Package ‘maSigPro’

Type Package

Title Significant Gene Expression Profile Differences in Time Course Gene Expression Data

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Description maSigPro is a regression based approach to find genes for which there are significant gene expression profile differences between experimental groups in time course microarray and RNA-Seq experiments.

Depends R (>= 2.3.1), stats, Biobase, MASS

Imports Biobase, graphics, grDevices, venn, mclust, stats, utils, MASS

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average.rows

Description

average.rows matches rownames of a matrix to a match vector and performs averaging of the rows by the index provided by an index vector.

Usage

average.rows(x, index, match, r = 0.7)

Arguments

- **x**: a matrix
- **index**: index vector indicating how rows must be averaged
- **match**: match vector for indexing rows
- **r**: minimal correlation value between rows to compute average

Details

rows will be averaged only if the pearson correlation coefficient between all rows of each given index is greater than r. If not, that group of rows is discarded in the result matrix.

Value

a matrix of averaged rows

Author(s)

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Examples

```r
# create data matrix for row averaging
x <- matrix(rnorm(30), nrow = 6, ncol = 5)
rownames(x) <- paste("ID", c(1, 2, 11, 12, 19, 20), sep = "")
i <- paste("g", rep(c(1:10), each = 2), sep = "") # index vector
m <- paste("ID", c(1:20), sep = "") # match vector
average.rows(x, i, m, r = 0)
```
**data.abiotic**

**Description**

`data.abiotic` contains gene expression of a time course microarray experiment where potato plants were submitted to 3 different abiotic stresses.

**Usage**

`data(data.abiotic)`

**Format**

A data frame with 1000 observations on the following 36 variables.

- `Control_3H_1` a numeric vector
- `Control_3H_2` a numeric vector
- `Control_3H_3` a numeric vector
- `Control_9H_1` a numeric vector
- `Control_9H_2` a numeric vector
- `Control_9H_3` a numeric vector
- `Control_27H_1` a numeric vector
- `Control_27H_2` a numeric vector
- `Control_27H_3` a numeric vector
- `Cold_3H_1` a numeric vector
- `Cold_3H_2` a numeric vector
- `Cold_3H_3` a numeric vector
- `Cold_9H_1` a numeric vector
- `Cold_9H_2` a numeric vector
- `Cold_9H_3` a numeric vector
- `Cold_27H_1` a numeric vector
- `Cold_27H_2` a numeric vector
- `Cold_27H_3` a numeric vector
- `Heat_3H_1` a numeric vector
- `Heat_3H_2` a numeric vector
- `Heat_3H_3` a numeric vector
- `Heat_9H_1` a numeric vector
- `Heat_9H_2` a numeric vector
- `Heat_9H_3` a numeric vector
- `Heat_27H_1` a numeric vector
- `Heat_27H_2` a numeric vector
- `Heat_27H_3` a numeric vector
edesign.abiotic

Salt_3H_1 a numeric vector
Salt_3H_2 a numeric vector
Salt_3H_3 a numeric vector
Salt_9H_1 a numeric vector
Salt_9H_2 a numeric vector
Salt_9H_3 a numeric vector
Salt_27H_1 a numeric vector
Salt_27H_2 a numeric vector
Salt_27H_3 a numeric vector

Details

This data set is part of a larger experiment in which gene expression was monitored in both roots and leaves using a 11K cDNA potato chip. This example data set contains a random subset of 1000 genes of the leaf study.

References


Examples

data(data.abiotic)

edesign.abiotic

Experimental design potato abiotic stress

Description

edesign.abiotic contains experimental set up of a time course microarray experiment where potato plants were submitted to 3 different abiotic stresses.

Usage

data(edesign.abiotic)

Format

A matrix with 36 rows and 6 columns

rows [1:36] "Control 3h 1" "Control 3h 2" "Control 3h 3" "Control 9h 1" ...
columns [1:6] "Time" "Replicates" "Control" "Cold" "Heat" "Salt"
**Details**

Arrays are given in rows and experiment descriptors are given in columns. Row names contain array names.

"Time" indicates the values that variable Time takes in each hybridization.

"Replicates" is an index indicating replicate hybridizations, i.e. hybridizations are numbered, giving replicates the same number.

"Control", "Cold", "Heat" and "Salt" columns indicate array assignment to experimental groups, coding with 1 and 0 whether each array belongs to that group or not.

**References**


**Examples**

```r
data(edesignCT)
```

---

**Description**

`edesignCT` contains the experimental setup of a time course microarray experiment where there is a common starting point for the different experimental groups.

**Usage**

```r
data(edesignCT)
```

**Format**

A matrix with 32 rows and 7 columns

rows [1:32] "Array1" "Array2" "Array3" "Array4" ...

columns [1:7] "Time" "Replicates" "Control" "Tissue1" "Tissue2" "Tissue3" "Tissue4"

**Details**

Arrays are given in rows and experiment descriptors are given in columns. Row names contain array names.

"Time" indicates the values that variable Time takes in each hybridization. There are 4 time points, which allows an up to 3 degree regression polynomial.

"Replicates" is an index indicating replicate hybridizations, i.e. hybridizations are numbered, giving replicates the same number.

"Control", "Tissue1", "Tissue2", "Tissue3" and "Tissue4" columns indicate array assignment to experimental groups, coding with 1 and 0 whether each array belongs to that group or not.

**Examples**

```r
data(edesignCT)
```
Experimental design with different replicates

Description

edesignDR contains experimental set up of a replicated time course microarray experiment where rats were submitted to 3 different dosis of a toxic compound. A control and an placebo treatments are also present in the experiment.

Usage

data(edesignDR)

Format

A matrix with 54 rows and 7 columns

rows [1:54] "Array1" "Array2" "Array3" "Array4" ...
columns [1:7] "Time" "Replicates" "Control" "Placebo" "Low" "Medium" "High"

Details

Arrays are given in rows and experiment descriptors are given in columns. Row names contain array names.

"Time" indicates the values that variable Time takes in each hybridization.

"Replicates" is an index indicating replicate hybridizations, i.e. hybridizations are numbered, giving replicates the same number.

"Control", "Placebo", "Low", "Medium" and "High" columns indicate array assignment to experimental groups, coding with 1 and 0 whether each array belongs to that group or not.

References


Examples

data(edesignDR)
**get.siggenes**

*Extract significant genes for sets of variables in time series gene expression experiments*

### Description

This function creates lists of significant genes for a set of variables whose significance value has been computed with the `T.fit` function.

### Usage

```r
get.siggenes(tstep, rsq = 0.7, add.IDs = FALSE, IDs = NULL, matchID.col = 1,
             only.names = FALSE, vars = c("all", "each", "groups"),
             significant.intercept = "dummy",
             groups.vector = NULL, trat.repl.spots = "none",
             index = IDs[, (matchID.col + 1)], match = IDs[, matchID.col],
             r = 0.7)
```

### Arguments

- **tstep**: a `T.fit` object
- **rsq**: cut-off level at the R-squared value for the stepwise regression fit. Only genes with R-squared more than rsq are selected
- **add.IDs**: logical indicating whether to include additional gene id's in the result
- **IDs**: matrix containing additional gene id information (required when `add.IDs` is `TRUE`)
- **matchID.col**: number of matching column in matrix IDs for adding genes ids
- **only.names**: logical. If `TRUE`, expression values are omitted in the results
- **vars**: variables for which to extract significant genes (see details)
- **significant.intercept**: experimental groups for which significant intercept coefficients are considered (see details)
- **groups.vector**: required when `vars` is "groups".
- **trat.repl.spots**: treatment given to replicate spots. Possible values are "none" and "average"
- **index**: argument of the `average.rows` function to use when `trat.repl.spots` is "average"
- **match**: argument of the `average.rows` function to use when `trat.repl.spots` is "average"
- **r**: minimum pearson correlation coefficient for replicated spots profiles to be averaged
get.siggenes

Details

There are 3 possible values for the vars argument:

"all": generates one single matrix or gene list with all significant genes.

"each": generates as many significant genes extractions as variables in the general regression model. Each extraction contains the significant genes for that variable.

"groups": generates a significant genes extraction for each experimental group.

The difference between "each" and "groups" is that in the first case the variables of the same group (e.g. "TreatmentA" and "time*TreatmentA") will be extracted separately and in the second case jointly.

When add.IDs is TRUE, a matrix of gene ids must be provided as argument of IDs, the matchID.col column of which having same levels as in the row names of sig.profiles. The option only.names is TRUE will generate a vector of significant genes or a matrix when add.IDs is set also to TRUE.

When trat.repl.spots is "average", match and index vectors are required for the average.rows function. In gene expression data context, the index vector would contain geneIDs and indicate which spots are replicates. The match vector is used to match these genesIDs to rows in the significant genes matrix, and must have the same levels as the row names of sig.profiles.

The argument significant.intercept modulates the treatment for intercept coefficients to apply for selecting significant genes when vars equals "groups". There are three possible values: "none", no significant intercept (differences) are considered for significant gene selection, "dummy", includes genes with significant intercept differences between control and experimental groups, and "all" when both significant intercept coefficient for the control group and significant intercept differences are considered for selecting significant genes.

add.IDs = TRUE and trat.repl.spots = "average" are not compatible argumet values. add.IDs = TRUE and only.names = TRUE are compatible argumet values.

Value

- summary: a vector or matrix listing significant genes for the variables given by the function parameters
- sig.genes: a list with detailed information on the significant genes found for the variables given by the function parameters. Each element of the list is also a list containing:
  - sig.profiles: expression values of significant genes
  - coefficients: regression coefficients of the adjusted models
  - groups.coeffs: regression coefficients of the implicit models of each experimental group
  - sig.pvalues: p-values of the regression coefficients for significant genes
  - g: number of genes
  - ...: arguments passed by previous functions

Author(s)

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References

get.siggenes

Examples

### GENERATE TIME COURSE DATA

```r
## generate n random gene expression profiles of a data set with
## one control plus 3 treatments, 3 time points and r replicates per time point.

tc.GENE <- function(n, r,
var11 = 0.01, var12 = 0.01, var13 = 0.01,
var21 = 0.01, var22 = 0.01, var23 = 0.01,
var31 = 0.01, var32 = 0.01, var33 = 0.01,
var41 = 0.01, var42 = 0.01, var43 = 0.01,
a1 = 0, a2 = 0, a3 = 0, a4 = 0,
b1 = 0, b2 = 0, b3 = 0, b4 = 0,
c1 = 0, c2 = 0, c3 = 0, c4 = 0)
{

tc.dat <- NULL
for (i in 1:n) {
  Ctl <- c(rnorm(r, a1, var11), rnorm(r, b1, var12), rnorm(r, c1, var13)) # Ctl group
  Tr1 <- c(rnorm(r, a2, var21), rnorm(r, b2, var22), rnorm(r, c2, var23)) # Tr1 group
  Tr2 <- c(rnorm(r, a3, var31), rnorm(r, b3, var32), rnorm(r, c3, var33)) # Tr2 group
  Tr3 <- c(rnorm(r, a4, var41), rnorm(r, b4, var42), rnorm(r, c4, var43)) # Tr3 group
gene <- c(Ctl, Tr1, Tr2, Tr3)
tc.dat <- rbind(tc.dat, gene)
}

tc.dat
}
```  

## Create 270 flat profiles
flat <- tc.GENE(n = 270, r = 3)
## Create 10 genes with profile differences between Ctl and Tr1 groups
twodiff <- tc.GENE(n = 10, r = 3, b2 = 0.5, c2 = 1.3)
## Create 10 genes with profile differences between Ctl, Tr2, and Tr3 groups
threediff <- tc.GENE(n = 10, r = 3, b3 = 0.8, c3 = -1, a4 = -0.1, b4 = -0.8, c4 = -1.2)
## Create 10 genes with profile differences between Ctl and Tr2 and different variance
vardiff <- tc.GENE(n = 10, r = 3, a3 = 0.7, b3 = 1, c3 = 1.2, var32 = 0.03, var33 = 0.03)
## Create dataset
tc.DATA <- rbind(flat, twodiff, threediff, vardiff)
rownames(tc.DATA) <- paste("feature", c(1:300), sep = "")
colnames(tc.DATA) <- paste("Array", c(1:36), sep = "")
tc.DATA[sample(c(1:(300*36)), 300)] <- NA # introduce missing values
```r
#### CREATE EXPERIMENTAL DESIGN

Time <- rep(c(rep(c(1:3), each = 3)), 4)
Replicates <- rep(c(1:12), each = 3)
Control <- c(rep(1, 9), rep(0, 27))
Treat1 <- c(rep(0, 9), rep(1, 9), rep(0, 18))
Treat2 <- c(rep(0, 18), rep(1, 9), rep(0, 9))
Treat3 <- c(rep(0, 27), rep(1, 9))
edesign <- cbind(Time, Replicates, Control, Treat1, Treat2, Treat3)
rownames(edesign) <- paste("Array", c(1:36), sep = ")

tc.p <- p.vector(tc.DATA, design = make.design.matrix(edesign), Q = 0.01)
tc.tstep <- T.fit(data = tc.p, alfa = 0.05)
```  

## This will obtain significant genes per experimental group
## which have a regression model Rsquared > 0.9
tc.sigs <- get.siggenes (tc.tstep, rsq = 0.9, vars = "groups")

## This will obtain all significant genes regardless the Rsquared value.
## Replicated genes are averaged.
IDs <- rbind(paste("feature", c(1:300), sep = ""),
             rep(paste("gene", c(1:150), sep = ""), each = 2))
tc.sigs.ALL <- get.siggenes (tc.tstep, rsq = 0, vars = "all", IDs = IDs)
tc.sigs.groups <- get.siggenes (tc.tstep, rsq = 0, vars = "groups", significant.intercept="dummy")

---

### i.rank

Ranks a vector to index

**Description**

Ranks the values in a vector to successive values. Ties are given the same value.

**Usage**

```r
i.rank(x)
```

**Arguments**

- `x`  
  vector

**Value**

Vector of ranked values

**Author(s)**

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**See Also**

`rank`, `order`

**Examples**

```r
i.rank(c(1, 1, 3, 5, 7, 7, 7))
```
Make a design matrix for regression fit of time series gene expression experiments

Description

make.design.matrix creates the design matrix of dummies for fitting time series microarray gene expression experiments.

Usage

make.design.matrix(edesign, degree = 2, time.col = 1, repl.col = 2, group.cols = c(3:ncol(edesign)))

Arguments

edesign matrix describing experimental design. Rows must be arrays and columns experiment descriptors
degree the degree of the regression fit polynomial. degree = 1 returns linear regression, degree = 2 returns quadratic regression, etc
time.col column number in edesign containing time values. Default is first column
repl.col column number in edesign containing coding for replicate arrays. Default is second column
group.cols column numbers in edesign indicating the coding for each experimental group (treatment, tissue, ...). See details

Details

rownames of edesign object should contain the arrays naming (i.e. array1, array2, ...). colnames of edesign must contain the names of experiment descriptors(i.e. "Time", "Replicates", "Treatment A", "Treatment B", etc.). for each experimental group a different column must be present in edesign, coding with 1 and 0 whether each array belongs to that group or not.

make.design.matrix returns a design matrix where rows represent arrays and column variables of time, dummies and their interactions for up to the degree given. Dummies show the relative effect of each experimental group related to the first one. Single dummies indicate the abscissa component of each group. $Time*dummy$ variables indicate slope changes, $Time^2*dummy$ indicates curvature changes. Higher grade values could model complex responses. In case experimental groups share a initial state (i.e. common time 0), no single dummies are modeled.

Value

dis design matrix of dummies for fitting time series

groups.vector vector coding the experimental group to which each variable belongs to

Author(s)

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References


Examples

data(edesign.abiotic, edesignCT)
make.design.matrix(edesign.abiotic) # quadratic model
make.design.matrix(edesignCT, degree = 3) # cubic model with common starting time point

maSigPro

Wrapping function for identifying significant differential gene expression profiles in microarray time course experiments

Description

maSigPro performs a whole maSigPro analysis for a times series gene expression experiment. The function successively calls the functions make.design.matrix(optional), p.vector, T.fit, get.siggenes and see.genes.

Usage

maSigPro(data, edesign, matrix = "AUTO", groups.vector = NULL, degree = 2, time.col = 1, repl.col = 2, group.cols = c(3:ncol(edesign)), Q = 0.05, alfa = 0, nvar.correction = FALSE, step.method = "backward", rsq = 0.7, min.obs = 3, vars = "groups", significant.intercept = "dummy", cluster.data = 1, add.IDs = FALSE, IDs = NULL, matchID.col = 1, only.names = FALSE, k = 9, cluster.method = "hclust", distance = "cor", agglo.method = "ward.D", iter.max = 500, summary.mode = "median", color.mode = "rainbow", trat.repl.spots = "none", index = IDs[, (matchID.col + 1)], match = IDs[, matchID.col], rs = 0.7, show.fit = TRUE, show.lines = TRUE, pdf = TRUE, cexlab = 0.8, legend = TRUE, main = NULL, ...)

Arguments

data

data with normalized gene expression data. Genes must be in rows and arrays in columns. Row names must contain geneIDs (argument of p.vector)
edesign

design matrix of experimental design. Row names must contain arrayIDs (argument of make.design.matrix and see.genes)
matrix

design matrix for regression analysis. By default design is calculated with make.design.matrix (argument of p.vector and T.fit, by default computed by make.design.matrix)
groups.vector

vector indicating experimental group of each variable (argument of get.siggenes and see.genes, by default computed by make.design.matrix)
degree

the degree of the regression fit polynomial. degree = 1 returns linear regression, degree = 2 returns quadratic regression, etc... (argument of make.design.matrix)
time.col column in edesign containing time values. Default is first column (argument of make.design.matrix and see.genes)
repl.col column in edesign containing coding for replicates arrays. Default is second column (argument of make.design.matrix and see.genes)
group.cols columns in edesign indicating the coding for each group of the experiment (see make.design.matrix) (argument of make.design.matrix and see.genes)
Q level of false discovery rate (FDR) control (argument of p.vector)
alfa significance level used for variable selection in the stepwise regression (argument of T.fit)
nvar.correction logical for indicating correcting of stepwise regression significance level (argument of T.fit)
step.method argument to be passed to the step function. Can be either "backward", "forward", "two.ways.backward" or "two.ways.forward"
rsq cut-off level at the R-squared value for the stepwise regression fit. Only genes with R-squared greater than rsq are selected
min.obs genes with less than this number of true numerical values will be excluded from the analysis (argument of p.vector and T.fit)
vars variables for which to extract significant genes (argument of get.siggenes)
significant.intercept experimental groups for which significant intercept coefficients are considered (argument of get.siggenes)
cluster.data Type of data used by the cluster algorithm (argument of get.siggenes)
add.IDs logical indicating whether to include additional gene id’s in the significant genes result (argument of get.siggenes)
IDs matrix containing additional gene id information (required when add.IDs is TRUE) (argument of get.siggenes)
matchID.col number of matching column in matrix IDs for adding genes ids (argument of get.siggenes)
only.names logical. If TRUE, expression values are ommited in the significant genes result (argument of get.siggenes)
k number of clusters (argument of see.genes)
cluster.method clustering method for data portioning (argument of see.genes)
distance distance measurement function used when cluster.method is "hclust" (argument of see.genes)
maSigPro finds and display genes with significant profile differences in time series gene expression experiments. The main, compulsory, input parameters for this function are a matrix of gene expression data (see `p.vector` for details) and a matrix describing experimental design (see `make.design.matrix` or `p.vector` for details). In case extended gene ID information is wanted to be included in the result of significant genes, a third IDs matrix containing this information will be required (see `get.siggenes` for details).

Basically in the function calls subsequent steps of the maSigPro approach which is:

- Make a general regression model with dummies to indicate different experimental groups.
- Select significant genes on the basis of this general model, applying FDR control.
- Find significant variables for each gene, using stepwise regression.
- Extract and display significant genes for any set of variables or experimental groups.

### Parameters

- **agglo.method**: aggregation method used when `cluster.method` is "hclust" (argument of `see.genes`)
- **iter.max**: number of iterations when `cluster.method` is "kmeans" (argument of `see.genes`)
- **summary.mode**: the method to condensate expression information when more than one gene is present in the data. Possible values are "representative" and "median" (argument of `PlotGroups`)
- **color.mode**: color scale for plotting profiles. Can be either "rainbow" or "gray" (argument of `PlotProfiles`)
- **trat.repl.spots**: treatment given to replicate spots. Possible values are "none" and "average" (argument of `get.siggenes`)
- **index**: argument of the `average.rows` function to use when `trat.repl.spots` is "average" (argument of `get.siggenes`)
- **match**: argument of the `link{average.rows}` function to use when `trat.repl.spots` is "average" (argument of `get.siggenes`)
- **rs**: minimum Pearson correlation coefficient for replicated spots profiles to be averaged (argument of `get.siggenes`)
- **show.fit**: logical indicating whether regression fit curves must be plotted (argument of `see.genes`)
- **show.lines**: logical indicating whether a line must be drawn joining plotted data points for each group (argument of `see.genes`)
- **pdf**: logical indicating whether a pdf results file must be generated (argument of `see.genes`)
- **cexlab**: graphical parameter magnification to be used for x labels in plotting functions
- **legend**: logical indicating whether legend must be added when plotting profiles (argument of `see.genes`)
- **main**: title for pdf results file
- **...**: other graphical function arguments

### Details

maSigPro finds and display genes with significant profile differences in time series gene expression experiments. The main, compulsory, input parameters for this function are a matrix of gene expression data (see `p.vector` for details) and a matrix describing experimental design (see `make.design.matrix` or `p.vector` for details). In case extended gene ID information is wanted to be included in the result of significant genes, a third IDs matrix containing this information will be required (see `get.siggenes` for details).

Basically in the function calls subsequent steps of the maSigPro approach which is:
**maSigPro**

**Value**

- **summary**: a vector or matrix listing significant genes for the variables given by the function parameters
- **sig.genes**: a list with detailed information on the significant genes found for the variables given by the function parameters. Each element of the list is also a list containing:
  - **sig.profiles**: expression values of significant genes. The cluster assignment of each gene is given in the last column
  - **coefficients**: regression coefficients for significant genes
  - **t.score**: value of the t statistics of significant genes
  - **sig.pvalues**: p-values of the regression coefficients for significant genes
- **g**: number of genes
- **input.data**: input analysis data
- **G**: number of input genes
- **edesign**: matrix of experimental design
- **dis**: regression design matrix
- **min.obs**: imputed value for minimal number of true observations
- **p.vector**: vector containing the computed p-values of the general regression model for each gene
- **variables**: variables in the general regression model
- **g**: number of significant genes
- **p.vector.alfa**: p-value at FDR = Q control
- **step.method**: imputed step method for stepwise regression
- **Q**: imputed value for false discovery rate (FDR) control
- **step.alfa**: inputed significance level in stepwise regression
- **influ.info**: data frame of genes containing influential data

**Author(s)**

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**References**


**See Also**

- `make.design.matrix`
- `p.vector`
- `T.fit`
- `get.siggenes`
- `see.genes`

**Examples**

```r
#### GENERATE TIME COURSE DATA
## generate n random gene expression profiles of a data set with
## one control plus 3 treatments, 3 time points and r replicates per time point.
```
tc.GENE <- function(n, r, 
  var11 = 0.01, var12 = 0.01, var13 = 0.01, 
  var21 = 0.01, var22 = 0.01, var23 = 0.01, 
  var31 = 0.01, var32 = 0.01, var33 = 0.01, 
  var41 = 0.01, var42 = 0.01, var43 = 0.01, 
  a1 = 0, a2 = 0, a3 = 0, a4 = 0, 
  b1 = 0, b2 = 0, b3 = 0, b4 = 0, 
  c1 = 0, c2 = 0, c3 = 0, c4 = 0) 
{
  tc.dat <- NULL
  for (i in 1:n) {
    Ctl <- c(rnorm(r, a1, var11), rnorm(r, b1, var12), rnorm(r, c1, var13)) # Ctl group
    Tr1 <- c(rnorm(r, a2, var21), rnorm(r, b2, var22), rnorm(r, c2, var23)) # Tr1 group
    Tr2 <- c(rnorm(r, a3, var31), rnorm(r, b3, var32), rnorm(r, c3, var33)) # Tr2 group
    Tr3 <- c(rnorm(r, a4, var41), rnorm(r, b4, var42), rnorm(r, c4, var43)) # Tr3 group
gene <- c(Ctl, Tr1, Tr2, Tr3)
tc.dat <- rbind(tc.dat, gene)
  }
  tc.dat
}
## Create 270 flat profiles
flat <- tc.GENE(n = 270, r = 3)
## Create 10 genes with profile differences between Ctl and Tr1 groups
twodiff <- tc.GENE(n = 10, r = 3, b2 = 0.5, c2 = 1.3)
## Create 10 genes with profile differences between Ctl, Tr2, and Tr3 groups
threediff <- tc.GENE(n = 10, r = 3, b3 = 0.8, c3 = -1, a4 = -0.1, b4 = -0.8, c4 = -1.2)
## Create 10 genes with profile differences between Ctl and Tr2 and different variance
vardiff <- tc.GENE(n = 10, r = 3, a3 = 0.7, b3 = 1, c3 = 1.2, var32 = 0.03, var33 = 0.03)
## Create dataset
tc.DATA <- rbind(flat, twodiff, threediff, vardiff)
rownames(tc.DATA) <- paste("feature", c(1:300), sep = "")
colnames(tc.DATA) <- paste("Array", c(1:36), sep = "")
tc.DATA[sample(c(1:(300*36)), 300)] <- NA # introduce missing values

### CREATE EXPERIMENTAL DESIGN
Time <- rep(c(rep(c(1:3), each = 3)), 4)
Replicates <- rep(c(1:12), each = 3)
Control <- c(rep(0, 9), rep(1, 9), rep(0, 18))
Treat1 <- c(rep(0, 9), rep(1, 9), rep(0, 18))
Treat2 <- c(rep(0, 18), rep(1, 9), rep(0, 9))
Treat3 <- c(rep(0, 27), rep(1, 9))
edesign <- cbind(Time, Replicates, Control, Treat1, Treat2, Treat3)
rownames(edesign) <- paste("Array", c(1:36), sep = "")

### RUN maSigPro
tc.test <- maSigPro (tc.DATA, edesign, degree = 2, vars = "groups", main = "Test")
tc.test$g # gives number of total significant genes
tc.test$summary # shows significant genes by experimental groups
tc.test$genes$Treat1$g$Treat1$g.pvalues # shows pvalues of the significant coefficients # in the regression models of the significant genes 
# for Control.vs.Treat1 comparison
maSigProUsersGuide

View maSigPro User's Guide

Description
Finds the location of the maSigPro User's Guide and opens it.

Usage
maSigProUsersGuide(view=TRUE)

Arguments
view logical, to specify if the document is opened using the PDF document reader.

Details
The function vignette("maSigPro") will find the short maSigPro Vignette which describes how to obtain the maSigPro User's Guide. The User's Guide is not itself a true vignette because it is not automatically generated using Sweave during the package build process. This means that it cannot be found using vignette, hence the need for this special function.

If the operating system is other than Windows, then the PDF viewer used is that given by Sys.getenv("R_PDFVIEWER"). The PDF viewer can be changed using Sys.putenv(R_PDFVIEWER=).

Value
If vignette(view=TRUE), the PDF document reader is started and the User's Guide is opened. If vignette(view=FALSE), returns the file location.

Examples
maSigProUsersGuide()
maSigProUsersGuide(view=FALSE)

NBdata

RNA-Seq dataset example

Description
NBdata contains a subset of a bigger normalized negative binomial simulated dataset.

Usage
data(NBdata)

Format
A data frame with 100 observations on 36 numeric variables.
Details

This dataset is part of a larger simulated and normalized dataset with 2 experimental groups, 6 time-points and 3 replicates. Simulation has been done by using a negative binomial distribution. The first 20 genes are simulated with changes among time.

Examples

```r
data(NBdata)
```

---

**NBdesign**

*Experimental design for RNA-Seq example*

---

Description

NBdesign contains a subset of a bigger normalized negative binomial simulated dataset.

Usage

```r
data(NBdesign)
```

Format

A matrix with 36 rows and 4 columns

- rows [1:36] "G1.T1.1" "G1.T1.2" "G1.T1.3" "G1.T2.1" ...
- columns [1:6] [1] "Time" "Replicates" "Group.1" "Group.2"

Details

Samples are given in rows and experiment descriptors are given in columns. Row names contain sample names.

"Time" indicates the values that variable Time takes in each experimental condition. There are 6 time points.

"Replicates" is an index indicating the same experimental condition.

"Group.1" and "Group.2" columns indicate assignment to experimental groups, coding with 1 and 0 whether each sample belongs to that group or not.

Examples

```r
data(NBdesign)
```
**Description**

`p.vector` performs a regression fit for each gene taking all variables present in the model given by a regression matrix and returns a list of FDR corrected significant genes.

**Usage**

```r
p.vector(data, design, Q = 0.05, MT.adjust = "BH", min.obs = 6, counts=FALSE, family=NULL, theta=10, epsilon=0.00001)
```

**Arguments**

- `data` matrix containing normalized gene expression data. Genes must be in rows and arrays in columns
- `design` design matrix for the regression fit such as that generated by the `make.design.matrix` function
- `Q` significance level
- `MT.adjust` argument to pass to `p.adjust` function indicating the method for multiple testing adjustment of p.value
- `min.obs` genes with less than this number of true numerical values will be excluded from the analysis. Minimum value to estimate the model is (degree+1)xGroups+1. Default is 6.
- `counts` a logical indicating whether your data are counts
- `family` the distribution function to be used in the glm model. It must be specified as a function: `gaussian()`, `poisson()`, `negative.binomial(theta)`... If NULL family will be `negative.binomial(theta)` when `counts=TRUE` or `gaussian()` when `counts=FALSE`
- `theta` theta parameter for `negative.binomial` family
- `epsilon` argument to pass to `glm.control`, convergence tolerance in the iterative process to estimate de glm model

**Details**

- `rownames(design)` and `colnames(data)` must be identical vectors and indicate array naming.
- `rownames(data)` should contain unique gene IDs.
- `colnames(design)` are the given names for the variables in the regression model.

**Value**

- `SELEC` matrix containing the expression values for significant genes
- `p.vector` vector containing the computed p-values
- `G` total number of input genes
- `g` number of genes taken in the regression fit
- `FDR` p-value at FDR Q control when Benajamini & Holderberg (BH) correction is used
i number of significant genes

dis design matrix used in the regression fit
dat matrix of expression value data used in the regression fit
...
additional values from input parameters

Author(s)
Ana Conesa, <aconesa@cipf.es>; Maria Jose Nueda, <mj.nueda@ua.es>

References

See Also
T.fit, lm

Examples

#### GENERATE TIME COURSE DATA
## generates n random gene expression profiles of a data set with
## one control plus 3 treatments, 3 time points and r replicates per time point.

tc.GENE <- function(n, r,
  var11 = 0.01, var12 = 0.01, var13 = 0.01,
  var21 = 0.01, var22 = 0.01, var23 = 0.01,
  var31 = 0.01, var32 = 0.01, var33 = 0.01,
  var41 = 0.01, var42 = 0.01, var43 = 0.01,
  a1 = 0, a2 = 0, a3 = 0, a4 = 0,
  b1 = 0, b2 = 0, b3 = 0, b4 = 0,
  c1 = 0, c2 = 0, c3 = 0, c4 = 0)
{
  tc.dat <- NULL
  for (i in 1:n) {
    Ctl <- c(rnorm(r, a1, var11), rnorm(r, b1, var12), rnorm(r, c1, var13)) # Ctl group
    Tr1 <- c(rnorm(r, a2, var21), rnorm(r, b2, var22), rnorm(r, c2, var23)) # Tr1 group
    Tr2 <- c(rnorm(r, a3, var31), rnorm(r, b3, var32), rnorm(r, c3, var33)) # Tr2 group
    Tr3 <- c(rnorm(r, a4, var41), rnorm(r, b4, var42), rnorm(r, c4, var43)) # Tr3 group
    gene <- c(Ctl, Tr1, Tr2, Tr3)
    tc.dat <- rbind(tc.dat, gene)
  }
  tc.dat
}

## Create 270 flat profiles
flat <- tc.GENE(n = 270, r = 3)

## Create 10 genes with profile differences between Ctl and Tr1 groups
twodiff <- tc.GENE(n = 10, r = 3, b2 = 0.5, c2 = 1.3)

## Create 10 genes with profile differences between Ctl, Tr2, and Tr3 groups
threediff <- tc.GENE(n = 10, r = 3, b3 = 0.8, c3 = -1, a4 = -0.1, b4 = -0.8, c4 = -1.2)

## Create 10 genes with profile differences between Ctl and Tr2 and different variance
vardiff <- tc.GENE(n = 10, r = 3, a3 = 0.7, b3 = 1, c2 = 1.3, var32 = 0.03, var33 = 0.03)
## Create dataset
tc.DATA <- rbind(flat, twodiff, threediff, vardiff)
rownames(tc.DATA) <- paste("feature", c(1:300), sep = "")
colnames(tc.DATA) <- paste("Array", c(1:36), sep = "")
tc.DATA[sample(c(1:(300*36)), 300)] <- NA # introduce missing values

#### CREATE EXPERIMENTAL DESIGN
Time <- rep(c(rep(c(1:3), each = 3)), 4)
Replicates <- rep(c(1:12), each = 3)
Control <- c(rep(1, 9), rep(0, 27))
Treat1 <- c(rep(0, 9), rep(1, 9), rep(0, 18))
Treat2 <- c(rep(0, 18), rep(1, 9), rep(0, 9))
Treat3 <- c(rep(0, 27), rep(1, 9))
edesign <- cbind(Time, Replicates, Control, Treat1, Treat2, Treat3)
rownames(edesign) <- paste("Array", c(1:36), sep = "")
tc.p <- p.vector(tc.DATA, design = make.design.matrix(edesign), Q = 0.05)
tc.p$i # number of significant genes
tc.p$SELEC # expression value of significant genes
tc.p$FDR # p.value at FDR control
tc.p$p.adjusted# adjusted p.values

---

**PlotGroups**

Function for plotting gene expression profile at different experimental groups

---

**Description**

This function displays the gene expression profile for each experimental group in a time series gene expression experiment.

**Usage**

PlotGroups(data, edesign = NULL, time = edesign[,1], groups = edesign[,c(3:ncol(edesign))],
repiect = edesign[,2], show.fit = FALSE, dis = NULL, step.method = "backward",
min.obs = 2, alfa = 0.05, nvar.correction = FALSE, summary.mode = "median", show.lines = TRUE,
xlab = "Time", ylab = "Expression value", cex.xaxis = 1, ylim = NULL, main = NULL, cexlab = 0.8,
legend = TRUE, sub = NULL)

**Arguments**

- **data**: vector or matrix containing the gene expression data
- **edesign**: matrix describing experimental design. Rows must be arrays and columns experiment descriptors
- **time**: vector indicating time assignment for each array
- **groups**: matrix indicating experimental group to which each array is assigned
- **repiect**: index vector indicating experimental replicates
- **show.fit**: logical indicating whether regression fit curves must be plotted
- **dis**: regression design matrix
- **step.method**: stepwise regression method to fit models for cluster mean profiles. It can be either "backward", "forward", "two.ways.backward" or "two.ways.forward"
min.obs  minimal number of observations for a gene to be included in the analysis
alfa  significance level used for variable selection in the stepwise regression
nvar.correction  argument for correcting stepwise regression significance level. See T.fit
summary.mode  the method to condensate expression information when more than one gene is present in the data. Possible values are "representative" and "median"
show.lines  logical indicating whether a line must be drawn joining plotted data points for reach group
groups.vector  vector indicating experimental group to which each variable belongs
xlab  label for the x axis
ylab  label for the y axis
cex.xaxis  graphical parameter magnification to be used for x axis in plotting functions
ylim  range of the y axis
main  plot main title
cexlab  graphical parameter magnification to be used for x axis label in plotting functions
legend  logical indicating whether legend must be added when plotting profiles
sub  plot subtitle

Details

To compute experimental groups either an edesign object must be provided, or separate values must be given for the time, repvect and groups arguments.

When data is a matrix, the average expression value is displayed.

When there are array replicates in the data (as indicated by repvect), values are averaged by repvect.

PlotGroups plots one single expression profile for each experimental group even if there are more than one genes in the data set. The way data is condensed for this is given by summary.mode. When this argument takes the value "representative", the gene with the lowest distance to all genes in the cluster will be plotted. When the argument is "median", then median expression value is computed.

When show.fit is TRUE the stepwise regression fit for the data will be computed and the regression curves will be displayed.

If data is a matrix of genes and summary.mode is "median", the regression fit will be computed for the median expression value.

Value

Plot of gene expression profiles by-group.

Author(s)

Ana Conesa, <aconesa@cipf.es>; Maria Jose Nueda, <mj.nueda@ua.es>

References

### GENERATE TIME COURSE DATA

#### Example 1

```r
## generate n random gene expression profiles of a data set with
## one control plus 3 treatments, 3 time points and r replicates per time point.

tc.GENE <- function(n, r,  
  var11 = 0.01, var12 = 0.01, var13 = 0.01,  
  var21 = 0.01, var22 = 0.01, var23 = 0.01,  
  var31 = 0.01, var32 = 0.01, var33 = 0.01,  
  var41 = 0.01, var42 = 0.01, var43 = 0.01,  
  a1 = 0, a2 = 0, a3 = 0, a4 = 0,  
  b1 = 0, b2 = 0, b3 = 0, b4 = 0,  
  c1 = 0, c2 = 0, c3 = 0, c4 = 0)
{
  tc.dat <- NULL
  for (i in 1:n) {
    Ctl <- c(rnorm(r, a1, var11), rnorm(r, b1, var12), rnorm(r, c1, var13)) # Ctl group
    Tr1 <- c(rnorm(r, a2, var21), rnorm(r, b2, var22), rnorm(r, c2, var23)) # Tr1 group
    Tr2 <- c(rnorm(r, a3, var31), rnorm(r, b3, var32), rnorm(r, c3, var33)) # Tr2 group
    Tr3 <- c(rnorm(r, a4, var41), rnorm(r, b4, var42), rnorm(r, c4, var43)) # Tr3 group
    gene <- c(Ctl, Tr1, Tr2, Tr3)
    tc.dat <- rbind(tc.dat, gene)
  }
  tc.dat
}
```

#### Example 2

```r
## create 10 genes with profile differences between Ctl, Tr2, and Tr3 groups

tc.DATA <- tc.GENE(n = 10, r = 3, b3 = 0.8, c3 = -1, a4 = -0.1, b4 = -0.8, c4 = -1.2)
rownames(tc.DATA) <- paste("gene", c(1:10), sep = "")
colnames(tc.DATA) <- paste("Array", c(1:36), sep = ")
```

#### Example 3

```r
## create experimental design

Time <- rep(c(rep(c(1:3), each = 3)), 4)
Replicates <- rep(c(1:12), each = 3)
Ctl <- c(rep(1, 9), rep(0, 27))  
Tr1 <- c(rep(0, 9), rep(1, 9), rep(0, 18))  
Tr2 <- c(rep(0, 18), rep(1, 9), rep(0, 9))  
Tr3 <- c(rep(0, 27), rep(1, 9))  
PlotGroups(tc.DATA, time = Time, repvect = Replicates, groups = cbind(Ctl, Tr1, Tr2, Tr3))
```

---

**PlotProfiles**  
*Function for visualization of gene expression profiles*

**Description**  
PlotProfiles displays the expression profiles of a group of genes.
Usage

PlotProfiles(data, cond, main = NULL, cex.xaxis = 0.5, ylim = NULL, repvect, sub = NULL, color.mode = "rainbow")

Arguments

data a matrix containing the gene expression data
cond vector for x axis labeling, typically array names
main plot main title
cex.xaxis graphical parameter magnification to be used for x axis in plotting functions
ylim index vector indicating experimental replicates
repvect index vector indicating experimental replicates
sub plot subtitle
color.mode color scale for plotting profiles. Can be either "rainbow" or "gray"

Details

The repvect argument is used to indicate with vertical lines groups of replicated arrays.

Value

Plot of experiment-wide gene expression profiles.

Author(s)

Ana Conesa, aconesa@ci.pf.es, Maria Jose Nueda, mj.nueda@ua.es

References


See Also

PlotGroups

Examples

#### GENERATE TIME COURSE DATA
## generate n random gene expression profiles of a data set with
## one control plus 3 treatments, 3 time points and r replicates per time point.

tc.GENE <- function(n, r,
  var11 = 0.01, var12 = 0.01, var13 = 0.01,
  var21 = 0.01, var22 = 0.01, var23 =0.01,
  var31 = 0.01, var32 = 0.01, var33 = 0.01,
  var41 = 0.01, var42 = 0.01, var43 = 0.01,
  a1 = 0, a2 = 0, a3 = 0, a4 = 0,
  b1 = 0, b2 = 0, b3 = 0, b4 = 0,
  c1 = 0, c2 = 0, c3 = 0, c4 = 0)
{
  tc.dat <- NULL

for (i in 1:n) {
  Ctl <- c(rnorm(r, a1, var11), rnorm(r, b1, var12), rnorm(r, c1, var13)) # Ctl group
  Tr1 <- c(rnorm(r, a2, var21), rnorm(r, b2, var22), rnorm(r, c2, var23)) # Tr1 group
  Tr2 <- c(rnorm(r, a3, var31), rnorm(r, b3, var32), rnorm(r, c3, var33)) # Tr2 group
  Tr3 <- c(rnorm(r, a4, var41), rnorm(r, b4, var42), rnorm(r, c4, var43)) # Tr3 group
  gene <- c(Ctl, Tr1, Tr2, Tr3)
  tc.dat <- rbind(tc.dat, gene)
}

tc.dat

## create 10 genes with profile differences between Ctl, Tr2, and Tr3 groups

# create 10 genes with profile differences between Ctl, Tr2, and Tr3 groups
tc.DATA <- tc.GENE(n = 10, r = 3, b3 = 0.8, c3 = -1, a4 = -0.1, b4 = -0.8, c4 = -1.2)
rownames(tc.DATA) <- paste("gene", c(1:10), sep = "")
colnames(tc.DATA) <- paste("Array", c(1:36), sep = "")

PlotProfiles(tc.DATA, cond = colnames(tc.DATA), main = "Time Course",
             repvect = rep(c(1:12), each = 3))

---

position

<table>
<thead>
<tr>
<th>Column position of a variable in a data frame</th>
</tr>
</thead>
</table>

**Description**

Finds the column position of a character variable in the column names of a data frame.

**Usage**

position(matrix, vari)

**Arguments**

- **matrix**: matrix or data.frame with character column names
- **vari**: character variable

**Value**

numerical. Column position for the given variable.

**Author(s)**

Ana Conesa, aconesa@cipf.es

**Examples**

x <- matrix(c(1, 1, 2, 2, 3, 3), ncol = 3, nrow = 2)
colnames(x) <- c("one", "two", "three")
position(x, "one")
**Description**

`reg.coeffs` calculates back regression coefficients for true variables (experimental groups) from dummy variables regression coefficients.

**Usage**

```r
reg.coeffs(coefficients, indepen = groups.vector[nchar(groups.vector)==min(nchar(groups.vector))][1], groups.vector, group)
```

**Arguments**

- `coefficients`: vector of regression coefficients obtained from a regression model with dummy variables
- `indepen`: independent variable of the regression formula
- `groups.vector`: vector indicating the true variable of each variable in `coefficients`
- `group`: true variable for which regression coefficients are to be computed

**Details**

Regression coefficients in coefficients vector should be ordered by polynomial degree in a regression formula, i.e.: intercept, $x$ term, $x^2$ term, $x^3$ term, and so on...

**Value**

- `reg.coeff`: vector of calculated regression coefficients

**Author(s)**

Ana Conesa, aconesa@cipf.es; Maria Jose Nueda, mj.nueda@ua.es

**References**


**Examples**

```r
groups.vector <- c("CT", "T1vsCT", "T2vsCT", "CT", "T1vsCT", "T2vsCT", "CT", "T1vsCT", "T2vsCT")
coefficients <- c(0.1, 1.2, -0.8, 1.7, 3.3, 0.4, 0.0, 2.1, -0.9)
## calculate true regression coefficients for variable "T1"
reg.coeffs(coefficients, groups.vector = groups.vector, group = "T1")
```
see.genes

Wrapper function for visualization of gene expression values of time course experiments

Description

This function provides visualisation tools for gene expression values in a time course experiment. The function first calls the heatmap function for a general overview of experiment results. Next a partioning of the data is generated using a clustering method. The results of the clustering are visualized both as gene expression profiles extended along all arrays in the experiment, as provided by the plot.profiles function, and as summary expression profiles for comparison among experimental groups.

Usage

see.genes(data, edesign = data$edesign, time.col = 1, repl.col = 2, group.cols = c(3:ncol(edesign)), names.groups = colnames(edesign)[3:ncol(edesign)], cluster.data = 1, groups.vector = data$groups.vector, k = 9, k.mclust=FALSE, cluster.method = "hclust", distance = "cor", agglo.method = "ward.D", show.fit = FALSE, dis = NULL, step.method = "backward", min.obs = 3, alfa = 0.05, nvar.correction = FALSE, show.lines = TRUE, iter.max = 500, summary.mode = "median", color.mode = "rainbow", cexlab = 1, legend = TRUE, newX11 = TRUE, ylim = NULL, main = NULL, ...)

Arguments

data either matrix or a list containing the gene expression data, typically a get.siggenes object
edesign matrix of experimental design
time.col column in edesign containing time values. Default is first column
repl.col column in edesign containing coding for replicates arrays. Default is second column
group.cols columns indicating the coding for each group (treatment, tissue,...) in the experiment (see details)
names.groups names for experimental groups
cluster.data type of data used by the cluster algorithm (see details)
groups.vector vector indicating the experimental group to which each variable belongs
k number of clusters for data partioning
k.mclust TRUE for computing the optimal number of clusters with Mclust algorithm
cluster.method clustering method for data partioning. Currently "hclust", "kmeans" and "Mclust" are supported
distance distance measurement function when cluster.method is hclust
agglo.method aggregation method used when cluster.method is hclust
show.fit logical indicating whether regression fit curves must be plotted
dis regression design matrix
step.method stepwise regression method to fit models for cluster mean profiles. Can be either "backward", "forward", "two.ways.backward" or "two.ways.forward"
min.obs  minimal number of observations for a gene to be included in the analysis
alfa  significance level used for variable selection in the stepwise regression
nvar.correction  argument for correcting T.fit significance level. See T.fit
show.lines  logical indicating whether a line must be drawn joining plotted data points for reach group
iter.max  maximum number of iterations when cluster.method is kmeans
summary.mode  the method PlotGroups takes to condensate expression information when more than one gene is present in the data. Possible values are "representative" and "median"
color.mode  color scale for plotting profiles. Can be either "rainblow" or "gray"
cexlab  graphical parameter magnification to be used for x labels in plotting functions
legend  logical indicating whether legend must be added when plotting profiles
main  plot title
ylim  range of the y axis to be used by PlotProfiles and PlotGroups
newX11  when TRUE, plot each type of plot in a diferent graphical device
...  other graphical function argument

Details

Data can be provided either as a single data matrix of expression values, or a get.siggenes object. In the later case the other argument of the function can be taken directly from data.

Data clustering can be done on the basis of either the original expression values, the regression coefficients, or the t.scores. In case data is a get.siggenes object, this is given by providing the element names of the list c("sig.profiles","coefficients","t.score") of their list position (1,2 or 3).

Value

Experiment wide gene profiles and by group profiles plots are generated for each data cluster in the graphical device.

cut  vector indicating gene partitioning into clusters
c.algo.used  clustering algorithm used for data partitioning
groups  groups matrix used for plotting functions

Author(s)

Ana Conesa, aconesa@cipf.es; Maria Jose Nueda, mj.nueda@ua.es

References


See Also

PlotProfiles, PlotGroups
### GENERATE TIME COURSE DATA

#### GENERATE TIME COURSE DATA

Examples

```r
### GENERATE TIME COURSE DATA
# generate n random gene expression profiles of a data set with
# one control plus 3 treatments, 3 time points and r replicates per time point.
tc.GENE <- function(n, r,
  var11 = 0.01, var12 = 0.01, var13 = 0.01,
  var21 = 0.01, var22 = 0.01, var23 = 0.01,
  var31 = 0.01, var32 = 0.01, var33 = 0.01,
  var41 = 0.01, var42 = 0.01, var43 = 0.01,
  a1 = 0, a2 = 0, a3 = 0, a4 = 0,
  b1 = 0, b2 = 0, b3 = 0, b4 = 0,
  c1 = 0, c2 = 0, c3 = 0, c4 = 0)
{
  tc.dat <- NULL
  for (i in 1:n) {
    Ctl <- c(rnorm(r, a1, var11), rnorm(r, b1, var12), rnorm(r, c1, var13)) # Ctl group
    Tr1 <- c(rnorm(r, a2, var21), rnorm(r, b2, var22), rnorm(r, c2, var23)) # Tr1 group
    Tr2 <- c(rnorm(r, a3, var31), rnorm(r, b3, var32), rnorm(r, c3, var33)) # Tr2 group
    Tr3 <- c(rnorm(r, a4, var41), rnorm(r, b4, var42), rnorm(r, c4, var43)) # Tr3 group
    gene <- c(Ctl, Tr1, Tr2, Tr3)
    tc.dat <- rbind(tc.dat, gene)
  }
  tc.dat
}

## Create 270 flat profiles
flat <- tc.GENE(n = 270, r = 3)

## Create 10 genes with profile differences between Ctl and Tr1 groups
twodiff <- tc.GENE(n = 10, r = 3, b2 = 0.5, c2 = 1.3)

## Create 10 genes with profile differences between Ctl, Tr2, and Tr3 groups
threediff <- tc.GENE(n = 10, r = 3, b3 = 0.8, c3 = -1, a4 = -0.1, b4 = -0.8, c4 = -1.2)

## Create 10 genes with profile differences between Ctl and Tr2 and different variance
vardiff <- tc.GENE(n = 10, r = 3, a3 = 0.7, b3 = 1, c3 = 1.2, var32 = 0.03, var33 = 0.03)

## Create dataset
tc.DATA <- rbind(flat, twodiff, threediff, vardiff)
rownames(tc.DATA) <- paste("feature", c(1:300), sep = "")
colnames(tc.DATA) <- paste("Array", c(1:36), sep = "")
tc.DATA [sample(c(1:(300*36)), 300)] <- NA # introduce missing values

### CREATE EXPERIMENTAL DESIGN

Time <- rep(c(rep(c(1:3), each = 3), 4)
Replicates <- rep(c(1:12), each = 3)
Control <- c(rep(1, 9), rep(0, 27))
Treat1 <- c(rep(0, 9), rep(1, 9), rep(0, 18))
Treat2 <- c(rep(0, 18), rep(1, 9), rep(0,9))
Treat3 <- c(rep(0, 27), rep(1, 9))
edesign <- cbind(Time, Replicates, Control, Treat1, Treat2, Treat3)
rownames(edesign) <- paste("Array", c(1:36), sep = "")

see.genes(tc.DATA, edesign = edesign, k = 4, main = "Time Course")

# This will show the regression fit curve
dise <- make.design.matrix(edesign)
```
stepback

Fitting a linear model by backward-stepwise regression

Description

stepback fits a linear regression model applying a backward-stepwise strategy.

Usage

stepback(y = y, d = d, alfa = 0.05, family = gaussian(), epsilon=0.00001 )

Arguments

y dependent variable
d data frame containing by columns the set of variables that could be in the selected model
alfa significance level to decide if a variable stays or not in the model
family the distribution function to be used in the glm model
epsilon argument to pass to glm.control, convergence tolerance in the iterative process to estimate de glm model

Details

The strategy begins analysing a model with all the variables included in d. If all variables are statistically significant (all variables have a p-value less than alfa) this model will be the result. If not, the less statistically significant variable will be removed and the model is re-calculated. The process is repeated up to find a model with all the variables statistically significant.

Value

stepback returns an object of the class lm, where the model uses y as dependent variable and all the selected variables from d as independent variables.

The function summary are used to obtain a summary and analysis of variance table of the results. The generic accessor functions coefficients, effects, fitted.values and residuals extract various useful features of the value returned by lm.

Author(s)

Ana Conesa, aconesa@cipf.es; Maria Jose Nueda, mj.nueda@ua.es

References


See Also

lm, step, stepfor, two.ways.stepback, two.ways.stepfor
stepfor

Examples

```r
## create design matrix
Time <- rep(c(rep(c(1:3), each = 3)), 4)
Replicates <- rep(c(rep(c(1:12), each = 3))
Control <- c(rep(1, 9), rep(0, 27))
Treat1 <- c(rep(0, 9), rep(1, 9), rep(0, 18))
Treat2 <- c(rep(0, 18), rep(1, 9), rep(0, 9))
Treat3 <- c(rep(0, 27), rep(1, 9))
edesign <- cbind(Time, Replicates, Control, Treat1, Treat2, Treat3)
rownames(edesign) <- paste("Array", c(1:36), sep = "")
dise <- make.design.matrix(edesign)
dis <- as.data.frame(dise$dis)

## expression vector
y <- c(0.082, 0.021, 0.010, 0.013, 0.077, 0.068, 0.042, -0.056, -0.014, -0.040,
-0.055, 0.150, -0.027, 0.064, -0.108, -0.220, 0.275, -0.130, 0.130, 1.018, 1.005, 0.931,
-1.009, -1.101, -1.014, -0.045, -0.110, -0.128, -0.643, -0.785, -1.077, -1.187, -1.249, -1.463)
s.fit <- stepback(y = y, d = dis)
summary(s.fit)
```

stepfor

### Fitting a linear model by forward-stepwise regression

**Description**

`stepfor` fits a linear regression model applying forward-stepwise strategy.

**Usage**

```r
stepfor(y = y, d = d, alfa = 0.05, family = gaussian(), epsilon=0.00001 )
```

**Arguments**

- `y`: dependent variable
- `d`: data frame containing by columns the set of variables that could be in the selected model
- `alfa`: significance level to decide if a variable stays or not in the model
- `family`: the distribution function to be used in the glm model
- `epsilon`: argument to pass to glm.control, convergence tolerance in the iterative process to estimate de glm model

**Details**

The strategy begins analysing all the possible models with only one of the variables included in d. The most statistically significant variable (with the lowest p-value) is included in the model and then it is considered to introduce in the model another variable analysing all the possible models with two
variables (the selected variable in the previous step plus a new variable). Again the most statistically significant variable (with lowest p-value) is included in the model. The process is repeated till there are no more statistically significant variables to include.

Value

stepfor returns an object of the class \texttt{lm}, where the model uses \texttt{y} as dependent variable and all the selected variables from \texttt{d} as independent variables.

The function \texttt{summary} are used to obtain a summary and analysis of variance table of the results. The generic accessor functions \texttt{coefficients}, \texttt{effects}, \texttt{fitted.values} and \texttt{residuals} extract various useful features of the value returned by \texttt{lm}.

Author(s)

Ana Conesa, aconesa@cipf.es; Maria Jose Nueda, mj.nueda@ua.es

References


See Also

\texttt{lm, step, stepback, two.ways.stepback, two.ways.stepfor}

Examples

```r
## create design matrix
Time <- rep(c(rep(c(1:3), each = 3)), 4)
Replicates <- rep(c(1:12), each = 3)
Control <- c(rep(1, 9), rep(0, 27))
Treat1 <- c(rep(0, 9), rep(1, 9), rep(0, 18))
Treat2 <- c(rep(0, 18), rep(1, 9), rep(0,9))
Treat3 <- c(rep(0, 27), rep(1, 9))
edesign <- cbind(Time, Replicates, Control, Treat1, Treat2, Treat3)
rownames(edesign) <- paste("Array", c(1:36), sep = "")
dise <- make.design.matrix(edesign)
dis <- as.data.frame(dise$dis)

## expression vector
y <- c(0.082, 0.021, 0.010, 0.013, 0.077, 0.068, 0.042, -0.056, -0.232, -0.014, -0.040, -0.055, 0.150, -0.027, 0.064, -0.108, -0.220, 0.275, -0.130, 0.130, 1.018, 1.005, 0.931, -1.009, -1.101, -1.014, -0.045, -0.110, -0.128, -0.643, -0.785, -1.077, -1.187, -1.249, -1.463)
s.fit <- stepfor(y = y, d = dis)
summary(s.fit)
```
suma2Venn

Creates a Venn Diagram from a matrix of characters

Description

suma2Venn transforms a matrix or a data frame with characters into a list to draw and display a Venn diagram with up to 7 sets.

Usage

suma2Venn(x, size = 30, cexil = 0.9, cexsn = 1, zcolor = heat.colors(ncol(x)), ...)

Arguments

- **x**: matrix or data frame of character values
- **size**: Plot size, in centimeters
- **cexil**: Character expansion for the intersection labels
- **cexsn**: Character expansion for the set names
- **zcolor**: A vector of colors for the custom zones
- **...**: Additional plotting arguments for the venn function

Details

suma2Venn creates a list with the columns of a matrix or a data frame of characters which can be taken by the venn to generate a Venn Diagram.

Value

suma2Venn returns a Venn Plot such as that created by the venn function.

Author(s)

Maria J. Nueda, mj.nueda@ua.es

See Also

venn

Examples

```
A <- c("a", "b", "c", "d", "e", NA, NA)
B <- c("a", "b", "f", NA, NA, NA, NA)
C <- c("a", "b", "e", "f", "h", "i", "j", "k")
x <- cbind(A, B, C)
suma2Venn(x)
```
T.fit

makes a stepwise regression fit for time series gene expression experiments

Description

T.fit selects the best regression model for each gene using stepwise regression.

Usage

T.fit(data, design = data$dis, step.method = "backward", min.obs = data$min.obs, alfa = data$Q, nvar.correction = FALSE, family = gaussian(), epsilon=0.00001)

Arguments

data can either be a p.vector object or a matrix containing expression data with the same requirements as for the p.vector function
design design matrix for the regression fit such as that generated by the make.design.matrix function. If data is a p.vector object, the same design matrix is used by default
step.method argument to be passed to the step function. Can be either "backward", "forward", "two.ways.backward" or "two.ways.forward"
min.obs genes with less than this number of true numerical values will be excluded from the analysis
alfa significance level used for variable selection in the stepwise regression
nvar.correction argument for correcting T.fit significance level. See details
family the distribution function to be used in the glm model. It must be the same used in p.vector
epsilon argument to pass to glm.control, convergence tolerance in the iterative process to estimate glm model

Details

In the maSigPro approach p.vector and T.fit are subsequent steps, meaning that significant genes are first selected on the basis of a general model and then the significant variables for each gene are found by step-wise regression.

The step regression can be "backward" or "forward" indicating whether the step procedure starts from the model with all or none variables. With the "two.ways.backward" or "two.ways.forward" options the variables are both allowed to get in and out. At each step the p-value of each variable is computed and variables get in/out the model when this p-value is lower or higher than given threshold alfa. When nvar.correction is TRUE the given significance level is corrected by the number of variables in the model

Value

sol matrix for summary results of the stepwise regression. For each selected gene the following values are given:
• p-value of the regression ANOVA
• R-squared of the model
• p-value of the regression coefficients of the selected variables

**sig.profiles**
expression values for the genes contained in sol

**coefficients**
matrix containing regression coefficients for the adjusted models

**groups.coeffs**
matrix containing the coefficients of the implicit models of each experimental group

**variables**
variables in the complete regression model

**G**
total number of input genes

**g**
number of genes taken in the regression fit

**dat**
input analysis data matrix

**dis**
regression design matrix

**step.method**
imputed step method for stepwise regression

**edesign**
matrix of experimental design

**influ.info**
data frame of genes containing influential data

**Author(s)**
Ana Conesa, <aconesa@cipf.es>; Maria Jose Nueda, <mj.nueda@ua.es>

**References**

**See Also**

* p.vector, step

**Examples**

```r
### GENERATE TIME COURSE DATA
## generate n random gene expression profiles of a data set with
## one control plus 3 treatments, 3 time points and r replicates per time point.

tc.GENE <- function(n, r,
  var11 = 0.01, var12 = 0.01,var13 = 0.01,
  var21 = 0.01, var22 = 0.01, var23 =0.01,
  var31 = 0.01, var32 = 0.01, var33 = 0.01,
  var41 = 0.01, var42 = 0.01, var43 = 0.01,
  a1 = 0, a2 = 0, a3 = 0, a4 = 0,
  b1 = 0, b2 = 0, b3 = 0, b4 = 0,
  c1 = 0, c2 = 0, c3 = 0, c4 = 0)
{
  tc.dat <- NULL
  for (i in 1:n) {
    Ctl <- c(rnorm(r, a1, var11), rnorm(r, b1, var12), rnorm(r, c1, var13)) # Ctl group
    Tr1 <- c(rnorm(r, a2, var21), rnorm(r, b2, var22), rnorm(r, c2, var23)) # Tr1 group
    Tr2 <- c(rnorm(r, a3, var31), rnorm(r, b3, var32), rnorm(r, c3, var33)) # Tr2 group
    Tr3 <- c(rnorm(r, a4, var41), rnorm(r, b4, var42), rnorm(r, c4, var43)) # Tr3 group
    gene <- c(Ctl, Tr1, Tr2, Tr3)
    tc.dat <- rbind(tc.dat, gene)
  }
}
```
two.ways.stepback

Fitting a linear model by backward-stepwise regression

description

two.ways.stepback fits a linear regression model applying backward-stepwise strategy.

Usage

two.ways.stepback(y = y, d = d, alfa = 0.05, family = gaussian(), epsilon=0.00001)

Arguments

y dependent variable
**two.ways.stepback**

- **d**: data frame containing by columns the set of variables that could be in the selected model.
- **alfa**: significance level to decide if a variable stays or not in the model.
- **family**: the distribution function to be used in the glm model.
- **epsilon**: argument to pass to glm.control, convergence tolerance in the iterative process to estimate the glm model.

**Details**

The strategy begins analysing a model with all the variables included in `d`. If all the variables are statistically significant (all the variables have a p-value less than `alfa`) this model will be the result. If not, the less statistically significant variable will be removed and the model is re-calculated. The process is repeated up to find a model with all the variables statistically significant (p-value < alpha). Each time that a variable is removed from the model, it is considered the possibility of one or more removed variables to come in again.

**Value**

`two.ways.stepback` returns an object of the class `lm`, where the model uses `y` as dependent variable and all the selected variables from `d` as independent variables.

The function `summary` are used to obtain a summary and analysis of variance table of the results. The generic accessor functions `coefficients, effects, fitted.values` and `residuals` extract various useful features of the value returned by `lm`.

**Author(s)**

Ana Conesa, aconesa@cipf.es; Maria Jose Nueda, mj.nueda@ua.es

**References**


**See Also**

`lm, step, stepfor, stepback, two.ways.stepfor`

**Examples**

```r
## create design matrix
Time <- rep(c(rep(c(1:3), each = 3), 4)
Replicates <- rep(c(1:12), each = 3)
Control <- c(rep(1, 9), rep(0, 27))
Treat1 <- c(rep(0, 9), rep(1, 9), rep(0, 18))
Treat2 <- c(rep(0, 18), rep(1, 9), rep(0,9))
Treat3 <- c(rep(0, 27), rep(1, 9))
edesign <- cbind(Time,Replicates,Control,Treat1,Treat2,Treat3)
rownames(edesign) <- paste("Array", c(1:36), sep = "")
dise <- make.design.matrix(edesign)
dis <- as.data.frame(dise$dis)

## expression vector
```
\begin{verbatim}
y <- c(0.082, 0.021, 0.010, 0.113, 0.013, 0.077, 0.068, 0.042, -0.056, -0.232, -0.014, -0.040, -0.055, 0.150, -0.027, 0.064, -0.108, 0.275, -0.130, 0.130, 1.018, 1.005, 0.931, -1.009, -1.101, -1.014, -0.045, -0.110, -0.128, -0.643, -0.785, -1.077, -1.187, -1.249, -1.463)
s.fit <- two.ways.stepback(y = y, d = dis)
summary(s.fit)
\end{verbatim}

\section*{two.ways.stepfor}

\textit{Fitting a linear model by forward-stepwise regression}

\section*{Description}

two.ways.stepfor fits a linear regression model applying forward-stepwise strategy.

\section*{Usage}

two.ways.stepfor(y = y, d = d, alfa = 0.05, family = gaussian(), epsilon=0.00001 )

\section*{Arguments}

\begin{itemize}
\item \textbf{y} \hspace{1cm} dependent variable
\item \textbf{d} \hspace{1cm} data frame containing by columns the set of variables that could be in the selected model
\item \textbf{alfa} \hspace{1cm} significance level to decide if a variable stays or not in the model
\item \textbf{family} \hspace{1cm} the distribution function to be used in the glm model
\item \textbf{epsilon} \hspace{1cm} argument to pass to glm.control, convergence tolerance in the iterative process to estimate de glm model
\end{itemize}

\section*{Details}

The strategy begins analysing all the possible models with only one of the variables included in d. The most statistically significant variable (with the lowest p-value) is included in the model and then it is considered to introduce in the model another variable analysing all the possible models with two variables (the selected variable in the previous step plus a new variable). Again the most statistically significant variable (with lowest p-value) is included in the model. The process is repeated till there are no more statistically significant variables to include. Each time that a variable enters the model, the p-values of the current model variables is recalculated and non significant variables will be removed.

\section*{Value}

two.ways.stepfor returns an object of the class \texttt{lm}, where the model uses y as dependent variable and all the selected variables from d as independent variables.

The function \texttt{summary} are used to obtain a summary and analysis of variance table of the results. The generic accessor functions \texttt{coefficients}, \texttt{effects}, \texttt{fitted.values} and \texttt{residuals} extract various useful features of the value returned by \texttt{lm}.

\section*{Author(s)}

Ana Conesa, aconesa@cif.es; Maria Jose Nueda, mj.nueda@ua.es
References


See Also

lm, step, stepback, stepfor, two.ways.stepback

Examples

```r
## create design matrix
Time <- rep(c(rep(c(1:3), each = 3)), 4)
Replicates <- rep(c(1:12), each = 3)
Control <- c(rep(1, 9), rep(0, 27))
Treat1 <- c(rep(0, 9), rep(1, 9), rep(0, 18))
Treat2 <- c(rep(0, 18), rep(1, 9), rep(0,9))
Treat3 <- c(rep(0, 27), rep(1, 9))
edesign <- cbind(Time, Replicates, Control, Treat1, Treat2, Treat3)
rownames(edesign) <- paste("Array", c(1:36), sep = "")
dise <- make.design.matrix(edesign)
dis <- as.data.frame(dise$dis)

## expression vector
y <- c(0.082, 0.021, 0.010, 0.113, 0.077, 0.042, -0.056, -0.232, -0.014, -0.040, 
-0.055, 0.150, -0.027, 0.064, -0.108, -0.220, 0.275, -0.130, 0.130, 1.018, 1.005, 0.931, 
-1.009, -1.101, -1.014, -0.045, -0.110, -0.128, -0.643, -0.785, -1.077, -1.187, -1.249, -1.463)
s.fit <- two.ways.stepfor(y = y, d = dis)
summary(s.fit)
```
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