Package ‘affyPLM’

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Title Methods for fitting probe-level models
Author Ben Bolstad <bmb@bmbolstad.com>
Maintainer Ben Bolstad <bmb@bmbolstad.com>
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Description A package that extends and improves the functionality of
         the base affy package. Routines that make heavy use of
         compiled code for speed. Central focus is on implementation of
         methods for fitting probe-level models and tools using these
         models. PLM based quality assessment tools.
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Contents

bg.correct.LESN .............................................................. 2
fitPLM ................................................................. 3
bg.correct.LESN

LESN - Low End Signal is Noise Background corrections

Description

This function background corrects PM probe data using LESN - Low End Signal is Noise concepts.

Usage

bg.correct.LESN(object, method=2, baseline=0.25, theta=4)

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>object</td>
<td>an AffyBatch</td>
</tr>
<tr>
<td>method</td>
<td>an integer code specifying which method to use</td>
</tr>
<tr>
<td>baseline</td>
<td>A baseline value to use</td>
</tr>
<tr>
<td>theta</td>
<td>A parameter used in the background correction process</td>
</tr>
</tbody>
</table>

Details

This method will be more formally documented at a later date.

The basic concept is to consider that the lowest end of intensites is most likely just noise (and should be heavily corrected) and the highest end signals are most likely signal and should have little adjustment. Low end signals are made much smaller while high end signals get less adjustment relative adjustment.

Value

An AffyBatch
**fitPLM**

**Author(s)**

Ben Bolstad <bmb@bmbolstad.com>

**References**


**Examples**

```r
if (require(affydata)) {
  data(Dilution)
  Dilution.example.bgcorrect <- bg.correct.LESN(Dilution)
}
```

---

**Description**

This function converts an *AffyBatch* into an *PLMset* by fitting a specified robust linear model to the probe level data.

**Usage**

```r
fitPLM(object, model=PM ~ -1 + probes + samples,
       variable.type=c(default="factor"),
       constraint.type=c(default="contr.treatment"),
       subset=NULL,
       background=TRUE, normalize=TRUE, background.method="RMA.2",
       normalize.method="quantile", background.param=list(),
       normalize.param=list(), output.param=verify.output.param(),
       model.param=verify.model.param(object, model),
       verbosity.level=0)
```

**Arguments**

- **object**: an *AffyBatch*
- **model**: A formula describing the model to fit. This is slightly different from the standard method of specifying formulae in R. Read the description below
- **variable.type**
- **constraint.type**: a way to specify whether variables in the model are factors or standard variables should factor variables sum to zero or have first variable set to zero (endpoint constraint)
- **subset**: a vector with the names of probesets to be used. If NULL then all probesets are used.
normalize logical value. If TRUE normalize data using quantile normalization
background logical value. If TRUE background correct using RMA background correction
background.method name of background method to use.
normalize.method name of normalization method to use.
background.param A list of parameters for background routines
normalize.param A list of parameters for normalization routines
output.param A list of parameters controlling optional output from the routine.
model.param A list of parameters controlling model procedure
verbosity.level An integer specifying how much to print out. Higher values indicate more verbose. A value of 0 will print nothing

Details
This function fits robust Probe Level linear Models to all the probesets in an AffyBatch. This is carried out on a probeset by probeset basis. The user has quite a lot of control over which model is used and what outputs are stored. For more details please read the vignette.

Value
An PLMset

Author(s)
Ben Bolstad <bmb@bmbolstad.com>

References

See Also
expresso, rma.threestep

Examples
if (require(affydata)) {
data(Dilution)
Pset <- fitPLM(Dilution, model=PM ~ -1 + probes + samples)
se(Pset)[1:5,]

image(Pset)
NUSE(Pset)
#now lets try a wider class of models
## Not run: Pset <- fitPLM(Dilution,model=PM ~ -1 + probes + liver, normalize=FALSE,background=FALSE)
## End(Not run)
## Not run: coefs(Pset)[1:10,]

## Not run: Pset <- fitPLM(Dilution,model=PM ~ -1 + probes + liver + scanner, normalize=FALSE,background=FALSE)
## End(Not run)
coefs(Pset)[1:10,]

#try liver as a covariate
logliver <- log2(c(20,20,10,10))
## Not run: Pset <- fitPLM(Dilution, model=PM~-1+probes+logliver+scanner, normalize=FALSE, background=FALSE, variable.type=c(logliver="covariate"))
## End(Not run)
coefs(Pset)[1:10,]

#try a different se.type
## Not run: Pset <- fitPLM(Dilution, model=PM~-1+probes+scanner, normalize=FALSE,background=FALSE, model.param=list(se.type=2))
## End(Not run)
se(Pset)[1:10,]

---

### internal functions

**Internal affyPLM functions**

**Description**

Internal affyPLM functions

**Details**

These are not to be called by the user and/or are undergoing testing

---

**MAplot**

**Relative M vs. A plots**

**Description**

Create boxplots of M or M vs A plots. Where M is determined relative to a specified chip or to a pseudo-median reference chip.
Arguments

... Additional parameters for the routine
A A vector to plot along the horizontal axis
M A vector to plot along vertical axis
subset A set of indices to use when drawing the loess curve
show.statistics If true some summary statistics of the M values are drawn
span span to be used for loess fit.
family.loess "guassian" or "symmetric" as in loess.
cex Size of text when writing summary statistics on plot

See Also
mva.pairs

normalize.ExpressionSet

Normalization applied to ExpressionSets

Description

Allows the user to apply normalization routines to ExpressionSets.

Usage

normalize.ExpressionSet.quantiles(eset, transfn=c("none","log","antilog"))
normalize.ExpressionSet.loess(eset, transfn=c("none","log","antilog"),...)
normalize.ExpressionSet.contrasts(eset, span = 2/3,
choose.subset=TRUE, subset.size=5000, verbose=TRUE, family="symmetric",
transfn=c("none","log","antilog"))
normalize.ExpressionSet.qspline(eset, transfn=c("none","log","antilog"),...)
normalize.ExpressionSet.invariantset(eset,prd.td=c(0.003, 0.007),
verbose=FALSE, transfn=c("none","log","antilog"),
baseline.type=c("mean","median","pseudo-mean","pseudo-median"))
normalize.ExpressionSet.scaling(eset, trim=0.02, baseline=-1,
transfn=c("none","log","antilog"))

Arguments

eset An ExpressionSet
span parameter to be passed to the function loess.
choose.subset use a subset of values to establish the normalization relationship
subset.size number to use for subset
### Details

This function carries out normalization of expression values. In general you should either normalize at the probe level or at the expression value level, not both.

Typing `normalize.ExpressionSet.methods` should give you a list of methods that you may use. Note that you can also use the `normalize` function on ExpressionSets. Use `method` to select the normalization method.

### Value

A normalized `ExpressionSet`.

### Author(s)

Ben Bolstad, <bmb@bmbolstad.com>

### References


### Examples

```r
if (require(affydata)) {
  data(Dilution)
  eset <- rma(Dilution, normalize=FALSE, background=FALSE)
  normalize(eset)
}
```
normalize.quantiles.probeset

Quantile Normalization applied to probesets

Description
Using a normalization based upon quantiles, this function normalizes a matrix of probe level intensities.

Usage
normalize.AffyBatch.quantiles.probeset(abatch, type=c("separate","pmonly","mmonly","together"), use.median=FALSE, use.log=TRUE)

Arguments
- abatch: An AffyBatch
- type: how should MM and PM values be handled
- use.median: use median rather than mean
- use.log: take logarithms, then normalize

Details
This function applies the quantile method in a probeset specific manner.
In particular a probeset summary is normalized using the quantile method and then the probes adjusted accordingly.

Value
A normalized AffyBatch.

Author(s)
Ben Bolstad, <bmb@bmbolstad.com>

References

See Also
normalize.quantiles
**normalize.scaling**

*Scaling normalization*

**Description**

Allows the user to apply scaling normalization.

**Usage**

```r
normalize.scaling(X, trim=0.02, baseline=-1, log.scalefactors=FALSE)
normalize.AffyBatch.scaling(abatch, 
    type=c("together","pmonly","mmonly","separate"), 
    trim=0.02, baseline=-1, log.scalefactors=FALSE)
```

**Arguments**

- `X`: A matrix. The columns of which are to be normalized.
- `abatch`: An `AffyBatch`
- `type`: A parameter controlling how normalization is applied to the Affybatch.
- `trim`: How much to trim from the top and bottom before computing the mean when using the scaling normalization.
- `baseline`: Index of array to use as baseline, negative values (-1,-2,-3,-4) control different baseline selection methods.
- `log.scalefactors`: Compute the scale factors based on log2 transformed data.

**Details**

These function carries out scaling normalization of expression values.

**Value**

A normalized `ExpressionSet`.

**Author(s)**

Ben Bolstad, <bmb@bmbolstad.com>

**Examples**

```r
if (require(affydata)) {
    data(Dilution)
    normalize.AffyBatch.scaling(Dilution)
}
```
**PLMset-class**

**Description**

This is a class representation for Probe level Linear Models fitted to Affymetrix GeneChip probe level data.

**Objects from the Class**

Objects can be created using the function `fitPLM`.

**Slots**

- `probe.coefs`: Object of class "matrix". Contains model coefficients related to probe effects.
- `se.probe.coefs`: Object of class "matrix". Contains standard error estimates for the probe coefficients.
- `chip.coefs`: Object of class "matrix". Contains model coefficients related to chip (or chip level) effects for each fit.
- `se.chip.coefs`: Object of class "matrix". Contains standard error estimates for the chip coefficients.
- `const.coefs`: Object of class "matrix". Contains model coefficients related to intercept effects for each fit.
- `se.const.coefs`: Object of class "matrix". Contains standard error estimates for the intercept estimates.
- `model.description`: Object of class "character". This string describes the probe level model fitted.
- `weights`: List of objects of class "matrix". Contains probe weights for each fit. The matrix has columns for chips and rows are probes.
- `phenoData`: Object of class "phenoData". This is an instance of class phenoData containing the patient (or case) level data. The columns of the pData slot of this entity represent variables and the rows represent patients or cases.
- `annotation`: A character string identifying the annotation that may be used for the ExpressionSet instance.
- `experimentData`: Object of class "MIAME". For compatibility with previous version of this class description can also be a "character". The class characterOrMIAME has been defined just for this.
- `cdfName`: A character string giving the name of the cdfFile.
- `nrow`: Object of class "numeric". Number of rows in chip.
- `ncol`: Object of class "numeric". Number of cols in chip.
- `narrays`: Object of class "numeric". Number of arrays used in model fit.
- `normVec`: Object of class "matrix". For storing normalization vector(s). Not currently used.
PLMset-class

varcov: Object of class "list". A list of variance/covariance matrices.
residualSE: Object of class "matrix". Contains residual standard error and df.
residuals: List of objects of class "matrix". Contains residuals from model fit (if stored).
model.call: Object of class "call"

Methods

weights <- signature(object = "PLMset"): replaces the weights.
weights signature(object = "PLMset"): extracts the model fit weights.
coefs <- signature(object = "PLMset"): replaces the chip coefs.
coefs signature(object = "PLMset"): extracts the chip coefs.
se <- signature(object = "PLMset"): extracts the standard error estimates of the chip coefs.
se signature(object = "PLMset"): replaces the standard error estimates of the chip coefs.
coefs.probe signature(object = "PLMset"): extracts the probe coefs.
se.probe signature(object = "PLMset"): extracts the standard error estimates of the probe coefs.
coefs.const signature(object = "PLMset"): extracts the intercept coefs.
se.const signature(object = "PLMset"): extracts the standard error estimates of the intercept coefs.
getCdfInfo signature(object = "PLMset"): retrieve the environment that defines the location of probes by probe set.
image signature(x = "PLMset"): creates an image of the robust linear model fit weights for each sample.
indexProbes signature(object = "PLMset", which = "character"): returns a list with locations of the probes in each probe set. The list names defines the probe set names. which can be "pm", "mm", or "both". If "both" then perfect match locations are given followed by mismatch locations.
Mbox signature(object = "PLMset"): gives a boxplot of M's for each chip. The M's are computed relative to a "median" chip.
normvec signature(x = "PLMset"): will return the normalization vector (if it has been stored).
residSE signature(x = "PLMset"): will return the residual SE (if it has been stored).
boxplot signature(x = "PLMset"): Boxplot of Normalized Unscaled Standard Errors (NUSE).
NUSE signature(x = "PLMset"): Boxplot of Normalized Unscaled Standard Errors (NUSE) or NUSE values.
RLE signature(x = "PLMset"): Relative Log Expression boxplot or values.

Note
This class is better described in the vignette.

Author(s)

B. M. Bolstad <bmb@bmbolstad.com>
References


---

**PLMset2exprSet**

*Convert a PLMset to an ExpressionSet*

**Description**

This function converts a PLMset to an ExpressionSet. This is often useful since many Bioconductor functions operate on ExpressionSet objects.

**Usage**

```r
PLMset2exprSet(pset)
pset2eset(pset)
```

**Arguments**

- `pset` The PLMset to convert to ExpressionSet.

**Details**

These functions convert PLMset objects to ExpressionSet objects. This is often useful since many Bioconductor functions operate on ExpressionSet objects. Note that the function `pset2eset` is a wrapper for `PLMset2exprSet`.

**Value**

returns a ExpressionSet

**Author(s)**

Ben Bolstad <bmb@bmbolstad.com>

**See Also**

ExpressionSet

**Examples**

```r
if (require(affydata)) {
  data(Dilution)
  Pset <- fitPLM(Dilution)
  eset <- pset2eset(Pset)
}
```
preprocess  

**Description**

This function pre-processes an *AffyBatch*.

**Usage**

```r
preprocess(object, subset=NULL, normalize=TRUE, background=TRUE,
           background.method="RMA.2", normalize.method="quantile",
           background.param=list(), normalize.param=list(),
           verbosity.level=0)
```

**Arguments**

- `object`: an *AffyBatch*
- `subset`: a vector with the names of probesets to be used. If NULL then all probesets are used.
- `normalize`: logical value. If TRUE normalize data using quantile normalization
- `background`: logical value. If TRUE background correct using RMA background correction
- `background.method`: name of background method to use.
- `normalize.method`: name of normalization method to use.
- `background.param`: list of parameters for background correction methods
- `normalize.param`: list of parameters for normalization methods
- `verbosity.level`: An integer specifying how much to print out. Higher values indicate more verbose. A value of 0 will print nothing

**Details**

This function carries out background correction and normalization pre-processing steps. It does not summarize to produce gene expression measures. All the same pre-processing methods supplied by *threeestep* are supported by this function.

**Value**

An *AffyBatch*

**Author(s)**

Ben Bolstad <bmb@bmbolstad.com>
pseudo.coloring

References


See Also

expresso, rma

Examples

```r
if (require(affydata)) {
  data(Dilution)

  # should be equivalent to the bg and norm of rma()
  abatch.preprocessed <- preprocess(Dilution)
}
```

---

pseudo.coloring  Coloring pseudo chip images

Description

These are routines used for coloring pseudo chip images.

Usage

```r
pseudoPalette(low = "white", high = c("green", "red"), mid = NULL,k =50)
pseudoColorBar(x, horizontal = TRUE, col = heat.colors(50), scale = 1:length(x),k = 11, log.ticks=FALSE)
```

Arguments

- `low`  color at low end of scale
- `high`  color at high end of scale
- `mid`  color at exact middle of scale
- `k`  number of colors to have
- `x`  A data series
- `horizontal`  If TRUE then color bar is to be draw horizontally
- `col`  colors for color bar
- `scale`  tickmarks for `x` if `x` is not numeric
- `log.ticks`  use a log type transformation to assign the colors
- `...`  additional parameters to plotting routine

Details

Adapted from similar tools in maPlots package.
ReadRMAExpress

Author(s)

Ben Bolstad <bmb@bmbolstad.com>

See Also

AffyBatch, read.affybatch

ReadRMAExpress

Read RMAExpress computed expression values

Description

Read RMAExpress computed binary output files into a matrix or ExpressionSet

Usage

ReadRMAExpress(filename, return.value=c("ExpressionSet","matrix"))

Arguments

filename The name of the file containing RMAExpress output to be read in
return.value should a matrix or an ExpressionSet be returned

Value

returns an ExpressionSet

Author(s)

Ben Bolstad <bmb@bmbolstad.com>

References

http://rmaexpress.bmbolstad.com
Fit a RMA to Affymetrix Genechip Data as a PLMset

Description

This function converts an AffyBatch into an PLMset by fitting a multichip model. In particular we concentrate on the RMA model.

Usage

rmaPLM(object, subset=NULL, normalize=TRUE, background=TRUE, background.method="RMA.2", normalize.method="quantile", background.param=list(), normalize.param=list(), output.param=list(), model.param=list(), verbosity.level=0)

Arguments

object an AffyBatch
subset a vector with the names of probesets to be used. If NULL then all probesets are used.
normalize logical value. If TRUE normalize data using quantile normalization
background logical value. If TRUE background correct using RMA background correction
background.method name of background method to use.
normalize.method name of normalization method to use.
background.param A list of parameters for background routines
normalize.param A list of parameters for normalization routines
output.param A list of parameters controlling optional output from the routine.
model.param A list of parameters controlling model procedure
verbosity.level An integer specifying how much to print out. Higher values indicate more verbose. A value of 0 will print nothing

Details

This function fits the RMA as a Probe Level Linear models to all the probesets in an AffyBatch.

Value

An PLMset
Author(s)

Ben Bolstad <bmb@bmbolstad.com>

References


See Also

`expresso`, `rma`, `threestep`, `fitPLM`, `threestepPLM`

Examples

```r
if (require(affydata)) {
  # A larger example testing weight image function
  data(Dilution)
  ## Not run: Pset <- rmaPLM(Dilution,output.param=list(weights=TRUE))
  ## Not run: image(Pset)
}
```

---

threestep

*Three Step expression measures*

Description

This function converts an `AffyBatch` into an `ExpressionSet` using a three step expression measure.

Usage

```r
threestep(object, subset=NULL, normalize=TRUE, background=TRUE,
  background.method="RMA.2", normalize.method="quantile",
  summary.method="median.polish", background.param=list(),
  normalize.param=list(), summary.param=list(), verbosity.level=0)
```

Arguments

- `object`: an `AffyBatch`
- `subset`: a vector with the names of probesets to be used. If `NULL`, then all probesets are used.
normalize    logical value. If TRUE normalize data using quantile normalization
background   logical value. If TRUE background correct using RMA background correction
background.method
             name of background method to use.
normalize.method
              name of normalization method to use.
summary.method
               name of summary method to use.
background.param
           list of parameters for background correction methods.
normalize.param
            list of parameters for normalization methods.
summary.param
             list of parameters for summary methods.
verbosity.level
              An integer specifying how much to print out. Higher values indicate more verbose. A value of 0 will print nothing.

Details

This function computes the expression measure using threestep methods. Greater details can be found in a vignette.

Value

An ExpressionSet

Author(s)

Ben Bolstad <bmb@bmbolstad.com>

References


See Also

expresso, rma

Examples

if (require(affydata)) {
  data(Dilution)

  # should be equivalent to rma()
  eset <- threestep(Dilution)

  # Using Tukey Biweight summarization
  eset <- threestep(Dilution, summary.method="tukey.biweight")
# Using Average Log2 summarization
eset <- threestep(Dilution, summary.method="average.log")

# Using IdealMismatch background and Tukey Biweight and no normalization.
eset <- threestep(Dilution, normalize=FALSE, background.method="IdealMM", summary.method="tukey.biweight")

# Using average.log summarization and no background or normalization.
eset <- threestep(Dilution, background=FALSE, normalize=FALSE, background.method="IdealMM", summary.method="tukey.biweight")

# Use threestep methodology with the rlm model fit
eset <- threestep(Dilution, summary.method="rlm")

# Use threestep methodology with the log of the average
# eset <- threestep(Dilution, summary.method="log.average")

# Use threestep methodology with log 2nd largest method
eset <- threestep(Dilution, summary.method="log.2nd.largest")

# Use threestep methodology with the rlm model fit
eset <- threestep(Dilution, background.method="LESN2")

---

threestepPLM

Three Step expression measures returned as a PLMset

Description

This function converts an AffyBatch into an PLMset using a three step expression measure.

Usage

threestepPLM(object, subset=NULL, normalize=TRUE, background=TRUE, background.method="RMA.2", normalize.method="quantile", summary.method="median.polish", background.param = list(), normalize.param=list(), output.param=list(), model.param=list(), verbosity.level=0)

Arguments

object an AffyBatch
subset a vector with the names of probesets to be used. If NULL then all probesets are used.
normalize logical value. If TRUE normalize data using quantile normalization
background logical value. If TRUE background correct using RMA background correction
background.method name of background method to use.
normalize.method
   name of normalization method to use.
summary.method
   name of summary method to use.
background.param
   list of parameters for background correction methods
normalize.param
   list of parameters for normalization methods
output.param
   list of parameters for output methods
model.param
   list of parameters for model methods
verbosity.level
   An integer specifying how much to print out. Higher values indicate more verbose. A value of 0 will print nothing

Details
   This function computes the expression measure using threestep methods. It returns a PLMset. The most important difference is that the PLMset allows you to access the residuals which the threestep function does not do.

Value
   An PLMset

Author(s)
   Ben Bolstad <bmb@bmbolstad.com>

References

See Also
   expresso, rma, threestep, rmaPLM, fitPLM

Examples
   if (require(affydata)) {
      data(Dilution)

      # should be equivalent to rma()
      ## Not run: eset <- threestepPLM(Dilution)
   }
Index

* classes
  PLMset-class, 10
* hplot
  MAplot, 5
* internal
  internal functions, 5
* manip
  bg.correct.LESN, 2
  fitPLM, 3
  normalize.ExpressionSet, 6
  normalize.quantiles.probeset, 8
  normalize.scaling, 9
  PLMset2exprSet, 12
  preprocess, 13
  pseudo.coloring, 14
  ReadRMAExpress, 15
  rmaPLM, 16
  threestep, 17
  threestepPLM, 19
  AffyBatch, 2–4, 8, 9, 13, 15–17, 19
  Annotation, PLMset-method (PLMset-class), 10
  bg.correct.gcrma (internal functions), 5
  bg.correct.LESN, 2
  bg.correct.shift (internal functions), 5
  bg.correct.stretch (internal functions), 5
  boxplot, PLMset-method (PLMset-class), 10
  cdfName, PLMset-method (PLMset-class), 10
  coefs (PLMset-class), 10
  coefs, PLMset-method (PLMset-class), 10
  coefs.const (PLMset-class), 10
  coefs.const, PLMset-method (PLMset-class), 10
  coefs.probe (PLMset-class), 10
  coefs.probe, PLMset-method (PLMset-class), 10
  coefs< (PLMset-class), 10
  coefs<, PLMset-method (PLMset-class), 10
  convert.LESN.param (internal functions), 5
  description, PLMset-method (PLMset-class), 10
  ExpressionSet, 6, 7, 9, 12, 15, 17, 18
  expresso, 4, 14, 17, 18, 20
  fitPLM, 3, 10, 17, 20
  fitPLM.old (internal functions), 5
  get.background.code (internal functions), 5
  get.default.psi.k (internal functions), 5
  get.normalization.code (internal functions), 5
  get.psi.code (internal functions), 5
  get.summary.code (internal functions), 5
 getCdfInfo, PLMset-method (PLMset-class), 10
  image, PLMset-method (PLMset-class), 10
  indexProbes, PLMset, character-method (PLMset-class), 10
  indexProbesProcessed (PLMset-class), 10
  indexProbesProcessed, PLMset-method (PLMset-class), 10
  internal functions, 5
  loess, 6, 7
  MAplot, 5
  MAplot, PLMset-method (MAplot), 5
  matrix, 15
  Mbox (PLMset-class), 10
  Mbox, PLMset-method (PLMset-class), 10
  model.description (PLMset-class), 10

21
INDEX

model.description(PLMset-method (PLMset-class), 10
mva.pairs, 6
normalize.AffyBatch.quantiles.chromosome (internal functions), 5
normalize.AffyBatch.quantiles.probeset (normalize.quantiles.probeset), 8
normalize.AffyBatch.scaling (normalize.scaling), 9
normalize.ExpressionSet, 6
normalize.quantiles, 8
normalize.quantiles.probeset, 8
normalize.scaling, 9
normvec (PLMset-class), 10
normvec,PLMset-method (PLMset-class), 10
NUSE (PLMset-class), 10
nuSE,PLMset-method (PLMset-class), 10
pData,PLMset-method (PLMset-class), 10
pData<-,PLMset,data.frame-method (PLMset-class), 10
phenoData,PLMset-method (PLMset-class), 10
phenoData<-,PLMset,AnnotatedDataFrame-method (PLMset-class), 10
PLM.designmatrix (internal functions), 5
PLM.designmatrix2 (internal functions), 5
PLM.designmatrix3 (internal functions), 5
PLMset, 3, 4, 12, 16, 19, 20
PLMset (PLMset-class), 10
PLMset-class, 10
PLMset2exprSet, 12
preprocess, 13
pset2eset (PLMset2exprSet), 12
pseudo.coloring, 14
pseudoColorBar (pseudo.coloring), 14
pseudoPalette (pseudo.coloring), 14
quantile, 8
read.affybatch, 15
readRMAExpress, 15
resid,PLMset-method (PLMset-class), 10
resid<- (PLMset-class), 10
resid<-,PLMset-method (PLMset-class), 10
residSE (PLMset-class), 10
residSE,PLMset-method (PLMset-class), 10
residuals,PLMset-method (PLMset-class), 10
residuals<-,PLMset-method (PLMset-class), 10
RLE (PLMset-class), 10
RLE,PLMset-method (PLMset-class), 10
rma, 4, 14, 17, 18, 20
rmaPLM, 16, 20
sampleNames,PLMset-method (PLMset-class), 10
sampleNames<-,PLMset,character-method (PLMset-class), 10
se (PLMset-class), 10
se,PLMset-method (PLMset-class), 10
se.const (PLMset-class), 10
se.const,PLMset-method (PLMset-class), 10
se.probe (PLMset-class), 10
se.probe,PLMset-method (PLMset-class), 10
se<-,PLMset-method (PLMset-class), 10
summary,PLMset-method (PLMset-class), 10
threestep, 4, 13, 17, 17, 20
threestepPLM, 17, 19
varcov (PLMset-class), 10
varcov,PLMset-method (PLMset-class), 10
verify.bg.param (internal functions), 5
verify.constraint.types (internal functions), 5
verify.model.param (internal functions), 5
verify.norm.param (internal functions), 5
verify.output.param (internal functions), 5
verify.variable.types (internal functions), 5
weights (PLMset-class), 10
weights, PLMset-method (PLMset-class), 10
weights<- (PLMset-class), 10
weights<-, PLMset-method (PLMset-class), 10