

# Package: methylInheritance (via r-universe)

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**Title** Permutation-Based Analysis associating Conserved Differentially Methylated Elements Across Multiple Generations to a Treatment Effect

**Description** Permutation analysis, based on Monte Carlo sampling, for testing the hypothesis that the number of conserved differentially methylated elements, between several generations, is associated to an effect inherited from a treatment and that stochastic effect can be dismissed.

**Depends** R (>= 3.5)

**Imports** methylKit, BiocParallel, GenomicRanges, IRanges, S4Vectors, methods, parallel, ggplot2, gridExtra, rebus

**Suggests** BiocStyle, BiocGenerics, knitr, rmarkdown, RUnit, methInheritSim, testthat (>= 3.0.0)

**Encoding** UTF-8

**License** Artistic-2.0

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**BugReports** <https://github.com/adeschen/methylInheritance/issues>

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---

calculateSignificantLevel

*Calculate significant level for hypo and hyper conserved elements*

---

### Description

Calculate significant level for hypo and hyper conserved elements using permutation results as well as observed results

### Usage

```
calculateSignificantLevel(formatForGraphDataFrame)
```

### Arguments

formatForGraphDataFrame

a data.frame containing the observation results (using real data) and the permutation results (using shuffled data). Both hyper and hypo differentially conserved methylation results must be present. The data.frame must have 3 columns: "TYPE", "RESULT" and "SOURCE". The "TYPE" can be either "HYPER" or "HYPO". The "RESULT" is the number of conserved differentially elements. The "SOURCE" can be either "OBSERVATION" or "PERMUTATION".

### Value

a list containing two elements:

- HYPER a double, the significant level for the hyper differentially methylated conserved elements
- HYPO a double, the significant level for the hypo differentially methylated conserved elements

**Author(s)**

Astrid Deschenes, Pascal Belleau

**Examples**

```
## Loading dataset containing all results
data(methylInheritanceResults)

## Extract information for the intersection between conserved differentially
## methylated sites (type = sites) between the intersection of 2
## generations (inter = i2): F2 and F3 (position = 2)
info <- extractInfo(allResults = methylInheritanceResults,
  type = "sites", inter="i2", 2)

## Create graph
methylInheritance:::calculateSignificantLevel(info)
```

---

demoForTransgenerationalAnalysis

*The methylation information from samples over three generations. Information for each generation is stored in a methylRawList format (for demo purpose).*

---

**Description**

The object is a list with 3 entries. Each entry corresponds to the information for one generation (first entry = first generation, etc..) stored in a methylRawList object. There are 12 samples (6 controls and 6 cases) for each generation. Each sample information is stored in a methylRaw object.

**Usage**

```
data(demoForTransgenerationalAnalysis)
```

**Format**

A list containing three methylRawList objects. Each methylRawList contains the information for one generation (first entry = first generation, etc..). Each sample information is stored in a methylRaw object. There is methylRaw objects (6 controls and 6 cases) in each generation.

**Details**

This dataset can be used to test runPermutation and runObservation functions.

**Value**

A list containing three methylRawList objects. Each methylRawList contains the information for one generation (first entry = first generation, etc..). Each sample information is stored in a methylRaw object. There is methylRaw objects (6 controls and 6 cases) in each generation.

**See Also**

[runPermutation](#) for running a permutation analysis, and optionally an observation analysis, using a multi-generational dataset

[runObservation](#) for running an observation analysis using methylKit info entry

**Examples**

```
## Loading dataset
data(demoForTransgenerationalAnalysis)

## Run a permutation analysis
runObservation(methylKitData = demoForTransgenerationalAnalysis,
  outputDir = "test_demo", type = "tiles", vSeed = 2001)

## Get results
result <- loadAllRDSResults(analysisResultsDir = "test_demo",
  permutationResultsDir = NULL, doingSites = FALSE,
  doingTiles = TRUE)

## Remove result directory
if (dir.exists("test_demo")) {
  unlink("test_demo", recursive = TRUE)
}
```

---

extractInfo

*Extract the information specific to a subsection of the permutation analysis*

---

**Description**

Extract the information specific to a subsection of the permutation analysis. The extracted information will be specific to one type of differential methylation analysis (tiles or sites), to one type of intersection (two consecutive generation or more) and to one specific group of generations.

**Usage**

```
extractInfo(
  allResults,
  type = c("sites", "tiles"),
  inter = c("i2", "iAll"),
  position = 1
)
```

**Arguments**

allResults	a list of class methylInheritanceAllResults as created by the runPermutation function. The list must contain two entries: "PERMUTATION" and "OBSERVATION". The "PERMUTATION" list must contain all results from all permutations while the "OBSERVATION" list must contain the result obtained with the observed dataset (not shuffled).
type	One of the "sites" or "tiles" strings. Specifies the type of differentially methylated elements should be returned. For retrieving differentially methylated bases type = "sites"; for differentially methylated regions type = "tiles". Default: "sites".
inter	One of the "i2" or "iAll" strings. Specifies the type of intersection should be returned. For retrieving intersection results between two consecutive generations inter = "i2"; for intersection results between three generations or more inter = "iAll". Default: "i2".
position	a positive integer, the position in the list where the information will be extracted. Default=1.

**Value**

a data.frame containing the observation results (using real data) and the permutation results (using shuffled data). Both hyper and hypo differentially conserved methylation results are present.

**Author(s)**

Astrid Deschenes, Pascal Belleau

**Examples**

```
## Get the name of the directory where files are stored
filesDir <- system.file("extdata", "TEST", package="methylInheritance")

## Load information from files
results <- loadAllRDSResults(analysisResultsDir = filesDir,
  permutationResultsDir = filesDir, doingSites = TRUE, doingTiles = TRUE)

## Extract information for the intersection between conserved differentially
## methylated sites (type = sites) between the intersection of 2
## generations (inter = i2): F1 and F2 (position = 1)
info <- extractInfo(allResults = results, type = "sites", inter="i2", 1)
```

---

loadAllRDSResults	<i>Load all RDS files created by the permutation and observation analysis</i>
-------------------	---

---

**Description**

Load all RDS files created by the permutation and observation analysis. The function returns an object of class "methylInheritanceAllResults" that holds all the pertinent information.

**Usage**

```
loadAllRDSResults(  
  analysisResultsDir,  
  permutationResultsDir,  
  doingSites = TRUE,  
  doingTiles = FALSE,  
  maxID = NA  
)
```

**Arguments**

analysisResultsDir	a character string, the path to the directory that contains the analysis results. The path can be the same as for the permutationResultsDir parameter. When NULL, the observation results are not loaded. Default = NULL.
permutationResultsDir	a character string, the path to the directory that contains the permutation results. The path can be the same as for the analysisResultsDir parameter. When NULL, the permutation results are not loaded. Default = NULL.
doingSites	a logical, the data related to differentially methylated sites are loaded when doingSites = TRUE. Default: TRUE.
doingTiles	a logical, the data related to differentially methylated tiles are loaded when doingTiles = TRUE. Default: TRUE.
maxID	NA or a positive integer, the maximum identification number of the permutation files to be loaded. When NA, all files present in the directory are loaded. Default: NA.

**Value**

a list of class methylInheritanceAllResults containing the result of the observation analysis as well as the results of all the permutations.

**Author(s)**

Astrid Deschenes, Pascal Belleau

**See Also**

[mergePermutationAndObservation](#) for detail description, in the Value section, of the methylInheritanceAllResults object.

## Examples

```
## Get the name of the directory where files are stored
filesDir <- system.file("extdata", "TEST", package="methylInheritance")

## Load information from files
results <- loadAllRDSResults(analysisResultsDir = filesDir,
  permutationResultsDir = filesDir, doingSites = TRUE, doingTiles = TRUE)

## Print the observation results
results

## Access the results for the first permutation only for sites
results$PERMUTATION[[1]]$SITES
```

---

loadConvergenceData    *Load convergence information from RDS files*

---

## Description

Load convergence information from RDS files.

## Usage

```
loadConvergenceData(
  analysisResultsDir,
  permutationResultsDir,
  type = c("sites", "tiles"),
  inter = c("i2", "iAll"),
  position,
  by = 100
)
```

## Arguments

**analysisResultsDir**  
a character string, the path to the directory that contains the analysis results. The path can be the same as for the permutationResultsDir parameter.

**permutationResultsDir**  
a character string, the path to the directory that contains the permutation results. The path can be the same as for the analysisResultsDir parameter.

**type**  
One of the "sites" or "tiles" strings. Specifies the type of differentially methylated elements should be returned. For retrieving differentially methylated bases type = "sites"; for differentially methylated regions type = "tiles". Default: "sites".

inter	One of the "i2" or "iAll" strings. Specifies the type of intersection should be returned. For retrieving intersection results between two consecutive generations inter = "i2"; for intersection results between three generations or more inter = "iAll". Default: "i2".
position	a positive integer, the position in the list where the information will be extracted.
by	a integer, the increment of the number of permutations where the significant level is tested. Default: 100.

**Value**

a graph showing the evolution of the significant level with the number of permutations

**Author(s)**

Astrid Deschenes, Pascal Belleau

**Examples**

```
## Get the name of the directory where files are stored
filesDir <- system.file("extdata", "TEST", package="methylInheritance")

## Load convergence information
results <- loadConvergenceData(analysisResultsDir = filesDir,
  permutationResultsDir = filesDir, type="sites", inter="i2", position=1,
  by=1)
```

---

mergePermutationAndObservation

*Merge the permutation results with the observation results.*

---

**Description**

Merge the permutation results with the observation results. The merging is only needed when permutation and observation have been processed separately. The returned value is a methylInheritanceAllResults object that can be used by the extractInfo function.

**Usage**

```
mergePermutationAndObservation(permutationResults, observationResults)
```

**Arguments**

permutationResults

a list with 1 entry called PERMUTATION. The PERMUTATION entry is a list with a number of entries corresponding to the number of permutations that have been processed. Each entry contains the result of one permutation.

observationResults

a list with 1 entry called OBSERVATION. The OBSERVATION entry is a list containing the result obtained with the observed dataset (not shuffled).

**Value**

a list of class methylInheritanceAllResults with 2 entries. The 2 entries are:

- PERMUTATION list with a number of entries corresponding to the number of permutations that have been processed. Each entry contains the result of one permutation. The elements in each entry are:
  - SITES Only present when a sites analysis has been achieved, a list containing:
    - \* i2 a list containing:
      - HYPER a list of integer, the number of conserved hyper differentially methylated sites between two consecutive generations. The first element represents the intersection of the first and second generations; the second element, the intersection of the second and third generations; etc.
      - HYPO a list of integer, the number of conserved hypo differentially methylated sites between two consecutive generations. The first element represents the intersection of the first and second generations; the second element, the intersection of the second and third generations; etc.
    - \* iAll a list containing:
      - HYPER a list of integer, the number of conserved hyper differentially methylated sites between three or more consecutive generations. The first element represents the intersection of the first three generations; the second element, the intersection of the first fourth generations; etc. The number of entries depends on the number of generations.
      - HYPO a list of integer, the number of conserved hypo differentially methylated sites between three or more consecutive generations. The first element represents the intersection of the first three generations; the second element, the intersection of the first fourth generations; etc. The number of entries depends on the number of generations.
  - TILES Only present when a tiles analysis has been achieved, a list containing:
    - \* i2 a list containing:
      - HYPER a list of integer, the number of conserved hyper differentially methylated positions between two consecutive generations. The first element represents the intersection of the first and second generations; the second element, the intersection of the second and third generations; etc.
      - HYPO a list of integer, the number of conserved hypo differentially methylated positions between two consecutive generations. The first element represents the intersection of the first and second generations; the second element, the intersection of the second and third generations; etc.

- \* iAll a list containing:
  - HYPER a list of integer, the number of conserved hyper differentially methylated positions between three or more consecutive generations. The first element represents the intersection of the first three generations; the second element, the intersection of the first fourth generations; etc. The number of entries depends on the number of generations.
  - HYPO a list of integer, the number of conserved hypo differentially methylated positions between three or more consecutive generations. The first element represents the intersection of the first three generations; the second element, the intersection of the first fourth generations; etc. The number of entries depends on the number of generations.
- OBSERVATION a list containing the result obtained with the observed dataset (not shuffled). The elements are:
  - SITES Only present when a sites analysis has been achieved, a list containing:
    - \* i2 a list containing:
      - HYPER a list of integer, the number of conserved hyper differentially methylated sites between two consecutive generations. The first element represents the intersection of the first and second generations; the second element, the intersection of the second and third generations; etc.
      - HYPO a list of integer, the number of conserved hypo differentially methylated sites between two consecutive generations. The first element represents the intersection of the first and second generations; the second element, the intersection of the second and third generations; etc.
    - \* iAll a list containing:
      - HYPER a list of integer, the number of conserved hyper differentially methylated sites between three or more consecutive generations. The first element represents the intersection of the first three generations; the second element, the intersection of the first fourth generations; etc. The number of entries depends on the number of generations.
      - HYPO a list of integer, the number of conserved hypo differentially methylated sites between three or more consecutive generations. The first element represents the intersection of the first three generations; the second element, the intersection of the first fourth generations; etc. The number of entries depends on the number of generations.
  - TILES Only present when a tiles analysis has been achieved, a list containing:
    - \* i2 a list containing:
      - HYPER a list of integer, the number of conserved hyper differentially methylated positions between two consecutive generations. The first element represents the intersection of the first and second generations; the second element, the intersection of the second and third generations; etc.
      - HYPO a list of integer, the number of conserved hypo differentially methylated positions between two consecutive generations. The first element represents the intersection of the first and second generations; the second element, the intersection of the second and third generations; etc.
    - \* iAll a list containing:

- HYPER a list of integer, the number of conserved hyper differentially methylated positions between three or more consecutive generations. The first element represents the intersection of the first three generations; the second element, the intersection of the first fourth generations; etc. The number of entries depends on the number of generations.
- HYPO a list of integer, the number of conserved hypo differentially methylated positions between three or more consecutive generations. The first element represents the intersection of the first three generations; the second element, the intersection of the first fourth generations; etc. The number of entries depends on the number of generations.

### Author(s)

Astrid Deschenes, Pascal Belleau

### Examples

```
## Create a observation result
observed <- list()
observed[["OBSERVATION"]] <- list()
observed[["OBSERVATION"]][["SITES"]] <- list()
observed[["OBSERVATION"]][["SITES"]][["i2"]] <- list(HYPER = list(11, 10),
  HYPO = list(13, 12))
observed[["OBSERVATION"]][["SITES"]][["iAll"]] <- list(HYPER = list(1),
  HYPO = list(3))

## Create a permutation result containing only 1 permutation result
## Real perumtations results would have more entries
permutated <- list()
permutated[["PERMUTATION"]] <- list()
permutated[["PERMUTATION"]][[1]] <- list()
permutated[["PERMUTATION"]][[1]][["SITES"]] <- list()
permutated[["PERMUTATION"]][[1]][["SITES"]][["i2"]] <- list(HYPER =
  list(11, 12), HYPO = list(8, 11))
permutated[["PERMUTATION"]][[1]][["SITES"]][["iAll"]] <- list(HYPER =
  list(0), HYPO = list(1))

## Merge permutation and observation results
mergePermutationAndObservation(permutationResults = permutated,
  observationResults = observed)
```

---

methylInheritanceResults

*All observed and permutation results formatted in a methylInheritanceResults class (for demo purpose).*

---

### Description

The object is a list with 2 entries: "OBSERVATION" and "PERMUTATION".

**Usage**

```
data(methylInheritanceResults)
```

**Format**

a list of class methylInheritanceAllResults containing the following elements:

- OBSERVATION a list containing:
  - SITES a list containing:
    - \* i2 a list containing:
      - HYPER a list of integer with 2 entries, the number of conserved hyper differentially methylated sites between two consecutive generations. The first element represents the intersection of the first and second generations; the second element, the intersection of the second and third generations.
      - HYPO a list of integer with 2 entries, the number of conserved hypo differentially methylated sites between two consecutive generations. The first element represents the intersection of the first and second generations; the second element, the intersection of the second and third generations.
    - \* iAll a list containing:
      - HYPER a list of integer with 1 entry, the number of conserved hyper differentially methylated sites between the three consecutive generations.
      - HYPO a list of integer with 1 entry, the number of conserved hypo differentially methylated sites between the three consecutive generations.
  - TILES a list containing:
    - \* i2 a list containing:
      - HYPER a list of integer with 2 entries, the number of conserved hyper differentially methylated positions between two consecutive generations. The first element represents the intersection of the first and second generations; the second element, the intersection of the second and third generations.
      - HYPO a list of integer with 2 entries, the number of conserved hypo differentially methylated positions between two consecutive generations. The first element represents the intersection of the first and second generations; the second element, the intersection of the second and third generations.
    - \* iAll a list containing:
      - HYPER a list of integer with 1 entry, the number of conserved hyper differentially methylated positions between the three consecutive generations.
      - HYPO a list of integer with 1 entry, the number of conserved hypo differentially methylated positions between the three consecutive generations.
- PERMUTATION a list containing nbrPermutations entries. Each entry is a list containing:
  - SITES a list containing:
    - \* i2 a list containing:
      - HYPER a list of integer with 2 entries, the number of conserved hyper differentially methylated sites between two consecutive generations. The first element represents the intersection of the first and second generations; the second element, the intersection of the second and third generations.

- HYPO a list of integer with 2 entries, the number of conserved hypo differentially methylated sites between two consecutive generations. The first element represents the intersection of the first and second generations; the second element, the intersection of the second and third generations.
- \* iAll a list containing:
  - HYPER a list of integer with 1 entry, the number of conserved hyper differentially methylated sites between the three consecutive generations.
  - HYPO a list of integer with 1 entry, the number of conserved hypo differentially methylated sites between the three consecutive generations.
- TILES a list containing:
  - \* i2 a list containing:
    - HYPER a list of integer with 2 entries, the number of conserved hyper differentially methylated positions between two consecutive generations. The first element represents the intersection of the first and second generations; the second element, the intersection of the second and third generations.
    - HYPO a list of integer with 2 entries, the number of conserved hypo differentially methylated positions between two consecutive generations. The first element represents the intersection of the first and second generations; the second element, the intersection of the second and third generations.
  - \* iAll a list containing:
    - HYPER a list of integer with 1 entry, the number of conserved hyper differentially methylated positions between the three consecutive generations.
    - HYPO a list of integer with 1 entry, the number of conserved hypo differentially methylated positions between the three consecutive generations.

## Details

This dataset can be used to test the `extractInfo` function. The extracted information can be used to calculate the significant level or to create a graph.

## Value

a list of class `methylInheritanceAllResults` containing the following elements:

- OBSERVATION a list containing:
  - SITES a list containing:
    - \* i2 a list containing:
      - HYPER a list of integer with 2 entries, the number of conserved hyper differentially methylated sites between two consecutive generations. The first element represents the intersection of the first and second generations; the second element, the intersection of the second and third generations.
      - HYPO a list of integer with 2 entries, the number of conserved hypo differentially methylated sites between two consecutive generations. The first element represents the intersection of the first and second generations; the second element, the intersection of the second and third generations.
    - \* iAll a list containing:

- HYPER a list of integer with 1 entry, the number of conserved hyper differentially methylated sites between the three consecutive generations.
- HYPO a list of integer with 1 entry, the number of conserved hypo differentially methylated sites between the three consecutive generations.
- TILES a list containing:
  - \* i2 a list containing:
    - HYPER a list of integer with 2 entries, the number of conserved hyper differentially methylated positions between two consecutive generations. The first element represents the intersection of the first and second generations; the second element, the intersection of the second and third generations.
    - HYPO a list of integer with 2 entries, the number of conserved hypo differentially methylated positions between two consecutive generations. The first element represents the intersection of the first and second generations; the second element, the intersection of the second and third generations.
  - \* iAll a list containing:
    - HYPER a list of integer with 1 entry, the number of conserved hyper differentially methylated positions between the three consecutive generations.
    - HYPO a list of integer with 1 entry, the number of conserved hypo differentially methylated positions between the three consecutive generations.
- PERMUTATION a list containing a number of entries corresponding to the number of permutations that have been produced. Each entry is a list containing:
  - SITES a list containing:
    - \* i2 a list containing:
      - HYPER a list of integer with 2 entries, the number of conserved hyper differentially methylated sites between two consecutive generations. The first element represents the intersection of the first and second generations; the second element, the intersection of the second and third generations.
      - HYPO a list of integer with 2 entries, the number of conserved hypo differentially methylated sites between two consecutive generations. The first element represents the intersection of the first and second generations; the second element, the intersection of the second and third generations.
    - \* iAll a list containing:
      - HYPER a list of integer with 1 entry, the number of conserved hyper differentially methylated sites between the three consecutive generations.
      - HYPO a list of integer with 1 entry, the number of conserved hypo differentially methylated sites between the three consecutive generations.
  - TILES a list containing:
    - \* i2 a list containing:
      - HYPER a list of integer with 2 entries, the number of conserved hyper differentially methylated positions between two consecutive generations. The first element represents the intersection of the first and second generations; the second element, the intersection of the second and third generations.
      - HYPO a list of integer with 2 entries, the number of conserved hypo differentially methylated positions between two consecutive generations. The first element represents the intersection of the first and second generations; the second element, the intersection of the second and third generations.

- \* iAll a list containing:
  - HYPER a list of integer with 1 entry, the number of conserved hyper differentially methylated positions between the three consecutive generations.
  - HYPO a list of integer with 1 entry, the number of conserved hypo differentially methylated positions between the three consecutive generations.

### See Also

[extractInfo](#) for extracting the information specific to a subsection of the permutation analysis

### Examples

```
## Loading dataset containing all results
data(methylInheritanceResults)

## Extract information for the intersection between conserved differentially
## methylated sites (type = sites) between the intersection of 2
## generations (inter = i2): F1 and F2 (position = 1)
extractInfo(allResults = methylInheritanceResults,
            type = "sites", inter="i2", 1)
```

---

plotConvergenceGraph *Generate a graph showing the convergence for a permutation analysis*

---

### Description

Generate a graph showing the convergence for a permutation analysis using observed and permuted results.

### Usage

```
plotConvergenceGraph(dataFrameConvergence)
```

### Arguments

dataFrameConvergence  
 a data.frame containing the significant levels at different number of cycles (total number of permuted data analysed). The data.frame must have 6 columns : "NBR\_PERMUTATIONS", "ELEMENT", "ANALYSIS", "POSITION", "TYPE" and "SIGNIFICANT\_LEVEL". The "ELEMENT" can be either "SITES" or "TILES". The "TYPE" can be either "HYPER" or "HYPO".

### Value

a ggplot object.

**Author(s)**

Astrid Deschenes, Pascal Belleau

**Examples**

```
## Get the name of the directory where files are stored
filesDir <- system.file("extdata", "TEST", package="methylInheritance")

## Extract convergenc information for F1 and F2 and F3
data <- loadConvergenceData(analysisResultsDir = filesDir,
  permutationResultsDir = filesDir, type = "sites", inter = "iAll",
  position = 1, by = 1)

## Create convergence graph
plotConvergenceGraph(data)
```

---

plotGraph

*Generate a graph for a permutation analysis*

---

**Description**

Generate a graph for a permutation analysis using observed and shuffled results.

**Usage**

```
plotGraph(formatForGraphDataFrame)
```

**Arguments**

formatForGraphDataFrame

a data.frame containing the observation results (using real data) and the permutation results (using shuffled data). Both hyper and hypo differentially conserved methylation results must be present. The data.frame must have 3 columns : "TYPE", "RESULT" and "SOURCE". The "TYPE" can be either "HYPER" or "HYPO". The "RESULT" is the number of conserved differentially elements. The "SOURCE" can be either "OBSERVATION" or "PERMUTATION".

**Value**

a graph showing the permutation analysis results

**Author(s)**

Astrid Deschenes, Pascal Belleau

**Examples**

```
## Loading dataset containing all results
data(methylInheritanceResults)

## Extract information for the intersection between conserved differentially
## methylated sites (type = sites) between the intersection of 2
## generations (inter = i2): F2 and F3 (position = 2)
info <- extractInfo(allResults = methylInheritanceResults,
  type = "sites", inter="i2", 2)

## Create graph
plotGraph(info)
```

---

```
print.methylInheritanceAllResults
  Print a methylInheritanceAllResults object
```

---

**Description**

Print a methylInheritanceAllResults object

**Usage**

```
## S3 method for class 'methylInheritanceAllResults'
print(x, ...)
```

**Arguments**

x	the output object from mergePermutationAndObservation function, runPermutationUsingRDSFile function (when runObservationAnalysis = TRUE and runPermutationUsingMethylKitInfo function (when runObservationAnalysis = TRUE to be printed
...	arguments passed to or from other methods

**Value**

an object of class methylInheritanceAllResults

**Examples**

```
## Load dataset
data("methylInheritanceResults")

## Print dataset
print(methylInheritanceResults)
```

---

runObservation	<i>Run a differential methylation analysis on multi-generational dataset</i>
----------------	--

---

### Description

Run a differential methylation analysis on each generation present in a dataset. The number of conserved differentially methylated elements (sites, tile or both) between generations is then calculated. The methylKit package is used to identify the differentially methylated elements.

The multi-generational dataset or the name of the RDS file that contains the dataset can be used as input.

The results can also be saved in RDS file (optional).

### Usage

```
runObservation(
  methylKitData,
  type = c("both", "sites", "tiles"),
  outputDir = "output",
  nbrCoresDiffMeth = 1,
  minReads = 10,
  minMethDiff = 10,
  qvalue = 0.01,
  maxPercReads = 99.9,
  destrand = FALSE,
  minCovBasesForTiles = 0,
  tileSize = 1000,
  stepSize = 1000,
  vSeed = -1,
  restartCalculation = FALSE,
  saveInfoByGeneration = FALSE
)
```

### Arguments

methylKitData	a list of methylRawList entries or the name of the RDS file containing the list. Each methylRawList contains all the methylRaw entries related to one generation (first entry = first generation, second entry = second generation, etc..). The number of generations must correspond to the number of entries in the methylKitData. At least 2 generations must be present to calculate the conserved elements. More information can be found in the methylKit package.
type	One of the "sites", "tiles" or "both" strings. Specifies the type of differentially methylated elements should be returned. For retrieving differentially methylated bases type="sites"; for differentially methylated regions type="tiles". Default: "both".
outputDir	a string, the name of the directory that will contain the results of the analysis. If the directory does not exist, it will be created. Default: "output".

nbrCoresDiffMeth	a positive integer, the number of cores to use for parallel differential methylation calculations. The parameter is used for both sites and tiles analysis. The parameter corresponds to the num.cores parameter in the package methylKit. Default: 1 and always 1 for Windows.
minReads	a positive integer Bases and regions having lower coverage than this count are discarded. The parameter correspond to the lo.count parameter in the package methylKit.
minMethDiff	a positive double between [0,100], the absolute value of methylation percentage change between cases and controls. The parameter corresponds to the difference parameter in the methylKit package. Default: 10.
qvalue	a positive double between [0,1], the cutoff for qvalue of differential methylation statistics. Default: 0.01.
maxPercReads	a double between [0,100], the percentile of read counts that is going to be used as an upper cutoff. Bases or regions having higher coverage than this percentile are discarded. The parameter is used for both CpG sites and tiles analysis. The parameter corresponds to the hi.perc parameter in the package methylKit. Default: 99.9.
destrand	a logical, when TRUE will merge reads on both strands of a CpG dinucleotide to provide better coverage. Only advised when looking at CpG methylation. Parameter used for both CpG sites and tiles analysis. Default: FALSE.
minCovBasesForTiles	a non-negative integer, the minimum number of bases to be covered in a given tiling window. The parameter corresponds to the cov.bases parameter in the package methylKit. Only used when doingTiles = TRUE. Default: 0.
tileSize	a positive integer, the size of the tiling window. The parameter corresponds to the win.size parameter in the package methylKit. Only used when doingTiles = TRUE. Default: 1000.
stepSize	a positive integer, the step size of tiling windows. The parameter corresponds to the stepSize parameter in the package methylKit. Only used when doingTiles = TRUE. Default: 1000.
vSeed	a integer, a seed used when reproducible results are needed. When a value inferior or equal to zero is given, a random integer is used. Default: -1.
restartCalculation	a logical, when TRUE, only permutations that don't have a RDS result final are run. Useful to restart a permutation analysis that has been interrupted. Beware that the parameters have to be identical except for this one.
saveInfoByGeneration	a logical, when TRUE, the information about differentially methylated sites and tiles for each generation is saved in a RDS file. The files are saved in the directory specified by the outputDir parameter.

**Value**

0.

**Author(s)**

Astrid Deschenes, Pascal Belleau

**See Also**

[mergePermutationAndObservation](#) for detail description, in the Value section, of the OBSERVATION section of the methylInheritanceAllResults object.

**Examples**

```
## Load methylation information
data(samplesForTransgenerationalAnalysis)

## Run an observation analysis
runObservation(methylKitData = samplesForTransgenerationalAnalysis,
               outputDir = "test", type = "sites", vSeed = 221)

## Load the results
results <- loadAllRDSResults(analysisResultsDir = "test",
                             permutationResultsDir = NULL, doingSites = TRUE, doingTiles = FALSE)

## Print the results
results

## Remove directory
if (dir.exists("test")) {
  unlink("test", recursive = TRUE, force = FALSE)
}
```

---

runPermutation

*Run all permutations on the specified multi-generational dataset*

---

**Description**

Run a permutation analysis, based on Monte Carlo sampling, for testing the hypothesis that the number of conserved differentially methylated elements (sites, tiles or both), between several generations, is associated to an effect inherited from a treatment and that stochastic effect can be dismissed.

The multi-generational dataset or the name of the RDS file that contains the dataset can be used as input.

The observation analysis can also be run (optional). All permutation results are saved in RDS files.

**Usage**

```
runPermutation(
  methylKitData,
  type = c("both", "sites", "tiles"),
  outputDir = "output",
  runObservationAnalysis = TRUE,
  nbrPermutations = 1000,
  nbrCores = 1,
  nbrCoresDiffMeth = 1,
  minReads = 10,
  minMethDiff = 10,
  qvalue = 0.01,
  maxPercReads = 99.9,
  destrand = FALSE,
  minCovBasesForTiles = 0,
  tileSize = 1000,
  stepSize = 1000,
  vSeed = -1,
  restartCalculation = FALSE,
  saveInfoByGeneration = FALSE
)
```

**Arguments**

**methylKitData** a list of methylRawList entries or the name of the RDS file containing the list. Each methylRawList entry must contain all the methylRaw entries related to one generation (first entry = first generation, second entry = second generation, etc..). The number of generations must correspond to the number of entries in the methylKitData. At least 2 generations must be present to make a permutation analysis. More information can be found in the methylKit package.

**type** One of the "sites", "tiles" or "both" strings. Specifies the type of differentially methylated elements should be returned. For retrieving differentially methylated bases type="sites"; for differentially methylated regions type="tiles". Default: "both".

**outputDir** a string, the name of the directory that will contain the results of the permutation. If the directory does not exist, it will be created. Default: "output".

**runObservationAnalysis** a logical, when runObservationAnalysis = TRUE, a CpG analysis on the observed dataset is done. Default: TRUE.

**nbrPermutations** a positive integer, the total number of permutations that is going to be done. Default: 1000.

**nbrCores** a positive integer, the number of cores to use when processing the analysis. Default: 1 and always 1 for Windows.

**nbrCoresDiffMeth** a positive integer, the number of cores to use for parallel differential methylation calculations. The parameter is used for both sites and tiles analysis. The

	parameter corresponds to the <code>num.cores</code> parameter in the package <code>methylKit</code> . Default: 1 and always 1 for Windows.
<code>minReads</code>	a positive integer Bases and regions having lower coverage than this count are discarded. The parameter corresponds to the <code>lo.count</code> parameter in the package <code>methylKit</code> .
<code>minMethDiff</code>	a positive double between [0,100], the absolute value of methylation percentage change between cases and controls. The parameter corresponds to the <code>difference</code> parameter in the <code>methylKit</code> package. Default: 10.
<code>qvalue</code>	a positive double between [0,1], the cutoff for qvalue of differential methylation statistics. Default: 0.01.
<code>maxPercReads</code>	a double between [0,100], the percentile of read counts that is going to be used as an upper cutoff. Bases or regions having higher coverage than this percentile are discarded. The parameter is used for both CpG sites and tiles analysis. The parameter corresponds to the <code>hi.perc</code> parameter in the package <code>methylKit</code> . Default: 99.9.
<code>destrand</code>	a logical, when TRUE will merge reads on both strands of a CpG dinucleotide to provide better coverage. Only advised when looking at CpG methylation. The parameter is used for both CpG sites and tiles analysis. Default: FALSE.
<code>minCovBasesForTiles</code>	a non-negative integer, the minimum number of bases to be covered in a given tiling window. The parameter corresponds to the <code>cov.bases</code> parameter in the package <code>methylKit</code> . Only used when <code>doingTiles = TRUE</code> . Default: 0.
<code>tileSize</code>	a positive integer, the size of the tiling window. The parameter corresponds to the <code>win.size</code> parameter in the package <code>methylKit</code> . Only used when <code>doingTiles = TRUE</code> . Default: 1000.
<code>stepSize</code>	a positive integer, the step size of tiling windows. The parameter corresponds to the <code>stepSize</code> parameter in the package <code>methylKit</code> . Only used when <code>doingTiles = TRUE</code> . Default: 1000.
<code>vSeed</code>	a integer, a seed used when reproducible results are needed. When a value inferior or equal to zero is given, a random integer is used. Default: -1.
<code>restartCalculation</code>	a logical, when TRUE, only permutations that don't have an associated RDS result file are run. Useful to restart a permutation analysis that has been interrupted. Beware that the parameters have to be identical except for this one.
<code>saveInfoByGeneration</code>	a logical, when TRUE, the information about differentially methylated sites and tiles for each generation is saved in a RDS file. The information is saved in a different file for each permutation. The files are saved in the directory specified by the <code>outputDir</code> parameter.

**Value**

0.

**Author(s)**

Astrid Deschenes, Pascal Belleau

**See Also**

[mergePermutationAndObservation](#) for detail description, in the Value section, of the methylInheritanceAllResults object as well as its PERMUTATION section.

**Examples**

```
## Load methylKit information
data(samplesForTransgenerationalAnalysis)

## Run a permutation analysis using the methylKit dataset
## A real analysis would require a much higher number of permutations
runPermutation(methylKitData = samplesForTransgenerationalAnalysis,
  outputDir = "test_01", runObservationAnalysis = FALSE, type = "sites",
  nbrPermutations = 2, vSeed = 221)

## Get results
results_01 <- loadAllRDSResults(analysisResultsDir = NULL,
  permutationResultsDir = "test_01", doingSites = TRUE,
  doingTiles = FALSE)

## Remove results directory
if (dir.exists("test_01")) {
  unlink("test_01", recursive = TRUE, force = TRUE)
}

## Path to a methylKit RDS file
methylFile <- system.file("extdata", "methylObj_001.RDS",
  package = "methylInheritance")

## Run a permutation analysis using RDS file name
## A real analysis would require a much higher number of permutations
runPermutation(methylKitData = methylFile, type = "tiles",
  outputDir = "test_02", nbrPermutations = 2, minCovBasesForTiles = 10,
  vSeed = 2001)

## Get results
results_02 <- loadAllRDSResults(analysisResultsDir = NULL,
  permutationResultsDir = "test_02", doingSites = FALSE,
  doingTiles = TRUE)

## Remove results directory
if (dir.exists("test_02")) {
  unlink("test_02", recursive = TRUE, force = TRUE)
}
```

---

samplesForTransgenerationalAnalysis

*All samples information, formatted by methylKit, in a methylRawList format (for demo purpose).*

---

**Description**

The object is a list with 3 entries. Each entry corresponds to the information for one generation (first entry = first generation, etc..) stored in a `methylRawList`. There are 12 samples (6 controls and 6 cases) for each generation. Each sample information is stored in a `methylRaw` object.

**Usage**

```
data(samplesForTransgenerationalAnalysis)
```

**Format**

A list containing three `methylRawList` objects. Each `methylRawList` contains the information for one generation (first entry = first generation, etc..). Each sample information is stored in a `methylRaw` object. There is `methylRaw` objects (6 controls and 6 cases) in each generation.

**Details**

This dataset can be used to test the `runPermutation` function.

**Value**

A list containing three `methylRawList` objects. Each `methylRawList` contains the information for one generation (first entry = first generation, etc..). Each sample information is stored in a `methylRaw` object. There is `methylRaw` objects (6 controls and 6 cases) in each generation.

**See Also**

[runPermutation](#) for running a permutation analysis, and optionally an observation analysis, using multi-generational dataset

**Examples**

```
## Loading dataset
data(samplesForTransgenerationalAnalysis)

## Run a permutation analysis
runPermutation(methylKitData = samplesForTransgenerationalAnalysis,
               type = "tiles", nbrPermutations = 2, vSeed = 2332)
```

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