# affy

# April 19, 2010

AffvBatch-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.

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#### Methods

```
cdfName signature(object = "AffyBatch"): obtains the cdfName slot.
pm<- signature(object = "AffyBatch"): replaces the perfect match intensities.</pre>
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 mis-
    match 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 ex-
    pression 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</pre>
    matrix of standard errors of expression values.
[<- signature(x = "AffyBatch"): replaces subsets.</pre>
[ 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 re-
    ferred 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</pre>
    slot, i.e. the intensities.
intensity signature (object = "AffyBatch"): extract the exprs slot, i.e. the intensi-
length signature (x = "AffyBatch"): returns the number of samples.
pmindex signature(object = "AffyBatch"): return the location of perfect matches in
    the intensity matrix.
```

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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 pro vides 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")</pre>
  ## 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)
```

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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 it self a list with two elements what and where. When looking for the informations 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)</pre>
```

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.

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#### Usage

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

#### **Arguments**

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

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

# Author(s)

Leslie Cope

#### **Examples**

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

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```
affy.scalevalue.exprSet

Scale normalization for expreSets
```

# **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

```
barplot.ProbeSet show a ProbeSet as barplots
```

#### **Description**

Displays the probe intensities in a ProbeSet as a barplots

```
## 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, ...)
```

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#### **Arguments**

```
an object of class ProbeSet.
height
                  label for x axis.
xlab
ylab
                  label for y axis.
                  main label for the figure.
main
col.pm
                  color for the 'pm' intensities.
col.mm
                  color for the 'mm' intensities.
                  bars beside each others or not.
beside
names.arg
                  names to be plotted below each bar or group of bars.
                  ask before ploting the next barplot.
ask
                  put all the barplot to the same scale.
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)
}</pre>
```

bg.adjust

Background adjustment (internal function)

# **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.

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#### Value

a matrix

#### See Also

```
bg.correct.rma
```

bg.correct

**Background Correction** 

# 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 bg.correct.methods.
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.

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#### **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")
}</pre>
```

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**

cdfname name of the CDF.

lib install directory for the CDF package.

where environment to search.

verbose logical controlling extra output.

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#### **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

Clean Affymetrix's CDF name

#### **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'x 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")</pre>
```

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debug.affy123 Debugging Flag

### **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
        bq.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

```
afbatch an AffyBatch object.

bg.correct a boolean to express whether background correction is wanted or not.

bgcorrect.method
the name of the background adjustment method.

bgcorrect.param
a list of parameters for bgcorrect.method (if needed/wanted).

normalize normalization step wished or not.
```

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```
normalize.method
                 the normalization method to use.
normalize.param
                 a list of parameters to be passed to the normalization method (if wanted).
pmcorrect.method
                 the name of the PM adjustment method.
pmcorrect.param
                 a list of parameters for pmcorrect.method (if needed/wanted).
summary.method
                 the method used for the computation of expression values.
summary.param
                 a list of parameters to be passed to the summary.method (if wanted).
summary.subset
                 a list of 'affyids'. If NULL, an expression summary value is computed for ev-
                 erything 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**

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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

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#### References

Documentations of affy package

#### See Also

```
expresso
```

#### **Examples**

```
if(interactive()) {
    require(widgetTools)
    expressoWidget(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

#### **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.

fit.li.wong

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 phis.

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)

convergence 1 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 \leftarrow 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

x a AffyBatch holding the probe level informations to generate the expression values, for computeExprSet, and for upDate.generateExprSet.methods it is a character vector..

```
pmcorrect.method
```

the method used to correct PM values (see section 'details').

summary.method

the method used to generate the expression value (see section 'details').

... 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**

```
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, mas$exprs, pch="p", col=4, type="b", lty=4)</pre>
```

```
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-E
```

# **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).

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#### Value

A vector of expression values.

#### Author(s)

Laurent <a href="mailto:laurent@cbs.dtu.dk">Laurent@cbs.dtu.dk</a> (Thanks to E. Lazaridris 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 Biosciences, Volume 176, Issue 1, March 2002, Pages 53-58

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))</pre>
```

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```
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))
}</pre>
```

hlog

Hybrid Log

# **Description**

Given a constant c this function returns x if x is less than c and sign(x) \* (c\*log(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**

```
x a number.constant 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 21

justRMA Read CEL files into an ExpressionSet	justRMA	Read CEL files into an ExpressionSet	
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#### **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.
                 file names in a character vector.
filenames
                 an AnnotatedDataFrame object.
phenoData
description a MIAME object.
notes
                 notes.
                 are the CEL files compressed?
compress
                 should the spots marked as 'MASKS' set to NA?
rm.mask
                 should the spots marked as 'OUTLIERS' set to NA?
rm.outliers
                 if TRUE, then overrides what is in rm.mask and rm.oultiers.
rm.extra
                 use of hdf5? (not available yet)
hdf5
hdf5FilePath a filename to use with hdf5 (not available yet).
                 verbosity flag.
verbose
                 a logical specifying if widgets should be used.
widget
celfile.path a character denoting the path ReadAffy should look for cel files.
```

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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 $\ensuremath{\mathtt{TRUE}},$ 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
	$2\colon$ use background similar to pure R rma background given in affy version $1.1$ and above
destructive	logical value. If ${\tt TRUE},$ 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 <code>NULL</code> , 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 <code>justRMA()</code>, then all the CEL files in the working directory are read, converted to an expression measure using RMA and put into an <code>ExpressionSet</code>. 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 <br/> bmb@bmbolstad.com>

#### See Also

rma, read.affybatch

list.celfiles 23

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()
```

loess.normalize

Normalize arrays

# Description

This function treats PM and MM as the raw data on each chip. It fits loess curves to MVA plots and tries to normalize the chips with respect to each other by forcing log ratios to be scattered around the same constant.

```
loess.normalize(mat, subset = sample(1:(dim(mat)[2]), 5000), epsilon = 10^-2, maxit = 1, log.it = TRUE, verbose = TRUE, span = 2/3, family.loess = "symmetric")
```

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#### **Arguments**

mat	a matrix with columns containing the values of the chips to normalize.
subset	a subset of the data to fit a loess to.
epsilon	small value used for the 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	span to be used by loess.
family.loess	"gaussian" or "symmetric" as in loess.

#### **Details**

Experience shows that you only need 1-2 iterations to obtain useful results. This function is not written in an efficient way. In order to make it faster, loess is fit to a sample of the data which we then use to predict the curve for all the data. By setting family.loess="gaussian" the function is faster, but you risk losing information on differentially expressed genes. The function normalize.quantiles is faster.

#### Value

A matrix with normalized values for chips in columns.

#### Author(s)

Rafael A. Irizarry

# See Also

```
normalize.quantiles, maffy.normalize, maffy.subset
```

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.

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#### **Arguments**

```
object
                  an AffyBatch-class.
                  additional parameters for the routine.
. . .
Α
                  a vector to plot along the horizontal axis.
                  a vector to plot along vertical axis.
M
                  a set of indices to use when drawing the loess curve.
subset
show.statistics
                  logical. If TRUE, some summary statistics of the M values are drawn.
                  span to be used for loess fit.
span
family.loess "guassian" or "symmetric" as in loess.
                  size of text when writing summary statistics on plot.
                  a string specifying how the plot is to be drawn. "normal" plots points, "smoothScatter"
plot.method
                  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.
                  width of loess line.
lwd
                  line type for loess line.
lty
                  color for loess line.
loess.col
```

#### See Also

```
mva.pairs
```

#### **Examples**

```
if (require(affydata)) {
   data(Dilution)
   MAplot(Dilution)
   Mbox(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.

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#### **Arguments**

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

level of 46000.

cont.correct logical controlling whether continuity correction is used in the p-value normal approximation.

... 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 Ri = (PMi - MMi) / (PMi + MMi) 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(observing a more "present looking" probe-set than data I 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

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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:

pval	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.
call	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)
}</pre>
```

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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)</pre>
```

merge.AffyBatch 29

merge.AffyBatch

merge two AffyBatch objects

### **Description**

merge two AffyBatch objects into one.

#### Usage

### **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

M vs. A Matrix

# 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

30 normalize.constant

#### **Arguments**

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

#### See Also

pairs

# **Examples**

```
x \leftarrow matrix(rnorm(4000), 1000, 4)

x[,1] \leftarrow x[,1]^2

dimnames(x) \leftarrow list(NULL, c("chip 1", "chip 2", "chip 3", "chip 4"))

mva.pairs(x, log=FALSE, main="example")
```

normalize.constant Scale probe intensities

# 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.

normalize.contrasts 31

#### Author(s)

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

### See Also

```
AffyBatch
```

```
normalize.contrasts
```

Normalize intensities using the contrasts method

# Description

Scale chip objects in an AffyBatch-class.

# Usage

# Arguments

```
abatch an AffyBatch-class object.

span parameter to be passed to the function loess.

choose.subset

subset.size

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
```

32 normalize.invariantset

```
normalize.invariantset
```

Invariant Set normalization

### Description

Normalize arrays in an AffyBatch using an invariant set.

# Usage

#### **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

normalize.loess 33

#### See Also

normalize to normalize AffyBatch objects.

```
normalize.loess Scale microarray data
```

# Description

Normalizes arrays using loess.

# Usage

# **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 normalize.loess 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
```

34 normalize-methods

#### **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)
}</pre>
```

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.pmcorrect.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. 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="invariant set").

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.

normalize.qspline 35

#### See Also

```
AffyBatch-class, normalize.
```

# **Examples**

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

```
normalize.qspline Normalize arrays
```

### **Description**

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

#### Usage

#### Arguments

```
a data.matrix of intensities
Х
abatch
                  an AffyBatch
                  numerical vector of intensity values to normalize to. (could be the name for one
target
                  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".
                  logical, if 'TRUE', smoothing splines are used on the quantiles.
smooth
                  smoothing parameter for 'splinefun', typically in (0,1].
spar
                  minimum percentile for the first quantile.
p.min
```

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p.max maximum percentile for the last quantile.

incl. ends include the minimum and maximum values from the normalized and target ar-

rays 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 = 0.02 (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 normal- ization method for reducing variability in dna microarray experiments. Genome Biology, accepted, 2002

normalize.quantiles

Quantile Normalization

#### **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", "tog
```

## **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, <br/>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**

## **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, <br/>com>

## See Also

normalize, normalize.quantiles

normalize 39

normalize

Normalize - generic

## **Description**

A generic function which normalizes microarray data. Normalization is intended to remove from the intensity measures any systematic trends which arise from the microarray technology rather than from differences between the probes or between the target RNA samples hybridized to the arrays.

# Usage

```
normalize(object, ...)
```

## **Arguments**

```
object a data object containing microarray data.any other arguments.
```

#### See Also

Type showMethods ("normalize") at the R prompt to see what methods are available. Help on individual methods is generally available as normalize. <class> where <class> is the class of the data object. For example, for the main class in the affy package use ?normalize.AffyBatch.

Other Bioconductor packages include some related generic functions: normalizeWithinArrays, and normalizeBetweenArrays, in the limma package.

```
pairs.AffyBatch plot intensities using 'pairs'
```

# Description

Plot intensities using the function 'pairs'

40 plotDensity

#### **Arguments**

```
an AffyBatch object.
Х
                 a function to produce a plot (see pairs).
panel
                 extra parameters for the 'panel' function.
                 a function to transform the intensity values before generating the plot. 'log' and
transfo
                  'log2' are popular choices.
                 title for the plot
main
oma
                 see 'oma' in par.
font.main
                 see pairs.
cex.main
                 see pairs.
cex.labels
                 see pairs.
                 a function to produce the plots in the lower triangle (see pairs).
lower.panel
upper.panel
                 a function to produce the plots in the upper triangle (see pairs).
                 a function to produce the plots in the diagonal (see pairs).
diag.panel
font.labels
                 see pairs.
row1attop
                 see pairs.
                 see pairs.
gap
```

## **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'.

```
plotDensity Plot Densities
```

## **Description**

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

plotLocation 41

# Arguments

mat	a matrix containing the values to make densities in the columns.
Х	an object of class AffyBatch.
log	logical value. If ${\tt TRUE}$ the log of the intensities in the ${\tt AffyBatch}$ are plotted.
which	should a histogram of the PMs, MMs, 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

Plot a location on a cel image

## **Description**

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

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

42 plot.ProbeSet

# Arguments

a 'location'. It can be obtained by the method of AffyBatch indexProbes, or made elsewhere (basically a location is nrows 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 celfile
   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)
}</pre>
```

plot.ProbeSet

plot a probe set

## **Description**

Plot intensities by probe set.

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

pmcorrect 43

## **Arguments**

#### Value

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

## See Also

ProbeSet

# Examples

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

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 perfromed 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.

44 ppsetApply

#### 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)
}</pre>
```

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**

## Value

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

probeMatch-methods 45

#### 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)</pre>
```

probeMatch-methods Methods for accessing perfect matches and mismatches

## **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 Methods for accessing the Probe Names

## **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.

46 ProbeSet-class

ProbeSet-class

Class ProbeSet

## **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]])
}</pre>
```

ProgressBarText-class 47

```
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)</pre>
```

48 read.affybatch

```
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))</pre>
  open (pbt)
  for (i in 1:x) {
   updateMe(pbt)
  close(pbt)
}
system.time(q(10000))
system.time(h(10000))
```

read.affybatch

Read CEL files into an AffyBatch

# **Description**

Read CEL files into an Affybatch.

```
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)
```

read.affybatch 49

#### **Arguments**

file names separated by comma. 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.oultiers.

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, MI-AME 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 metadata. 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)

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#### 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(filenames=fns[1])
    ##read a text celfile
    abatch <- ReadAffy(filenames=fns[2])
    ##read all files in that dir
    abatch <- ReadAffy(celfile.path=celpath)
}</pre>
```

read.probematrix Read CEL file data into PM or MM matrices

## **Description**

Read CEL data into matrices.

## Usage

## **Arguments**

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

rma 51

#### 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 ${\tt TRUE},$ 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.

.setAffyOptions

#### Value

An ExpressionSet

## Author(s)

Ben Bolstad <br/>
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)
}</pre>
```

.setAffyOptions

~~function to set options ~~

## **Description**

~~ Set the options for the package

## Usage

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

#### **Arguments**

 $\hbox{affy.opt} \qquad \quad A \ list \ structure \ of \ options. \ If \ {\tt NA}, \ the \ default \ options \ are \ set.$ 

## **Details**

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

SpikeIn 53

#### Value

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

#### Author(s)

Laurent

## **Examples**

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

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

54 tukey.biweight

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

One-step Tukey's biweight

## **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 55

wha	+ 0	·df
WILL		·u_

Find which CDF corresponds

## **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, nr = NULL, cel = NULL, abatch = NULL, cdf = NULL, xy.offset = Null, cel = NULL, abatch = NULL, cdf = NULL, xy.offset = NULL
```

## **Arguments**

xy.offset

Х	X position for the probes.
У	Y position for the probes.
i	indices in the AffyBatch for the probes.
nr	total number of Xs on the chip.
cel	a corresponding object of class Cel.
abatch	a corresponding object of class AffyBatch.
cdf	character - the name of the corresponding cdf package.

an eventual offset for the XY coordinates. See Details.

56 xy2indices

#### **Details**

The probes intensities for given probe set ids are extracted from an AffyBatch object using the indices stored in the corresponding cdfenv.

The parameter xy.offset is there for compatibility. For historical reasons, the xy-coordinates for the features on Affymetrix chips 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)

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

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