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

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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

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`anno_matrix` *IlluminaHumanMethylation450kanno*

Description

IlluminaHumanMethylation450kanno

Usage

```
data(anno_matrix)
```

Format

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

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

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

`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

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

Examples

```
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'. 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

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)
```

`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

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" )
```

`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.

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])
```

---

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.

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()
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

```
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.
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

```
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)
```

`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 = TRUE
)
```

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 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

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
```

`IdentifyMCB_parallel` *Identification of methylation correlated blocks with parallel algorithm*

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 (default) 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

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

MCBset	Methylation Correlation Block information returned by the IndentifyMCB function.
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.

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 <= 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.

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()
```

```
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(MCBset = demo_MCBinformation,
               training_set = datamatrix[,trainingset],
               Surv = demo_survival_data[trainingset],
               testing_set = datamatrix[,testingset],
               Surv.new = demo_survival_data[testingset],
               Method = "cox"
               )
```

`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 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 precedure.
`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

```
#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 = "EnMCB")
demo_set<-demo_data$realdata
res<-multi_coxph(t(demo_set),demo_survival_data)
```

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)
```

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)
```

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

---

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() 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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