Package 'hummingbird'

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Type Package

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

Version 1.20.0

Description A package for detecting differential methylation. It exploits a Bayesian hid-
den Markov model that incorporates location dependence among genomic loci, unlike most ex-
isting methods that assume independence among observations. Bayesian priors are ap-
plied to permit information sharing across an entire chromosome for improved power of detec-
tion. The direct output of our software package is the best sequence of methylation states, elimi-
nating the use of a subjective, and most of the time an arbitrary, threshold of p-value for deter-
mining significance. At last, our methodology does not require replication in ei-
ther or both of the two comparison groups.

License GPL (>=2)

Depends R (>= 4.0)

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

Maintainer Eleni Adam <eadam002@odu.edu>

Contents

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.

Author(s)

Eleni Adam, Tieming Ji, Desh Ranjan

Maintainer: Eleni Adam <eadam002@odu.edu>

References

Ji (2019) A Bayesian hidden Markov model for detecting differentially methylated regions. Biometrics 75(2):663-673.

---

abnormM *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

```
data(exampleHummingbird)
abnormM
```

---

abnormUM *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

```
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

```
data("exampleHummingbird")
```

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

```
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

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

---

exampleSEControl　　　　*Sample input data*

Description

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

Usage

```
exampleSEControl
```

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

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

---

hummingbirdEM   *EM Algorithm for Fitting the Hidden Markov Model*

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 pre-
               dicted 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

```
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

```
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.

postAdjInfoDMRs

               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

```
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

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

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)
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.

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 hyper-methylation, 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

```
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$obs,
    pos = pos, minCpGs = 10, minLength = 100, maxGap = 300)
```

---

normM   *Sample matrix*

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 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)
normM
```

---

normUM   *Sample matrix*

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)
normUM
```

---

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
```

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