Package ‘simpleaffy’

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Title Very simple high level analysis of Affymetrix data

Version 2.50.0

Author Crispin J Miller

Description Provides high level functions for reading Affy .CEL files, phenotypic data, and then computing simple things with it, such as t-tests, fold changes and the like. Makes heavy use of the affy library. Also has some basic scatter plot functions and mechanisms for generating high resolution journal figures...

Maintainer Crispin Miller <cmiller@picr.man.ac.uk>

Depends R (>= 2.0.0), methods, utils, grDevices, graphics, stats, BiocGenerics (>= 0.1.12), Biobase, affy (>= 1.33.6), genefilter, gcrma

Imports methods, utils, grDevices, graphics, stats, BiocGenerics, Biobase, affy, genefilter, gcrma

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URL http://www.bioconductor.org,
    http://bioinformatics.picr.man.ac.uk/simpleaffy/

LazyLoad yes

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NeedsCompilation yes

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**Description**

must be present in at least no arrays to be called present

**Usage**

```r
## S3 method for class 'present'
all(x, calls, no = "all")
```

**Arguments**

- `x` An object to filter
- `calls` A matrix of PMA calls
- `no` How many in a row to pass the filter? If 'all' then all must be present

**Value**

A probesetid
all.present.in.group

Author(s)
Crispin J Miller

Examples

```r
## Not run:
all.present(eset,calls,dim(calls)[2])
## End(Not run)
```

---

**all.present.in.group**  \textit{Filter by PMA call}

Description

Filters an object by PMA calls. Must be called present in at least `no` elements in at least one of the replicate sets in the factor `group`.

Usage

```r
## S3 method for class 'present.in.group'
all(x,group,members,calls,no = "all")
```

Arguments

- **x**: An object to filter
- **group**: The factor to filter by
- **members**: The members in the group to check. If null, checks all possible ones
- **calls**: A matrix of PMA calls
- **no**: How many in a row to pass the filter? If ’all’ then all must be present

Value

A probesetid

Author(s)
Crispin J Miller

Examples

```r
## Not run:
all.present.in.group(eset,calls,"line",dim(calls)[2])
## End(Not run)
```
**bg.correct.sa**

*Simpleaffy Implementation of Mas5 Background Correction*

**Description**

Implements the MAS5.0 background correction functions as described in Affy’s ‘Statistical Algorithms Description Document’.

**Usage**

```r
bg.correct.sa(unnormalised, grid=c(4, 4))
```

**Arguments**

- `unnormalised` An unnormalised AffyBatch object
- `grid` The dimensions of the grid to divide the chip into for background correction.

**Value**

An AffyBatch object

**Author(s)**

Crispin J Miller

**References**


**See Also**


**Examples**

```r
## Not run:
eset.bg.mas <- bg.correct.sa(eset);
## End(Not run)
```
**blue.white.red.cols**

*Generate colourings for heatmaps*

**Description**

Produces standard colourings for heatmaps.

**Usage**

```r
blue.white.red.cols(x)
red.black.green.cols(x)
red.yellow.white.cols(x)
```

**Arguments**

- `x` How many colours to make

**Value**

A vector of colors

**Author(s)**

Crispin J Miller

**See Also**

hmap hmap.eset hmap.pc

**Examples**

```r
## Not run:
cols <- blue.white.red.cols(21)
## End(Not run)
```

---

**call.exprs**

*Generate Expression Summaries for Affymetrix Data*

**Description**

Generates expression summaries and normalizes Affymetrix data using either MAS5.0, GCRMA or RMA algorithms.

**Usage**

```r
call.exprs(x, algorithm = "rma", do.log = TRUE, sc = 100, method = NA)
```
Arguments

x an AffyBatch object
algorithm one of "rma","rma-R","gcrma", "mas5", "mas5-R". "rma" and "mas5" make use of a native C-library and are faster than "rma-R" and "mas5-R".
do.log return logged data if true
sc if the mas5 algorithm is being used, sets the target intensity to which the chips should be scaled.
method The algorithm used to normalise the data. Has no effect for "rma", defaults to quantile normalisation for "rma" and no normalisation for "mas5"

Value

An AffyBatch object containing expression summaries.

Author(s)

Crispin J Miller

References

http://bioinformatics.picr.man.ac.uk/

See Also

read.affy, expresso, justRMA, justMAS

Examples

```r
## Not run:
eset.rma <- call.exprs(eset,"rma");
eset.mas5 <- call.exprs(eset,"mas5");
## End(Not run)
```

detection.p.val Calculate Detection p-values

Description

Calculate MAS5 detection pvalues and Present Marginal Absent calls. This is an implementation based on the algorithm described in Liu, Mei et al. (2002) 'Analysis of high density expression microarrays with signed-rank call algorithms', Bioinformatics 18(12) pp1593-1599.

Usage

detection.p.val(x, tau = NULL,calls=TRUE,alpha1=NULL,alpha2=NULL,ignore.saturated=TRUE)
Arguments

- **x**: An unnormalised AffyBatch object
- **tau**: Errrrmmm... tau
- **alpha1**: Present-Marginal threshold
- **alpha2**: Marginal-Absent threshold
- **calls**: if true, generate PMA calls
- **ignore.saturated**: if true do the saturation correction described in the paper, with a saturation level of 46000

Value

A list:

- **pval**: A matrix of detection p values
- **call**: A matrix of PMA calls

Note

alpha1 and alpha2 are parameters that change according to the chip type you are using. If they are not specified, the function uses the current QC environment to find them, and attempts to set one up if it is not there. This is done with an internal call to the function `setQCEnvironment`. If it is unable to find the appropriate config files, this will cause an error. See `setQCEnvironment` for more details.

Author(s)

Crispin J Miller

References

http://bioinformatics.picr.man.ac.uk/

See Also

`setQCEnvironment`

Examples

```r
## Not run:
dpv <- detection.p.val(eset);
## End(Not run)
```
get.annotation

Get annotation data for a gene list

Description

Takes a vector of probeset names and a CDF name. Produces a table of annotations, containing gene name, description, sequence accession number and unigene accession number for each probeset. In addition, write.annotation is a utility function that outputs the annotation data in a form suitable for loading into excel and results.summary takes the output of pairwise.comparison or pairwise.filter and spits out a table with the means of the replicates the fold-change between them (log2) and t-test p-values. This is followed by a table of annotation (produced by get.annotation).

Usage

get.annotation(x, cdfname, verbose=FALSE)
write.annotation(summary, file="results/annotation.table.xls")
results.summary(results, cdfname)

Arguments

x  
a vector of probe names

cdfname  
the name of the chip (as produced by cdfName(AffyBatch)

verbose  
print out information if problems are found looking things up in the annotation data

summary  
a table of data to write in a format appropriate to read into Excel

file  
a table delimited file

results  
a PairComp object, as produced by pairwise.comparison and pairwise.filter

Value

A table containing annotation data

Author(s)

Crispin J Miller

References

http://bioinformatics.picr.man.ac.uk/

Examples

```r
## Not run:
pw <- pairwise.comparison(eset.rma,"group",c("A","P"))
pw.filtered <- pairwise.filter(pw)
summary <- results.summary(pw.filtered,"hgu133a")
write.annotation(file="spreadsheet.xls",summary)
## End(Not run)
```
**get.array.indices**

*Find arrays in an AffyBatch object defined by their phenoData*

**Description**

Given an AffyBatch object, looks at its phenoData slot to find the factor, or column specified by 'group' and searches that column for entries supplied in 'members'. Returns the indices of these rows. For example, in a six chip AffyBatch object, x, with a column 'treatment' containing 'c','c',t1','t2','t1','t2', a call to get.array.indices(x,"treatment",c("c","t1")) would return c(1,2,3,5).

**Usage**

get.array.indices(x,group,members)

**Arguments**

- **x** An ExpressionSet or AffyBatch object.
- **group** The name of the pData column to use.
- **members** The labels within the pData column to match against.

**Author(s)**

Crispin J Miller

**Examples**

```r
## Not run:
indices3 <- get.array.indices(eset.rma,"group","A")
## End(Not run)
```

**get.array.subset**

*Get a subset of arrays from an affybatch object, split by phnotypic data*

**Description**

Looks at a factor in the phenotypic data for an AffyBatch or ExpressionSet object and uses it to select a subset of arrays, as defined by 'members'.

**Usage**

get.array.subset(x,group,members)

**Arguments**

- **x** An ExpressionSet or AffyBatch object.
- **group** The name of the pData column to use.
- **members** The labels within the pData column to match against.
get.array.subset.affybatch

Author(s)
Crispin J Miller

See Also
get.array.subset.affybatch get.array.subset.exprset

Examples

```r
## Not run:
subset1 <- get.array.subset.affybatch(eset.rma,"group","A")
subset2 <- get.array.subset.exprset(eset.rma,"group",c("A","P"))
subset3 <- get.array.subset(eset.rma,"group","A")

## End(Not run)
```

get.array.subset.affybatch

*Get a subset of arrays from an affybatch object, split by phenotypic data*

Description

Looks at a factor in the phenotypic data for an AffyBatch or ExpressionSet object and uses it to select a subset of arrays, as defined by ‘members’.

Usage

```r
get.array.subset.affybatch(x, group, members)
get.array.subset.exprset(x, group, members)
```

Arguments

- `x`: An AffyBatch or ExpressionSet object.
- `group`: The name of the pData column to use.
- `members`: The labels within the pData column to match against.

Details

Subsetting an AffyBatch object by array is achieved using `[x,]`, while the same is achieved for an ExpressionSet by `[,x]`. Hence the two different functions. In general the generic method `get.array.subset` should be used - since it sorts this all out automatically.

Value

An AffyBatch or ExpressionSet (as appropriate) containing the selected subset of chips.

Author(s)
Crispin J Miller
get.fold.change.and.t.test

Examples

```r
## Not run:
subset1 <- get.array.subset.affybatch(eset.rma,"group","A")
subset2 <- get.array.subset.exprset(eset.rma,"group",c("A","P"))
subset3 <- get.array.subset(eset.rma,"group","A")
## End(Not run)
```

get.fold.change.and.t.test

*Compute fold change and t-test statistics between two experimental groups*

Description

Generate fold changes (and possibly means) for a pair of experimental groups

Usage

```r
get.fold.change.and.t.test(x,group,members,logged = TRUE,a.order=NULL,b.order=NULL,method=c("unlogged","logged","median"));
```

Arguments

- `x` an ExpressionSet object.
- `group` column in pData(x).
- `members` labels in group.
- `logged` is the AffyBatch data logged?
- `a.order` For a pairwise comparison the ordering of the first group of replicates
- `b.order` For a pairwise comparison the ordering of the second group of replicates
- `method` What method should be used to calculate the average for the fold-change - can be either "logged", "unlogged", "median"

Details

Given an ExpressionSet object, generate quick stats for pairwise comparisons between a pair of experimental groups. If `a.order` and `b.order` are specified then a paired sample t-test will be conducted between the groups, with the arrays in each group sorted according to the ordering specified.

The fold-changes are computed from the average values across replicates. By default this is done using the mean of the unlogged values. The parameter, `method` allows the mean of the logged values or the median to be used instead. T-tests are always computed with the logged data.

Value

An object of class PairComp

Author(s)

Crispin J Miller
hmap.eset

Draw a heatmap from an AffyBatch object

Description

Given either an AffyBatch draw a heatmap.

Usage

hmap.eset(x, probesets, samples = 1:length(sampleNames(x)), scluster = standard.pearson, pcluster = standard.pearson, slabs = sampleNames(x), plabs = geneNames(x), col = 1:4, min.val, max.val, scale, spread = 6, by.fc = TRUE, sdev, show.legend = TRUE, title = NULL, cex = 0.5)

Arguments

x The AffyBatch object to get the expression data from
probesets What probesets to plot, defaults to all of them
samples Which samples to plot
scluster The function to use to cluster the samples by. Can also be a dendrogram object.
pcluster The function to use to cluster the probesets by. Can also be a dendrogram object.
slabs Labels for the sample axis
plabs Labels for the probeset axis defaults to geneNames(x)
col Vector of colour values to use (see below)
min.val The minimum intensity to plot
max.val The maximum intensity to plot
scale Scale each gene’s clouring based on standard deviation (See below)
spread If the data is scaled, how many standard deviations (or fold changes) either way should we show. If no scaling, then does nothing
by.fc If the data is scaled, scale by s.d. or by fold.change?
sdev A vector of standard deviations for each gene to be plotted. If no value is supplied these are worked out from the data.
show.legend Draw a scale on the graph and show the title if supplied
title The title of the graph
cex Character expansion
Details

Takes an AffyBatch object and plots a heatmap. At its simplest, all that is required is an AffyBatch object (as calculated by `call.exprs`) and a vector supplying the probesets to plot. These can be specified by name, as an integer index or as a vector of TRUEs and FALSES. The function will try to do something sensible with the labels. If it fails you will need to specify this with plabs. The function will then draw a heatmap, coloured blue-white-red in increasing intensity, scaled so that 100 Col can be used to change the colouring. "bwr" specifies blue-white-red, "rbg" specifies red-black-green, and "ryw" specifies red-yellow-white. Alternatively, a vector of arbitrary colours can be supplied (try `rainbow(21)`, for example).

The clustering method can also be changed by supplying, either, a function that takes a matrix of expression values and returns an `hclust` or `dendrogram` object, or alternatively, an `hclust` or `dendrogram` object itself. Setting either of these to NULL will stop the heatmap being clustered on that axis.

Scaling is somewhat more complex. If scale is TRUE, then each gene is coloured independently, on a scale based on its standard deviation. By default this is calculated for the samples that are being plotted, unless a value is supplied for sdev – in which case this should be a vector of standard deviations, one for each probeset being plotted (and in the same order). This scaling is done after the clustering. For more details on how all of this works see the website http://bioinf.picr.man.ac.uk/simpleaffy and also look at `hmap.pc` which uses the scaling to plot transcripts identified as being differentially expressed.

Value

Returns an (invisible) list containing the dendrograms used for samples and probesets

Author(s)

Crispin J Miller

See Also

`hmap.pc` `blue.white.red.cols` `standard.pearson`

Examples

```r
## Not run:
eset.mas <- call.exprs(eset,"mas5")
hmap.eset(eset.mas,1:100,1:6,col="rbg")
## End(Not run)
```

hmap.pc Draw a heatmap from an PairComp object

Description

Given either a PairComp object draw a heatmap.
Usage

hmap.pc(x, eset, samples=rownames(pData(x)), scluster=standard.pearson, pcluster=standard.pearson, slabs, plabs, col="rbg", scale=T, spread=10, by.fc=F, gp=group(x), mbrs=members(x), show.legend=T, title=NULL, cex=0.1)

Arguments

x The PairComp object to get the probeset list (and other data) from
eset The AffyBatch object containing expression data
samples Which samples to plot – defaults to those used to calculate ‘x’, but can be any of the samples in eset
scluster The function to use to cluster the samples by. Can also be a dendrogram object.
pcluster The function to use to cluster the probesets by. Can also be a dendrogram object.
slabs Labels for the sample axis
plabs Labels for the probeset axis
col Vector of colour values to use (see below)
scale Scale each gene’s clouring based on standard deviation (See below)
spread If the data is scaled, how many standard deviations (or fold changes) either way should we show. If no scaling, then does nothing
by.fc If the data is scaled, do it by fold change?
gp The column in the expression set’s pData object used to select the samples to plot. By default this is the one used to calculate x.
mbrrs The members of the ‘group’ column that we wish to plot. By default these are the pair used to calculate x. If ‘all’ is supplied then all samples are used.
show.legend Draw a scale on the graph and show the title if supplied
title The title of the graph
cex Character expansion

details

Takes a PairComp object and an AffyBatch object and plots a heatmap. At its simplest, all that is required are these two objects. The function will then draw a heatmap, coloured red-black-green in increasing intensity, scaled for each gene based on standard deviation. The legend shows how these colours translate into intensity.

Col can be used to change the colouring. ”bwr” specifies blue-white-red, ”rbg” specifies red-black-green, and ”ryw” specifies red-yellow-white. Alternatively, a vector of arbitrary colours can be supplied (try rainbow(21), for example).

Scaling is somewhat complex. If scale is TRUE, then each gene is coloured independently, on a scale based on its standard deviation. This is calculated as follows: ’group’ supplies a column in the pData object of ’eset’ that is used to collect samples together (generally as replicate groups). ’members’ supplies the entries within this column that are to be used. (Unless specified, the function uses the same value for ‘group’ and ‘members’ used to calculate the PairComp object). The function uses these data to calculate the standard deviation for each probeset within each set of replicates, and then calculates the average sd for each gene. This is then used to scale the data so that each probeset is plotted on a scale that shows the number of standard deviations away from the mean it is for that sample. For more details on how all of this works see the website http://bioinf.picr.man.ac.uk/simpleaffy.

Alternatively, by setting by.fc to FALSE, scaling can be done simply in terms of fold-change, in which case, spread defines the maximum and minimum fold changes to show.
journalpng

Value
Returns an (invisible) list containing the dendrograms used for samples and probesets

Author(s)
Crispin J Miller

See Also
hmap.eset blue.white.red.cols standard.pearson

Examples
## Not run:
pc <- pairwise.comparison(eset.mas,group="group",members=c("a","b"),spots=eset)
pf <- pairwise.filter(pc)
hmap.pc(pf,eset.mas)

## End(Not run)

---

journalpng

Produce a device for producing artwork for presentations and journals

Description

journalpng generates a device to print a 4 x 4 inch 300 dpi figure (by default). screenpng does the same, but 72dpi.

Usage

journalpng(file="figure.png",width=4, height=4,res=300)
screenpng(file="figure.png",width=4, height=4,res=72)

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>file</td>
<td>the file to write the figure to</td>
</tr>
<tr>
<td>width</td>
<td>the width of the figure</td>
</tr>
<tr>
<td>height</td>
<td>its height</td>
</tr>
<tr>
<td>res</td>
<td>resolution in dots-per-inch</td>
</tr>
</tbody>
</table>

Value

A table containing annotation data

Author(s)
Crispin J Miller

References

http://bioinformatics.picr.man.ac.uk/
justMAS

Generate Expression calls using a C implementation of the MAS 5.0 Algorithm

Description

Implements the MAS5.0 background correction, expression summary and scaling functions as described in Affy’s 'Statistical Algorithms Description Document'

Usage

justMAS(unnormalised,tgt=100,scale=TRUE)

Arguments

- unnormalised: An unnormalised AffyBatch object
- tgt: The target intensity to scale array to, if scaling.
- scale: Scale the data to the specified target intensity.

Details


Value

An AffyBatch object, with, in addition, scale-factors for each array stored in the object's description@preprocessing@sfs slot, and the target intensity the arrays were scaled to in description@preprocessing@tgt

Author(s)

Crispin J Miller

References

http://bioinformatics.picr.man.ac.uk/

See Also


Examples

```r
## Not run:
journalpng(file="results/figure1.png"); # starts a new device
trad.scatter.plot(exprs(eset)[,1],exprs(eset)[,2])
dev.off(); # writes the file at this point.

## End(Not run)
```
Examples

```r
## Not run:
eset.mas <- justMAS(eset.mas);
## End(Not run)
```

PairComp-class

Class "PairComp" Represents the results of pairwise comparison between two experimental factors

Description

Holds fold-change, ttest p-score and detection p-value calls(if used) between a pair of experimental factors.

Slots

- `means`: Object of class "matrix" Mean values for each of the experimental factors.
- `fc`: Object of class "numeric" Fold change between the means.
- `tt`: Object of class "numeric" P-score between the factors.
- `calls`: Object of class "matrix" Detection p-values for each probeset on each array.
- `group`: Object of class "character" The name of the factor that was compared.
- `members`: Object of class "character" A list containing the two levels compared between.
- `pData`: Object of class "pData" The phenoData for the members that were compared.
- `calculated.from`: Object of class "ExpressionSet" The original expression set that was being compared.

Methods

- `[]` signature(x = "PairComp"): get the values for the specified gene(s).
- `[<-` signature(x = "PairComp"): not supported.
- `calls` signature(object = "PairComp"): the detection.p.values.
- `fc` signature(object = "PairComp"): the fold-changes.
- `group` signature(object = "PairComp"): the name of the group that was compared.
- `means` signature(object = "PairComp"): the means of the two experimental factors that were compared.
- `members` signature(object = "PairComp"): the members of that group that were compared.
- `pairwise.filter` signature(object = "PairComp"): Take a PairComp object and filter it to yield probesets that pass the specified criteria.
- `tt` signature(object = "PairComp"): the results of a ttest between groups.
- `pData` signature(object = "pData"): The phenoData from the members that were compared.
- `calculated.from` signature(object = "ExpressionSet"): The original expression set.

Author(s)

Crispin Miller
pairwise.comparison

Compute pairwise comparison statistics between two experimental groups

Description
Generate fold changes, t-tests and means for a pair of experimental groups

Usage
pairwise.comparison(x, group, members=NULL, spots=NULL, a.order=NULL, b.order=NULL, method="unlogged", logged=TRUE)

Arguments
x an ExpressionSet object.
group column in pData(x).
members labels in group.
spots unnormalised AffyBatch data for this experiment - if included, results in PMA calls and detection p-values being generated
a.order For a comparison with matched pairs, the ordering of the first group of replicates
b.order For a comparison with matched pairs, the ordering of the second group of replicates
method What method should be used to calculate the average for the fold-change - can be either "logged", "unlogged", "median"
logged Whether the input data is logged or not

Details
Given an ExpressionSet object, generate quick stats for pairwise comparisons between a pair of experimental groups. If a.order and b.order are specified then a paired sample t-test will be conducted between the groups, with the arrays in each group sorted according to the ordering specified. By default, the function assumes that the expression values are logged (this can be changed with the parameter "logged"). The fold-changes are computed from the average values across replicates. Unless you specify otherwise, this is done using the mean of the unlogged values (i.e. logged data is first unlogged, the mean calculated, and the result re-logged). The parameter "method", allows the mean of the logged values or their median to be used instead. T-tests are always computed with the logged data.

Value
A Pairwise comparison object.

Author(s)
Crispin J Miller

References
http://bioinformatics.picr.man.ac.uk/
pairwise.filter

Examples

```r
## Not run:
pc <- pairwise.comparison(eset.rma,"group",c("A","P"))
## End(Not run)
```

Description

Given the results of a pairwise.comparison, filter the resulting gene list on expression level, PMA calls (if available), fold change and t-test statistic.

min.exp and min.exp.no allow the data to be filtered on intensity (where min.exp.no specifies the minimum number of arrays that must be above the threshold 'min.exp' to be allowed through the filter).

PMA filtering is done when min.present.number is greater than 0.

min.present.no allows arrays to be filtered by PMA call. A number or 'all' must be specified. If a number, then the at least this many arrays must be called present, if 'all', then all arrays must be called present.

present.by.group specifies whether PMA filtering is to be done on a per-group basis or for all arrays at once. If 'false' then the experiment is treated as a single group (i.e. a probeset passes the filter if it is called present on at least min.present.number arrays in the whole experiment. If 'true' then it must be called present on at least this many arrays in one or more groups. See the vignette for more details.

Usage

`pairwise.filter(object,min.exp=log2(100),min.exp.no=0,min.present.no=0,present.by.group=T,fc=1,tt=0.001)`

Arguments

- `object`: a `PairComp` object
- `min.exp`: Filter genes using a minimum expression cut off
- `min.exp.no`: A gene must have an expression intensity greater than 'min.exp' in at least this number of chips
- `min.present.no`: A gene must be called present on at least this number of chips
- `present.by.group`: If true, then the probeset must be called Present on at least min.present.number arrays in any of the replicate sets used to generate the PairComp object being filtered. If false, then must be called present on at least min.present.no of the arrays in the whole experiment
- `fc`: A gene must show a log2 fold change greater than this to be called significant
- `tt`: A gene must be changing with a p-score less than this to be called significant
Value

A 'PairComp' object filtered to contain only the genes that pass the specified filter parameters.

Author(s)

Crispin J Miller

References

http://bioinformatics.picr.man.ac.uk/

Examples

```
## Not run:
pc <- pairwise.comparison(eset.rma,"group",c("A","P"))
pf <- pairwise.filter(pc,tt=0.01);
## End(Not run)
```

---

**plot.pairwise.comparison**

Plots a PairComp object

Description

Draws a scatter plot between means from a pairwise comparison. Colours according to PMA calls and identifies 'significant' genes yielded by a filtering

Usage

```
## S3 method for class 'pairwise.comparison'
plot(x,y=NULL,labels=colnames(means(x)),showPMA=TRUE,type="scatter",...)
```

Arguments

- `x` A PairComp object
- `y` A PairComp object
- `labels` A list containing x and y axis labels
- `showPMA` True if PMA calls are to be identified
- `type` Can be 'scatter', 'ma' or 'volcano'
- `...` Additional arguments to plot
plot.qc.stats

Details

Takes a PairComp object (as produced by pairwise.comparison and plots a scatter plot between the sample means. If PMA calls are present in the calls slot of the object then it uses them to colour the points. Present on all arrays: red; absent on all arrays: yellow; present in all some arrays; orange. In addition, if a second PairComp object is supplied, it identifies spots in that object, by drawing them as black circles. This allows, for example, the results of a pairwise.filter to be plotted on the same graph.

If type is 'scatter' does a simple scatter plot. If type is 'volcano' does a volcano plot. If type is 'ma' does an MA plot.

Author(s)

Crispin J Miller

See Also

pairwise.comparison pairwise.filter trad.scatter.plot

Examples

```r
## Not run:
pc <- pairwise.comparison(eset.mas, group="group", members=c("a","b"), spots=eset)
pf <- pairwise.filter(pc)
plot(pc,pf)
## End(Not run)
```

plot.qc.stats

Plots a QCStats object

Description

Generates a visual summary of the various QC statistics recommended by Affymetrix in their 'Data Analysis Fundamentals' handbook.

Arguments

- `x` A QCStats object
- `fc.line.col` The colour to mark fold change lines with
- `sf.ok.region` The colour to mark the region in which scale factors lie within appropriate bounds
- `chip.label.col` The colour to label the chips with
- `sf.thresh` Scale factors must be within this fold-range
- `gdh.thresh` Gapdh ratios must be within this range
- `ba.thresh` Beta actin must be within this range
- `present.thresh` The percentage of genes called present must lie within this range
- `bg.thresh` Array backgrounds must lie within this range
- `label` What to call the chips
The title for the plot
If true use 3'/M ratios for the GAPDH and beta actin probes
Value to scale character size by (e.g. 0.5 means that the text should be plotted half size)
... Other parameters to pass through to plot

Details

A lot of information is presented in this one figure. By default, each array is represented by a separate line in the figure. The central vertical line corresponds to 0 fold change, the dotted lines on either side correspond to 3 fold up and down regulation. The blue bar represents the region in which all arrays have scale factors within, by default, three-fold of each other. Its position is found by calculating the mean scale factor for all chips and placing the center of the region such that the borders are -1.5 fold up or down from the mean value.

Each array is plotted as a line from the 0-fold line to the point that corresponds to its scale factor. If the ends of all of the lines are in the blue region, their scale-factors are compatible. The lines are coloured blue if OK, red if not.

The figure also shows GAPDH and beta-actin 3’/5’ ratios. These are represented as a pair of points for each chip. Affy state that beta actin should be within 3, gapdh around 1. Any that fall outside these thresholds (1.25 for gapdh) are coloured red; the rest are blue.

Written along the left hand side of the figure are the number of genes called present on each array and the average background. These will vary according to the samples being processed, and Affy’s QC suggests simply that they should be similar. If any chips have significantly different values this is flagged in red, otherwise the numbers are displayed in blue. By default, ‘significant’ means that %-present are within 10% of each other; background intensity, 20 units. These last numbers are somewhat arbitrary and may need some tweaking to find values that suit the samples you’re dealing with, and the overall nature of your setup.

Finally, if BioB is not present on a chip, this will be flagged by printing ’BioB’ in red.

In short, everything in the figure should be blue - red highlights a problem!

Usage

plot.qc.stats(x, fc.line.col = "black", sf.ok.region = "light blue", chip.label.col = "black", sf.thresh = 3, gdh.thresh = 1.25, ba.thresh = 3, present.thresh = 10, bg.thresh = 20, label = NULL, title="QC Stats", spread=c(-8,8), usemid=F, type="l", cex=1, ...)

Author(s)

Crispin J Miller

See Also

qc

Examples

data(qcs)
plot(qcs)
Generate QC stats from an AffyBatch object

Description

Generate QC metrics for Affymetrix data.

Usage

qc(unnormalised, ...)

Arguments

unnormalised An AffyBatch object with nothing done to it
... Any other parameters

Details

Affymetrix recommend a series of QC metrics that should be used to check that arrays have hybridised correctly and that sample quality is acceptable. These are discussed in the document 'QC and Affymetrix data' accompanying this package, and on the web at http://bioinformatics.picr.man.ac.uk.

They are described in detail in the 'Expression Analysis Fundamentals' manual available from Affymetrix.

Before using this function you are strongly encouraged to read the 'QC and Affymetrix data' document, which contains detailed examples.

This function takes an AffyBatch object and generates a QCStats object containing a set of QC metrics. See qc.affy for more details.

Author(s)

Crispin J Miller

See Also

qc.affy setQCEnvironment

Examples

```r
## Not run:
qcs <- qc(eset, eset.mas)

## End(Not run)
data(qcs)
ratios(qcs)
avbg(qcs)
maxbg(qcs)
minbg(qcs)
spikeInProbes(qcs)
qcProbes(qcs)
percent.present(qcs)
plot(qcs)
sfs(qcs)
```
qc.affy

Generate Affymetrix Style QC Statistics

Description

Generate QC data for Affymetrix arrays

Usage

qc.affy(unnormalised, normalised=NULL, tau=0.015, logged=TRUE, cdfn=cdfName(unnormalised))

Arguments

unnormalised An unnormalised raw AffyBatch object to call qc stats on
normalised The same one, processed using justMAS (contains scale factors etc.). If not supplied, then the object gets calculated internally.
tau used by detection p value
logged True if the data is logged
cdfn The cdf name for the array - can be used to specify a different set of probes to the default

Details

Affymetrix recommend a series of QC metrics that should be used to check that arrays have hybridised correctly and that sample quality is acceptable. These are discussed in the document 'QC and Affymetrix data' accompanying this package, and on the web at http://bioinformatics.picr.man.ac.uk. They are described in detail in the 'Expression Analysis Fundamentals' manual available from Affymetrix.

This function takes an (unnormalised) AffyBatch object, and (optionally) an ExprSet, with MAS expression calls produced by call.exprs and generates QC metrics. If the MAS calls are not supplied these are calculated internally.

Value

A QCStats object describing the supplied AffyBatch

Author(s)

Crispin J Miller
qc.get.alpha1

Examples

### Not run:
```r
cqs <- qc(eset)
```
```
## End(Not run)
data(cqs)
ratios(cqs)
avbg(cqs)
maxbg(cqs)
minbg(cqs)
spikeInProbes(cqs)
qcProbes(cqs)
percent.present(cqs)
plot(cqs)
sfs(cqs)
target(cqs)
ratios(cqs)
```

### qc.get.alpha1

*Get or set the alpha values for the current QC environment*

Description

Alpha1 and Alpha2 are used to define the P/M/A thresholds for detection calling algorithm see `detection.p.val`. These are array dependent, these functions set or get their values. Tau is a constant parameter within the calculation and is not array specific.

Usage

```r
qc.get.alpha1()
qc.set.alpha1(value)
qc.get.alpha2()
qc.set.alpha2(value)
qc.get.tau()
```

Arguments

```r
value A double representing the alpha1 or alpha2 threshold for defining detection calls.
```

See `detection.p.val` for more details.

Value

### qc.set.alpha1 and `qc.set.alpha2` return nothing. `qc.get.alpha1` and `qc.get.alpha2` return a double.

Author(s)

Crispin J Miller

References

http://bioinformatics.picr.man.ac.uk/
qc.get.array

**See Also**

detection.p.val

**Examples**

```r
setQCEnvironment("hgu133plus2cdf")
qc.get.alpha1()
qc.get.alpha2()
qc.set.alpha1(0.05)
qc.get.alpha1()
qc.set.alpha2(0.05)
qc.get.alpha2()
```

```
qc.set.array("plus2")
qc.get.array()
```

---

**qc.get.array**

Get or set the name of the array for which the current QC environment is valid. Currently not used for anything important; is a free text identifier.

**Description**

The array name is simply a free text name for the array of interest.

**Usage**

```r
qc.get.array()
qc.set.array(name)
```

**Arguments**

- `name` a free text name for the array of interest

**Value**

a string

**Author(s)**

Crispin J Miller

**References**

[http://bioinformatics.picr.man.ac.uk/](http://bioinformatics.picr.man.ac.uk/)

**See Also**

setQCEnvironment

**Examples**

```r
qc.set.array("plus2")
qc.get.array()
```
qc.get.probes

Retrieve QC probeset names for the current array type

Description

Get the names of probesets used to calculate 3'/5' ratios for the current array type. qc.get.spikes is used to set the spike probe names (i.e. bioB, bioC, etc.)

Usage

qc.get.probes()
qc.get.probe(name)
qc.add.probe(name,probeset)

Arguments

name A name for the given probeset. By default, this is the probeset identifier
probeset A probeset ID

Value

A character array of probeset IDs, or the requested probeset ID, as appropriate.

Author(s)

Crispin J Miller

References

http://bioinformatics.picr.man.ac.uk/

See Also

setQCEnvironment qc.get.spikes

Examples

setQCEnvironment("hgu133plus2cdf")
qc.get.probes()
qc.add.probe("my.name","a.probesetid_at")
qc.add.probe("another.name","another.probesetid_at")
qc.get.probes()
qc.get.ratios

*Retrieve pairs of probesets used for calculating 3'/5' ratios*

**Description**

Get the names of the qc probesets used to define the 3'/5' ratios.

**Usage**

```r
qc.get.ratios()
qc.get.ratio(name)
qc.add.ratio(name,probeset1,probeset2)
```

**Arguments**

- *name*  
  A name for the given ratio calculation (such as gapdh3/5)
- *probeset1*  
  A probeset ID
- *probeset2*  
  A probeset ID

**Value**

A list, each element with a name like gapdh3/5 and comprising of a two-element character vector of probeset IDs.

**Author(s)**

Crispin J Miller

**References**

http://bioinformatics.picr.man.ac.uk/

**See Also**

`setQCEnvironment qc.get.probes`

**Examples**

```r
setQCEnvironment("hgu133plus2cdf")
qc.get.ratios()
qc.add.ratio("a.name","probeset1.id","probeset2.id")
qc.get.ratio("a.name")
```
qc.get.spikes

Retrieve QC spike probeset names for the current array type

Description
Get the names of spike probesets for bioB, bioC, etc. ratios for the current array type. `qc.get.probes` is used to define the 3'/5' ratio probesets.

Usage
```
qc.get.spikes()
qc.get.spike(name)
qc.add.spike(name,probeset)
```

Arguments
- `name` A name for the given probeset. By default, this is the probeset identifier
- `probeset` A probeset ID

Value
A character array of probeset IDs, or the requested probeset ID, as appropriate.

Author(s)
Crispin J Miller

References
http://bioinformatics.picr.man.ac.uk/

See Also
- `setQCEnvironment`
- `qc.get.probes`

Examples
```
qc.get.spikes()
qc.add.spike("my.name","a.probesetid_at")
qc.add.spike("another.name","another.probesetid_at")
qc.get.spikes()
```
qc.have.params

Does simpleaffy have a QC definition file for the specified array?

Description

Simpleaffy requires a definition file describing the qc probes, spikes, alpha values, etc. for the array of interest. This is used to initialize the QC environment for the array (usually implicitly within the qc function), by a call to setQCEnvironment. This function can be used to see if the specified array has a definition file.

Usage

qc.have.params(name)

Arguments

name

The 'clean' CDF name of the array (i.e. the result of calling cleancdfname on the cdfName of the AffyBatch object containing the array data of interest).

Value

True or False

Author(s)

Crispin J Miller

References

http://bioinformatics.picr.man.ac.uk/

See Also

setQCEnvironment, qc, qc.ok, cdfName, cleancdfname

Examples

qc.have.params("nosucharraycdf")
qc.have.params("hgu133plus2cdf")
setQCEnvironment("hgu133plus2cdf")
qc.have.params(cleancdfname("HG-U133_Plus_2"))
qc.ok

Has simpleaffy’s QC environment been set up?

Description

Simpleaffy requires a definition file describing the qc probes, spikes, alpha values, etc. for the array of interest. This is used to initialize the QC environment for the array (usually implicitly within the qc function), by a call to `setQCEnvironment`. This function can be used to check if the qc environment has been set up for the current session.

Usage

qc.ok()

Value

True or False

Author(s)

Crispin J Miller

References

http://bioinformatics.picr.man.ac.uk/

See Also

`setQCEnvironment` `qc` `qc.have.params` `cdfName`

Examples

```r
qc.ok()
setQCEnvironment("hgu133plus2cdf")
qc.ok()
```

qc.read.file

Read a file defining the QC parameters for a specified array and set up the QC Environment

Description

Affymetrix define a series of QC parameters for their arrays. Many of these rely on specific probeset that differ between arrays and are used to calculate things like 3’/5’ ratios. See `qc.affy` for more details. This is usually done by a call to `setQCEnvironment`; the function described here is the one that does the actual loading of the configuration file. See the package vignette for details of the config file’s syntax.

Usage

qc.read.file(fn)
Arguments

fn  full path and name of the file to load

Value

none.

Author(s)

Crispin J Miller

References

http://bioinformatics.picr.man.ac.uk/

See Also

setQCEnvironment

Examples

fn <- system.file("extdata","hgu133plus2cdf.qcdef",package="simpleaffy")
qc.read.file(fn)
qc.get.spikes()
qc.get.probes()
qc.get.ratios()

qcs

an example QC Stats object

Description

This dataset gives sample QC data for 25 array hgu133a comparison between two cell lines (MCF7 and MCF10A) and a variety of protocols.

Usage

qcs

Format

a QCStats object

Examples

data(qcs)
plot(qcs)
Class "QCStats"

Description

Holds Quality Control data for a set of Affymetrix arrays

Objects from the Class

Objects can be created by calls of the form `qc(AffyBatch)`.

Slots

- `scale.factors`: Object of class "numeric" Scale factors used to scale the chips to the specified target intensity
- `target`: Object of class "numeric" The target intensity to which the chips were scaled
- `percent.present`: Object of class "numeric" Number of genes called present
- `average.background`: Object of class "numeric" The average background for the arrays
- `minimum.background`: Object of class "numeric" The minimum background for the arrays
- `maximum.background`: Object of class "numeric" The maximum background for the arrays
- `bioB Calls`: Object of class "character" The detection PMA (present / marginal / absent) calls of bioB spike-in probes
- `spikes`: Object of class "list" spiked in probes (e.g. biob, bioc...)
- `qc.probes`: Object of class "list" qc probes (e.g. gapdh 3,5,M,...)
- `arraytype`: The `cdfName` of the AffyBatch object used to create the object

Methods

- `avbg` signature(object = "QCStats"): average background
- `maxbg` signature(object = "QCStats"): maximum background
- `minbg` signature(object = "QCStats"): minimum background
- `spikeInProbes` signature(object = "QCStats"): the spike-in QC probes
- `qcProbes` signature(object = "QCStats"): the gapdh and actin QC probes
- `percent.present` signature(object = "QCStats"): no probesets called present
- `plot` signature(x = "QCStats"): Plot a QCStats object
- `sfs` signature(object = "QCStats"): scale factors
- `target` signature(object = "QCStats"): target scaling
- `ratios` signature(object = "QCStats"): 5’3’ and 5’M ratios for QC Probes
- `arrayType` signature(object = "QCStats"): The type of array for which this QC stats object was generated

Author(s)

Crispin J Miller

See Also

`qc`
Description

Reads the specified file, which defines phenotypic data for a set of .CEL files. Reads the specified files into an AffyBatch object and then creates a phenoData object, defining the experimental factors for those chips.

Usage

read.affy(covdesc = "covdesc", path=".", ...)

Arguments

covdesc A white space delimited file suitable for reading as a data.frame. The first column (with no column name) contains the names(or paths to) the .CEL files to read. Remaining columns (with names) represent experimental factors for each chip. these become elements of the phenoData object.

... extra functions to pass on to ReadAffy

path The path to prefix the filenames with before calling ReadAffy

Value

An AffyBatch object

Author(s)

Crispin J Miller

References

http://bioinformatics.picr.man.ac.uk/

See Also

ReadAffy, AffyBatch data.frame phenoData

Examples

## Not run:
eset <- read.affy(); # read a set of CEL files
eset.rma <- call.exprs(eset,"rma");

## End(Not run)
\textbf{read.affy.mixed} \hspace{1cm} \textit{Read a Set of .CEL Files and Phenotypic Data from mixed chip types}

\textbf{Description}

Reads the specified file, which defines phenotypic data for a set of .CEL files. Reads the specified files into an \texttt{AffyBatch} object and then creates a \texttt{phenoData} object, defining the experimental factors for those chips. This function deals with different array types by generating a pseudo arrayset containing only the probes in common. It does this by finding the smallest chip type in the set, and using this as a template. Probesets that aren’t shared are set to 0. Other probesets are copied in. Note that this means that spots that were in one place on one array, appear to be at a different place on another. What this does to position specific background correction algorithms (such as mas5) is left as an exercise to the reader. Beware...

\textbf{Usage}

\begin{verbatim}
read.affy.mixed(covdesc = "covdesc",path=".", ...) \end{verbatim}

\textbf{Arguments}

- \texttt{covdesc} A white space delimited file suitable for reading as a \texttt{data.frame}. The first column (with no column name) contains the names(or paths to) the .CEL files to read. Remaining columns (with names) represent experimental factors for each chip. these become elements of the \texttt{phenoData} object.
- \texttt{...} extra functions to pass on to \texttt{ReadAffy}
- \texttt{path} The path to prefix the filenames with before calling \texttt{ReadAffy}

\textbf{Value}

An \texttt{AffyBatch} object

\textbf{Author(s)}

Crispin J Miller

\textbf{References}

http://bioinformatics.picr.man.ac.uk/

\textbf{See Also}

\texttt{ReadAffy, AffyBatch data.frame phenoData}

\textbf{Examples}

\begin{verbatim}
## Not run:
eset <- read.affy.mixed(); # read a set of CEL files
eset.rma <- call.exprs(eset,"rma");
## End(Not run)
\end{verbatim}
setQCEnvironment

Establish the appropriate QC environment for the specified array

Description

Affymetrix define a series of QC parameters for their arrays. Many of these rely on specific probeset that differ between arrays and are used to calculate things like 3'/5' ratios. See qc.affy for more details. These functions are used to set up the appropriate QC environment for the specified array. This is done by loading a configuration file, either from the packages data directory, or from the specified path. See the package vignette for details of the config file’s syntax.

Usage

setQCEnvironment(array,path=NULL)

Arguments

array
This should be the 'clean' cdf name of the array as generated by cleancdfname in the affy package.

path
Path to the file. By default, checks the package's own data directory - only needed if a definition file is being specified manually, as described in the vignette.

Details

The usual way to get the 'clean' cdfname is as follows: cleancdfname(cdfName(eset)), where eset is an AffyBatch object.

Value

none.

Author(s)

Crispin J Miller

References

http://bioinformatics.picr.man.ac.uk/

See Also

qc

Examples

setQCEnvironment("hgu133plus2cdf")
setQCEnvironment(cleancdfname("HG-U133_Plus_2"))
Does `simpleaffy` have a QC definition file for the specified array?

**Description**

The underlying implementation of `simpleaffy` has changed significantly and it now represents QC parameters differently. In particular, it loads only the QC data for the specified array type. A call to any of these functions loads the appropriate environment specified by `name`. They therefore been deprecated and WILL disappear from `simpleaffy` in the future.

**Usage**

```r
getTao(name)
getAlpha1(name)
getAlpha2(name)
getActin3(name)
getActinM(name)
getActin5(name)
getGapdh3(name)
getGapdhM(name)
getGapdh5(name)
getAllQCProbes(name)
getBioB(name)
getBioC(name)
getBioD(name)
getCreX(name)
getAllSpikeProbes(name)
haveQCParams(name)
```

**Arguments**

- `name` The 'clean' CDF name of the array (i.e. the result of calling `cleancdfname` on the `cdfName` of the AffyBatch object containing the array data of interest).

**Details**

Each of these functions has been replaced by a new function of the form `qc.get..` In order to support ratios other than gapdh and beta-actin, the appropriate way to get ratios is now to use `qc.get.ratios`, which will return a table containing all suggested ratio calculations for the array. Similarly, `qc.get.spikes` will return a table containing all spike probesets for the array.

**Value**

None.

**Author(s)**

Crispin J Miller

**References**

http://bioinformatics.picr.man.ac.uk/
standard.pearson

See Also
setQCEnvironment qc qc.ok cdfName cleancdfname qc.get.ratios qc.get.spikes qc.get.probes

Examples

#old
getBioB("hgu133plus2cdf")
getActin3("hgu133plus2cdf")
getActinM("hgu133plus2cdf")
getActin5("hgu133plus2cdf")
#new
setQCEnvironment("hgu133plus2cdf")
qc.get.spikes()["bioB"]
r <- qc.get.probes()
r["actin3"]
r["actinM"]
r["actin5"]

standard.pearson  A clustering function based on pearson correlation

Description

Given a matrix of values, uses hclust and cor to generate a clustering based on 1-Pearson correlation

Usage

standard.pearson(x)

Arguments

x  A matrix of data

Value

The result of performing an hclust

Author(s)

Crispin J Miller

See Also

hmap hmap.eset hmap.pc

Examples

## Not run:
y <- standard.pearson(x)
## End(Not run)
Does a Traditional Scatter Plot of Expression Data

Description
Plots expression data as a scatter plot with optional fold-change lines.

Usage
```r
trad.scatter.plot(x, y, add = FALSE, fc.lines = log2(c(2, 4, 6, 8)), draw.fc.lines = TRUE, draw.fc.line.labels = TRUE, fc.line.col = "lightgrey", pch = 20, xlim = NULL, ylim = NULL, ...)
```

Arguments
- `x`: x coords
- `y`: y coords
- `add`: add this data to an existing graph
- `fc.lines`: Vector of intervals at which to draw fold-change lines
- `draw.fc.lines`: Draw fold change lines?
- `draw.fc.line.labels`: Label the fold change lines with the fold changes they represent?
- `fc.line.col`: The colour to draw fold change lines
- `pch`: Plotting character to use for the scatter data (see `plot` for more details)
- `xlim`: Range for the xaxis
- `ylim`: Range for the yaxis
- `...`: Additional parameters to pass through to the underlying `plot` function

Author(s)
Crispin J Miller

References
http://bioinformatics.picr.man.ac.uk/

See Also
`plot`

Examples
```r
## Not run:
trad.scatter.plot(exprs(eset.rma)[,1], exprs(eset.rma)[,4])
## End(Not run)
```
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