

Package ‘xmsPANDA’

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Type Package

Title Predictive And Network Discovery Analysis (xmsPANDA): R package for biomarker discovery and biomarker-driven network analysis for studying disease mechanisms using metabolomics

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Description This package includes functions to perform biomarker discovery for classification and regression scenarios and to perform correlation based network analysis (all metabolites and/or targeted metabolites eg: glucose metabolism pathway).

License GPL2.0

LazyLoad yes

Depends limma, GO.db, statmod, rgl, gplots, snow, mixOmics, doSNOW, foreach, e1071, WGCNA, randomForest, party, qvalue, fdrtool, GeneNet, corpcor, earth, pROC, multcomp, RColorBrewer, nlme, pls, plsgenomics, igraph, ROCR, flashClust, data.table, dplyr, plyr, mclust, parallel, RankAggreg, pcaMethods, pamr, sandwich, Boruta

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Description

R pacakge for biomarker discovery, supervised and unsupervised learning, and network analysis.

Details

Package:	xmsPANDA
Type:	Package
Version:	1.0.7.5
Date:	2018-05-01
License:	gpl2.0
LazyLoad:	yes

Author(s)

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data_preprocess	<i>data_preprocess</i>
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Description

This function performs data transformation, normalization

Usage

```
data_preprocess(Xmat=NA,Ymat=NA,feature_table_file, parentoutput_dir, class_labels_file,
num_replicates = 3,
feat.filt.thresh = NA, summarize.replicates = TRUE, summary.method = "mean",
all.missing.thresh=0.5,
group.missing.thresh = 0.7, log2transform = TRUE,
medcenter = TRUE, znormtransform = FALSE, quantile_norm = TRUE,
lowess_norm = FALSE,madscaling = FALSE, missing.val = 0, samplerindex = NA,
rep.max.missing.thresh = 0.5,summary.na.replacement = "zeros",featselmethod=NA)
```

Arguments

Xmat	R object for feature table. If this is given, then feature table can be set to NA.
Ymat	R object for response/class labels matrix. If this is given, then class can be set to NA.

feature_table_file	Feature table that includes the mz, retention time, and measured intensity in each sample for each analyte. The first 2 columns should be the mz and time. The remaining columns should correspond to the samples in the class labels file with each column including the intensity profile of a sample. Full path required. Eg: C:/My Documents/test.txt The feature table should be in a tab-delimited format. An example of the input file is provided under the "example" folder.
parentoutput_dir	Provide full path of the folder where you want the results to be written. Eg: C:/My Documents/ProjectA/results/
class_labels_file	File with class labels information for each sample. Samples should be in the same order as in the feature table. Please use the same format as in the example folder.
num_replicates	Number of technical replicates
feat.filt.thresh	Percent Intensity Difference or Coefficient of variation threshold; feature filtering Use NA to skip this step.
summarize.replicates	Do the technical replicates per sample need to be averaged or median summarized?
summary.method	Method for summarizing the replicates. Options: "mean" or "median"
summary.na.replacement	How should the missing values be represented? Options: "zeros", "halffeaturemin", "halfsamplemin", "halfdatamin", "none" "zeros": replaces missing values by 0 "halfsamplemin": replaces missing value by one-half of the lowest signal intensity in the corresponding sample "halfdatamin": replaces missing value by one-half of the lowest signal intensity in the complete dataset "halffeaturemin": replaces missing value by one-half of the lowest signal intensity for the current feature "none": keeps missing values as NAs Users are recommended to perform imputation prior to performing biomarker discovery.
missing.val	How are the missing values represented in the input data? Options: "0" or "NA"
samplerindex	Column index of any additional or irrelevant columns to be deleted. Options: "NA" or list of column numbers. eg: c(1,3,4) Default=NA
rep.max.missing.thresh	What proportion of replicates are allowed to have missing values during the averaging or median summarization step of each biological sample? If the number of replicates with missing values is greater than the defined threshold, then the summarized value is represented by the "missing.val" parameter. If the number of replicates with missing values is less than or equal to the defined threshold, then the summarized value is equal to the mean or the median of the non-missing values. Default: 0.5
all.missing.thresh	What proportion of total number of samples should have an intensity? Default: 0.5
group.missing.thresh	What proportion of samples in either of the two groups should have an intensity? If at least x for further analysis. Default: 0.7

log2transform	Data transformation: Please refer to http://www.biomedcentral.com/1471-2164/7/142 Try different combinations; such as log2transform=TRUE, znormtransform=FALSE or log2transform=FALSE, znormtransform=TRUE
medcenter	Median centering of metabolites
znormtransform	Auto scaling; each metabolite will have a mean of 0 and unit variance
quantile_norm	Performs quantile normalization. Normalization options: Please set only one of the options to be TRUE
lowess_norm	Performs lowess normalization. Normalization options: Please set only one of the options to be TRUE
madscaling	Performs median adjusted scale normalization. Normalization options: Please set only one of the options to be TRUE

Value

Pre-processed data matrix.

Author(s)

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diffexp

diffexp

Description

This function performs biomarker discovery and generates a correlation network based on the metabolome-wide (and targeted) correlation analysis of the differentially expressed features. The "featselmethod" allows users to select the method for selecting discriminatory features. The function evaluates the k-fold cross-validation accuracy using Support Vector Machine, performs hierarchical clustering analysis, PCA analysis (R2/Q2 diagnostics), and generates boxplots for discriminatory features identified at each relative standard deviation (coefficient of variation) threshold across all samples (if one feature selection method is used). An optimization score that minimizes the number of false positives and increases the classification accuracy is used to select the best set of features. The best set is then used for correlation (complete or partial) based metabolome-wide network analysis. Additionally, users have the option to provide a list of mzs corresponding to chemicals of interest such as (phenylalanine, choline, etc). The function uses the getVenn function in xMSanalyzer to find the mzs matching the target list based on a user defined mass search threshold (+/- ppm).

Usage

```
diffexp(Xmat = NA, Ymat = NA, feature_table_file, parentoutput_dir, class_labels_file,
num_replicates = 3, summarize.replicates = TRUE, summary.method = "mean",
summary.na.replacement = "zeros", missing.val = 0, rep.max.missing.thresh = 0.3,
all.missing.thresh = 0.5, group.missing.thresh = 0.8, input.intensity.scale = "raw",
log2transform = TRUE, medcenter = FALSE, znormtransform = FALSE, quantile_norm = TRUE,
lowess_norm = FALSE, madscaling = FALSE, rsd.filt.list = seq(0, 75, 5), pairedanalysis = FALSE,
featselmethod = "limma", pvalue.thresh = 0.05, fdrthresh = 0.05, fdrmethod = "BH",
cor.method = "spearman", networktype = "complete", abs.cor.thresh = 0.4, cor.fdrthresh = 0.05,
kfold = 10, pred.eval.method = "BER", globalcor = TRUE, target.metab.file = NA,
```

```

target.mzmatch.diff = 10, target.rtmatch.diff = NA, max.cor.num = 100,
numtrees = 20000, analysismode = "classification", net_node_colors = c("green", "red"),
net_legend = TRUE, svm_kernel = "radial", heatmap.col.opt = "redblue",
manhattanplot.col.opt = c("darkblue", "red3"), boxplot.col.opt = c("grey57"),
sample.col.opt = "rainbow", rf_selmethod = "absVIMthresh", pls_vip_thresh = 1,
num_nodes = 2, max_varsel = 100, pls_ncomp = 5, pca.stage2.eval = TRUE,
scoreplot_legend = TRUE, pca.global.eval = TRUE, rocfeatlist = seq(2, 11, 1),
rocfeatincrement = TRUE, rocclassifier = "svm", foldchangethresh = 2, wgcnaresdthresh = 20,
WGCNAmolecules = TRUE, optselect = TRUE, max_comp_sel = 1, saveRda = TRUE,
legendlocation = "topleft", pca.cex.val = 4, pca.ellipse = FALSE, ellipse.conf.level = 0.95,
permutations.count = 1000, svm.acc.tolerance = 5, limmadecideTests = TRUE,
pls.vip.selection = "max", globalclustering = TRUE, plots.res = 600, plots.width = 8,
plots.height = 8, plots.type = "cairo", output.device.type = "pdf", ,
individualsampleplot.col.opt = NA, pamr.threshold.select.max = FALSE,
aggregation.method = "RankAggreg", aggregation.max.iter=1000, mars.gcv.thresh=10, ...)

```

Arguments

Xmat	R object for feature table. If this is given, then feature table can be set to NA.
Ymat	R object for response/class labels matrix. If this is given, then class can be set to NA.
feature_table_file	Feature table that includes the mz, retention time, and measured intensity in each sample for each analyte. The first 2 columns should be the mz and time. The remaining columns should correspond to the samples in the class labels file with each column including the intensity profile of a sample. Full path required. Eg: C:/My Documents/test.txt The feature table should be in a tab-delimited format. An example of the input file is provided under the "example" folder.
parentoutput_dir	Provide full path of the folder where you want the results to be written. Eg: C:/My Documents/ProjectA/results/
class_labels_file	File with class labels information for each sample. Samples should be in the same order as in the feature table. Please use the same format as in the example folder. If you want to adjust for covariates in "lmreg" option, then you can add additional columns, one per covariate. Categorical variables should be strings (eg: "male", "female"). Please see "classlabels_gender.txt" file as an example.
num_replicates	Number of technical replicates
feat.filt.thresh	Percent Intensity Difference or Coefficient of variation threshold; feature filtering Use NA to skip this step.
summarize.replicates	Do the technical replicates per sample need to be averaged or median summarized?
summary.method	Method for summarizing the replicates. Options: "mean" or "median"
summary.na.replacement	How should the missing values be represented? Options: "zeros", "halffeaturemin", "halfsamplemin", "halfdatamin", "none" "zeros": replaces missing values by 0 "halfsamplemin": replaces missing value by one-half of the lowest signal intensity in the corresponding sample "halfdatamin": replaces missing

	value by one-half of the lowest signal intensity in the complete dataset "halffeaturemin": replaces missing value by one-half of the lowest signal intensity for the current feature "none": keeps missing values as NAs Users are recommended to perform imputation prior to performing biomarker discovery.
missing.val	How are the missing values represented in the input data? Options: "0" or "NA"
rep.max.missing.thresh	What proportion of replicates are allowed to have missing values during the averaging or median summarization step of each biological sample? If the number of replicates with missing values is greater than the defined threshold, then the summarized value is represented by the "missing.val" parameter. If the number of replicates with missing values is less than or equal to the defined threshold, then the summarized value is equal to the mean or the median of the non-missing values. Default: 0.5
all.missing.thresh	What proportion of total number of samples should have an intensity? Default: 0.5
input.intensity.scale	Are the intensities in the input feature table at raw scale or log2 scale? eg: "raw" or "log2" Default: "raw"
group.missing.thresh	What proportion of samples in either of the two groups should have an intensity? If at least x for further analysis. Default: 0.7
log2transform	Data transformation: Please refer to http://www.biomedcentral.com/1471-2164/7/142 Try different combinations; such as log2transform=TRUE, znormtransform=FALSE or log2transform=FALSE, znormtransform=TRUE
medcenter	Median centering of metabolites
znormtransform	Auto scaling; each metabolite will have a mean of 0 and unit variance
quantile_norm	Performs quantile normalization. Normalization options: Please set only one of the options to be TRUE
lowess_norm	Performs lowess normalization. Normalization options: Please set only one of the options to be TRUE
madscaling	Performs median adjusted scale normalization. Normalization options: Please set only one of the options to be TRUE
rsd.filt.list	This parameter allows to perform feature filtering based on overall variance (across all samples) prior to performing hypothesis testing. Eg: seq(0,30,5).
pairedanalysis	Is this a paired-study design? TRUE or FALSE If samples are paired, then the feature table and the class labels file should be organized so that the paired samples are arranged in the same order in each group. For example, the first sample in group A and the first sample in group B should be paired.
featselmethod	Options: "limma": for one-way ANOVA using LIMMA (mode=classification) "limma2way": for two-way ANOVA using LIMMA (mode=classification) "limma1wayrepeat": for one-way ANOVA repeated measures using LIMMA (mode=classification) "limma2wayrepeat": for two-way ANOVA repeated measures using LIMMA (mode=classification) "lm1wayanova": for one-way ANOVA using linear model (mode=classification) "lm2wayanova": for two-way ANOVA using linear model (mode=classification) "lm1wayanovarepeat": for one-way ANOVA repeated measures using linear model (mode=classification) "lm2wayanovarepeat": for two-way ANOVA repeated measures using linear model (mode=classification) "lm-reg": variable selection based on p-values calculated using a linear regression

	<p>model; allows adjustment for covariates (mode= regression or classification) "logitreg": variable selection based on p-values calculated using a logistic regression model; allows adjustment for covariates (mode= classification) "rfesvm": uses recursive feature elimination SVM algorithm for variable selection; (mode=classification) "RF": for random forest based feature selection (mode= regression or classification) "RFconditional": for conditional random forest based feature selection (mode= regression or classification) "pamr": for prediction analysis for microarrays algorithm based on the nearest shrunken centroid method (mode= classification) "MARS": for multiple adaptive regression splines (MARS) based feature selection (mode= regression or classification) "pls": for partial least squares (PLS) based feature selection (mode= regression or classification) "spl": for sparse partial least squares (PLS) based feature selection (mode= regression or classification) "spl1wayrepeat": for sparse partial least squares (PLS) based feature selection for one-way repeated measures (mode= regression or classification) "spl2wayrepeat": for sparse partial least squares (PLS) based feature selection for two-way repeated measures (mode= regression or classification) "o1pls": for orthogonal partial least squares (OPLS) based feature selection (mode= regression or classification)</p>
fdrthresh	False discovery rate threshold. Eg: 0.05
fdrmethod	Options: "BH", "ST", "Strimmer", "none" "BH": Benjamini-Hochberg (1995) (Default: more conservative than "ST" and "Strimmer") "ST": Storey & Tibshirani (Storey 2001, PNAS) algorithm implemented in the qvalue package "Strimmer": (Strimmer 2008, Bioinformatics) algorithm implemented in the fdrtool package "none": No FDR correction will be performed. fdrthresh will be treated as raw p-value cutoff
cor.method	Correlation method. Options: "pearson" or "spearman". Default: "spearman"
networktype	Options: "complete" or "GGM" "complete": performs network analysis using ordinary Pearson or Spearman correlation statistic "GGM": generates network based on partial correlation analysis using the GeneNet package
abs.cor.thresh	Absolute Pearson correlation coefficient for network analysis. Default: 0.4
cor.fdrthresh	False discovery rate threshold for correlation analysis. Default: 0.05
kfold	Number of subsets in which the data should be divided for cross-validation. If kfold=10, then the data set will be divided into 10 subsets of size (N/10), where N is the total number of samples. 9 subsets are used for training and the remaining 1 is used for testing. This process is repeated 10 times and the CV-accuracy would be the mean of the classification accuracy from the 10 iterations. The same will be true for any other value of k. WARNING: The kfold value should be less than or equal to the total number of samples.
pred.eval.method	Criteria for evaluating the performance of the model. CV: Overall Cross-validation classification accuracy, balanced error rate (BER): (sum of accuracy in each class)/(number of classes) area under the curve (AUC) Eg: "CV", "BER", or "AUC". Default: "BER"
globalcor	Do you want to perform correlation analysis after biomarker discovery? Options: "TRUE" or "FALSE"
target.metab.file	File that includes the mz and/or retention time of the targeted metabolites. See example.
target.mzmatch.diff	+/- ppm mass tolerance for searching the target m/z in the current feature table

target.rtmatch.diff	+/- retention time tolerance for searching the target m/z in the current feature table
max.cor.num	Maximum number of correlated metabolites to be included in the network figure. Default: 100
pcacenter	Data centering for PCA. Options: "TRUE" or "FALSE". Default=TRUE
pcascale	Data scaling for PCA. Options: "TRUE" or "FALSE". Default=TRUE
samplermindex	Column index of any additional or irrelevant columns to be deleted. Options: "NA" or list of column numbers. eg: c(1,3,4) Default=NA
numtrees	Number of trees to be used for random forest method. Default=500
analysismode	"classification" for group-wise comparison (case vs control) or "regression" for continuous response variables. Default: "classification"
net_node_colors	Colors of nodes in the correlation networks. Eg: c("pink", "skyblue"), or ("red", "green")
net_legend	Should the network be displayed for the correlation network? eg: TRUE or FALSE
max_var	Max number of variables to be used for sPLS, rfesvm, and Random Forest? eg:150
svm_kernel	SVM kernel eg: "radial" or "linear"
heatmap.col.opt	Color scheme for HCA heatmap eg: "RdBu", "topo", "heat", or "terrain"
sample.col.opt	Color scheme for PCA and heatmap sample axis eg: "rainbow", "heat" or "topo"
rf_selmethod	Random forest VIP based selection method. If rankbased option is selected, variables are ranked based on the Variable Importance Measure. Only the top "max_varsel" variables are selected. If absVIMthresh is selected, then all features with VIM greater than the absolute value of the lowest VIM are selected. eg: "absVIMthresh" or "rankbased"
pls_vip_thresh	Threshold for VIP score from PLS/OIPLS. eg: 1
max_varsel	Maximum number of variables to keep if "rankbased" RF or spls is used. eg: 100
pls_ncomp	Maximum number of components to be considered during the PLS optimal number of components selection step. eg: 2
pca.stage2.eval	Should PCA diagnostics be performed in stage 2? eg: TRUE or FALSE
scoreplot_legend	Should legends be included in score plots? eg: TRUE or FALSE
pca.global.eval	Should global PCA evaluation be performed? Default:TRUE eg: TRUE or FALSE
rocfeatlist	Vector indicating number of features to be used for ROC evaluation: eg: c(2,4,6) will generate ROC for top 2, top 4, and top 6 feautres. Default: seq(2,10,1)
rocclassifier	Classifier to be used for ROC evaluation. Options: "svm" or "logitreg". Default: "svm"
foldchangethresh	Secondary feature selection criteria based on fold change threshold. This is performed after statistical significance or importance evaluation.

wgcnarsdthresh	Relative standard deviation or coefficient of variation (across all samples) based filtering threshold before performing WGCNA module preservation analysis. Default: 20
WGCNAmodules	Perform WGCNA module preservation analysis. TRUE or FALSE Default: TRUE
optselect	Determine optimal number of PLS components. Default: TRUE
max_comp_sel	Number of PLS components to use for VIP or sparse loading selection (sPLS). Default=1
saveRda	Should the results be saved in a binary R object. Default: TRUE
legendlocation	Legend location for PLS or PCA plots
pca.cex.val	Size of points on PCA plots. eg: 4
pca.ellipse	Should ellipse be plotted on PCA plots? eg: TRUE or FALSE
ellipse.conf.level	Confidence interval for PCA ellipses eg: 0.95
pls.permut.count	Number of permutations for calculating p-values for PLS or sPLS models. eg: 1000
svm.acc.tolerance	Stopping criteria for forward feature selection using "rfeSVM" method. If the difference between best accuracy and current accuracy based on the newly added feature drops below the tolerance level, the forward selection process is terminated. eg: 5
pamr.threshold.select.max	If two or more thresholds for shrinking the d statistic in the PAM algorithm (Tibshirani et al. Statistical Science 2003) have equal accuracy, should the maximum value (lowest number of features) be used? Default: FALSE
aggregation.method	Method for combining the results from multiple feature selection methods Options: Consensus: will only keep features that are selected in all methods Rank-Aggreg: will use the cross entropy algorithm with Spearman footrule distance as the distance measure (RankAggreg; Pihur et al. BMC Bioinformatics 2009) aggregation.max.iter=1000,mars.gcv.thresh=10
aggregation.max.iter	Maximum number of iterations used in the rank aggregation step. Default: 1000 \itemmars.gcv.thresh Minimum generalized cross-validation value (range: 0 to 100) for a feature to be selected. Default: 10 This function performs data transformation, normalization, FDR analysis using LIMMA, variable selection using random forests, evaluates the predictive accuracy of the FDR significant features using k-fold cross-validation with a Support Vector Machine classifier, performs two-way hierarchical clustering analysis, and principal component analysis. An optimization scheme is used to select the best set of features from different log2 fold change filtering thresholds. Finally, metabolome-wide and targeted correlation based network analysis of the FDR significant features is performed. Following files are generated in the parent output location: Manhattan plots: showing metabolome wide p-values; Heatmap from Two-way hierarchical clustering analysis; Pairwise score plots from Principal Component Analysis; PCA score distribution plots; ROC plots; List of differentially expressed metabolites; Boxplots of differentially expressed metabolites; Correlation network figure and

matrix; Pairwise correlation matrix CIRCOS format ready to be uploaded to: <http://mkweb.bcgsc.ca/tableviewer/visualize/> Or uploaded to Cytoscape gml format

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do_wgcna

do_wgcna

Description

This function performs module preservation analysis using WGCNA.

Usage

```
do_wgcna(feature_table_file = NA, class_labels_file = NA, X = NA,
Y = NA, sigfeats = NA)
```

Arguments

`feature_table_file`

Path and name of feature table that includes the mz, retention time, and measured intensity in each sample for each analyte. The first 2 columns should be the mz and time. The remaining columns should correspond to the samples in the class labels file with each column including the intensity profile of a sample. Full path required. Eg: C:/My Documents/test.txt The feature table should be in a tab-delimited format. An example of the input file is provided under the "example" folder.

`class_labels_file`

File with class labels information for each sample. Samples should be in the same order as in the feature table. Please use the same format as in the example folder. If you want to adjust for covariates in "lmreg" option, then you can add additional columns, one per covariate. Categorical variables should be strings (eg: "male", "female"). Please see "classlabels_gender.txt" file as an example.

`X`

R object for feature table. If this is given, then feature table can be set to NA.

`Y`

R object for response/class labels matrix. If this is given, then class labels file can be set to NA.

`sigfeats`

List of differentially expressed features. Default: NA

Details

This function calls WGCNA to perform module preservation analysis between different classes or groups.

Value

PDF plots for module preservation from WGCNA and preservation matrix

Author(s)

Karan Uppal

References

WGCNA (Horvath 2007)

```
get.classification.accuracy
      get.classification.accuracy
```

Description

This function performs classification accuracy analysis using the training and test sets. Users can choose support vector machine, logistic regression, random forest, and naive bayes as classifiers. The performance evaluation is determined based on the total classification rate, balanced accuracy rate, and AUC.

Usage

```
get.classification.accuracy(kfold, featuretable, classlabels,
  kernelname = "radial", errortype = "AUC", conflevel = 95,
  classifier = "svm", seednum = 555,
  testfeaturetable = NA, testclasslabels = NA)
```

Arguments

kfold	Number of folds for cross-validation. e.g. 5 or 10
featuretable	R object for feature table with only differentially expressed features. This is the training set. The first two columns should be m/z and time.
classlabels	Class labels vector. e.g. c("case","control","case")
kernelname	Kernel for SVM: e.g. "radial" or "linear"
errortype	total: total classification accuracy rate; (number of correct classifications/total N) BAR: balanced accuracy rate; accounts of number of correct classification per class; $BAR = (1/m) * ((C1/N1) + (C2/N2) + \dots + (Cm/Nm))$ where m is the number of classes, Cm is the number of correct classifications in class m, and Nm is the total number of subjects in class m. AUC: area under the curve
conflevel	Confidence level for k-fold classification accuracy e.g: 95
classifier	Classification algorithm to be used for ROC analysis. svm: Support Vector Machine logitreg: Logistic Regression rf: random forest naivebayes: naive bayes eg: "svm", "logitreg", "rf", "naivebayes"
seednum	Starting point used in the generation of a sequence of random numbers. e.g. 555
testfeaturetable	R object for test feature table with only differentially expressed features. This is the test set. The first two columns should be m/z and time. The order of features should be same as the training set.
testclasslabels	Class labels vector for samples in the test set.

Details

Function to evaluate classification. This function performs classification accuracy analysis using the training and test sets. Users can choose support vector machine, logistic regression, random forest, and naive bayes as classifiers. The performance evaluation is determined based on the total classification rate, balanced accuracy rate, and AUC.

Value

Classification accuracy in training and test sets

Author(s)

Karan Uppal; kuppal2@emory.edu

get_boxplots	<i>get_boxplots</i>
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Description

This function generates boxplots for m/z features. The input intensity matrix could be transformed or non-transformed intensities. Sample labels in the class labels file should be in the same order as the intensity matrix or feature table.

Usage

```
get_boxplots(feature_table_file, parentoutput_dir, class_labels_file, sample.col.opt = "rainbow",
```

Arguments

- feature_table_file**
Feature table that includes the mz, retention time, and measured intensity in each sample for each analyte. The first 2 columns should be the mz and time. The remaining columns should correspond to the samples in the class labels file with each column including the intensity profile of a sample. Full path required. Eg: C:/My Documents/test.txt The feature table should be in a tab-delimited format. An example of the input file is provided under the "example" folder.
- parentoutput_dir**
Provide full path of the folder where you want the results to be written. Eg: C:/My Documents/ProjectA/results/
- class_labels_file**
File with class labels information for each sample. Samples should be in the same order as in the feature table. Please use the same format as in the example folder.
- sample.col.opt** Color scheme for PCA and heatmap sample axis eg: "heat" or "topo"
- alphacol=0.3** Color scaling parameter eg:0.3

Value

Creates a PDF with boxplots for each m/z feature.

Author(s)

Karan Uppal <kuppal2@emory.edu>

*get_hca**get_hca*

Description

This function performs two-way hierarchical clustering analysis and generates a heatmap showing the clustering results. The input intensity matrix could be transformed or non-transformed intensities. Sample labels in the class labels file should be in the same order as the intensity matrix or feature table.

Usage

```
get_hca(feature_table_file, parentoutput_dir, class_labels_file, heatmap.col.opt = "RdBu", cor.met
is.data.znorm = FALSE, analysismode = "classification", sample.col.opt = "rainbow", plots.width = 2000,
plots.height = 2000, plots.res = 300, alphacol = 0.3, hca_type="two-way")
```

Arguments**feature_table_file**

Feature table that includes the mz, retention time, and measured intensity in each sample for each analyte. The first 2 columns should be the mz and time. The remaining columns should correspond to the samples in the class labels file with each column including the intensity profile of a sample. Full path required. Eg: C:/My Documents/test.txt The feature table should be in a tab-delimited format. An example of the input file is provided under the "example" folder.

parentoutput_dir

Provide full path of the folder where you want the results to be written. Eg: C:/My Documents/ProjectA/results/

class_labels_file

File with class labels information for each sample. Samples should be in the same order as in the feature table. Please use the same format as in the example folder.

heatmap.col.opt

Color scheme for HCA heatmap eg: "RdBu", "topo", "heat", or "terrain"

cor.method

Correlation method. Options: "person" or "spearman". Default: "spearman"

is.data.znorm

Is the data already auto-scaled or z-scaled? eg: TRUE or FALSE

analysismode

"classification" for group-wise comparison (case vs control) or "regression" for continuous response variables. Default: "classification"

sample.col.opt

Color scheme for PCA and heatmap sample axis eg: "heat" or "topo" or "rainbow"

plots.width

Width of the tiff file. eg: 2000

plots.height

Height of the tiff file. eg: 2000

plots.res

Resolution of the tiff file. eg: 300

alphacol

Color scaling parameter eg:0.3

hcatype

Color scaling parameter eg:"two-way" or "one-way"

Value

Heatmap from Two-way hierarchical clustering analysis; Intensity matrix in the same order as the dendrograms in heatmap; Sample cluster labels

Author(s)

Karan Uppal <kuppal2@emory.edu>

get_manhattanplots	<i>get_manhattanplots</i>
--------------------	---------------------------

Description

Function to generate Manhattan plots.

Usage

```
get_manhattanplots(xvec, yvec, up_or_down, maintext = "", ythresh = 0.05,
y2thresh=NA, ylab, xlab, colorvec = c("darkgreen", "firebrick1"),
col_seq = c("brown", "chocolate3", "orange3", "coral", "pink", "skyblue",
"blue", "darkblue", "purple", "violet"), xincrement = 150, yincrement = 1)
```

Arguments

xvec	Vector with values for the x-axis. eg: m/z or retention time values
yvec	Vector with values for the y-axis. eg: (-)Log10 of p-values, VIP, loadings, regression coefficients, etc.
up_or_down	Vector indicating directionality of change. eg: Fold change values
maintext	Text for the plot title
ylab	Y-axis label
xlab	X-axis label
colorvec	Vector of colors for representing up-regulation and down-regulation. eg: c("darkgreen", "firebrick1") In this case, features that are up-regulated in class A will have "darkgreen" color, and features that are up-regulated in class B will have "firebrick1" color.
col_seq	Vector of colors for plotting different segments of the x-axis
xincrement	Window size for breaking the x-axis into different segments for visualization purposes. eg: 150
yincrement	Window size for breaking the y-axis into different segments for visualization purposes. eg: 1

Details

This function can be used to generate Type 1 Manhattan plots: significance vs m/z Type 2 Manhattan plots: significance vs retention time Type 3 Manhattan plots: significance vs intensity

Value

Manhattan plots

Note

```
#Example pdf("Manhattanplot.pdf") get_manhattanplots(...) #pass arguments dev.off()
```

Author(s)

Karan Uppal

get_pca	<i>Performs PCA analysis</i>
---------	------------------------------

Description

This function uses the `pca` function implemented in the `mixOmics` package for PCA analysis

Usage

```
get_pca(X, samplelabels, legendlocation = "topright", filename = NA,
        ncomp = 5, center = TRUE, scale = TRUE, legendcex = 0.5,
        outloc = getwd(), col_vec = NA, sample.col.opt = "default",
        alphacol = 0.3, class_levels = NA, pca.cex.val = 3,
        pca.ellipse = TRUE, ellipse.conf.level = 0.5, samplenames = FALSE)
```

Arguments

<code>X</code>	Data matrix without m/z and time.
<code>samplelabels</code>	Vector with class label for each sample.
<code>legendlocation</code>	Location of the legend on PCA score plots
<code>filename</code>	eg: "all", "significantfeats"
<code>ncomp</code>	Number of components; please use <code>?pca</code> for more information
<code>center</code>	Should the data be centered?; please use <code>?pca</code> for more information
<code>scale</code>	Should the data be scaled?; please use <code>?pca</code> for more information
<code>legendcex</code>	Size of the legend text in the PCA score plots. e.g.: 0.5 or 0.7
<code>outloc</code>	Output folder location
<code>col_vec</code>	Provide vector of colors for each group. eg: NA or <code>c("red", "green")</code> for cases and controls, respectively. This argument is ignored if <code>sample.col.opt</code> is provided
<code>sample.col.opt</code>	Select R color palette. eg: "rainbow", "terrain", "topo". "heat", "default"
<code>alphacol</code>	Semi-transparent colors eg: 0.2
<code>class_levels</code>	Vector with names of different sample groups. eg: <code>c("case", "control")</code> or NA
<code>pca.cex.val</code>	Size of dots in PCA score plots. eg: 0.4
<code>pca.ellipse</code>	Should the score confidence interval for each group be drawn? eg: TRUE or FALSE
<code>ellipse.conf.level</code>	Confidence interval level eg: 0.95
<code>samplenames</code>	Should the sample names be included in PCA plots? eg: TRUE or FALSE

Details

This function performs PCA analysis. The results are saved in a RDA file.

Value

The function returns PCA results as an object and generates pairwise score plots for the first three components

Author(s)

Karan Uppal

References

mixOmics

get_pcascoredistplots *get_pcascoredistplots*

Description

PCA score distribution (25th percentile, median, 75th percentile) plots

Usage

```
get_pcascoredistplots(X = NA, Y = NA, feature_table_file, parentoutput_dir,
  class_labels_file, sample.col.opt = "rainbow", plots.width = 2000,
  plots.height = 2000, plots.res = 300, alphacol = 0.3, col_vec,
  pairedanalysis = FALSE, pca.cex.val = 3, legendlocation = "topright",
  pca.ellipse = TRUE, ellipse.conf.level = 0.5, filename = "all")
```

Arguments

- | | |
|--------------------|--|
| X | R object for feature table. If this is given, then feature table can be set to NA. |
| Y | R object for response/class labels matrix. If this is given, then class labels file can be set to NA. |
| feature_table_file | Path and name of feature table that includes the mz, retention time, and measured intensity in each sample for each analyte. The first 2 columns should be the mz and time. The remaining columns should correspond to the samples in the class labels file with each column including the intensity profile of a sample. Full path required. Eg: C:/My Documents/test.txt The feature table should be in a tab-delimited format. An example of the input file is provided under the "example" folder. |
| parentoutput_dir | Provide full path of the folder where you want the results to be written. Eg: C:/My Documents/ProjectA/results/ |

<code>class_labels_file</code>	File with class labels information for each sample. Samples should be in the same order as in the feature table. Please use the same format as in the example folder. If you want to adjust for covariates in "lmreg" option, then you can add additional columns, one per covariate. Categorical variables should be strings (eg: "male", "female"). Please see "classlabels_gender.txt" file as an example.
<code>sample.col.opt</code>	Color scheme for PCA and heatmap sample axis eg: "rainbow", "heat" or "topo"
<code>alphacol</code>	Color scaling parameter eg:0.3
<code>col_vec</code>	Vector of colors for each sample.
<code>pairedanalysis</code>	Is this a paired-study design? TRUE or FALSE If samples are paired, then the feature table and the class labels file should be organized so that the paired samples are arranged in the same order in each group. For example, the first sample in group A and the first sample in group B should be paired.
<code>pca.cex.val</code>	Size of points on PCA plots. eg: 4
<code>legendlocation</code>	Legend location on PCA plots
<code>pca.ellipse</code>	Should ellipse be plotted on PCA plots? eg: TRUE or FALSE
<code>ellipse.conf.level</code>	Confidence interval for PCA ellipses eg: 0.95
<code>filename</code>	Name of output PDF file

Details

This function performs PCA and generates pairwise score plots as well as score distribution plots (per group). It uses the Y vector and classlabels for color coding the samples in the pairwise score plots.

Value

The output includes: Pairwise PCA score plots, PCA score distribution plots, PCA scores and loadings text files.

Note

The plots can be sent to an external device by running the following commands: `pdf("get_pcascoredistplots.pdf")`
`get_pcascoredistplots(...) dev.off()`

Author(s)

Karan Uppal

get_roc

get_roc

Description

This function generates Receiver Operating Characteristic curves using SVM and Logistic Regression as classifiers.

Usage

```
get_roc(dataA, classlabels, classifier = "svm", kname = "radial",
rocfeatlist = seq(2, 10, 1), rocfeatincrement = TRUE,
testset = NA, testclasslabels = NA, mainlabel = NA)
```

Arguments

dataA	R object for feature table with only differentially expressed features. This is the training set. The first two columns should be m/z and time.
classlabels	Class labels vector. e.g. c("case","control","case")
classifier	Classification algorithm to be used for ROC analysis. svm: Support Vector Machine logitreg: Logistic Regression eg: "svm" or "logitreg"
kname	Kernel for SVM. eg: "radial"
rocfeatlist	Vector indicating number of features to be used for ROC evaluation: eg: c(2,4,6) will generate ROC for top 2, top 4, and top 6 feautres. Default: seq(2,10,1)
rocfeatincrement	Turns on or off forward selection. eg: TRUE or FALSE
testset	R object for test feature table with only differentially expressed features. This is the test set. The first two columns should be m/z and time. The order of features should be same as the training set.
testclasslabels	Class labels vector for samples in the test set.
mainlabel	Main text label for the ROC plot. eg: "Group A vs B ROC curve"

Details

Function to perform ROC curve analysis using only traning set or using both training and test set.

Value

PDF file with ROC plot

Author(s)

Karan Uppal; kuppal2@emory.edu

get_volcanoplots

get_volcanoplots

Description

Function to generate volcano plots

Usage

```
get_volcanoplots(xvec, yvec, up_or_down, maintext = "", ythresh = 0.05, y2thresh=NA, ylab, xlab, col
```

Arguments

xvec	Vector with log2 fold change values for the x-axis.
yvec	Vector with values for the y-axis. eg: $(-)\log_{10}$ of p-values, VIP, loadings, regression coefficients.
up_or_down	Same as xvec.
maintext	Text for the plot title
ythresh	Y-axis threshold for significance or differential expression. eg: 3 for $p=0.001$; $y=(-1)*\log_{10}(0.001)$ or 2 for VIP from PLS
y2thresh	Optional secondary Y-axis threshold for significance or differential expression. eg: 1.3 for $p=0.05$; $y=(-1)*\log_{10}(0.05)$ or 1 for VIP from PLS
ylab	Y-axis label
xlab	X-axis label
colorvec	Vector of colors for representing up-regulation and down-regulation. eg: <code>c("darkgreen", "firebrick1")</code> In this case, features that are up-regulated in class A will have "darkgreen" color, and features that are up-regulated in class B will have "firebrick1" color.
xincrement	Window size for breaking the x-axis into different segments for visualization purposes. eg: 150
yincrement	Window size for breaking the y-axis into different segments for visualization purposes. eg: 1
xthresh	Absolute value of the threshold for log2 fold change. e.g. 0, 1, 2

Details

This function generates volcano plots.

Value

Volcano plot

Author(s)

Karan Uppal; kuppal2@emory.edu

metabnet

metabnet

Description

Function for correlation (complete or partial) based metabolome-wide network analysis. Additionally, users have the option to provide a matrix of m/z features corresponding to chemicals of interest such as (phenylalanine, choline, etc) and/or a matrix of m/z features corresponding to discriminatory metabolites.

Usage

```
metabnet(feature_table_file, target.metab.file, sig.metab.file, class_labels_file=NA, parentoutput_dir,
num_replicates=3, cor.method="spearman", abs.cor.thresh=0.4,
cor.fdrthresh=0.05, target.mzmatch.diff=10, target.rtmatch.diff=NA,
max.cor.num=100, feat.filt.thresh=NA, summarize.replicates=TRUE,
summary.method="mean", all.missing.thresh=0.5,
group.missing.thresh=0.7,
log2transform=TRUE, medcenter=TRUE, znormtransform=FALSE,
quantile_norm=TRUE, lowess_norm=FALSE, madscaling=FALSE,
missing.val=0, networktype="complete", samplerindex=NA,
rep.max.missing.thresh=0.3, summary.na.replacement="zeros",
net_node_colors = c("pink", "skyblue"),
net_legend = FALSE)
```

Arguments

feature_table_file
Feature table that includes the mz, retention time, and measured intensity in each sample for each analyte. The first 2 columns should be the mz and time. The remaining columns should correspond to the samples in the class labels file with each column including the intensity profile of a sample. Full path required. Eg: C:/My Documents/test.txt The feature table should be in a tab-delimited format. An example of the input file is provided under the "example" folder.

target.metab.file
File that includes the mz and/or retention time of the targeted metabolites corresponding to pathways or chemicals of interest. See example.

sig.metab.file File that includes the mz and/or retention time of the discriminatory metabolites. See example.

class_labels_file
File with class labels information for each sample. Samples should be in the same order as in the feature table. Please use the same format as in the example folder.

parentoutput_dir
Provide full path of the folder where you want the results to be written. Eg: C:/My Documents/ProjectA/results/

num_replicates Number of technical replicates

cor.method Correlation method. Options: "pearson" or "spearman". Default: "spearman"

abs.cor.thresh Absolute Pearson correlation coefficient for network analysis. Eg: 0.5

cor.fdrthresh False discovery rate threshold for correlation analysis. Eg: 0.05

target.mzmatch.diff
+/- ppm mass tolerance for searching the target m/z in the current feature table

target.rtmatch.diff
+/- retention time tolerance for searching the target m/z in the current feature table

max.cor.num Maximum number of correlated metabolites to be included in the network figure. Default: 100

feat.filt.thresh
Percent Intensity Difference or Coefficient of variation threshold; feature filtering Use NA to skip this step.

summarize.replicates	Do the technical replicates per sample need to be averaged or median summarized?
summary.method	Method for summarizing the replicates. Options: "mean" or "median"
summary.na.replacement	How should the missing values be represented? Options: "zeros", "halfsamplemin", "halfdatamin", "none" "zeros": replaces missing values by 0 "halfsamplemin": replaces missing value by one-half of the lowest signal intensity in the corresponding sample "halfdatamin": replaces missing value by one-half of the lowest signal intensity in the complete dataset "none": keeps missing values as NAs Users are recommended to perform imputation prior to performing biomarker discovery.
all.missing.thresh	What proportion of total number of samples should have an intensity? Default: 0.5
group.missing.thresh	What proportion of samples in either of the two groups should have an intensity? If at least x for further analysis. Default: 0.7
log2transform	Data transformation: Please refer to http://www.biomedcentral.com/1471-2164/7/142 Try different combinations; such as log2transform=TRUE, znormtransform=FALSE or log2transform=FALSE, znormtransform=TRUE
medcenter	Median centering of metabolites
znormtransform	Auto scaling; each metabolite will have a mean of 0 and unit variance
quantile_norm	Performs quantile normalization. Normalization options: Please set only one of the options to be TRUE
lowess_norm	Performs lowess normalization. Normalization options: Please set only one of the options to be TRUE
madscaling	Performs median adjusted scale normalization. Normalization options: Please set only one of the options to be TRUE
missing.val	How are the missing values represented in the input data? Options: "0" or "NA"
networktype	Options: "complete" or "GGM" "complete": performs network analysis using ordinary Pearson or Spearman correlation statistic "GGM": generates network based on partial correlation analysis using the GeneNet package
samplerindex	Column index of any additional or irrelevant columns to be deleted. Options: "NA" or list of column numbers. eg: c(1,3,4) Default=NA
rep.max.missing.thresh	What proportion of replicates are allowed to have missing values during the averaging or median summarization step of each biological sample? If the number of replicates with missing values is greater than the defined threshold, then the summarized value is represented by the "missing.val" parameter. If the number of replicates with missing values is less than or equal to the defined threshold, then the summarized value is equal to the mean or the median of the non-missing values. Default: 0.5
net_node_colors	Colors of nodes in the correlation networks. Eg: c("pink", "skyblue"), or ("red", "green")
net_legend	Should the network be displayed for the correlation network? eg: TRUE or FALSE

Details

Function for metabolomic network analysis

Value

Correlation matrix and network of metabolites.

Author(s)

Karan Uppal <kuppal2@emory.edu>

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