

# Package ‘methylCC’

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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** rmarkdown, knitr, testthat (>= 2.1.0), BiocGenerics, BiocStyle, tidy, 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>
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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`*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,
  bumphunter_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

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

---

*.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**

n	Number of samples
K	Number of cell types
alpha0	Default NULL. Initial mean methylation level in unmethylated regions
alpha1	Default NULL. Initial mean methylation level in methylated regions
sig0	Default NULL. Initial var methylation level in unmethylated regions
sig1	Default NULL. Initial var methylation level in methylated regions
tau	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**

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
epsilon	Add here.
max_iter	Add here.

**Value**

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

---

.methyloc\_estep      *Expectation step*

---

### Description

Expectation step in EM algorithm for methyloc

### Usage

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

### 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
meth_status	Indicator function corresponding to regions that are unmethylated (meth_status=0) or methylated (meth_status=1)

### Value

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

---

.methyloc\_mstep      *Maximization step*

---

### Description

Maximization step in EM Algorithm for methyloc

### Usage

```
.methyloc_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 .methyloc\_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**

`object` an object can be a `RGChannelSet`, `GenomicMethylSet` or `BSseq` object  
`verbose` TRUE/FALSE argument specifying if verbose messages should be returned or not. Default is TRUE.  
`init_param_method` 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".  
`celltype_specific_dmrs` cell type specific differentially methylated regions (DMRs).

**Value**

A list of object to be used in estimatecc

---

<code>.splitit</code>	<code>.splitit</code>
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---

**Description**

helper function to split along a variable

**Usage**

```
.splitit(x)
```

**Arguments**

x                    a vector

**Value**

A list to be used in find\_dmrs()

---

<code>.WFun</code>	<i>Helper function to take the product of Z and cell composition estimates</i>
--------------------	--

---

**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	<i>Generic function that returns the cell composition estimates</i>
-------------	---

---

**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	<i>Estimate cell composition from DNAm data</i>
------------	---

---

**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**

<code>object</code>	an object can be a <code>RGChannelSet</code> , <code>GenomicMethylSet</code> or <code>BSseq</code> object
<code>find_dmrs_object</code>	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> .
<code>verbose</code>	TRUE/FALSE argument specifying if verbose messages should be returned or not. Default is TRUE.
<code>epsilon</code>	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 <code>object</code> 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	<i>the estimatecc class</i>
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---

## 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

*Unmethylated regions for all celltypes*

---

### 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

*Methylated regions for all celltypes*

---

### 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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