Package ‘EnMCB’

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Type Package

Title Predicting Disease Progression Based on Methylation Correlated Blocks using Ensemble Models

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Encoding UTF-8

Imports survivalROC, glmnet, rms, mboost, Matrix, igraph, methods, survivalsvm, ggplot2, boot, e1071, survival, BiocFileCache

VignetteBuilder knitr

Suggests SummarizedExperiment, testthat, Biobase, survminer, affycoretools, knitr, plotROC, limma, rmarkdown

Description Creation of the correlated blocks using DNA methylation profiles. Machine learning models can be constructed to predict differentially methylated blocks and disease progression.

License GPL-2

BugReports https://github.com/whirlsyu/EnMCB/issues

biocViews Normalization, DNAMethylation, MethylationArray, SupportVectorMachine

LazyData FALSE

RoxygenNote 7.2.3

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Date/Publication 2024-05-28
 anno_matrix

## Description

IlluminaHumanMethylation450kanno

## Usage

data(anno_matrix)

## Format

IlluminaHumanMethylation450kanno.ilmn12.hg19 annotation file. This data have several columns
as.data.frame.ridgemat

Description

data frame ridge matrix

Usage

## S3 method for class 'ridgemat'
as.data.frame(x, ...)

Arguments

x       data vector
...
other parameters pass to as.data.frame.model.matrix()

as.ridgemat

Description

as.matrix attempts to turn its argument

Usage

as.ridgemat(x)

Arguments

x       data vector
CompareMCB

Compare multiple methylation correlated blocks lists

Description

This function is used to find the Methylation correlated blocks that differentially expressed between groups. This function calculates attractors of all the MCBs among the groups and find the attractor MCBs.

Usage

CompareMCB(
  MCBs,
  method = c("attractors")[1],
  p_value = 0.05,
  min_CpGs = 5,
  platform = "Illumina Methylation 450K"
)

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCBs</td>
<td>Methylation correlated blocks list.</td>
</tr>
<tr>
<td>method</td>
<td>method used for calculation of differential expression, should be one of &quot;attractors&quot;,&quot;t-test&quot;. Default is &quot;attractors&quot;.</td>
</tr>
<tr>
<td>p_value</td>
<td>p value threshold for the test.</td>
</tr>
<tr>
<td>min_CpGs</td>
<td>threshold for minimum CpGs must included in the individual MCBs.</td>
</tr>
<tr>
<td>platform</td>
<td>This parameter indicates the platform used to produce the methylation profile.</td>
</tr>
</tbody>
</table>

Details

Currently, only illumina 450k platform is supported, the methylation profile need to convert into matrix format.

Value

Object of class list with elements:

- **MCBsites** Character set contains all CpG sites in MCBs.
- **MCBinformation** Matrix contains the information of results.

Author(s)

Xin Yu
create_demo

References

Xin Yu, De-Xin Kong. EnMCB: an R/bioconductor package for predicting disease progression based on methylation correlated blocks using ensemble models, Bioinformatics, 2021, btab415

Examples

data('demo_data', package = "EnMCB")

demo_set<-create_demo()
demo_data  

Expression matrix of demo dataset.

Description

A Expression matrix containing the 10020 CpGs beta value of 455 samples in TCGA lung Adenocarcinoma dataset. This will call from create_demo() function.

Usage

data(demo_data)

Format

ExpressionSet:

rownames  rownames of 10020 CpG features
colnames  colnames of 455 samples
realdata  Real data matrix for demo.

demo_MCBinformation  

MCB information.

Description

A dataset containing the number and other attributes of 94 MCBs; This results was created by the identification function IdentifyMCB. This data used for metricMCB function.

Usage

data(demo_MCBinformation)

Format

A data frame with 94 rows and 8 variables:

MCB_no  MCB code
start  Start point of this MCB in the chromosome.
end  End point of this MCB in the chromosome.
CpGs  All the CpGs probe names in the MCB.
location  Start, end point and the chromosome number of this MCB.
chromosomes  the chromosome number of this MCB.
length  the length of bps of this MCB in the chromosome.
CpGs_num  number of CpG probes of this MCB.
**demo_survival_data**

Survival data of demo dataset.

**Description**

A Surv containing survival value of 455 samples in TCGA lung Adenocarcinoma dataset.

**Usage**

data(demo_survival_data)

**Format**

Surv data created by Surv() function in survival package. This data have two unnamed arguments, they will match time and event.

---

**DiffMCB**

Differential expressed methylation correlated blocks

**Description**

This function is used to find the Methylation correlated blocks that differentially expressed between groups based on the attractor framework. This function calculates attractors of all the MCBs among the groups and find the attractor MCBs.

**Usage**

diffMCB(
  methylation_matrix, 
  class_vector, 
  mcb_matrix = NULL, 
  min.cpgsize = 5, 
  pVals_num = 0.05, 
  base_method = c("Fstat", "Tstat", "eBayes")[1], 
  sec_method = c("ttest", "kstest")[1], 
  ...
)

**Arguments**

- **methylation_matrix**
  - methylation profile matrix.
- **class_vector**
  - class vectors that indicated the groups.
- **mcb_matrix**
  - dataframe or matrix results returned by IdentifyMCB function.
min.cpgsize: threshold for minimum CpGs must included in the individual MCBs.

pVals_num: p value threshold for the test.

base_method: base method used for calculation of differentially methylated regions, should be one of 'Fstat','Tstat','eBayes'. Default is Fstat.

sec_method: secondly method in attractor framework, should be one of 'ktest','ttest'. Default is ttest.

... other parameters pass to the function.

Details

Currently, only illumina 450k platform is supported.
If you want to use other platform, please provide the annotation file with CpG’s chromosome and loci.
The methylation profile need to convert into matrix format.

Value

Object of class list with elements:

  global Character set contains statistical value for all CpG sites in MCBs.
  tab Matrix contains the information of results.

Author(s)

Xin Yu

References

Xin Yu, De-Xin Kong. EnMCB: an R/bioconductor package for predicting disease progression based on methylation correlated blocks using ensemble models, Bioinformatics, 2021, btab415

Examples

data('demo_data', package = "EnMCB")
data('demo_survival_data', package = "EnMCB")
data('demo_MCBinformation', package = "EnMCB")
#Using survival censoring as group label just for demo,
#this may replace with disease and control group in real use.
diffMCB_results <- DiffMCB(demo_data$realdata,demo_survival_data[,2],
demo_MCBinformation,
pVals_num = 1)
**Description**

Draw a survival curve based on survminer package. This is a wrapper function of ggsurvplot.

**Usage**

```r
draw_survival_curve(
  exp,
  living_days,
  living_events,
  write_name,
  title_name = "",
  threshold = NA,
  file = FALSE
)
```

**Arguments**

- `exp` expression level for variable.
- `living_days` The survival time (days) for each individual.
- `living_events` The survival event for each individual, 0 indicates alive and 1 indicates death. Other choices are TRUE/FALSE (TRUE = death) or 1/2 (2=death). For interval censored data, the status indicator is 0=right censored, 1=event at time, 2=left censored, 3=interval censored.
- `write_name` The name for pdf file which contains the result figure.
- `title_name` The title for the result figure.
- `threshold` Threshold used to indicate the high risk or low risk.
- `file` If True, function will automatic generate a result pdf, otherwise it will return a ggplot object. Default is FALSE.

**Value**

This function will generate a pdf file with 300dpi which compare survival curves using the Kaplan-Meier (KM) test.

**Author(s)**

Xin Yu
**Examples**

```r
data(demo_survival_data)
library(survival)
demo_set<-create_demo()
draw_survival_curve(demo_set[,1],
               living_days = demo_survival_data[,1],
               living_events = demo_survival_data[,2],
               write_name = "demo_data")
```

**Description**

Method for training a stacking ensemble model for Methylation Correlation Block.

**Usage**

```r
ensemble_model(single_res,training_set,Surv_training,testing_set,
               Surv_testing,ensemble_type)
```

**Arguments**

- `single_res`: Methylation Correlation Block information returned by the `IdentifyMCB` function.
- `training_set`: Methylation matrix used for training the model in the analysis.
- `Surv_training`: Survival function contain the survival information for training.
- `testing_set`: Methylation matrix used for testing the model in the analysis.
- `Surv_testing`: Survival function contain the survival information for testing.
- `ensemble_type`: Secondary model use for ensemble, one of "Cox", "C-index" and "feature weighted linear regression". "feature weighted linear regression" only uses two meta-features namely kurtosis and S.D.

**Value**

Object of class `list` with elements (XXX represents the model you choose):

- `cox`: Model object for the cox model at first level.
- `svm`: Model object for the svm model at first level.
- `enet`: Model object for the enet model at first level.
- `mboost`: Model object for the mboost model at first level.
- `stacking`: Model object for the stacking model.
ensemble_prediction

Author(s)
Xin Yu

References
Xin Yu et al. 2019 Predicting disease progression in lung adenocarcinoma patients based on methylation correlated blocks using ensemble machine learning classifiers (under review)

Examples

```r
# import datasets
library(survival)
data(demo_survival_data)
datamatrix<-create_demo()
data(demo_MCBinformation)
# select MCB with at least 3 CpGs.
demo_MCBinformation<-demo_MCBinformation[demo_MCBinformation[, "CpGs_num"] > 2, ]
trainingset<-colnames(datamatrix) %in% sample(colnames(datamatrix), 0.6 * length(colnames(datamatrix)))
select_single_one=1
em<-ensemble_model(t(demo_MCBinformation[select_single_one, ]),
                    training_set=datamatrix[, trainingset],
                    Surv_training=demo_survival_data[trainingset])
```

---

**ensemble_prediction**

_fitting function using stacking ensemble model for Methylation Correlation Block_

---

Description
predict is a generic function for predictions from the results of stacking ensemble model fitting functions. The function invokes particular methods which is the ensemble model described in the reference.

Usage

```r
ensemble_prediction(ensemble_model, prediction_data, multiple_results = FALSE)
```

Arguments

- **ensemble_model**
  - ensemble model which built by ensemble_model() function
- **prediction_data**
  - A vector, matrix, list, or data frame containing the predictions (input).
- **multiple_results**
  - Boolean vector, True for including the single model results.
Value

Object of numeric class double

References

Xin Yu et al. 2019 Predicting disease progression in lung adenocarcinoma patients based on methylation correlated blocks using ensemble machine learning classifiers (under review)

Examples

```r
library(survival)
# import datasets
data(demo_survival_data)
datamatrix<-create_demo()
data(demo_MCBinformation)
# select MCB with at least 3 CpGs.
demo_MCBinformation<-demo_MCBinformation[demo_MCBinformation[,"CpGs_num"]>2,]
trainingset<-colnames(datamatrix) %in% sample(colnames(datamatrix),0.6*length(colnames(datamatrix)))
testingset<-!trainingset
# select one MCB
select_single_one=1
em<-ensemble_model(t(demo_MCBinformation[select_single_one,]),
training_set=datamatrix[,trainingset],
Surv_training=demo_survival_data[trainingset])
em_prediction_results<-ensemble_prediction(ensemble_model = em,
prediction_data = datamatrix[,testingset])
```

---

**fast_roc_calculation**

Fast calculation of AUC for ROC using parallel strategy

Description

This function is used to create time-dependent ROC curve from censored survival data using the Kaplan-Meier (KM) or Nearest Neighbor Estimation (NNE) method of Heagerty, Lumley and Pepe, 2000

Usage

```r
fast_roc_calculation(test_matrix, y_surv, predict_time = 5, roc_method = "NNE")
```

Arguments

- `test_matrix`: Test matrix used in the analysis. Columns are samples, rows are markers.
- `y_surv`: Survival information created by Surv function in survival package.
- `predict_time`: Time point of the ROC curve, default is 5 year.
- `roc_method`: Method for fitting joint distribution of (marker, t), either of KM or NNE, the default method is NNE.
**Value**

This will retrun a numeric vector contains AUC results for each row in test_matrix.

**Author(s)**

Xin Yu

**Examples**

```r
data(demo_survival_data)
data('demo_data', package = "EnMCB")
demo_set <- demo_data$realdata
demo_survival_data
res <- fast_roc_calculation(demo_set[1:2,], demo_survival_data)
```

---

**IdentifyMCB**

**Identification of methylation correlated blocks**

**Description**

This function is used to partition the genome into blocks of tightly co-methylated CpG sites, Methylation correlated blocks. This function calculates Pearson correlation coefficients between the beta values of any two CpGs < CorrelationThreshold was used to identify boundaries between any two adjacent markers indicating uncorrelated methylation. Markers not separated by a boundary were combined into MCB. Pearson correlation coefficients between two adjacent CpGs were calculated.

**Usage**

```r
IdentifyMCB(
  MethylationProfile,
  method = c("pearson", "spearman", "kendall")[[1]],
  CorrelationThreshold = 0.8,
  PositionGap = 1000,
  platform = "Illumina Methylation 450K",
  verbose = T
)
```

**Arguments**

- **MethylationProfile**
  Methylation matrix is used in the analysis.
- **method**
  method used for calculation of correlation, should be one of "pearson", "spearman", "kendall". Defualt is "pearson".
- **CorrelationThreshold**
  coef correlation threshold is used for define boundaries.
PositionGap: CpG Gap between any two CpGs positioned CpG sites less than 1000 bp (default) will be calculated.

platform: This parameter indicates the platform used to produce the methylation profile. You can use your own annotation file.

verbose: True as default, which will print the block information for each chromosome.

Details:
Currently, only illumina 450k platform is supported, the methylation profile need to convert into matrix format.

Value:
Object of class list with elements:

- MCBsites: Character set contains all CpG sites in MCBs.
- MCBinformation: Matrix contains the information of results.

Author(s):
Xin Yu

References:
Xin Yu, De-Xin Kong. EnMCB: an R/bioconductor package for predicting disease progression based on methylation correlated blocks using ensemble models, Bioinformatics, 2021, btab415

Examples:
```
data('demo_data', package = 'EnMCB')

# import the demo TCGA data with 10000+ CpGs site and 455 samples
# remove # to run
res<-IdentifyMCB(demo_data$realdata)
demo_MCBinformation<-res$MCBinformation
```
**Description**

This function is used to partition the genome into blocks of tightly co-methylated CpG sites, Methylation correlated blocks parallelly. This function calculates Pearson correlation coefficients between the beta values of any two CpGs < CorrelationThreshold was used to identify boundaries between any two adjacent markers indicating uncorrelated methylation. Markers not separated by a boundary were combined into MCB. Pearson correlation coefficients between two adjacent CpGs were calculated.

**Usage**

```r
IdentifyMCB_parallel(
  MethylationProfile,
  method = c("pearson", "spearman", "kendall")[1],
  CorrelationThreshold = 0.8,
  PositionGap = 1000,
  platform = "Illumina Methylation 450K",
  verbose = T
)
```

**Arguments**

- **MethylationProfile**: Methylation matrix is used in the analysis.
- **method**: method used for calculation of correlation, should be one of "pearson", "spearman", "kendall". Default is "pearson".
- **CorrelationThreshold**: coef correlation threshold is used for define boundaries.
- **PositionGap**: CpG Gap between any two CpGs positioned CpG sites less than 1000 bp (default) will be calculated.
- **platform**: This parameter indicates the platform used to produce the methylation profile. You can use your own annotation file.
- **verbose**: True as default, which will print the block information for each chromosome.

**Details**

Currently, only illumina 450k platform is supported, the methylation profile need to convert into matrix format.

**Value**

Object of class list with elements:

- **MCBsites**: Character set contains all CpG sites in MCBs.
- **MCBinformation**: Matrix contains the information of results.
Author(s)
Xin Yu

References
Xin Yu, De-Xin Kong. EnMCB: an R/bioconductor package for predicting disease progression based on methylation correlated blocks using ensemble models. Bioinformatics, 2021, btab415

Examples

data('demo_data', package = "EnMCB")

# import the demo TCGA data with 10000+ CpGs site and 455 samples
# remove # to run
res<-IdentifyMCB_parallel(demo_data$realdata)
demo_MCBinformation<-res$MCBinformation

metricMCB

Calculation of the metric matrix for Methylation Correlation Block

Description
To enable quantitative analysis of the methylation patterns within individual Methylation Correlation Blocks across many samples, a single metric to define the methylated pattern of multiple CpG sites within each block. Compound scores which calculated all CpGs within individual Methylation Correlation Blocks by linear, SVM or elastic-net model. Predict values were used as the compound methylation values of Methylation Correlation Blocks.

Usage
metricMCB(MCBset, training_set, Surv, testing_set, Surv.new, Method, predict_time, ci, silent, alpha, n_mstop, n_nu, theta)

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCBset</td>
<td>Methylation Correlation Block information returned by the IdentifyMCB function.</td>
</tr>
<tr>
<td>training_set</td>
<td>methylation matrix used for training the model in the analysis.</td>
</tr>
<tr>
<td>Surv</td>
<td>Survival function contain the survival information for training.</td>
</tr>
<tr>
<td>testing_set</td>
<td>methylation matrix used in the analysis. This can be missing then training set itself will be used as testing set.</td>
</tr>
<tr>
<td>Surv.new</td>
<td>Survival function contain the survival information for testing.</td>
</tr>
</tbody>
</table>
**Method**

Model used to calculate the compound values for multiple Methylation correlation blocks. Options include "svm" "cox" "mboost" and "enet". The default option is SVM method.

**predict_time**

time point of the ROC curve used in the AUC calculations, default is 5 years.

**ci**

if True, the confidence intervals for AUC under area under the receiver operating characteristic curve will be calculated. This will be time consuming. default is False.

**silent**

True indicates that processing information and progress bar will be shown.

**alpha**

The elasticnet mixing parameter, with \(0 \leq \alpha \leq 1\). \(\alpha=1\) is the lasso penalty, and \(\alpha=0\) the ridge penalty.

It works only when "enet" Method is selected.

**n_mstop**

an integer giving the number of initial boosting iterations. If mstop = 0, the offset model is returned.

It works only when "mboost" Method is selected.

**n_nu**

a double (between 0 and 1) defining the step size or shrinkage parameter in mboost model.

It works only when "mboost" Method is selected.

**theta**

penalty used in the penalized coxph model, which is \(\theta/2\) time sum of squared coefficients. default is 1.

It works only when "cox" Method is selected.

**Value**

Object of class list with elements (XXX will be replaced with the model name you choose):

- MCB_XXX_matrix_training: Prediction results of model for training set.
- MCB_XXX_matrix_test_set: Prediction results of model for test set.
- XXX_auc_results: AUC results for each model.
- best_XXX_model: Model object for the model with best AUC.
- maximum_auc: Maximum AUC for the whole generated models.

**Author(s)**

Xin Yu

**References**

Xin Yu et al. 2019 Predicting disease progression in lung adenocarcinoma patients based on methylation correlated blocks using ensemble machine learning classifiers (under review)

**Examples**

```r
# import datasets
data(demo_survival_data)
datamatrix<-create_demo()
```
calculeaza metrica pentru blocuri de correlare a metilatii

Description
To enable quantitative analysis of the methylation patterns within individual Methylation Correlation Blocks across many samples, a single metric to define the methylated pattern of multiple CpG sites within each block. Compound scores which calculated all CpGs within individual Methylation Correlation Blocks by SVM model were used as the compound methylation values of Methylation Correlation Blocks.

Usage
metricMCB.cv(MCBset, data_set, Surv, nfold, Method, predict_time, alpha, n_mstop, n_nu, theta, silent)

Arguments
- **MCBset**: Methylation Correlation Block information returned by the IndentifyMCB function.
- **data_set**: methylation matrix used for training the model in the analysis.
- **Surv**: Survival function contain the survival information for training.
- **nfold**: fold used in the cross validation procedure.
- **Method**: model used to calculate the compound values for multiple Methylation correlation blocks. Options include "svm", "cox", "mboost", and "enet". The default option is SVM method.
- **predict_time**: time point of the ROC curve used in the AUC calculations, default is 3 years.
- **alpha**: The elasticnet mixing parameter, with 0 <= alpha <= 1. alpha=1 is the lasso penalty, and alpha=0 the ridge penalty. It works only when "enet" Method is selected.
n_mstop an integer giving the number of initial boosting iterations. If mstop = 0, the offset model is returned. It works only when "mboost" Method is selected.

n_nu a double (between 0 and 1) defining the step size or shrinkage parameter in mboost model. It works only when "mboost" Method is selected.

theta penalty used in the penalized coxph model, which is theta/2 time sum of squared coefficients. default is 1. It works only when "cox" Method is selected.

silent Ture indicates that processing information and progress bar will be shown.

Value

Object of class list with elements (XXX will be replaced with the model name you choose):

- MCB_matrix Prediction results of model.
- auc_results AUC results for each model.

Author(s)

Xin Yu

References

Xin Yu et al. 2019 Predicting disease progression in lung adenocarcinoma patients based on methylation correlated blocks using ensemble machine learning classifiers (under review)

Examples

```r
#import datasets
data(demo_survival_data)
datamatrix<-create_demo()
data(demo_MCBinformation)
#select MCB with at least 3 CpGs.
demo_MCBinformation<-demo_MCBinformation[demo_MCBinformation[,"CpGs_num"]>2,]

trainingset<-colnames(datamatrix) %in% sample(colnames(datamatrix),0.6*length(colnames(datamatrix)))
testingset<!trainingset
#create the results using Cox regression.
mcb_cox_res<-metricMCB.cv(MCBset = demo_MCBinformation,
data_set = datamatrix,
Surv = demo_survival_data,
Method = "cox")
```
multi_coxph

Multivariate survival analysis using coxph

Description

Multivariate survival analysis using coxph

Usage

multi_coxph(dataframe, y_surv, digits = 4, asnumeric = TRUE)

Arguments

dataframe Clinic data and covariates ready to be tested. Note that Rows are samples and columns are variables.
y_surv Survival function contain survival data, usually are obtained form Surv() function in survival package.
digits Integer indicating the number of decimal places.
asnumeric indicator that the data will be (True) / not (False) transformed into numeric. Default is true.

Value

Object of class matrix with results.

Author(s)

Xin Yu

Examples

data(demo_survival_data)
data('demo_data',package = "EnMIB")
demo_set<-demo_data$realdata
res<-multi_coxph(t(demo_set),demo_survival_data)
**predict.mcb.coxph.penal**

`predict coxph penal using MCB`

**Description**

Compute fitted values and regression terms for a model fitted by coxph

**Usage**

```r
## S3 method for class 'mcb.coxph.penal'
predict(object, newdata, ...)
```

**Arguments**

- `object`: the results of a coxph fit.
- `newdata`: Optional new data at which to do predictions. If absent predictions are for the data frame used in the original fit. When coxph has been called with a formula argument created in another context, i.e., coxph has been called within another function and the formula was passed as an argument to that function, there can be problems finding the data set. See the note below.
- `...`: other parameters pass to predict.coxph

**Value**

prediction values of regression.

**Author(s)**

Xin Yu

---

**pre_process_methylation**

`Preprocess the Beta value matrix`

**Description**

This process is optional for the pipeline. This function pre-process the Beta matrix and transform the Beta value into M value.

**Usage**

```r
pre_process_methylation(met,Mvalue,constant_offset,remove_na,remove_percentage)
```
Arguments

- met: Methylation matrix for CpGs. Rows are the CpG names, columns are samples.
- Mvalue: Boolean value, TRUE for the M transformation.
- constant_offset: The constant offset used in the M transformation formula.
- remove_na: Boolean value, if TRUE, CpGs with NA values will be removed.
- remove_percentage: If percentage of NA value exceed the threshold (percentage), the whole CpG probe will be removed. Otherwise, the NA values are replaced with row means.

Value

Object of class `matrix`.

Examples

```r
demo_set<-create_demo()
pre_process_methylation(demo_set,Mvalue=FALSE)
```

univ_coxph

Univariate and multivariate survival analysis using coxph

Description

Univariate and multivariate survival analysis using coxph

Usage

```r
univ_coxph(dataframe, y_surv, digits = 4, asnumeric = TRUE)
```

Arguments

- dataframe: Clinic data and covariates ready to be tested. Rows are variables and columns are samples.
- y_surv: Survival function contain survival data, usually are obtained form `Surv()` function in survival package.
- digits: Integer indicating the number of decimal places.
- asnumeric: Indicator that the data will be (True) / not (False) transformed into numeric. Default is true.

Value

Object of class `matrix` with results.
Author(s)
Xin Yu

Examples

data(demo_survival_data)
data('demo_data',package = "EnMCB")
demo_set<-demo_data$realdata
res<-univ_coxph(demo_set,demo_survival_data)
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