# Package 'EnMCB'

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Type Package Title Predicting Disease Progression Based on Methylation Correlated Blocks using Ensemble Models **Version** 1.16.0 Date 2023-3-13 Author Xin Yu Maintainer Xin Yu <whirlsyu@gmail.com> **Depends** R (>= 4.0) **Encoding UTF-8** Imports survivalROC, glmnet, rms, mboost, Matrix, igraph, methods, survivalsym, 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 git\_url https://git.bioconductor.org/packages/EnMCB git\_branch RELEASE\_3\_19 git\_last\_commit d9ee2d5 git\_last\_commit\_date 2024-04-30 **Repository** Bioconductor 3.19

Date/Publication 2024-05-14

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Illumina Human Methylation 450 kanno

## Description

 $\verb"anno_matrix"$ 

Illumina Human Methylation 450 kanno

### Usage

data(anno\_matrix)

### **Format**

IlluminaHumanMethylation450kanno.ilmn12.hg19 annotation file. This data have several columns

as.data.frame.ridgemat 3

```
as.data.frame.ridgemat
```

data frame ridge matrix

### 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

ridge matrix

### Description

as.matrix attempts to turn its argument

## Usage

```
as.ridgemat(x)
```

### Arguments

Х

data vector

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CompareMCB	Compare multiple methylation correlated blocks lists
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**

MCBs Methylation correlated blocks list.

method method used for calculation of differential expression,

should be one of "attractors", "t-test". Defualt is "attractors".

p\_value p value threshold for the test.

min\_CpGs threshold for minimum CpGs must included in the individual MCBs.

platform This parameter indicates the platform used to produce the methlyation profile.

#### **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

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### 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")
```

create\_demo

create demo matrix

### Description

Demo matrix for methylation matrix.

### Usage

```
create_demo(model = c("all", "short")[1])
```

## Arguments

model

Two options, 'all' or 'short' for creating full dataset or very brief demo.

#### Value

This function will generate a demo data.

### Author(s)

Xin Yu

```
demo_set<-create_demo()</pre>
```

demo\_MCBinformation

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 7

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.
```

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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', 'Estat', 'eBayes'. Defualt is Fstat.
sec_method	secondly method in attractor framework, should be one of 'kstest', 'ttest'. Defualt 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

draw\_survival\_curve 9

draw\_survival\_curve draw survival curve

#### **Description**

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

#### Usage

```
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

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#### **Examples**

```
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")</pre>
```

ensemble\_model

Trainging stacking ensemble model for Methylation Correlation Block

### Description

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

### Usage

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

#### **Arguments**

single_res	Methylation Correlation Block information returned by the IndentifyMCB 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 repesents 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.

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#### 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**

```
#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])</pre>
```

 $ensemble\_prediction$ 

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

```
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.
```

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#### 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**

```
library(survival)
#import datasets
data(demo_survival_data)
datamatrix<-create_demo()</pre>
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)))</pre>
testingset<-!trainingset
#select one MCB
select_single_one=1
em<-ensemble_model(t(demo_MCBinformation[select_single_one,]),</pre>
    training_set=datamatrix[,trainingset],
    Surv_training=demo_survival_data[trainingset])
em_prediction_results<-ensemble_prediction(ensemble_model = em,
prediction_data = datamatrix[,testingset])
```

 $fast\_roc\_calculation \quad \textit{Fast calculation of AUC for ROC using parallel strategy}$ 

### **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

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

### **Arguments**

test\_matrix Test matrix used in the analysis. Colmuns 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.

Method for fitting joint distribution of (marker,t), either of KM or NNE, the default method is NNE.

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#### Value

This will retrun a numeric vector contains AUC results for each row in test\_matrix.

#### Author(s)

Xin Yu

#### **Examples**

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

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

```
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 (de-

fault) will be calculated.

platform This parameter indicates the platform used to produce the methlyation 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

```
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</pre>
```

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#### **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

```
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". 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 (de-

fault) will be calculated.

platform This parameter indicates the platform used to produce the methlyation 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.

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#### 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</pre>
```

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**

MCBset Methylation Correlation Block information returned by the IndentifyMCB func-

tion.

training\_set methylation matrix used for training the model in the analysis.

Surv

Survival function contain the survival information for training.

testing\_set methylation matrix used in the analysis. This can be missing then training set

itself will be used as testing set.

Surv. new Survival function contain the survival information for testing.

metricMCB 17

Method model used to calculate the compound values for multiple Methylation correla-

tion 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  $0 \le \text{alpha} \le 1$ . alpha=1 is the lasso

penalty, and alpha=0 the ridge penalty.

It works only when "enet" Method is selected.

 $n_m$ stop 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**

#import datasets
data(demo\_survival\_data)
datamatrix<-create\_demo()</pre>

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metricMCB.cv

Calculation of model AUC for Methylation Correlation Blocks using cross validation

#### **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 func-

tion.

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 precedure.

Method model used to calculate the compound values for multiple Methylation correla-

tion 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  $0 \le \text{alpha} \le 1$ . alpha=1 is the lasso

penalty, and alpha=0 the ridge penalty. It works only when "enet" Method is

selected.

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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)

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multi_coxph	multivariate survival analysis using coxph	
marti_coxpn	mutivariate survivat anatysis using coxpn	

### 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

```
data(demo_survival_data)
data('demo_data',package = "EnMCB")
demo_set<-demo_data$realdata
res<-multi_coxph(t(demo_set),demo_survival_data)</pre>
```

```
predict.mcb.coxph.penal
```

predict coxph penal using MCB

### **Description**

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

#### Usage

```
## 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

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

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#### 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 precentage of NA value exceed the threshold(percentage), the whole CpG probe will be removed. Otherwise, the NA values are replaced with rowmeans.

#### Value

Object of class matrix.

### **Examples**

```
demo_set<-create_demo()
pre_process_methylation(demo_set,Mvalue=FALSE)</pre>
```

univ\_coxph

univariate and multivariate survival analysis using coxph

### **Description**

univariate and multivariate survival analysis using coxph

### Usage

```
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() func-

tion 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.

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### Author(s)

Xin Yu

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

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