Package ‘hummingbird’

May 29, 2024

Type Package

Title Bayesian Hidden Markov Model for the detection of differentially methylated regions

Version 1.14.0

Description A package for detecting differential methylation. It exploits a Bayesian hidden Markov model that incorporates location dependence among genomic loci, unlike most existing methods that assume independence among observations. Bayesian priors are applied to permit information sharing across an entire chromosome for improved power of detection. The direct output of our software package is the best sequence of methylation states, eliminating the use of a subjective, and most of the time an arbitrary, threshold of p-value for determining significance. At last, our methodology does not require replication in either or both of the two comparison groups.

License GPL (>=2)

Depends R (>= 4.0)

Encoding UTF-8

LazyData true

Imports Rcpp, graphics, GenomicRanges, SummarizedExperiment, IRanges

Suggests knitr, rmarkdown, BiocStyle

LinkingTo Rcpp

biocViews HiddenMarkovModel, Bayesian, DNA Methylation, BiomedicalInformatics, Sequencing, GeneExpression, DifferentialExpression, DifferentialMethylation

VignetteBuilder knitr

git_url https://git.bioconductor.org/packages/hummingbird

git_branch RELEASE_3_19

git_last_commit a78fbf8

git_last_commit_date 2024-04-30

Repository Bioconductor 3.19

Date/Publication 2024-05-29
hummingbird-package

**Author**  Eleni Adam [aut, cre],
Tieming Ji [aut],
Desh Ranjan [aut]

**Maintainer**  Eleni Adam <eadam002@odu.edu>

**Contents**

hummingbird-package ............................................ 2
abnormM .......................................................... 3
abnormUM ......................................................... 4
exampleHummingbird ........................................... 4
e.exampleSECase ............................................... 5
exampleSEControl .............................................. 6
hummingbirdEM .................................................. 7
hummingbirdEMInternal ...................................... 8
hummingbirdGraph .............................................. 9
hummingbirdPostAdjustment ................................ 10
hummingbirdPostAdjustmentInternal ...................... 11
normM ............................................................ 13
normUM .......................................................... 13
pos ................................................................. 14

**Index**

15

hummingbird-package  *A Bayesian Hidden Markov Model for the detection of differentially methylated regions*

**Description**

A package for identifying differentially methylated regions (DMRs) between case and control groups using whole genome bisulfite sequencing (WGBS) or reduced representative bisulfite sequencing (RRBS) experiment data.

The hummingbird package uses a Bayesian hidden Markov model (HMM) for detecting DMRs. It fits a Bayesian HMM for one chromosome at a time. The final output of hummingbird are the detected DMRs with start and end positions in a given chromosome, directions of the DMRs (hyper- or hypo-), and the numbers of CpGs in these DMRs.

The hummingbird package implements the algorithm described in the publication below.

**Details**

The main functions of the package are: hummingbirdEM, hummingbirdPostAdjustment and hummingbirdGraph.
**abnormM**

**Author(s)**

Eleni Adam, Tieming Ji, Desh Ranjan
Maintainer: Eleni Adam <eadam002@odu.edu>

**References**


---

**Sample matrix**

**Description**

A matrix containing the methylated read count data of the case group. It is part of the sample dataset exampleHummingbird.

**Usage**

`abnormM`

**Details**

Each column of the matrix represents a replicate and each row represents a CpG position.

**Source**

Chen et al. (2017) Global misregulation of genes largely uncoupled to DNA methylome epimutations characterizes a congenital overgrowth syndrome. Scientific Reports 7, 12667

The raw FASTQ files of the WGBS experiment from this study are publicly available at Gene Expression Omnibus (GEO) database with accession no. GSE93775.

**Examples**

```r
data(exampleHummingbird)
abnormM
```
AbnormalUM

**Sample matrix**

**Description**

A matrix containing the unmethylated read count data of the case group. It is part of the sample dataset exampleHummingbird.

**Usage**

abnormUM

**Details**

Each column of the matrix represents a replicate and each row represents a CpG position.

**Source**

Chen et al. (2017) Global misregulation of genes largely uncoupled to DNA methylome epimutations characterizes a congenital overgrowth syndrome. Scientific Reports 7, 12667

The raw FASTQ files of the WGBS experiment from this study are publicly available at Gene Expression Omnibus (GEO) database with accession no. GSE93775.

**Examples**

```r
data(exampleHummingbird)
abnormUM
```

---

**ExampleHummingbird**

**Sample dataset**

**Description**

Example of input data for the hummingbird package.

The sample dataset is partial data of chromosome 29 in the large offspring syndrome (LOS) study described in Chen Z. et al (2017).

**Usage**

```r
data("exampleHummingbird")
```
exampleSECase

Format

**experimentSEControl**  A SummarizedExperiment object containing the input data for the control group: The two assays: normM, normUM and the CpG position information: pos.

**experimentSECase**  A SummarizedExperiment object containing the input data for the case group: The two assays: abnormM, abnormUM and the CpG position information: pos.

**normM**  A matrix containing the methylated read count data of the control group. Each column of the matrix represents a replicate and each row represents a CpG position.

**normUM**  A matrix containing the unmethylated read count data of the control group. Each column of the matrix represents a replicate and each row represents a CpG position.

**abnormM**  A matrix containing the methylated read count data of the case group. Each column of the matrix represents a replicate and each row represents a CpG position.

**abnormUM**  A matrix containing the unmethylated read count data of case group. Each column of the matrix represents a replicate and each row represents a CpG position.

**pos**  The CpG positions.

Source

Chen et al. (2017) Global misregulation of genes largely uncoupled to DNA methylome epimutations characterizes a congenital overgrowth syndrome. Scientific Reports 7, 12667

The raw FASTQ files of the WGBS experiment from this study are publicly available at Gene Expression Omnibus (GEO) database with accession no. GSE93775.

Examples

```r
library(SummarizedExperiment)
data(exampleHummingbird)

exampleSECase

---

Sample input data

---

Description

A SummarizedExperiment object containing the input data for the case group. It is part of the sample dataset exampleHummingbird.

Usage

exampleSECase

Details

It contains the two assays: abnormM, abnormUM and the CpG position information: pos.
Source

Chen et al. (2017) Global misregulation of genes largely uncoupled to DNA methylome epimutations characterizes a congenital overgrowth syndrome. Scientific Reports 7, 12667

The raw FASTQ files of the WGBS experiment from this study are publicly available at Gene Expression Omnibus (GEO) database with accession no. GSE93775.

Examples

```r
library(SummarizedExperiment)
data(exampleHummingbird)
exampleSECase
```

<table>
<thead>
<tr>
<th>exampleSEControl</th>
<th>Sample input data</th>
</tr>
</thead>
</table>

Description

A SummarizedExperiment object containing the input data for the control group. It is part of the sample dataset exampleHummingbird.

Usage

```r
eexampleSEControl
```

Details

It contains the two assays: normM, normUM and the CpG position information: pos.

Source

Chen et al. (2017) Global misregulation of genes largely uncoupled to DNA methylome epimutations characterizes a congenital overgrowth syndrome. Scientific Reports 7, 12667

The raw FASTQ files of the WGBS experiment from this study are publicly available at Gene Expression Omnibus (GEO) database with accession no. GSE93775.

Examples

```r
library(SummarizedExperiment)
data(exampleHummingbird)
exampleSEControl
```
Description

This function reads input data, sets initial values, executes the Expectation-Maximization (EM) algorithm for the Bayesian HMM and infers the best sequence of methylation states.

Usage

hummingbirdEM(experimentInfoControl, experimentInfoCase, binSize)

Arguments

experimentInfoControl
A SummarizedExperiment object containing the input data for the control group:
The two assays: normM, normUM and the CpG position information: pos.
normM is a matrix containing the methylated read count data of the normal group and normUM is a matrix containing the unmethylated read count data of the normal group. Each column of a matrix represents a replicate and each row represents a CpG position.

experimentInfoCase
A SummarizedExperiment object containing the input data for the case group:
The two assays: abnormM, abnormUM and the CpG position information: pos.
abnormM is a matrix containing the methylated read count data of the abnormal group and abnormUM is a matrix containing the unmethylated read count data of the abnormal group. Each column of a matrix represents a replicate and each row represents a CpG position.

binSize
The size of a bin. Default value is: 40.

Value

A GenomicRanges object that contains the start and end positions of each bin, the distance between the current bin the bin ahead of it, the average methylation rate of normal and abnormal groups and the predicted direction of methylation change ("0" means a predicted normal bin; "1" means a predicted hypermethylated bin; "-1" means a predicted hypomethylated bin).

Examples

library(GenomicRanges)
library(SummarizedExperiment)
data(exampleHummingbird)

# CpG position vector
pos <- pos[,1]
# Assays for the normal group
assaysControl <- list(normM = normM, normUM = normUM)
Assays for the case group

assaysCase <- list(abnormM = abnormM, abnormUM = abnormUM)

# SummarizedExperiment objects
exampleSEControl <- SummarizedExperiment(assaysControl, rowRanges = GPos("chr29", pos))
exampleSECase <- SummarizedExperiment(assaysCase, rowRanges = GPos("chr29", pos))

emInfo <- hummingbirdEM(experimentInfoControl = exampleSEControl, experimentInfoCase = exampleSECase, binSize = 40)

hummingbirdEMinternal

**EM Algorithm (internal)**

**Description**

Expectation-Maximization Algorithm for Fitting the Hidden Markov Model. This function reads in methylated and unmethylated read count data, transforms it into logarithm bin-wise data, sets up initial values and implements the EM algorithm to estimate HMM parameters and find the best sequence of hidden states based on model fitting.

**Usage**

hummingbirdEMinternal(normM, normUM, abnormM, abnormUM, pos, binSize)

**Arguments**

- **normM**: A matrix containing the methylated read count data of the normal group. Each column of a matrix represents a replicate and each row represents a CpG position.
- **normUM**: A matrix containing the unmethylated read count data of the normal group. Each column of a matrix represents a replicate and each row represents a CpG position.
- **abnormM**: A matrix containing the methylated read count data of the abnormal group. Each column of a matrix represents a replicate and each row represents a CpG position.
- **abnormUM**: A matrix containing the unmethylated read count data of the abnormal group. Each column of a matrix represents a replicate and each row represents a CpG position.
- **pos**: The CpG position information.
- **binSize**: The size of a bin.

**Details**

Users do not need to call this function directly. This is a low-level function used by the higher-level function in the hummingbird package, the hummingbirdEM.
**Value**

- **obs**
  For each bin: The predicted direction of methylation change ("0" means a predicted normal bin; "1" means a predicted hypermethylated bin; "-1" means a predicted hypomethylated bin). The distance between the current bin and the bin ahead of it, the start and end positions of each bin.

- **normAbnorm**
  The average methylation rate of normal and abnormal groups.

**See Also**

Users may call the `hummingbirdEM` function.

**Examples**

```r
library(GenomicRanges)
library(SummarizedExperiment)

# Load sample dataset containing input data
data(exampleHummingbird)

# Run the EM (internal) function
hmmbirdEMinternalOutput <- hummingbirdEMinternal(
  normM = assays(exampleSEControl)[["normM"]],
  normUM = assays(exampleSEControl)[["normUM"]],
  abnormM = assays(exampleSECase)[["abnormM"]],
  abnormUM = assays(exampleSECase)[["abnormUM"]],
  pos = pos, binSize = 40)
```

**hummingbirdGraph**

**Observations and Predictions Graphs**

**Description**

This function generates observation and prediction graphs for a user specified region.

**Usage**

```r
hummingbirdGraph(experimentInfoControl, experimentInfoCase, postAdjInfoDMRs, coord1, coord2)
```

**Arguments**

- **experimentInfoControl**
  A SummarizedExperiment object containing the input data for the control group:
  The two assays: normM, normUM and the CpG position information: pos.

- **experimentInfoCase**
  A SummarizedExperiment object containing the input data for the case group:
  The two assays: abnormM, abnormUM and the CpG position information: pos.
The DMRs GenomicRanges object output of the hummingbirdPostAdjustment function.

coord1 The start coordinate of the genomic region to plot.
coord2 The end coordinate of the genomic region to plot.

Value

The function outputs two graphs: The Observations graph and the Predictions graph. The observation figure shows bin-wise average methylation rate for case and control groups. The prediction figure shows bin-wise prediction, where "0" denotes a predicted normal bin; "1" denotes a predicted hypermethylated bin; and "-1" denotes a predicted hypomethylated bin.

Examples

```r
library(GenomicRanges)
library(SummarizedExperiment)
data(exampleHummingbird)
emInfo <- hummingbirdEM(experimentInfoControl = exampleSEControl,
                         experimentInfoCase = exampleSECase, binSize = 40)
postAdjInfo <- hummingbirdPostAdjustment(
    experimentInfoControl = exampleSEControl, experimentInfoCase = exampleSECase, emInfo = emInfo, minCpGs = 10,
    minLength = 100, maxGap = 300)
hummingbirdGraph(experimentInfoControl = exampleSEControl,
                 experimentInfoCase = exampleSECase, postAdjInfoDMRs = postAdjInfo$DMRs,
                 coord1 = 107991, coord2 = 108350)
```

hummingbirdPostAdjustment

Post Adjustment algorithm for the output of the EM

Description

This function adjusts HMM output. It enables three additional requirements on DMRs: 1) the minimum length of a DMR, 2) the minimum number of CpGs in a DMR, and 3) the maximum distance (in base pairs) between any two adjacent CpGs in a DMR.

Usage

```r
hummingbirdPostAdjustment(experimentInfoControl, experimentInfoCase, emInfo,
                           minCpGs, minLength, maxGap)
```
hummingbirdPostAdjustmentInternal

Arguments

experimentInfoControl
A SummarizedExperiment object containing the input data for the control group:
The two assays: normM, normUM and the CpG position information: pos.

experimentInfoCase
A SummarizedExperiment object containing the input data for the case group:
The two assays: abnormM, abnormUM and the CpG position information: pos.

emInfo
The output GenomicRanges object of the hummingbirdEM function.

minCpGs
The minimum number of CpGs contained in a DMR. Default value: 10.

minLength
The minimum length of a DMR. Default value: 500.

maxGap
The maximum gap between any two CpGs. Default value: 300.

Value

A list of two GenomicRanges objects, the DMRs and the obsPostAdj.

DMRs
Contains the detected regions based on the user-defined arguments (minLength,
minCpGs, and maxGap). It contains the refined DMRs with the start genomic
position, the end genomic position, length of the region, direction of predicted
methylation change ("0" indicates no significant change, "1" indicates predicted
hyper-methylation, and "-1" indicates predicted hypo-methylation), and the num-
ber of CpGs.

obsPostAdj
The methylation status of each CpG site.

Examples

library(GenomicRanges)
l library(SummarizedExperiment)
data(exampleHummingbird)
emInfo <- hummingbirdEM(experimentInfoControl = exampleSEControl,
experimentInfoCase = exampleSECase, binSize = 40)
postAdjInfo <- hummingbirdPostAdjustment(
    experimentInfoControl = exampleSEControl,
    experimentInfoCase = exampleSECase,
    emInfo = emInfo, minCpGs = 10,
    minLength = 100, maxGap = 300)

hummingbirdPostAdjustmentInternal

Post Adjustment algorithm (internal)

Description

Post Adjustment algorithm for the output of the EM. This function adjusts HMM output such that
each detected DMR has a minimum length and a minimum number of CpGs in each DMR.
Usage

`hummingbirdPostAdjustmentInternal(em, pos, minCpGs, minLength, maxGap)`

Arguments

- **em**: The output of the `hummingbirdEMinternal` function, specifically the `obs` object.
- **pos**: The CpG position information.
- **minCpGs**: The minimum number of CpGs contained in a DMR.
- **minLength**: The minimum length of a DMR.
- **maxGap**: The maximum gap between any two CpGs.

Details

Users do not need to call this function directly. This is a low-level function used by the higher-level function in the `hummingbird` package, the `hummingbirdPostAdjustment` function.

Value

- **DMRs**: The detected regions based on the user-defined arguments (minLength, minCpGs, and maxGap).
  - It contains the (numbered) refined DMRs with the start genomic position, the end genomic position, length of the region, direction of predicted methylation change ("0" indicates no significant change, "1" indicates predicted hypermethylation, and "-1" indicates predicted hypo-methylation) and the number of CpGs.
- **obsPostAdj**: The methylation status of each CpG site.

See Also

Users may call the `hummingbirdPostAdjustment` function.

Examples

```r
library(GenomicRanges)
library(SummarizedExperiment)

# Load sample dataset containing input data
data(exampleHummingbird)

# Run the EM (internal) function
hmmbirdEMinternalOutput <- hummingbirdEMinternal(
  normM = assays(exampleSEControl)[["normM"]],
  normUM = assays(exampleSEControl)[["normUM"]],
  abnormM = assays(exampleSECase)[["abnormM"]],
  abnormUM = assays(exampleSECase)[["abnormUM"]],
  pos = pos, binSize = 40)

# Run the Post Adjustment (internal) function
hmmbirdPAinternalOutput <- hummingbirdPostAdjustmentInternal(
  em = hmmbirdEMinternalOutput[, "em"],
  pos = pos, minCpGs = 2, minLength = 1000, maxGap = 500)
```
### normM

\[
\text{em} = \text{hmmbirdEMinternalOutput}\$\text{obs}, \\
\text{pos} = \text{pos}, \text{minCpGs} = 10, \text{minLength} = 100, \text{maxGap} = 300
\]

<table>
<thead>
<tr>
<th>normM</th>
<th>Sample matrix</th>
</tr>
</thead>
</table>

**Description**

A matrix containing the methylated read count data of the control group. It is part of the sample dataset exampleHummingbird.

**Usage**

normM

**Details**

Each column of the matrix represents a replicate and each row represents a CpG position.

**Source**

Chen et al. (2017) Global misregulation of genes largely uncoupled to DNA methylene epimutations characterizes a congenital overgrowth syndrome. Scientific Reports 7, 12667

The raw FASTQ files of the WGBS experiment from this study are publicly available at Gene Expression Omnibus (GEO) database with accession no. GSE93775.

**Examples**

```r
data(exampleHummingbird)
normM
```

<table>
<thead>
<tr>
<th>normUM</th>
<th>Sample matrix</th>
</tr>
</thead>
</table>

**Description**

A matrix containing the unmethylated read count data of the control group. It is part of the sample dataset exampleHummingbird.

**Usage**

normUM

**Details**

Each column of the matrix represents a replicate and each row represents a CpG position.
Source

Chen et al. (2017) Global misregulation of genes largely uncoupled to DNA methylome epimutations characterizes a congenital overgrowth syndrome. Scientific Reports 7, 12667.

The raw FASTQ files of the WGBS experiment from this study are publicly available at Gene Expression Omnibus (GEO) database with accession no. GSE93775.

Examples

data(exampleHummingbird)

pos

pos Sample matrix

Description

The CpG positions. It is part of the sample dataset exampleHummingbird.

Usage

pos

Source

Chen et al. (2017) Global misregulation of genes largely uncoupled to DNA methylome epimutations characterizes a congenital overgrowth syndrome. Scientific Reports 7, 12667.

The raw FASTQ files of the WGBS experiment from this study are publicly available at Gene Expression Omnibus (GEO) database with accession no. GSE93775.

Examples

data(exampleHummingbird)

pos
Index

* datasets
  abnormM, 3
  abnormUM, 4
  exampleHummingbird, 4
  exampleSECase, 5
  exampleSEControl, 6
  normM, 13
  normUM, 13
  pos, 14

  abnormM, 3
  abnormUM, 4

  exampleHummingbird, 4
  exampleSECase, 5
  exampleSEControl, 6

  hummingbird (hummingbird-package), 2
  hummingbird-package, 2
  hummingbirdEM, 7, 9
  hummingbirdEMinternal, 8
  hummingbirdGraph, 9
  hummingbirdPostAdjustment, 10, 12
  hummingbirdPostAdjustmentInternal, 11

  normM, 13
  normUM, 13

  pos, 14