

Package ‘PAA’

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Title PAA (Protein Array Analyzer)

Author Michael Turewicz [aut, cre], Martin Eisenacher [ctb, cre]

Maintainer Michael Turewicz <michael.turewicz@rub.de>, Martin Eisenacher <martin.eisenacher@rub.de>

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LinkingTo Rcpp

Suggests BiocStyle, RUnit, BiocGenerics, vsn

Description PAA imports single color (protein) microarray data that has been saved in gpr file format - esp. ProtoArray data. After pre-processing (background correction, batch filtering, normalization) univariate feature pre-selection is performed (e.g., using the "minimum M statistic" approach - hereinafter referred to as "mMs"). Subsequently, a multivariate feature selection is conducted to discover biomarker candidates. Therefore, either a frequency-based backwards elimination approach or ensemble feature selection can be used. PAA provides a complete toolbox of analysis tools including several different plots for results examination and evaluation.

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URL <http://www.medizinisches-proteom-center.de/PAA>

SystemRequirements C++ software package Random Jungle

biocViews Classification, Microarray, OneChannel, Proteomics

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batchAdjust	<i>Adjust microarray data for batch effects.</i>
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Description

Adjusts EListRaw or EList data for batch/lot effects.

Usage

```
batchAdjust(elist=NULL, log=NULL)
```

Arguments

elist	EList or EListRaw object containing the data to be adjusted (mandatory).
log	string ("TRUE" or "FALSE") indicating whether the data is in log scale (mandatory).

Details

This is a wrapper to sva's function ComBat() for batch adjustment using the empirical Bayes approach. To use batchAdjust the targets information of the EList or EListRaw object must contain the columns "Batch" (containing batch/lot information for each particular array) and "Group" (containing experimental group information for each particular array).

Value

An EListRaw or EList object with the adjusted data in log scale is returned.

Note

The targets information of the EListRaw or EList object must contain the columns "Batch" and "Group".

Author(s)

Michael Turewicz, <michael.turewicz@rub.de>

References

The package sva by Jeffrey T. Leek et al. can be downloaded from Bioconductor (<http://www.bioconductor.org/>).

Johnson WE, Li C, and Rabinovic A (2007) Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostatistics* 8:118-27.

Examples

```
cwd <- system.file(package="PAA")
load(paste(cwd, "/extdata/Alzheimer.RData", sep=""))
elist <- elist[elist$genes$Block < 10,]
elist <- batchAdjust(elist=elist, log=FALSE)
```

batchFilter

Remove differential features regarding array batches/lots.

Description

Finds differential features regarding array batches/lots and removes them.

Usage

```
batchFilter(elist=NULL, lot1=NULL, lot2=NULL, p.thresh=0.05, fold.thresh=1.5,
output.path=NULL)
```

Arguments

elist	EList or EListRaw object (mandatory).
lot1	vector of column names for group 1 (mandatory).
lot2	vector of column names for group 2 (mandatory).
p.thresh	positive float number between 0 and 1 indicating the maximum Student's t-test p-value for features to be considered as differential (e.g., "0.5").
fold.thresh	float number indicating the minimum fold change for features to be considered as differential (e.g., "1.5").
output.path	string indicating a path for saving results (optional).

Details

This function takes an EList or EListRaw object (see limma documentation) and the batch-specific column name vectors lot1 and lot2 to find differential features regarding batches/lots. For this purpose, thresholds for p-values (Student's t-test) and fold changes can be defined. To visualize the differential features a volcano plot is drawn. Then, differential features are removed and the remaining data are returned. When an output path is defined (via output.path) volcano plots and result files are saved on the hard disk.

Value

An EList or EListRaw object without differential features regarding array batches/lots.

Author(s)

Michael Turewicz, <michael.turewicz@rub.de>

Examples

```

cwd <- system.file(package="PAA")
load(paste(cwd, "/extdata/Alzheimer.RData", sep=""))
elist <- elist[elist$genes$Block < 10,]
lot1 <- elist$targets[elist$targets$Batch==Batch1,ArrayID]
lot2 <- elist$targets[elist$targets$Batch==Batch2,ArrayID]
elist <- batchFilter(elist=elist, lot1=lot1, lot2=lot2, p.thresh=0.001,
  fold.thresh=3)

```

diffAnalysis

Differential analysis.

Description

Performs a univariate differential analysis.

Usage

```

diffAnalysis(input=NULL, label1=NULL, label2=NULL, class1=NULL, class2=NULL,
  output.path=NULL, mMs.matrix1=NULL, mMs.matrix2=NULL, above=1500,
  between=400, features=NULL, feature.names=NULL)

```

Arguments

input	EList\$E- or EListRaw\$E-matrix extended by row names comprising BRC-IDs of the corresponding features (mandatory).
label1	vector of column names for group 1 (mandatory).
label2	vector of column names for group 2 (mandatory).
class1	label of group 1 (mandatory).
class2	label of group 2 (mandatory).
output.path	string indicating a path for saving the results (optionally).
mMs.matrix1	pre-computed mMs reference matrix (see mMsMatrix()) for group 1 (mandatory).
mMs.matrix2	pre-computed mMs reference matrix (see mMsMatrix()) for group 2 (mandatory).
above	mMs above parameter (integer). Default is "1500".
between	mMs between parameter (integer). Default is "400".
features	vector of row indices (optional).
feature.names	vector of corresponding feature names (additionally to features).

Details

This function takes an `EList` or `EListRaw` matrix (e.g., `temp <- elist$E`) extended by row names comprising BRC-IDs of the corresponding features. The BRC-IDs can be created via:

```
brc <- paste(elist$genes[,1], elist$genes[,3], elist.$genes[,2]).
```

The BRC-row names can be defined as follows: `rownames(temp) <- brc`. Furthermore, the corresponding column name vectors, group labels and mMs-parameters are needed to perform the univariate differential analysis. This analysis covers inter alia p-value computation, p-value adjustment (method: Benjamini & Hochberg, 1995), and fold change computation. Since the results table is usually large, a path for saving the results can be defined via `output.path`. Optionally, a vector of row indices (features) and additionally (not mandatory for subset analysis) a vector of corresponding feature names (`feature.names`) can be forwarded to perform the analysis for a feature subset.

Value

A matrix containing the analysis results is returned.

Author(s)

Michael Turewicz, < michael.turewicz@rub.de >

Examples

```
cwd <- system.file(package="PAA")
load(paste(cwd, "/extdata/Alzheimer.RData", sep=""))
elist <- elist[elist$genes$Block < 10,]
c1 <- paste(rep("AD",20), 1:20, sep="")
c2 <- paste(rep("NDC",20), 1:20, sep="")
mMs.matrix1 <- mMs.matrix2 <- mMsMatrix(x=20, y=20)
temp <- elist$E
rownames(temp) <- paste(elist$genes[,1], elist$genes[,3], elist$genes[,2])
diffAnalysis(input=temp, label1=c1, label2=c2, class1="AD", class2="NDC",
  mMs.matrix1=mMs.matrix1, mMs.matrix2=mMs.matrix2, above=1500,
  between=400)
```

loadGPR

A function to read raw data from gpr files.

Description

Reads an `EListRaw` from a set of `gpr` files containing `ProtoArray` data or other one-color data.

Usage

```
loadGPR(gpr.path = NULL, targets.path = NULL, array.type = "ProtoArray",
  protoarray.aggregation = "min", array.columns = list(E = "F635 Median",
  Eb = "B635 Median"),
  array.annotation = c("Block", "Column", "Row", "Description", "Name", "ID"))
```

Arguments

<code>gpr.path</code>	string indicating the path to a folder containing gpr files (mandatory).
<code>targets.path</code>	string indicating the path to targets file (see <code>limma</code> , mandatory).
<code>array.type</code>	string indicating whether the gpr files contain ProtoArray data (default is "ProtoArray"). In any other case no duplicate aggregation will be performed (mandatory).
<code>protoarray.aggregation</code>	string indicating which type of ProtoArray spot duplicate aggregation should be performed. If "min" is chosen, the value for the corresponding feature will be the minimum of both duplicate values. If "mean" is chosen, the arithmetic mean will be computed. The default is "min" (optional).
<code>array.columns</code>	list containing the column names for foreground intensities (E) and background intensities (Eb) in the gpr files that is passed to <code>limma</code> 's "read.maimages" function (optional).
<code>array.annotation</code>	string vector containing further mandatory column names that are passed to <code>limma</code> (optional).

Details

This function is partially a wrapper to `limma`'s function `read.maimages()` featuring optional duplicate aggregation for ProtoArray data. Paths to a target file and to a folder containing gpr files (all gpr files in that folder that are listed in the targets file will be read) are mandatory. The folder "`R_HOME/library/PAA/extdata`" contains an exemplary target file that can be used as a template. If `array.type` is set to "ProtoArray" (default), duplicate spots will be aggregated. The corresponding method ("min" or "mean") can be specified via `protoarray.aggregation`. Arguments `array.columns` and `array.annotation` define the columns where `read.maimages()` will find foreground and background intensity values as well as other important columns.

Value

An object of class `EListRaw` (see the documentation of `limma` for details) is returned.

Note

Don't forget to check column names in your gpr files. They may differ from the default settings of `loadGPR()`. Note that if `array.type` is not "ProtoArray", no aggregation will be done and all computations will be performed by `limma`'s `read.maimages()`.

Author(s)

Michael Turewicz, <michael.turewicz@rub.de>

References

The package `limma` by Gordon Smyth et al. can be downloaded from Bioconductor (<http://www.bioconductor.org/>).

Smyth, G. K. (2005). Limma: linear models for microarray data. In: Bioinformatics and Computational Biology Solutions using R and Bioconductor, R. Gentleman, V. Carey, S. Dudoit, R. Irizarry, W. Huber (eds.), Springer, New York, pages 397-420.

Examples

```
gpr <- system.file("extdata", package="PAA")
targets <- list.files(system.file("extdata", package="PAA"),
  pattern = "dummy_targets", full.names=TRUE)
elist <- loadGPR(gpr.path=gpr, targets.path=targets)
```

mMsMatrix

Compute a reference minimum M statistic (n1 x n2)-matrix.

Description

Computes a reference minimum M statistic (n1 x n2)-matrix (mMs matrix).

Usage

```
mMsMatrix(x, y)
```

Arguments

x	integer, first dimension (i.e., number of samples in group 1) of the mMs matrix to be computed (mandatory).
y	integer, second dimension (i.e., number of samples in group 2) of the mMs matrix to be computed (mandatory).

Details

For feature pre-selection the "minimum M Statistic" (mMs) proposed by Love B. is used. The mMs is a univariate measure that is sensitive to population subgroups. To avoid redundant mMs computations for a large number of features (e.g., ca. 9500 features on ProtoArray v5) a reference matrix containing all relevant mMs values can be pre-computed. Therefore, only two parameters are needed: the number of samples in group 1 (n1) and the number of samples in group 2 (n2). According to mMs definition for each matrix element (i,m) a mMs value (= the probability of) for having m values in group 1 larger than the i-th largest value in group 2 is computed.

Value

A (n1 x n2)-matrix containing all mMs values for group 1 and group 2.

Note

To check whether a feature is more prevalent in group 1 or in group 2, PAA needs both the mMs for having m values in group 1 larger than the i-th largest element in group 2 as well as the mMs for having m values in group 2 larger than the i-th largest element in group 1. Hence, always both needs be computed `mMsMatrix(n1, n2)` and `mMsMatrix(n2, n1)`.

Author(s)

Michael Turewicz, <michael.turewicz@rub.de>

References

Love B: The Analysis of Protein Arrays. In: Functional Protein Microarrays in Drug Discovery. CRC Press; 2007: 381-402.

Examples

```
#exemplary computation for a group 1 comprising 10 arrays and a group 2
#comprising 12 arrays
mMs.matrix1 <- mMsMatrix(x=10, y=12)
mMs.matrix2 <- mMsMatrix(x=12, y=10)
```

normalizeArrays	<i>Normalize microarray data.</i>
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Description

Normalizes EListRaw data and returns an EList object containing normalized data in log scale.

Usage

```
normalizeArrays(elist = NULL, method = "quantile", cyclicloess.method = "pairs",
group1 = NULL, group2 = NULL, controls.elist=NULL, gpr.path=NULL,
targets.path=NULL, contr.names=NULL, output.path=NULL)
```

Arguments

elist	EListRaw object containing raw data to be normalized (mandatory).
method	string indicating the normalization method ("cyclicloess", "quantile" or "vsn") to be used (mandatory).
cyclicloess.method	string indicating which type of cyclicloess normalization ("pairs", "fast", "affy") should be performed (optional).
group1	vector of integers (column indices) indicating all group 1 samples (optional).
group2	vector of integers (column indices) indicating all group 2 samples (optional).
controls.elist	EListRaw object containing intensity values of control features for RLM normalization for ProtoArrays (optional).
gpr.path	when controls.elist is not defined: path to original gpr files for loading control feature intensities for ProtoArray RLM normalization (optional).
targets.path	when controls.elist is not defined: path to original targets file for loading control feature intensities for ProtoArray RLM normalization (optional).
contr.names	character vector containing the names of the control features for ProtoArray RLM normalization (optional).
output.path	output.path for ProtoArray RLM normalization (optional).

Details

This function is partially a wrapper to limma's function `normalizeBetweenArrays()` for inter-array normalization featuring optional groupwise normalization when the arguments `group1` AND `group2` are assigned. For more information on "cyclicloess", "quantile" or "vsn" see the documentation of the limma package. Furthermore, for ProtoArrays robust linear normalization ("r1m", see Sboner A. et al.) is provided.

Value

An EList object with the normalized data in log scale is returned.

Note

Groupwise normalization will be performed when the arguments `group1` AND `group2` are assigned.

Author(s)

Michael Turewicz, <michael.turewicz@rub.de>

References

The package limma by Gordon Smyth et al. can be downloaded from Bioconductor (<http://www.bioconductor.org/>).

Smyth, G. K. (2005). Limma: linear models for microarray data. In: Bioinformatics and Computational Biology Solutions using R and Bioconductor, R. Gentleman, V. Carey, S. Dudoit, R. Irizarry, W. Huber (eds.), Springer, New York, pages 397-420.

Sboner A. et al., Robust-linear-model normalization to reduce technical variability in functional protein microarrays. J Proteome Res 2009, 8(12):5451-5464.

Examples

```
cwd <- system.file(package="PAA")
load(paste(cwd, "/extdata/Alzheimer.RData", sep=""))
elist <- elist[elist$genes$Block < 10,]
normalized.elist <- normalizeArrays(elist=elist, method="quantile")
```

plotFeatures

Plot intensities of selected features.

Description

Plots intensities of all selected features (one sub-plot per feature) in group- specific colors.

Usage

```
plotFeatures(features = NULL, elist = NULL, n1 = NULL, n2 = NULL,
  group1 = "group1", group2 = "group2", output.path = NULL)
```

Arguments

features	vector containing the selected features as "BRC"-IDs (mandatory).
elist	EListRaw or EList object containing all intensity data in log scale (mandatory).
n1	integer indicating the sample size of group 1 (mandatory).
n2	integer indicating the sample size of group 2 (mandatory).
group1	class label of group 1.
group2	class label of group 2.
output.path	string indicating the folder where the figure will be saved (optional).

Details

Plots intensities of all features selected by the function `selectFeatures()` (one sub-plot per feature) in group-specific colors. All sub-plots are aggregated to one figure. When `output.path` is not NULL this figure will be saved in a tiff file in `output.path`. This function can be used to check whether the selected features are differential.

Value

No value is returned.

Author(s)

Michael Turewicz, <michael.turewicz@rub.de>

Examples

```

cwd <- system.file(package="PAA")
load(paste(cwd, "/extdata/Alzheimer.RData", sep=""))
elist <- elist[elist$genes$Block < 10,]

c1 <- paste(rep("AD",20), 1:20, sep="")
c2 <- paste(rep("NDC",20), 1:20, sep="")

pre.sel.results <- preselect(elist=elist, columns1=c1, columns2=c2, label1="AD",
  label2="NDC", discard.threshold=0.1, fold.thresh=2, discard.features=TRUE,
  method="tTest")
elist <- elist[-pre.sel.results$discard,]

selectFeatures.results <- selectFeatures(elist,n1=20,n2=20,label1="AD",
  label2="NDC",selection.method="rf.rfe",preselection.method="none",subruns=2,
  k=2,candidate.number=20,method="frequency")

plotFeatures(features=selectFeatures.results$features, elist=elist, n1=20,
  n2=20, group1="AD", group2="NDC")

```

plotFeaturesHeatmap *Plot feature intensities as a heatmap.*

Description

Plots intensities of given features as a heatmap.

Usage

```
plotFeaturesHeatmap(features = NULL, elist = NULL, n1 = NULL, n2 = NULL,  
  output.path = NULL, description=FALSE)
```

Arguments

features	vector containing the selected features as "BRC"-IDs (mandatory).
elist	EListRaw or EList object containing all intensity data in log scale (mandatory).
n1	integer indicating the sample size of group 1 (mandatory).
n2	integer indicating the sample size of group 2 (mandatory).
output.path	path for saving the heatmap as a tiff file (default: NULL).
description	if TRUE, features will be described via protein names instead of uniprot accessions (default: FALSE).

Details

Plots intensities of all features given in the vector features via their corresponding "BRC"-IDs as a heatmap. If description is TRUE (default: FALSE), features will be described via protein names instead of uniprot accessions. Furthermore, if output.path is not NULL, the heatmap will be saved as a tiff file in output.path. This function can be used to check whether the selected features are differential.

Value

No value is returned.

Author(s)

Michael Turewicz, <michael.turewicz@rub.de>

Examples

```
cwd <- system.file(package="PAA")  
load(paste(cwd, "/extdata/Alzheimer.RData", sep=""))  
elist <- elist[elist$genes$Block < 10,]  
  
c1 <- paste(rep("AD",20), 1:20, sep="")  
c2 <- paste(rep("NDC",20), 1:20, sep="")
```

```
pre.sel.results <- preselect(elist=elist, columns1=c1, columns2=c2, label1="AD",
  label2="NDC", discard.threshold=0.1, fold.thresh=2, discard.features=TRUE,
  method="tTest")
elist <- elist[~pre.sel.results$discard,]

selectFeatures.results <- selectFeatures(elist,n1=20,n2=20,label1="AD",
  label2="NDC",selection.method="rf.rfe",preselection.method="none",subruns=2,
  k=2,candidate.number=20,method="frequency")

plotFeaturesHeatmap(features=selectFeatures.results$features, elist=elist,
  n1=20, n2=20, description=TRUE)
```

plotMAPlots

Check normalization results with MA plots.

Description

Draws MA plots of raw data and data after all kinds of normalization provided by PAA.

Usage

```
plotMAPlots(elist = NULL, idx="all", include.rlm=FALSE, controls.elist=NULL,
  gpr.path=NULL, targets.path=NULL, contr.names=NULL, output.path = NULL)
```

Arguments

<code>elist</code>	EListRaw object containing raw data (mandatory).
<code>idx</code>	integer indicating the column index of the sample for drawing MA plots or the string 'all' for drawing MA plots for all samples (default: all).
<code>include.rlm</code>	boolean indicating whether RLM normalization should be included (for ProtoArrays only, default: FALSE).
<code>controls.elist</code>	EListRaw object containing intensity values of control features for RLM normalization for ProtoArrays (optional).
<code>gpr.path</code>	when <code>controls.elist</code> is not defined: path to original gpr files for loading control feature intensities for ProtoArray RLM normalization (optional).
<code>targets.path</code>	when <code>controls.elist</code> is not defined: path to original targets file for loading control feature intensities for ProtoArray RLM normalization (optional).
<code>contr.names</code>	character vector containing the names of the control features for ProtoArray RLM normalization (optional).
<code>output.path</code>	string indicating the folder where the tiff files will be saved (mandatory when <code>idx='all'</code>).

Details

When `idx="all"` (default) for each microarray a tiff file containing MA plots for raw data, cyclocoess normalized data, quantile normalized data and vsn normalized data (and, optionally, for ProtoArrays, rlm normalized data) will be created. When `idx` is an integer indicating the column index of a particular sample MA plots only for this sample will be created. For A and M value computation the artificial median array is used as reference signal. All figures can be saved in `output.path` (mandatory when `idx="all"`). The resulting MA plots can be used to compare the results of the different normalization methods.

Value

No value is returned.

Author(s)

Michael Turewicz, <michael.turewicz@rub.de>

Examples

```
cwd <- system.file(package="PAA")
load(paste(cwd, "/extdata/Alzheimer.RData", sep=""))
elist <- elist[elist$genes$Block < 10,]
plotMAPlots(elist=elist, idx=1)
```

plotNormMethods

Check normalization results with boxplots.

Description

Draws boxplots (one boxplot per sample) of raw data and data after all kinds of normalization provided by PAA.

Usage

```
plotNormMethods(elist = NULL, include.rlm=FALSE, controls.elist=NULL,
gpr.path=NULL, targets.path=NULL, contr.names=NULL, output.path = NULL)
```

Arguments

<code>elist</code>	EListRaw object containing raw data (mandatory).
<code>include.rlm</code>	boolean indicating whether RLM normalization should be included (for ProtoArrays only, default: FALSE).
<code>controls.elist</code>	EListRaw object containing intensity values of control features for RLM normalization for ProtoArrays (optional).
<code>gpr.path</code>	when <code>controls.elist</code> is not defined: path to original gpr files for loading control feature intensities for ProtoArray RLM normalization (optional).

targets.path	when controls.elist is not defined: path to original targets file for loading control feature intensities for ProtoArray RLM normalization (optional).
contr.names	character vector containing the names of the control features for ProtoArray RLM normalization (optional).
output.path	string indicating a folder for saving the boxplots as tiff files (optional).

Details

For each normalization approach sample-wise boxplots are created. All boxplots can be saved as high-quality tiff files (when an output path has been specified via the argument `output.path`). The resulting boxplots can be used to compare the results of different normalization methods.

Value

No value is returned.

Author(s)

Michael Turewicz, <michael.turewicz@rub.de>

Examples

```

cwd <- system.file(package="PAA")
load(paste(cwd, "/extdata/Alzheimer.RData", sep=""))
elist <- elist[elist$genes$Block < 10,]
plotNormMethods(elist=elist)

```

```
preselect
```

```
Score and preselect features.
```

Description

Iterates all features to score them via mMs, Student's t-test, or mRMR. Optionally, a list of not informative features can be obtained (for discarding them).

Usage

```

preselect(elist=NULL, columns1=NULL, columns2=NULL, label1="A", label2="B",
  discard.threshold=0.5, fold.thresh=1.5, discard.features=TRUE,
  mMs.above=1500, mMs.between=400, mMs.matrix1=NULL,
  mMs.matrix2=NULL, method=NULL)

```

Arguments

<code>elist</code>	EListRaw or EList object (mandatory).
<code>columns1</code>	column name vector (string vector) of group 1 (mandatory).
<code>columns2</code>	column name vector (string vector) of group 2 (mandatory).
<code>label1</code>	class label of group 1.
<code>label2</code>	class label of group 2.
<code>discard.threshold</code>	positive numeric between 0 and 1 indicating the maximum mMs or, respectively, the maximum t-test p-value for features to be included for further analysis. Default is "0.5".
<code>fold.thresh</code>	numeric indicating the minimum fold change for features to be included for further analysis. Default is "1.5".
<code>discard.features</code>	boolean indicating whether merely feature scores (i.e., mMs or t-test p-values) (= "FALSE") or feature scores and a discard list "TRUE" should be returned. Default is "FALSE".
<code>mMs.above</code>	mMs above parameter (integer). Default is "1500".
<code>mMs.between</code>	mMs between parameter (integer). Default is "400".
<code>mMs.matrix1</code>	pre-computed mMs reference matrix (see <code>mMsMatrix()</code>) for group 1 (mandatory).
<code>mMs.matrix2</code>	pre-computed mMs reference matrix (see <code>mMsMatrix()</code>) for group 2 (mandatory).
<code>method</code>	preselection method ("mMs", "tTest", "mrmr"). Default is "mMs".

Details

This function takes an EListRaw or EList object and group-specific column vectors. Furthermore, the class labels of group 1 and group 2 are needed. If `discard.features` is "TRUE" (default), all features that are considered as not differential will be collected and returned for discarding.

If `method = "mMs"`, additionally pre-computed mMs reference matrices (see `mMsMatrix()`) for group 1 and group 2 will be needed to compute mMs values (see Love B.) as scoring method. All mMs parameters (`mMs.above` and `mMs.between`) can be set. The defaults are "1500" for `mMs.above` and "400" for `mMs.between`. Features having an mMs value larger than `discard.threshold` (here: numeric between 0.0 and 1.0) or do not satisfy the minimal absolute fold change `fold.thresh` are considered as not differential.

If `method = "tTest"`, Student's t-test will be used as scoring method. Features having a p-value larger than `discard.threshold` (here: numeric between 0.0 and 1.0) or do not satisfy the minimal absolute fold change `fold.thresh` are considered as not differential.

If `method = "mrmr"`, mRMR scores for all features will be computed as scoring method (using the function `mRMR.classic()` of the CRAN R package `mRMRe`). Features that are not the `discard.threshold` (here: integer indicating a number of features) best features regarding their mRMR score are considered as not differential.

Value

If `discard.features` is "FALSE": matrix containing metadata, feature scores and intensity values for the whole data set.

If `discard.features` is "TRUE", a list containing:

<code>results</code>	matrix containing metadata, feature scores and intensity values for the whole data set.
<code>discard</code>	vector containing row indices (= features) for discarding features considered as not differential.

Author(s)

Michael Turewicz, < michael.turewicz@rub.de >

References

Love B: The Analysis of Protein Arrays. In: Functional Protein Microarrays in Drug Discovery. CRC Press; 2007: 381-402.

The software "Prospector" for ProtoArray analysis can be downloaded from Life Technologies web site (<http://www.lifetechnologies.com/>).

The R package mRMRe can be downloaded from CRAN. See also: De Jay N, Papillon-Cavanagh S, Olsen C, El-Hachem N, Bontempi G, Haibe-Kains B. mRMRe: an R package for parallelized mRMR ensemble feature selection. Bioinformatics 2013.

The package limma by Gordon Smyth et al. can be downloaded from Bioconductor (<http://www.bioconductor.org/>).

Smyth, G. K. (2005). Limma: linear models for microarray data. In: Bioinformatics and Computational Biology Solutions using R and Bioconductor, R. Gentleman, V. Carey, S. Dudoit, R. Irizarry, W. Huber (eds.), Springer, New York, pages 397-420.

Examples

```
cwd <- system.file(package="PAA")
load(paste(cwd, "/extdata/Alzheimer.RData", sep=""))
elist <- elist[elist$genes$Block < 10,]
c1 <- paste(rep("AD",20), 1:20, sep="")
c2 <- paste(rep("NDC",20), 1:20, sep="")
preselect(elist, columns1=c1, columns2=c2, label1="AD", label2="NDC",
  discard.threshold=0.5, fold.thresh=1.5, discard.features=TRUE, method="tTest")
```

printFeatures

Print biomarker candidate panel into a table.

Description

Creates a table containing the selected biomarker candidate panel.

Usage

```
printFeatures(features = NULL, elist = NULL, output.path = NULL)
```

Arguments

features vector containing the selected features as "BRC"-IDs (mandatory).
elist EListRaw or EList object containing all intensity data in log scale (mandatory).
output.path string indicating the folder where the table will be saved as a txt file (optional).

Details

Creates a table containing the selected biomarker candidate panel as well as additional information. When output.path is defined this table will be saved in a txt file ("candidates.txt").

Value

Table containing the selected biomarker panel.

Author(s)

Michael Turewicz, <michael.turewicz@rub.de>

Examples

```
cwd <- system.file(package="PAA")
load(paste(cwd, "/extdata/Alzheimer.RData", sep=""))
elist <- elist[elist$genes$Block < 10,]

c1 <- paste(rep("AD",20), 1:20, sep="")
c2 <- paste(rep("NDC",20), 1:20, sep="")

pre.sel.results <- preselect(elist=elist, columns1=c1, columns2=c2, label1="AD",
  label2="NDC", discard.threshold=0.1, fold.thresh=2, discard.features=TRUE,
  method="tTest")
elist <- elist[-pre.sel.results$discard,]

selectFeatures.results <- selectFeatures(elist,n1=20,n2=20,label1="AD",
  label2="NDC",selection.method="rf.rfe",preselection.method="none",subruns=2,
  k=2,candidate.number=20,method="frequency")

printFeatures(features=selectFeatures.results$features, elist=elist)
```

pvaluePlot	<i>Draw a p-value plot.</i>
------------	-----------------------------

Description

Draws a p-value plot to visualize the p-values for all features stored in a `EList` or `EListRaw` object.

Usage

```
pvaluePlot(elist=NULL, group1=NULL, group2=NULL, method="tTest",  
output.path=NULL, tag="", mMs.matrix1=NULL, mMs.matrix2=NULL, above=1500,  
between=400, adjust=FALSE)
```

Arguments

<code>elist</code>	<code>EList</code> or <code>EListRaw</code> object (mandatory).
<code>group1</code>	vector of column names for group 1 (mandatory).
<code>group2</code>	vector of column names for group 2 (mandatory).
<code>method</code>	method for p-value computation: "tTest" or "mMs". Default is "tTest".
<code>output.path</code>	string indicating a path for saving the plot (optional).
<code>tag</code>	string that can be used for tagging the saved plot (optional).
<code>mMs.matrix1</code>	pre-computed M score reference matrix (see <code>mMsMatrix()</code>) for group 1 (mandatory when <code>method = "mMs"</code>).
<code>mMs.matrix2</code>	pre-computed M score reference matrix (see <code>mMsMatrix()</code>) for group 2 (mandatory when <code>method = "mMs"</code>).
<code>above</code>	M score above parameter (integer). Default is "1500".
<code>between</code>	M score between parameter (integer). Default is "400".
<code>adjust</code>	Boolean indicating whether p-values should be adjusted. Default is FALSE.

Details

This function takes an `EList` or `EListRaw` object and the corresponding column name vectors to draw a plot of p-values for all features stored in `elist` (sorted in increasing order and in log2 scale). The p-value computation method ("tTest" or "mMs") can be set via the argument `method`. Furthermore, when `adjust=TRUE` adjusted p-values (method: Benjamini & Hochberg, 1995, computed via `p.adjust()`) will be used. When an output path is defined (via `output.path`) the plot will be saved as a tiff file.

Value

No value is returned.

Author(s)

Michael Turewicz, <michael.turewicz@rub.de>

Examples

```

cwd <- system.file(package="PAA")
load(paste(cwd, "/extdata/Alzheimer.RData", sep=""))
elist <- elist[elist$genes$Block < 10,]
c1 <- paste(rep("AD",20), 1:20, sep="")
c2 <- paste(rep("NDC",20), 1:20, sep="")
pvaluePlot(elist=elist, group1=c1, group2=c2, method="tTest", tag="_tTest",
  adjust=FALSE)

```

selectFeatures	<i>Select features using frequency-based or ensemble feature selection.</i>
----------------	---

Description

Performs a multivariate feature selection using frequency-based feature selection (based on RF-RFE, RJ-RFE or SVM-RFE) or ensemble feature selection (based on SVM-RFE).

Usage

```

selectFeatures(elist = NULL, n1 = NULL, n2 = NULL, label1 = "A", label2 = "B",
  cutoff = 10, selection.method = "rf.rfe", preselection.method = "mMs",
  subruns = 100, k = 10, subsamples = 10, bootstraps = 10,
  candidate.number = 300, above=1500, between=400,
  panel.selection.criterion="accuracy", importance.measure="MDA", ntree = 500,
  mtry = NULL, plot = FALSE, output.path = NULL, verbose = FALSE,
  method = "frequency")

```

Arguments

elist	EListRaw or EList object containing all microarray data (mandatory).
n1	integer indicating the sample number in group 1 (mandatory).
n2	integer indicating the sample number in group 2 (mandatory).
label1	class label of group 1 (default: "A").
label2	class label of group 2 (default: "B").
cutoff	integer indicating how many features will be selected (default: 10).
selection.method	string indicating the feature selection method: "rf.rfe" (default), "svm.rfe" or "rj.rfe".
preselection.method	string indicating the feature pre-selection method: "mMs" (default), "tTest", "mrmr" or "none".
subruns	integer indicating the number of re-sampling repeats to be performed (default: 100).
k	integer indicating the number of k-fold cross validation subsets (default: 10, i.e., 10-fold CV).

subsamples	integer indicating the number of subsamples for ensemble feature selection (default: 10).
bootstraps	integer indicating the number of bootstrap samples for ensemble feature selection (default: 10).
candidate.number	integer indicating how many features shall be pre-selected. Default is "300".
above	mMs above parameter (integer). Default is "1500".
between	mMs between parameter (integer). Default is "400".
panel.selection.criterion	string indicating the panel selection criterion: "accuracy" (default), "sensitivity" or "specificity".
importance.measure	string indicating the random forest importance measure: "MDA" (default) or "MDG".
ntrree	random forest parameter ntrree (default: "500").
mtry	random forest parameter mtry (default: sqrt(p) where p is the number of predictors).
plot	indicates whether performance plots shall be plotted (default: FALSE).
output.path	string indicating the results output folder (optional).
verbose	logical, indicating whether additional information shall be printed to the console (default: FALSE).
method	the feature selection method: "frequency" (default) for frequency-based and "ensemble" for ensemble feature selection.

Details

This function takes an `EListRaw` or `EList` object, group-specific sample numbers, group labels and parameters choosing and setting up a univariate feature pre-selection method as well as a multivariate feature selection method (frequency-based or ensemble feature selection) to select a panel of differential features. When an output path is defined (via `output.path`) results will be saved on the hard disk and when `verbose` is `TRUE` additional information will be printed to the console.

Frequency-based feature selection (`method="frequency"`): The whole data is splitted in k cross validation training and test set pairs. For each training set a multivariate feature selection procedure is performed. The resulting k feature subsets are tested using the corresponding test sets (via classification). As a result, `selectFeatures()` returns the average k -fold cross validation classification accuracy as well as the selected feature panel (i.e., the union set of the k particular feature subsets). As multivariate feature selection methods random forest recursive feature elimination (RF-RFE), random jungle recursive feature elimination (RJ-RFE) and support vector machine recursive feature elimination (SVM-RFE) are supported. To reduce running times, optionally, univariate feature pre-selection can be performed (usage via `preselection.method`). As univariate pre-selection methods `mMs` "mMs", Student's t-test "tTest" and mRMR "mrmr" are supported. Alternatively, no pre-selection can be chosen "none". This approach is similar to the method proposed in Baek et al.

Ensemble feature selection (`method="ensemble"`): From the whole data the previously defined number of subsamples is drawn defining pairs of training and test sets. Moreover, for each training set a previously defined number of bootstrap samples is drawn. Then, for each bootstrap sample

SVM-RFE is performed and a feature ranking is obtained. To obtain a final ranking for a particular training set, all associated bootstrap rankings are aggregated to a single ranking. To score the cutoff best features, for each subsample a classification of the test set is performed (using a svm trained with the cutoff best features from the training set) and the classification accuracy is determined. Finally, the stability of the subsample-specific panels is assessed (via Kuncheva index, Kuncheva LI, 2007), all subsample-specific rankings are aggregated, the top n features (defined by cutoff) are selected, the average classification accuracy is computed, and all these results are returned in a list. This approach has been proposed and is described in Abeel et al.

Value

If method is "frequency", the results list contains the following elements:

accuracy	average k-fold cross validation accuracy.
sensitivity	average k-fold cross validation sensitivity.
specificity	average k-fold cross validation specificity.
features	selected feature panel.
all.results	complete cross validation results.

If method is "ensemble", the results list contains the following elements:

accuracy	average accuracy regarding all subsamples.
sensitivity	average sensitivity regarding all subsamples.
specificity	average specificity regarding all subsamples.
features	selected feature panel.
all.results	all feature ranking results.
stability	stability of the feature panel (i.e., Kuncheva index for the subrun-specific panels).

Author(s)

Michael Turewicz, <michael.turewicz@rub.de>

References

- Baek S, Tsai CA, Chen JJ.: Development of biomarker classifiers from high- dimensional data. *Brief Bioinform.* 2009 Sep;10(5):537-46.
- Abeel T, Helleputte T, Van de Peer Y, Dupont P, Saeys Y: Robust biomarker identification for cancer diagnosis with ensemble feature selection methods. *Bioinformatics.* 2010 Feb 1;26(3):392-8.
- Kuncheva, LI: A stability index for feature selection. *Proceedings of the IASTED International Conference on Artificial Intelligence and Applications.* February 12-14, 2007. Pages: 390-395.

Examples

```

cwd <- system.file(package="PAA")
load(paste(cwd, "/extdata/Alzheimer.RData", sep=""))
elist <- elist[elist$genes$Block < 10,]

c1 <- paste(rep("AD",20), 1:20, sep="")
c2 <- paste(rep("NDC",20), 1:20, sep="")

pre.sel.results <- preselect(elist=elist, columns1=c1, columns2=c2, label1="AD",
  label2="NDC", discard.threshold=0.1, fold.thresh=2, discard.features=TRUE,
  method="tTest")
elist <- elist[-pre.sel.results$discard,]

selectFeatures.results <- selectFeatures(elist,n1=20,n2=20,label1="AD",
  label2="NDC",selection.method="rf.rfe",preselection.method="none",subruns=2,
  k=2,candidate.number=20,method="frequency")

```

shuffleData

Shuffles class labels to obtain random groups.

Description

Shuffles class labels of an EList or EListRaw object randomly to obtain two random groups (e.g. "A" and "B").

Usage

```
shuffleData(elist=NULL, n1=NULL, n2=NULL, label1="A", label2="B")
```

Arguments

elist	EList or EListRaw object (mandatory).
n1	sample size of random group 1 (mandatory).
n2	sample size of random group 2 (mandatory).
label1	class label of random group 1 (default: "A").
label2	class label of random group 2 (default: "B").

Details

Shuffles class labels of an EList or EListRaw object randomly to obtain two random groups (e.g. "A" and "B").

Value

EList or EListRaw object with random groups.

Author(s)

Michael Turewicz, <michael.turewicz@rub.de>

Examples

```

cwd <- system.file(package="PAA")
load(paste(cwd, "/extdata/Alzheimer.RData", sep=""))
shuffleData(elist=elist, n1=20, n2=20, label1="A", label2="B")

```

volcanoPlot	<i>Draw a volcano plot.</i>
-------------	-----------------------------

Description

Draws a volcano plot to visualize differential features.

Usage

```

volcanoPlot(elist=NULL, group1=NULL, group2=NULL, method="tTest", p.thresh=NULL,
fold.thresh=NULL, output.path=NULL, tag="", mMs.matrix1=NULL, mMs.matrix2=NULL,
above=1500, between=400)

```

Arguments

elist	EList or EListRaw object (mandatory).
group1	vector of column names for group 1 (mandatory).
group2	vector of column names for group 2 (mandatory).
method	method for p-value computation: "tTest" or "mMs". Default is "tTest".
p.thresh	positive float number between 0 and 1 indicating the maximum p-value for features to be considered as differential (e.g., "0.5"). This argument is optional.
fold.thresh	float number indicating the minimum fold change for features to be considered as differential (e.g., "1.5"). This argument is optional.
output.path	string indicating a path for saving the plot (optional).
tag	string that can be used for tagging the saved plot (optional).
mMs.matrix1	a pre-computed M score reference matrix (see <code>mMsMatrix()</code>) for group 1 (mandatory when method = "mMs").
mMs.matrix2	a pre-computed M score reference matrix (see <code>mMsMatrix()</code>) for group 2 (mandatory when method = "mMs").
above	M score above parameter (integer). Default is "1500".
between	M score between parameter (integer). Default is "400".

Details

This function takes an EList or EListRaw object and the corresponding column name vectors to draw a volcano plot. To visualize differential features, thresholds for p-values and fold changes can be defined. Furthermore, the p-value computation method ("mMs" or "tTest") can be set. When an output path is defined (via output.path) the plot will be saved as a tiff file.

Value

No value is returned.

Author(s)

Michael Turewicz, <michael.turewicz@rub.de>

Examples

```
cwd <- system.file(package="PAA")
load(paste(cwd, "/extdata/Alzheimer.RData", sep=""))
elist <- elist[elist$genes$Block < 10,]
c1 <- paste(rep("AD",20), 1:20, sep="")
c2 <- paste(rep("NDC",20), 1:20, sep="")
volcanoPlot(elist=elist, group1=c1, group2=c2, method="tTest", p.thresh=0.01,
  fold.thresh=2)
```


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