Package ‘affyPLM’

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Title Methods for fitting probe-level models
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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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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**

```r
top.correct.LESN(object, method=2, baseline=0.25, theta=4)
```

**Arguments**

- **object**: an `AffyBatch`
- **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`
fitPLM

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

References

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
```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**: 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>

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)
internal functions

### Internal affyPLM functions

#### Description

Internal affyPLM functions

#### Details

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

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**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.
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

See Also

mva.pairs
normalize.ExpressionSet

**verbose**
verbosity flag

**family**
parameter to be passed to the function `loess`.

**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>

**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

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>abatch</td>
<td>An AffyBatch</td>
</tr>
<tr>
<td>type</td>
<td>how should MM and PM values be handled</td>
</tr>
<tr>
<td>use.median</td>
<td>use median rather than mean</td>
</tr>
<tr>
<td>use.log</td>
<td>take logarithms, then normalize</td>
</tr>
</tbody>
</table>

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

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

if (require(affydata)) {
    data(Dilution)
    normalize.AffyBatch.scaling(Dilution)
}
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 \texttt{fitPLM}

Slots

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

This function pre-processes an AffyBatch.

Usage

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 ver-
bose. 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


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), k = 11, log.ticks = FALSE, ...)

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>low</td>
<td>color at low end of scale</td>
</tr>
<tr>
<td>high</td>
<td>color at high end of scale</td>
</tr>
<tr>
<td>mid</td>
<td>color at exact middle of scale</td>
</tr>
<tr>
<td>k</td>
<td>number of colors to have</td>
</tr>
<tr>
<td>x</td>
<td>A data series</td>
</tr>
<tr>
<td>horizontal</td>
<td>If TRUE then color bar is to be draw horizontally</td>
</tr>
<tr>
<td>col</td>
<td>colors for color bar</td>
</tr>
<tr>
<td>scale</td>
<td>tickmarks for x if x is not numeric</td>
</tr>
<tr>
<td>log.ticks</td>
<td>use a log type transformation to assign the colors</td>
</tr>
<tr>
<td>...</td>
<td>additional parameters to plotting routine</td>
</tr>
</tbody>
</table>

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
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.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
threestep

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

References


See Also
treestep, rma, 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")

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

```r
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)
}
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