

# Package ‘M3D’

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**Type** Package

**Title** Identifies differentially methylated regions across testing groups

**Version** 1.18.0

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**Author** Tom Mayo

**Maintainer** Tom Mayo <t.mayo@ed.ac.uk>

**Description** This package identifies statistically significantly differentially methylated regions of CpGs. It uses kernel methods (the Maximum Mean Discrepancy) to measure differences in methylation profiles, and relates these to inter-replicate changes, whilst accounting for variation in coverage profiles.

**License** Artistic License 2.0

**Imports** parallel, Rcpp, BiocGenerics, S4Vectors, IRanges, GenomicRanges, SummarizedExperiment, BiSeq

**LinkingTo** Rcpp

**Depends** R (>= 3.3.0)

**VignetteBuilder** knitr

**Suggests** BiocStyle, knitr, testthat

**biocViews** DNAMethylation, DifferentialMethylation, Coverage, CpGIsland

**RoxygenNote** 5.0.1

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M3D-package	<i>Non-parametric statistical testing</i>
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## Description

This package identifies statistically significantly differentially methylated regions of CpGs. It uses kernel methods, specifically the Maximum Mean Discrepancy (Gretton et al. 2006), to measure differences in methylation profiles, and relates these to inter-replicate changes, whilst accounting for variation in coverage profiles.

## Details

Package: M3D  
 Type: Package  
 Version: 0.99.0  
 Date: 2014-07-17  
 License: Artistic License 2.0

This package works on RRBS data as processed by the BiSeq package. The starting point is an rrbs object, a class defined by the BiSeq package (Hebestreit et al. 2013), and a GRanges object outlining the regions to test. The maximum mean discrepancy (MMD) (Gretton et al. 2006) is calculated over each region for each pair of samples, once with respect to methylation levels and once respecting only coverage. These two values are subtracted to form a test-statistic and between-group values are compared to inter-replicate values to provide p-values. These reflect the empirical probability of observing the between-group methylation differences among the replicates.

Function list:

determineGroupComps: returns a vector of the sample comparisons findComps: returns the indices of the M3D test-statistic that corresponding to particular samples M3D\_Single: Computes the two components of the M3D test-statistic over 1 island for 1 sample pair. M3D\_Wrapper: Computes the two components of the M3D test-statistic over all sample pairs over all islands. medianFreq:

Returns the median of data summarised by unique values and the frequency with which they occur.  
 pvals: Returns empirical p-values for the regions based on the M3D test-statistic.

### Author(s)

Tom Mayo

Maintainer: Tom Mayo <t.mayo@ed.ac.uk>

### References

Gretton, A., Borgwardt, K. M., Rasch, M., Scholkopf, B., Smola, A. J. (2006). A kernel method for the two-sample-problem. In Advances in neural information processing systems (pp. 513-520).

Hebestreit, K., Dugas, M., Klein, H. U. (2013). Detection of significantly differentially methylated regions in targeted bisulfite sequencing data. Bioinformatics, 29(13), 1647-1653.

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CpGsDemo	<i>Toy data for the package - 1000 CpG regions to be tested in a GRanges object</i>
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### Description

Toy data for the package - 1000 CpG regions to be tested in a GRanges object

### Author(s)

Tom Mayo

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determineGroupComps	<i>Creates strings of sample pair comparisons</i>
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### Description

Takes in a vector of strings of sample names and returns strings of all the comparisons, either within a testing group or between testing groups. This is not intended to be called directly by the user.

### Usage

```
determineGroupComps(samples1, samples2 = NULL, type)
```

### Arguments

samples1	A vector of sample names from one group
samples2	A vector of sample names from the other group, if we want to specify between-group comparisons
type	'within' or 'between'. 'within' returns all the sample pairs within samples1, 'between' returns all the sample pairs between samples1 and samples2

**Value**

A vector of sample pair comparisons of the form 'sample1 vs sample2' for use with the M3D functions

**Author(s)**

Tom Mayo <t.mayo@ed.ac.uk>

**Examples**

```
library(BiSeq)
data(rrbsDemo)
samples1 <- rownames(colData(rrbsDemo))[colData(rrbsDemo)[,]== 'H1=hESC']
within1 <- determineGroupComps(samples1,type='within')
```

---

findComps

*Finds columns in the M3D test-statistic matrix*

---

**Description**

Returns the columns of the test-statistic matrix that refer to specific samples. This is not intended to be called directly by the user.

**Usage**

```
findComps(MMD, samples)
```

**Arguments**

MMD	A matrix containing the M3D test-statistic, the difference the full and methylation blind metrics, for each region in the CpGs object. Each column is a comparison between two samples, which are described in the column names.
samples	A vector of sample pairs of the form 'sample1 vs sample2' as returned from determineGroupComps

**Value**

Returns the indices of the M3D test-statistic components that contain the sample pair comparisons in 'samples'

**Author(s)**

Tom Mayo <t.mayo@ed.ac.uk>

**Examples**

```
library(BiSeq)
data(MMDlistDemo)
data(rrbsDemo)
group1 <- 'H1=hESC'
samples1 <- rownames(colData(rrbsDemo))[colData(rrbsDemo)[,]==group1]
within1 <- M3D::determineGroupComps(samples1,type='within')
findComps(MMDlistDemo, within1)
```

---

M3D_Single	<i>Computes the components of the M3D test-statistic over one region for 2 samples</i>
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---

**Description**

Returns the two components of the M3D test-statistic - the MMD (Gretton et al. 2006) for the full data and the coverage only data, respectively. This is not intended to be called directly by the user.

**Usage**

```
M3D_Single(test_data, loc_mx, locInds, method = "MinusCovMMD")
```

**Arguments**

test_data	Contains the methylation data over two samples for a given region.
loc_mx	The matrix of distances between the CpG sites.
locInds	The indices of the non-zero entries of loc_mx.
method	This specifies whether to return the full MMD and the methylation blind MMD (focusing only on the coverage) or just the former. if method = 'MinusCovMMD' it is both, all other values return just the full MMD.

**Value**

This returns the value of the MMD for the region between the two samples as a numeric. If method is set to 'MinusCovMMD', a list is returned of the full MMD and the coverage only MMD. Subtracting them gives the M3D test-statistic.

**Author(s)**

Tom Mayo <t.mayo@ed.ac.uk>

**References**

Gretton, A., Borgwardt, K. M., Rasch, M., Scholkopf, B., Smola, A. J. (2006). A kernel method for the two-sample-problem. In Advances in neural information processing systems (pp. 513-520).

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M3D_Wrapper	<i>Computes the components of the M3D test-statistic over all regions for all sample-pairs.</i>
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**Description**

Returns the two components of the M3D test-statistic - the MMD (Gretton et al. 2006) for the full data and the coverage only data, respectively - for all regions and all samples pairs, as a matrix.

**Usage**

```
M3D_Wrapper(rrbs, overlaps, para = FALSE)
```

**Arguments**

rrbs	An rrbs object containing methylation and coverage data as created using the BiSeq package
overlaps	The overlaps between the list of testing regions and the methylation data. This is obtained using the function <code>findOverlaps(CpGs,rrbs)</code> for a GRanges object CpGs detailing the testing regions.
para	Set to true if called via M3D_Para

**Value**

This returns the two components of the M3D test-statistic for each region over all sample pairs as a matrix. Subtracting them gives the M3D test-statistic. This is processed with the function `pvals`.

**Author(s)**

Tom Mayo <t.mayo@ed.ac.uk>

**References**

Gretton, A., Borgwardt, K. M., Rasch, M., Scholkopf, B., Smola, A. J. (2006). A kernel method for the two-sample-problem. In *Advances in neural information processing systems* (pp. 513-520).

**Examples**

```
data(rrbsDemo)
data(CpGsDemo)
CpGsDemo <- CpGsDemo[1:5]
overlaps <- GenomicRanges::findOverlaps(CpGsDemo,rrbsDemo)
M3D_list <- M3D_Wrapper(rrbsDemo,overlaps)
head(M3D_list$Full-M3D_list$Coverage)
```

---

M3D_Wrapper_lite	<i>Computes the components of the M3D test-statistic over all regions for all sample-pairs.</i>
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---

**Description**

Returns the M3D test-statistic, without the components, for all regions with the cross group sample pairs averaged to save memory (in column one), as a matrix.

**Usage**

```
M3D_Wrapper_lite(rrbs, overlaps, group1 = NaN, group2 = NaN,
  verbose = TRUE)
```

**Arguments**

rrbs	An rrbs object containing methylation and coverage data as created using the BiSeq package
overlaps	The overlaps between the list of testing regions and the methylation data. This is obtained using the function <code>findOverlaps(CpGs,rrbs)</code> for a GRanges object CpGs detailing the testing regions.
group1	The name of the first group for the comparison. This is stored in <code>colData(rrbs)</code> . Default finds first unique group in <code>colData(rrbs)</code> .
group2	The name of the second group for the comparison. This is stored in <code>colData(rrbs)</code> . Default finds second unique group in <code>colData(rrbs)</code> .
verbose	Logical vector. If true, the function prints a progress bar.

**Value**

This returns the two components of the M3D test-statistic for each region over all sample pairs as a matrix. Subtracting them gives the M3D test-statistic. This is processed with the function `pvals`.

**Author(s)**

Tom Mayo <t.mayo@ed.ac.uk>

**References**

Getton, A., Borgwardt, K. M., Rasch, M., Scholkopf, B., Smola, A. J. (2006). A kernel method for the two-sample-problem. In *Advances in neural information processing systems* (pp. 513-520).

**Examples**

```
data(rrbsDemo)
data(CpGsDemo)
CpGsDemo <- CpGsDemo[1:5]
overlaps <- GenomicRanges::findOverlaps(CpGsDemo,rrbsDemo)
M3D_list <- M3D_Wrapper_lite(rrbsDemo,overlaps)
head(M3D_list)
```

---

medianFreq

*Finds the median*

---

**Description**

Returns the median of a list of values with corresponding frequencies. This is not intended to be called directly by the user.

**Usage**

```
medianFreq(values, freqs)
```

**Arguments**

values	A vector of the unique values that occur
freqs	A vector of the number of occurrences of each value

**Value**

Returns the median value of the data comprising each entry in values repeated the corresponding entry in freqs number of times, as a numeric.

**Author(s)**

Tom Mayo <t.mayo@ed.ac.uk>

---

median_freq	<i>Finds the median</i>
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---

**Description**

Returns the median of a list of values with corresponding frequencies. This is not intended to be called directly by the user.

**Usage**

```
median_freq(values, freqs)
```

**Arguments**

values	A vector of the unique values that occur
freqs	A vector of the number of occurrences of each value

**Value**

Returns the median value of the data comprising each entry in values repeated the corresponding entry in freqs number of times, as a numeric.

**Author(s)**

Tom Mayo <t.mayo@ed.ac.uk>

**Examples**

```
median_freq(c(1,2,3), c(3,6,10))
```

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MMDlistDemo	<i>Toy data for the package - the output of the M3D_Wrapper function.</i>
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**Description**

Toy data for the package - the output of the M3D\_Wrapper function.

**Author(s)**

Tom Mayo



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PDemo

*Toy data for the package - the output of the pvals function.*

---

### Description

Toy data for the package - the output of the pvals function.

### Author(s)

Tom Mayo

---

plotCoverageSampleProfiles

*Plots coverage profiles over a specific region*

---

### Description

Plots the profile for each of the two testing groups. Within each group, the mean of methylation level is taken, smoothed and plotted, along with the individual values.

### Usage

```
plotCoverageSampleProfiles(rrbs, CpGs, group1, group2, CpGindex,  
  samples = NaN, plot_title = NaN)
```

### Arguments

rrbs	An rrbs object containing methylation and coverage data as created using the BiSeq package
CpGs	A GRanges object with each row being a testing region
group1	The name of the first testing group
group2	The name of the second testing group
CpGindex	The index within the CpGs object of the region we are plotting
samples	Names of specific samples in the groups to plot, defaults to all samples in both groups
plot_title	Optional title for the plot, overrides automatic title

### Value

NULL, the function plots the profiles

### Author(s)

Tom Mayo <t.mayo@ed.ac.uk>

### Examples

```
# plot the 9th region in the Toy Data Set  
data(rrbsDemo)  
data(CpGsDemo)  
plotMethProfile(rrbsDemo, CpGsDemo, 'H1-hESC', 'K562', 9)
```

---

plotMethProfile      *Plots methylation profiles over a specific region*

---

### Description

Plots a smoothed methylation profile for each of the two testing groups. Within each group, the mean of methylation level is taken, smoothed and plotted, along with the individual values.

### Usage

```
plotMethProfile(rrbs, CpGs, group1, group2, CpGindex, plot_title = NaN)
```

### Arguments

rrbs	An rrbs object containing methylation and coverage data as created using the BiSeq package
CpGs	A GRanges object with each row being a testing region
group1	The name of the first testing group
group2	The name of the second testing group
CpGindex	The index within the CpGs object of the region we are plotting
plot_title	Optional title for the plot, overrides automatic title

### Value

NULL, the function plots the profiles

### Author(s)

Tom Mayo <t.mayo@ed.ac.uk>

### Examples

```
# plot the 9th region in the Toy Data Set
data(rrbsDemo)
data(CpGsDemo)
plotMethProfile(rrbsDemo, CpGsDemo, 'H1-hESC', 'K562', 9)
```

---

plotMethSampleProfiles      *Plots methylation profiles over a specific region*

---

### Description

Plots a smoothed methylation profile for each of the two testing groups. Within each group, the mean of methylation level is taken, smoothed and plotted, along with the individual values.

### Usage

```
plotMethSampleProfiles(rrbs, CpGs, group1, group2, CpGindex, samples = NaN,
  plot_title = NaN)
```

**Arguments**

rrbs	An rrbs object containing methylation and coverage data as created using the BiSeq package
CpGs	A GRanges object with each row being a testing region
group1	The name of the first testing group
group2	The name of the second testing group
CpGindex	The index within the CpGs object of the region we are plotting
samples	Names of specific samples in the groups to plot, defaults to all samples in both groups
plot_title	Optional title for the plot, overrides automatic title

**Value**

NULL, the function plots the profiles

**Author(s)**

Tom Mayo <t.mayo@ed.ac.uk>

**Examples**

```
# plot the 9th region in the Toy Data Set
data(rrbsDemo)
data(CpGsDemo)
plotMethProfile(rrbsDemo, CpGsDemo, 'H1-hESC', 'K562', 9)
```

---

pvals

*Computes p-values*

---

**Description**

Returns p-values for each region reflecting the probability of observing the mean test-statistic of the between group comparisons among the inter-replicate comparisons.

**Usage**

```
pvals(rrbs, CpGs, MMD, group1, group2, smaller = FALSE,
      comparison = "allReps", method = "empirical", outlier_test = FALSE,
      thresh = NA, cut_off = 0.975, sds = 8, closePara = 0.005)
```

**Arguments**

rrbs	An rrbs object containing methylation and coverage data as created using the BiSeq package
CpGs	A GRanges object with each row being a testing region
MMD	A matrix containing the M3D test-statistic, the difference the full and methylation blind metrics, for each region in the CpGs object. Each column is a comparison between two samples, which are described in the column names.
group1	The name of the first group for the comparison. This is stored in colData(rrbs)

group2	The name of the second group for the comparison. This is stored in colData(rrbs)
smaller	Determines whether the p-value is computed whether the test-statistic is greater or lesser than inter-replicate values. For our purposes, it should be set to FALSE.
comparison	Details which groups we are using to define our empirical testing distribution. The default is to use all of them, however, should the user find one group contains unusually high variability, then that group can be selected. Values are either 'allReps', 'Group1' or 'Group2'.
method	Determines which method is used to calculate p-values. 'empirical' uses the empirical distribution directly, without modelling. This is the default. 'model', fits an exponential distribution to the tail of our null distribution.
outlier_test	Logical, indicating whether to screen for outliers in null distribution. Use only when comparison is to one group. Default is FALSE
thresh	The threshold for cutting off regions as highly variable. This is only to be used if results are being standardised across multiple tests. Default is NA and is calculated using cut_off and sds parameters.
cut_off	In the outlier test, we require any outlier to be in this quartile, as a minimum, set to 0.975 by default
sds	In the outlier test, we require any outlier in the null to be greater than the mean of the null plus this many standard deviations, set to 8 by default
closePara	Sets a threshold for how close the exponential curve should fit the empirical distribution in the 'model' method. If the method produces errors, consider raising this parameter.

### Value

Returns a list P, with 2 entries. 'FDRmean' is the Benjamini-Hochberg adjusted p-values. The unadjusted p-values are stored in 'Pmean'. If outlier\_test is TRUE, we also have the highly variable regions as a third entry

### Author(s)

Tom Mayo <t.mayo@ed.ac.uk>

### Examples

```
data(rrbsDemo)
data(CpGsDemo)
data(MMDlistDemo)
library(GenomicRanges)
library(BiSeq)
M3Dstat <- MMDlistDemo$Full-MMDlistDemo$Coverage
group1 <- unique(colData(rrbsDemo)$group)[1]
group2 <- unique(colData(rrbsDemo)$group)[2]
PDemo <- pvals(rrbsDemo, CpGsDemo, M3Dstat,
              group1, group2, smaller=FALSE, comparison='allReps')
head(PDemo)
```

---

pvals_lite	<i>Computes p-values</i>
------------	--------------------------

---

### Description

Returns p-values for each region reflecting the probability of observing the mean test-statistic of the between group comparisons among the inter-replicate comparisons. The 'lite' version works with the outcome of M3D\_Wrapper\_lite and the 'lite' parallel version.

### Usage

```
pvals_lite(rrbs, CpGs, M3D_stat_lite, group1, group2, smaller = FALSE,
           comparison = "allReps", method = "empirical", outlier_test = FALSE,
           thresh = NA, cut_off = 0.975, sds = 8, closePara = 0.005)
```

### Arguments

rrbs	An rrbs object containing methylation and coverage data as created using the BiSeq package
CpGs	A GRanges object with each row being a testing region
M3D_stat_lite	A matrix containing the M3D test-statistic, as returned from the M3D_Wrapper_lite function. This has the mean of the MMDs for the comparisons between groups in the first column, and the within groups MMDs in the others, for each region in the CpGs object.
group1	The name of the first group for the comparison. This is stored in colData(rrbs)
group2	The name of the second group for the comparison. This is stored in colData(rrbs)
smaller	Determines whether the p-value is computed whether the test-statistic is greater or lesser than inter-replicate values. For our purposes, it should be set to FALSE.
comparison	Details which groups we are using to define our empirical testing distribution. The default is to use all of them, however, should the user find one group contains unusually high variability, then that group can be selected. Values are either 'allReps', 'Group1' or 'Group2'.
method	Determines which method is used to calculate p-values. 'empirical' uses the empirical distribution directly, without modelling. This is the default. 'model', fits an exponential distribution to the tail of our null distribution.
outlier_test	Logical, indicating whether to screen for outliers in null distribution. Use only when comparison is to one group. Default is FALSE
thresh	The threshold for cutting off regions as highly variable. This is only to be used if results are being standardised across multiple tests. Default is NA and is calculated using cut_off and sds parameters.
cut_off	In the outlier test, we require any outlier to be in this quartile, as a minimum, set to 0.975 by default
sds	In the outlier test, we require any outlier in the null to be greater than the mean of the null plus this many standard deviations, set to 8 by default
closePara	Sets a threshold for how close the exponential curve should fit the empirical distribution in the 'model' method. If the method produces errors, consider raising this parameter.

**Value**

Returns a list P, with 2 entries. 'FDRmean' is the Benjamini-Hochberg adjusted p-values. The unadjusted p-values are stored in 'Pmean'. If we are test for outliers, we also have the highly variable regions as a third entry

**Author(s)**

Tom Mayo <t.mayo@ed.ac.uk>

**Examples**

```
data(rrbsDemo)
data(CpGsDemo)
library(GenomicRanges)
library(BiSeq)
CpGsDemo <- CpGsDemo[1:5]
overlaps <- GenomicRanges::findOverlaps(CpGsDemo,rrbsDemo)
M3D_list <- M3D_Wrapper_lite(rrbsDemo,overlaps)
group1 <- unique(colData(rrbsDemo)$group)[1]
group2 <-unique(colData(rrbsDemo)$group)[2]
PDemo <- pvals_lite(rrbsDemo, CpGsDemo, M3D_list,
                  group1, group2, smaller=FALSE,comparison='allReps')
head(PDemo)
```

---

readBedFiles

*Reads in bed file to make RRBS data*


---

**Description**

Reads in RRBS data in bed file format from various styles and outputs an rrbs data structure. Adapted from readBismark in the BiSeq package.

**Usage**

```
readBedFiles(files, colData, bed_type = "Encode", eData = NaN)
```

**Arguments**

files	A character pointing the the rrbs files downloads from the ENCODE database.
colData	Samples' names plus additional sample information as character, data.frame or DataFrame.
bed_type	Character string representing the style of the bed file. Options are
eData	Experiment data to describe the work. This is used to create the BSraw object as in the BiSeq package.

**Value**

Returns a BSraw object storing methylation and coverage data - the underlying structure for this package.

**Author(s)**

Tom Mayo <t.mayo@ed.ac.uk>

**Examples**

```
# download the files and change the working directory
# to that location
files <- c('wgEncodeHaibMethylRrbsH1heschHaibSitesRep1.bed.gz',
          'wgEncodeHaibMethylRrbsH1heschHaibSitesRep2.bed.gz',
          'wgEncodeHaibMethylRrbsK562HaibSitesRep1.bed.gz',
          'wgEncodeHaibMethylRrbsK562HaibSitesRep2.bed.gz')
group <- factor(c('H1-hESC', 'H1-hESC', 'K562', 'K562'))
samples <- c('H1-hESC1', 'H1-hESC2', 'K562-1', 'K562-2')
colData <- DataFrame(group, row.names= samples)
rrbs <- readBedFiles(files, colData)
```

---

readBedRaw

*Reads in RRBS data in raw format to be processed later*

---

**Description**

Reads in RRBS data in bed file format from a variety of formats and outputs a list that is turned into an rrbs data structure in the wrapper function. Adapted from readBismark in the BiSeq package.

**Usage**

```
readBedRaw(files, colData, bed_type = "Encode")
```

**Arguments**

files	A character pointing the the rrbs files downloads from the ENCODE database.
colData	Samples' names plus additional sample information as character, data.frame or DataFrame.
bed_type	Character string representing the style of the bed file. Options are

**Value**

Returns a list with all the data that will be turned into a BSraw object - the underlying structure for this package.

**Author(s)**

Tom Mayo <t.mayo@ed.ac.uk>

**Examples**

```
# download the files and change the working directory
# to that location
files <- c('wgEncodeHaibMethylRrbsH1heschaibSitesRep1.bed.gz',
'wgEncodeHaibMethylRrbsH1heschaibSitesRep2.bed.gz',
'wgEncodeHaibMethylRrbsK562HaibSitesRep1.bed.gz',
'wgEncodeHaibMethylRrbsK562HaibSitesRep2.bed.gz')
group <- factor(c('H1-hESC', 'H1-hESC', 'K562', 'K562'))
samples <- c('H1-hESC1', 'H1-hESC2', 'K562-1', 'K562-2')
colData <- DataFrame(group, row.names= samples)
rrbs <- readBedRaw(files, colData)
```

---

rrbsDemo	<i>Toy data for the package - methylation data for cytosines sites within the testing regions only, in an rrbs object.</i>
----------	--

---

**Description**

Toy data for the package - methylation data for cytosines sites within the testing regions only, in an rrbs object.

**Author(s)**

Tom Mayo

---

test\_colData\_structure

*Tests that the files are specified in a workable order for M3D to run*

---

**Description**

M3D requires that the files be specified group by group (all of condition 1, then all of condition 2 etc.). This function tests this, and is used before loading so that the error is caught before time is wasted.

**Usage**

```
test_colData_structure(colData)
```

**Arguments**

colData	Samples' names plus additional sample information as character, data.frame or DataFrame.
---------	--

**Value**

Returns TRUE if the colData is structured correctly for downstream analysis, FALSE otherwise

**Author(s)**

Tom Mayo <t.mayo@ed.ac.uk>



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