

Package ‘methyICC’

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

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

Suggests 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

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| | |
|-------------------|-------------------------|
| .extract_raw_data | <i>Extract raw data</i> |
|-------------------|-------------------------|

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 *Finding differentially methylated regions*

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> |
|------------------------------|------------------------|

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()`.

.initialize_theta *.initialize_theta*

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()`.

.methylcc_engine *.methylcc_engine*

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> |
|------------------------|-------------------------|

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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