

Package ‘methyLCC’

November 26, 2024

Title Estimate the cell composition of whole blood in DNA methylation samples

Version 1.21.0

Imports Biobase, GenomicRanges, IRanges, S4Vectors, dplyr, magrittr, minfi, bsseq, quadprog, plyranges, stats, utils, bumphunter, genefilter, methods, IlluminaHumanMethylation450kmanifest, IlluminaHumanMethylation450kanno.ilmn12.hg19

Depends R (>= 3.6), FlowSorted.Blood.450k

Suggests rmarkdown, knitr, testthat (>= 2.1.0), BiocGenerics, BiocStyle, tidyr, ggplot2

Description A tool to estimate the cell composition of DNA methylation whole blood sample measured on any platform technology (microarray and sequencing).

biocViews Microarray, Sequencing, DNAMethylation, MethylationArray, MethylSeq, WholeGenome

VignetteBuilder knitr

RoxygenNote 6.1.1

Encoding UTF-8

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BugReports <https://github.com/stephaniehicks/methyLCC/>

URL <https://github.com/stephaniehicks/methyLCC/>

git_url <https://git.bioconductor.org/packages/methyLCC>

git_branch devel

git_last_commit 109b2f6

git_last_commit_date 2024-10-29

Repository Bioconductor 3.21

Date/Publication 2024-11-25

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<code>.extract_raw_data</code>	<i>Extract raw data</i>
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Description

Extract the methylation values and GRanges objects

Usage

```
.extract_raw_data(object)
```

Arguments

`object` an object can be a `RGChannelSet`, `GenomicMethylSet` or `BSseq` object

Value

A list preprocessed objects from the `RGChannelSet`, `GenomicMethylSet` or `BSseq` objects to be used in `.preprocess_estimatecc()`.

.find_dmrs	<i>Finding differentially methylated regions</i>
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Description

This function uses the FlowSorted.Blood.450k whole blood reference methylomes with six cell types to identify differentially methylated regions.

Usage

```
.find_dmrs(verbose = TRUE, gr_target = NULL, include_cpgs = FALSE,
  include_dmrs = TRUE, num_cpgs = 50, num_regions = 50,
  bumhunter_beta_cutoff = 0.2, dmr_up_cutoff = 0.5,
  dmr_down_cutoff = 0.4, dmr_pval_cutoff = 1e-11,
  cpg_pval_cutoff = 1e-08, cpg_up_dm_cutoff = 0,
  cpg_down_dm_cutoff = 0, pairwise_comparison = FALSE,
  mset_train_flow_sort = NULL)
```

Arguments

verbose	TRUE/FALSE argument specifying if verbose messages should be returned or not. Default is TRUE.
gr_target	Default is NULL. However, the user can provide a GRanges object from the object in estimatecc. Before starting the procedure to find differentially methylated regions, the intersection of the gr_target and GRanges object from the reference methylomes (FlowSorted.Blood.450k).
include_cpgs	TRUE/FALSE. Should individual CpGs be returned. Default is FALSE.
include_dmrs	TRUE/FALSE. Should differentially methylated regions be returned. Default is TRUE. User can turn this to FALSE and search for only CpGs.
num_cpgs	The max number of CpGs to return for each cell type. Default is 50.
num_regions	The max number of DMRs to return for each cell type. Default is 50.
bumhunter_beta_cutoff	The cutoff threshold in bumhunter() in the bumhunter package.
dmr_up_cutoff	A cutoff threshold for identifying DMRs that are methylated in one cell type, but not in the other cell types.
dmr_down_cutoff	A cutoff threshold for identifying DMRs that are not methylated in one cell type, but methylated in the other cell types.
dmr_pval_cutoff	A cutoff threshold for the p-values when identifying DMRs that are methylated in one cell type, but not in the other cell types (or vice versa).
cpg_pval_cutoff	A cutoff threshold for the p-values when identifying differentially methylated CpGs that are methylated in one cell type, but not in the other cell types (or vice versa).

<code>cpg_up_dm_cutoff</code>	A cutoff threshold for identifying differentially methylated CpGs that are methylated in one cell type, but not in the other cell types.
<code>cpg_down_dm_cutoff</code>	A cutoff threshold for identifying differentially methylated CpGs that are not methylated in one cell type, but are methylated in the other cell types.
<code>pairwise_comparison</code>	TRUE/FAISE of whether all pairwise comparisons (e.g. methylated in Granulocytes and Monocytes, but not methylated in other cell types). Default if FALSE.
<code>mset_train_flow_sort</code>	Default is NULL. However, a user can provide a <code>MethylSet</code> object after processing the <code>FlowSorted.Blood.450k</code> dataset. The default normalization is <code>preprocessIllumina()</code> .

Value

A list of data frames and GRanges objects.

<code>.initializeMLEs</code>	<i>.initializeMLEs</i>
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Description

Helper functions to initialize MLEs in `estimatecc()`.

Usage

```
.initializeMLEs(init_param_method, n, K, Ys, Zs, a0init, a1init, sig0init,
  sig1init, tauinit)
```

Arguments

<code>init_param_method</code>	method to initialize parameter estimates. Choose between "random" (randomly sample) or "known_regions" (uses unmethylated and methylated regions that were identified based on Reinus et al. (2012) cell sorted data.). Defaults to "random".
<code>n</code>	Number of samples
<code>K</code>	Number of cell types
<code>Ys</code>	observed methylation levels in samples provided by user of dimension $R \times n$
<code>Zs</code>	Cell type specific regions of dimension $R \times K$
<code>a0init</code>	Default NULL. Initial mean methylation level in unmethylated regions
<code>a1init</code>	Default NULL. Initial mean methylation level in methylated regions
<code>sig0init</code>	Default NULL. Initial var methylation level in unmethylated regions
<code>sig1init</code>	Default NULL. Initial var methylation level in methylated regions
<code>tauinit</code>	Default NULL. Initial var for measurement error

Value

A list of MLE estimates to be used in `estimatecc()`.

<code>.initialize_theta</code>	<code>.initialize_theta</code>
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Description

Creates a container with initial theta parameter estimates

Usage

```
.initialize_theta(n, K, alpha0 = NULL, alpha1 = NULL, sig0 = NULL, sig1 = NULL, tau = NULL)
```

Arguments

<code>n</code>	Number of samples
<code>K</code>	Number of cell types
<code>alpha0</code>	Default NULL. Initial mean methylation level in unmethylated regions
<code>alpha1</code>	Default NULL. Initial mean methylation level in methylated regions
<code>sig0</code>	Default NULL. Initial var methylation level in unmethylated regions
<code>sig1</code>	Default NULL. Initial var methylation level in methylated regions
<code>tau</code>	Default NULL. Initial var for measurement error

Value

A data frame with initial parameter estimates to be used in `.initializeMLEs()`.

<code>.methylcc_engine</code>	<code>.methylcc_engine</code>
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Description

Helper function for `estimatecc`

Usage

```
.methylcc_engine(Ys, Zs, current_pi_mle, current_theta, epsilon, max_iter)
```

Arguments

<code>Ys</code>	observed methylation levels in samples provided by user of dimension $R \times n$
<code>Zs</code>	Cell type specific regions of dimension $R \times K$
<code>current_pi_mle</code>	cell composition MLE estimates of dimension $K \times n$
<code>current_theta</code>	other parameter estimates in EM algorithm
<code>epsilon</code>	Add here.
<code>max_iter</code>	Add here.

Value

A list of MLE estimates that is used in `estimatecc()`.

<i>.methylcc_estep</i>	<i>Expectation step</i>
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Description

Expectation step in EM algorithm for methylCC

Usage

```
.methylcc_estep(Ys, Zs, current_pi_mle, current_theta, meth_status = 0)
```

Arguments

<code>Ys</code>	observed methylation levels in samples provided by user of dimension $R \times n$
<code>Zs</code>	Cell type specific regions of dimension $R \times K$
<code>current_pi_mle</code>	cell composition MLE estimates of dimension $K \times n$
<code>current_theta</code>	other parameter estimates in EM algorithm
<code>meth_status</code>	Indicator function corresponding to regions that are unmethylated (<code>meth_status=0</code>) or methylated (<code>meth_status=1</code>)

Value

List of expected value of the first two moments of the random effects (or the E-Step in the EM algorithm) used in `.methylcc_engine()`

.methylcc_mstep *Maximization step*

Description

Maximization step in EM Algorithm for methylCC

Usage

```
.methylcc_mstep(Ys, Zs, current_pi_mle, current_theta, estep0, estep1)
```

Arguments

Ys	observed methylation levels in samples provided by user of dimension R x n
Zs	Cell type specific regions of dimension R x K
current_pi_mle	cell composition MLE estimates of dimension K x n
current_theta	other parameter estimates in EM algorithm
estep0	Results from expectation step for unmethylated regions
estep1	Results from expectation step for methylated regions

Value

A list of the updated MLEs (or the M-Step in the EM algorithm) used in .methylcc_engine()

.pick_target_positions
Pick target positions

Description

Pick probes from target data using the indices in dmp_regions

Usage

```
.pick_target_positions(target_granges, target_object = NULL,  
                      target_cvg = NULL, dmp_regions)
```

Arguments

target_granges	add more here.
target_object	an optional argument which contains the meta-data for target_granges. If target_granges already contains the meta-data, do not need to supply target_object.
target_cvg	coverage reads for the target object
dmp_regions	differentially methylated regions

Value

A list of GRanges objects to be used in `.preprocess_estimatecc()`

```
.preprocess_estimatecc
      .preprocess_estimatecc
```

Description

This function preprocesses the data before the `estimatecc()` function

Usage

```
.preprocess_estimatecc(object, verbose = TRUE,
  init_param_method = "random",
  celltype_specific_dmrs = celltype_specific_dmrs)
```

Arguments

<code>object</code>	an object can be a <code>RGChannelSet</code> , <code>GenomicMethylSet</code> or <code>BSseq</code> object
<code>verbose</code>	TRUE/FALSE argument specifying if verbose messages should be returned or not. Default is TRUE.
<code>init_param_method</code>	method to initialize parameter estimates. Choose between "random" (randomly sample) or "known_regions" (uses unmethylated and methylated regions that were identified based on Reinus et al. (2012) cell sorted data.). Defaults to "random".
<code>celltype_specific_dmrs</code>	cell type specific differentially methylated regions (DMRs).

Value

A list of object to be used in `estimatecc`

```
.splitit      .splitit
```

Description

helper function to split along a variable

Usage

```
.splitit(x)
```


Arguments

x a vector

Value

A list to be used in find_dmrs()

.WFun *Helper function to take the product of Z and cell composition estimates*

Description

Helper function which is the product of Z and pi_mle

Usage

.WFun(Zs, pi_mle)

Arguments

Zs Cell type specific regions of dimension R x K
pi_mle cell composition MLE estimates

Value

A list of output after taking the product of Z and cell composition mle estimates to be used in .methylcc_estep().

cell_counts *Generic function that returns the cell composition estimates*

Description

Given a estimatecc object, this function returns the cell composition estimates
Accessors for the 'cell_counts' slot of a estimatecc object.

Usage

cell_counts(object)

S4 method for signature 'estimatecc'
cell_counts(object)

Arguments

object an object of class estimatecc.

Value

Returns the cell composition estimates

Examples

```
# This is a reduced version of the FlowSorted.Blood.450k
# dataset available by using BiocManager::install("FlowSorted.Blood.450k"),
# but for purposes of the example, we use the smaller version
# and we set \code{demo=TRUE}. For any case outside of this example for
# the package, you should set \code{demo=FALSE} (the default).

dir <- system.file("data", package="methylCC")
files <- file.path(dir, "FlowSorted.Blood.450k.sub.RData")
if(file.exists(files)){
  load(file = files)

  set.seed(12345)
  est <- estimatecc(object = FlowSorted.Blood.450k.sub, demo = TRUE)
  cell_counts(est)
}
```

estimatecc

Estimate cell composition from DNAm data

Description

Estimate cell composition from DNAm data

Usage

```
estimatecc(object, find_dmrs_object = NULL, verbose = TRUE,
  epsilon = 0.01, max_iter = 100, take_intersection = FALSE,
  include_cpgs = FALSE, include_dmrs = TRUE,
  init_param_method = "random", a0init = NULL, a1init = NULL,
  sig0init = NULL, sig1init = NULL, tauinit = NULL, demo = FALSE)
```

Arguments

object	an object can be a RGChannelSet, GenomicMethylSet or BSseq object
find_dmrs_object	If the user would like to supply different differentially methylated regions, they can use the output from the <code>find_dmrs</code> function to supply different regions to <code>estimatecc</code> .
verbose	TRUE/FALSE argument specifying if verbose messages should be returned or not. Default is TRUE.
epsilon	Threshold for EM algorithm to check for convergence. Default is 0.01.

<code>max_iter</code>	Maximum number of iterations for EM algorithm. Default is 100 iterations.
<code>take_intersection</code>	TRUE/FALSE asking if only the CpGs included in object should be used to find DMRs. Default is FALSE.
<code>include_cpgs</code>	TRUE/FALSE. Should individual CpGs be returned. Default is FALSE.
<code>include_dmrs</code>	TRUE/FALSE. Should differentially methylated regions be returned. Default is TRUE.
<code>init_param_method</code>	method to initialize parameter estimates. Choose between "random" (randomly sample) or "known_regions" (uses unmethylated and methylated regions that were identified based on Reinus et al. (2012) cell sorted data.). Defaults to "random".
<code>a0init</code>	Default NULL. Initial mean methylation level in unmethylated regions
<code>a1init</code>	Default NULL. Initial mean methylation level in methylated regions
<code>sig0init</code>	Default NULL. Initial var methylation level in unmethylated regions
<code>sig1init</code>	Default NULL. Initial var methylation level in methylated regions
<code>tauinit</code>	Default NULL. Initial var for measurement error
<code>demo</code>	TRUE/FALSE. Should the function be used in demo mode to shorten examples in package. Defaults to FALSE.

Value

A object of the class `estimatecc` that contains information about the cell composition estimation (in the `summary` slot) and the cell composition estimates themselves (in the `cell_counts` slot).

Examples

```
# This is a reduced version of the FlowSorted.Blood.450k
# dataset available by using BiocManager::install("FlowSorted.Blood.450k"),
# but for purposes of the example, we use the smaller version
# and we set \code{demo=TRUE}. For any case outside of this example for
# the package, you should set \code{demo=FALSE} (the default).

dir <- system.file("data", package="methylCC")
files <- file.path(dir, "FlowSorted.Blood.450k.sub.RData")
if(file.exists(files)){
  load(file = files)

  set.seed(12345)
  est <- estimatecc(object = FlowSorted.Blood.450k.sub, demo = TRUE)
  cell_counts(est)
}
```

estimatecc-class *the estimatecc class*

Description

Objects of this class store all the values needed information to work with a estimatecc object

Value

summary returns the summary information about the cell composition estimate procedure and cell_counts
returns the cell composition estimates

Slots

summary information about the samples and regions used to estimate cell composition
cell_counts cell composition estimates

Examples

```
# This is a reduced version of the FlowSorted.Blood.450k
# dataset available by using BiocManager::install("FlowSorted.Blood.450k"),
# but for purposes of the example, we use the smaller version
# and we set \code{demo=TRUE}. For any case outside of this example for
# the package, you should set \code{demo=FALSE} (the default).

dir <- system.file("data", package="methylCC")
files <- file.path(dir, "FlowSorted.Blood.450k.sub.RData")
if(file.exists(files)){
  load(file = files)

  set.seed(12345)
  est <- estimatecc(object = FlowSorted.Blood.450k.sub, demo = TRUE)
  cell_counts(est)
}
```

FlowSorted.Blood.450k.sub

A reduced size of the FlowSorted.Blood.450k dataset

Description

A reduced size of the FlowSorted.Blood.450k dataset

The object was created using the script in /inst and located in the /data folder.

Format

A RGset object with 2e5 rows (probes) and 6 columns (whole blood samples).

offMethRegions	<i>Unmethylated regions for all celltypes</i>
----------------	---

Description

This is the script used to create the offMethRegions data set. The purpose is use in the estimate_cc() function.

The object was created using the script in /inst and located in the /data folder.

Format

add more here.

onMethRegions	<i>Methylated regions for all celltypes</i>
---------------	---

Description

This is the script used to create the onMethRegions data set. The purpose is use in the estimate_cc() function.

The object was created using the script in /inst and located in the /data folder.

Format

add more here.

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