

Package ‘Melissa’

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Type Package

Title Bayesian clustering and imputation of single cell methylomes

Version 1.2.0

Description Melissa is a Bayesian probabilistic model for jointly clustering and imputing single cell methylomes. This is done by taking into account local correlations via a Generalised Linear Model approach and global similarities using a mixture modelling approach.

Depends R (>= 3.5.0), BPRMeth, GenomicRanges

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binarise_files	<i>Binarise CpG sites</i>
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Description

Script for binarising CpG sites and formatting the coverage file so it can be directly used from the BPRMeth package. The format of each file is the following: <chr> <start> <met_level>, where met_level can be either 0 or 1.

Usage

```
binarise_files(indir, outdir = NULL, no_cores = NULL)
```

Arguments

indir	Directory containing the coverage files, output from Bismark.
outdir	Directory to store the output files for each cell with exactly the same name. If NULL, then a directory called 'binarised' inside 'indir' will be create by default.
no_cores	Number of cores to use for parallel processing. If NULL, no parallel processing is used.

Value

No value is returned, the binarised data are stored in the outdir.

Author(s)

C.A.Kapourani <C.A.Kapourani@ed.ac.uk>

See Also

[create_melissa_data_obj](#), [melissa](#), [filter_regions](#)

Examples

```
## Not run:
# Met directory
met_dir <- "name_of_met_dir"

binarise_files(met_dir)

## End(Not run)
```

cluster_ari	<i>Compute clustering ARI</i>
-------------	-------------------------------

Description

cluster_ari computes the clustering performance in terms of the Adjusted Rand Index (ARI) metric.

Usage

```
cluster_ari(C_true, C_post)
```

Arguments

C_true	True cluster assignemnts.
C_post	Posterior responsibilities of predicted cluster assignemnts.

Value

The clustering ARI.

Author(s)

C.A.Kapourani <C.A.Kapourani@ed.ac.uk>

cluster_error	<i>Compute clustering assignment error cluster_error computes the clustering assignment error, i.e. the average number of incorrect cluster assignments:</i>
---------------	--

$$OE = \sum_{n=1}^N I(LT_n \neq LP_n) / N$$

Description

Compute clustering assignment error

cluster_error computes the clustering assignment error, i.e. the average number of incorrect cluster assignments:

$$OE = \sum_{n=1}^N (I(LT_n \neq LP_n)) / N$$

Usage

```
cluster_error(C_true, C_post)
```

Arguments

C_true	True cluster assignemnts.
C_post	Posterior mean of predicted cluster assignemnts.

Value

The clustering assignment error

Author(s)

C.A.Kapourani <C.A.Kapourani@ed.ac.uk>

```
create_melissa_data_obj
```

Create methylation regions for all cells

Description

Wrapper function for creating methylation regions for all cells, which is the input object for Melissa prior to filtering.

Usage

```
create_melissa_data_obj(met_dir, anno_file, chrom_size_file = NULL,
  chr_discarded = NULL, is_centre = FALSE, is_window = TRUE,
  upstream = -5000, downstream = 5000, cov = 5, sd_thresh = -1,
  no_cores = NULL)
```

Arguments

met_dir	Directory of (binarised) methylation files, each file corresponds to a single cell.
anno_file	The annotation file with 'tab' delimited format: "chromosome", "start", "end", "strand", "id", "name" (optional). Read the 'BPRMeth' documentation for more details.
chrom_size_file	Optional file name to read genome chromosome sizes.
chr_discarded	Optional vector with chromosomes to be discarded.
is_centre	Logical, whether 'start' and 'end' locations are pre-centred. If TRUE, the mean of the locations will be chosen as centre. If FALSE, the 'start' will be chosen as the center; e.g. for genes the 'start' denotes the TSS and we use this as centre to obtain K-bp upstream and downstream of TSS.
is_window	Whether to consider a predefined window region around centre. If TRUE, then 'upstream' and 'downstream' parameters are used, otherwise we consider the whole region from start to end location.
upstream	Integer defining the length of bp upstream of 'centre' for creating the genomic region. If is_window = FALSE, this parameter is ignored.

downstream	Integer defining the length of bp downstream of 'centre' for creating the genomic region. If is_window = FALSE, this parameter is ignored.
cov	Integer defining the minimum coverage of CpGs that each region must contain.
sd_thresh	Optional numeric defining the minimum standard deviation of the methylation change in a region. This is used to filter regions with no methylation variability.
no_cores	Number of cores to be used, default is max_no_cores - 1.

Value

A melissa_data_obj object, with the following elements:

- met: A list of elements of length N, where N are the total number of cells. Each element in the list contains another list of length M, where M is the total number of genomic regions, e.g. promoters. Each element in the inner list is an I x 2 matrix, where I are the total number of observations. The first column contains the input observations x (i.e. CpG locations) and the 2nd column contains the corresponding methylation level.
- anno_region: The annotation object.
- opts: A list with the parameters that were used for creating the object.

Author(s)

C.A.Kapourani <C.A.Kapourani@ed.ac.uk>

See Also

[binarise_files](#), [melissa](#), [filter_regions](#)

Examples

```
## Not run:
# Met directory
met_dir <- "name_of_met_dir"
# Annotation file name
anno_file <- "name_of_anno_file"

obj <- create_melissa_data_obj(met_dir, anno_file)

# Extract annotation regions
met <- obj$met

# Extract annotation regions
anno <- obj$anno_region

## End(Not run)
```

eval_cluster_performance

Evaluate clustering performance

Description

eval_cluster_performance is a wrapper function for computing clustering performance in terms of ARI and clustering assignment error.

Usage

```
eval_cluster_performance(obj, C_true)
```

Arguments

obj Output of Melissa inference object.
C_true True cluster assignments.

Value

The 'melissa' object, with an additional slot named 'clustering', containing the ARI and clustering assignment error performance.

Author(s)

C.A.Kapourani <C.A.Kapourani@ed.ac.uk>

See Also

[create_melissa_data_obj](#), [melissa](#), [filter_regions](#), [eval_imputation_performance](#), [eval_cluster_performance](#)

Examples

```
## Extract synthetic data
dt <- melissa_synth_dt

# Partition to train and test set
dt <- partition_dataset(dt)

# Create basis object from BPRMeth package
basis_obj <- BPRMeth::create_rbf_object(M = 3)

# Run Melissa
melissa_obj <- melissa(X = dt$met, K = 2, basis = basis_obj, vb_max_iter = 10,
  vb_init_nstart = 1, is_parallel = FALSE, is_verbose = FALSE)

# Compute cluster performance
melissa_obj <- eval_cluster_performance(melissa_obj, dt$opts$C_true)

cat("ARI: ", melissa_obj$clustering$ari)
```

`eval_imputation_performance`*Evaluate imputation performance*

Description

`eval_imputation_performance` is a wrapper function for computing imputation/clustering performance in terms of different metrics, such as AUC and precision recall curves.

Usage

```
eval_imputation_performance(obj, imputation_obj)
```

Arguments

`obj` Output of Melissa inference object.
`imputation_obj` List containing two vectors of equal length, corresponding to true methylation states and predicted/imputed methylation states.

Value

The ‘melissa’ object, with an additional slot named ‘imputation’, containing the AUC, F-measure, True Positive Rate (TPR) and False Positive Rate (FPR), and Precision Recall (PR) curves.

Author(s)

C.A.Kapourani <C.A.Kapourani@ed.ac.uk>

See Also

[create_melissa_data_obj](#), [melissa](#), [filter_regions](#), [eval_imputation_performance](#), [eval_cluster_performa](#)

Examples

```
# First take a subset of cells to efficiency
# Extract synthetic data
dt <- melissa_synth_dt

# Partition to train and test set
dt <- partition_dataset(dt)

# Create basis object from BPRMeth package
basis_obj <- BPRMeth::create_rbf_object(M = 3)

# Run Melissa
melissa_obj <- melissa(X = dt$met, K = 2, basis = basis_obj, vb_max_iter = 10,
  vb_init_nstart = 1, is_parallel = FALSE, is_verbose = FALSE)

imputation_obj <- impute_met_state(obj = melissa_obj, test = dt$met_test)

melissa_obj <- eval_imputation_performance(obj = melissa_obj,
  imputation_obj = imputation_obj)
```

```
cat("AUC: ", melissa_obj$imputation$auc)
```

extract_y	<i>Extract responses y</i>
-----------	----------------------------

Description

Given a list of observations, extract responses y

Usage

```
extract_y(X, coverage_ind)
```

Arguments

X	Observations
coverage_ind	Which observations have coverage

Value

The design matrix H

filter_regions	<i>Filtering process prior to running Melissa</i>
----------------	---

Description

Fuctions for filter genomic regions due to (1) low CpG coverage, (2) low coverage across cells, or (3) low mean methylation variability.

Usage

```
filter_by_cpg_coverage(obj, min_cpgcov = 10)
```

```
filter_by_coverage_across_cells(obj, min_cell_cov_prcg = 0.5)
```

```
filter_by_variability(obj, min_var = 0.1)
```

Arguments

obj	Melissa data object.
min_cpgcov	Minimum CpG coverage for each genomic region.
min_cell_cov_prcg	Threshold on the proportion of cells that have coverage for each region.
min_var	Minimum variability of mean methylation across cells, measured in terms of standard deviation.

Details

The (1) ‘filter_by_cpg_coverage’ function does not actually remove the region, it only sets NA to those regions. The (2) ‘filter_by_coverage_across_cells’ function keeps regions from which we can share information across cells. The (3) ‘filter_by_variability’ function keeps variable regions which are informative for cell subtype identification.

Value

The filtered Melissa data object

Author(s)

C.A.Kapourani <C.A.Kapourani@ed.ac.uk>

See Also

[melissa, create_melissa_data_obj](#)

Examples

```
# Run on synthetic data from Melissa package
filt_obj <- filter_by_cpg_coverage(melissa_encode_dt, min_cpgcov = 20)

# Run on synthetic data from Melissa package
filt_obj <- filter_by_coverage_across_cells(melissa_encode_dt,
                                           min_cell_cov_prcg = 0.7)

# Run on synthetic data from Melissa package
filt_obj <- filter_by_variability(melissa_encode_dt, min_var = 0.1)
```

impute_met_state

Impute/predict methylation states

Description

Make predictions of missing methylation states, i.e. perform imputation using Melissa. This requires keeping a subset of data as a held out test set during Melissa inference.

Usage

```
impute_met_state(obj, test, basis = NULL, is_predictive = TRUE,
                 return_test = FALSE)
```

Arguments

obj	Output of Melissa inference object.
test	Test data to evaluate performance.
basis	Basis object, if NULL we perform imputation using Melissa, otherwise using BPRMeth.
is_predictive	Logical, use predictive distribution for imputation, or choose the cluster label with the highest responsibility.
return_test	Whether or not to return a list with the predictions.

Value

A list containing two vectors, the true methylation state and the predicted/imputed methylation states.

Author(s)

C.A.Kapourani <C.A.Kapourani@ed.ac.uk>

See Also

[create_melissa_data_obj](#), [melissa](#), [filter_regions](#), [eval_imputation_performance](#), [eval_cluster_performance](#)

Examples

```
# Extract synthetic data
dt <- melissa_synth_dt

# Partition to train and test set
dt <- partition_dataset(dt)

# Create basis object from BPRMeth package
basis_obj <- BPRMeth::create_rbf_object(M = 3)

# Run Melissa
melissa_obj <- melissa(X = dt$met, K = 2, basis = basis_obj, vb_max_iter = 10,
  vb_init_nstart = 1, is_parallel = FALSE, is_verbose = FALSE)

imputation_obj <- impute_met_state(obj = melissa_obj, test = dt$met_test)
```

init_design_matrix *Initialise design matrices*

Description

Given a list of observations, initialise design matrices H for computational efficiency.

Usage

```
init_design_matrix(basis, X, coverage_ind)
```

Arguments

basis	Basis object.
X	Observations
coverage_ind	Which observations have coverage

Value

The design matrix H

log_sum_exp	<i>Compute stable log-sum-exp</i>
-------------	-----------------------------------

Description

log_sum_exp computes the log sum exp trick for avoiding numeric underflow and have numeric stability in computations of small numbers.

Usage

```
log_sum_exp(x)
```

Arguments

x A vector of observations

Value

The logs-sum-exp value

Author(s)

C.A.Kapourani <C.A.Kapourani@ed.ac.uk>

References

<https://hips.seas.harvard.edu/blog/2013/01/09/computing-log-sum-exp/>

Melissa	Melissa: <i>Bayesian clustering and imputation of single cell methylomes</i>
---------	--

Description

Bayesian clustering and imputation of single cell methylomes

Usage

```
.datatable.aware
```

Format

An object of class logical of length 1.

Value

Melissa main package documentation.

Author(s)

C.A.Kapourani <kapouranis.andreas@gmail.com>

See Also

[melissa](#), [create_melissa_data_obj](#), [partition_dataset](#), [plot_melissa_profiles](#), [filter_regions](#)

melissa

Cluster and impute single cell methylomes using VB

Description

melissa clusters and imputes single cells based on their methylome landscape on specific genomic regions, e.g. promoters, using the Variational Bayes (VB) EM-like algorithm.

Usage

```
melissa(X, K = 3, basis = NULL, delta_0 = NULL, w = NULL,
        alpha_0 = 0.5, beta_0 = NULL, vb_max_iter = 300,
        epsilon_conv = 1e-05, is_kmeans = TRUE, vb_init_nstart = 10,
        vb_init_max_iter = 20, is_parallel = FALSE, no_cores = 3,
        is_verbose = TRUE)
```

Arguments

X	The input data, which has to be a list of elements of length N, where N are the total number of cells. Each element in the list contains another list of length M, where M is the total number of genomic regions, e.g. promoters. Each element in the inner list is an I X 2 matrix, where I are the total number of observations. The first column contains the input observations x (i.e. CpG locations) and the 2nd columns contains the corresponding methylation level.
K	Integer denoting the total number of clusters K.
basis	A 'basis' object. E.g. see <code>create_basis</code> function from BPRMeth package. If NULL, will an RBF object with 3 basis functions will be created.
delta_0	Parameter vector of the Dirichlet prior on the mixing proportions pi.
w	Optional, an Mx(D)xK array of the initial parameters, where first dimension are the genomic regions M, 2nd the number of covariates D (i.e. basis functions), and 3rd are the clusters K. If NULL, will be assigned with default values.
alpha_0	Hyperparameter: shape parameter for Gamma distribution. A Gamma distribution is used as prior for the precision parameter tau.
beta_0	Hyperparameter: rate parameter for Gamma distribution. A Gamma distribution is used as prior for the precision parameter tau.
vb_max_iter	Integer denoting the maximum number of VB iterations.
epsilon_conv	Numeric denoting the convergence threshold for VB.
is_kmeans	Logical, use Kmeans for initialization of model parameters.
vb_init_nstart	Number of VB random starts for finding better initialization.
vb_init_max_iter	Maximum number of mini-VB iterations.
is_parallel	Logical, indicating if code should be run in parallel.
no_cores	Number of cores to be used, default is <code>max_no_cores - 1</code> .
is_verbose	Logical, print results during VB iterations.

Value

An object of class `melissa` with the following elements:

- `W`: An $(M+1) \times K$ matrix with the optimized parameter values for each cluster, M are the number of basis functions. Each column of the matrix corresponds a different cluster k .
- `W_Sigma`: A list with the covariance matrices of the posterior parameter W for each cluster k .
- `r_nk`: An $(N \times K)$ responsibility matrix of each observations being explained by a specific cluster.
- `delta`: Optimized Dirichlet parameter for the mixing proportions.
- `alpha`: Optimized shape parameter of Gamma distribution.
- `beta`: Optimized rate parameter of the Gamma distribution
- `basis`: The basis object.
- `lb`: The lower bound vector.
- `labels`: Cluster assignment labels.
- `pi_k`: Expected value of mixing proportions.

Details

The modelling and mathematical details for clustering profiles using mean-field variational inference are explained here: <http://rpubs.com/cakapourani/> . More specifically:

- For Binomial/Bernoulli observation model check: <http://rpubs.com/cakapourani/vb-mixture-bpr>
- For Gaussian observation model check: <http://rpubs.com/cakapourani/vb-mixture-lr>

Author(s)

C.A.Kapourani <C.A.Kapourani@ed.ac.uk>

See Also

[create_melissa_data_obj](#), [partition_dataset](#), [plot_melissa_profiles](#), [filter_regions](#)

Examples

```
# Example of running Melissa on synthetic data

# Create RBF basis object with 4 RBFs
basis_obj <- BPRMeth::create_rbf_object(M = 4)

set.seed(15)
# Run Melissa
melissa_obj <- melissa(X = melissa_synth_dt$met, K = 2, basis = basis_obj,
  vb_max_iter = 10, vb_init_nstart = 1, vb_init_max_iter = 5,
  is_parallel = FALSE, is_verbose = FALSE)

# Extract mixing proportions
print(melissa_obj$pi_k)
```

melissa_encode_dt	<i>Synthetic ENCODE single cell methylation data</i>
-------------------	--

Description

Small synthetic ENCODE data generated by inferring methylation profiles from bulk ENCODE data, and subsequently generating single cells. It consists of $N = 200$ cells and $M = 100$ genomic regions. The data are in the required format for directly running Melissa and are used as a case study for the vignette.

Usage

```
melissa_encode_dt
```

Format

A list object containing methylation regions, annotation data and the options used for creating the data. This in general would be the output of the `create_melissa_data_obj` function. It has the following three objects:

- `met`: A list containing the methylation data, each element of the list is a different cell.
- `anno_region`: Corresponding annotation data for each genomic region.
- `opts`: Parameters/options used to generate the data.

Value

Synthetic ENCODE methylation data

See Also

[create_melissa_data_obj](#)

melissa_gibbs	<i>Gibbs sampling algorithm for Melissa model</i>
---------------	---

Description

`melissa_gibbs` implements the Gibbs sampling algorithm for performing clustering of single cells based on their DNA methylation profiles, where the observation model is the Bernoulli distributed Probit Regression likelihood. NOTE: that Gibbs sampling is really slow and we recommend using the VB implementation: [melissa](#).

Usage

```
melissa_gibbs(X, K = 2, pi_k = rep(1/K, K), w = NULL, basis = NULL,  
w_0_mean = NULL, w_0_cov = NULL, dir_a = rep(1, K), lambda = 1/2,  
gibbs_nsim = 1000, gibbs_burn_in = 200, inner_gibbs = FALSE,  
gibbs_inner_nsim = 50, is_parallel = TRUE, no_cores = NULL,  
is_verbose = FALSE)
```

Arguments

<code>X</code>	A list of length <code>I</code> , where <code>I</code> are the total number of cells. Each element of the list contains another list of length <code>N</code> , where <code>N</code> is the total number of genomic regions. Each element of the inner list is an <code>L x 2</code> matrix of observations, where 1st column contains the locations and the 2nd column contains the methylation level of the corresponding CpGs.
<code>K</code>	Integer denoting the number of clusters <code>K</code> .
<code>pi_k</code>	Vector of length <code>K</code> , denoting the mixing proportions.
<code>w</code>	A <code>N x M x K</code> array, where each column contains the basis function coefficients for the corresponding cluster.
<code>basis</code>	A 'basis' object. E.g. see <code>create_rbf_object</code> from <code>BPRMeth</code> package
<code>w_0_mean</code>	The prior mean hyperparameter for <code>w</code>
<code>w_0_cov</code>	The prior covariance hyperparameter for <code>w</code>
<code>dir_a</code>	The Dirichlet concentration parameter, prior over <code>pi_k</code>
<code>lambda</code>	The complexity penalty coefficient for penalized regression.
<code>gibbs_nsim</code>	Argument giving the number of simulations of the Gibbs sampler.
<code>gibbs_burn_in</code>	Argument giving the burn in period of the Gibbs sampler.
<code>inner_gibbs</code>	Logical, indicating if we should perform Gibbs sampling to sample from the augmented BPR model.
<code>gibbs_inner_nsim</code>	Number of inner Gibbs simulations.
<code>is_parallel</code>	Logical, indicating if code should be run in parallel.
<code>no_cores</code>	Number of cores to be used, default is <code>max_no_cores - 1</code> .
<code>is_verbose</code>	Logical, print results during EM iterations

Value

An object of class `melissa_gibbs`.

Author(s)

C.A.Kapourani <C.A.Kapourani@ed.ac.uk>

See Also

[melissa](#), [create_melissa_data_obj](#), [partition_dataset](#), [filter_regions](#)

Examples

```
# Example of running Melissa Gibbs on synthetic data

# Create RBF basis object with 4 RBFs
basis_obj <- BPRMeth::create_rbf_object(M = 4)

set.seed(15)
# Run Melissa Gibbs
melissa_obj <- melissa_gibbs(X = melissa_synth_dt$met, K = 2, basis = basis_obj,
  gibbs_nsim = 10, gibbs_burn_in = 5, is_parallel = FALSE, is_verbose = FALSE)
```

```
# Extract mixing proportions
print(melissa_obj$pi_k)
```

melissa_synth_dt	<i>Synthetic single cell methylation data</i>
------------------	---

Description

Small synthetic data for quick analysis. It consists of $N = 50$ cells and $M = 50$ genomic regions.

Usage

```
melissa_synth_dt
```

Format

A list object containing methylation regions, annotation data and the options used for creating the data. This in general would be the output of the [create_melissa_data_obj](#) function. It has the following three objects:

- met: A list containing the methylation data, each element of the list is a different cell.
- anno_region: Corresponding annotation data for each genomic region.
- opts: Parameters/options used to generate the data.

Value

Synthetic methylation data

See Also

[create_melissa_data_obj](#)

partition_dataset	<i>Partition synthetic dataset to training and test set</i>
-------------------	---

Description

Partition synthetic dataset to training and test set

Usage

```
partition_dataset(dt_obj, data_train_prcg = 0.5,
  region_train_prcg = 0.95, cpg_train_prcg = 0.5, is_synth = FALSE)
```


Arguments

dt_obj Melissa data object

data_train_prcg Percentage of genomic regions that will be fully used for training, i.e. across the whole region we will have no CpGs missing.

region_train_prcg Fraction of genomic regions to keep for training set, i.e. some genomic regions will have no coverage at all during training.

cpg_train_prcg Fraction of CpGs in each genomic region to keep for training set.

is_synth Logical, whether we have synthetic data or not.

Value

The Melissa object with the following changes. The 'met' element will now contain only the 'training' data. An additional element called 'met_test' will store the data that will be used during testing to evaluate the imputation performance. These data will not be seen from Melissa during inference.

Author(s)

C.A.Kapourani <C.A.Kapourani@ed.ac.uk>

See Also

[create_melissa_data_obj](#), [melissa](#), [filter_regions](#)

Examples

```
# Partition the synthetic data from Melissa package
dt <- partition_dataset(melissa_encode_dt)
```

plot_melissa_profiles *Plot predictive methylation profiles*

Description

This function plots the predictive distribution of the methylation profiles inferred using the Melissa model. Each colour corresponds to a different cluster.

Usage

```
plot_melissa_profiles(melissa_obj, region = 1,
  title = "Melissa profiles", x_axis = "genomic region",
  y_axis = "met level", x_labels = c("Upstream", "", "Centre", "",
  "Downstream"), ...)
```

Arguments

<code>melissa_obj</code>	Clustered cell subtypes using Melissa inference functions.
<code>region</code>	Genomic region number.
<code>title</code>	Plot title
<code>x_axis</code>	x axis label
<code>y_axis</code>	x axis label
<code>x_labels</code>	x axis ticks labels
<code>...</code>	Additional parameters

Value

A ggplot2 object.

Author(s)

C.A.Kapourani <C.A.Kapourani@ed.ac.uk>

See Also

[create_melissa_data_obj](#), [melissa](#), [filter_regions](#), [eval_imputation_performance](#), [eval_cluster_performance](#)

Examples

```
# Extract synthetic data
dt <- melissa_synth_dt

# Create basis object from BPRMeth package
basis_obj <- BPRMeth::create_rbf_object(M = 3)

# Run Melissa
melissa_obj <- melissa(X = dt$met, K = 2, basis = basis_obj, vb_max_iter = 10,
  vb_init_nstart = 1, is_parallel = FALSE, is_verbose = FALSE)

gg <- plot_melissa_profiles(melissa_obj, region = 10)
```

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