

Package ‘PAA’

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Title PAA (Protein Array Analyzer)

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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 preprocessing (background correction, batch filtering, normalization) univariate feature preselection 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.ruhr-uni-bochum.de/mpc/software/PAA/>

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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	logical indicating whether the data is in log scale (mandatory; note: if TRUE log2 scale is expected).

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, log=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).
log	logical indicating whether the data is in log scale (mandatory; note: if TRUE log2 scale is expected).

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, log=FALSE,
  p.thresh=0.001, fold.thresh=3)
```

batchFilter.anova	<i>Remove features which are differential regarding microarray batches / lots in a multi-batch scenario.</i>
-------------------	--

Description

Finds features which are differential regarding at least two microarray batches / lots in a multi-batch scenario (i.e., > 2 batches) via one-way analysis of variance (ANOVA) and removes them.

Usage

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

Arguments

<code>elist</code>	EList or EListRaw object (mandatory).
<code>log</code>	logical indicating whether the data is in log scale (mandatory; note: if TRUE log2 scale is expected).
<code>p.thresh</code>	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").
<code>fold.thresh</code>	float number indicating the minimum fold change for features to be considered as differential (e.g., "1.5").
<code>output.path</code>	string indicating a path for saving results (optional).

Details

This function takes an EList or EListRaw object (see limma documentation) to find features which are differential regarding at least two microarray batches / lots in a multi-batch scenario (i.e., more than two batches). For this purpose, thresholds for p-values obtained from an one-way analysis of variance (ANOVA) 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 at least two microarray batches / lots.

Author(s)

Ivan Grishagin (Rancho BioSciences LLC, San Diego, CA, USA), John Obenauer (Rancho BioSciences LLC, San Diego, CA, USA) and Michael Turewicz (Ruhr-University Bochum, Bochum, Germany), <michael.turewicz@rub.de>

Examples

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

<code>input</code>	EList\$E- or EListRaw\$E-matrix extended by row names comprising BRC-IDs of the corresponding features (mandatory; note: it is expected that this matrix is in original scale and not in log2 scale).
<code>label1</code>	vector of column names for group 1 (mandatory).
<code>label2</code>	vector of column names for group 2 (mandatory).
<code>class1</code>	label of group 1 (mandatory).
<code>class2</code>	label of group 2 (mandatory).
<code>output.path</code>	string indicating a path for saving the results (optionally).
<code>mMs.matrix1</code>	precomputed mMs reference matrix (see <code>mMsMatrix()</code>) for group 1 (mandatory).
<code>mMs.matrix2</code>	precomputed mMs reference matrix (see <code>mMsMatrix()</code>) for group 2 (mandatory).
<code>above</code>	mMs above parameter (integer). Default is "1500".
<code>between</code>	mMs between parameter (integer). Default is "400".
<code>features</code>	vector of row indices (optional).
<code>feature.names</code>	vector of corresponding feature names (additionally to features).

Details

This function takes an EList\$E- or EListRaw\$E-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

Importing raw data from gpr files.

Description

Constructs an EListRaw object from a set of gpr files containing ProtoArray data or other protein microarray data.

Usage

```
loadGPR(gpr.path = NULL, targets.path = NULL, array.type = NULL,
  aggregation = "none", array.columns = list(E = "F635 Median",
  Eb = "B635 Median"),
  array.annotation = c("Block", "Column", "Row", "Description", "Name", "ID"),
  description = NULL, description.features = NULL, description.discard = NULL)
```

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 limma, mandatory).
<code>array.type</code>	string indicating the microarray type of the imported gpr files. Only for ProtoArrays duplicate aggregation will be performed. The possible options are: "ProtoArray", "HuProt" and "other" (mandatory).
<code>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. Alternatively, no aggregation will be performed, if "none" is chosen. 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 limma's "read.maimages" function (optional).
<code>array.annotation</code>	string vector containing further mandatory column names that are passed to limma (optional).

description string indicating the column name of an alternative column containing the information which spot is a feature, control or to be discarded for gpr files not providing the column "Description" (optional).
description.features string containing a regular expression identifying feature spots. Mandatory when description has been defined.
description.discard string containing a regular expression identifying spots to be discarded (e.g., empty spots). Mandatory when description has been defined.

Details

This function is partially a wrapper to limma's function `read.maimages()` featuring optional duplicate aggregation for ProtoArray data. Paths to a targets 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 targets file that can be used as a template. If `array.type` (also mandatory) is set to `"ProtoArray"`, duplicate spots can be aggregated. The corresponding method (`"min"`, `"mean"` or `"none"`) can be specified via the argument `aggregation`. As another ProtoArray-specific feature, control spot data and information will be stored in additional components of the returned object (see below). 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. For `array.annotation` the default columns `"Block"`, `"Column"`, `"Row"`, `"Description"`, `"Name"` and `"ID"` are mandatory.

If the column `"Description"` is not provided by the gpr files for ProtoArrays a makeshift column will be constructed from the column `"Name"` automatically. For other microarrays the arguments `description`, `description.features` and `description.discard` can be used to provide the mandatory information (see the example below).

Value

An extended object of class `EListRaw` (see the documentation of limma for details) is returned. If `array.type` is set to `"ProtoArray"` (default), the object provides additional components for control spot data: `C`, `Cb` and `cgenes` which are analogous to the probe spot data `E`, `Eb` and `genes`. Moreover, the returned object always provides the additional component `array.type` indicating the type of the imported protein microarray data (e.g., `"ProtoArray"`).

Note

Don't forget to check column names in your gpr files. They may differ from the default settings of `loadGPR()` and should be renamed to the default column names (see also the exemplary gpr files accompanying PAA as a reference for the default column names). At worst, important columns in your gpr files may be completely missing and should be added in order to provide all information needed by PAA.

Note that if `array.type` is not `"ProtoArray"`, neither aggregation will be done nor controls components will be added to the returned object of class `EListRaw`.

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, array.type="ProtoArray")

# Example showing how to use the arguments description, description.features and
# description.discard in order to construct a makeshift column 'Description'
# for gpr files without this column. Please see also the exemplary gpr files
# coming with PAA.
targets2 <- list.files(system.file("extdata", package="PAA"),
  pattern = "dummy_no_descr_targets", full.names=TRUE)
elist2 <- loadGPR(gpr.path=gpr, targets.path=targets2, array.type="other",
  description="Name", description.features="^Hs~", description.discard="Empty")
```

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 preselection the "minimum M Statistic" (mMs) proposed by Love B. can be 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 precomputed. For this purpose, 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 must 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>
-----------------	-----------------------------------

Description

Normalizes `EListRaw` data and returns an `EList` object containing normalized data in log2 scale.

Usage

```
normalizeArrays(elist = NULL, method = "quantile", cyclicloess.method = "pairs",
controls="internal", group1 = NULL, group2 = NULL, output.path=NULL)
```

Arguments

<code>elist</code>	EListRaw object containing raw data to be normalized (mandatory).
<code>method</code>	string indicating the normalization method ("cyclicloess", "quantile", "vsn" or "rlm") to be used (mandatory).
<code>cyclicloess.method</code>	string indicating which type of cyclicloess normalization ("pairs", "fast", "affy") should be performed (optional).
<code>controls</code>	string indicating the ProtoArray controls for rlm normalization (optional). Valid options are "internal" (default), "external", "both" or a regular expression defining a specific control or a specific set of controls.
<code>group1</code>	vector of integers (column indices) indicating all group 1 samples (optional).
<code>group2</code>	vector of integers (column indices) indicating all group 2 samples (optional).
<code>output.path</code>	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 ("rlm", see Sboner A. et al.) is provided.

For rlm normalization (`method = "rlm"`) the additional argument `controls` needs to be specified in order to select a set of controls used for normalization. Valid options are "internal" (default), "external" and "both" which refer to the following sets of ProtoArray controls:

- `internal`: The set of all internal controls spotted on the ProtoArray. The human-IgG series and anti-human-IgG series, which respond to serum and secondary antibodies.
- `external`: The V5-CMK1 series spotted on the ProtoArray which responds to exogenously added anti-V5 antibody (external control).
- `both`: The combined set of both the internal and the external controls (i.e., the human-IgG and anti-human-IgG series and the V5-CMK1 series).

Moreover, via `controls` a regular expression can be passed in order to select a more specific group of controls. Please check the column "Name" in your gpr files in order to obtain the complete list of names of all controls spotted on the ProtoArray. In the following some examples of valid regular expressions are given:

- `"^HumanIg"` Only human IgGs and IgAs are selected (esp., no anti-human Igs).
- `"Anti-HumanIgA"` Only anti-human-IgAs are selected (esp., no human IgGs and IgAs).
- `"(Anti-HumanIg|^V5control|BSA|ERa)"` Only anti-human IgGs and anti-human IgAs, the V5-CMK1 series, BSA and ERa are selected.
- `"HumanIgG"` Only human IgGs and anti-human IgGs are selected.
- `"V5control"` Only the V5-CMK1 series is selected.

Value

An EList object with the normalized data in log2 scale is returned.

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")
```

plotArray

Plot ProtoArray expression intensities in the original arrangement mimicking the original scan image.

Description

Uses the "Block", "Row" and "Column" information of an EList or EListRaw object to resemble the original positions on the array(s). The resulting plot is similar to the original scan image of the considered array(s). Thus, this function is a visualization tool that can be used to visualize protein microarrays for which the original scan image is not available. Visual inspection of the spatial expression pattern can then identify possible local tendencies and strong spatial biases. Moreover, the array can be inspected at all stages of the preprocessing workflow in order to check the impact of the particular methods that have been applied.

Usage

```
plotArray(elist=NULL, idx=NULL, data.type="fg", log=NULL, normalized=NULL,
          aggregation=NULL, colpal="heat.colors", graphics.device="tiff",
          output.path=NULL)
```

Arguments

elist	EList or EListRaw object (mandatory).
idx	integer, vector of integers or the string "all" indicating the column indices of the sample(s) for drawing the plot(s) (mandatory).

<code>data.type</code>	string indicating whether the foreground ("fg") or background ("bg") data should be plotted. The default is "fg" (optional).
<code>log</code>	logical indicating whether the input data is logarithmized. If TRUE the log2 scale is expected. If FALSE a log2-transformation will be performed (mandatory).
<code>normalized</code>	logical indicating whether elist was normalized (mandatory).
<code>aggregation</code>	string indicating whether the data stored in elist has been aggregated and, if this is the case, which method has been used by the function loadGPR(). Possible values are "min", "mean" and "none"(mandatory).
<code>colpal</code>	string indicating the color palette for the plot(s). The default is "heat.colors" (optional).
<code>graphics.device</code>	string indicating the file format for the plot(s) saved in <code>output.path</code> . Accepted values are "tiff" and "png". The default is "tiff" (optional).
<code>output.path</code>	string indicating the output path for the plots (optional).

Details

This function allows plotting of protein microarray data using the `gplots` function `heatmap.2()` for visual quality control. The data obtained from an `EList` or `EListRaw` object is re-ordered and represented in the same way the spots are ordered on the actual microarray. Consequently, the resulting plot is similar to the original scan image of the considered array. This allows for visual control and assessment of possible patterns in spatial distribution.

Mandatory arguments are `elist`, `idx`, `log`, `normalized` and `aggregation`. While `elist` specifies the `EList` or `EListRaw` object to be used, `idx` designates the array column index in `elist` to plot a single array from the `EList` object. Alternatively, a vector (e.g., 1:5) or the string "all" can be designated to include multiple, respectively, all arrays that were imported.

Furthermore, `data.type` allows for plotting of "fg", foreground data (i.e., `elist$E` and `elist$C`), which is the default or "bg", background data (i.e., `elist$Eb` and `elist$Cb`).

The normalization approaches of PAA which comprise also data logarithmization do not include control data. With `normalized=TRUE` it is indicated that the input data was normalized, so the control data will be logarithmized (log2) before plotting as well. However, since the complete data (foreground and background values of protein features and control spots) can be logarithmized regardless of normalization the argument `log` states whether the designated data is already logarithmized (note: log2 scale is always expected).

The parameter `aggregation` indicates whether the protein microarray data has been aggregated by `loadGPR()` and, if so, which method has been used.

Moreover, the parameter `colpal` defines the color palette that will be used for the plot. Some exemplary values are "heat.colors" (default), "terrain.colors", "topo.colors", "greenred" and "bluered".

Finally, the output path optionally can be specified with the argument `output.path` to save the plot(s). Then, one or more tiff or png file(s) containing the corresponding plot(s) are saved into the subfolder "array_plots".

Value

No value is returned.

Note

Please note the instructions of the PAA function `loadGPR()`. Note that the data has to be imported including controls to avoid annoying gaps in the plot (for `ProtoArrays` this is done automatically and for other types of arrays the arguments `description`, `description.features` and `description.discard` must be defined). Note that the data can be imported without aggregation by `loadGPR()` (when `aggregation="none"`) in order to inspect the array visually with `plotArray()` before duplicate aggregation.

Author(s)

Daniel Bemmerl and Michael Turewicz <michael.turewicz@rub.de>

References

The package `gplots` by Gregory R. Warnes et al. can be downloaded from CRAN (<http://CRAN.R-project.org/package=gplots>).

Gregory R. Warnes, Ben Bolker, Lodewijk Bonebakker, Robert Gentleman, Wolfgang Huber, Andy Liaw, Thomas Lumley, Martin Maechler, Arni Magnusson, Steffen Moeller, Marc Schwartz and Bill Venables (2015). `gplots`: Various R Programming Tools for Plotting Data. R package version 2.17.0. <http://CRAN.R-project.org/package=gplots>

Examples

```
cwd <- system.file(package="PAA")
load(paste(cwd, "/extdata/BadData.RData", sep=""))
plotArray(elist=bad.elist, idx=1, data.type="bg", log=FALSE, normalized=FALSE,
  aggregation="none")
```

plotFeatures

Plot intensities of features.

Description

Plots intensities of all given 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 "BRC"-IDs (mandatory).
elist	EListRaw or EList object containing all intensity data in log2 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 given features (e.g., selected by the function `selectFeatures()`) in group-specific colors (one sub-plot per feature). All sub-plots are aggregated to one figure. When the argument `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=1.9, 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")

load(paste(cwd, "/extdata/selectFeaturesResultsFreq.RData", sep=""))
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 "BRC"-IDs (mandatory).
elist	EListRaw or EList object containing all intensity data in log2 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 UniProtKB 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 UniProtKB 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=1.9, 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")

load(paste(cwd, "/extdata/selectFeaturesResultsFreq.RData", sep=""))
plotFeaturesHeatmap(features=selectFeatures.results$features, elist=elist,
  n1=20, n2=20, description=TRUE)
```

plotFeaturesHeatmap.2 *Alternative function to plot feature intensities as a heatmap.*

Description

This function is an alternative to plotFeaturesHeatmap() and is based on the function heatmap.2() provided by the package gplots.

Usage

```
plotFeaturesHeatmap.2(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 log2 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 png file (default: NULL).
description	if TRUE, features will be described via protein names instead of UniProtKB 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 UniProtKB accessions. Furthermore, if output.path is not NULL, the heatmap will be saved as a png file in output.path. This function can be used to check whether the selected features are differential.

plotFeaturesHeatmap.2() is an alternative to plotFeaturesHeatmap() and is based on the function heatmap.2() provided by the package gplots.

Value

No value is returned.

Author(s)

Ivan Grishagin (Rancho BioSciences LLC, San Diego, CA, USA), John Obenauer (Rancho BioSciences LLC, San Diego, CA, USA) and Michael Turewicz (Ruhr-University Bochum, Bochum, Germany), <michael.turewicz@rub.de>

References

The package gplots by Gregory R. Warnes et al. can be downloaded from CRAN (<http://CRAN.R-project.org/package=gplots>).

Gregory R. Warnes, Ben Bolker, Lodewijk Bonebakker, Robert Gentleman, Wolfgang Huber, Andy Liaw, Thomas Lumley, Martin Maechler, Arni Magnusson, Steffen Moeller, Marc Schwartz and Bill Venables (2015). gplots: Various R Programming Tools for Plotting Data. R package version 2.17.0. <http://CRAN.R-project.org/package=gplots>

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=1.9, 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")

load(paste(cwd, "/extdata/selectFeaturesResultsFreq.RData", sep=""))
plotFeaturesHeatmap.2(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="internal",
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>	logical indicating whether RLM normalization should be included (for ProtoArrays only; default: FALSE).
<code>controls</code>	string indicating the ProtoArray controls for rlm normalization (optional). Valid options are "internal" (default), "external", "both" or a regular expression defining a specific control or a specific set of controls.
<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 == 1,]
plotMAPlots(elist=elist, idx=1)
```

plotNormMethods	<i>Check normalization results with boxplots.</i>
-----------------	---

Description

Draws sample-wise boxplots of raw data and data after all kinds of normalization provided by PAA.

Usage

```
plotNormMethods(elist = NULL, include.rlm=FALSE, controls="internal",  
output.path = NULL)
```

Arguments

<code>elist</code>	EListRaw object containing raw data (mandatory).
<code>include.rlm</code>	logical indicating whether RLM normalization should be included (for ProtoArrays only, default: FALSE).
<code>controls</code>	string indicating the ProtoArray controls for <code>rlm</code> normalization (optional). Valid options are "internal" (default), "external", "both" or a regular expression defining a specific control or a specific set of controls.
<code>output.path</code>	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 == 1,]  
plotNormMethods(elist=elist)
```

preselect	<i>Score and preselect features.</i>
-----------	--------------------------------------

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",
  log=NULL, 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

elist	EListRaw or EList object (mandatory).
columns1	column name vector (string vector) of group 1 (mandatory).
columns2	column name vector (string vector) of group 2 (mandatory).
label1	class label of group 1.
label2	class label of group 2.
log	indicates whether the data is in log scale (mandatory; note: if TRUE log2 scale is expected).
discard.threshold	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".
fold.thresh	numeric indicating the minimum fold change for features to be included for further analysis. Default is "1.5".
discard.features	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 "TRUE".
mMs.above	mMs above parameter (integer). Default is "1500".
mMs.between	mMs between parameter (integer). Default is "400".
mMs.matrix1	precomputed mMs reference matrix (see mMsMatrix()) for group 1 (mandatory).
mMs.matrix2	precomputed mMs reference matrix (see mMsMatrix()) for group 2 (mandatory).
method	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 precomputed 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 the Thermo Fisher Scientific web page (<https://www.thermofisher.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 (<https://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", log=FALSE,
  discard.threshold=0.5, fold.thresh=1.5, discard.features=TRUE, method="tTest")
```

printFeatures	<i>Print features into a table.</i>
---------------	-------------------------------------

Description

Creates a table containing the given features (e.g., the selected biomarker candidate panel).

Usage

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

Arguments

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

Details

Creates a table containing the given features (e.g., 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 given features.

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=1.9, 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")

load(paste(cwd, "/extdata/selectFeaturesResultsFreq.RData", sep=""))
printFeatures(features=selectFeatures.results$features, elist=elist)
```

pvaluePlot

Draw a p-value plot.

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, log=NULL, method="tTest",
output.path=NULL, tag="", mMs.matrix1=NULL, mMs.matrix2=NULL, above=1500,
between=400, adjust=FALSE)
```

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).
log	indicates whether the data is in log scale (mandatory; note: if TRUE log2 scale is expected).
method	method for p-value computation: "tTest" or "mMs". Default is "tTest".
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	precomputed M score reference matrix (see mMsMatrix()) for group 1 (mandatory when method = "mMs").

mMs.matrix2	precomputed M score reference matrix (see mMsMatrix()) 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".
adjust	logical 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, log=FALSE, 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",
log=NULL, 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

<code>elist</code>	EListRaw or EList object containing all microarray data (mandatory).
<code>n1</code>	integer indicating the sample number in group 1 (mandatory).
<code>n2</code>	integer indicating the sample number in group 2 (mandatory).
<code>label1</code>	class label of group 1 (default: "A").
<code>label2</code>	class label of group 2 (default: "B").
<code>log</code>	indicates whether the data is in log scale (mandatory; note: if TRUE log2 scale is expected).
<code>cutoff</code>	integer indicating how many features will be selected (default: 10).
<code>selection.method</code>	string indicating the feature selection method: "rf.rfe" (default), "svm.rfe" or "rj.rfe". Has no effect when method="ensemble".
<code>preselection.method</code>	string indicating the feature preselection method: "mMs" (default), "tTest", "mrmr" or "none". Has no effect when method="ensemble".
<code>subruns</code>	integer indicating the number of resampling repeats to be performed (default: 100). Has no effect when method="ensemble".
<code>k</code>	integer indicating the number of k-fold cross validation subsets (default: 10, i.e., 10-fold CV).
<code>subsamples</code>	integer indicating the number of subsamples for ensemble feature selection (default: 10). Has no effect when method="frequency".
<code>bootstraps</code>	integer indicating the number of bootstrap samples for ensemble feature selection (default: 10). Has no effect when method="frequency" only.
<code>candidate.number</code>	integer indicating how many features shall be preselected. Default is "300". Has no effect when method="ensemble".
<code>above</code>	mMs above parameter (integer). Default is "1500". There will be no effect when method="ensemble".
<code>between</code>	mMs between parameter (integer). Default is "400". There will be no effect when method="ensemble".
<code>panel.selection.criterion</code>	indicating the panel selection criterion: "accuracy" (default), "sensitivity" or "specificity". No effect for method="ensemble".

importance.measure	string indicating the random forest importance measure: "MDA" (default) or "MDG". Has no effect when method="ensemble".
ntree	random forest parameter ntree (default: "500"). There will be no effect when method="ensemble".
mtry	random forest parameter mtry (default: sqrt(p) where p is the number of predictors). Has no effect when method="ensemble".
plot	logical indicating 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 or "ensemble" for ensemble feature selection.

Details

This function takes an `EListRaw` or `EList` object, group-specific sample numbers, group labels and parameters choosing and configuring 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 preselection can be performed (control via `preselection.method`). As univariate preselection methods `mMs` ("mMs"), Student's t -test ("tTest") and `mRMR` ("mrmr") are supported. Alternatively, no preselection 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 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", log=FALSE, discard.threshold=0.1, fold.thresh=1.9,
  discard.features=TRUE, method="tTest")
elist <- elist[-pre.sel.results$discard,]

selectFeatures.results <- selectFeatures(elist, n1=20, n2=20, label1="AD",
  label2="NDC", log=FALSE, subsamples=2, bootstraps=1, candidate.number=20,
  method="ensemble")
```

shuffleData	<i>Shuffles class labels to obtain random groups.</i>
-------------	---

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, log=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).
log	indicates whether the data is in log scale (mandatory; note: if TRUE log2 scale is expected; 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 precomputed M score reference matrix (see mMsMatrix()) for group 1 (mandatory when method = "mMs").
mMs.matrix2	a precomputed M score reference matrix (see mMsMatrix()) 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.

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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, log=FALSE, method="tTest",
  p.thresh=0.01, fold.thresh=2)
```

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