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Description The purpose of this package is to analyze (i.e. Normalize and select significant spots) data issued from 2D GEI experiments

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prot2D-package

Statistics Tools for data issued from a 2D Gel Electrophoresis

Description

The purpose of this package is to analyze (i.e. Normalize and select significant spots) data issued from 2D GEI experiments

Details

This package provides a simple interface for analysing data from 2D gel experiments. Functions for performing normalization as well as selecting significant spots are provided. All the functions for selecting significant spots are adapted from functions for microarray analysis provided by Bioconductor and CRAN, and all credits go to the authors of these functions. For analyzing 2D gel experiments data, users are advised to follow these steps:

1. Normalize data using `Norm.qt`
2. Coerce data into an `ExpressionSet` using `ES.prot`
3. Use normalized data to find differentially expressed proteins with FDR-controlled functions: `modT.Prot` was find to be the more efficient for 2-DE (see Artigaud et al, 2013) but other functions are provided (`ttest.Prot`, `samT.Prot`, `efronT.Prot`, `shrinkT.Prot`)

Author(s)

Sebastien Artigaud <sebastien.artigaud@gmx.com>

References


See Also

`ExpressionSet`, `fdrtool`

Examples

data(pecten)
data(pecten.fac)

pecten.norm <- Norm.qt(pecten, n1=6, n2=6, plot=TRUE) #Quantiles normalization of the data
ES.p <- ES.prot(pecten.norm, n1=6, n2=6, f=pecten.fac)
x <- modT.Prot(ES.p, fdr.thr=0.1, plot=TRUE)
featureNames(x) # Names of the spots selected for a moderated t-test with a fdr of 0.1
fData(x) # Displaying fold change (as log2(ratio)) for selected spots
exprs(x) # Normalized volume data for all the selected spots
## Not run: heatplot(x) #Great heatmap of the selected spots (requires made4 Bioconductor package )
ES.prot

Create an ExpressionSet containing 2D Gel Volume Data

Description
This function creates an ExpressionSet containing 2D Gel Volume Data and associated metadata. It requires a matrix of 2D Gel Volume data with Gels as columns and Spots as rows as well as a dataframe describing the data.

Usage
ES.prot(data, n1, n2, f)

Arguments
data
a matrix of spots intensities. data should be intensities displayed with gels as columns with the name of columns corresponding to the names of the gels and spots as rows with the names of the rows corresponding to the name of the spots. The replicates for each condition should be ordered in following columns.
n1
an integer. Number of replicates in condition 1.
n2
an integer. Number of replicates in condition 2.
f
a dataframe giving the factors for data. f dataframe should have only 1 column giving the 2 levels of factor with rownames corresponding to the names of gels (in the same order as data).

Details
Usually data matrix should be the output of Norm.qt and therefore is log2-transformed. In data the columns (i.e. Gels) must be ordered with replicates from condition 1 before those from condition 2.

Value
The function returns an ExpressionSet. The matrix of spots intensities (i.e. Volume) is stored in the assayData slot of the ExpressionSet and can be retrieved with exprs. In the FeatureData slot the log2-ratio is computed and can be retrieved with fData.

Author(s)
Sebastien Artigaud <sebastien.artigaud@gmx.com>

See Also
Norm.qt, Norm.vsn, ExpressionSet

Examples
data(pecten)
data(pecten.fac)
pecten.norm <- Norm.qt(pecten, n1=6, n2=6, plot=TRUE) #Quantiles normalization of the data
ES.p <- ES.prot(pecten.norm, n1=6, n2=6, f=pecten.fac)
head(exprs(ES.p))
Description

This function allows to normalize 2D Gel Volume data using the Quantiles Normalization.

Usage

Norm.qt(data, n1, n2, plot = T)

Arguments

data a dataframe of raw 2D Gel Volume data. data should be raw intensities displayed with gel as columns with the name of columns corresponding to the names of the gels and spots as rows with the names of the rows corresponding to the name of the spots. The replicates for each condition should be ordered in following columns.
n1 an integer. Number of replicates in condition 1.
n2 an integer. Number of replicates in condition 2.
plot logical. if TRUE (default) displaying two RIplot, one with the raw data, another with normalized data.

Details

2D Gel Volume data must be normalized in order to remove systemic variation prior to data analysis. The principle of the "quantiles normalization" is to set each quantile of each column (i.e. the spots volume data of each gels) to the mean of that quantile across gels. The intention is to make all the normalized columns have the same empirical distribution. This function is based on normalizeQuantiles from limma package.

Value

The function returns a matrix of log2 transformed quantiles normalized data

Author(s)

Sebastien Artigaud <sebastien.artigaud@gmx.com>

References

Examples

```r
data(pecten)
pecten.norm <- Norm.qt(pecten, n1=6, n2=6, plot=TRUE)
```

Description

This function allows to normalize 2D Gel Volume data using the "Variance Stabilizing Normalization".

Usage

```r
Norm.vsn(data, n1, n2, plot = T)
```

Arguments

- `data`: a dataframe of raw 2D Gel Volume data. Data should be raw intensities displayed with gel as columns with the name of columns corresponding to the names of the gels and spots as rows with the names of the rows corresponding to the name of the spots. The replicates for each condition should be ordered in following columns.
- `n1`: an integer. Number of replicates in condition 1.
- `n2`: an integer. Number of replicates in condition 2.
- `plot`: logical. If TRUE (default) displaying two `RIplot`, one with the raw data, another with normalized data.

Details

The "Variance Stabilizing Normalization" relies on a transformation $h$, of the parametric form $h(x) = \text{arsinh}(a+bx)$ (for details see, Huber et al., 2002). The parameters of $h$ together with those of the calibration between experiments are estimated with a robust variant of maximum-likelihood estimation. This function is based on `normalizeVSN` from `limma` package.

Value

The function returns a matrix of vsn normalized data

Author(s)

Sebastien Artigaud <sebastien.artigaud@gmx.com>

References

See Also

normalizeVSN, RIplot, ES.prot

Examples

data(pecten)

pecten.norm <- Norm.vsn(pecten, n1=6, n2=6, plot=TRUE)

Description

Raw Volume data from a 2-DE experiment performed on proteins from *Pecten maximus* gills subjected to a temperature challenge (15C = control vs 25C). 766 spots were identified with 6 replicates per condition, therefore the dataset is a dataframe of 12 columns and 766 rows (for details, see Artigaud et al, 2013).

Author(s)

Sebastien Artigaud <sebastien.artigaud@gmx.com>

References


Description

Dataframe with 1 column describing factors for *pecten* dataset. It gives the names of the gels (as rownames) and the condition for the temperature challenge (15C = control vs 25C) in the "Condition" column (for details, see Artigaud et al, 2013).

Author(s)

Sebastien Artigaud <sebastien.artigaud@gmx.com>

References

Description

R-I plots consist in plotting the intensity log2-ratio (R) against mean log10 intensity (I) of spot’s volume data from 2D Gel experiments.

Usage

RIplot(data, n1, n2, ...)

Arguments

data: either a dataframe or a matrix of 2D Gel Volume data. data should be spots intensities displayed with gel as columns with the name of columns corresponding to the names of the gels and spots as rows with the names of the rows corresponding to the name of the spots. The replicates for each condition should be ordered in following columns.
n1: an integer. Number of replicates in condition 1.
n2: an integer. Number of replicates in condition 2.
...: Further arguments to be passed to methods, such as graphical parameters (see par).

Details

Dudoit et al (2002) proposed a method for visualization of artifacts in microarray datasets, called the MA-plot, which was transposed for proteomics data as the Ratio-Intensity plot by Meunier et al (2005). R-I plots allow to directly visualize artifacts in the original data set as well as the effects of normalization. The log2-ratio (R) is the log2 of the mean of volume data in condition 2 upon the mean of volume data in condition 1:

$$ R = \log_2 \frac{\text{mean}(V_{\text{Cond}2})}{\text{mean}(V_{\text{Cond}1})} $$

The intensity (I) is the log10 of the mean of volume data in condition 2 by the mean of volume data in condition 1

$$ I = \log_{10}(\text{mean}(V_{\text{Cond}2}) \times \text{mean}(V_{\text{Cond}1})) $$

Value

Display the RI-plot of data, the values on the plot are the number of spots with a Ratio greater then 1 (upper values) or lesser then -1 (lower value). A list is invisibly returns with components:

RI: a dataframe with ratio and intensity values calculated.
diff: a vector with number of spots with a Ratio greater then 1 or lesser then -1 (in that order).

Author(s)

Sebastien Artigaud <sebastien.artigaud@gmx.com>
robust.fdr

References


See Also

Norm.qt, Norm.vsn

Examples

data(pecten)

RIplot(pecten, 6, 6, main="Without Normalization")

robust.fdr "robust FDR" estimation

Description

Implements robust method of FDR estimation (Pounds and Cheng 2006, Bioinformatics)

Usage

robust.fdr(p, sides = 1, p2 = 1 - p, discrete = F, use8 = T)

Arguments

p vector of p-values from the analysis.
sides indicate whether p-values are 1-sided (set sides=1) or 2-sided (set sides=2), default=1.
p2 for one-sided testing, p-values from testing the "other alternative", default=1-p.
discrete logical. Indicates whether p-values are discrete
use8 indicates whether the constant 8 should be used if p-values are discrete, see Pounds and Cheng (2006) for more details.

Details

This function uses the code from Stan Pounds available at http://www.stjuderesearch.org/depts/biostats/documents/robust-fdr.R and is included in prot2D package for convenience and comparison purpose.
Sim.Prot.2D

Value
A list with components:

- \( p \) the vector of p-values provided by the user.
- \( fdr \) the vector of smoothed FDR estimates.
- \( q \) the vector of q-values based on the smoothed FDR estimates.
- \( cdf \) the vector with p-value empirical distribution function at corresponding entry of \( p \).
- \( loc.fdr \) the local (unsmoothed) FDR estimates.
- \( fp \) the estimated number of false positives at p-value cutoff in \( p \).
- \( fn \) the estimated number of false negatives at p-value cutoff in \( p \).
- \( te \) the total of \( fp \) and \( fn \).
- \( pi \) the null proportion estimate.
- \( ord \) a vector of indices to order the vectors above by ascending p-value.

Author(s)
Stan Pounds. Edited by Sebastien Artigaud for prot2D package.

References

Sim.Prot.2D Simulate 2D Gel volume data

Description
This function simulates realistic 2D Gel volume data, based on parameters estimate from real dataset.

Usage
Sim.Prot.2D(data, nsp=nrow(data), nr=10, p0=0.1, s2_0=0.2, d0=3)

Arguments

data 
either a dataframe or a matrix of 2D Gel Volume data. Usually the output of Norm.qt.

nsp 
an integer. Number of spots (i.e. rows) to generate (should be equal or less than the number of rows of data).
nr 
an integer. Number of replicates for each conditions.
p0 
proportion of spots to be differentially expressed (defaults to 0.1).
s2_0 
estimator of the standard deviation of the spots distribution (defaults to 0.2).
d0 
degree of freedom of the Chi-squared distribution used in the calculation of the distribution (defaults to 3).
Details

Volume data are computed following these steps (see Smyth, 2004 and Artigaud et al, 2013 for details):

1. Log2 mean volumes from data are computed for each spot.
2. Means are used as input parameters in order to simulate a normal distribution (with no differential expression between conditions) for each spots with standard deviations, computed as described by Smyth (2004).
3. p0 of the spots are randomly picked for introducing differential expression in both conditions (p0/2 in each condition).

If nsp is less than the number of rows in data, the nsp first columns of data are used as input parameters.

Value

returns an ExpressionSet of simulated volume data (log2 transformed) with 2 conditions ("Cond1" and "Cond2" in phenoData) slot of the ExpressionSet. The spots differentially generated can be retrieve with notes.

Author(s)

Sebastien Artigaud <sebastien.artigaud@gmx.com>

References


See Also

variousT.Prot,

Examples

#Simulate data based on "pecten"
data(pecten)
data(pecten.fac)
pecten.norm <- Norm.qt(pecten, n1=6, n2=6, plot=FALSE)
Sim.data <- Sim.Prot.2D(data=pecten.norm, nsp=700, nr=10, p0=0.1, s2_0=0.2, d0=3)

#Compare different methods for finding differentially expressed proteins
res.stud <- ttest.Prot(Sim.data, fdr.thr=0.1, plot=FALSE)
res.mo <- modT.Prot(Sim.data, fdr.thr=0.1, plot=FALSE)
a <- featureNames(res.stud)
a # Names of the spots selected for a student's t-test with an FDR of 0.1
b <- featureNames(res.mo)
b # Names of the spots selected for a modT-test with an FDR of 0.1
c <- notes(Sim.data)$SpotSig
c # Names of the really differentially generated spots

# Compare between t-test and moderate t-test (requires made4 package)
# Just for illustration purpose (only 1 simulation !)
## Not run:
c.l.t <- comparelists(c,a)
c.l.m <- comparelists(c,b)

fp <- matrix(c(c(length(a)- (c.l.t$Length.Y-length(c.l.t$intersect))),
c(cl.t$Length.Y-length(cl.t$intersect))),
c(length(b)- (c.l.m$Length.Y-length(cl.m$intersect))),
c(cl.m$Length.Y-length(cl.m$intersect))),
nrow=2,ncol=2,byrow=F)

barplot(fp, main="spots declared significant",names.arg=c("t-test","modT"))
legend("topleft",c("False Positives","True Positives"), fill=c("grey","black"), bty="n")
## End(Not run)

### variousT.Prot

Various Tests for finding differentially expressed proteins in 2-DE experiments

#### Description

These functions provides simple methods for finding differentially expressed proteins in 2-DE experiments.

#### Usage

- `ttest.Prot(data, plot = T, fdr.thr = 0.1, Fold2 = F, method.fdr = "BH", var.equal = F)`
- `modT.Prot(data, plot = T, fdr.thr = 0.1, Fold2 = F, method.fdr = "BH", col=1)`
- `samT.Prot(data, plot = T, fdr.thr = 0.1, Fold2 = F, method.fdr = "BH")`
- `efronT.Prot(data, plot = T, fdr.thr = 0.1, Fold2 = F, method.fdr = "BH")`
- `shrinkT.Prot(data, plot = T, fdr.thr = 0.1, Fold2 = F, method.fdr = "BH", var.equal = F)`

#### Arguments

- **data**: an `ExpressionSet` of volume data. Usually as returned by `ES.prot`.
- **fdr.thr**: threshold value for the FDR (defaults to 0.1).
- **method.fdr**: method for FDR estimator. 4 methods are implemented: "BH" for the Classical FDR from Benjamini & Hochberg, "Strimmer", "Storey" and "Pounds" (see references for details).
- **Fold2**: logical. If true only spots with an absolute ratio of 2 are selected.
- **plot**: logical. If true (default) draws a plot fdr values depending on the p-values with a line indicating the FDR threshold.
var.equal logical. If false (default) assume unequal variances in each condition and uses a proper correction. Only for ttest.Prot and shrinkT.Prot.
col An integer (default to 1). Only for modT.Prot. If there is more than one factor in pData, indicates the column to use for the analysis.

Details

As described in Artigaud et al (2013) these are functions adapted from microarray analysis. 2-DE experiments analysis requires a variant of the t-statistic that is suitable for high-dimensional data and large-scale multiple testing. For this purpose, in the last few years, various test procedures have been suggested.

These functions provides:
- the classical Student’s t-test (adapted from studentt.stat).
- two tests especially modified for micro-array analysis : Efron’s t-test (adapted from efront.stat, Efron et al, 2001) and the modified t-test used in Significance Analysis for Microarray (adapted from samr, Tusher et al, 2001)
- two methods that take advantage of hierarchical Bayes methods for estimation of the variance across genes: the moderate t-test from Smyth (using limma; see Smyth, 2004) and the "Shrinkage t" statistic test from Opge-Rhein & Strimmer (adapted from shrinkcat.stat; see Opge-Rhein & Strimmer, 2007).

As statistical tests allowing the identification of differentially expressed proteins must take into account a correction for multiple tests in order to avoid false conclusions. These functions also provides different methods to estimate the False Discovery Rate :
- the classical FDR estimator of Benjamini & Hochberg (using p.adjust; see Benjamini & Hochberg, 1995)
- the Fdr estimator of Strimmer (based on local fdr calculation) (using fdrtool; see Strimmer 2008)
- the "robust FDR" estimator of Pounds & Cheng (implemented in robust.fdr for the prot2D package; see Pounds & Cheng, 2006)
- Fdr method of Storey and Tibshirani (2003), also known as "q-values" (using qvalue).

Value

returns an ExpressionSet containing only the significant spots (see Examples).

Author(s)

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References


See Also

Norm.qt, ES.prot, fdrtool, limma, samr, student.t.stat, shrink.t.stat, efront.stat, qvalue

Examples

data(pecten)
data(pecten.fac)

pecten.norm <- Norm.qt(pecten, n1=6, n2=6, plot=TRUE) #Quantiles normalization of the data
ES.p <- ES.prot(pecten.norm, n1=6, n2=6, f=pecten.fac)
ES.diff <- modT.Prot(ES.p, fdr.thr=0.1, plot=TRUE)
featureNames(ES.diff) # Names of the spots selected for a moderated t-test with a fdr of 0.1
fData(ES.diff) # Displaying fold change (as log2(ratio)) for selected spots
exprs(ES.diff) # Volume normalized data for all the selected spots
## Not run: heatplot(ES.diff) #Great heatmap of the selected spots (require made4 Bioconductor package)
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