

Package ‘affy’

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Title Methods for Affymetrix Oligonucleotide Arrays

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Imports affyio (>= 1.13.3), BiocManager, graphics, grDevices, methods,
preprocessCore, stats, utils

Suggests tkWidgets (>= 1.19.0), affydata, widgetTools, hgu95av2cdf

LinkingTo preprocessCore

Description The package contains functions for exploratory
oligonucleotide array analysis. The dependence on tkWidgets
only concerns few convenience functions. 'affy' is fully
functional without it.

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Collate ProgressBarText.R ppset.ttest.R ppsetApply.R expressoWidget.R
getCDFenv.R AffyRNAdeg.R avdiff.R barplot.ProbeSet.R
bg.Affy.chipwide.R bg.R expresso.R fit.li.wong.R
generateExprVal.method.avgdif.R
generateExprVal.method.liwong.R generateExprVal.method.mas.R
generateExprVal.method.medianpolish.R
generateExprVal.method.playerout.R hlog.R justma.R
loess.normalize.R maffy.R mas5.R merge.AffyBatch.R
normalize.constant.R normalize.contrasts.R
normalize.invariantset.R normalize.loess.R normalize.qspline.R

normalize.quantiles.R pairs.AffyBatch.R plot.density.R
 plotLocation.R plot.ProbeSet.R pmcorrect.mas.R AffyBatch.R
 mva.pairs.R ProbeSet.R read.affybatch.R rma.R summary.R
 tukey.biweight.R whatcdf.R xy2indices.R zzz.R

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<code>.setAffyOptions</code>	<i>~~function to set options~~</i>
------------------------------	------------------------------------

Description

~~ Set the options for the package

Usage

```
.setAffyOptions(affy.opt = NA)
```

Arguments

`affy.opt` A list structure of options. If NA, the default options are set.

Details

See the vignettes to know more. This function could disappear in favor of a more general one the package Biobase.

Value

The function is used for its side effect. Nothing is returned.

Author(s)

Laurent

Examples

```
affy.opt <- getOption("BioC")$affy
.setAffyOptions(affy.opt)
```

affy-deprecated

Deprecated functions in package 'affy'

Description

These functions are provided for compatibility with older versions of affy only, and will be defunct at the next release.

Details

The following functions are deprecated and will be made defunct; use the replacement indicated below:

- loess.normalize: [normalize.loess](#)
- maffy.normalize
- multiloess
- simplemultiLoess

affy-options

Options for the affy package

Description

Description of the options for the affy package.

Note

The affy package options are contained in the Bioconductor options. The options are:

- use.widgets: a logical used to decide on the default of widget use.
- compress.cel: a logical
- compress.cdf: a logical
- probes.loc: a list. Each element of the list is itself a list with two elements *what* and *where*. When looking for the information about the locations of the probes on the array, the elements in the list will be looked at one after the other. The first one for which *what* and *where* lead to the matching locations information is used. The element *what* can be one of *package*, *environment* or *file*. The element *where* depends on the corresponding element *what*.
 - if *package*: location for the package (like it would be for the argument `lib.loc` for the function `library`.)
 - if *environment*: an environment to look for the information (like the argument `env` for the function `get`).
 - if *file*: a character with the path in which a CDF file can be found.

Examples

```
## get the options
opt <- getOption("BioC")
affy.opt <- opt$affy

## list their names
names(affy.opt)

## set the option compress.cel
affy.opt$compress.cel <- TRUE
options(BioC=opt)
```

affy.scalevalue.exprSet

Scale normalization for exprSets

Description

Normalizes expression values using the method described in the Affymetrix user manual.

Usage

```
affy.scalevalue.exprSet(eset, sc = 500, analysis="absolute")
```

Arguments

eset	An ExpressionSet object.
sc	Value at which all arrays will be scaled to.
analysis	Should we do absolute or comparison analysis, although "comparison" is still not implemented.

Details

This is function was implemented from the Affymetrix technical documentation for MAS 5.0. It can be downloaded from the website of the company. Please refer to this document for details.

Value

A normalized [ExpressionSet](#).

Author(s)

Laurent

AffyBatch-class

*Class AffyBatch***Description**

This is a class representation for Affymetrix GeneChip probe level data. The main component are the intensities from multiple arrays of the same CDF type. It extends [eSet](#).

Objects from the Class

Objects can be created using the function [read.affybatch](#) or the wrapper [ReadAffy](#).

Slots

cdfName: Object of class character representing the name of CDF file associated with the arrays in the AffyBatch.

nrow: Object of class integer representing the physical number of rows in the arrays.

ncol: Object of class integer representing the physical number of columns in the arrays.

assayData: Object of class AssayData containing the raw data, which will be at minimum a matrix of intensity values. This slot can also hold a matrix of standard errors if the 'sd' argument is set to TRUE in the call to ReadAffy.

phenoData: Object of class AnnotatedDataFrame containing phenotypic data for the samples.

annotation A character string identifying the annotation that may be used for the ExpressionSet instance.

protocolData: Object of class AnnotatedDataFrame containing protocol data for the samples.

featureData Object of class AnnotatedDataFrame containing feature-level (e.g., probeset-level) information.

experimentData: Object of class "MIAME" containing experiment-level information.

__classVersion__: Object of class Versions describing the R and Biobase version number used to create the instance. Intended for developer use.

Extends

Class "eSet", directly.

Methods

cdfName signature(object = "AffyBatch"): obtains the cdfName slot.

pm<- signature(object = "AffyBatch"): replaces the perfect match intensities.

pm signature(object = "AffyBatch"): extracts the pm intensities.

mm<- signature(object = "AffyBatch"): replaces the mismatch intensities.

mm signature(object = "AffyBatch"): extracts the mm intensities.

probes signature(object = "AffyBatch", which): extract the perfect match or mismatch probe intensities. Uses which can be "pm" and "mm".

exprs signature(object = "AffyBatch"): extracts the expression matrix.

exprs<- signature(object = "AffyBatch", value = "matrix"): replaces the expression matrix.

- se.exprs** signature(object = "AffyBatch"): extracts the matrix of standard errors of expression values, if available.
- se.exprs<-** signature(object = "AffyBatch", value = "matrix"): replaces the matrix of standard errors of expression values.
- [<-** signature(x = "AffyBatch"): replaces subsets.
- [** signature(x = "AffyBatch"): subsets by array.
- boxplot** signature(x = "AffyBatch"): creates a **boxplots** of log base 2 intensities (pm, mm or both). Defaults to both.
- hist** signature(x = "AffyBatch"): creates a plot showing all the histograms of the pm,mm or both data. See [plotDensity](#).
- computeExprSet** signature(x = "AffyBatch", summary.method = "character"): For each probe set computes an expression value using summary.method.
- featureNames** signature(object = "AffyBatch"): return the probe set names also referred to as the Affymetrix IDs. Notice that one can not assign featureNames. You must do this by changing the cdfenvs.
- geneNames** signature(object="AffyBatch"): deprecated, use featureNames.
- getCdfInfo** signature(object = "AffyBatch"): retrieve the environment that defines the location of probes by probe set.
- image** signature(x = "AffyBatch"): creates an image for each sample.
- indexProbes** signature(object = "AffyBatch", which = "character"): returns a list with locations of the probes in each probe set. The affyID corresponding to the probe set to retrieve can be specified in an optional parameter genenames. By default, all the affyIDs are retrieved. The names of the elements in the list returned are the affyIDs. which can be "pm", "mm", or "both". If "both" then perfect match locations are given followed by mismatch locations.
signature(object = "AffyBatch", which = "missing") (i.e., calling indexProbes without a "which" argument) is the same as setting "which" to "pm".
- intensity<-** signature(object = "AffyBatch"): a replacement method for the exprs slot, i.e. the intensities.
- intensity** signature(object = "AffyBatch"): extract the exprs slot, i.e. the intensities.
- length** signature(x = "AffyBatch"): returns the number of samples.
- pmindex** signature(object = "AffyBatch"): return the location of perfect matches in the intensity matrix.
- mmindex** signature(object = "AffyBatch"): return the location of the mismatch intensities.
- dim** signature(x = "AffyBatch"): Row and column dimensions.
- ncol** signature(x = "AffyBatch"): An accessor function for ncol.
- nrow** signature(x = "AffyBatch"): an accessor function for nrow.
- normalize** signature(object = "AffyBatch"): a method to [normalize](#). The method accepts an argument method. The default methods is specified in package options (see the main vignette).
- normalize.methods** signature(object = "AffyBatch"): returns the normalization methods defined for this class. See [normalize](#).
- probeNames** signature(object = "AffyBatch"): returns the probe set associated with each row of the intensity matrix.
- probeset** signature(object = "AffyBatch", genenames=NULL, locations=NULL): Extracts [ProbeSet](#) objects related to the probe sets given in genenames. If an alternative set of locations defining pms and mms a list with those locations should be passed via the locations argument.

bg.correct signature(object = "AffyBatch", method="character") applies background correction methods defined by method.

updateObject signature(object = "AffyBatch", ..., verbose=FALSE): update, if necessary, an object of class AffyBatch to its current class definition. verbose=TRUE provides details about the conversion process.

Note

This class is better described in the vignette.

See Also

related methods [merge.AffyBatch](#), [pairs.AffyBatch](#), and [eSet](#)

Examples

```
if (require(affydata)) {
  ## load example
  data(Dilution)

  ## nice print
  print(Dilution)

  pm(Dilution)[1:5,]
  mm(Dilution)[1:5,]

  ## get indexes for the PM probes for the affyID "1900_at"
  mypmindex <- pmindex(Dilution,"1900_at")
  ## same operation using the primitive
  mypmindex <- indexProbes(Dilution, which="pm", genenames="1900_at")[[1]]
  ## get the probe intensities from the index
  intensity(Dilution)[mypmindex, ]

  description(Dilution) ##we can also use the methods of eSet
  sampleNames(Dilution)
  abstract(Dilution)
}
```

AffyRNAdeg

Function to assess RNA degradation in Affymetrix GeneChip data.

Description

Uses ordered probes in probeset to detect possible RNA degradation. Plots and statistics used for evaluation.

Usage

```
AffyRNAdeg(abatch, log.it=TRUE)
summaryAffyRNAdeg(rna.deg.obj, signif.digits=3)
plotAffyRNAdeg(rna.deg.obj, transform = "shift.scale", cols = NULL, ...)
```


Arguments

<code>abatch</code>	An object of class <code>AffyBatch-class</code> .
<code>log.it</code>	A logical argument: If <code>log.it=T</code> , then probe data is log2 transformed.
<code>rna.deg.obj</code>	Output from <code>AffyRNAdeg</code> .
<code>signif.digits</code>	Number of significant digits to show.
<code>transform</code>	Possible choices are "shift.scale", "shift.only", and "neither". "Shift" vertically staggers the plots for individual chips, to make the display easier to read. "Scale" normalizes so that standard deviation is equal to 1.
<code>cols</code>	A vector of colors for plot, length = number of chips.
<code>...</code>	further arguments for <code>plot</code> function.

Details

Within each probeset, probes are numbered directionally from the 5' end to the 3' end. Probe intensities are averaged by probe number, across all genes. If `log.it=FALSE` and `transform="Neither"`, then `plotAffyRNAdeg` simply shows these means for each chip. Shifted and scaled versions of the plot can make it easier to see.

Value

`AffyRNAdeg` returns a list with the following components:

<code>sample.names</code>	names of samples, derived from affy batch object
<code>means.by.number</code>	average intensity by probe position
<code>ses</code>	standard errors for probe position averages
<code>slope</code>	from linear regression of <code>means.by.number</code>
<code>pvalue</code>	from linear regression of <code>means.by.number</code>

Author(s)

Leslie Cope

Examples

```
if (require(affydata)) {
  data(Dilution)
  RNAdeg<-AffyRNAdeg(Dilution)
  plotAffyRNAdeg(RNAdeg)
}
```

barplot.ProbeSet	<i>show a ProbeSet as barplots</i>
------------------	------------------------------------

Description

Displays the probe intensities in a ProbeSet as a barplots

Usage

```
## S3 method for class 'ProbeSet'
barplot(height, xlab = "Probe pair", ylab = "Intensity",
        main = NA, col.pm = "red", col.mm = "blue", beside = TRUE, names.arg = "pp",
        ask = TRUE, scale, ...)
```

Arguments

height	an object of class ProbeSet.
xlab	label for x axis.
ylab	label for y axis.
main	main label for the figure.
col.pm	color for the ‘pm’ intensities.
col.mm	color for the ‘mm’ intensities.
beside	bars beside each others or not.
names.arg	names to be plotted below each bar or group of bars.
ask	ask before plotting the next barplot.
scale	put all the barplot to the same scale.
...	extra parameters to be passed to barplot .

Examples

```
if (require(affydata)) {
  data(Dilution)
  gn <- geneNames(Dilution)
  pps <- probeset(Dilution, gn[1]][[1]]

  barplot.ProbeSet(pps)
}
```

bg.adjust	<i>Background adjustment (internal function)</i>
-----------	--

Description

An internal function to be used by [bg.correct.rma](#).

Usage

```
bg.adjust(pm, n.pts = 2^14, ...)
bg.parameters(pm, n.pts = 2^14)
```

Arguments

pm	a pm matrix
n.pts	number of points to use in call to density.
...	extra arguments to pass to bg.adjust.

Details

Assumes PMs are a convolution of normal and exponential. So we observe $X+Y$ where X is background and Y is signal. `bg.adjust` returns $E[Y|X+Y, Y>0]$ as our background corrected PM. `bg.parameters` provides ad hoc estimates of the parameters of the normal and exponential distributions.

Value

a matrix

See Also

[bg.correct.rma](#)

bg.correct	<i>Background Correction</i>
------------	------------------------------

Description

Background corrects probe intensities in an object of class [AffyBatch](#).

Usage

```
bg.correct(object, method, ...)

bg.correct.rma(object, ...)
bg.correct.mas(object, griddim)
bg.correct.none(object, ...)
```

Arguments

object	An object of class AffyBatch .
method	A character that defines what background correction method will be used. Available methods are given by <code>bg.correct.methods</code> .
griddim	grid dimension used for mas background estimate. The array is divided into griddim equal parts. Default is 16.
...	arguments to pass along to the engine function.

Details

The name of the method to apply must be double-quoted. Methods provided with the package are currently:

- `bg.correct.none`: returns object unchanged.
- `bg.correct.chipwide`: noise correction as described in a ‘white paper’ from Affymetrix.
- `bg.correct.rma`: the model based correction used by the RMA expression measure.

They are listed in the variable `bg.correct.methods`. The user must supply the word after "bg.correct", i.e none, subtractmm, rma, etc...

More details are available in the vignette.

R implementations similar in function to the internal implementation used by `bg.correct.rma` are in [bg.adjust](#).

Value

An [AffyBatch](#) for which the intensities have been background adjusted. For some methods (RMA), only PMs are corrected and the MMs remain the same.

Examples

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

  ##bgc will be the bg corrected version of Dilution
  bgc <- bg.correct(Dilution, method="rma")

  ##This plot shows the tranformation
  plot(pm(Dilution)[,1],pm(bgc)[,1],log="xy",
       main="PMs before and after background correction")
}
```

cdfenv.example

Example cdfenv

Description

Example cdfenv (environment containing the probe locations).

Usage

```
data(cdfenv.example)
```

Format

An `environment` `cdfenv.example` containing the probe locations

Source

Affymetrix CDF file for the array Hu6800

`cdfFromBioC`*Functions to obtain CDF files*

Description

A set of functions to obtain CDF files from various locations.

Usage

```
cdfFromBioC(cdfname, lib = .libPaths()[1], verbose = TRUE)
cdfFromLibPath(cdfname, lib = NULL, verbose=TRUE)
cdfFromEnvironment(cdfname, where, verbose=TRUE)
```

Arguments

<code>cdfname</code>	name of the CDF.
<code>lib</code>	install directory for the CDF package.
<code>where</code>	environment to search.
<code>verbose</code>	logical controlling extra output.

Details

These functions all take a requested CDF environment name and will attempt to locate that environment in the appropriate location (a package's data directory, as a CDF package in the `.libPaths()`, from a loaded environment or on the Bioconductor website. If the environment can not be found, it will return a list of the methods tried that failed.

Value

The CDF environment or a list detailing the failed locations.

Author(s)

Jeff Gentry

cleancdfname	<i>Clean Affymetrix's CDF name</i>
--------------	------------------------------------

Description

This function converts Affymetrix's names for CDF files to the names used in the annotation package and in all Bioconductor.

Usage

```
cleancdfname(cdfname, addcdf = TRUE)
```

Arguments

cdfname	A character denoting Affymetrix's CDF file name
addcdf	A logical. If TRUE it adds the string "cdf" at the end of the cleaned CDF name. This is used to name the cdfenvs packages.

Details

This function takes a CDF filename obtained from an Affymetrix file (from a CEL file for example) and convert it to a convention of ours: all small caps and only alphanumeric characters. The details of the rule can be seen in the code. We observed exceptions that made us create a set of special cases for mapping CEL to CDF. The object mapCdfName holds information about these cases. It is a data.frame of three elements: the first is the name as found in the CDF file, the second the name in the CEL file and the third the name in Bioconductor. mapCdfName can be loaded using data(mapCdfName).

Value

A character

Examples

```
cdf.tags <- c("HG_U95Av2", "HG-133A")
for (i in cdf.tags)
  cat(i, "becomes", cleancdfname(i), "\n")
```

debug.affy123	<i>Debugging Flag</i>
---------------	-----------------------

Description

For developmental use only

expresso

From raw probe intensities to expression values

Description

Goes from raw probe intensities to expression values

Usage

```
expresso(
  afbatch,
  # background correction
  bg.correct = TRUE,
  bgcorrect.method = NULL,
  bgcorrect.param = list(),
  # normalize
  normalize = TRUE,
  normalize.method = NULL,
  normalize.param = list(),
  # pm correction
  pmcorrect.method = NULL,
  pmcorrect.param = list(),
  # expression values
  summary.method = NULL,
  summary.param = list(),
  summary.subset = NULL,
  # misc.
  verbose = TRUE,
  widget = FALSE)
```

Arguments

<code>afbatch</code>	an AffyBatch object.
<code>bg.correct</code>	a boolean to express whether background correction is wanted or not.
<code>bgcorrect.method</code>	the name of the background adjustment method.
<code>bgcorrect.param</code>	a list of parameters for <code>bgcorrect.method</code> (if needed/wanted).
<code>normalize</code>	normalization step wished or not.
<code>normalize.method</code>	the normalization method to use.
<code>normalize.param</code>	a list of parameters to be passed to the normalization method (if wanted).
<code>pmcorrect.method</code>	the name of the PM adjustment method.
<code>pmcorrect.param</code>	a list of parameters for <code>pmcorrect.method</code> (if needed/wanted).
<code>summary.method</code>	the method used for the computation of expression values.
<code>summary.param</code>	a list of parameters to be passed to the <code>summary.method</code> (if wanted).

`summary.subset` a list of 'affyids'. If NULL, an expression summary value is computed for everything on the chip.

`verbose` logical value. If TRUE, it writes out some messages.

`widget` a boolean to specify the use of widgets (the package `tkWidget` is required).

Details

Some arguments can be left to NULL if the `widget=TRUE`. In this case, a widget pops up and let the user choose with the mouse. The arguments are: `AffyBatch`, `bgcorrect.method`, `normalize.method`, `pmcorrect.method` and `summary.method`.

For the mas 5.0 and 4.0 methods ones need to normalize after obtaining expression. The function [affy.scalevalue.exprSet](#) does this.

For the Li and Wong summary method notice you will not get the same results as you would get with dChip. dChip is not open source so it is not easy to reproduce. Notice also that this iterative algorithm will not always converge. If you run the algorithm on thousands of probes expect some non-convergence warnings. These are more likely when few arrays are used. We recommend using this method only if you have 10 or more arrays. Please refer to the [fit.li.wong](#) help page for more details.

Value

An object of class [ExpressionSet](#), with an attribute `pps.warnings` as returned by the method [computeExprSet](#).

See Also

[AffyBatch](#)

Examples

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

  eset <- expresso(Dilution, bgcorrect.method="rma",
                  normalize.method="constant", pmcorrect.method="pmonly",
                  summary.method="avgdiff")

  ##to see options available for bg correction type:
  bgcorrect.methods()
}
```

`expressoWidget`

A widget for users to pick correction methods

Description

This widget is called by `expresso` to allow users to select correction methods that will be used to process affy data.

Usage

```
expressoWidget(BGMethods, normMethods, PMMethods, expMethods, BGDefault,
normDefault, PMDefault, expDefault)
```

Arguments

BGMethods	a vector of character strings for the available methods that can be used as a background correction method of affy data.
normMethods	a vector of character strings for the available methods that can be used as a normalization method of affy data.
PMMethods	a vector of character strings for the available methods that can be used as a PM correction method of affy data.
expMethods	a vector of character strings for the available methods that can be used as a summary method of affy data.
BGDefault	a character string for the name of a default background correction method.
normDefault	a character string for the name of a default normalization method.
PMDefault	a character string for the name of a default PM correction method.
expDefault	a character string for the name of a default summary method.

Details

The widget will be invoked when `expresso` is called with argument "widget" set to TRUE. Default values can be changed using the drop down list boxes. Double clicking on an option from the drop-down list makes an selection. The first element of the list for available methods will be the default method if no default is provided.

Value

The widget returns a list of selected correction methods.

BG	background correction method
NORM	normalization method
PM	PM correction method
EXP	summary method

Author(s)

Jianhua Zhang

References

Documentations of affy package

See Also

[expresso](#)

Examples

```
if(interactive()){
  require(widgetTools)
  espressoWidget(c("mas", "none", "rma"), c("constant", "quantiles"),
c("mas", "pmonly"), c("liwong", "playerout"))
}
```

fit.li.wong

Fit Li and Wong Model to a Probe Set

Description

Fits the model described in Li and Wong (2001) to a probe set with I chips and J probes.

Usage

```
fit.li.wong(data.matrix, remove.outliers=TRUE, normal.array.quantile=0.5,
normal.resid.quantile=0.9, large.threshold=3, large.variation=0.8,
outlier.fraction=0.14, delta=1e-06, maxit=50,
outer.maxit=50, verbose=FALSE, ...)
```

```
li.wong(data.matrix, remove.outliers=TRUE, normal.array.quantile=0.5,
normal.resid.quantile=0.9, large.threshold=3, large.variation=0.8,
outlier.fraction=0.14, delta=1e-06, maxit=50,
outer.maxit=50, verbose=FALSE)
```

Arguments

data.matrix	an I x J matrix containing the probe set data. Typically the i,j entry will contain the PM-MM value for probe pair j in chip i. Another possible use, is to use PM instead of PM-MM.
remove.outliers	logical value indicating if the algorithm will remove outliers according to the procedure described in Li and Wong (2001).
large.threshold	used to define outliers.
normal.array.quantile	quantile to be used when determining what a normal SD is. probes or chips having estimates with SDs bigger than the quantile normal.array.quantile of all SDs x large.threshold.
normal.resid.quantile	any residual bigger than the normal.resid.quantile quantile of all residuals x large.threshold is considered an outlier.
large.variation	any probe or chip describing more than this much total variation is considered an outlier.
outlier.fraction	this is the maximum fraction of single outliers that can be in the same probe or chip.
delta	numerical value used to define the stopping criterion.

maxit	maximum number of iterations when fitting the model.
outer.maxit	maximum number of iterations of defined outliers.
verbose	logical value. If TRUE information is given of the status of the algorithm.
...	additional arguments.

Details

This is Bioconductor's implementation of the Li and Wong algorithm. The Li and Wong PNAS 2001 paper was followed. However, you will not get the same results as you would get with dChip. dChip is not open source so it is not easy to reproduce.

Notice that this iterative algorithm will not always converge. If you run the algorithm on thousands of probes expect some non-convergence warnings. These are more likely when few arrays are used. We recommend using this method only if you have 10 or more arrays.

Please refer to references for more details.

Value

li.wong returns a vector of expression measures (or column effects) followed by their respective standard error estimates. It was designed to work with express which is no longer part of the package.

fit.li.wong returns much more. Namely, a list containing the fitted parameters and relevant information.

theta	fitted thetas.
phi	fitted phis.
sigma.eps	estimated standard deviation of the error term.
sigma.theta	estimated standard error of theta.
sigma.phi	estimated standard error of phi.
theta.outliers	logical vector describing which chips (thetas) are considered outliers (TRUE).
phi.outliers	logical vector describing which probe sets (phis) are considered outliers (TRUE)
convergence1	logical value. If FALSE the algorithm did not converge when fitting the phis and thetas.
convergence2	logical value. If FALSE the algorithm did not converge in deciding what are outliers.
iter	number of iterations needed to achieve convergence.
delta	difference between thetas when iteration stopped.

Author(s)

Rafael A. Irizarry, Cheng Li, Fred A. Wright, Ben Bolstad

References

Li, C. and Wong, W.H. (2001) *Genome Biology* **2**, 1–11.

Li, C. and Wong, W.H. (2001) *Proc. Natl. Acad. Sci USA* **98**, 31–36.

See Also

[li.wong](#), [expresso](#)

Examples

```
x <- sweep(matrix(2^rnorm(600),30,20),1,seq(1,2,len=30),FUN="+")
fit1 <- fit.li.wong(x)
plot(x[1,])
lines(fit1$theta)
```

generateExprSet-method

generate a set of expression values

Description

Generate a set of expression values from the probe pair information. The set of expression is returned as an [ExpressionSet](#) object.

Usage

```
computeExprSet(x, pmcorrect.method, summary.method, ...)
```

```
generateExprSet.methods()
```

```
upDate.generateExprSet.methods(x)
```

Arguments

<code>x</code>	a AffyBatch holding the probe level informations to generate the expression values, for <code>computeExprSet</code> , and for <code>upDate.generateExprSet.methods</code> it is a character vector..
<code>pmcorrect.method</code>	the method used to correct PM values (see section 'details').
<code>summary.method</code>	the method used to generate the expression value (see section 'details').
<code>...</code>	any of the options of the normalization you would like to modify.

Details

An extra argument `ids=` can be passed. It must be a vector of affids. The expression values will only be computed and returned for these affyids.

The different methods available through this mechanism can be accessed by calling the method `generateExprSet.methods` with an object of call `Cel.container` as an argument.

In the Affymetrix design, *MM* probes were included to measure the noise (or background signal). The original algorithm for background correction was to subtract the *MM* signal to the *PM* signal. The methods currently included in the package are "bg.correct.subtractmm", "bg.correct.pmonly" and "bg.correct.adjust".

To alter the available methods for generating ExprSets use `upDate.generateExprSet.methods`.

See Also

method generateExprSet of the class [AffyBatch](#)
[expresso](#)

Examples

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

  ids <- c( "1000_at", "1001_at")

  eset <- computeExprSet(Dilution, pmcorrect.method="pmonly",
                        summary.method="avgdiff", ids=ids)
}
```

generateExprVal

Compute a summary expression value from the probes intensities

Description

Compute a summary expression value from the probes intensities

Usage

```
express.summary.stat(x, pmcorrect, summary, ...)
express.summary.stat.methods() # vector of names of methods
update.express.summary.stat.methods(x)
```

Arguments

x	a (ProbeSet
pmcorrect	the method used to correct the PM values before summarizing to an expression value.
summary	the method used to generate the expression value.
...	other parameters the method might need... (see the corresponding methods below...)

Value

Returns a vector of expression values.

Examples

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

  p <- probeset(Dilution, "1001_at")[[1]]

  par(mfcol=c(5,2))
  mymethods <- express.summary.stat.methods()
  nmet <- length(mymethods)
  nc <- ncol(pm(p))
```

```

layout(matrix(c(1:nc, rep(nc+1, nc)), nc, 2), width = c(1, 1))

barplot(p)

results <- matrix(0, nc, nmet)
rownames(results) <- paste("sample", 1:nc)
colnames(results) <- mymethods

for (i in 1:nmet) {
  ev <- express.summary.stat(p, summary=mymethods[i], pmcorrect="pmonly")
  if (mymethods[[i]] != "medianpolish")
    results[, i] <- 2^(ev$exprs)
  else
    results[, i] <- ev$exprs
}

dotchart(results, labels=paste("sample", 1:nc))
}

```

generateExprVal.method.avgdiff

Generate an expression value from the probes informations

Description

Generate an expression from the probes

Usage

```

generateExprVal.method.avgdiff(probes, ...)
generateExprVal.method.medianpolish(probes, ...)
generateExprVal.method.liwong(probes, ...)
generateExprVal.method.mas(probes, ...)

```

Arguments

probes	a matrix of probe intensities with rows representing probes and columns representing samples. Usually pm(probeset) where probeset is a of class ProbeSet .
...	extra arguments to pass to the respective function.

Value

A list containing entries:

exprs	The expression values.
se.exprs	The standard error estimate.

See Also

[generateExprSet-methods](#), [generateExprVal.method.playerout](#), [fit.li.wong](#)

Examples

```

data(SpikeIn) ##SpikeIn is a ProbeSets
probes <- pm(SpikeIn)
avgdiff <- generateExprVal.method.avgdiff(probes)
medianpolish <- generateExprVal.method.medianpolish(probes)
liwong <- generateExprVal.method.liwong(probes)
playerout <- generateExprVal.method.playerout(probes)
mas <- generateExprVal.method.mas(probes)

concentrations <- as.numeric(sampleNames(SpikeIn))
plot(concentrations,avgdiff$exprs,log="xy",ylim=c(50,10000),pch="a",type="b")
points(concentrations,2^medianpolish$exprs,pch="m",col=2,type="b",lty=2)
points(concentrations,liwong$exprs,pch="l",col=3,type="b",lty=3)
points(concentrations,playerout$exprs,pch="p",col=4,type="b",lty=4)
points(concentrations,mass$exprs,pch="p",col=4,type="b",lty=4)

```

```
generateExprVal.method.playerout
```

Generate an expression value from the probes informations

Description

Generate an expression from the probes

Usage

```
generateExprVal.method.playerout(probes, weights=FALSE, optim.method="L-BFGS-B")
```

Arguments

probes	a list of probes slots from PPSet.container
weights	Should the resulting weights be returned ?
optim.method	see parameter 'optim' for the function optim

Details

A non-parametric method to weight each perfect match probe in the set and to compute a weighted mean of the perfect match values. One will notice this method only makes use of the perfect matches. (see function `playerout.costfunction` for the cost function).

Value

A vector of expression values.

Author(s)

Laurent <laurent@cbs.dtu.dk>
(Thanks to E. Lazaridis for the original playerout code and the discussions about it)

References

Emmanuel N. Lazaridis, Dominic Sinibaldi, Gregory Bloom, Shrikant Mane and Richard Jove
A simple method to improve probe set estimates from oligonucleotide arrays, Mathematical Bio-sciences, Volume 176, Issue 1, March 2002, Pages 53-58

hlog	<i>Hybrid Log</i>
------	-------------------

Description

Given a constant c this function returns x if x is less than c and $\text{sign}(x) * (c * \log(\text{abs}(x)/c) + c)$ if its not. Notice this is a continuous odd ($f(-x) = -f(x)$) function with continuous first derivative. The main purpose is to perform log transformation when one has negative numbers, for example for PM-MM.

Usage

```
hlog(x, constant=1)
```

Arguments

<code>x</code>	a number.
<code>constant</code>	the constant c (see description).

Details

If constant is less than or equal to 0 $\log(x)$ is returned for all x . If constant is infinity x is returned for all x .

Author(s)

Rafael A. Irizarry

justRMA	<i>Read CEL files into an ExpressionSet</i>
---------	---

Description

Read CEL files and compute an expression measure without using an AffyBatch.

Usage

```
just.rma(..., filenames = character(0),
         phenoData = new("AnnotatedDataFrame"),
         description = NULL,
         notes = "",
         compress = getOption("BioC")$affy$compress.cel,
         rm.mask = FALSE, rm.outliers = FALSE, rm.extra = FALSE,
         verbose=FALSE, background=TRUE, normalize=TRUE,
         bgversion=2, destructive=FALSE, cdfname = NULL)

justRMA(..., filenames=character(0),
        widget=getOption("BioC")$affy$use.widgets,
        compress=getOption("BioC")$affy$compress.cel,
        celfile.path=getwd(),
        sampleNames=NULL,
        phenoData=NULL,
        description=NULL,
        notes="",
        rm.mask=FALSE, rm.outliers=FALSE, rm.extra=FALSE,
        hdf5=FALSE, hdf5FilePath=NULL, verbose=FALSE,
        normalize=TRUE, background=TRUE,
        bgversion=2, destructive=FALSE, cdfname = NULL)
```

Arguments

...	file names separated by comma.
filenames	file names in a character vector.
phenoData	an AnnotatedDataFrame object.
description	a MIAME object.
notes	notes.
compress	are the CEL files compressed?
rm.mask	should the spots marked as 'MASKS' set to NA?
rm.outliers	should the spots marked as 'OUTLIERS' set to NA?
rm.extra	if TRUE, then overrides what is in rm.mask and rm.outliers.
hdf5	use of hdf5 ? (not available yet)
hdf5FilePath	a filename to use with hdf5 (not available yet).
verbose	verbosity flag.
widget	a logical specifying if widgets should be used.
celfile.path	a character denoting the path ReadAffy should look for cel files.
sampleNames	a character vector of sample names to be used in the AffyBatch.
normalize	logical value. If TRUE, then normalize data using quantile normalization.
background	logical value. If TRUE, then background correct using RMA background correction.
bgversion	integer value indicating which RMA background to use 1: use background similar to pure R rma background given in affy version 1.0 - 1.0.2 2: use background similar to pure R rma background given in affy version 1.1 and above

destructive	logical value. If TRUE, then works on the PM matrix in place as much as possible, good for large datasets.
cdfname	Used to specify the name of an alternative cdf package. If set to NULL, then the usual cdf package based on Affymetrix' mappings will be used.

Details

justRMA is a wrapper for `just.rma` that permits the user to read in phenoData, MIAME information, and CEL files using widgets. One can also define files where to read phenoData and MIAME information.

If the function is called with no arguments `justRMA()`, then all the CEL files in the working directory are read, converted to an expression measure using RMA and put into an [ExpressionSet](#). However, the arguments give the user great flexibility.

phenoData is read using [read.AnnotatedDataFrame](#). If a character is given, it tries to read the file with that name to obtain the AnnotatedDataFrame object as described in [read.AnnotatedDataFrame](#). If left NULL and widget=FALSE (widget=TRUE is not currently supported), then a default object is created. It will be an object of class [AnnotatedDataFrame](#) with its pData being a data.frame with column x indexing the CEL files.

description is read using [read.MIAME](#). If a character is given, it tries to read the file with that name to obtain a MIAME instance. If left NULL but widget=TRUE, then widgets are used. If left NULL and widget=FALSE, then an empty instance of MIAME is created.

The arguments `rm.masks`, `rm.outliers`, `rm.extra` are passed along to the function `read.celfile`.

Value

An ExpressionSet object, containing expression values identical to what one would get from running `rma` on an AffyBatch.

Author(s)

In the beginning: James MacDonald <jmacdon@med.umich.edu> Supporting routines, maintenance and just.rma: Ben Bolstad <bmb@bmbolstad.com>

See Also

[rma](#), [read.affybatch](#)

list.celfiles

List the Cel Files in a Directory/Folder

Description

This function produces a vector containing the names of files in the named directory/folder ending in .cel or .CEL.

Usage

```
list.celfiles(...)
```

Arguments

... arguments to pass along to [list.files](#)

Value

A character vector of file names.

See Also

[list.files](#)

Examples

```
list.celfiles()
```

maffy.subset	<i>Select Subset</i>
--------------	----------------------

Description

Select a subset of rows with small rank-range over columns.

Usage

```
maffy.subset(data, subset.size=5000, maxit=100,
             subset.delta=max(round(subset.size/100), 25), verbose=FALSE)
```

Arguments

data	a matrix
subset.size	desired size of subset
maxit	maximum number of iterations
subset.delta	maximum deviation from subset.size
verbose	logical value.

Details

Please refer to references.

Value

A list with component subset, the indexes for subset.

Author(s)

Magnus Astrand

References

Astrand, M. (2001) <http://www.math.chalmers.se/~magnusaa/maffy/>

See Also[maffy.normalize](#)**Examples**

```

if (require(affydata)) {
  #data(Dilution)
  #x <- log2(pm(Dilution)[,1:3])
  #Index <- maffy.subset(x,subset.size=100)$subset
  #mva.pairs(x[Index,])
}

```

MAplot	<i>Relative M vs. A plots</i>
--------	-------------------------------

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.

Usage

```

MAplot(object,...)
Mbox(object,...)
ma.plot(A, M, subset = sample(1:length(M), min(c(10000, length(M))))),
  show.statistics = TRUE, span = 2/3, family.loess = "gaussian",
  cex = 2, plot.method = c("normal","smoothScatter","add"),
  add.loess = TRUE, lwd = 1, lty = 1, loess.col = "red", ...)

```

Arguments

object	an AffyBatch-class .
...	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	logical. 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.
plot.method	a string specifying how the plot is to be drawn. "normal" plots points, "smoothScatter" uses the smoothScatter function. Specifying "add" means that the MAplot should be added to the current plot.
add.loess	add a loess line to the plot.
lwd	width of loess line.
lty	line type for loess line.
loess.col	color for loess line.

See Also[mva.pairs](#)**Examples**

```

if (require(affydata)) {
  data(Dilution)
  MAplot(Dilution)
  Mbox(Dilution)
}

```

mas5

*MAS 5.0 expression measure***Description**

This function converts an instance of [AffyBatch](#) into an instance of [ExpressionSet](#) using our implementation of Affymetrix's MAS 5.0 expression measure.

Usage

```
mas5(object, normalize = TRUE, sc = 500, analysis = "absolute", ...)
```

Arguments

object	an instance of AffyBatch
normalize	logical. If TRUE scale normalization is used after we obtain an instance of ExpressionSet
sc	Value at which all arrays will be scaled to.
analysis	should we do absolute or comparison analysis, although "comparison" is still not implemented.
...	other arguments to be passed to expresso .

Details

This function is a wrapper for [expresso](#) and [affy.scalevalue.exprSet](#).

Value[ExpressionSet](#)

The methods used by this function were implemented based upon available documentation. In particular a useful reference is Statistical Algorithms Description Document by Affymetrix. Our implementation is based on what is written in the documentation and, as you might appreciate, there are places where the documentation is less than clear. This function does not give exactly the same results. All source code of our implementation is available. You are free to read it and suggest fixes.

For more information visit this URL: <http://stat-www.berkeley.edu/users/bolstad/>

See Also[expresso](#), [affy.scalevalue.exprSet](#)

Examples

```
if (require(affydata)) {
  data(Dilution)
  eset <- mas5(Dilution)
}
```

mas5calls

*MAS 5.0 Absolute Detection***Description**

Performs the Wilcoxon signed rank-based gene expression presence/absence detection algorithm first implemented in the Affymetrix Microarray Suite version 5.

Usage

```
mas5calls(object,...)

mas5calls.AffyBatch(object, ids = NULL, verbose = TRUE, tau = 0.015,
  alpha1 = 0.04, alpha2 = 0.06,
  ignore.saturated=TRUE)

mas5calls.ProbeSet(object, tau = 0.015, alpha1 = 0.04, alpha2 = 0.06,
  ignore.saturated=TRUE)

mas5.detection(mat, tau = 0.015, alpha1 = 0.04, alpha2 = 0.06,
  exact.pvals = FALSE, cont.correct = FALSE)
```

Arguments

<code>object</code>	an object of class <code>AffyBatch</code> or <code>ProbeSet</code> .
<code>ids</code>	probeset IDs for which you want to compute calls.
<code>mat</code>	an n-by-2 matrix of paired values (pairs in rows), PMs first col.
<code>verbose</code>	logical. If TRUE, status of processing is reported.
<code>tau</code>	a small positive constant.
<code>alpha1</code>	a significance threshold in (0, alpha2).
<code>alpha2</code>	a significance threshold in (alpha1, 0.5).
<code>exact.pvals</code>	logical controlling whether exact p-values are computed (irrelevant if n<50 and there are no ties). Otherwise the normal approximation is used.
<code>ignore.saturated</code>	if TRUE, do the saturation correction described in the paper, with a saturation level of 46000.
<code>cont.correct</code>	logical controlling whether continuity correction is used in the p-value normal approximation.
<code>...</code>	any of the above arguments that applies.

Details

This function performs the hypothesis test:

H0: median(Ri) = tau, corresponding to absence of transcript
H1: median(Ri) > tau, corresponding to presence of transcript

where $R_i = (PM_i - MM_i) / (PM_i + MM_i)$ for each i a probe-pair in the probe-set represented by data.

Currently `exact.pvals=TRUE` is not supported, and `cont.correct=TRUE` works but does not give great results (so both should be left as `FALSE`). The defaults for tau, alpha1 and alpha2 correspond to those in MAS5.0.

The p-value that is returned estimates the usual quantity:

$\Pr(\text{observing a more "present looking" probe-set than data} \mid \text{data is absent})$

So that small p-values imply presence while large ones imply absence of transcript. The detection call is computed by thresholding the p-value as in:

call "P" if p-value < alpha1 call "M" if alpha1 <= p-value < alpha2 call "A" if alpha2 <= p-value

This implementation has been validated against the original MAS5.0 implementation with the following results (for `exact.pvals` and `cont.correct` set to F):

Average Relative Change from MAS5.0 p-values:38% Proportion of calls different to MAS5.0 calls:1.0%

where "average/proportion" means over all probe-sets and arrays, where the data came from 11 bacterial control probe-sets spiked-in over a range of concentrations (from 0 to 150 pico-mols) over 26 arrays. These are the spike-in data from the GeneLogic Concentration Series Spikein Dataset.

Clearly the p-values computed here differ from those computed by MAS5.0 – this will be improved in subsequent releases of the affy package. However the p-value discrepancies are small enough to result in the call being very closely aligned with those of MAS5.0 (99 percent were identical on the validation set) – so this implementation will still be of use.

The function `mas5.detect` is no longer the engine function for the others. C code is no available that computes the Wilcox test faster. The function is kept so that people can look at the R code (instead of C).

Value

`mas5.detect` returns a list containing the following components:

<code>pval</code>	a real p-value in [0,1] equal to the probability of observing probe-level intensities that are more present looking than data assuming the data represents an absent transcript; that is a transcript is more likely to be present for p-values closer 0.
<code>call</code>	either "P", "M" or "A" representing a call of present, marginal or absent; computed by simply thresholding pval using alpha1 and alpha2.

The `mas5calls` method for `AffyBatch` returns an `ExpressionSet` with calls accessible with `exprs(obj)` and p-values available with `assayData(obj)[["se.exprs"]]`. The code `mas5calls` for `ProbeSet` returns a list with vectors of calls and p-values.

Author(s)

Crispin Miller, Benjamin I. P. Rubinstein, Rafael A. Irizarry

References

- Liu, W. M. and Mei, R. and Di, X. and Ryder, T. B. and Hubbell, E. and Dee, S. and Webster, T. A. and Harrington, C. A. and Ho, M. H. and Baid, J. and Smeekens, S. P. (2002) Analysis of high density expression microarrays with signed-rank call algorithms, *Bioinformatics*, 18(12), pp. 1593–1599.
- Liu, W. and Mei, R. and Bartell, D. M. and Di, X. and Webster, T. A. and Ryder, T. (2001) Rank-based algorithms for analysis of microarrays, *Proceedings of SPIE, Microarrays: Optical Technologies and Informatics*, 4266.
- Affymetrix (2002) Statistical Algorithms Description Document, Affymetrix Inc., Santa Clara, CA, whitepaper. http://www.affymetrix.com/support/technical/whitepapers/sadd_whitepaper.pdf, http://www.affymetrix.com/support/technical/whitepapers/sadd_whitepaper.pdf

Examples

```
if (require(affydata)) {
  data(Dilution)
  PACalls <- mas5calls(Dilution)
}
```

merge.AffyBatch	<i>merge two AffyBatch objects</i>
-----------------	------------------------------------

Description

merge two AffyBatch objects into one.

Usage

```
## S3 method for class 'AffyBatch'
merge(x, y, annotation = paste(annotation(x),
                                annotation(y)), description = NULL, notes =
                                character(0), ...)
```

Arguments

x	an AffyBatch object.
y	an AffyBatch object.
annotation	a character vector.
description	a characterORmiame, eventually NULL.
notes	a character vector.
...	additional arguments.

Details

To be done.

Value

A object if class `AffyBatch`.

See Also[AffyBatch-class](#)

mva.pairs	<i>M vs. A Matrix</i>
-----------	-----------------------

Description

A matrix of M vs. A plots is produced. Plots are made on the upper triangle and the IQR of the Ms are displayed in the lower triangle

Usage

```
mva.pairs(x, labels=colnames(x), log.it=TRUE, span=2/3, family.loess="gaussian",
          digits=3, line.col=2, main="MVA plot", cex=2, ...)
```

Arguments

x	a matrix containing the chip data in the columns.
labels	the names of the variables.
log.it	logical. If TRUE, uses log scale.
span	span to be used for loess fit.
family.loess	"gaussian" or "symmetric" as in loess .
digits	number of digits to use in the display of IQR.
line.col	color of the loess line.
main	an overall title for the plot.
cex	size for text.
...	graphical parameters can be given as arguments to <code>mva.plot</code>
.	

See Also[pairs](#)**Examples**

```
x <- matrix(rnorm(4000), 1000, 4)
x[,1] <- x[,1]^2
dimnames(x) <- list(NULL, c("chip 1", "chip 2", "chip 3", "chip 4"))
mva.pairs(x, log=FALSE, main="example")
```

 normalize-methods

 Normalize Affymetrix Probe Level Data - methods

Description

Method for normalizing Affymetrix Probe Level Data

Usage

```
normalize.methods(object)
bgcorrect.methods()
update.bgcorrect.methods(x)
pmcorrect.methods()
update.pmccorrect.methods(x)
```

Arguments

object	An AffyBatch .
x	A character vector that will replace the existing one.

Details

If object is an [AffyBatch](#) object, then `normalize(object)` returns an [AffyBatch](#) object with the intensities normalized using the methodology specified by `getOption("BioC")$affy$normalize.method`. The affy package default is quantiles.

Other methodologies can be used by specifying them with the `method` argument. For example to use the invariant set methodology described by Li and Wong (2001) one would type: `normalize(object, method="invariantset")`.

Further arguments passed by `...`, apart from `method`, are passed along to the function responsible for the methodology defined by the `method` argument.

A character vector of *nicknames* for the methodologies available is returned by `normalize.methods(object)`, where object is an [AffyBatch](#), or simply by typing `normalize.AffyBatch.methods`. If the nickname of a method is called "loess", the help page for that specific methodology can be accessed by typing `?normalize.loess`.

For more on the normalization methodologies currently implemented please refer to the vignette 'Custom Processing Methods'.

To add your own normalization procedures please refer to the `customMethods` vignette.

The functions: `bgcorrect.methods`, `pmcorrect.methods`, provide access to internal vectors listing the corresponding capabilities.

See Also

[AffyBatch-class](#), [normalize](#).

Examples

```
if (require(affydata)) {  
  data(Dilution)  
  normalize.methods(Dilution)  
  generateExprSet.methods()  
  bgcorrect.methods()  
  pmcorrect.methods()  
}
```

normalize.constant	<i>Scale probe intensities</i>
--------------------	--------------------------------

Description

Scale array intensities in a [AffyBatch](#).

Usage

```
normalize.AffyBatch.constant(abatch, refindex=1, FUN=mean, na.rm=TRUE)  
normalize.constant(x, refconstant, FUN=mean, na.rm=TRUE)
```

Arguments

abatch	an instance of the AffyBatch-class .
x	a vector of intensities on a chip (to normalize to the reference).
refindex	the index of the array used as a reference.
refconstant	the constant used as a reference.
FUN	a function generating a value from the intensities on an array. Typically mean or median.
na.rm	parameter passed to the function FUN.

Value

An [AffyBatch](#) with an attribute "constant" holding the value of the factor used for scaling.

Author(s)

L. Gautier <laurent@cbs.dtu.dk>

See Also

[AffyBatch](#)

normalize.contrasts	<i>Normalize intensities using the contrasts method</i>
---------------------	---

Description

Scale chip objects in an [AffyBatch-class](#).

Usage

```
normalize.AffyBatch.contrasts(abatch, span=2/3, choose.subset=TRUE,
                             subset.size=5000, verbose=TRUE,
                             family="symmetric",
                             type=c("together", "pmonly", "mmonly", "separate"))
```

Arguments

abatch	an AffyBatch-class object.
span	parameter to be passed to the function loess .
choose.subset	Boolean. Defaults to TRUE
subset.size	Integer. Number of probesets to use in each subset.
verbose	verbosity flag.
family	parameter to be passed to the function loess .
type	a string specifying how the normalization should be applied.

Value

An object of the same class as the one passed.

See Also

[maffy.normalize](#)

normalize.invariantset	<i>Invariant Set normalization</i>
------------------------	------------------------------------

Description

Normalize arrays in an [AffyBatch](#) using an invariant set.

Usage

```
normalize.AffyBatch.invariantset(abatch, prd.td = c(0.003, 0.007),
                                verbose = FALSE,
                                baseline.type = c("mean", "median", "pseudo-mean", "pseudo-median"),
                                type = c("separate", "pmonly", "mmonly", "together"))

normalize.invariantset(data, ref, prd.td=c(0.003,0.007))
```

Arguments

abatch	an AffyBatch object.
data	a vector of intensities on a chip (to normalize to the reference).
ref	a vector of reference intensities.
prd.td	cutoff parameter (details in the bibliographic reference).
baseline.type	specifies how to determine the baseline array.
type	a string specifying how the normalization should be applied. See details for more.
verbose	logical indicating printing throughout the normalization.

Details

The set of invariant intensities between data and ref is found through an iterative process (based on the respective ranks the intensities). This set of intensities is used to generate a normalization curve by smoothing.

The type argument should be one of "separate", "pmonly", "mmonly", "together" which indicates whether to normalize only one probe type (PM,MM) or both together or separately.

Value

Respectively a [AffyBatch](#) of normalized objects, or a vector of normalized intensities, with an attribute "invariant.set" holding the indexes of the 'invariant' intensities.

Author(s)

L. Gautier <laurent@cbs.dtu.dk> (Thanks to Cheng Li for the discussions about the algorithm.)

References

Cheng Li and Wing Hung Wong, Model-based analysis of oligonucleotides arrays: model validation, design issues and standard error application. *Genome Biology* 2001, 2(8):research0032.1-0032.11

See Also

[normalize](#) to normalize [AffyBatch](#) objects.

normalize.loess	<i>Scale microarray data</i>
-----------------	------------------------------

Description

Normalizes arrays using loess.

Usage

```
normalize.loess(mat, subset = sample(1:(dim(mat)[1]), min(c(5000,
  nrow(mat)))), epsilon = 10^-2, maxit = 1, log.it =
  TRUE, verbose = TRUE, span = 2/3, family.loess =
  "symmetric")
normalize.AffyBatch.loess(abatch,type=c("together","pmonly","mmonly","separate"), ...)
```

Arguments

mat	a matrix with columns containing the values of the chips to normalize.
abatch	an AffyBatch object.
subset	a subset of the data to fit a loess to.
epsilon	a tolerance value (supposed to be a small value - used as a stopping criterion).
maxit	maximum number of iterations.
log.it	logical. If TRUE it takes the log2 of mat
verbose	logical. If TRUE displays current pair of chip being worked on.
span	parameter to be passed the function loess
family.loess	parameter to be passed the function loess . "gaussian" or "symmetric" are acceptable values for this parameter.
type	A string specifying how the normalization should be applied. See details for more.
...	any of the options of <code>normalize.loess</code> you would like to modify (described above).

Details

The type argument should be one of "separate", "pmonly", "mmonly", "together" which indicates whether to normalize only one probe type (PM,MM) or both together or separately.

See Also

[normalize](#)

Examples

```
if (require(affydata)) {
  #data(Dilution)
  #x <- pm(Dilution[,1:3])
  #mva.pairs(x)
  #x <- normalize.loess(x,subset=1:nrow(x))
  #mva.pairs(x)
}
```

normalize.qspline	<i>Normalize arrays</i>
-------------------	-------------------------

Description

normalizes arrays in an `AffyBatch` each other or to a set of target intensities

Usage

```
normalize.AffyBatch.qspline(abatch,type=c("together", "pmonly", "mmonly",
"separate"), ...)

normalize.qspline(x, target = NULL, samples = NULL,
  fit.iters = 5, min.offset = 5,
  spline.method = "natural", smooth = TRUE,
  spar = 0, p.min = 0, p.max = 1.0,
  incl.ends = TRUE, converge = FALSE,
  verbose = TRUE, na.rm = FALSE)
```

Arguments

x	a data.matrix of intensities
abatch	an AffyBatch
target	numerical vector of intensity values to normalize to. (could be the name for one of the celfiles in 'abatch').
samples	numerical, the number of quantiles to be used for spline. if (0,1], then it is a sampling rate.
fit.iters	number of spline interpolations to average.
min.offset	minimum span between quantiles (rank difference) for the different fit iterations.
spline.method	specifies the type of spline to be used. Possible values are "fmm", "natural", and "periodic".
smooth	logical, if 'TRUE', smoothing splines are used on the quantiles.
spar	smoothing parameter for 'splinefun', typically in (0,1].
p.min	minimum percentile for the first quantile.
p.max	maximum percentile for the last quantile.
incl.ends	include the minimum and maximum values from the normalized and target arrays in the fit.
converge	(currently unimplemented)
verbose	logical, if 'TRUE' then normalization progress is reported.
na.rm	logical, if 'TRUE' then handle NA values (by ignoring them).
type	a string specifying how the normalization should be applied. See details for more.
...	optional parameters to be passed through.

Details

This normalization method uses the quantiles from each array and the target to fit a system of cubic splines to normalize the data. The target should be the mean (geometric) or median of each probe but could also be the name of a particular chip in the abatch object.

Parameters setting can be of much importance when using this method. The parameter `fit.iter` is used as a starting point to find a more appropriate value. Unfortunately the algorithm used do not converge in some cases. If this happens, the `fit.iter` value is used and a warning is thrown. Use of different settings for the parameter `samples` was reported to give good results. More specifically, for about 200 data points use `samples = 0.33`, for about 2000 data points use `samples = 0.05`, for about 10000 data points use `samples = 0.02` (thanks to Paul Boutros).

The `type` argument should be one of "separate", "pmonly", "mmonly", "together" which indicates whether to normalize only one probe type (PM,MM) or both together or separately.

Value

a normalized AffyBatch.

Author(s)

Laurent and Workman C.

References

Christopher Workman, Lars Juhl Jensen, Hanne Jarmer, Randy Berka, Laurent Gautier, Henrik Bjorn Nielsen, Hans-Henrik Saxild, Claus Nielsen, Soren Brunak, and Steen Knudsen. A new non-linear normalization method for reducing variability in dna microarray experiments. Genome Biology, accepted, 2002

normalize.quantiles	<i>Quantile Normalization</i>
---------------------	-------------------------------

Description

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

Usage

```
normalize.AffyBatch.quantiles(abatch, type=c("separate", "pmonly", "mmonly", "together"))
```

Arguments

abatch	an AffyBatch object.
type	A string specifying how the normalization should be applied. See details for more.

Details

This method is based upon the concept of a quantile-quantile plot extended to n dimensions. No special allowances are made for outliers. If you make use of quantile normalization either through [rma](#) or [expresso](#) please cite Bolstad et al, Bioinformatics (2003).

The type argument should be one of "separate", "pmonly", "mmonly", "together" which indicates whether to normalize only one probe type (PM,MM) or both together or separately.

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://bmbolstad.com/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://bmbolstad.com/misc/normalize/normalize.html>

See Also

[normalize](#)

normalize.quantiles.robust

Robust Quantile Normalization

Description

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

Usage

```
normalize.AffyBatch.quantiles.robust(abatch,
                                     type = c("separate", "pmonly", "mmonly", "together"),
                                     weights = NULL,
                                     remove.extreme = c("variance", "mean", "both", "none"),
                                     n.remove = 1, use.median = FALSE,
                                     use.log2 = FALSE)
```

Arguments

abatch	an AffyBatch object.
type	a string specifying how the normalization should be applied. See details for more.
weights	a vector of weights, one for each chip.
remove.extreme	if weights is NULL, then this will be used for determining which chips to remove from the calculation of the normalization distribution. See details for more info.
n.remove	number of chips to remove.
use.median	if TRUE, the use the median to compute normalization chip; otherwise uses a weighted mean.
use.log2	work on log2 scale. This means we will be using the geometric mean rather than ordinary mean.

Details

This method is based upon the concept of a quantile-quantile plot extended to n dimensions. Note that the matrix is of intensities not log intensities. The function performs better with raw intensities.

Choosing **variance** will remove chips with variances much higher or lower than the other chips, **mean** removes chips with the mean most different from all the other means, **both** removes first extreme variance and then an extreme mean. The option **none** does not remove any chips, but will assign equal weights to all chips.

The type argument should be one of "separate", "pmonly", "mmonly", "together" which indicates whether to normalize only one probe type (PM,MM) or both together or separately.

Value

a matrix of normalized intensities

Note

This function is still experimental.

Author(s)

Ben Bolstad, <bmb@bmbolstad.com>

See Also

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

pairs.AffyBatch	<i>plot intensities using 'pairs'</i>
-----------------	---------------------------------------

Description

Plot intensities using the function 'pairs'

Usage

```
## S3 method for class 'AffyBatch'
pairs(x, panel=points, ..., transfo=I, main=NULL, oma=NULL,
      font.main = par("font.main"),
      cex.main = par("cex.main"), cex.labels = NULL,
      lower.panel=panel, upper.panel=NULL, diag.panel=NULL,
      font.labels = 1, rowlattop = TRUE, gap = 1)
```

Arguments

x	an AffyBatch object.
panel	a function to produce a plot (see pairs).
...	extra parameters for the 'panel' function.
transfo	a function to transform the intensity values before generating the plot. 'log' and 'log2' are popular choices.
main	title for the plot

oma	see 'oma' in par .
font.main	see pairs .
cex.main	see pairs .
cex.labels	see pairs .
lower.panel	a function to produce the plots in the lower triangle (see pairs).
upper.panel	a function to produce the plots in the upper triangle (see pairs).
diag.panel	a function to produce the plots in the diagonal (see pairs).
font.labels	see pairs .
rowlattice	see pairs .
gap	see pairs .

Details

Plots with several chips can represent zillions of points. They require a lot of memory and can be very slow to be displayed. You may want to try to split of the plots, or to plot them in a device like 'png' or 'jpeg'.

plot.ProbeSet	<i>plot a probe set</i>
---------------	-------------------------

Description

Plot intensities by probe set.

Usage

```
## S3 method for class 'ProbeSet'
plot(x, which=c("pm", "mm"), xlab = "probes", type = "l", ylim = NULL, ...)
```

Arguments

x	a ProbeSet object.
which	get the PM or the MM.
xlab	x-axis label.
type	plot type.
ylim	range of the y-axis.
...	optional arguments to be passed to <code>matplot</code> .

Value

This function is only used for its (graphical) side-effect.

See Also

[ProbeSet](#)

Examples

```
data(SpikeIn)
plot(SpikeIn)
```

plotDensity

*Plot Densities***Description**

Plots the non-parametric density estimates using values contained in the columns of a matrix.

Usage

```
plotDensity(mat, ylab = "density", xlab="x", type="l", col=1:6,
            na.rm = TRUE, ...)
```

```
plotDensity.AffyBatch(x, col = 1:6, log = TRUE,
                      which=c("pm", "mm", "both"),
                      ylab = "density",
                      xlab = NULL, ...)
```

Arguments

mat	a matrix containing the values to make densities in the columns.
x	an object of class AffyBatch .
log	logical value. If TRUE the log of the intensities in the AffyBatch are plotted.
which	should a histogram of the PMs, MM, or both be made?
col	the colors to use for the different arrays.
ylab	a title for the y axis.
xlab	a title for the x axis.
type	type for the plot.
na.rm	handling of NA values.
...	graphical parameters can be given as arguments to plot .

Details

The list returned can be convenient for plotting large input matrices with different colors/line types schemes (the computation of the densities can take some time).

To match other functions in base R, this function should probably be called `matdensity`, as it is sharing similarities with `matplot` and `matlines`.

Value

It returns invisibly a list of two matrices 'x' and 'y'.

Author(s)

Ben Bolstad and Laurent Gautier

Examples

```
if (require(affydata)) {
  data(Dilution)
  plotDensity(exprs(Dilution), log="x")
}
```

plotLocation	<i>Plot a location on a cel image</i>
--------------	---------------------------------------

Description

Plots a location on a previously plotted cel image. This can be used to locate the physical location of probes on the array.

Usage

```
plotLocation(x, col="green", pch=22, ...)
```

Arguments

x	a 'location'. It can be obtained by the method of AffyBatch indexProbes, or made elsewhere (basically a location is n rows and two columns array. The first column corresponds to the x positions and the second columns corresponds to the y positions of n elements to locate).
col	colors for the plot.
pch	plotting type (see function plot).
...	other parameters passed to the function points.

Author(s)

Laurent

See Also

[AffyBatch](#)

Examples

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

  ## image of the cel file
  image(Dilution[, 1])

  ## genenames, arbitrarily pick the 101th
  n <- geneNames(Dilution)[101]

  ## get the location for the gene n
  l <- indexProbes(Dilution, "both", n)[[1]]
  ## convert the index to X/Y coordinates
  xy <- indices2xy(l, abatch=Dilution)

  ## plot
  plotLocation(xy)
}
```

pmcorrect

*PM Correction***Description**

Corrects the PM intensities in a [ProbeSet](#) for non-specific binding.

Usage

```
pmcorrect.pmonly(object)
```

```
pmcorrect.subtractmm(object)
```

```
pmcorrect.mas(object, contrast.tau=0.03, scale.tau=10, delta=2^(-20))
```

Arguments

object	An object of class ProbeSet .
contrast.tau	a number denoting the contrast tau parameter in the MAS 5.0 pm correction algorithm.
scale.tau	a number denoting the scale tau parameter in the MAS 5.0 pm correction algorithm.
delta	a number denoting the delta parameter in the MAS 5.0 pm correction algorithm.

Details

These are the pm correction methods performed by Affymetrix MAS 4.0 (subtractmm) and MAS 5.0 (mas). See the Affymetrix Manual for details. pmonly does what you think: does not change the PM values.

Value

A [ProbeSet](#) for which the pm slot contains the corrected PM values.

References

Affymetrix MAS 4.0 and 5.0 manual

Examples

```
if (require(affydata)) {
  data(Dilution)
  gn <- geneNames(Dilution)
  pps <- probeset(Dilution, gn[1]][[1]]

  pps.pmonly <- pmcorrect.pmonly(pps)
  pps.subtractmm <- pmcorrect.subtractmm(pps)
  pps.mas5 <- pmcorrect.mas(pps)
}
```

ppsetApply

*Apply a function over the ProbeSets in an AffyBatch***Description**

Apply a function over the ProbeSets in an AffyBatch

Usage

```
ppsetApply(abatch, FUN, genenames = NULL, ...)
```

```
ppset.ttest(ppset, covariate, pmcorrect.fun = pmcorrect.pmonly, ...)
```

Arguments

abatch	an object inheriting from AffyBatch.
ppset	an object of class ProbeSet.
covariate	the name a covariate in the slot phenoData.
pmcorrect.fun	a function to correct PM intensities.
FUN	a function working on a ProbeSet.
genenames	a list of Affymetrix probesets ids to work with. All probe set ids used when NULL.
...	optional parameters to the function FUN.

Value

Returns a list of objects, or values, as returned by the function FUN for each ProbeSet it processes.

Author(s)

Laurent Gautier <laurent@cbs.dtu.dk>

See Also

[ProbeSet-class](#)

Examples

```
ppset.ttest <- function(ppset, covariate, pmcorrect.fun = pmcorrect.pmonly, ...) {
  probes <- do.call("pmcorrect.fun", list(ppset))
  my.ttest <- function(x) {
    y <- split(x, get(covariate))
    t.test(y[[1]], y[[2]])$p.value
  }
  r <- apply(probes, 1, my.ttest)
  return(r)
}
##this takes a long time - and rowttests is a good alternative
## eg: rt = rowttests(exprs(Dilution), Dilution$liver)
## Not run:
data(Dilution)
```

```
all.ttest <- ppsetApply(Dilution, ppset.ttest, covariate="liver")

## End(Not run)
```

probeMatch-methods	<i>Methods for accessing perfect matches and mismatches</i>
--------------------	---

Description

Methods for perfect matches and mismatches probes

Methods

object = AffyBatch All the *perfect match* (pm) or *mismatch* (mm) probes on the arrays the object represents are returned.

object = ProbeSet The pm or mm of the object are returned.

probeNames-methods	<i>Methods for accessing the Probe Names</i>
--------------------	--

Description

Methods for accessing Probe Names

Methods

object = Cdf an accessor function for the name slot.

object = probeNames returns the probe names associated with the rownames of the intensity matrices one gets with the pm and mm methods.

ProbeSet-class	<i>Class ProbeSet</i>
----------------	-----------------------

Description

A simple class that contains the PM and MM data for a probe set from one or more samples.

Objects from the Class

Objects can be created by applying the method [probeset](#) to instances of AffyBatch.

Slots

id: Object of class "character" containing the probeset ID.

pm: Object of class "matrix" containing the PM intensities. Columns represent samples and rows the different probes.

mm: Object of class "matrix" containing the MM intensities.

Methods

colnames signature(`x = "ProbeSet"`): the column names of the pm matrices which are the sample names

express.summary.stat signature(`x = "ProbeSet"`, `pmcorrect = "character"`, `summary = "character"`): applies a summary statistic to the probe set.

sampleNames signature(`object = "ProbeSet"`): the column names of the pm matrices which are the sample names.

Note

More details are contained in the vignette.

See Also

[probeset](#), [AffyBatch-class](#)

Examples

```
if (require(affydata)) {
  data(Dilution)
  ps <- probeset(Dilution, geneNames(Dilution)[1:2])
  names(ps)
  print(ps[[1]])
}
```

ProgressBarText-class *Class "ProgressBarText"*

Description

A class to handle progress bars in text mode.

Objects from the Class

Objects can be created by calls of the form `new("ProgressBarText", steps)`.

Slots

steps: Object of class "integer". The total number of steps the progress bar should represent.

barsteps: Object of class "integer". The size of the progress bar.

internals: Object of class "environment". For internal use.

Methods

close signature(`con = "ProgressBarText"`): Terminate the progress bar (i.e. print what needs to be printed). Note that closing the instance will ensure the progress bar is plotted to its end.

initialize signature(`.Object = "ProgressBarText"`): initialize a instance.

open signature(`con = "ProgressBarText"`): Open a progress bar (i.e. print things). In the case open is called on a progress bar that was 'progress', the progress bar is resumed (this might be useful when one wishes to insert text output while there is a progress bar running).

updateMe signature(`object = "ProgressBarText"`): Update the progress bar (see examples).

Author(s)

Laurent

Examples

```
f <- function(x, header = TRUE) {
  pbt <- new("ProgressBarText", length(x), barsteps = as.integer(20))

  open(pbt, header = header)

  for (i in x) {
    Sys.sleep(i)
    updateMe(pbt)
  }
  close(pbt)
}

## if too fast on your machine, change the number
x <- runif(15)

f(x)
f(x, header = FALSE)

## 'cost' of the progress bar:
g <- function(x) {
  z <- 1
  for (i in 1:x) {
    z <- z + 1
  }
}
h <- function(x) {
  pbt <- new("ProgressBarText", as.integer(x), barsteps = as.integer(20))
  open(pbt)
  for (i in 1:x) {
    updateMe(pbt)
  }
  close(pbt)
}

system.time(g(10000))
system.time(h(10000))
```

read.affybatch*Read CEL files into an AffyBatch*

Description

Read CEL files into an Affybatch.

Usage

```
read.affybatch(..., filenames = character(0),
               phenoData = new("AnnotatedDataFrame"),
```

```

description = NULL,
notes = "",
compress = getOption("BioC")$affy$compress.cel,
rm.mask = FALSE, rm.outliers = FALSE, rm.extra = FALSE,
verbose = FALSE, sd=FALSE, cdfname = NULL)

ReadAffy(..., filenames=character(0),
widget=getOption("BioC")$affy$use.widgets,
compress=getOption("BioC")$affy$compress.cel,
celfile.path=NULL,
sampleNames=NULL,
phenoData=NULL,
description=NULL,
notes="",
rm.mask=FALSE, rm.outliers=FALSE, rm.extra=FALSE,
verbose=FALSE, sd=FALSE, cdfname = NULL)

```

Arguments

...	file names separated by comma.
filenames	file names in a character vector.
phenoData	an AnnotatedDataFrame object, a character of length one, or a data.frame.
description	a MIAME object.
notes	notes.
compress	are the CEL files compressed?
rm.mask	should the spots marked as 'MASKS' set to NA?
rm.outliers	should the spots marked as 'OUTLIERS' set to NA?
rm.extra	if TRUE, then overrides what is in rm.mask and rm.outliers.
verbose	verbosity flag.
widget	a logical specifying if widgets should be used.
celfile.path	a character denoting the path ReadAffy should look for cel files.
sampleNames	a character vector of sample names to be used in the AffyBatch.
sd	should the standard deviation values in the CEL file be read in? Since these are typically not used default is not to read them in. This also save lots of memory.
cdfname	used to specify the name of an alternative cdf package. If set to NULL, then the usual cdf package based on Affymetrix's mappings will be used.

Details

ReadAffy is a wrapper for read.affybatch that permits the user to read in phenoData, MIAME information, and CEL files using widgets. One can also define files where to read phenoData and MIAME information.

If the function is called with no arguments ReadAffy() all the CEL files in the working directory are read and put into an AffyBatch. However, the arguments give the user great flexibility.

If phenoData is a character vector of length 1, the function [read.AnnotatedDataFrame](#) is called to read a file of that name and produce the AnnotationDataFrame object with the sample meta-data. If phenoData is a data.frame, it is converted to an AnnotatedDataFrame. If it is NULL and widget=FALSE (widget=TRUE is not currently supported), then a default object of class [AnnotatedDataFrame](#)

is created, whose pData is a data.frame with rownames being the names of the CEL files, and with one column sample with an integer index.

AllButCelsForReadAffy is an internal function that gets called by ReadAffy. It gets all the information except the cel intensities.

description is read using [read.MIAME](#). If a character is given, then it tries to read the file with that name to obtain a MIAME instance. If left NULL but widget=TRUE, then widgets are used. If left NULL and widget=FALSE, then an empty instance of MIAME is created.

Value

An AffyBatch object.

Author(s)

Ben Bolstad <bmb@bmbolstad.com> (read.affybatch), Laurent Gautier, and Rafael A. Irizarry (ReadAffy)

See Also

[AffyBatch](#)

Examples

```
if(require(affydata)){
  celpath <- system.file("celfiles", package="affydata")
  fns <- list.celfiles(path=celpath,full.names=TRUE)

  cat("Reading files:\n",paste(fns,collapse="\n"),"\n")
  ##read a binary celfile
  abatch <- ReadAffy(filename=fns[1])
  ##read a text celfile
  abatch <- ReadAffy(filename=fns[2])
  ##read all files in that dir
  abatch <- ReadAffy(celfile.path=celpath)
}
```

read.probematrix

Read CEL file data into PM or MM matrices

Description

Read CEL data into matrices.

Usage

```
read.probematrix(..., filenames = character(0),
  phenoData = new("AnnotatedDataFrame"),
  description = NULL,
  notes = "",
  compress = getOption("BioC")$affy$compress.cel,
  rm.mask = FALSE, rm.outliers = FALSE, rm.extra = FALSE,
  verbose = FALSE, which = "pm", cdname = NULL)
```

Arguments

...	file names separated by comma.
filenames	file names in a character vector.
phenoData	a AnnotatedDataFrame object.
description	a MIAME object.
notes	notes.
compress	are the CEL files compressed?
rm.mask	should the spots marked as 'MASKS' set to NA?
rm.outliers	should the spots marked as 'OUTLIERS' set to NA?
rm.extra	if TRUE, overrides what is in rm.mask and rm.outliers.
verbose	verbosity flag.
which	should be either "pm", "mm" or "both".
cdfname	Used to specify the name of an alternative cdf package. If set to NULL, the usual cdf package based on Affymetrix's mappings will be used.

Value

A list of one or two matrices. Each matrix is either PM or MM data. No [AffyBatch](#) is created.

Author(s)

Ben Bolstad <bmb@bmbolstad.com>

See Also

[AffyBatch](#), [read.affybatch](#)

rma

Robust Multi-Array Average expression measure

Description

This function converts an [AffyBatch](#) object into an [ExpressionSet](#) object using the robust multi-array average (RMA) expression measure.

Usage

```
rma(object, subset=NULL, verbose=TRUE, destructive=TRUE, normalize=TRUE,
     background=TRUE, bgversion=2, ...)
```

Arguments

object	an AffyBatch object.
subset	a character vector with the the names of the probesets to be used in expression calculation.
verbose	logical value. If TRUE, it writes out some messages indicating progress. If FALSE nothing should be printed.
destructive	logical value. If TRUE, works on the PM matrix in place as much as possible, good for large datasets.
normalize	logical value. If TRUE, normalize data using quantile normalization.
background	logical value. If TRUE, background correct using RMA background correction.
bgversion	integer value indicating which RMA background to use 1: use background similar to pure R rma background given in affy version 1.0 - 1.0.2 2: use background similar to pure R rma background given in affy version 1.1 and above
...	further arguments to be passed (not currently implemented - stub for future use).

Details

This function computes the RMA (Robust Multichip Average) expression measure described in Irizarry et al Biostatistics (2003).

Note that this expression measure is given to you in log base 2 scale. This differs from most of the other expression measure methods.

Please note that the default background adjustment method was changed during the lead up to the Bioconductor 1.2 release. This means that this function and [expresso](#) should give results that directly agree.

Value

An [ExpressionSet](#)

Author(s)

Ben Bolstad <bmb@bmbolstad.com>

References

Rafael. A. Irizarry, Benjamin M. Bolstad, Francois Collin, Leslie M. Cope, Bridget Hobbs and Terence P. Speed (2003), Summaries of Affymetrix GeneChip probe level data Nucleic Acids Research 31(4):e15

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):185-193

Irizarry, RA, Hobbs, B, Collin, F, Beazer-Barclay, YD, Antonellis, KJ, Scherf, U, Speed, TP (2003) Exploration, Normalization, and Summaries of High Density Oligonucleotide Array Probe Level Data. Biostatistics .Vol. 4, Number 2: 249-264

See Also

[expresso](#)

Examples

```
if (require(affydata)) {
  data(Dilution)
  eset <- rma(Dilution)
}
```

SpikeIn

*SpikeIn Experiment Data: ProbeSet Example***Description**

This [ProbeSet](#) represents part of SpikeIn experiment data set.

Usage

```
data(SpikeIn)
```

Format

SpikeIn is [ProbeSet](#) containing the \$PM\$ and \$MM\$ intensities for a gene spiked in at different concentrations (given in the vector `colnames(pm(SpikeIn))`) in 12 different arrays.

Source

This comes from an experiments where 11 different cRNA fragments have been added to the hybridization mixture of the GeneChip arrays at different pM concentrations. The 11 control cRNAs were BioB-5, BioB-M, BioB-3, BioC-5, BioC-3, BioDn-5 (all *E. coli*), CreX-5, CreX-3 (phage P1), and DapX-5, DapX-M, DapX-3 (*B. subtilis*). The cRNA were chosen to match the target sequence for each of the Affymetrix control probe sets. For example, for DapX (a *B. subtilis* gene), the 5', middle and 3' target sequences (identified by DapX-5, DapX-M, DapX-3) were each synthesized separately and spiked-in at a specific concentration. Thus, for example, DapX-3 target sequence may be added to the total hybridization solution of 200 micro-liters to give a final concentration of 0.5 pM.

For this example we have the \$PM\$ and \$MM\$ for BioB-5 obtained from the arrays where it was spiked in at 0.0, 0.5, 0.75, 1, 1.5, 2, 3, 5, 12.5, 25, 50, and 150 pM.

For more information see Irizarry, R.A., et al. (2001) <http://biosun01.biostat.jhsph.edu/~ririzarr/papers/index.html>

summary

*Probe Set Summarizing Functions***Description**

These were used with the function `express`, which is no longer part of the package. Some are still used by the `generateExprVal` functions, but you should avoid using them directly.

See Also

[expresso](#)

tukey.biweight	<i>One-step Tukey's biweight</i>
----------------	----------------------------------

Description

One-step Tukey's biweight on a matrix.

Usage

```
tukey.biweight(x, c = 5, epsilon = 1e-04)
```

Arguments

x	a matrix.
c	tuning constant (see details).
epsilon	fuzzy value to avoid division by zero (see details).

Details

The details can be found in the given reference.

Value

a vector of values (one value per column in the input matrix).

References

Statistical Algorithms Description Document, 2002, Affymetrix.

See Also

[pmcorrect.mas](#) and [generateExprVal.method.mas](#)

whatcdf	<i>Find which CDF corresponds</i>
---------	-----------------------------------

Description

Find which kind of CDF corresponds to a CEL file.

Usage

```
whatcdf(filename, compress = getOption("BioC")$affy$compress.cel)
```

Arguments

filename	a '.CEL' file name.
compress	logical (file compressed or not).

Details

Information concerning the corresponding CDF file seems to be found in CEL files. This allows us to try to link CDF information automatically.

Value

a character with the name of the CDF.

See Also

`getInfoInAffyFile`, `read.celfile`

xy2indices

Functions to convert indices to x/y (and reverse)

Description

Functions to convert indices to x/y (and reverse)

Usage

```
xy2indices(x, y, nc = NULL, cel = NULL, abatch = NULL, cdf = NULL, xy.offset = NULL)
indices2xy(i, nc = NULL, cel = NULL, abatch = NULL, cdf = NULL, xy.offset = NULL)
```

Arguments

x	A numeric vector of X (column) position(s) for the probes.
y	A numeric vector of Y (row) position(s) for the probes.
i	A numeric vector of indices in the AffyBatch for the probes.
nc	total number of columns on the chip. It is usually better to specify either the cdf or abatch arguments rather than the number of columns.
cel	a corresponding object of class <code>Cel</code> . This has been deprecated. Use abatch or cdf instead.
abatch	a corresponding object of class AffyBatch .
cdf	character - the name of the corresponding cdf package.
xy.offset	an eventual offset for the XY coordinates. See Details.

Details

The Affymetrix scanner reads data from a GeneChip by row, and exports those data to a CEL file. When we read in the CEL file data to an AffyBatch object, we store data for each GeneChip as a single column in a matrix of probe-wise intensity values.

The CDF files that Affymetrix make available for various GeneChips map individual probes to probesets based on their (x,y) coordinates on the GeneChip. Note that these coordinates are zero-based, and (x,y) is the same as (column, row). In other words, the x coordinate indicates the horizontal location of the probe, and the y coordinate indicates the vertical location of the probe. By convention, (0,0) is the coordinate location for the top left position, and (ncol-1, nrow-1) is the coordinate location of the lower right position.

For most users, the mapping of probes to probeset is handled internally by various functions (rma, espresso, etc), and in general usage it is never necessary for a user to convert probe index position in an AffyBatch to the corresponding (x,y) coordinates on the GeneChip. These functions are only useful for those who wish to know more about the internal workings of the Affymetrix GeneChip.

The parameter `xy.offset` is there for compatibility. For historical reasons, the xy-coordinates for the features on Affymetrix GeneChips were decided to start at 1 (one) rather than 0 (zero). One can set the offset to 1 or to 0. Unless the you *_really_* know what you are doing, it is advisable to let it at the default value NULL. This way the package-wide option `xy.offset` is always used.

Value

A vector of indices or a two-columns matrix of Xs and Ys.

Warning

Even if one really knows what is going on, playing with the parameter `xy.offset` could be risky. Changing the package-wide option `xy.offset` appears much more sane.

Author(s)

L.

See Also

[indexProbes](#)

Examples

```
if (require(affydata)) {
  data(Dilution)
  pm.i <- indexProbes(Dilution, which="pm", genenames="AFFX-BioC-5_at")[[1]]
  mm.i <- indexProbes(Dilution, which="mm", genenames="AFFX-BioC-5_at")[[1]]

  pm.i.xy <- indices2xy(pm.i, abatch = Dilution)
  mm.i.xy <- indices2xy(mm.i, abatch = Dilution)

  ## and back to indices
  i.pm <- xy2indices(pm.i.xy[,1], pm.i.xy[,2], cdf = "hgu95av2cdf")
  i.mm <- xy2indices(mm.i.xy[,1], mm.i.xy[,2], cdf = "hgu95av2cdf")

  identical(pm.i, as.integer(i.pm))
  identical(mm.i, as.integer(i.mm))

  image(Dilution[1], transfo=log2)
  ## plot the pm in red
  plotLocation(pm.i.xy, col="red")
  plotLocation(mm.i.xy, col="blue")
}
```

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