

# Package ‘prot2D’

April 5, 2014

**Type** Package

**Title** Statistical Tools for volume data from 2D Gel Electrophoresis

**Version** 1.0.0

**Date** 2013-04-10

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

**Depends** R (>= 2.15),fdrtool,st,samr,Biobase,limma,Mulcom,impute,MASS,qvalue

**Suggests** made4,affy

**License** GPL (>= 2)

**biocViews** Bioinformatics, DifferentialExpression, MultipleComparisons,Proteomics

## R topics documented:

prot2D-package . . . . .	2
ES.prot . . . . .	3
Norm.qt . . . . .	4
Norm.vsn . . . . .	5
pecten . . . . .	6
pecten.fac . . . . .	7
RIplot . . . . .	7
robust.fdr . . . . .	9
Sim.Prot.2D . . . . .	10
variousT.Prot . . . . .	12

<b>Index</b>	<b>15</b>
--------------	-----------

## Description

The purpose of this package is to analyze (i.e. Normalize and select significant spots) data issued from 2D GEL 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](mailto:sebastien.artigaud@gmx.com)>

## References

- Dudoit, S., Yang, Y.H., Callow, M.J., & Speed, T.P. (2002) "Statistical methods for identifying differentially expressed genes in replicated cDNA microarray experiments" *Statistica Sinica*, vol. 12: 111-139.
- Strimmer, K. (2008) "A unified approach to false discovery rate estimation." *BMC Bioinformatics*, vol. 9: 303.
- Artigaud, S., Gauthier, O. & Pichereau, V. (2013) "Identifying differentially expressed proteins in 2-DE experiments: Inputs from transcriptomics statistical tools." Submitted

## 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: heatmap(x) #Great heatmap of the selected spots (requires made4 Bioconductor package )
```

---

ES.prot

*Create an ExpressionSet containing 2D Gel Volume Data*

---

## Description

This function create an [ExpressionSet](#) containing 2D Gel Volume Data and associated meta-data. 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))
```

---

Norm.qt

*Normalize 2D Gel Volume data using Quantiles Normalization*

---

**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 <a href="#">Riplot</a> , 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 log<sub>2</sub> transformed quantiles normalized data

**Author(s)**

Sebastien Artigaud <sebastien.artigaud@gmx.com>

**References**

Artigaud, S., Gauthier, O. & Pichereau, V. (2013) "Identifying differentially expressed proteins in 2-DE experiments: Inputs from transcriptomics statistical tools." Submitted

**See Also**

[normalizeQuantiles,RIplot,ES.prot](#)

**Examples**

```
data(pecten)

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

---

Norm.vsn

*Normalize 2D Gel Volume data using VSN*

---

**Description**

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

**Usage**

```
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 <a href="#">RIplot</a> , 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**

- Artigaud, S., Gauthier, O. & Pichereau, V. (2013) "Identifying differentially expressed proteins in 2-DE experiments: Inputs from transcriptomics statistical tools." Submitted
- Huber, W., Heydebreck, von, A., Sultmann, H., Poustka, A., & Vingron, M. (2002) "Variance stabilization applied to microarray data calibration and to the quantification of differential expression" *Bioinformatics*, vol. 18 (Suppl 1): S96-S104.

**See Also**

[normalizeVSN,RIplot,ES.prot](#)

**Examples**

```
data(pecten)
```

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

---

pecten

*Pecten Dataset*

---

**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**

Artigaud, S., Gauthier, O. & Pichereau, V. (2013) "Identifying differentially expressed proteins in 2-DE experiments: Inputs from transcriptomics statistical tools." Submitted

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pecten.fac

*pecten.fac Dataset*

---

### 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

Artigaud, S., Gauthier, O. & Pichereau, V. (2013) "Identifying differentially expressed proteins in 2-DE experiments: Inputs from transcriptomics statistical tools." Submitted

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RIplot

*Ratio-Intensity Plot for 2D Gel Volume data*

---

### Description

R-I plots consist in plotting the intensity log<sub>2</sub>-ratio (R) against mean log<sub>10</sub> 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 <a href="#">par</a> ).

## 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:

$$R = \log_2 \frac{\text{mean}(V_{\text{Cond2}})}{\text{mean}(V_{\text{Cond1}})}$$

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

$$I = \log_{10}(\text{mean}(V_{\text{Cond2}}) \times \text{mean}(V_{\text{Cond1}}))$$

## 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>

## References

- Dudoit, S., Yang, Y.H., Callow, M.J., & Speed, T.P. (2002) "Statistical methods for identifying differentially expressed genes in replicated cDNA microarray experiments" *Statistica Sinica*, vol. 12: 111-139.
- Meunier, B., Bouley, J., Piec, I., Bernard, C., Picard, B., & Hocquette, J.-F. (2005) "Data analysis methods for detection of differential protein expression in two-dimensional gel electrophoresis" *Analytical Biochemistry*, vol. 340 (2): 226-230.

## See Also

[Norm.qt](#), [Norm.vsn](#)

## Examples

```
data(pecten)

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



---

robust.fdr	<i>"robust FDR" estimation</i>
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---

### 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.stjude.com/depts/biostats/documents/robust-fdr.R> and is included in `prot2D` package for convenience and comparison purpose.

### 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**

Pounds, S. & Cheng, C. (2006) "Robust estimation of the false discovery rate" *Bioinformatics*, vol. 22 (16): 1979-1987.

---

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 <a href="#">Norm.qt</a> .
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**

- Smyth, G.K. (2004) "Linear models and empirical bayes methods for assessing differential expression in microarray experiments." *Statistical Applications in Genetics and Molecular Biology*, vol. 3: Article 3.
- Artigaud, S., Gauthier, O. & Pichereau, V. (2013) "Identifying differentially expressed proteins in 2-DE experiments: Inputs from transcriptomics statistical tools." Submitted

**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 diffenrentially 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 students 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:
cl.t <- comparelists(c,a)
cl.m <- comparelists(c,b)

fp <- matrix(c(c(length(a)- (cl.t$Length.Y-length(cl.t$intersect))),
c(cl.t$Length.Y-length(cl.t$intersect)),
c(length(b)- (cl.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	<i>Various Tests for finding differentially expressed proteins in 2-DE experiments</i>
---------------	--

---

### 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 <a href="#">ExpressionSet</a> of volume data. Usually as returned by <a href="#">ES.prot</a> .
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 <code>ttest.Prot</code> and <code>shrinkT.Prot</code> .
col	An integer (default to 1). Only for <code>modT.Prot</code> . If there is more than one factor in <a href="#">pData</a> , 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 Opgen-Rhein & Strimmer (adapted from [shrinkcat.stat](#); see Opgen-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)

Sebastien Artigaud <[sebastien.artigaud@gmx.com](mailto:sebastien.artigaud@gmx.com)>

## References

- Benjamini, Y. & Hochberg, Y. (1995) "Controlling the false discovery rate: a practical and powerful approach to multiple testing" *Journal of the Royal Statistical Society. Series B. Methodological.*: 289-300.
- Efron, B., Tibshirani, R., Storey, J.D., & Tusher, V. (2001) "Empirical Bayes Analysis of a Microarray Experiment" *Journal of the American Statistical Association*, vol. 96 (456): 1151-1160.
- Tusher, V.G., Tibshirani, R., & Chu, G. (2001) "Significance analysis of microarrays applied to the ionizing radiation response" *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98 (9): 5116-5121.
- Smyth, G.K. (2004) "Linear models and empirical bayes methods for assessing differential expression in microarray experiments." *Statistical Applications in Genetics and Molecular Biology*, vol. 3: Article 3.

- Pounds, S. & Cheng, C. (2006) "Robust estimation of the false discovery rate" *Bioinformatics*, vol. 22 (16): 1979-1987.
- Strimmer, K. (2008) "A unified approach to false discovery rate estimation." *BMC Bioinformatics*, vol. 9: 303.
- Opgen-Rhein, R. & Strimmer, K. (2007) "Accurate Ranking of Differentially Expressed Genes by a Distribution-Free Shrinkage Approach" *Statistical Applications in Genetics and Molecular Biology*, vol. 6 (1).
- Artigaud, S., Gauthier, O. & Pichereau, V. (2013) "Identifying differentially expressed proteins in 2-DE experiments: Inputs from transcriptomics statistical tools." Submitted

### See Also

[Norm.qt](#), [ES.prot](#), [fdrtool](#), [limma](#), [samr](#), [studentt.stat](#), [shrinkt.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: heatmap(ES.diff) #Great heatmap of the selected spots (require made4 Bioconductor package )
```

# Index

- \*Topic **2DGel**
  - prot2D-package, 2
- \*Topic **biology**
  - prot2D-package, 2
- \*Topic **package**
  - prot2D-package, 2
- \*Topic **prot2D**
  - ES.prot, 3
  - Norm.qt, 4
  - Norm.vsn, 5
  - pecten, 6
  - pecten.fac, 7
  - prot2D-package, 2
  - RIplot, 7
  - robust.fdr, 9
  - Sim.Prot.2D, 10
  - variousT.Prot, 12
- \*Topic **proteomic**
  - prot2D-package, 2
- assayData, 3
- efrontT.Prot, 2
- efrontT.Prot (variousT.Prot), 12
- efront.stat, 13, 14
- ES.prot, 2, 3, 5, 6, 12, 14
- ExpressionSet, 2–4, 11–13
- exprs, 3
- fData, 3
- fdrtool, 2, 13, 14
- featureData, 3
- limma, 13, 14
- modT.Prot, 2
- modT.Prot (variousT.Prot), 12
- Norm.qt, 2, 3, 4, 4, 8, 10, 14
- Norm.vsn, 4, 5, 8
- normalizeQuantiles, 4, 5
- normalizeVSN, 5, 6
- notes, 11
- p.adjust, 13
- par, 7
- pData, 12
- pecten, 6, 7
- pecten.fac, 7
- phenoData, 11
- prot2D, 9, 10, 13
- prot2D (prot2D-package), 2
- prot2D-package, 2
- qvalue, 13, 14
- RIplot, 4–6, 7
- robust.fdr, 9, 13
- samr, 13, 14
- samT.Prot, 2
- samT.Prot (variousT.Prot), 12
- shrinkcat.stat, 13
- shrinkT.Prot, 2
- shrinkT.Prot (variousT.Prot), 12
- shrinkt.stat, 14
- Sim.Prot.2D, 10
- studentt.stat, 13, 14
- ttest.Prot, 2
- ttest.Prot (variousT.Prot), 12
- variousT.Prot, 11, 12