Package 'affycoretools'

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Title Functions useful for those doing repetitive analyses with Affymetrix GeneChips.
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Description Various wrapper functions that have been written to streamline the more common analyses that a core Biostatistician might see.
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Depends affy, Biobase, GO.db, KEGG.db
Imports biomaRt, limma, GOstats, annotate, annaffy, genefilter, gcrma, splines, xtable, AnnotationDbi, lattice, gplots, R2HTML, oligoClasses, ReportingTools, hwriter
Suggests affydata, hgfocuscdf, rgl
biocViews ReportWriting, Microarray, OneChannel, GeneExpression
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 ${\it affystart}$

Pre-processing for Affymetrix Data

Description

This function is designed to automatically read in all cel files in a directory, make all pre-processing QC plots and compute expression measures.

Usage

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```
affystart(..., filenames=NULL, groups = NULL, groupnames = NULL, plot =
TRUE, pca = TRUE, squarepca = FALSE, plottype="pdf", express = c("rma",
"mas5", "gcrma"), addname = NULL, output = "txt", annotate =
FALSE, ann.vec = c("SYMBOL", "GENENAME", "ENTREZID", "UNIGENE", "REFSEQ"))
```

Arguments

	Requires that all variables be named.
filenames	If not all cel files in a directory will be used, pass a vector of filenames.
groups	An integer vector indicating the group assignments for the PCA plot.
groupnames	A character vector with group names for PCA legend.
plottype	What type of plot to save. Can be "pdf", "postscript", "png", "jpeg", or "bmp". Defaults to "pdf". Note that "png" and "jpeg" may not be available on a given computer. See the help page for capabilities and png for more information.
plot	Should density and degradation plots be made? Defaults to TRUE.
рса	Should a PCA plot be made? Defaults to TRUE.

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squarepca	Should the y-axis of the PCA plot be made comparable to the x-axis? This may aid in interpretation of the PCA plot. Defaults to FALSE.
express	One of either rma, mas5, gcrma. Defaults to rma. Partial matching OK.
addname	Used to append something to the name of the pca plot and the expression values output file (e.g., if function is run twice using different methods to compute expression values).
output	What format to use for the output of expression values. Currently only supports text format.
annotate	Boolean. Add annotation data to the output file?
ann.vec	A character vector of annotation data to add to the output file.

Value

Returns an ExpressionSet.

Author(s)

James W. MacDonald <jmacdon@u.washington.edu>

See Also

```
plotHist, plotDeg, plotPCA
```

annBM	Select Available Annotation from a Biomart

Description

These functions are designed to do two things that are useful for an end user. If called with no arguments, they will output a character vector of annotation sources that are typically available from a Biomart database. If called with a 'mart' connection (typically created by a call to useMart), they will return a character vector of annotation sources that exist for that particular Biomart and species. If called with a 'mart' connection and a character vector of annotation sources, they will return a list that is intended to be used by other functions for creating HTML pages. This last function doesn't have any real utility for the end user.

Usage

```
annBM(mart, annot, species)
linksBM(mart, annot, affyid = FALSE, ann.source = NULL)
```

Arguments

mart A 'mart' connection, typically created by a call to useMart.

annot A character vector of annotation sources. This is not typically useful for an end

user to specify.

affyid Boolean. Are the IDs being annotated Affymetrix IDs?

ann.source Character. The filter name to use if we are annotating Affymetrix IDs.

species A species name, of the form e.g., 'hsapiens'

Details

The purpose of these functions is to either give an example of typical annotation sources that may be available at a particular Biomart, or to output those sources that are known to exist at a Biomart.

linksBM is intended to list those annotation sources that may be turned into hyperlinks whereas annBM is intended to list those annotation sources that will not be linked.

These functions have only a few of the possible annotation sources, and currently there is no simple way to extend these sources. Additions to the list are possible, however. Please contact me if there is something in particular that should be included in either list.

Value

Normally called by an end user to output a character vector of annotation sources.

Author(s)

James W. MacDonald <jmacdon@u.washington.edu>

Examples

annBM()

data And Heatmap Page	A function to create an annotated HTML table for all genes in a sig-
	nificant gene set as well as a heatmap of these data.

Description

This is intended to be an internal function to runRomer. It is documented here only because it may be necessary to pass alternative arguments to this function from runRomer.

Usage

dataAndHeatmapPage(eset, fit, ind, columns = NULL, annot = NULL, fname, heatmap, title, key = TRUE, fiti

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Arguments

eset	An ExpressionSet containing normalized, summarized gene expression data.
fit	An MArrayLM object.
ind	Numeric vector indicating which rows of the ExpressionSet to use.
columns	Numeric vector indicating which columns of the ExpressionSet to use. If NULL, all columns will be used.
annot	Character. The name of the annotation package for the array used. If NULL, data will be output without any additional annotation.
fname	The filename of the resulting output, without the 'html' file extension.
heatmap	Character. The filename of the heatmap to append to the bottom of the HTML page.
title	Title to be placed at the top of the resulting HTML page.
key	Character. The filename of the heatmap key to append to the bottom of the HTML page.
fitind	Numeric. Which column of the MArrayLM object to use for output in the HTML table.

Details

This function creates an annotation table using probes2table if an annotation file is used, otherwise data will be output in a simple HTML table. A heatmap showing the expression values for all the genes in the gene set is then placed below this table, along with a key that indicates the range of the expression values.

Author(s)

James W. MacDonald

	foldFilt	Output Fold Change Data	
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Description

This function is designed to take an ExpressionSet and some comparisons and output either HTML tables, text files, or both.

Usage

```
foldFilt(object, fold = 1, groups, comps, compnames, save = FALSE, text
= TRUE, html = TRUE, filterfun = NULL)
```

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Arguments

object An ExpressionSet object

The log fold change cutoff to use. Note that this is log base two.

groups A vector of group identifiers. Probably easiest to use a numeric vector

comps A list containing all the comparisons to be made. Each list item should be a

vector of length two. See details for more information.

compnames A character vector of the names for each of the comparisons to be made. This

will be the name of the resulting HTML or text file.

save Boolean. If TRUE, a list will be returned. The first item in the list will be a vector

showing the number of 'significant' genes for each comparison. The second item will be a matrix of -1's, 0's and 1's indicating a significant difference, and the direction of the difference. The first item is useful for creating Sweave - based reports and the second is useful for making Vennn diagrams using the

vennDiagram from the limma package.

html Boolean - if TRUE, output HTML tables
text Boolean - if TRUE, output text tables

filterfun A filtering function, created by genefilter to filter the data using additional

criteria. See details for more information

Details

This function is useful for outputting annotated gene lists for multiple fold change comparisons. The genes will be ordered by the absolute fold change. Note that this function is essentially a wrapper to call annaffy, so is only useful for Affymetrix GeneChips for which there is an annotation package.

Without attaching a data file to this package, it is not possible to give a working example. Instead, here is a 'for instance'.

Say you have an ExpressionSet containing four Affy HG-U133Plus2 chips. There is no replication, and you simply want to output genes with a two-fold or greater difference between the first chip and each of the last three (the first chip is the control, and the other three are experimentals). The ExpressionSet is called eset.

Additionally, say we don't want any genes called significant if both of the samples have very low expression. We can set up a filter using the **genefilter** package.

f1 <- kOverA(1,6)

filt <- filterfun(f1)

foldFilt(eset, groups=1:4, comps=list(c(2, 1), c(3, 1), c(4, 1)), compnames=c("Expt1-Cont", "Expt2-Cont", "Expt3-Cont"), filterfun = filt)

This will output three HTML tables called 'Expt1-Cont.html', etc., each containing sorted genes that have two-fold or greater differences between the two samples.

Value

Returns a list; see above for the elements of the list. This function is mainly called for the side effect of outputting HTML or text files containing annotated 'significant' gene lists.

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Author(s)

James W. MacDonald <jmacdon@u.washington.edu>

Description

This function is designed to take an ExpressionSet and some comparisons and output HTML tables. It is very similar to foldFilt except it uses the biomaRt package to annotate genes and the annotate package to create the HTML table(s).

Usage

```
foldFiltBM(object, fold = 1, groups, comps, compnames, species, links =
linksBM()[1:3], otherann = annBM()[1:3], filterfun = NULL, ann.source =
"entrezgene", affyid = FALSE, html = TRUE, text = TRUE, save = FALSE)
```

Arguments

object	An ExpressionSet object
fold	The log fold change cutoff to use. Note that this is log base two.
groups	A vector of group identifiers. Probably easiest to use a numeric vector
comps	A list containing all the comparisons to be made. Each list item should be a vector of length two. See details for more information.
compnames	A character vector of the names for each of the comparisons to be made. This will be the name of the resulting HTML or text file.
species	The species name. This must be in a particular format for biomaRt. An example for human is "hsapiens" or for mouse is "mmusculus".
links	A character vector of things to annotate with hyperlinks to online databases. See linksBM for possible values.
otherann	A character vector of things to annotate with text only (i.e., no hyperlinks). See annBM for possible values.
filterfun	A filtering function created by genefilter to filter the data using additional criteria. See details for more information
ann.source	The annotation source of the IDs that will be used to annotate the genes. The default value is "entrezgene". See details for other possibilities.
affyid	Boolean. Are the IDs used to annotate these data Affymetrix IDs?
html	Boolean. Output HTML tables? Defaults to TRUE
text	Boolean. Output text tables? Defaults to TRUE
save	Boolean. If TRUE, a list will be returned. The first item in the list will be a vector showing the number of 'significant' genes for each comparison. The second item will be a matrix of -1's, 0's and 1's indicating a significant difference, and the direction of the difference. The first item is useful for creating Sweave - based reports and the second is useful for making Vennn diagrams using vennDiagram from the limma package.

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Details

This function is useful for outputting annotated gene lists for multiple fold change comparisons. The genes will be ordered by the absolute fold change.

This function currently only supports Affymetrix data. It is designed for Affymetrix chips that don't have an annotation package, which includes data that have been analyzed using the 're-mapped' CDFs supplied to BioC by MBNI at University of Michigan.

The IDs that will be used to annotate the genes depend on the source of the data. If, for example, one is using an Affymetrix chip that doesn't have a BioC annotation package, then the IDs will be Affymetrix IDs. To find out the correct name to use for the ann.source argument, one can create a connection to a Biomart database using useMart and then get a list of available Affy arrays using getAffyArrays.

If one is using one of the re-mapped CDFs from MBNI at University of Michigan, then the IDs to use depend on the mapping used to create the CDF. At this time, only three types of CDFs can be used; EntrezGene, UniGene, and RefSeq. One can determine the correct ann.source argument by creating a connection to a Biomart database, and then calling linksBM(mart, linksBM())[[3]].

One can also protect against selecting probesets that have very small expression values for all samples (which likely have a large fold change due to noise, rather than signal) by using the filterfun argument. An example would be:

```
f <- kOverA(1, 6)
filt <- filterfun(f)
Then add filterfun = filt as an argument to the call to foldFilt.</pre>
```

Value

Returns a list; see above for the elements of the list. This function is mainly called for the side effect of outputting HTML or text files containing annotated 'significant' gene lists.

Author(s)

James W. MacDonald <jmacdon@u.washington.edu>

geneSetPage A function to create an HTML page for each gene set, as well as the HTML pages for each significant gene set.

Description

This is intended to be an internal function to runRomer, and is not intended to be called by end users. However, the ... argument to runRomer allows one to pass arguments to lower level functions, so the arguments are described here.

Usage

```
geneSetPage(rslts, genesets, eset, fit, file, cutoff = 0.05, dir = ".",
subdir = ".", columns = NULL, colnames = NULL, col = NULL, caption =
NULL, annot = NULL, nam = NULL, fitind = NULL, bline = NULL, ...)
```

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Arguments

rslts	The results from running romer on one gene set.
genesets	Character. A vector of gene symbols for one gene set.
eset	An ExpressionSet containing normalized, summarized gene expression data.
fit	An MArrayLM object, containing the fitted data.
file	Filename for the resulting HTML page.
cutoff	Numeric. The cutoff for significance for a given gene set. Defaults to 0.05.
dir	The directory to write the results. Defaults to the working directory.
subdir	The subdirectory to write the individual gene set results. Defaults to the working directory.
columns	Numeric. The columns of the ExpressionSet to use for the individual gene set output pages. See dataAndHeatmapPage for more information.
colnames	Character. Alternative column names for the resulting heatmap. See dataAndHeatmapPage for more information.
col	A vector of colors for the heatmap. Defaults to bluered.
caption	Caption to put at the top of the HTML page.
annot	Character. The name of the annotation package for the arrray used. If NULL, the row names of the heatmap will be the probeset IDs unless the 'nam' argument below is used.
nam	Character. The row names for the resulting heatmap. If NULL and no 'annot' argument is used, the probeset IDs will be used for the row names.
fitind	Numeric. The columns of the MArrayLM object to use for the individual HTML tables.
bline	Defaults to NULL. Otherwise, a numeric vector indicating which columns of the data are the baseline samples. The data used for the heatmap will be centered by subtracting the mean of these columns from all data.
•••	Allows arguments to be passed to lower-level functions. See dataAndHeatmapPage and gsHeatmap for available arguments.

Details

This function creates a 'midlevel' HTML table that contains each gene set that was significant, with a link to an HTML table that shows data for each gene in that gene set (with annotation), as well as a heatmap showing the expression levels. Normally this is not run by end users, but is called as part of the runRomer function.

Value

Nothing is returned. Called only for the side effect of creating HTML tables.

Author(s)

James W. MacDonald <jmacdon@u.washington.edu>

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getMainProbes

Remove control probesets from ST arrays

Description

This function is designed to remove all but the 'main' type of probesets from the Gene ST array types.

Usage

```
getMainProbes(input)
```

Arguments

input

Either a character string (e.g., "pd.hugene.1.0.st.v1") or a FeatureSet object.

Value

If the argument is a character string, returns a data frame containing probeset IDs along with the probeset type, that can be used to subset e.g., an ExpressionSet of Gene ST data, or an MArrayLM object created from Gene ST data. Note that the order of the probesets is not guaranteed to match the order in your ExpressionSet or MArrayLM object, so that should be checked first. If the argument is a FeatureSet object, it returns a FeatureSet object with only main probes remaining.

Author(s)

James W. MacDonald <jmacdon@u.washington.edu>

getUniqueLL

Subset a Vector of Probesets

Description

This function will take a vector of Affy IDs and return a vector of Entrez IDs that have replicated IDs removed. The resulting vector will still have the corresponding Affy IDs appended as names, which is important for some functions.

Usage

```
getUniqueLL(probes, annot)
```

Arguments

probes A vector of probe IDs

annot The annotation package for the chip used

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Details

Subsetting a set of Affy IDs to unique Entrez Gene IDs is a common thing to do prior to doing a hypergeometric test. Functions such as hyperGTest can use un-named vectors of Entrez IDs (e.g., unique(getLL(probeIDs, annot))), but there is some functionality that requires the Entrez Gene IDs to be in a named vector, with the names being the associated Probeset IDs.

As an example, hyperGoutput will only work correctly if the input Entrez ID vector is named with the associated Probeset IDs.

Value

A named vector of unique Entrez IDs

Author(s)

James W. MacDonald <jmacdon@u.washington.edu>

gsHeatmap	A function to create a simple heatmap and key.

Description

This is an internal function called by runRomer and is not intended to be used directly. It is documented here only because arguments may be passed down via the dots argument.

Usage

```
gsHeatmap(eset, ind, filename, columns = NULL, colnames = NULL, col =
NULL, annot = NULL, nam = NULL, scale.row = FALSE, key = TRUE, bline
= NULL)
```

Arguments

eset	An ExpressionSet containing normalized, summarized gene expression data.
ind	Numeric vector indicating which rows of the ExpressionSet to use.
filename	The filename for the heatmap and associated key.
columns	Numeric vector indicating which columns of the ExpressionSet to use. If NULL, all columns will be used.
colnames	Character. Substitute column names for the heatmap. If NULL, the sampleNames will be used.
col	A vector of colors to use for the heatmap. If NULL, the bluered function will be used.
annot	Character. The name of the array annotation package to use to convert probeset IDs to gene symbols. If NULL, either the probeset IDs will be used, or the input from the 'nam' argument below will be used.

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nam Character. A vector of row.names to use for the heatmap. If NULL, and the above

'annot' argument is NULL as well, probeset IDs will be used.

scale.row Boolean. Should the data be scaled by row? Defaults to FALSE.

key Boolean. Should a key be produced that shows the numeric range for the colors

of the heatmap? Defaults to TRUE.

bline A numeric vector, usually extracted from a contrast matrix, used to 'sweep' the

mean baseline sample means from the heatmap data. The end result will be a heatmap in which the colors correspond to log fold changes from the baseline

samples.

Details

As noted above, this is only intended to be called indirectly by runRomer. However, certain arguments such as scale.row, or col, etc, can be passed down to this function via the dots argument, allowing the end user to have more control over the finished product.

Value

Nothing is returned. Called only for the side effect of creating heatmaps in 'png' format.

Author(s)

James W. MacDonald <jmacdon@u.washington.edu>

hyperG2annaffy HTML tables from GOIDs

Description

Output HTML tables containing the 'enriched' genes for each GO term resulting from a call to hyperGtable.

Usage

```
hyperG2annaffy(probids, lib, eset, fit = NULL, subset = NULL, comp = 1, type = "MF", pvalue = 0.05, min.count = 10)
```

Arguments

probids	A vector of Affymetrix probe IDs
lib	An annotation package (e.g., hgu95av2)
eset	An ExpressionSet
fit	An lmFit object. Only necessary if statistics are desired in the resulting table. Defaults to NULL.
subset	A numeric vector used to select GO terms to output (see description for more

information). Defaults to NULL

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comp Which contrast/parameter estimate should be used to extract the relevant statis-

tics? Only used if fit is not NULL. See description for more information.

type One of "MF", "CC", "BP", indicating molecular function, cellular component,

or biological process, respectively.

pvalue The significance level used to choose GO terms

min.count The minimum number of a given GO term that must be on the chip in order to

choose that GO term. This protects against very low p-values that result from the situation where there are very few genes with a given GO term on the chip,

but one or two are found in the set of significant genes.

Details

This function is used to create HTML tables based on the output of hyperGtable. The basic idea is as follows; as part of an analysis, say hyperGtable was used to create a table of 'enriched' GO terms. Unfortunately, the table only lists GO terms and the number of probesets that are annotated to those GO terms, and the client may be interested in knowing what probesets are enriched for each (or some) GO term.

The default behaviour is to output an HTML table for each GO term, containing the probesets that are annotated at that term (and that are in the set of significant genes). If only some of the GO terms are of interest, one may use the subset argument to select only particular rows. In addition, if the relevant t-statistics, p-values and fold changes are of interest, one can also use the fit argument to point to an lmFit object that contains these data, as well as the comp argument to indicate which parameter or contrast to use. Note that the comp argument defaults to 1, so the first parameter or contrast will be extracted by default.

Value

This function is used only for the side effect of creating HTML tables.

Author(s)

James W. MacDonald <jmacdon@u.washington.edu>

hyperGoutput	Output Tables Based on Hypergeometric Test	
hyperGoutput	Output Tables Based on Hypergeometric Test	

Description

This function will output various tables containing probesets that are annotated to a particular GO, KEGG, or PFAM term. The tables are based on the results from a call to hyperGtest.

Usage

```
hyperGoutput(hyptObj, eset, pvalue, categorySize, sigProbesets, fit = NULL, subset = NULL,comp = 1, output = c("significant", "all", "split"), statistics = c("tstat", "pval", "FC"), html = TRUE, text = TRUE, ...)
```

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Arguments

hypt0bj A HyperGResult object, usually produced by a call to hyperGTest

eset An ExpressionSet object

pvalue The p-value cutoff used for selecting significant GO terms. If not specified, it

will be extracted from the HyperGResult object

categorySize Number of terms in the universe required for a term to be significant. See details

for more information

sigProbesets Vector of probeset IDs that were significant in the original analysis.

fit An MArrayLM object, produced from a call to eBayes

subset Numeric vector used to select particular tables to output. The default is to output

tables for all terms. See details for more information

comp Numeric vector of length one, used to indicate which comparison in the MArrayLM

object to use for extracting relevant statistics. See details for more information

output One of 'selected', 'all', or 'split'. See details for more information

statistics Which statistics to output in the resulting tables. Choices include 'tstat', 'pval',

or 'FC', corresponding to t-statistics, p-values, and fold change, respectively

html Boolean. Output HTML tables? Defaults to TRUE text Boolean. Output text tables? Defaults to TRUE

... Allows end user to pass further arguments. The most notable would be an

anncols argument, passed to probes2table to control the hyperlinked anno-

tation columns. See aaf.handler for more information

Details

This function is designed to be used to output the results from a hypergeometric test for overrepresented terms. This function would be used at the end of an analysis such as:

1.) Compute expression values 2.) Fit a model using 1imma 3.) Output significant probesets using 1imma2annaffy 4.) Perform hypergeometric test using hyperGTest

At step 4, one can output a list of the over-represented terms using htmlReport. One might then be interested in knowing which probesets contributed to the significance of a particular term, which is what this function is designed to do.

One argument that can be passed to <a href="https://html.ncb.ncb.nlm.n

This function will output HTML and/or text tables containing annotation information about each probeset as well as the expression values. In addition, if limma were used to fit the model, the relevant statistics (t-statistic, p-value, fold change) can also be output in the table by passing the MArrayLM object that resulted from a

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call to eBayes. The statistics argument can

be used to control which statistics are output.

By default hyperGoutput will output tables for all significant terms, which may end up being quite a few tables. Usually only a few terms are of interest, so there is a subset argument that can be used to select only those terms. This argument follows directly from the order of the table output by htmlReport or summary. For instance, if the first, third and fifth terms in the HTML table output by htmlReport were of interest, one would use subset=c(1,3,5).

One critical step prior to the hypergeometric test is to subset the probesets to unique Entrez Gene IDs. It should be noted however, that the functions used by hypergOutput will output all the probesets annotated to a particular term. The output argument is used to control this behavior. If output = "significant" (the default), then only those probesets that correspond to the original subsetting will be output. If output = "all", then all probesets will be output (grouped by Entrez ID), with the 'significant' probeset first. If output = "split", then all the probesets will be output, with all the 'significant' probesets first, followed by the other probesets, grouped by Entrez ID.

Note that the 'significant' probesets come from one of two sources. First, one can pass a character vector of probeset IDs corresponding to those that were significant in the original analysis (recommended). Second, if the geneIds slot of the GOHyperGParams object containes a named vector of Entrez Gene IDs, then the names from that vector will be used. This can be accomplished by using either findLargest or getUniqueLL.

Since the geneIds are by definition a unique set of Entrez Gene IDs, any duplicate probeset IDs will have been removed, so the first method is to be preferred for accuracy.

Value

This function returns no value, and is called solely for the side effect of outputting HTML and/or text tables.

Author(s)

James W. MacDonald <imacdon@u.washington.edu>

See Also

hyperGTest, htmlReport, probeSetSummary

limma2annaffy

Function to Create HTML Tables from limma Objects

Description

This function is designed to take an ExpressionSet and an lmFit, model.matrix, and contrast object from limma and convert into HTML tables using annaffy. The alternate function limma2annaffy.na is designed to be run without user intervention.

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Usage

```
limma2annaffy(eset, fit, design, contrast, lib, adjust = "fdr",
anncols = aaf.handler()[c(1:3, 6:7, 9:12)], number = 30, pfilt = NULL,
fldfilt= NULL,tstat = TRUE, pval = TRUE, FC = TRUE,
expression = TRUE, html = TRUE, text = FALSE, save = FALSE, addname =
NULL, addtitle = NULL, interactive = TRUE, natFC = FALSE)
```

Arguments

eset An ExpressionSet containing affymetrix expression values.

fit An lmFit object.

design A model.matrix object.

contrast A contrasts matrix from limma.

1ib An annotation package for the Affy chips used.

adjust Multiplicity adjustment. Choices are "fdr", "holm", "hommel", "bonferroni", or

"none". Partial matching allowed.

anncols A vector of things to annotate, produced by a call to aaf.handler().

Number of genes to output to table. See details for more information.

pfilt A p-value to filter output. See details for more information.

fldfilt A fold change to filter output. See details for more information.

tstat Boolean: Output t-statistics in table? Defaults to FALSE.

pval Boolean: Output (adjusted) p-values in table? Defaults to FALSE.

FC Boolean: Output fold changes in table? Defaults to FALSE.

expression Boolean: Output expression values in table? Defaults to TRUE.

html Boolean: Output data in HTML tables? Defaults to TRUE.
text Boolean: Output data in text tables? Defaults to TRUE.

save Boolean: Save tables as R objects for further processing? Defaults to FALSE.

addname A character vector to add to the end of the automatically generated output file

names. Useful for multiple calls to eliminate over-writing of existing HTML or

text tables.

addtitle A character vector to add to the title for the HTML table. By default the title will

be the same as the filename. If the addname argument is not NULL, then that will be appended to the filename (and will be used as the HTML title). If addtitle is not NULL, it will be appended to the filename and that will then be used as the

HTML table title.

interactive Boolean: Is this an interactive call, or run as part of a script (e.g., in an Sweave

document)? Defaults to TRUE

natFC Boolean: Add 'unlogged' fold change to output? This is intended for people

who don't understand logs or fractions. If the fold change is positive, it is simply exponentiated (e.g., 2^x where x is the log fold change). If negative, we use -

 2^{-1} , so e.g., a log fold change of -2 will result in a -4.

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Details

This function is designed to automatically output HTML or text tables, with filenames taken from the column names of the contrast matrix. The number of genes output can be controlled several different ways. First, if pfilt and fldfilt are both NULL, the top genes will be output based on the number variable. Otherwise, the genes are filtered based on p-value, fold change, or both. If the genes are filtered this way, the number of genes to be output will be listed and the filter(s) can then be adjusted if necessary.

This function currently only supports Affymetrix data.

Value

If save is TRUE, a list of tables from topTable will be output.

Author(s)

James W. MacDonald <jmacdon@u.washington.edu>

See Also

topTable, aafTableAnn

 ${\it limma2biomaRt} \qquad \qquad {\it Function~to~Create~HTML~Tables~from~limma~Objects~using~biomaRt} \\ {\it for~Annotation}$

Description

This function is designed to take an ExpressionSet and an lmFit, model.matrix, and contrast object from limma and convert into HTML and text tables using biomaRt. The alternate function limma2biomaRt.na is designed to be run without user intervention.

Usage

```
limma2biomaRt(eset, fit, design, contrast, species, links = linksBM()[1:3],
otherdata = annBM()[1:3], ann.source = "entrezgene", adjust = "fdr",
number = 30, pfilt = NULL, fldfilt = NULL, tstat = TRUE,
pval = TRUE, FC = TRUE, expression = TRUE, html = TRUE, text = TRUE,
save = FALSE, addname = NULL, interactive = TRUE, affyid = FALSE)
```

Arguments

eset An ExpressionSet containing affymetrix expression values.

fit An lmFit object.
design A model.matrix object.

contrast A contrasts matrix from limma.

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species The species name. This must be in a particular format for biomaRt. An example for human is "hsapiens", or for mouse "mmusculus". links A character vector of things to annotate with hyperlinks to online databases. See linksBM for possible values. otherdata A character vector of things to annotate with text only (i.e., no hyperlinks). See annBM for possible values. The annotation source of the IDs that will be used to annotate the genes. The ann.source default value is "entrezgene". See details for other possibilities. Multiplicity adjustment. Choices are "fdr", "holm", "hommel", "bonferroni", or adjust "none". Partial matching allowed. number Number of genes to output to table. See details for more information. pfilt A p-value to filter output. See details for more information. fldfilt A fold change to filter output. See details for more information. Boolean: Output t-statistics in table? Defaults to FALSE. tstat pval Boolean: Output (adjusted) p-values in table? Defaults to FALSE. FC Boolean: Output fold changes in table? Defaults to FALSE. expression Boolean: Output expression values in table? Defaults to TRUE. Boolean: Output data in HTML tables? Defaults to TRUE. html Boolean: Output data in text tables? Defaults to TRUE text save Boolean: Save tables as R objects for further processing? Defaults to FALSE. A character vector to add to the end of the automatically generated output file addname names. Useful for multiple calls to eliminate over-writing of existing HTML or text tables. Boolean: Is this an interactive call, or run as part of a script (e.g., in an Sweave interactive document)? Defaults to TRUE affyid Boolean. Are the IDs used to annotate these data Affymetrix IDs?

Details

This function is designed to automatically output HTML tables, with filenames taken from the column names of the contrast matrix. The number of genes output can be controlled several different ways. First, if pfilt and fldfilt are both NULL, the top genes will be output based on the number variable. Otherwise, the genes are filtered based on p-value, fold change, or both. If the genes are filtered this way, the number of genes to be output will be listed and the filter(s) can then be adjusted if necessary.

This function currently only supports Affymetrix data. It is designed for Affymetrix chips that don't have an annotation package, which includes data that have been analyzed using the 're-mapped' CDFs supplied to BioC by MBNI at University of Michigan.

The IDs that will be used to annotate the genes depend on the source of the data. If, for example, one is using an Affymetrix chip that doesn't have a BioC annotation package, then the IDs will be Affymetrix IDs. To find out the correct name to use for the ann.source argument, one can create a connection to a Biomart database using useMart and then get a list of available Affy arrays using listFilters.

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If one is using one of the re-mapped CDFs from MBNI at University of Michigan, then the IDs to use depend on the mapping used to create the CDF. At this time, only three types of CDFs can be used; EntrezGene, UniGene, and RefSeq. One can determine the correct ann.source argument by creating a connection to a Biomart database, and then calling linksBM(mart, linksBM())[[3]].

Value

If save is TRUE, a list of tables from topTable will be output.

Author(s)

James W. MacDonald <jmacdon@u.washington.edu>

See Also

topTable, aafTableAnn

makeHmap	A function to create a heatmap-like object or matrix of correlations between miRNA and mRNA data.

Description

This function is intended for use when both miRNA and mRNA data are available for the same samples. In this situation it may be advantageous to compute correlations between the two RNA types, in order to detect mRNA transcripts that are targeted by miRNA.

Usage

makeHmap(mRNAdat, miRNAdat, mRNAlst, mRNAvec = NULL, miRNAvec = NULL, chipPkg, header, plot = TRUE, out

Arguments

mRNAdat	An ExpressionSet, data. frame or matrix of mRNA expression values. The row.names for these data should correspond to the manufacturer's probe ID. Currently, the only manufacturer supported is Affymetrix.
miRNAdat	An ExpressionSet, data.frame or matrix of mRNA expression values. The row.names for these data should correspond to the manufacturer's probe ID. Currently, the only manufacturer supported is Affymetrix.
mRNAlst	A list of mRNA probe IDs where the names of each list item are mirBase miRNA IDs. Usually this will be the output from mirna2mrna.
mRNAvec	A numeric vector used to subset or reorder the mRNA data, by column. If NULL, this will simply be 1:ncol(mRNAdat).
miRNAvec	A numeric vector used to subset or reorder the miRNA data, by column. If NULL, this will simply be 1:ncol(miRNAdat).
chipPkg	Character. The name of the chip-specific annotation package (e.g., "hgu133plus2.db").

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header	Character. The plot title if a heatmap is output.
plot	Boolean. Should a heatmap be generated?

out Boolean. Should the matrix of correlation coefficients be output?

Details

As noted above, this function is intended to generate output from simultaneous analyses of miRNA and mRNA data for the same samples, the goal being either a heatmap like plot of correlations, or the data (or both).

If creating a plot, note that if the number of significant mRNA probes is large, the resulting heatmap will have many rows and will not plot correctly on the usual graphics device within R. In order to visualize, it is almost always better to output as a pdf. In addition, the dimensions of this pdf will have to be adjusted so the row names for the heatmap will be legible. As an example, a heatmap with 10 miRNA transcripts and 100 mRNA transcripts will likely need a pdf with a width argument of 6 and a height argument of 25 or 30. It may require some experimentation to get the correct arguments to the pdf function.

Also please note that this function by necessity outputs rectangular data. However, there will be many instances in which a given miRNA isn't thought to target a particular mRNA. Whenever this occurs, the heatmap will have a white cell, and the output data for that combination will be NA.

Value

This function will output a numeric matrix if the 'out' argument is TRUE.

Author(s)

James W. MacDonald

See Also

mirna2mrna

makeVenn

High-level function for making Venn diagrams and outputting the results from the diagrams in HTML and CSV files.

Description

This function is designed to output CSV and HTML tables based on an analysis using the limma package, with output generated using the ReportingTools package.

Usage

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Arguments

fit	An MArrayLM object, from a call to eBayes.
contrast	A contrasts matrix, produced either by hand, or by a call to makeContrasts
design	A design matrix.
eset	An ExpressionSet object.
groups	This argument is used when creating a legend for the resulting HTML pages. If NULL, the groups will be generated using the column names of the design matrix. In general it is best to leave this NULL.
collist	A list containing numeric vectors indicating which columns of the fit, contrast and design matrix to use. If NULL, all columns will be used.
p.value	A p-value to filter the results by.
lfc	A log fold change to filter the results by.
method	One of "same", "both", "up", "down", "sameup", or "samedown". See details for more information.
adj.meth	Method to use for adjusting p-values. Default is 'BH', which corresponds to 'fdr'. Ideally one would set this value to be the same as was used for decideTests
titleadd	Additional text to add to the title of the HTML tables. Default is NULL, in which case the title of the table will be the same as the filename.
fileadd	Additional text to add to the name of the HTML and CSV tables. Default is NULL.
baseUrl	A character string giving the location of the page in terms of HTML locations. Defaults to "."
reportDirectory	
	A character string giving the location that the results will be written. Defaults to "./venns"
	Used to pass other arguments to probes2table, in particular, to change the argument to anncols which controls the columns of hyperlinks to online databases (e.g., Entrez Gene, etc.). See aaf.handler for more information.

Details

The purpose of this function is to output HTML and text tables with lists of genes that fulfill the criteria of a call to decideTests as well as the direction of differential expression. This is a high-level function that calls vennSelect2 internally, and is intended to be used with vennPage to create a set of Venn diagrams (on an HTML page) that have clickable links in each cell of the diagram. The links will then pass the end user to individual HTML pages that contain the genes that are represented by the counts in a given cell of the Venn diagram.

In general, the only thing that is needed to create a set of Venn diagrams is a list of numeric vectors that indicate the columns of the contrast matrix that are to be used for a given diagram. See the example below for a better explanation.

Some important things to note: First, the names of the HTML and text tables are extracted from the colnames of the TestResults object, which come from the contrasts matrix, so it is important to use something descriptive. Second, the method argument is analogous to the include argument

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from vennCounts or vennDiagram. Choosing "both" will select genes that are differentially expressed in one or more comparisons, regardless of direction. Choosing "up" or "down" will select genes that are only differentially expressed in one direction. Choosing "same" will select genes that are differentially expressed in the same direction. Choosing "sameup" or "samedown" will select genes that are differentially expressed in the same direction as well as 'up' or 'down'.

Note that this is different than sequentially choosing "up" and then "down". For instance, a gene that is upregulated in one comparison and downregulated in another comparison will be listed in the intersection of those two comparisons if "both" is chosen, it will be listed in only one comparison for both the "up" and "down" methods, and it will be listed in the union (e.g., not selected) if "same" is chosen.

Unlike vennSelect, this function automatically creates both HTML and CSV output files.

Value

A list containing the output from calling vennSelect2 on the columns specified by the collist argument. This is intended as input to vennPage, which will use those data to create the HTML page with Venn diagrams with clickable links.

Author(s)

James W. MacDonald <jmacdon@u.washington.edu>

Examples

```
## Not run:
   mat <- matrix(rnorm(1e6), ncol = 20)</pre>
    design <- model.matrix(~factor(1:4, each=5))</pre>
    colnames(design) <- LETTERS[1:4]</pre>
    contrast <- matrix(c(1,-1,0,0,1,0,-1,0,1,0,0,-1,0,1,-1,0,0,1,0,-1),
    ncol = 5)
    colnames(contrast) <- paste(LETTERS[c(1,1,1,2,2)],</pre>
    LETTERS[c(2,3,4,3,4)], sep = " vs ")
    fit <- lmFit(mat, design)</pre>
    fit2 <- contrasts.fit(fit, contrast)</pre>
    fit2 <- eBayes(fit2)</pre>
    ## two Venn diagrams - a 3-way Venn with the first three contrasts
    ## and a 2-way Venn with the last two contrasts
    collist <- list(1:3,4:5)</pre>
    venn <- makeVenn(fit2, contrast, design, eset, collist = collist)</pre>
    vennPage(venn, "index.html", "Venn diagrams")
## End(Not run)
```

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Description

This function creates an MA plot for all arrays in either an ExpressionSet or a matrix. A 'baseline' array is created using the median expression for each gene, and each array is then compared to the baseline array.

Usage

```
maplot(object, layout = NULL, ...)
```

Arguments

object An ExpressionSet or matrix containing log-transformed array data.

layout A numeric vector, length two. Best results will be obtained if both values are

the same, and between 2 and 5 (e.g., c(3,3))

... Other arguments that will be passed down to the xyplot function from the lattice

package.

Value

No output. Used only for the side effect of creating MA plots.

Author(s)

James W. MacDonald <jmacdon@u.washington.edu>

na A function to map miRNA to mRNA.

Description

This function is intended use when there are miRNA and mRNA data for the same subjects, and the goal is to detect mRNAs that appear to be targeted by the miRNA.

Usage

```
mirna2mrna(miRNAids, miRNAannot, mRNAids, orgPkg, chipPkg, sanger =
TRUE, miRNAcol = NULL, mRNAcol = NULL, transType = "ensembl")
```

Arguments

miRNAids	A character vector of miRNA IDs. Currently only supports Affymetrix platform.
miRNAannot	Character. The filename (including path if not in working directory) for the file containing miRNA to mRNA mappings.
mRNAids	A character vector of mRNA IDs. Currently only supports Affymetrix platform.
orgPkg	Character. The Bioconductor organism package (e.g., org.Hs.eg.db) to be used for mapping.

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chipPkg	Character. The Bioconductor chip-specific package (e.g., hgu133plus2.db) to be used for mapping.
sanger	Boolean. Is the miRNAannot file a Sanger miRBase targets file? These can be downloaded from http://www.ebi.ac.uk/enright-srv/microcosm/cgi-bin/targets/v5/download.pl
miRNAcol	Numeric. If using a Sanger miRBase targets file, leave NULL. Otherwise, use this to indicate which column of the miRNAannot file contains miRNA IDs.
mRNAcol	Numeric. If using Sanger miRBase targets file, leave NULL. Otherwise, use this to indicate which column of the miRNAannot file contains mRNA IDs.
transType	Character. Designates the type of transcript ID for mRNA supplied by the miR-NAannot file. If using the Sanger miRBase files, this is ensembl. Other choices include refseq and accnum.

Details

This function is intended to take a vector of miRNA IDs that are significantly differentially expressed in a given experiment and then map those IDs to putative mRNA transcripts that the miRNAs are supposed to target. The mRNA transcript IDs are then mapped to chip-specific probeset IDs, which are then subsetted to only include those probesets that were also significantly differentially expressed.

The output from this function is intended as input for makeHmap.

Value

A list with names that correspond to each significant miRNA, and the mRNA probeset IDs that are targeted by that miRNA.

Author(s)

James W. MacDonald

See Also

makeHmap

outputRomer	A function to create HTML output from the results of running romer on a set of contrasts.

Description

This function is actually intended to be a sub-function of runRomer, but can hypothetically run by itself if the romer step has already been done.

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Usage

```
outputRomer(rsltlst, genesetlst, eset, fit, design = NULL, contrast =
   NULL, changenames = TRUE, dir = "genesets", explanation = NULL,
   annot = NULL, baseline.hmap = TRUE, file = "indexRomer.html", ...)
```

Arguments

rsltlst A list of results, generated by the romer function. See discussion for more

information.

geneset1st A list of genesets, usually created by loading in the RData files that can be

downloaded from http://bioinf.wehi.edu.au/software/MSigDB/. See details for

more information.

eset An ExpressionSet containing normalized, summarized gene expression data.

fit An MArrayLM object, containing the fitted data.

design A design matrix describing the model.

contrast A contrast matrix describing the contrasts that were fit. This matrix should have

colnames, which will be used to name subdirectories containing results.

changenames Boolean. When creating heatmaps of the gene sets, should the columns be ap-

pended with the colnames from the design matrix? If FALSE, the sampleNames

will be used.

dir Character. The subdirectory to use for the output data. Defaults to 'genesets'.

explanation If NULL, a generic paragraph will be placed at the top of the indexRomer.html

page, giving a brief explanation of the analysis. Alternatively, this can be replaced with other text. Please note that this text should conform to HTML standards (e.g., will be pasted into the HTML document as-is, so should contain any

required HTML markup).

annot Character. The name of the annotation package for the array being used. This

is used to add annotation data to the data tables for each gene set. If NULL (the

default), the tables will only contain probeset IDs and statistics.

baseline.hmap Boolean. If TRUE, then the resulting heatmaps will be centered by subtracting

the mean of the baseline sample. As an example, in a contrast of treatment A - treatment B, the mean of the treatment B samples will be subtracted. The heatmap colors then represent the fold change between the A and B samples.

file Character. The filename to output. Defaults to 'indexRomer.html'.

... Arguments to be passed to lower-level functions. See geneSetPage, dataAndHeatmapPage

and gsHeatmap for available arguments.

Details

This function is intended to be an internal function for runRomer. However, it is possible that runRomer errored out after saving the results from running romer on a set of contrasts, and all that remains is to create the output HTML.

Please note that the first two arguments to this function have certain expectations. The rsltlst should be the output from running romer. If using the saved output from runRomer, one should first load

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the 'romer.Rdata' file, which will introduce a list object with the name 'romerlst' into the working directory, so the first argument should be rsltlst = romerlst.

Second, see the code for runRomer, specifically the line that creates the 'sets' object, which will show how to create the correct genesetlst object.

Value

Nothing is returned. The function is run only for the side effect of creating HTML tables with output for each significant gene set.

Author(s)

James W. MacDonald < jmacdon@u.washington.edu>

plotDeg

Functions to Plot Density and RNA Degradation Plots

Description

These functions make density and RNA degradation plots with automatic placement of legends.

Usage

```
plotDeg(dat, filenames = NULL)
plotHist(dat, filenames = NULL)
```

Arguments

dat An AffyBatch object, or in the case of plotHist, a matrix (e.g., from a call to

read.probematrix. Note that plotDeg requires an AffyBatch object to work

correctly.

filenames Filenames that will be used in the legend of the resulting plot. If NULL (the de-

fault), these names will be extracted from the sampleNames slot of the AffyBatch

object.

Value

These functions are called only for the side effect of making the plots. Nothing else is returned.

Author(s)

James W. MacDonald <jmacdon@u.washington.edu>

Examples

```
library("affydata")
data(Dilution)
plotDeg(Dilution)
plotHist(Dilution)
```

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plotPCA	A Function to Make a PCA Plot from an ExpressionSet

Description

This function makes a PCA plot from an ExpressionSet or matrix

Usage

```
plotPCA(object, groups = NULL, groupnames = NULL, addtext = NULL, x.coord = NULL,
y.coord = NULL, screeplot = FALSE, squarepca = FALSE, pch = NULL, col =
NULL, pcs = c(1,2), legend = TRUE, main = "Principal Components Plot",
plot3d = FALSE, outside = FALSE, ...)
```

Arguments

object	An ExpressionSet, matrix or prcomp object.
groups	A numeric vector delineating group membership for samples. Default is NULL, in which case default plotting symbols and colors will be used.
groupnames	A character vector describing the different groups. Default is NULL, in which case the sample names will be used.
addtext	A character vector of additional text to be placed just above the plotting symbol for each sample. This is helpful if there are a lot of samples for identifying e.g., outliers.
x.coord	Pass an x-coordinate if automatic legend placement fails
y.coord	Pass a y-coordinate if automatic legend placement fails.
screeplot	Boolean: Plot a screeplot instead of a PCA plot? Defaults to FALSE.
squarepca	Should the y-axis of the PCA plot be made comparable to the x-axis? This may aid in interpretation of the PCA plot. Defaults to FALSE.
pch	A numeric vector indicating what plotting symbols to use. Default is NULL, in which case default plotting symbols will be used. Note that this argument will override the 'groups' argument.
col	A numeric or character vector indicating what color(s) to use for the plotting symbols. Default is NULL in which case default colors will be used. Note that this argument will override the 'groups' argument.
pcs	A character vector of length two (or three if plot3d is TRUE), indicating which principal components to plot. Defaults to the first two principal components.
legend	Boolean. Should a legend be added to the plot? Defaults to TRUE.
main	A character vector for the plot title.
plot3d	Boolean. If TRUE, then the PCA plot will be rendered in 3D using the rgl package. Defaults to FALSE. Note that the pcs argument should have a length of three in this case.

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outside Boolean. If TRUE the legend will be placed outside the plotting region, at the top

right of the plot.

... Further arguments to be passed to plot. See the help page for plot for further

information.

Value

This function returns nothing. It is called only for the side effect of producing a PCA plot or screeplot.

Author(s)

James W. MacDonald <jmacdon@u.washington.edu>

Examples

```
library("affy")
data(sample.ExpressionSet)
plotPCA(sample.ExpressionSet, groups =
   as.numeric(pData(sample.ExpressionSet)[,2]), groupnames =
   levels(pData(sample.ExpressionSet)[,2]))
```

probes2table

Convert Affy Probe ids to Annotated HTML Table

Description

A function to convert a vector of Affy ids to an annotated HTML table.

Usage

```
probes2table(eset, probids, lib, otherdata = NULL,
anncols = aaf.handler()[c(1:3, 6:7, 9:12)], html = TRUE, text = FALSE,
express = TRUE, save = FALSE, filename, title = NULL)
```

Arguments

eset An ExpressionSet containing Affy expression values.

probids A vector of probe ids.

1ib An annotation package for the Affy chips used.

otherdata A *named* list of additional information to include in the resulting table. Ex-

amples would be t-statistics, p-values, fold change, etc. Each list item should be a vector the same length as the probids vector. The name associated with each

list item will be used as the column name in the resulting table.

anncols A vector of things to annotate, produced by a call to aaf.handler().

html Output data in HTML tables? Defaults to TRUE.

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text	Output data in text tables? Defaults to TRUE.

express Output expression values in table? Defaults to TRUE.

save Should tables be saved as R objects for further processing? Defaults to FALSE.

filename Filename of the resulting HTML table.

title Title for HTML table. If NULL, the filename will be used.

Value

If save is TRUE, a data. frame is saved containing the data.

Author(s)

James W. MacDonald <jmacdon@u.washington.edu>

See Also

```
topTable, aafTableAnn
```

probes2tableBM	Convert Affy Probe ids to Annotated HTML Table using biomaRt
----------------	--

Description

A function to convert a vector of Affy ids to an annotated HTML or text table. This function is very similar to probes2table, except it uses the biomaRt package to annotate genes, and the annotate package to create the HTML table.

Usage

```
probes2tableBM(eset, probids, species, filename, otherdata = NULL,
links = linksBM()[1:3], otherann = annBM()[1:3], ann.source = "entrezgene",
express = TRUE, html = TRUE, text = TRUE, affyid = FALSE)
```

Arguments

eset An ExpressionSet cont	taining Affy expression values.
----------------------------	---------------------------------

probids A vector of probe ids.

species The species name. This must be in a particular format for biomaRt. An example

for human is "hsapiens" or for mouse is "mmusculus".

filename File name of the resulting HTML table.

otherdata A *named* list of additional information to include in the resulting table. Ex-

amples would be t-statistics, p-values, fold change, etc. Each list item should be a vector the same length as the probids vector. The name associated with each

list item will be used as the column name in the resulting table.

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A character vector of things to annotate with hyperlinks to online databases. See links linksBM for possible values. otherann A character vector of things to annotate with text only (i.e., no hyperlinks). See annBM for possible values. The annotation source of the IDs that will be used to annotate the genes. The ann.source default value is "entrezgene". See details for other possibilities. express Output expression values in table? Defaults to TRUE. html Boolean. Output HTML table? Defaults to TRUE Boolean. Output text table? Defaults to TRUE text affyid Boolean. Are the IDs used to annotate these data Affymetrix IDs?

Details

This function is designed to output HTML tables based on a set of IDs. This function currently only supports Affymetrix data. It is designed for Affymetrix chips that don't have an annotation package, which includes data that have been analyzed using the 're-mapped' CDFs supplied to BioC by MBNI at University of Michigan.

The IDs that will be used to annotate the genes depend on the source of the data. If, for example, one is using an Affymetrix chip that doesn't have a BioC annotation package, then the IDs will be Affymetrix IDs. To find out the correct name to use for the ann.source argument, one can create a connection to a Biomart database using useMart and then get a list of available Affy arrays using listFilters.

If one is using one of the re-mapped CDFs from MBNI at University of Michigan, then the IDs to use depend on the mapping used to create the CDF. At this time, only three types of CDFs can be used; EntrezGene, UniGene, and RefSeq. One can determine the correct ann.source argument by creating a connection to a Biomart database, and then calling linksBM(mart, linksBM())[[3]].

Value

This function is only used for the side effect of outputting an HTML table.

Author(s)

James W. MacDonald <jmacdon@u.washington.edu>

See Also

topTable

runRomer 31

runRomer	A function to run the romer function on a set of contrasts.
Turitoillet	11 junction to run the romer junction on a set of contrasts.

Description

This function automates both running romer on a set of contrasts as well as the creation of output HTML tables that can be used to explore the results. The basic idea here is that one might have used limma to fit a model and compute some contrasts, and then want to do a GSEA using romer.

Usage

```
runRomer(setloc, annot = NULL, eset, design = NULL, contrast = NULL,
fit, wts = NULL, save = TRUE, baseline.hmap = TRUE, ...)
```

Arguments

setloc	A character vector giving the path for gene set RData files (see description for more information).
annot	Character. The name of the array annotation package.
eset	An ExpressionSet containing normalized expression data.
design	A design matrix describing the model fit to the data.
contrast	A contrast matrix describing the contrasts that were computed from the data. This contrast should have colnames, which will be used to create parts of the resulting directory structure.
fit	An MArrayLM object, containing the fitted model data.
wts	Optional weights vector - if array weights were used to fit the model, they should be supplied here as well.
save	Boolean. If true, after running the romer step, the results will be saved in a file 'romer.Rdata', which can be used as input for outputRomer to create HTML tables. Since romer can take a long time to run, it is advantageous to keep the default.
baseline.hmap	Boolean. If TRUE, then the resulting heatmaps will be centered by subtracting the mean of the baseline sample. As an example, in a contrast of treatment A - treatment B, the mean of the treatment B samples will be subtracted. The heatmap colors then represent the fold change between the A and B samples.
•••	$Used \ to \ pass \ arguments \ to \ lower-level \ functions. \ See \ output Romer \ gene Set Page, \\ data And Heatmap Page \ and \ gs Heatmap \ for \ available \ arguments.$

Details

The romer expects as input a list or lists of gene symbols that represent individual gene sets. One example is the various gene sets from the Broad Institute that are available at http://bioinf.wehi.edu.au/software/MSigDB/, which are distributed as RData files. The default assumption for this function is that the end user will have downloaded these files, and the setloc argument simply tells runRomer where to find them.

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Alternatively, user-based gene sets could be created (these should consist of lists of character vectors of gene symbols - see one of the Broad gene sets for an example).

This function will run romer using all the gene sets in the referenced directory, on all the contrasts supplied, and then output the results in a (default) 'genesets' subdirectory. There will be an HTML file in the working directory with a (default) filename of 'indexRomer.html' that will point to individual HTML files in the genesets subdirectory, which will point to individual files in subdirectories within the genesets subdirectory (named after the colnames of the contrast matrix).

Value

Nothing is returned. This function is called only for the side-effects of creating output HTML files in the working and sub-directories.

Author(s)

James W. MacDonald <jmacdon@u.washington.edu>

vennCounts2

Compute Counts for Venn Diagram

Description

This function is designed to compute counts for a Venn diagram. It is slightly different from vennCounts in the additional ability to compute counts for genes that are differentially expressed in the same direction.

Usage

```
vennCounts2(x, method = "same", fit = NULL, foldFilt = NULL)
```

Arguments

A TestResults object, produced by a call to decideTests or foldFilt.

Method One of "same", "both", "up", "down". See details for more information.

An MArrayLM object, produced by a call to lmFit and eBayes. Only necessary if 'foldFilt' = TRUE.

A fold change to filter samples. This is primarily here for consistency with the corresponding argument in vennSelect.

Details

The function vennCounts will return identical results except for the "same" method. This will only select those genes that both pass the criteria of decideTests as well as being differentially expressed in the same direction. Note that this is different from the "both" method, which simply requires that a given gene be differentially expressed in e.g., two different comparisons without any requirement that the direction be the same.

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Value

A VennCounts object.

Author(s)

James W. MacDonald <jmacdon@u.washington.edu>

Examples

```
library("limma")
tstat <- matrix(rt(300,df=10),100,3)
tstat[1:33,] <- tstat[1:33,]+2
clas <- classifyTestsF(tstat,df=10,p.value=0.05)
a <- vennCounts2(clas)
print(a)
vennDiagram(a)</pre>
```

vennPage

High-level function for making Venn diagrams with clickable links to HTML pages with the underlying genes.

Description

This function is designed to be used in conjunction with the makeVenn function, to first create a set of HTML pages containing the genes that are represented by the cells of a Venn diagram, and then create an HTML page with the same Venn diagrams, with clickable links that will point the end user to the HTML pages.

Usage

Arguments

vennlst	The output from makeVenn.
pagename	Character. The file name for the resulting HTML page. Something like 'venns' is reasonable. Note that the .html will automatically be appended.
pagetitle	Character. The heading for the HTML page.
cex.venn	Numeric. Adjusts the size of the font in the Venn diagram. Usually the default is OK.
shift.title	Boolean. Should the right contrast name of the Venn diagram be shifted down? Useful for long contrast names. If a two-way Venn diagram, this will shift the right name down so they don't overlap. If a three-way Venn diagram, this will shift the top right name down.

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baseUrl Character. The base URL for the resulting HTML page. The default of "." is usually optimal.

reportDirectory

If NULL, the reportDirectory will be extracted from the vennlst. This is usually what one should do.

Details

This function is intended to be used as part of a pipeline, by first calling makeVenn and then using the output from that function as input to this function to create the HTML page with clickable links.

Value

An HTMLReport object. If used as input to the ReportingTools publish function, this will create a link on an index page to the Venn diagram HTML page. See e.g., the microarray analysis vignette for ReportingTools for more information.

Author(s)

James W. MacDonald < jmacdon@u.washington.edu>

Examples

```
## Not run:
   mat <- matrix(rnorm(1e6), ncol = 20)</pre>
    design <- model.matrix(~factor(1:4, each=5))</pre>
   colnames(design) <- LETTERS[1:4]</pre>
    contrast <- matrix(c(1,-1,0,0,1,0,-1,0,1,0,0,-1,0,1,-1,0,0,1,0,-1),
   ncol = 5)
    colnames(contrast) <- paste(LETTERS[c(1,1,1,2,2)],</pre>
   LETTERS[c(2,3,4,3,4)], sep = " vs ")
    fit <- lmFit(mat, design)</pre>
    fit2 <- contrasts.fit(fit, contrast)</pre>
    fit2 <- eBayes(fit2)</pre>
    ## two Venn diagrams - a 3-way Venn with the first three contrasts
    ## and a 2-way Venn with the last two contrasts
    collist <- list(1:3,4:5)</pre>
    venn <- makeVenn(fit2, contrast, design, eset, collist = collist)</pre>
    vennreport <- vennPage(venn, "index.html", "Venn diagrams")</pre>
    indexPage <- HTMLReport("index", "My results", reportDirectory =</pre>
    ".", baseUrl = ".")
    publish(vennreport)
    finish(indexPage)
## End(Not run)
```

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Description

This function is designed to output text and/or HTML tables based on the results of a call to decideTests.

Usage

```
vennSelect(eset, design, x, contrast, fit, method = "same", adj.meth =
"BH", stat = "fstat", otherstats = c("pval", "FC"), order.by = "pval",
foldFilt = NULL, save = FALSE, titleadd = NULL, ...)
```

Arguments

eset	An ExpressionSet object.
design	A design matrix.
x	A TestResults object, usually from a call to decideTests.
contrast	A contrasts matrix, produced either by hand, or by a call to makeContrasts
fit	An MArrayLM object, from a call to eBayes.
method	One of "same", "both", "up", "down", "sameup", or "samedown". See details for more information.
adj.meth	Method to use for adjusting p-values. Default is 'BH', which corresponds to 'fdr'. Ideally one would set this value to be the same as was used for decideTests.
stat	The statistic to report in the resulting HTML tables. Choices are 'fstat', 'tstat', and NULL. Ideally, the statistic chosen would correspond to the method used in decideTests. In other words, if one used methods such as 'separate' or 'hierarchical', which are based on a t-statistic, one should choose 'tstat', however, if one used 'nestedF', the logical choice would be 'fstat'.
otherstats	Other statistics to be included in the HTML tables. Choices include 'pval' and 'FC'.
order.by	Which statistic should be used to order the probesets? Choices include 'fstat', 'tstat', 'pval', and 'FC'. Note that if 'FC' is chosen and there are more than one set of fold changes, the first will be used.
foldFilt	A log fold change to filter results.
save	Boolean. Save the results for further processing?
titleadd	Additional text to add to the title of the HTML tables. Default is NULL, in which case the title of the table will be the same as the filename.
	Used to pass other arguments to probes2table, in particular, to change the argument to anncols which controls the columns of hyperlinks to online databases (e.g., Entrez Gene, etc.). See aaf.handler for more information.

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Details

The purpose of this function is to output HTML and text tables with lists of genes that fulfill the criteria of a call to decideTests as well as the direction of differential expression.

Some important things to note: First, the names of the HTML and text tables are extracted from the colnames of the TestResults object, which come from the contrasts matrix, so it is important to use something descriptive. Second, the method argument is analogous to the include argument from vennCounts or vennDiagram. Choosing "both" will select genes that are differentially expressed in one or more comparisons, regardless of direction. Choosing "up" or "down" will select genes that are only differentially expressed in one direction. Choosing "same" will select genes that are differentially expressed in the same direction. Choosing "sameup" or "samedown" will select genes that are differentially expressed in the same direction as well as 'up' or 'down'.

Note that this is different than sequentially choosing "up" and then "down". For instance, a gene that is upregulated in one comparison and downregulated in another comparison will be listed in the intersection of those two comparisons if "both" is chosen, it will be listed in only one comparison for both the "up" and "down" methods, and it will be listed in the union (e.g., not selected) if "same" is chosen.

Calling the function normally will result in the output of HTML and text tables:

vennSelect(eset, fit, design, x)

Calling the function with save set to TRUE will output both HTML and text tables as well as a vector of counts for each comparison. This is useful when using the function programmatically (e.g., when making reports using Sweave).

out <- vennSelect(eset, fit, design, x, save = TRUE)

An alternative would be to use vennCounts2 and vennDiagram to output a Venn diagram, which is probably more reasonable since the tables being output are supposed to be based on a Venn diagram.

Value

Normally called only for the side effect of producing HTML and text tables. However, setting save to TRUE will output a vector of counts that can be used for making Sweave-style reports.

Author(s)

James W. MacDonald <jmacdon@u.washington.edu>

vennSelect2

Select and Output Genelists Based on Venn Diagrams

Description

This function is designed to output text and/or HTML tables based on the results of a call to decideTests, using the ReportingTools package.

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Usage

```
vennSelect2(fit, contrast, design, eset, groups = NULL, cols = NULL,
p.value = 0.05, lfc = 0, method = "same", adj.meth =
"BH", titleadd = NULL, fileadd = NULL, baseUrl = ".", reportDirectory =
"./venns", ...)
```

Arguments

fit	An MArrayLM object, from a call to eBayes.	
contrast	A contrasts matrix, produced either by hand, or by a call to makeContrasts	
design	A design matrix.	
eset	An ExpressionSet object.	
groups	This argument is used when creating a legend for the resulting HTML pages. If NULL, the groups will be generated using the column names of the design matrix.	
cols	A numeric vector indicating which columns of the fit, contrast and design matrix to use. If NULL, all columns will be used.	
p.value	A p-value to filter the results by.	
lfc	A log fold change to filter the results by.	
method	One of "same", "both", "up", "down", "sameup", or "samedown". See details for more information.	
adj.meth	Method to use for adjusting p-values. Default is 'BH', which corresponds to 'fdr'. Ideally one would set this value to be the same as was used for $decideTests$.	
titleadd	Additional text to add to the title of the HTML tables. Default is NULL, in which case the title of the table will be the same as the filename.	
fileadd	Additional text to add to the name of the HTML and CSV tables. Default is NULL.	
baseUrl	A character string giving the location of the page in terms of HTML locations. Defaults to "."	
reportDirectory		
	A character string giving the location that the results will be written. Defaults to "./venns"	
	Used to pass other arguments to probes2table, in particular, to change the argument to anncols which controls the columns of hyperlinks to online databases	

Details

The purpose of this function is to output HTML and text tables with lists of genes that fulfill the criteria of a call to decideTests as well as the direction of differential expression.

(e.g., Entrez Gene, etc.). See aaf.handler for more information.

Some important things to note: First, the names of the HTML and text tables are extracted from the colnames of the TestResults object, which come from the contrasts matrix, so it is important to use something descriptive. Second, the method argument is analogous to the include argument

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from vennCounts or vennDiagram. Choosing "both" will select genes that are differentially expressed in one or more comparisons, regardless of direction. Choosing "up" or "down" will select genes that are only differentially expressed in one direction. Choosing "same" will select genes that are differentially expressed in the same direction. Choosing "sameup" or "samedown" will select genes that are differentially expressed in the same direction as well as 'up' or 'down'.

Note that this is different than sequentially choosing "up" and then "down". For instance, a gene that is upregulated in one comparison and downregulated in another comparison will be listed in the intersection of those two comparisons if "both" is chosen, it will be listed in only one comparison for both the "up" and "down" methods, and it will be listed in the union (e.g., not selected) if "same" is chosen.

Unlike vennSelect, this function automatically creates both HTML and CSV output files.

Value

A list with two items. First, a list of HTMLReport objects from the ReportingTools package, which can be used to create an index page with links to the HTML pages created by this function. See the help page for HTMLReport in ReportingTools as well as the vignettes for more information. The second item is a vennCounts object from limma, which can be used to create a Venn diagram, e.g., in a report if this function is called within a Sweave or knitR pipeline.

Author(s)

James W. MacDonald <jmacdon@u.washington.edu>

vennSelectBM

Select and Output Genelists Based on Venn Diagrams using biomaRt

Description

This function is designed to output HTML and text tables based on the results of a call to decideTests. This function is very similar to vennSelect, except it uses the biomaRt package to annotate genes, and the annotate package to create the HTML table.

Usage

```
vennSelectBM(eset, design, x, contrast, fit, method = "same", adj.meth = "BH",
stat = "fstat", otherstats = c("pval", "FC"), order.by = "pval",
foldFilt = NULL, save = FALSE, species, links = linksBM()[1:3],
otherdata = annBM()[1:3], ann.source = "entrezgene",
html = TRUE, text = TRUE, affyid = FALSE, ...)
```

Arguments

eset A ExpressionSet object.

design A design matrix, usually from a call to model.matrix. See details for more

information.

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x	A TestResults object, usually from a call to decideTests.
contrast	A contrasts matrix, produced either by hand, or by a call to makeContrasts
fit	An MArrayLM object, from a call to eBayes.
method	One of "same", "both", "up", "down", "sameup", or "samedown". See details for more information.
adj.meth	Method to use for adjusting p-values. Default is 'BH', which corresponds to 'fdr'. Ideally one would set this value to be the same as was used for decideTests.
stat	The statistic to report in the resulting HTML tables. Choices are 'fstat', 'tstat', and NULL. Ideally, the statistic chosen would correspond to the method used in decideTests. In other words, if one used methods such as 'separate' or 'hierarchical', which are based on a t-statistic, one should choose 'tstat', however, if one used 'nestedF', the logical choice would be 'fstat'.
otherstats	Other statistics to be included in the HTML tables. Choices include 'pval' and 'FC'.
order.by	Which statistic should be used to order the probesets? Choices include 'fstat', 'tstat', 'pval', and 'FC'. Note that if 'FC' is chosen and there are more than one set of fold changes, the first will be used.
foldFilt	A fold change to use for filtering. Default is NULL, meaning no filtering will be done.
save	Boolean - If TRUE, output a count of genes that fulfill the criteria. Useful for e.g., Sweave-type reports.
species	The species name. This must be in a particular format for biomaRt. An example for human is "hsapiens", or for mouse "mmusculus".
links	A character vector of things to annotate with hyperlinks to online databases. See linksBM for possible values.
otherdata	A character vector of things to annotate with text only (i.e., no hyperlinks). See annBM for possible values.
ann.source	The annotation source of the IDs that will be used to annotate the genes. The default value is "entrezgene". See details for other possibilities.
html	Boolean. Output HTML tables? Defaults to TRUE
text	Boolean. Output text tables? Defaulst to TRUE
affyid	Boolean. Are the IDs used to annotate these data Affymetrix IDs?
	Used to pass other variables to e.g., htmlpage.

Details

The purpose of this function is to output HTML tables with lists of genes that fulfill the criteria of a call to decideTests as well as the direction of differential expression.

The IDs that will be used to annotate the genes depend on the source of the data. If, for example, one is using an Affymetrix chip that doesn't have a BioC annotation package, then the IDs will be Affymetrix IDs. To find out the correct name to use for the ann.source argument, one can create a connection to a Biomart database using useMart and then deduce the correct argument by the output from listFilters(mart). It will usually be something starting with 'affy', and contain the name of the chip.

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If one is using one of the re-mapped CDFs from MBNI at University of Michigan, then the IDs to use depend on the mapping used to create the CDF. At this time, only three types of CDFs can be used; EntrezGene, UniGene, and RefSeq. One can determine the correct ann.source argument by creating a connection to a Biomart database, and then calling linksBM(mart, linksBM())[[3]].

Some important things to note: First, the names of the HTML tables are extracted from the colnames of the TestResults object, which come from the contrasts matrix, so it is important to use something descriptive. Second, the method argument is analogous to the include argument from vennCounts or vennDiagram. Choosing "both" will select genes that are differentially expressed in one or more comparisons, regardless of direction. Choosing "up" or "down" will select genes that are only differentially expressed in one direction. Choosing "same" will select genes that are differentially expressed in the same direction. Choosing "sameup" or "samedown" will select genes that are differentially expressed in the same direction as well as 'up' or 'down'.

Note that this is different than sequentially choosing "up" and then "down". For instance, a gene that is upregulated in one comparison and downregulated in another comparison will be listed in the intersection of those two comparisons if "both" is chosen, it will be listed in only one comparison for both the "up" and "down" methods, and it will be listed in the union (e.g., not selected) if "same" is chosen.

Calling the function normally will result in the output of HTML tables.

Calling the function with save set to TRUE will output HTML tables as well as a vector of counts for each comparison. This is useful when using the function programmatically (e.g., when making reports using Sweave).

out <- vennSelectBM(eset, fit, design, x, <other arguments>, save = TRUE)

An alternative would be to use vennCounts2 and vennDiagram to output a Venn diagram, which is probably more reasonable since the tables being output are supposed to be based on a Venn diagram.

Value

Normally called only for the side effect of producing HTML tables. However, setting save to TRUE will output a vector of counts that can be used for making Sweave-style reports.

Author(s)

James W. MacDonald <jmacdon@u.washington.edu>

vennSelectFC

Select and Output Gene Lists Based on Venn Diagrams

Description

This function is designed to output text and/or HTML tables based on the results of a call to foldFilt. The general idea being that one might want to create a Venn diagram showing probesets that are unique to particular comparisons, or consistent between comparisons, and then might want to output the probesets that are contained in each cell of the Venn diagram.

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Usage

```
vennSelectFC(eset, x, comps, order.by = "sum", method =
"same", text = TRUE, html = TRUE, ...)
```

Arguments

eset	A ExpressionSet object.
х	An indicator matrix showing up or down regulation based on fold change, usually from a call to foldFilt. See details for more information.
comps	A list containing all the comparisons to be made. Each list item should be a vector of length two. This should be identical to the 'comps' argument used in the call to foldFilt See details for more information.
order.by	One of 'sum', 'max', 'median', or 'mean'. This orders the output for those tables that have multiple fold change values based on the summary statistic chosen. Defaults to 'sum'.
method	One of "same", "both", "up", "down", "sameup", or "samedown". See details for more information.
text	Boolean. Output text tables? Defaults to TRUE
html	Boolean. Output HTML tables? Defaults to TRUE
	Used to pass other arguments to probes2table, in particular, to change the argument to anncols which controls the columns of hyperlinks to online databases (e.g., Entrez Gene, etc.). See aaf.handler for more information.

Details

The purpose of this function is to output the probesets listed in a Venn diagram that has been produced by a call to foldFilt. A small example would be as follows:

Assume an ExpressionSet exists that contains expression values for three Affymetrix chips, say a control, and two experimentals. One might want to know what probesets are different between each of the experimentals and the control, and those that are different between both of the experimentals and the control. We first make the comparisons, based on a fold change of 2 (or a difference of 1 on the log scale).

```
comps <- list(c(1,2), c(1,3))
```

This list indicates what comparisons we want. In this case 1vs2 and 1vs3.

out <- foldFilt(eset, fold = 1, groups = 1:3, comps = comps, compnames=c("Control vs experimental1", "Control vs experimental2"), save = TRUE)

By setting save = TRUE, we are saving a list, the first item being a vector of the number of probesets in each comparison, the second item being an indicator matrix showing up or down regulation based on a two-fold difference. We could make a Venn diagram using this matrix with vennCounts2 and vennDiagram. If we then wanted to output the probesets in each cell of that Venn diagram, we could use vennSelectFC as follows:

```
vennSelectFC(eset, out[[2]], comps)
```

One thing to note here is that the names of the resulting tables as well as the columns containing the fold change values will be extracted from the column names of the indicator matrix. This matrix will

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get its column names from the 'compnames' argument to foldFilt, so it is best to use reasonable names here. Also note that any character used in the 'compnames' argument that is not a valid character for a file name will be stripped out.

Value

Called only for the side effect of outputting HTML and/or text tables.

Author(s)

James W. MacDonald <jmacdon@u.washington.edu>

writeFit

Function to output annotated fit data from limma

Description

This function is designed to take an ExpressionSet an annotation package and an 1mFit object, and output an annotated text file containing t-statistics, p-values, and fold change data for all contrasts.

Usage

```
writeFit(fit, annotation, eset, touse =
c("symbol", "genename", "accnum", "entrezid", "unigene"))
```

Arguments

fit A lmFit object, created by the limma package.

annotation An annotation package, specific for the chip used in the analysis.

eset An ExpressionSet object containing expression values.

touse Character vector of BiMaps from annotation package. As an example, if the

annotation package is the hgu133plus2.db package, then 'symbol' refers to the

hgu133plus2SYMBOL BiMap.

Details

This function is designed to output annotation data as well as statistics (p-values, fold change, t-statistics) for all probes on a chip.

Value

A data.frame is returned.

Author(s)

James W. MacDonald < jmacdon@u.washington.edu>

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See Also

write.fit

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