents the quantile threshold: for example, percentage = 0.05 keeps the top 5% or bottom 5% of bins based on weighted standard deviation, depending on the selected method.

parallel — Logical; run in parallel if TRUE.

BPPARAM — A BiocParallelParam object controlling parallel execution. This value will automatically set when parallel is TRUE, also able to set as manually.

cores — Integer number of workers (must not exceed BPPARAM$workers). This value will automatically set as the maximum number of system workers, also able to set as manually.

## Value

a GRanges object with 9 metadata columns that contain the VMDs; see computeVMDs.

## Author(s)

Nicolae Radu Zabet and Young Jun Kim

## See Also

computeVMDs and analyseReadsInsideRegionsForCondition

## Examples

```
# load the ONT methylation data
data(ontSampleGRangesList)
# load the gene annotation data
data(GEs_hg38)

# select the transcript
transcript <- GEs_hg38[which(GEs_hg38$type == "transcript")]

# the regions where to compute the VMDs
regions <- GRanges(seqnames = Rle("chr1"), ranges = IRanges(1E6+5E5,2E6))
transcript <- transcript[overlapsAny(transcript, regions)]

# filter genes that are variance methylated in the two conditions
VMDsGenesCG <- filterVMDs(ontSampleGRangesList[["GM18501"]],
               potentialVMDs = transcript,
               context = "CG", sdCutoffMethod = "per.high", percentage = 0.05,
               minCytosinesCount = 4, minReadsPerCytosine = 3, cores = 1)
```

`filterVMRsONT` *Filter VMRs for ONT Data*

## Description

Filter VMRs with ONT-specific variance tests and CI filters

## Usage

```
filterVMRsONT(
  methylationData1,
  methylationData2,
  potentialVMRs,
  context = "CG",
  pValueThreshold = 0.01,
  minCytosinesCount = 4,
  minProportionDifference = 0.4,
  minReadsPerCytosine = 3,
  ciExcludesOne = TRUE,
  varRatioFc = NULL,
  parallel = FALSE,
  BPPARAM = NULL,
  cores = NULL
)
```

## Arguments

| | |
|---|---|
| `methylationData1` | A `GRanges` of methylation calls for condition 1 (see `ontSampleGRangesList`). |
| `methylationData2` | A `GRanges` of methylation calls for condition 2. |
| `potentialVMRs` | A `GRanges` of candidate VMR regions (genes, TEs, CpG islands, etc.). |
| `context` | Character string specifying cytosine context ("CG", "CHG", or "CHH"). |
| `pValueThreshold` | Numeric p-value threshold (0<value<1) for both Wilcoxon and F-tests after FDR adjustment. |
| `minCytosinesCount` | Integer minimum number of cytosines per region. |
| `minProportionDifference` | Numeric minimum methylation difference between conditions (0<value<1). |
| `minReadsPerCytosine` | Integer minimum average coverage per cytosine. |
| `ciExcludesOne` | Logical; if `TRUE`, filter out regions whose F-test 95% confidence interval spans 1 (i.e., no significant variance change). |

| | |
|---|---|
| varRatioFc | Optional; numeric fold-change cutoff on variance ratio (e.g., 2 for twofold variance difference). Regions with variance ratio outside [1/varRatioFc, varRatioFc] are kept when set. |
| parallel | Logical; run in parallel if TRUE. |
| BPPARAM | A BiocParallelParam object controlling parallel execution. This value will automatically set when parallel is TRUE, also able to set as manually. |
| cores | Integer number of workers (must not exceed BPPARAM$workers). This value will automatically set as the maximum number of system workers, also able to set as manually. |

## Details

This function verifies whether a set of potential VMRs (e.g., genes, transposons, CpG islands) are differentially methylated or not in ONT data, adding per-read Wilcoxon and F-tests on per-site proportions, confidence interval filtering, and optional variance-fold change cutoffs.

For each potential VMR, per-site methylation proportions are aggregated per read, then a two-sample Wilcoxon rank-sum test compares means (wilcox_pvalue), and an F-test compares variances (f_pvalue). You may further filter by requiring the 95 apply a fold-change cutoff on the variance ratio (varRatioFc).

## Value

A GRanges with the same ranges as regions, plus these metadata:

**sumReadsM1** total methylated reads in condition 1

**sumReadsN1** total reads in condition 1

**proportion1** methylation proportion (sumReadsM1/sumReadsN1)

**variance1** variance of per-read methylation proportions in condition 1

**sumReadsM2** total methylated reads in condition 2

**sumReadsN2** total reads in condition 2

**proportion2** methylation proportion (sumReadsM2/sumReadsN2)

**variance2** variance of per-read methylation proportions in condition 2

**cytosinesCount** number of cytosines observed in each region

**wilcox_pvalue** FDR adjusted p-value from Wilcoxon rank-sum test comparing per-read proportions

**f_pvalue** FDR adjusted p-value from F-test comparing variances of per-read proportions

**var_ratio** Ratio of variances (variance1 / variance2)

**wilcox_result** Full htest object returned by wilcox.test

**F_test_result** Full htest object returned by var.test

**direction** a number indicating whether the region lost (-1) or gain (+1) methylation in condition 2 compared to condition 1.

**regionType** a string indicating whether the region lost ("loss") or gained ("gain") methylation in condition 2 compared to condition 1.

**is_DMR** logical; TRUE if region passed the wilcox.test

**is_VMR** logical; TRUE if region passed the var.test

## Author(s)

Nicolae Radu Zabet and Young Jun Kim

## See Also

readONTbam, computePMDs, computeCoMethylatedPositions, ontSampleGRangesList, GEs_hg38

## Examples

```
## Not run:
# load the ONT methylation data
data(ontSampleGRangesList)
# load the gene annotation data
data(GEs_hg38)

# select the transcript
transcript <- GEs_hg38[which(GEs_hg38$type == "transcript")]

# the regions where to compute the PMDs
regions <- GRanges(seqnames = Rle("chr1"), ranges = IRanges(1E6+5E5,2E6))
transcript <- transcript[overlapsAny(transcript, regions)]

# filter genes that are differntially methylated in the two conditions
VMRsGenesCG <- filterVMRsONT(ontSampleGRangesList[["GM18501"]],
               ontSampleGRangesList[["GM18876"]], potentialVMRs = transcript,
               context = "CG", pValueThreshold = 0.01,
               minCytosinesCount = 4, minProportionDifference = 0.01,
               minReadsPerCytosine = 3, ciExcludesOne = TRUE,
               varRatioFc = NULL, parallel = TRUE) # parallel recommended

## End(Not run)
```

---

# 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

## GEs_hg38 *The genetic elements data of GRCh38 Genome Reference*

### Description

A GRanges object containing the annotation of the Homo sapiens (hg38)

### Format

A GRanges object

### Source

The object was created by loading the gtf file from https://hgdownload.soe.ucsc.edu/goldenPath/hg38/bigZips/genes/hg38.refGene.gtf.gz. and concatenated by range in 1.5 ~ 2 Mbp from Chromosome 1

## getWholeChromosomes *Get whole chromosomes from methylation data*

### Description

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

### Usage

```
getWholeChromosomes(methylationData)
```

### Arguments

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

### Value

a GRanges object will all chromosomes.

### Author(s)

Nicolae Radu Zabet

## Examples

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

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

---

`joinReplicates` *Joins together two GRange objects in a single containing all the replicates*

---

## Description

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

## Usage

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

## Arguments

| | |
|---|---|
| `methylationData1` | the methylation data stored as a `GRanges` object with four metadata columns (see `methylationDataList`). |
| `methylationData2` | the methylation data stored as a `GRanges` object with four metadata columns (see `methylationDataList`). |
| `usecomplete` | Boolean, determine wheter, when the two dataset differ for number of cytosines, if the smaller dataset should be added with zero reads to match the bigger dataset. |

## Value

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

## Author(s)

Alessandro Pio Greco and Nicolae Radu Zabet

## Examples

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

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

---

`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",
  parallel = FALSE,
  BPPARAM = NULL,
  cores = NULL
)
```

## Arguments

| | |
|---|---|
| `DMRs` | the list of DMRs as a `GRanges` object; e.g. see `computeDMRs` |
| `minGap` | DMRs separated by a gap of at least `minGap` are not merged. |
| `respectSigns` | logical value indicating whether to respect the sign when joining DMRs. |
| `methylationData1` | the methylation data in condition 1 (see `methylationDataList`). |
| `methylationData2` | the methylation data in condition 2 (see `methylationDataList`). |

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

`minProportionDifference`
two adjacent DMRs are merged only if the difference in methylation proportion of the new DMR is higher than `minProportionDifference`.

`minReadsPerCytosine`
two adjacent DMRs are merged only if the number of reads per cytosine of the new DMR is higher than `minReadsPerCytosine`.

`pValueThreshold`
two adjacent DMRs are merged only if the p-value of the new DMR (see `test` below) is lower than `pValueThreshold`. Note that we adjust the p-values using the Benjamini and Hochberg's method to control the false discovery rate.

`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"`.

`parallel` Logical; run in parallel if `TRUE`.

`BPPARAM` A `BiocParallelParam` object controlling parallel execution. This value will automatically set when parallel is `TRUE`, also able to set as manually.

`cores` Integer number of workers (must not exceed BPPARAM$workers). This value will automatically set as the maximum number of system workers, also able to set as manually.

## Value

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

## Author(s)

Nicolae Radu Zabet

## See Also

`filterDMRs`, `computeDMRs`, `analyseReadsInsideRegionsForCondition` and `DMRsNoiseFilterCG`

## Examples

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

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


#merge the DMRs
DMRsNoiseFilterCGLarger <- mergeDMRsIteratively(DMRsNoiseFilterCG[1:100],
                           minGap = 500, respectSigns = TRUE,
                           methylationDataList[["WT"]],
                           methylationDataList[["met1-3"]],
```

```
                            context = "CG", minProportionDifference=0.4,
                            minReadsPerCytosine = 1, pValueThreshold=0.01,
                            test="score",alternative = "two.sided")


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

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

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)
```

---

`mergePMDsIteratively` *Merge PMDs iteratively*

---

## Description

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

## Usage

```
mergePMDsIteratively(
  PMDs,
  minGap = 200,
  respectSigns = TRUE,
  methylationData,
  context = "CG",
  minReadsPerCytosine = 4,
  minMethylation = 0.4,
  maxMethylation = 0.6,
  parallel = FALSE,
  BPPARAM = NULL,
  cores = NULL
)
```

## Arguments

| | |
|---|---|
| PMDs | the list of PMDs as a GRanges object; e.g. see computePMDs |
| minGap | PMDs separated by a gap of at least minGap are not merged. |
| respectSigns | logical value indicating whether to respect the sign when joining PMDs. |
| methylationData | the methylation data in GRanges (see ontSampleGRangesList). |
| context | the context in which the PMDs are computed ("CG", "CHG" or "CHH"). |
| minReadsPerCytosine | two adjacent PMDs are merged only if the number of reads per cytosine of the new DMR is higher than minReadsPerCytosine. |
| minMethylation | Numeric [0,1]; minimum mean methylation proportion. |
| maxMethylation | Numeric [0,1]; maximum mean methylation proportion. |
| parallel | Logical; run in parallel if TRUE. |
| BPPARAM | A BiocParallelParam object controlling parallel execution. This value will automatically set when parallel is TRUE, also able to set as manually. |
| cores | Integer number of workers (must not exceed BPPARAM$workers). This value will automatically set as the maximum number of system workers, also able to set as manually. |

## Value

the reduced list of PMDs as a GRanges object; e.g. see computePMDs

## Author(s)

Nicolae Radu Zabet and Young Jun Kim

## See Also

filterPMDs, computePMDs, analyseReadsInsideRegionsForCondition and PMDsNoiseFilterCG

## Examples

```
# load the ONT methylation data
data(ontSampleGRangesList)

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


# merge the PMDs
PMDsNoiseFilterCGLarger <- mergePMDsIteratively(PMDsNoiseFilterCG[1:100],
                           minGap = 500, respectSigns = TRUE,
                           ontSampleGRangesList[["GM18501"]], context = "CG",
                           minReadsPerCytosine = 4, minMethylation = 0.4,
                           maxMethylation = 0.6, cores = 1)

## Not run:
# set genomic coordinates where to compute PMDs
chr1_ranges <- GRanges(seqnames = Rle("chr1"), ranges = IRanges(1E6+5E5,2E6))

# compute PMDs and remove gaps smaller than 200 bp
PMDsNoiseFilterCG200 <- computePMDs(ontSampleGRangesList[["GM18501"]],
                        regions = chr1_ranges, context = "CG", method = "noise_filter",
                        windowSize = 100, kernelFunction = "triangular",
                        minCytosinesCount = 1, minMethylation = 0.4,
                        maxMethylation = 0.6, minGap = 0, minSize = 200,
                        minReadsPerCytosine = 1, cores = 1)
PMDsNoiseFilterCG0 <- computePMDs(ontSampleGRangesList[["GM18501"]],
                        regions = chr1_ranges, context = "CG", method = "noise_filter",
                        windowSize = 100, kernelFunction = "triangular",
                        minCytosinesCount = 1, minMethylation = 0.4,
                        maxMethylation = 0.6, minGap = 0, minSize = 0,
                        minReadsPerCytosine = 1, cores = 1)
PMDsNoiseFilterCG0Merged200 <- mergePMDsIteratively(PMDsNoiseFilterCG0,
                               minGap = 200, respectSigns = TRUE,
                               ontSampleGRangesList[["GM18501"]], context = "CG",
                               minReadsPerCytosine = 4, minMethylation = 0.4,
                               maxMethylation = 0.6, cores = 1)

#check that all newley computed PMDs are identical
print(all(PMDsNoiseFilterCG200 == PMDsNoiseFilterCG0Merged200))


## 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

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).

---

# ontSampleGRangesList *The ONT methylation data list*

---

## Description

A GRangesList object containing the methylation data at each cytosine location in the genome in GM18501 and GM18876 B-Lymphocyte cell lines in Homo sapiens. The data only contains the 1.5 ~ 2 Mbp from Chromosome 1.

## Format

The GRanges elements contain six metadata columns

**context** the context in which the DMRs are computed "CG".

**trinucleotide_context** the specific context of the cytosine (H is replaced by the actual nucleotide).

**ONT_Cm** comma-delimited read-indices called modified

**ONT_C** comma-delimited read-indices covering but unmodified

**readsM** the number of methylated reads.

**readsN** the total number of reads.

## Source

Each element was created by calling bam files with readONTbam function which in DMRcaller package. The sample pod5 files were from the nanopore dataset from 1000 genome project https://pmc.ncbi.nlm.nih.gov/articles/PMC10942501/. https://s3.amazonaws.com/1000g-ont/index.html?prefix=pod5_data/GM18501_R9/ .pod5 files in the case of GM18501 and https://s3.amazonaws.com/1000g-ont/index.html?prefix=pod5_data/GM18876_R9/ .pod5 files in the case of GM18876 cell line. For base-calling and alignment, run dorado, ver.0.9.6 https://github.com/nanoporetech/dorado?tab=readme-ov-file#dna-models to generate the bam files with dna_r10.4.1_e8.2_400bps_hac@v5.2.0 as basecalling model.

---

# ont_gr_GM18870_chr1_PMD_bins_1k

*Partially Methilated Domains example*

---

## Description

Partially methylated domains called on chr1 in GM18870 cells called in 1Kb bins with computePMDs function.

## Format

The GRanges elements contain seven metadata columns:

**context** the context in which the PMDs was computed ("CG", "CHG" or "CHH").

**sumReadsM** the number of methylated reads.

**sumReadsN** the total number of reads.

**proportion** the proportion methylated reads filtered between minMethylation and maxMethylation.

**cytosinesCount** the number of cytosines in the PMDs.

## Source

data from https://genome.cshlp.org/content/34/11/2061.

---

# ont_gr_GM18870_chr1_sorted_bins_1k

*The ONT methylation data example*

---

## Description

A GRanges object containing cytosine sites, annotated with per-site ONT methylation calls

### Format

The GRanges elements contain four additional metadata columns:

**ONT_Cm** comma-delimited read-indices called modified

**ONT_C** comma-delimited read-indices covering but unmodified

**readsM** integer count of modified reads per site

**readsN** integer count of same-strand reads covering each site

### Source

data from https://genome.cshlp.org/content/34/11/2061.

---

## 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. |
| 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"). |
| 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")]

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

# load the DMRs in CG context
data(DMRsNoiseFilterCG)

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


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

---

plotMethylationDataCoverage

*Plot methylation data coverage*

---

## Description

This function plots the coverage for the bisulfite sequencing data.

## Usage

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

## Arguments

| | |
|---|---|
| methylationData1 | the methylation data in condition 1 (see methylationDataList). |
| methylationData2 | the methylation data in condition 2 (see methylationDataList). This is optional. |
| breaks | a numeric vector specifing the different values for the thresholds when computing the coverage. |
| regions | a GRanges object with the regions where to compute the coverage. If NULL, the coverage is computed genome-wide. |
| conditionsNames | a vector of character with the names of the conditions for methylationData1 and methylationData2. |
| context | the context in which the DMRs are computed ("CG", "CHG" or "CHH"). |
| proportion | a logical value indicating whether proportion or counts will be plotted. |
| labels | a vector of character used to add a subfigure character to the plot. If NULL nothing is added. |
| col | a character vector with the colors. It needs to contain a minimum of 2 colors per condition. If not or if NULL, the 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. |
| 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 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

## See Also

computeMethylationDataCoverage, methylationDataList

## Examples

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

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

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

## End(Not run)
```

---

# plotMethylationDataSpatialCorrelation

*Plot methylation data spatial correlation*

---

## Description

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

## Usage

```
plotMethylationDataSpatialCorrelation(
  methylationData1,
  methylationData2 = NULL,
  distances,
  regions = NULL,
  conditionsNames = NULL,
  context = "CG",
  labels = NULL,
  col = NULL,
```

```
  pch = c(1, 0, 16, 2, 15, 17),
  lty = c(4, 1, 3, 2, 6, 5),
  contextPerRow = FALSE,
  log = ""
)
```

## Arguments

| | |
|---|---|
| methylationData1 | the methylation data in condition 1 (see methylationDataList). |
| methylationData2 | the methylation data in condition 2 (see methylationDataList). This is optional. |
| distances | a numeric vector specifing the different values for the distances when computing the correlation. |
| regions | a GRanges object with the regions where to compute the correlation. If NULL, the coverage is computed genome-wide. |
| conditionsNames | a vector of character with the names of the conditions for methylationData1 and methylationData2. |
| context | the context in which the DMRs are computed ("CG", "CHG" or "CHH"). |
| labels | a vector of character used to add a subfigure character to the plot. If NULL nothing is added. |
| col | a character vector with the colors. It needs to contain a minimum of 2 colors per condition. If not or if NULL, the 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. |
| 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 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. |
| log | a character indicating if any of the axes will be displayed on log scale. This argument will be passed to plot function. |

## Details

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

## Value

Invisibly returns NULL

## Author(s)

Nicolae Radu Zabet

## See Also

computeMethylationDataSpatialCorrelation, methylationDataList

## Examples

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

# plot the spatial correlation in CG context
par(mar=c(4, 4, 3, 1)+0.1)
plotMethylationDataSpatialCorrelation(methylationDataList[["WT"]],
                          methylationDataList[["met1-3"]],
                          distances = c(1,5,10,15), regions = NULL,
                          conditionsNames = c("WT","met1-3"),
                          context = c("CG"),
                          labels = LETTERS, col = NULL,
                          pch = c(1,0,16,2,15,17), lty = c(4,1,3,2,6,5),
                          contextPerRow = FALSE)
## Not run:
# plot the spatial correlation in all three contexts
plotMethylationDataSpatialCorrelation(methylationDataList[["WT"]],
                          methylationDataList[["met1-3"]],
                          distances = c(1,5,10,15,20,50,100,150,200,500,1000),
                          regions = NULL, conditionsNames = c("WT","met1-3"),
                          context = c("CG", "CHG", "CHH"),
                          labels = LETTERS, col = NULL,
                          pch = c(1,0,16,2,15,17), lty = c(4,1,3,2,6,5),
                          contextPerRow = FALSE, log="x")

## End(Not run)
```

---

# plotMethylationProfile

*Plot Methylation Profile*

---

## Description

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

## Usage

```
plotMethylationProfile(
  methylationProfiles,
  autoscale = FALSE,
  labels = NULL,
  title = "",
  col = NULL,
```

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

## Arguments

| | |
|---|---|
| methylationProfiles | a GRangesList object. Each GRanges object in the list is generated by calling the function computeMethylationProfile. |
| autoscale | a logical value indicating whether the values are 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. |
| 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
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))

## 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 optional. |
| regions | a GRanges object with the regions where to plot the profiles. |
| conditionsNames | the names of the two conditions. This will be used to plot the legend. |
| context | a vector with all contexts in which the DMRs are computed ("CG", "CHG" or "CHH"). |
| windowSize | a numeric value indicating the size of the window in which methylation is averaged. |
| autoscale | a logical value indicating whether the values are 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. |
| 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 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

## 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. |
| 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

## 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
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)
```

---

PMDsBinsCG *The PMDs between GM18501 and GM18876 using Bins method*

---

## Description

A GRangesList object containing the PMDs between GM18501 and GM18876 B-Lymphocyte cell lines in Homo sapiens (see ontSampleGRangesList). The PMDs were computed on the 1.5 ~ 2 Mbp from Chromosome 1 with bins method using binSize of 1 kbp

## Format

The GRanges element contain 5 metadata columns; see computePMDs

## See Also

filterPMDs, computePMDs, analyseReadsInsideRegionsForConditionPMD and mergePMDsIteratively

---

`PMDsNoiseFilterCG` *The PMDs between GM18501 and GM18876 using Noise_filter method*

---

## Description

A `GRangesList` object containing the PMDs between GM18501 and GM18876 B-Lymphocyte cell lines in Homo sapiens (see `ontSampleGRangesList`). The PMDs were computed on the 1.5 ~ 2 Mbp from Chromosome 1 with noise filter method using a triangular kernel and a windowSize of 100 bp

## Format

The `GRanges` element contain 5 metadata columns; see `computePMDs`

## See Also

`filterPMDs`, `computePMDs`, `analyseReadsInsideRegionsForConditionPMD` and `mergePMDsIteratively`

---

`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`).

## Value

the methylation data stored as a `GRanges` object with four metadata columns (see `methylationDataList`). If the Granges are from ONT datasets, its have six metedata columns include as ONT_Cm and ONT_C (see `readONTbam`).

## Author(s)

Nicolae Radu Zabet updated by Young Jun Kim

## 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,
  sample1_name = NULL,
  sample2_name = NULL
)
```

## Arguments

| | |
|---|---|
| `methylationData1` | a `GRanges` object with the methylation data (see `methylationDataList`). |
| `methylationData2` | a `GRanges` object with the methylation data (see `methylationDataList`). |
| `sample1_name` | the label used for sample 1. |
| `sample2_name` | the label used for sample 2. |

## Value

the methylation data stored as a `GRanges` object with four metadata columns (see `methylationDataList`). If the Granges are from ONT datasets, its have six metedata columns include as ONT_Cm and ONT_C (see `readONTbam`).

## Author(s)

Nicolae Radu Zabet updated by Young Jun Kim

## 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

```
readBismark(file)
```

## Arguments

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

## Value

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

## Author(s)

Nicolae Radu Zabet and Jonathan Michael Foonlan Tsang

## Examples

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

# save the 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

```
readBismarkPool(files)
```

## Arguments

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

## Value

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

## Author(s)

Nicolae Radu Zabet and Jonathan Michael Foonlan Tsang

## Examples

```
# 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)
```

`readONTbam` *Load ONT BAM, decode MM/ML, and count modified vs. unmodified reads*

## Description

`readONTbam()` takes an indexed Nanopore BAM file with MM/ML tags, decodes each read's per-C modification probabilities, and overlays them on a `GRanges` of candidate cytosine sites. It returns a copy of `ref_gr` augmented with:

- `ONT_Cm` — comma-delimited read-indices called "modified"
- `ONT_C` — comma-delimited read-indices covering but _not_ modified
- `readsM` — count of modified reads at each site
- `readsN` — total same-strand coverage at each site

You can either supply your own `ref_gr` (e.g.\ from `selectCytosine()`) or leave it `NULL` and pass `context`, `chr`, `region` to build `ref_gr` on the fly.

## Usage

```
readONTbam(
  bamfile,
  ref_gr = NULL,
  modif = "C+m?",
  prob_thresh = 0.5,
  genome = BSgenome.Hsapiens.UCSC.hg38,
  context = "CG",
  chr = NULL,
  region = NULL,
  synonymous = FALSE,
  parallel = FALSE,
  BPPARAM = NULL,
  cores = NULL
)
```

## Arguments

| | |
|---|---|
| `bamfile` | Path to an indexed ONT BAM with MM/ML tags. |
| `ref_gr` | A `GRanges` of genomic cytosine positions to annotate. If `NULL`, will be created via `selectCytosine()` using `context`, `chr`, `region`. |
| `modif` | Character vector of MM codes to treat as "modified" (e.g. `"C+m?"`, `"C+h?"`, `"C+m."`). |
| `prob_thresh` | Numeric in \[0,1\] — minimum ML probability to call a read "modified." |
| `genome` | A `BSgenome` object such as `BSgenome.Hsapiens.UCSC.hg38`. This is used to extract sequence context and must be loaded in advance. **Note:** When running on an HPC system, please ensure that the required `BSgenome` package is already installed and loaded in advance. |

| | |
|---|---|
| context | Sequence context for selectCytosine() (e.g. "CG", "CHG", "CHH"). |
| chr | Chromosome names to restrict selectCytosine(). |
| region | A GRanges to further subset selectCytosine(). |
| synonymous | Logical (default: FALSE). If TRUE, include modified calls that match the specified context sequence (e.g. CGG), even if the site was previously excluded due to deletion or mismatch. For example, if a deletion occurs at position 234523, but the surrounding context still forms CGG, then the modified C at 234523 will be retained (Nmod=1). |
| parallel | Logical. If TRUE, automatically detect your system condition and decoding will use parallel threads via BiocParallel::. If FALSE (default), decoding is done serially. |
| BPPARAM | A BiocParallelParam object controlling parallel execution. This value will automatically set when parallel is TRUE, also able to set as manually. |
| cores | Integer number of workers (must not exceed BPPARAM$workers). This value will automatically set as the maximum number of system workers, also able to set as manually. |

## Details

This function read and annotate ONT MM/ML tags against a cytosine reference

## Value

A GRanges of the same length as ref_gr, with four additional metadata columns:

**ONT_Cm** comma-delimited read-indices called modified

**ONT_C** comma-delimited read-indices covering but unmodified

**readsM** integer count of modified reads per site

**readsN** integer count of same-strand reads covering each site

## Author(s)

Nicolae Radu Zabet and Young Jun Kim

## See Also

selectCytosine, computeDMRs, computePMDs, computeCoMethylatedPositions, computeCoMethylatedRegions, filterVMRsONT, ontSampleGRangesList, scanBamChr1Random5

## Examples

```
## Not run:
library(DMRcaller)
library(BSgenome.Hsapiens.UCSC.hg38)

# set the bam file directory
bam_path <- system.file("extdata", "scanBamChr1Random5.bam", package="DMRcaller")
```

```
# read ONTbam file (chromosome 1 only) in CG context with BSgenome.Hsapiens.UCSC.hg38
ONTSampleGRanges <- readONTbam(bamfile = bam_path, ref_gr = NULL, modif = "C+m?",
                         prob_thresh = 0.50,genome = BSgenome.Hsapiens.UCSC.hg38,
                         context = "CG", chr = "chr1", region = NULL,
                         synonymous = FALSE, parallel = FALSE, BPPARAM = NULL)

## End(Not run)
```

---

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). |
| 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")
```

## scanBamChr1Random5 *The bam file from ONT nanopore .pod5 files*

### Description

A .bam file containing the basecalling result of 5 sequences containing with the MM, ML tag from dorado.

### Format

A .bam object

### Source

The object was created by base-calling and alignment by dorado, ver.0.9.6 https://github.com/nanoporetech/dorado?tab=readme-ov-file#dna-models to generate the bam files with dna_r10.4.1_e8.2_400bps_hac@v5.2.0 as basecalling model from GM18501 cell line https://s3.amazonaws.com/1000g-ont/index.html?prefix=pod5_data/GM18501_R9/. To subset, call the bam file by scanBam function from Rsamtools package and randomly select the 5 sequences. and repackaging the subsetted list to the bam file for test running.

## selectCytosine *Select Cytosine Positions*

### Description

Constructs a GRanges of all cytosine positions in the specified BSgenome (or BSgenome package name), optionally filtering by methylation context ("CG", "CHG", "CHH"), by chromosome, and by genomic region.

### Usage

```
selectCytosine(
  genome = BSgenome.Hsapiens.UCSC.hg38,
  context = c("CG", "CHG", "CHH"),
  chr = NULL,
  region = NULL
)
```

### Arguments

| | |
|---|---|
| genome | A BSgenome object or the name (character) of a BSgenome package to use as the reference genome. If a package name is given, it will be loaded automatically (default: BSgenome.Hsapiens.UCSC.hg38). **Note:** When running on an HPC system, please ensure that the required BSgenome package is already installed and loaded in advance. |

| | |
|---|---|
| context | A character vector of one or more methylation contexts to include: "CG", "CHG", and/or "CHH". Defaults to all three. |
| chr | An optional character vector of chromosome names to restrict the enumeration. If NULL, all sequences in genome are used. |
| region | An optional GRanges object specifying subregions to keep. Requires chr to be non-NULL. |

## Details

This function enumerate cytosine positions in a BSgenome reference

## Value

A GRanges object with one 1-bp range per cytosine, and two metadata columns:

**context** A factor indicating the context ("CG", "CHG", or "CHH").

**trinucleotide_context** Character or factor giving the surrounding trinucleotide sequence (or NA for "CG").

## Author(s)

Nicolae Radu Zabet and Young Jun Kim

## See Also

readONTbam, ontSampleGRangesList

## Examples

```
## Not run:
library(BSgenome.Hsapiens.UCSC.hg38)

# Only CpG sites on chr1 and chr2:
gr_chr1_2 <- selectCytosine(context="CG", chr=c("chr1","chr2"))

# CHH sites in a specific region on chr3:
my_region <- GRanges("chr3", IRanges(1e6, 1e6 + 1e5))
gr_region <- selectCytosine(context="CHH", chr="chr3", region=my_region)

## End(Not run)
```

syntheticDataReplicates

*Simulated data for biological replicates*

## Description

A GRanges object containing simulated date for methylation in four samples. The conditions asscciated witch each sample are a, a, b and b.

## Format

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

## Source

The object was created by calling joinReplicates function.

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