

# Package ‘SOMNiBUS’

September 16, 2021

**Title** Smooth modeling of bisulfite sequencing

**Version** 1.0.0

**Description** This package aims to analyse count-based methylation data on predefined genomic regions, such as those obtained by targeted sequencing, and thus to identify differentially methylated regions (DMRs) that are associated with phenotypes or traits. The method is built a rich flexible model that allows for the effects, on the methylation levels, of multiple covariates to vary smoothly along genomic regions. At the same time, this method also allows for sequencing errors and can adjust for variability in cell type mixture.

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**URL** <https://github.com/kaiqiong/SOMNiBUS>

**BugReports** <https://github.com/kaiqiong/SOMNiBUS/issues>

**Depends** R (>= 4.1.0)

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binomRegMethModel	<i>A smoothed-EM algorithm to estimate covariate effects and test regional association in Bisulfite Sequencing-derived methylation data</i>
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### Description

This function fits a (dispersion-adjusted) binomial regression model to regional methylation data, and reports the estimated smooth covariate effects and regional p-values for the test of DMRs (differentially methylation regions). Over or under dispersion across loci is accounted for in the model by the combination of a multiplicative dispersion parameter (or scale parameter) and a sample-specific random effect.

This method can deal with outcomes, i.e. the number of methylated reads in a region, that are contaminated by known false methylation calling rate ( $p_0$ ) and false non-methylation calling rate ( $1-p_1$ ).

The covariate effects are assumed to smoothly vary across genomic regions. In order to estimate them, the algorithm first represents the functional parameters by a linear combination of a set of restricted cubic splines (with dimension  $n.k$ ), and a smoothness penalization term which depends on the smoothing parameters  $\lambda$  is also added to control smoothness. The estimation is performed by an iterated EM algorithm. Each M step constitutes an outer Newton's iteration to estimate smoothing parameters  $\lambda$  and an inner P-IRLS iteration to estimate spline coefficients  $\alpha$  for the covariate effects. Currently, the computation in the M step depends the implementation of `gam()` in package `mgcv`.

### Usage

```
binomRegMethModel(
  data,
  n.k,
  p0 = 0.003,
  p1 = 0.9,
  Quasi = TRUE,
  epsilon = 10^(-6),
  epsilon.lambda = 10^(-3),
  maxStep = 200,
  detail = FALSE,
  binom.link = "logit",
```

```

method = "REML",
covs = NULL,
RanEff = TRUE,
reml.scale = FALSE,
scale = -2
)

```

## Arguments

<code>data</code>	a data frame with rows as individual CpGs appeared in all the samples. The first 4 columns should contain the information of <code>Meth_Counts</code> (methylated counts), <code>Total_Counts</code> (read depths), <code>Position</code> (Genomic position for the CpG site) and <code>ID</code> (sample ID). The covariate information, such as disease status or cell type composition, are listed in column 5 and onwards.
<code>n.k</code>	a vector of basis dimensions for the intercept and individual covariates. <code>n.k</code> specifies an upper limit of the degrees of each functional parameters.
<code>p0</code>	the probability of observing a methylated read when the underlying true status is unmethylated. <code>p0</code> is the rate of false methylation calls, i.e. false positive rate.
<code>p1</code>	the probability of observing a methylated read when the underlying true status is methylated. <code>1-p1</code> is the rate of false non-methylation calls, i.e. false negative rate.
<code>Quasi</code>	whether a Quasi-likelihood estimation approach will be used; in other words, whether a multiplicative dispersion is added in the model or not.
<code>epsilon</code>	numeric; stopping criterion for the closeness of estimates of spline coefficients from two consecutive iterations.
<code>epsilon.lambda</code>	numeric; stopping criterion for the closeness of estimates of smoothing parameter <code>lambda</code> from two consecutive iterations.
<code>maxStep</code>	the algorithm will stop if the iteration steps exceed <code>maxStep</code>
<code>detail</code>	indicate whether print the number of iterations
<code>binom.link</code>	the link function used in the binomial regression model; the default is the logit link
<code>method</code>	the method used to estimate the smoothing parameters. The default is the 'REML' method which is generally better than prediction based criterion <code>GCV.cp</code>
<code>covs</code>	a vector of covariate names. The covariates with names in <code>covs</code> will be included in the model and their covariate effects will be estimated. The default is to fit all covariates in <code>data</code>
<code>RanEff</code>	whether sample-level random effects are added or not
<code>reml.scale</code>	whether a REML-based scale (dispersion) estimator is used. The default is Fletcher-based estimator
<code>scale</code>	negative values mean scale parameter should be estimated; if a positive value is provided, a fixed scale will be used.

**Value**

This function return a list including objects:

- `est`: estimates of the spline basis coefficients `alpha`
- `lambda`: estimates of the smoothing parameters for each functional parameters
- `est.pi`: predicted methylation levels for each row in the input data
- `ite.points`: estimates of `est`, `lambda` at each EM iteration
- `cov1`: estimated variance-covariance matrix of the basis coefficients `alphas`
- `reg.out`: regional testing output obtained using Fletcher-based dispersion estimate; an additional 'ID' row would appear if `RanEff` is TRUE
- `reg.out.reml.scale`: regional testing output obtained using REML-based dispersion estimate;
- `reg.out.gam`: regional testing output obtained using (Fletcher-based) dispersion estimate from `mgev` package;
- `phi_fletcher`: Fletcher-based estimate of the (multiplicative) dispersion parameter
- `phi_reml`: REML-based estimate of the (multiplicative) dispersion parameter
- `phi_gam`: Estimated dispersion parameter reported by `mgev`
- `SE.out`: a matrix of the estimated pointwise Standard Errors (SE); number of rows are the number of unique CpG sites in the input data and the number of columns equal to the total number of covariates fitted in the model (the first one is the intercept)
- `SE.out.REML.scale`: a matrix of the estimated pointwise Standard Errors (SE); the SE calculated from the REML-based dispersion estimates
- `uni.pos`: the genomic positions for each row of CpG sites in the matrix `SE.out`
- `Beta.out`: a matrix of the estimated covariate effects `beta(t)`, here `t` denotes the genomic positions.
- `ncovs`: number of functional parameters in the model (including the intercept)
- `sigma00`: estimated variance for the random effect if `RanEff` is TRUE; NA if `RanEff` is FALSE

**Author(s)**

Kaiqiong Zhao

**See Also**

[gam](#)

**Examples**

```
#-----#
data(RAdat)
head(RAdat)
RAdat.f <- na.omit(RAdat[RAdat$Total_Counts != 0, ])
out <- binomRegMethModel(
  data=RAdat.f, n.k=rep(5, 3), p0=0.003307034, p1=0.9,
  epsilon=10^(-6), epsilon.lambda=10^(-3), maxStep=200,
  detail=FALSE
)
```

---

binomRegMethModelPlot *Plot the smooth covariate effect*

---

## Description

This function accepts an output object from function `binomRegMethModel` and print out a plot of the estimated covariate effect across the region for each test covariate.

## Usage

```
binomRegMethModelPlot(BEM.obj, mfrow = NULL, same.range = FALSE)
```

## Arguments

<code>BEM.obj</code>	an output object from function <code>binomRegMethModel</code>
<code>mfrow</code>	the plot parameters to specify the layout of each plot
<code>same.range</code>	specify whether the plots should be in the same vertical scale

## Value

This function prints out a plot of smooth covariate effects and its pointwise confidence intervals

## Author(s)

Kaiqiong Zhao

## Examples

```
#-----#
head(RAdat)
RAdat.f <- na.omit(RAdat[RAdat$Total_Counts != 0, ])
out <- binomRegMethModel(
  data=RAdat.f, n.k=rep(5, 3), p0=0.003307034, p1=0.9,
  epsilon=10-6, epsilon.lambda=10-3, maxStep=200, detail=FALSE,
  Quasi = FALSE, RanEff = FALSE
)
binomRegMethModelPlot(out, same.range=FALSE)
```

---

binomRegMethModelPred *A smoothed-EM algorithm to estimate covariate effects and test regional association in Bisulfite Sequencing-derived methylation data*

---

## Description

This function returns the predicted methylation levels

## Usage

```
binomRegMethModelPred(BEM.obj, newdata = NULL, type = "proportion")
```

## Arguments

BEM.obj	an output from the function binomRegMethModel
newdata	the data set whose predictions are calculated; with columns 'Position', and covariate names that can be matched to the BEM.obj
type	return the predicted methylation proportion or the predicted response (in logit or other binom.link scale)

## Value

This function returns the predicted methylation levels

## Author(s)

Kaiqiong Zhao

## Examples

```
#-----#
head(RAdat)
RAdat.f <- na.omit(RAdat[RAdat$Total_Counts != 0, ])
out <- binomRegMethModel(
  data=RAdat.f, n.k=rep(5, 3), p0=0.003307034, p1=0.9,
  epsilon=10^(-6), epsilon.lambda=10^(-3), maxStep=200, detail=FALSE,
  Quasi = FALSE, RanEff = FALSE
)
binomRegMethModelPred(out)
```

---

binomRegMethModelSim *Simulate Bisulfite sequencing data from specified smooth covariate effects*

---

### Description

Simulate Bisulfite sequencing data from a Generalized Additive Model with functional parameters varying with the genomic position. Both the true methylated counts and observed methylated counts are generated, given the error/conversion rate parameters  $p_0$  and  $p_1$ . In addition, the true methylated counts can be simulated from a binomial or a dispersed binomial distribution (Beta-binomial distribution).

### Usage

```
binomRegMethModelSim(
  n,
  posit,
  theta.0,
  beta,
  phi,
  random.eff = FALSE,
  mu.e = 0,
  sigma.ee = 1,
  p0 = 0.003,
  p1 = 0.9,
  X,
  Z,
  binom.link = "logit"
)
```

### Arguments

n	sample size
posit	genomic position; a numeric vector of size p (the number of CpG sites in the considered region).
theta.0	a functional parameter for the intercept of the GAMM model; a numeric vector of size p.
beta	a functional parameter for the slope of cell type composition. a numeric vector of size p
phi	multiplicative dispersion parameter for each loci in a region. a vector of length p. The dispersed-Binomial counts are simulated from beta-binomial distribution, so each element of phi has to be greater than 1.
random.eff	indicate whether adding the subject-specific random effect term e.
mu.e	the mean of the random effect; a single number.
sigma.ee	variance of the random effect; a single positive number.

$p_0$	the probability of observing a methylated read when the underlying true status is unmethylated. $p_0$ is the rate of false methylation calls, i.e. false positive rate.
$p_1$	the probability of observing a methylated read when the underlying true status is methylated. $1-p_1$ is the rate of false non-methylation calls, i.e. false negative rate.
$X$	the matrix of the read coverage for each CpG in each sample; a matrix of $n$ rows and $p$ columns
$Z$	numeric matrix with $p$ columns and $n$ rows storing the covariate information
<code>binom.link</code>	the link function used for simulation

### Value

The function returns a list of following objects

- $S$  the true methylation counts; a numeric matrix of  $n$  rows and  $p$  columns
- $Y$  the observed methylation counts; a numeric matrix of  $n$  rows and  $p$  columns
- $\theta$  the methylation parameter (after the logit transformation); a numeric matrix of  $n$  rows and  $p$  columns
- $\pi$  the true methylation proportions used to simulate the data; a numeric matrix of  $n$  rows and  $p$  columns

### Author(s)

Kaiqiong Zhao

### Examples

```
#-----#
data(RAdat)
RAdat.f <- na.omit(RAdat[RAdat$Total_Counts != 0, ])
out <- binomRegMethModel(
  data=RAdat.f, n.k=rep(5, 3), p0=0, p1=1,
  epsilon=10^(-6), epsilon.lambda=10^(-3), maxStep=200,
  detail=FALSE, RanEff = FALSE
)
Z = as.matrix(RAdat.f[match(unique(RAdat.f$ID), RAdat.f$ID),
c('T_cell', 'RA')])
set.seed(123)
X = matrix(sample(80, nrow(Z)*length(out$uni.pos), replace = TRUE),
nrow = nrow(Z), ncol = length(out$uni.pos))+10
simdat = binomRegMethModelSim(n=nrow(Z), posit= out$uni.pos,
theta.0=out$Beta.out[,1], beta= out$Beta.out[,-1], random.eff=FALSE,
mu.e=0,sigma.ee=1, p0=0.003, p1=0.9,X=X , Z=Z, binom.link='logit',
phi = rep(1, length(out$uni.pos)))
```



---

RAdat

*Methylation data from a rheumatoid arthritis study*

---

### Description

A dataset containing methylation levels on one targeted region on chromosome 4 near gene BANK1 from cases with rheumatoid arthritis (RA) and controls

### Usage

RAdat

### Format

A data frame of 5289 rows and 6 columns. Each row represents a CpG site for a sample. Columns include in order

**Meth\_Counts** Number of methylated reads

**Total\_Counts** Total number of reads; read-depth

**Position** Genomic position (in bp) for the CpG site

**ID** indicates which sample the CpG site belongs to

**T\_cell** whether a sample is from T cell or monocyte

**RA** whether a sample is an RA patient or control

### Details

This example data include methylation levels of cell type separated blood samples of 22 rheumatoid arthritis (RA) patients and 21 healthy individuals. In the data set, 123 CpG sites are measured and there are 25 samples from circulating T cells and 18 samples from monocytes.

### Source

Dr. Marie Hudson (McGill University)

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RAdat2

*A simulated methylation dataset based on a real data.*

---

### Description

This example data include methylation levels on a region with 208 CpGs for 116 blood samples.

### Usage

RAdat2

**Format**

A data frame of 6064 rows and 13 columns. Each row represents a CpG site for a sample. Columns include in order

**Meth\_Counts** Number of methylated reads

**Total\_Counts** Total number of reads; read-depth

**Position** Genomic position (in bp) for the CpG site

**ID** indicates which sample the CpG site belongs to

**ACPA4** binary indicator for a biomarker anti-citrullinated protein antibody

**Age** Age

**Sex** 2-female; 1-male

**Smoking** 1-current or ex-smoker; 0-non-smoker

**Smoking\_NA** 1-Smoking info is NA; 0-Smoking info is available

**PC1** PC1 for the cell type proportions

**PC2** PC2 for the cell type proportions

**PC3** PC3 for the cell type proportions

**PC4** PC4 for the cell type proportions

**Source**

simulation is based a real data set provided by PI Dr. Sasha Bernatsky (McGill University)

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