

# Package: BisRNA (via r-universe)

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**Type** Package

**Title** Analysis of RNA Cytosine-5 Methylation

**Version** 0.2.2

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**Description** Bisulfite-treated RNA non-conversion in a set of samples is analysed as follows : each sample's non-conversion distribution is identified to a Poisson distribution. P-values adjusted for multiple testing are calculated in each sample. Combined non-conversion P-values and standard errors are calculated on the intersection of the set of samples. For further details, see C Legrand, F Tuorto, M Hartmann, R Liebers, D Jakob, M Helm and F Lyko (2017) <[doi:10.1101/gr.210666.116](https://doi.org/10.1101/gr.210666.116)>.

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**License** GPL (>= 2)

**LazyData** TRUE

**Depends** R (>= 3.3.2)

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**Suggests** IHW, testthat

**NeedsCompilation** no

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BisRNA-package	<i>Analysis of RNA Cytosine-5 Methylation from bisulfite sequencing experiments</i>
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## Description

BisRNA analyses non-conversion of bisulfite-treated RNA of a set of samples, in order to distinguish actual cytosine-5 methylation marks from artifacts. Calculations are done as follows:

- each sample's ratio (Poisson parameter / coverage) is determined, assuming a null Poisson distribution (`RNAmeth.poisson.par`),
- non-conversion p-values are calculated for each sample (`RNAmeth.poisson.test`),
- combined non-conversion p-values and standard error of the non-conversion ratios are calculated on the intersection of the set of samples `samples.combine`.

A small non-conversion p-value points to methylation of a RNA cytosine, or another event blocking bisulfite conversion.

## Examples

```
## Load data
data(Bisdata,package="BisRNA")

## Ratio (Poisson parameter / coverage), derived p-values,
# adjusted for multiple testing using either BH (here), or IHW if available.
lambda1 <- RNAmeth.poisson.par(Bisdata1)$estimate
BisXP1 <- RNAmeth.poisson.test(Bisdata1,lambda1,method="BH")
#
lambda2 <- RNAmeth.poisson.par(Bisdata2)$estimate
BisXP2 <- RNAmeth.poisson.test(Bisdata2,lambda2,method="BH")
#
```

```
lambda3 <- RNAmeth.poisson.par(Bisdata3)$estimate
BisXP3 <- RNAmeth.poisson.test(Bisdata3,lambda3,method="BH")

## Combined p-values ; median and standard error of
## bisulfite non-conversion ratio
BisXP.combined <- samples.combine(BisXP1,BisXP2,BisXP3)
```

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Bisdata	<i>Three synthetic samples of RNA bisulfite sequencing for examples and tests</i>
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## Description

A dataset containing the RNA names, C position, coverage and non-conversion ratio for three synthetic samples of a RNA bisulfite sequencing experiment.

## Usage

```
data(Bisdata)
```

## Format

Three data frames with 330 to 345 rows and the 4 following variables:

**RNA** RNA name or identifier

**Cpos** Cytosine position in RNA

**coverage** Number of available reads at Cpos

**ncratio** Bisulfite non-conversion ratio at Cpos

## Examples

```
data(Bisdata)
ls(pattern="Bisdata")
head(Bisdata1)
```

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Bisdata1	<i>Synthetic sample 1 of RNA bisulfite sequencing</i>
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**Description**

A dataset containing the RNA names, C position, coverage and non-conversion ratio for synthetic sample 1 of a RNA bisulfite sequencing experiment.

**Usage**

```
data(Bisdata)
```

**Format**

Data frame with 330 to 345 rows and the 4 following variables:

**RNA** RNA name or identifier

**Cpos** Cytosine position in RNA

**coverage** Number of available reads at Cpos

**ncratio** Bisulfite non-conversion ratio at Cpos

**Examples**

```
data(Bisdata)
ls(pattern="Bisdata")
head(Bisdata1)
```

---

Bisdata2	<i>Synthetic sample 2 of RNA bisulfite sequencing</i>
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**Description**

A dataset containing the RNA names, C position, coverage and non-conversion ratio for synthetic sample 2 of a RNA bisulfite sequencing experiment.

**Usage**

```
data(Bisdata)
```

**Format**

Data frame with 330 to 345 rows and the 4 following variables:

**RNA** RNA name or identifier

**Cpos** Cytosine position in RNA

**coverage** Number of available reads at Cpos

**ncratio** Bisulfite non-conversion ratio at Cpos

**Examples**

```
data(Bisdata)
ls(pattern="Bisdata")
head(Bisdata2)
```

---

**Bisdata3***Synthetic sample 3 of RNA bisulfite sequencing*

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**Description**

A dataset containing the RNA names, C position, coverage and non-conversion ratio for synthetic sample 3 of a RNA bisulfite sequencing experiment.

**Usage**

```
data(Bisdata)
```

**Format**

Data frame with 330 to 345 rows and the 4 following variables:

**RNA** RNA name or identifier

**Cpos** Cytosine position in RNA

**coverage** Number of available reads at Cpos

**ncratio** Bisulfite non-conversion ratio at Cpos

**Examples**

```
data(Bisdata)
ls(pattern="Bisdata")
head(Bisdata3)
```

---

**class.BisXP***Cast bisulfite experiment data into a BisXP object*

---

**Description**

`class.BisXP` creates a `BisXP` object from a table containing RNA name, C position, bisulfite non-conversion ratio and adjusted p-value.

**Usage**

```
class.BisXP(BisData)
```

**Arguments**

BisData            A data frame with 4 columns:

- RNA name
- C position (integer, in [1,+Inf])
- bisulfite non-conversion ratio (numeric, in [0,1])
- pvalue.adj (numeric, in [0,1])

**Details**

This function takes bisulfite experiment data as an input table, performs checks, and casts the table into a BisXP object, with rows labelled after a RNA\_C.position pattern.

**Value**

If the input table is correct, then the output will be a BisXP object corresponding to a consolidated data frame with rows labelled after a RNA\_C.position pattern.

**Examples**

```
RNA      <- c("NM_00001", "NM_00001", "NM_00002")
Cpos     <- as.integer(c(1,5,1))
ncratio  <- c(0.1,0.5,0.3)
pv.adj   <- c(0.001,0.1,0.3)
BSdata   <- data.frame(RNA, Cpos, ncratio, pv.adj, stringsAsFactors = FALSE)
bsXP     <- class.BisXP(BSdata)
```

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fisher.method	<i>Function implementing Fisher's method to combine independent p-values</i>
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**Description**

fisher.method takes a list of independent p-values and combines them using Fisher's method.

**Usage**

```
fisher.method(pvalues)
```

**Arguments**

pvalues            A list of p-values

**Details**

This function takes a list of p-values as input, determines the number of degrees of freedom (2 \* number of p-values), combines the p-values using Fisher's method and returns the combined p-value in output.

**Value**

The p-value combined using Fisher's method.

**References**

Fisher RA (1925) Statistical Methods for Research Workers. Edinburg: Oliver and Boyd.

Fisher RA (1948) Questions and Answers #14. In: Mosteller F, Fisher RA (1948) The American Statistician, 2:30-31 <http://www.jstor.org/stable/2681650>

**Examples**

```
list.pv <- c(0.0001,0.0142,0.0150)
p.combined <- fisher.method(list.pv)
```

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intersectMatrix	<i>Take intersection of 2 tables</i>
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**Description**

intersectMatrix takes 2 matrices and outputs their intersection based on common row.names.

**Usage**

```
intersectMatrix(Tab1, Tab2)
```

**Arguments**

Tab1            A matrix or data frame with defined row.names

Tab2            A matrix or data frame with defined row.names

**Details**

This function takes 2 matrices as input, determines the intersection of their row names, and returns a single matrix containing the rows in the intersection and concatenated columns of the initial matrices.

**Value**

A matrix with rows common to both Tab1 and Tab2, and concatenated columns.

---

read.BisXP	<i>Read RNA bisulfite experiment data and cast it into a BisXP object</i>
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## Description

read.BisXP reads a table containing RNA name, C position, bisulfite non-conversion ratio and adjusted p-value from a file and casts it into a BisXP object.

## Usage

```
read.BisXP(filename)
```

## Arguments

filename	Address of the file containing data from a bisulfite experiment in 4 columns separated by a tabulation, with header on the first line: <ul style="list-style-type: none"><li>• RNA name</li><li>• C position (integer, in [1,+Inf])</li><li>• bisulfite non-conversion ratio (numeric, in [0,1])</li><li>• pvalue.adj (numeric, in [0,1])</li></ul>
----------	---

## Details

This function takes a file name as input, reads the bisulfite data table contained in this file, performs checks, and casts the data into a BisXP object, which contain input data with rows labelled after a RNA\_C.position pattern.

## Value

If the input table is correct, then the output will be a BisXP object corresponding to a consolidated data frame with rows labelled after a RNA\_C.position pattern.

## Examples

```
RNAs      <- c("NM_00001", "NM_00001", "NM_00002")
Cpos      <- c(1,5,1)
ncratio   <- c(0.1,0.5,0.3)
pv.adj    <- c(0.001,0.1,0.3)
BSdata    <- data.frame(RNAs, Cpos, ncratio, pv.adj)
```

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RNAmeth.poisson.par	<i>Find ratio (Poisson parameter / coverage) for one sample of bisulfite-converted RNA</i>
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## Description

RNAmeth.poisson.par determines the ratio (Poisson parameter / coverage), based on the assumption that bisulfite-treated RNA's non-conversion ratios follow a Poisson distribution.

## Usage

```
RNAmeth.poisson.par(BSRna)
```

## Arguments

BSRna            A dataframe containing RNA name, C position, coverage and non-conversion ratio, in this order.

## Details

This function takes RNA bisulfite sequencing data from one sample as input, restricts the dataset to RNAs for which coverage is at least 10, and divides data into coverage bins. Then, the Poisson parameter is estimated at each coverage bin using `fitdistr` from package `MASS`. The ratio (Poisson parameter / coverage) (median and 95% confidence interval) is returned in output.

## Value

This function returns a list containing:

**estimate** The ratio (Poisson parameter / coverage) (median taken over coverage bins)

**bca.ci** A confidence interval for the ratio (Poisson parameter / coverage) (bootstrap confidence interval of the median, type "bca")

## Examples

```
## Load data, find out ratio (Poisson parameter / coverage).
data(Bisdata,package="BisRNA")
lambda1 <- RNAmeth.poisson.par(Bisdata1)$estimate

## P-values adjusted for multiple testing, using either BH (here) or IHW method.
BisXP1 <- RNAmeth.poisson.test(Bisdata1,lambda1,method="BH")

## Print BisXP1
BisXP1

## Display BisXP1 as a data frame
BisXP1.df <- data.frame(BisXP1$nonconv.ratio, BisXP1$pv.adj, row.names=BisXP1$RNA.pos)
BisXP1.df
```

---

RNAmeth.poisson.test *Test RNA Cytosine methylation based on Poisson model distribution*

---

## Description

RNAmeth.poisson.test tests RNA bisulfite sequencing non-conversion based on a sample and the ratio (Poisson parameter / coverage) for this sample.

## Usage

```
RNAmeth.poisson.test(BisRNA, lambda, method="BH")
```

## Arguments

BisRNA	A dataframe containing RNA name, C position, coverage and non-conversion ratio
lambda	Ratio (Poisson parameter / coverage)
method	Adjustment method for multiple testing, either "BH" (Benjamini-Hochberg) or "IHW" (Independent Hypothesis Weighting, from R package IHW)

## Details

This function takes RNA bisulfite sequencing data from one sample and the ratio (Poisson parameter / coverage) as inputs. Then, the dataset is restricted to those C positions where non-conversion ratio is larger than (Poisson parameter / coverage). Finally, it carries out poisson.test and a correction for multiple testing. The output of function RNAmeth.poisson.test is a BisXP object which contains RNA names, C positions, non-conversion ratios and adjusted p-values. The formatting into a BisXP object guarantees that some consistency checks are satisfied.

## Value

This function returns a BisXP object whose elements correspond to RNAs where non-conversion ratio is higher than  $\lambda = \text{ratio (Poisson parameter / coverage)}$ . The variables contained in this object are the non-conversion ratio and the adjusted p-value.

## Examples

```
## Load data, find out ratio Poisson parameter / coverage,
## and produce corresponding adjusted p-values.
data(Bisdata,package="BisRNA")
lambda1 <- RNAmeth.poisson.par(Bisdata1)$estimate
BisXP1 <- RNAmeth.poisson.test(Bisdata1,lambda1,method="BH")

## Print BisXP1
BisXP1

## Display BisXP1 as a data frame
BisXP1.df <- data.frame(BisXP1$nonconv.ratio, BisXP1$pv.adj, row.names=BisXP1$RNA.pos)
```

```
BisXP1.df
```

---

samples.combine	<i>Combine samples p-values and ratios</i>
-----------------	--

---

## Description

samples.combine takes RNA bisulfite sequencing samples as input, combines their p-values using Fisher's method, and produces median and standard error of the bisulfite non-conversion ratios.

## Usage

```
samples.combine(BisXP1, ...)
```

## Arguments

BisXP1	A BisXP object containing non-conversion ratio and p-value
...	One or more additional samples, in the form of BisXP objects

## Details

This function takes several bisulfite sequencing samples, in form of BisXP objects, as inputs. It is recommended to provide at least 3 samples and in any case all available, relevant samples. Using RNA and C positions present in all samples, the adjusted p-values of each sample are combined using Fisher's method. Median and standard error of the non-conversion ratio are also given in output.

## Value

This function returns a data frame whose row names correspond to the RNA and C position which are present in all samples, and the following variables:

**p.adj.combined** p-value adjusted (done in the preparation of the BisXP object) and combined (done here)

**nonconv.ratio.med** Median of bisulfite non-conversion ratio for a specific RNA and C positions

**nonconv.ratio.se** Standard error of bisulfite non-conversion ratio for a specific RNA and C positions

## References

Fisher RA (1925) Statistical Methods for Research Workers. Edinburg: Oliver and Boyd.

Fisher RA (1948) Questions and Answers #14. In: Mosteller F, Fisher RA (1948) The American Statistician, 2:30-31 <http://www.jstor.org/stable/2681650>

## Examples

```
## Load data
data(Bisdata,package="BisRNA")

## Obtain the ratio (Poisson parameter / coverage), and p-values
## adjusted for multiple testing using BH (here) or IHW method.
lambda1 <- RNAmeth.poisson.par(Bisdata1)$estimate
BisXP1 <- RNAmeth.poisson.test(Bisdata1,lambda1,method="BH")
lambda2 <- RNAmeth.poisson.par(Bisdata2)$estimate
BisXP2 <- RNAmeth.poisson.test(Bisdata2,lambda2,method="BH")
lambda3 <- RNAmeth.poisson.par(Bisdata3)$estimate
BisXP3 <- RNAmeth.poisson.test(Bisdata3,lambda3,method="BH")

## Combine samples
BisXP.combined <- samples.combine(BisXP1,BisXP2,BisXP3)
```

---

testMeth

*Apply poisson.test to BS coverage and non-conversion ratio.*

---

## Description

testMeth applies `poisson.test` to a sample from bisulfite-treated RNA experiment taking into account the ratio (Poisson parameter / coverage) characteristic of this sample.

## Usage

```
testMeth(X, lambda)
```

## Arguments

X	A list containing coverage as 1st element and non-conversion ratio as 2nd element, for one C position.
lambda	ratio (Poisson parameter / coverage)

## Details

This function takes RNA bisulfite sequencing coverage and non-conversion ratio, applies `poisson.test` and outputs the p-value.

## Value

This function returns the p-value from `poisson.test`.

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