

# affyPLM

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internal functions *Internal affyPLM functions*

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### Description

Internal affyPLM functions

### Details

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

---

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

object	an <a href="#">AffyBatch</a>
method	an integer code specifying which method to use
baseline	A baseline value to use
theta	A parameter used in the background correction process

### Details

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

The basic concept is to consider that the lowest end of intensities 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](#)

### Author(s)

Ben Bolstad (bmb@bmbolstad.com)

### References

Bolstad, BM (2004) *Low Level Analysis of High-density Oligonucleotide Array Data: Background, Normalization and Summarization*. PhD Dissertation. University of California, Berkeley.

### Examples

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

fitPLM

*Fit a Probe Level Model to Affymetrix Genechip Data.***Description**

This function converts an [AffyBatch](#) into an [PLMset](#) by fitting a specified robust linear model to the probe level data.

**Usage**

```
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 =
verify.output.param(), model.param =
verify.model.param(object, model),
verbosity.level=0)
```

**Arguments**

object	an <a href="#">AffyBatch</a>
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	a way to specify whether variables in the model are factors or standard variables
constraint.type	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](mailto:bmb@bmbolstad.com))

## References

Bolstad, BM (2004) *Low Level Analysis of High-density Oligonucleotide Array Data: Background, Normalization and Summarization*. PhD Dissertation. University of California, Berkeley.

## 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,backg
  ## Not run: coefs(Pset) [1:10,]

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

  #try a different se.type
  ## Not run: Pset <- fitPLM(Dilution,model=PM~-1+probes+scanner,normalize=FALSE,backgrou
  se(Pset) [1:10,]
}
```

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 <a href="#">loess</a> .
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,subs
normalize.ExpressionSet.qspline(eset,transfn=c("none","log","antilog"),...)
normalize.ExpressionSet.invariantset(eset,prd.td = c(0.003,0.007),verbose=FALSE)
normalize.ExpressionSet.scaling(eset,trim=0.02,baseline=-1,transfn=c("none","log"))
```

**Arguments**

eset	An <a href="#">ExpressionSet</a>
span	parameter to be passed to the function <a href="#">loess</a> .
choose.subset	
subset.size	
verbose	verbosity flag
family	parameter to be passed to the function <a href="#">loess</a> .
prd.td	cutoff parameter (details in the bibliographic reference)
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
transfn	Transform the ExpressionSet before normalizing. Useful when dealing with expression values that are log-scale
baseline.type	A method of selecting the baseline array
...	Additional parameters that may be passed to the normalization routine

**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](mailto:bmb@bmbolstad.com))

**References**

Bolstad, BM (2004) *Low Level Analysis of High-density Oligonucleotide Array Data: Background, Normalization and Summarization*. PhD Dissertation. University of California, Berkeley.

**See Also**

[normalize](#)

**Examples**

```
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"))
```

## Arguments

<code>abatch</code>	An <a href="#">AffyBatch</a>
<code>type</code>	how should MM and PM values be handled
<code>use.median</code>	use median rather than mean
<code>use.log</code>	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](mailto:bmb@bmbolstad.com))

## References

Bolstad, B (2001) *Probe Level Quantile Normalization of High Density Oligonucleotide Array Data*. Unpublished manuscript <http://oz.berkeley.edu/~bolstad/stuff/qnorm.pdf>

Bolstad, B. M., Irizarry R. A., Astrand, M, and Speed, T. P. (2003) *A Comparison of Normalization Methods for High Density Oligonucleotide Array Data Based on Bias and Variance*. *Bioinformatics* 19(2), pp 185-193. <http://www.stat.berkeley.edu/~bolstad/normalize/normalize.html>

## See Also

[normalize](#), [normalize.quantiles](#)

---

normalize.scaling *Scaling normalization*

---

### Description

Allows the user to apply scaling normalization.

### Usage

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

### Arguments

X	A matrix. The columns of which are to be normalized.
abatch	An <a href="#">AffyBatch</a>
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)

### See Also

[normalize](#)

### Examples

```
if (require(affydata)) {
  data(Dilution)
  normalize.AffyBatch.scaling(Dilution)
}
```



---

PLMset2exprSet	<i>Convert a PLMset to an ExpressionSet</i>
----------------	---

---

### Description

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

### Usage

```
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

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

PLMset-class

Class *PLMset***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.

**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.

**description:** 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.

**notes:** Object of class "character" Vector of explanatory text.

**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).

**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.

**RLEl** 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**

Bolstad, BM (2004) *Low Level Analysis of High-density Oligonucleotide Array Data: Background, Normalization and Summarization*. PhD Dissertation. University of California, Berkeley.

preprocess

*Background correct and Normalize***Description**

This function pre-processes an [AffyBatch](#).

**Usage**

```
preprocess(object, subset=NULL, normalize=TRUE, background=TRUE, background.method=
```

**Arguments**

object	an <a href="#">AffyBatch</a>
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 [threestep](#) are supported by this function.

**Value**

An [AffyBatch](#)

**Author(s)**

Ben Bolstad <bmb@bmbolstad.com>

**References**

Bolstad, BM (2004) *Low Level Analysis of High-density Oligonucleotide Array Data: Background, Normalization and Summarization*. PhD Dissertation. University of California, Berkeley.

**See Also**

[expresso](#), [rma](#)

**Examples**

```
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**

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

**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.

**Author(s)**

Ben Bolstad ([bmb@bmbolstad.com](mailto: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>

---

rmaPLM      *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")
```

### 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 ver-
    bose. 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](mailto:bmb@bmbolstad.com))

### References

Bolstad, BM (2004) *Low Level Analysis of High-density Oligonucleotide Array Data: Background, Normalization and Summarization*. PhD Dissertation. University of California,

Irizarry RA, Bolstad BM, Collin F, Cope LM, Hobbs B and Speed TP (2003) *Summaries of Affymetrix GeneChip probe level data* Nucleic Acids Research 31(4):e15

Bolstad, BM, Irizarry RA, Astrand, M, and Speed, TP (2003) *A Comparison of Normalization Methods for High Density Oligonucleotide Array Data Based on Bias and Variance*. Bioinformatics 19(2):185-193

### See Also

[expresso](#), [rma](#), [threestep](#), [fitPLM](#), [threestepPLM](#)

### Examples

```

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)
}

```

---

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)
```

### 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

Bolstad, BM (2004) *Low Level Analysis of High-density Oligonucleotide Array Data: Background, Normalization and Summarization*. PhD Dissertation. University of California, Berkeley.

## See Also

[expresso](#), [rma](#), [threestep](#), [rmaPLM](#), [fitPLM](#)

## Examples

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

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

---

threestep	<i>Three Step expression measures</i>
-----------	---------------------------------------

---

## Description

This function converts an [AffyBatch](#) into an [ExpressionSet](#) using a three step expression measure.

## Usage

```
threestep(object, subset=NULL, normalize=TRUE, background=TRUE, background.method="")
```

## Arguments

object	an <a href="#">AffyBatch</a> .
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**

Bolstad, BM (2004) *Low Level Analysis of High-density Oligonucleotide Array Data: Background, Normalization and Summarization*. PhD Dissertation. University of California, Berkeley.

**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")

  eset <- threestep(Dilution, background.method="LESN2")
}
```

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