Package 'affyPLM'

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Title Methods for fitting probe-level models

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Depends R (>= 2.6.0), BiocGenerics (>= 0.3.2), affy (>= 1.11.0), Biobase (>= 2.17.8), gcrma, stats, preprocessCore (>= 1.5.1)

Imports graphics, grDevices, methods

Suggests affydata, MASS, hgu95av2cdf

LinkingTo preprocessCore

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.

License GPL (>= 2)

URL https://github.com/bmbolstad/affyPLM

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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

```
bg.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 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`

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="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

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,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,m odel.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 horizonal 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

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

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)
  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.
```

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

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.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)
}
```

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.

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 currentl 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

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

`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)
}
```

preprocess *Background correct and Normalize*

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 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),k = 11, log.ticks=FALS
```

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 pacakge.

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

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,

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)
}
```

---

threestep *Three Step expression measures*

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="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

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")
}
```

---

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

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)
}
```

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