## Package 'GOexpress'

June 24, 2025

**Title** Visualise microarray and RNAseq data using gene ontology annotations

**Version** 1.43.0 **Date** 2017-04-04

Description The package contains methods to visualise the expression profile of genes from a microarray or RNA-seq experiment, and offers a supervised clustering approach to identify GO terms containing genes with expression levels that best classify two or more predefined groups of samples. Annotations for the genes present in the expression dataset may be obtained from Ensembl through the biomaRt package, if not provided by the user. The default random forest framework is used to evaluate the capacity of each gene to cluster samples according to the factor of interest. Finally, GO terms are scored by averaging the rank (alternatively, score) of their respective gene sets to cluster the samples. P-values may be computed to assess the significance of GO term ranking. Visualisation function include gene expression profile, gene ontology-based heatmaps, and hierarchical clustering of experimental samples using gene expression data.

**Depends** R (>= 3.4), grid, stats, graphics, Biobase (>= 2.22.0)

**Imports** biomaRt (>= 2.18.0), stringr (>= 0.6.2), ggplot2 (>= 0.9.0), RColorBrewer (>= 1.0), gplots (>= 2.13.0), randomForest (>= 4.6), RCurl (>= 1.95)

**Suggests** BiocStyle **License** GPL (>= 3)

**biocViews** Software, GeneExpression, Transcription,

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## **Description**

Integrates gene expression data with gene ontology annotations to score and visualise genes and gene ontologies best clustering groups of experimental samples. Supports custom annotations, or alternatively provides an interface to the Ensembl annotations using the biomaRt package. The default scoring approach is based on the random forest framework, while a one-way ANOVA is available as an alternative. GO term are scored and ranked according to the average rank (alternatively, average power) of all associated genes. P-values can be generated to assess the significance of GO term ranking. The ranked list of GO terms is returned, with tools allowing to visualise the statistics on a gene- and ontology-basis.

#### **Details**

Package: GOexpress Type: Package Version: 1.7.1 Date: 2016-02-06

License: GPL (>= 3)

This package requires only two input variables

1. An ExpressionSet containing assayData and phenoData. The former should be a gene-by-sample matrix providing gene expression values for each gene in each sample. The latter should be an AnnotatedDataFrame from the Biobase package providing phenotypic information and grouping factors with two or more levels.

2. The name of the grouping factor to investigate, which must be a valid column name in the phenoData slot of the above ExpressionSet.

Following analysis, visualisation methods include:

- Histogram and quantiles representations of the scores of GO terms
- Permutation-based P-values to assess the significance of GO term ranking
- Filtering of results on various criteria (e.g. number of genes annotated to GO term)
- Re-ordering of GO terms and gene result tables based on score or rank metric
- Table of statistics for genes annotated to a given GO term
- Hierarchical clustering of samples based on the expression level of genes annotated to a given GO term
- Heatmap of samples and genes based on the expression level of genes annotated to a given GO term
- Expression profile of a gene against one given factor (e.g. Time) while grouping samples on another given factor (e.g. Treatment)
- Univariate analysis of the expression level of a gene in the different groups of each experimental factor.
- Venn diagram of the counts of genes shared between a list of GO terms.

#### Author(s)

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#### See Also

Main method for an example usage: GO\_analyse.

Packages Biobase, randomForest, RColorBrewer, VennDiagram.

Methods biomaRt:getBM, ggplot2:ggplot, gplots:heatmap.2, gplots:bluered, gplots:greenred, grid:grid.newpage, grid:grid.layout, stringr:str\_extract.

#### **Examples**

```
# Sample input data available with package:
data(AlvMac)

# Sample output data available with package:
data(AlvMac_results)

# Supported species and microarrays:
data(microarray2dataset)
data(prefix2dataset)
```

AlvMac

Sample data from a RNAseq experiment.

## **Description**

An example ExpressionSet including expression data and phenotypic information about the samples.

The expression data is saved in the assayData slot of the ExpressionSet. It is a gene-by-sample matrix, containing a subset of data from an *in vitro* stimulation of bovine macrophages with different mycobacterial strains. Column names are sample names, and row names are Ensembl gene identifiers of the *Bos taurus* species. Each cell contains the log2-transformed normalised expression level of each gene in each sample.

The phenotypic information is saved in the phenoData slot of the ExpressionSet. Row names are sample names and columns contain descriptive information about each sample, including experimental factors(e.g. Treatment, Timepoint, Animal).

#### Usage

data(AlvMac)

#### **Details**

Gene expression was measured in poly-A purified strand-specific RNA libraries using the RNA-Sequencing Illumina(R) HiSeq(R) 2000 platform as paired-end 2 x 90 nucleotide reads. Raw reads from pooled RNA libraries were first deconvoluted according to sample-specific nucleotide barcodes. Read pairs containing adapter sequence in either read mate were discarded, and similarly read pairs of low overall quality in either mate were also discarded. Paired-end reads from each filtered individual library were aligned to the *Bos taurus* reference genome (*B. taurus* UMD3.1.71 genome release) using the STAR aligner software. For each library, raw counts for each gene based on sense strand data were obtained using the featureCounts software from the Subread package. The featureCounts parameters were set to unambiguously assign uniquely aligned paired-end reads in a stranded manner to the exons of genes within the *Bos taurus* reference genome annotation (*B*.

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*taurus* UMD3.1.71 genome annotation). The gene count outputs were further processed using the edgeR Bioconductor package.

The gene expression quantitation pipeline within the edgeR package was customised to: (1) filter out all bovine rRNA genes; (2) filter out genes displaying expression levels below the minimally-set threshold of one count per million [CPM] in at least ten individual libraries (number of biological replicates); (3) calculate normalisation factors for each library using the trimmed mean of M-values method; (4) log2-transform CPM values based on the normalised library size.

To generate this test data subset, we extracted 100 genes from the original dataset of 12,121 genes. All 7 genes associated with the GO term "GO:0034142" (i.e. "toll-like receptor 4 signaling pathway") present in the original full-size filtered-normalised dataset were kept, all 3 Ensembl gene identifiers annotated to the gene symbol 'RPL36A', and finally another random 90 random genes, making a total of 100 genes measured in 117 samples. Samples include all 10 biological replicates collected at four different time-points, see data(targets). The TLR4 pathway was found in the full dataset as the top-ranking biological pathway discriminating the different mycobacterial infections (unpublished observations).

#### Value

assayData is a matrix of expression levels for 100 genes (rows) measured in 117 samples (columns).

- rownames are Ensembl gene identifiers of the *Bos taurus* species.
- colnames are samples identifiers.

phenoData is a data frame with 117 samples and 7 descriptive fields (e.g. experimental factors) in the columns listed below:

- rownames are unique identifiers. Here, sample names.
- File contains local filenames where the RNAseq counts were obtained from.
- Sample contains individual sample name.
- Animal contains the unique identifier of the animal corresponding to the biological replicate, stored as a factor.
- Treatment contains the infection status of the sample, stored as a factor (CN: Control, MB: *M. bovis*, TB: *M. tuberculosis*)
- Time contains the time of measurement in hours post-infection, stored as a factor.
- Group contains a combination of the Treatment and Time factors above, stored as a factor itself.
- Timepoint contains the time of measurement, stored as a numeric value. This field is useful to use on the X-axis of expression plots. See function expression\_plot().

## Source

Publication in review process.

#### **Examples**

```
# Load the data
data(AlvMac)

# Structure of the data
str(AlvMac)

# Dimensions (rows, columns) of the data
```

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```
dim(AlvMac)

# Subset of first 5 features and 5 samples
AlvMac[1:5, 1:5]

# Phenotypic information
pData(AlvMac)

# Phenotypic information about factor "Group"
AlvMac$Group

# Conversion of a factor to a character vector
as.character(AlvMac$Group)

# Number of samples (rows) and annotations (columns)
dim(pData(AlvMac))
```

AlvMac\_allgenes

Example of custom gene feature annotations.

#### **Description**

An example data. frame providing the identifier, name and description corresponding to Ensembl gene identifiers present in the AlvMac example ExpressionSet.

## Usage

```
data("AlvMac_allgenes")
```

#### **Details**

This data-frame includes only Ensembl gene identifiers present in the AlvMac example ExpressionSet. See the help page of the GO\_analyse function for an example usage.

#### Value

A data frame detailing information about the 100 gene features present in the AlvMac example ExpressionSet:

- gene\_id are Ensembl gene identifiers.
- $\bullet$  external\_gene\_name contains the corresponding gene name.
- description contains the corresponding description.

## Source

These annotations were obtained from the Ensembl BioMart server using the biomaRt package to access the Ensembl release 75 http://feb2014.archive.ensembl.org.

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## **Examples**

```
# Load the data
data(AlvMac_allgenes)
# Structure of the data
str(AlvMac_allgenes)
# First few rows
head(AlvMac_allgenes)
```

AlvMac\_allGO

Example of custom gene ontology annotations.

## **Description**

An example data. frame providing the identifier, name and namespace corresponding to gene ontology identifiers, compatible with the AlvMac example ExpressionSet.

## Usage

```
data("AlvMac_allGO")
```

#### **Details**

This data-frame includes all gene ontologies present in the btaurus\_gene\_ensembl dataset of the Ensembl BioMart server, including those associated with no gene identifier in the AlvMac example ExpressionSet.

See the help page of the GO\_analyse function for an example usage.

## Value

A data frame detailing information about 13,302 gene ontologies:

- go\_id are gene ontology identifiers.
- name\_1006 contains the corresponding gene ontology name.
- namespace\_1003 contains the corresponding gene ontology namespace (i.e. "biological\_process", "molecular\_function", or "cellular\_component")

## **Source**

These annotations were obtained from the Ensembl BioMart server using the biomaRt package to access the Ensembl release 75 http://feb2014.archive.ensembl.org.

## **Examples**

```
# Load the data
data(AlvMac_allGO)

# Structure of the data
str(AlvMac_allGO)

# First few rows
head(AlvMac_allGO)
```

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AlvMac_GOgenes	Example of custom mapping between gene ontology identifiers and gene features.
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## **Description**

An example data.frame associating Ensembl gene identifiers to gene ontology identifiers, compatible with the AlvMac example ExpressionSet.

## Usage

```
data("AlvMac_GOgenes")
```

#### **Details**

This data-frame includes all annotations between Ensembl gene identifiers and gene ontology identifiers present in the btaurus\_gene\_ensembl dataset of the Ensembl BioMart server, including gene identifiers absent from the AlvMac example ExpressionSet.

Importantly, gene identifiers present in these annotations but absent from the ExpressionSet will be given a score of 0 (minimum valid score; indicates no power to discriminates the predefined groups of sample) and a rank equal to the number of genes present in the entire dataset plus one (worst rank, while preserving discrete continuity of the ranking). This is helpful where features considered uninformative were filtered out of the ExpressionSet.

See the help page of the GO\_analyse function for an example usage.

#### Value

A data frame with 191,614 associations between the following 2 variables.

- gene\_id are Ensembl gene identifiers of the Bos taurus species.
- go\_id are gene ontology identifiers.

## **Source**

These annotations were obtained from the Ensembl BioMart server using the biomaRt package to access the Ensembl release 75 http://feb2014.archive.ensembl.org.

## **Examples**

```
# Load the data
data(AlvMac_GOgenes)

# Structure of the data
str(AlvMac_GOgenes)

# First few rows
head(AlvMac_GOgenes)
```

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AlvMac_results	Sample output from the GO_analyse() function on an RNAseq experiment.

## Description

This variable may be used to test the filtering and visualisation methods implemented in the package. It contains the output of the command AlvMac\_results = GO\_analyse(eSet=AlvMac, f="Treatment") applied to the toy input data AlvMac.

#### Usage

```
data(AlvMac_results)
```

#### Value

A list of 9 slots summarising the input and results of the analysis:

- GO contains a table ranking all GO terms related to genes in the expression dataset based on the average ability of their related genes to cluster the samples according to the predefined grouping factor.
- mapping contains the table mapping genes present in the dataset to GO terms.
- genes contains a table ranking all genes present in the expression dataset based on their ability to cluster the samples according to the predefined grouping factor (see 'factor' below).
- factor contains the grouping factor analysed.
- method contains the statistical framework used.
- subset contains the filters used to select a subset of samples from the original ExpressionSet for analysis.
- rank.by contains the metric used to rank the scoring tables.
- ntree contains number of trees built during the randomForest analysis.
- mtry contains the number of features randomly sampled as candidates at each split in each tree built during the randomForest analysis.

#### Warning

Running the above command again, you might obtain slightly different scores and ranks due to the stochastic process of sampling used by the random forest algorithm. However, the ranking metric was found to be robust and stable across run, given adequate number of trees and predictor variables sampled.

To produce reproducible results, use the set.seed() function prior to running any randomising or sampling function.

## Source

Source data are part of a publication in review.

#### **Examples**

```
data(AlvMac_results)
str(AlvMac_results)
head(AlvMac_results$GO, n=20)
head(AlvMac_results$GO$genes, n=20)
```

AlvMac\_results.pVal

Sample output from the pValue\_GO() function on an RNAseq experiment.

## **Description**

This variable may be used to test the filtering and visualisation methods implemented in the package. It contains the output of successively applying the commands AlvMac\_results = GO\_analyse(eSet=AlvMac, f="Treatment") and AlvMac\_results.pVal = pValue\_GO(AlvMac\_results, N=100, ranked.by=result\$rank.by, rank.by='P') to the toy input data AlvMac.

#### Usage

```
data("AlvMac_results.pVal")
```

#### Value

A list of 9 slots summarising the input and results of the analysis:

- GO contains a table ranking all GO terms related to genes in the expression dataset based on the average ability of their related genes to cluster the samples according to the predefined grouping factor.
- mapping contains the table mapping genes present in the dataset to GO terms.
- genes contains a table ranking all genes present in the expression dataset based on their ability to cluster the samples according to the predefined grouping factor (see 'factor' below).
- factor contains the grouping factor analysed.
- method contains the statistical framework used.
- subset contains the filters used to select a subset of samples from the original ExpressionSet for analysis.
- rank. by contains the metric used to rank the scoring tables.
- ntree contains number of trees built during the randomForest analysis.
- mtry contains the number of features randomly sampled as candidates at each split in each tree built during the randomForest analysis.
- p.iterations contains the number of permutations performed to compute the P-value in the GO slot.

## Warning

Running the above command again, you might obtain slightly different scores and ranks due to the stochastic process of sampling used by the random forest algorithm. However, the ranking metric was found to be robust and stable across run, given adequate number of trees and predictor variables sampled.

To produce reproducible results, use the set.seed() function prior to running any randomising or sampling function.

cluster\_GO

#### Source

Source data are part of a publication in review.

## **Examples**

```
data(AlvMac_results.pVal)
str(AlvMac_results.pVal)
head(AlvMac_results.pVal, n=20)
```

cluster\_G0

Generates a hierarchical clustering of the samples

## Description

Clusters the samples using only the expression levels of genes associated with a given go\_id.

## Usage

```
cluster_GO(
    go_id, result, eSet, f=result$factor, subset=NULL,
    method_dist="euclidean", method_hclust="average", cex=0.8,
    main=paste(go_id, result$GO[result$GO$go_id == go_id, "name_1006"]),
    xlab="Distance", cex.main=1, main.Lsplit=NULL, ...)
```

## Arguments

go_id	A Gene Ontology (GO) identifier.
result	The output of GO_analyse() or a subset of it obtained from subset_scores().
eSet	ExpressionSet of the Biobase package including a gene-by-sample expression matrix in the AssayData slot, and a phenotypic information data-frame in the phenodata slot. In the expression matrix, row names are Ensembl gene identifiers or probeset identifiers, and column names are sample identifiers. In the phentypic data-frame, row names are sample identifiers, column names are grouping factors and phenotypic traits usable for the one-way ANOVA.
f	The grouping factor in phenodata to label the samples by.
subset	A named list to subset eSet. Names must be column names existing in colnames(pData(eSet)). Values must be vectors of values existing in the corresponding column of pData(eSet).
method_dist	The method used to calculate distance between samples. See the dist() method from package stats.
method_hclust	The method used to cluster samples. See the hclust() method from package stats.
cex	A numeric value defining the character expansion of text in the plot.
main	A character string for the main title of the plot.
xlab	A label for the x axis, defaults to "Distance".
cex.main	Scaling factor of the main title font size. Default is 1. We suggest to use it in combination with the argument main.Lsplit for GO terms with long names.

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main.Lsplit Number of characters after which a new-line character will be inserted in the main title. If this would occur within a word, the new-line character will be inserted before this word. Default is NULL, leaving the title on a single line.... Additional parameters passed on to dist(), hclust() and plot().

#### Value

Returns the output of the plot() function.

## Author(s)

Kevin Rue-Albrecht

#### See Also

Method GO\_analyse.

## **Examples**

```
# load the sample output data
data(AlvMac_results)

# Hierarchical clustering of samples based on the same GO term
cluster_GO(
    go_id="GO:0034142", result=AlvMac_results, eSet=AlvMac, cex=0.7
)

# Re-label sample by another factor
cluster_GO(
    go_id="GO:0034142", result=AlvMac_results, eSet=AlvMac, cex=0.7, f="Group"
)
```

expression\_plot

Plots the expression profile of a gene by levels of a factor

#### **Description**

This function will plot the expression profile of a gene across a valid X-axis variable in the phenodata while representing the mean and confidence interval of groups of samples defined by levels of another valid grouping factor in the phenodata.

## Usage

```
expression_plot(
   gene_id, result, eSet, x_var, f=result$factor, subset=NULL,
   xlab=x_var, ylab="log2(cpm)", ylim=range(exprs(eSet)),
   col.palette="Accent",
   col=brewer.pal(n=length(levels(pData(eSet)[,f])), name=col.palette),
   level=0.95, title=NULL, title.size=2, axis.title.size=20,
   axis.text.size=15, axis.text.angle=0,
   legend.title.size=20, legend.text.size=15, legend.key.size=30)
```

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#### **Arguments**

x\_var

An gene or probeset identifier present in rownames(expr\_data). gene\_id An output of the GO\_analyse() or subset\_scores() function. result

eSet ExpressionSet of the Biobase package including a gene-by-sample expres-

> sion matrix in the AssayData slot, and a phenotypic information data-frame in the phenodate slot. In the expression matrix, row names are Ensembl gene identifiers or probeset identifiers, and column names are sample identifiers. In the phentypic data-frame, row names are sample idenfifiers, column names are

grouping factors and phenotypic traits usable for the one-way ANOVA.

A column name in phenodata to plot on the X-axis. If representing time on the X-axis, users should store the time-points as numeric values in the AnnotatedDataFrame

to adequately space the time-points.

f A column name in phenodata to group the samples when representing mean

and confidence interval. The factor specified in the initial GO\_analyse() call is used by default. Unexpected grouping factors of samples can reveal interesting

trends (e.g. "Animal", "Tissue", "CellType", ...).

subset A named list to subset eSet. Names must be column names existing in col-

names(pData(eSet)). Values must be vectors of values existing in the corre-

sponding column of pData(eSet).

xlab Title of the X-axis. Default is tha value of x\_var.

ylab Title of the Y-axis. Default is "log2(cpm)".

ylim Numeric vector of length 2 specifying the lower and upper bounds of the Y axis.

> Default is scaled to the full range of expression values in the expression dataset, to ease comparison of different genes. If set to NULL, the axis will be scaled to

fit the plotted data only.

A valid RColorBrewer palette name to fetch the colormap from. Default is col.palette

palette "Accent".

A vector of color names or codes. The number of colors provided must match col

the number of levels of the grouping factor. If specified, overrides argument

col.palette.

level The confidence interval level to visualise around the mean of each group. De-

fault is 0.95.

title Changes the plot title. Default is a combination of the gene id and the associated

title.size Changes the font size of the title. Default is 2.

axis.title.size

Changes the font size of the axes title. Defalt is 20.

axis.text.size Changes the font size of the axes text labels. Default is 15.

axis.text.angle

Changes the angle of the X axis text labels. Default is 0 (horizontal).

legend.title.size

Changes the font size of the legend title. Default is 20.

legend.text.size

Changes the font size of the legend text labels. Default is 15.

legend.key.size

Changes the size of the legend keys (in points). Default is 30.

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#### Value

The ggplot object.

## Warning

Common issues:

• It may not be possible to produce plots where the combination of X-axis variable and grouping factor leaves too few replicates to compute a confidence interval for each X value. This is a limitation imposed by the ggplot2 package to produce proper statistics and confidence intervals. In such cases, it may be preferrable to use the expression\_profiles() method.

#### Author(s)

Kevin Rue-Albrecht

#### References

• ggplot2 package.

#### See Also

Package Biobase, methods expression\_plot\_symbol, GO\_analyse and ggplot.

## **Examples**

```
# load the sample output data
data(AlvMac_results)
# Expression by gene identifier (TNIP3)
expression_plot(
   gene_id="ENSBTAG00000047107",
   result=AlvMac_results, eSet=AlvMac, x_var="Timepoint"
# Same gene, plotted by animal and grouped by treatment (merging time points)
expression_plot(
   gene_id="ENSBTAG00000047107",
   result=AlvMac_results, eSet=AlvMac, x_var="Animal",
   f="Treatment")
# Same gene, plotted by animal and grouped by time-point (merging treatments)
expression_plot(
   gene_id="ENSBTAG00000047107",
   result=AlvMac_results, eSet=AlvMac, x_var="Animal",
   f="Time")
```

```
expression_plot_symbol
```

Plots the expression profile of a gene by levels of a factor

## Description

This function will plot the expression profile of a gene across a valid X-axis variable from the AnnotatedDataFrame while representing the mean and confidence interval of groups of samples defined by levels of a valid grouping factor from the AnnotatedDataFrame.

In the case of a gene name represented by multiple gene or probeset identifiers in the dataset, a lattice of plots will be produced. Each of the plots in this lattice can subsequently be plotted separately using its associated index.

## Usage

```
expression_plot_symbol(
   gene_symbol, result, eSet, x_var, f=result$factor, subset=NULL,
   index=0, xlab=x_var, ylab="log2(cpm)", ylim=range(exprs(eSet)),
   col.palette="Accent",
   col=brewer.pal(n=length(levels(pData(eSet)[,f])), name=col.palette),
   level=0.95, titles=c(), title.size=2, axis.title.size=20,
   axis.text.size=15, axis.text.angle=0,
   legend.title.size=20, legend.text.size=20, legend.key.size=30)
```

## **Arguments**

gene_symbol	A gene name present in rownames(AlvMac_results\$genes).
result	An output of the GO_analyse() or subset_scores() function.
eSet	ExpressionSet of the Biobase package including a gene-by-sample expression matrix in the AssayData slot, and a phenotypic information data-frame in the phenodate slot. In the expression matrix, row names are Ensembl gene identifiers or probeset identifiers, and column names are sample identifiers. In the phentypic data-frame, row names are sample idenfifiers, column names are grouping factors and phenotypic traits usable for the one-way ANOVA.
x_var	A column name in phenodata to plot on the X-axis. If representing time on the X-axis, users should store the time-points as numeric values in the AnnotatedDataFrame to adequately space the time-points.
f	A column name in phenodata to group the samples when representing mean and confidence interval. The factor specified in the initial GO_analyse() call is used by default. Unexpected grouping factors of samples can reveal interesting trends (e.g. "Animal", "Tissue", "CellType",).
subset	A named list to subset eSet. Names must be column names existing in colnames(pData(eSet)). Values must be vectors of values existing in the corresponding column of pData(eSet).
index	In the case where multiple gene or probeset identifiers are associated with the gene name, index=2 will plot the expression profile of the second feature identifier alone, for instance.
xlab	Title of the X-axis. Default is the value of x_var.

ylab Title of the Y-axis. Default is "log2(cpm)".

ylim Numeric vector of length 2 specifying the lower and upper bounds of the Y axis.

Default is scaled to the full range of expression values in the expression dataset, to ease comparison of different genes. If set to NULL, the axis will be scaled to

fit the plotted data only.

col.palette A valid RColorBrewer palette name to fetch the colormap from.

col A vector of color names or codes. The number of colors provided must match

the number of levels of the grouping factor. Default to a palette with an adequate

set of colors. If specified, overrides argument col.palette.

level The confidence interval level to visualise around the mean of each group. De-

fault is 0.95.

titles Character vector providing as many titles as there are plots to replace the default

titles. Default is a combination of the gene id and the associated gene.

title.size Changes the font size of the title. Default is 2.

axis.title.size

Changes the font size of the axes title. Defalt is 20.

axis.text.size Changes the font size of the axes text labels. Default is 15.

axis.text.angle

Changes the angle of the X axis text labels. Default is 0 (horizontal).

legend.title.size

Changes the font size of the legend title. Default is 20.

legend.text.size

Changes the font size of the legend text labels. Default is 15.

legend.key.size

Changes the size of the legend keys (in points). Default is 30.

#### Value

The ggplot object, or the vector of feature identifiers if multiple features are annotated to the gene symbol.

#### Warning

## Common issues:

• It may not be possible to produce plots where the combination of X-axis variable and grouping factor leaves too few replicates to compute a confidence interval for each X value. This is a limitation imposed by the ggplot2 package to produce proper statistics and confidence intervals. In such cases, it may be preferrable to use the expression\_profiles\_symbol() method.

## Author(s)

Kevin Rue-Albrecht

#### References

• ggplot2 package.

## See Also

Package Biobase, methods expression\_plot, GO\_analyse and ggplot.

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#### **Examples**

```
# load the sample output data
data(AlvMac_results)
# Expression by gene identifier (TNIP3)
expression_plot_symbol(
    gene_symbol="PIK3AP1"
    result=AlvMac_results, eSet=AlvMac, x_var="Timepoint"
# Same gene, plotted by animal and grouped by treatment (merging time points)
expression_plot_symbol(
    gene_symbol="PIK3AP1",
    result=AlvMac_results, eSet=AlvMac, x_var="Animal",
    f="Treatment"
    )
# Same gene, plotted by animal and grouped by time-point (merging treatments)
expression_plot_symbol(
    gene_symbol="PIK3AP1";
    result=AlvMac_results, eSet=AlvMac, x_var="Animal",
    f="Time")
```

expression\_profiles

Plots the individual expression profile of a gene in samples series

## Description

This function will plot the expression profile of a gene in individual samples series across a valid X-axis variable in the phenodata, while colouring sample groups according to another variable in the phenodata, and using different line types according to yet another (or the same) variable in the phenodata.

## Usage

#### **Arguments**

```
gene_id An gene or probeset identifier present in rownames(expr_data).

result An output of the GO_analyse() or subset_scores() function.
```

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eSet ExpressionSet of the Biobase package including a gene-by-sample expression matrix in the AssayData slot, and a phenotypic information data-frame in the phenodate slot. In the expression matrix, row names are Ensembl gene identifiers or probeset identifiers, and column names are sample identifiers. In the phentypic data-frame, row names are sample idenfifiers, column names are grouping factors and phenotypic traits usable for the one-way ANOVA. A column name in phenodata to plot on the X-axis. If representing time on the x\_var X-axis, users should store the time-points as numeric values in the AnnotatedDataFrame to adequately space the time-points. seriesF A column name in phenodata to track samples of an identical series across the X-axis. The combination of seriesF and x\_var should uniquely identify each sample in eSet. subset A named list to subset eSet. Names must be column names existing in colnames(pData(eSet)). Values must be vectors of values existing in the corresponding column of pData(eSet). colourF A column name in phenodata to colour the corresponding groups of samples. The factor specified in the initial GO\_analyse() call is used by default. linetypeF A column name in phenodata to assign a different line type to the corresponding groups of samples. Default mirrors colourF. line.size The width of the plotted lines. Default is 1.5. xlab Title of the X-axis. Default is the value of x\_var. Title of the Y-axis. Default is "log2(cpm)". ylab ylim Numeric vector of length 2 specifying the lower and upper bounds of the Y axis. Default is scaled to the full range of expression values in the expression dataset, to ease comparison of different genes. If set to NULL, the axis will be scaled to fit the plotted data only. col.palette A valid RColorBrewer palette name to fetch the colormap from. Default is palette "Accent". A vector of color names or codes. The number of colors provided must match col the number of levels of the factor colourF. If specified, overrides argument col.palette. Default colours are obtained from the col.palette. A vector of numeric values corresponding to line types. The number of line lty types provided must match the number of levels of the factor linetypeF. Default line types are 1:length(levels(pData(eSet)[, linetypeF])). title Changes the plot title. Default is a combination of the gene id and the associated gene. title.size Changes the font size of the title. Default is 2. axis.title.size Changes the font size of the axes title. Defalt is 20. axis.text.size Changes the font size of the axes text labels. Default is 15. axis.text.angle Changes the angle of the X axis text labels. Default is 0 (horizontal). legend.title.size Changes the font size of the legend title. Default is 20. legend.text.size

Changes the font size of the legend text labels. Default is 15.

Changes the size of the legend keys (in points). Default is 30.

legend.key.size

#### **Details**

In order to track and visualise individual sample series, each sample in the ExpressionSet should be associated with a unique combination of seriesF and x\_var. This may require the generation of a new factor in the phenodata, combining all experimental factors except that plotted on the X-axis. See below for an example on the training dataset.

#### Value

The ggplot object.

#### Author(s)

Kevin Rue-Albrecht

#### References

• ggplot2 package.

#### See Also

Package Biobase, methods expression\_plot\_symbol, expression\_plot\_symbol and ggplot.

## **Examples**

```
# load the sample output data
data(AlvMac_results)

AlvMac$Series <- paste(AlvMac$Animal, AlvMac$Treatment, sep="_")
expression_profiles(
   gene_id = "ENSBTAG00000047107", result = AlvMac_results,
   eSet=AlvMac, x_var = "Timepoint", seriesF="Series",
   legend.title.size=10, legend.text.size=10, legend.key.size=15)

expression_profiles(
   gene_id = "ENSBTAG00000047107", result = AlvMac_results,
   eSet=AlvMac, x_var = "Timepoint", seriesF="Series",
   linetypeF="Animal",
   legend.title.size=10, legend.text.size=10, legend.key.size=15)</pre>
```

expression\_profiles\_symbol

Plots the individual expression profile of a gene in samples series

## **Description**

This function will plot the expression profile of a gene in individual samples series across a valid X-axis variable in the phenodata, while colouring sample groups according to another variable in the phenodata, and using different line types according to yet another (or the same) variable in the phenodata.

## Usage

## **Arguments**

gene_symbol	A gene name present in rownames(AlvMac_results\$genes).
result	An output of the GO_analyse() or subset_scores() function.
eSet	ExpressionSet of the Biobase package including a gene-by-sample expression matrix in the AssayData slot, and a phenotypic information data-frame in the phenodate slot. In the expression matrix, row names are Ensembl gene identifiers or probeset identifiers, and column names are sample identifiers. In the phentypic data-frame, row names are sample idenfifiers, column names are grouping factors and phenotypic traits usable for the one-way ANOVA.
x_var	A column name in phenodata to plot on the X-axis. If representing time on the X-axis, users should store the time-points as numeric values in the AnnotatedDataFrame to adequately space the time-points.
seriesF	A column name in phenodata to track samples of an identical series across the X-axis. The combination of seriesF and x_var should uniquely identify each sample in eSet.
subset	A named list to subset eSet. Names must be column names existing in colnames(pData(eSet)). Values must be vectors of values existing in the corresponding column of pData(eSet).
colourF	A column name in phenodata to colour the corresponding groups of samples. The factor specified in the initial GO_analyse() call is used by default.
linetypeF	A column name in phenodata to assign a different line type to the corresponding groups of samples. Default mirrors colourF.
line.size	The width of the plotted lines. Default is 1.5.
index	In the case where multiple gene or probeset identifiers are associated with the gene name, index=2 will plot the expression profile of the second feature identifier alone, for instance.
xlab	Title of the X-axis. Default is tha value of x_var.
ylab	Title of the Y-axis. Default is "log2(cpm)".
ylim	Numeric vector of length 2 specifying the lower and upper bounds of the Y axis. Default is scaled to the full range of expression values in the expression dataset, to ease comparison of different genes. If set to NULL, the axis will be scaled to fit the plotted data only.
col.palette	A valid RColorBrewer palette name to fetch the colormap from. Default is

palette "Accent".

col	A vector of color names or codes. The number of colors provided must match the number of levels of the factor colourF. If specified, overrides argument col.palette. Default colours are obtained from the col.palette.	
lty	A vector of numeric values corresponding to line types. The number of line types provided must match the number of levels of the factor linetypeF. Default line types are 1:length(levels(pData(eSet)[, linetypeF])).	
titles	Character vector providing as many titles as there are plots to replace the default titles. Default is a combination of the gene id and the associated gene.	
title.size	Changes the font size of the title. Default is 2.	
axis.title.size	e	
	Changes the font size of the axes title. Defalt is 20.	
axis.text.size	Changes the font size of the axes text labels. Default is 15.	
axis.text.angle		
	Changes the angle of the X axis text labels. Default is 0 (horizontal).	
legend.title.size		
	Changes the font size of the legend title. Default is 20.	
legend.text.size		
	Changes the font size of the legend text labels. Default is 15.	
legend.key.size		
	Changes the size of the legend keys (in points). Default is 30.	

#### **Details**

In order to track and visualise individual sample series, each sample in the ExpressionSet should be associated with a unique combination of seriesF and x\_var. This may require the generation of a new factor in the phenodata, combining all experimental factors except that plotted on the X-axis. See below for an example on the training dataset.

## Value

The ggplot object, or the vector of feature identifiers if multiple features are annotated to the gene symbol.

## Author(s)

Kevin Rue-Albrecht

## References

• ggplot2 package.

#### See Also

Package Biobase, methods expression\_plot\_symbol, expression\_plot\_symbol and ggplot.

#### **Examples**

```
# load the sample output data
data(AlvMac_results)

AlvMac$Series <- paste(AlvMac$Animal, AlvMac$Treatment, sep="_")</pre>
```

```
expression_profiles_symbol(
   gene_symbol="TNIP3", result = AlvMac_results,
   eSet=AlvMac, seriesF="Series", x_var = "Timepoint", linetypeF="Animal",
   line.size=1.5, title.size=1.5, legend.title.size=10, legend.text.size=10,
   legend.key.size=30)

expression_profiles_symbol(
   gene_symbol="TNIP3", result = AlvMac_results,
   eSet=AlvMac, seriesF="Series", x_var = "Timepoint", linetypeF="Animal",
   line.size=1.5, lty=rep(1,10), title.size=1.5, legend.title.size=10,
   legend.text.size=10, legend.key.size=30)
```

GO\_analyse

Identifies gene ontologies clustering samples according to predefined factor.

## Description

Combines gene expression data with Gene Ontology (GO) annotations to rank and visualise genes and GO terms enriched for genes best clustering predefined groups of samples based on gene expression levels.

This methods semi-automatically retrieves the latest information from Ensembl using the biomaRt package, except if custom GO annotations are provided. Custom GO annotations have two main benefits: firstly they allow the analysis of species not supported in the Ensembl BioMart server, and secondly they save time skipping calls to the Ensembl BioMart server for species that are supported. The latter also presents the possiblity of using an older release of the Ensembl annotations, for example.

Using default settings, a random forest analysis is performed to evaluate the ability of each gene to cluster samples according to a predefined grouping factor (one-way ANOVA available as an alteranative). Each GO term is scored and ranked according to the average rank (alternatively, average power) of all associated genes to cluster the samples according to the factor. The ranked list of GO terms is returned, with tools allowing to visualise the statistics on a gene- and ontology-basis.

## Usage

```
GO_analyse(
    eSet, f, subset=NULL, biomart_name = "ENSEMBL_MART_ENSEMBL",
    biomart_dataset="", microarray="",
    method="randomForest", rank.by="rank", do.trace=100, ntree=1000,
    mtry=ceiling(2*sqrt(nrow(eSet))), GO_genes=NULL, all_GO=NULL,
    all_genes=NULL, FUN.GO=mean, ...)
```

### **Arguments**

eSet

ExpressionSet of the Biobase package including a gene-by-sample expression matrix in the AssayData slot, and a phenotypic information data-frame in the phenodate slot. In the expression matrix, row names are identifiers of expressed features, and column names are identifiers of the individual samples. In the phenotypic data-frame, row names are sample idenfifiers, column names are grouping factors and phenotypic traits usable for statistical tests and visualisation methods.

f A column name in phenodata used as the grouping factor for the analysis.

A named list to subset eSet for the analysis. Names must be column names existing in colnames(pData(eSet)). Values must be vectors of values existing in the corresponding column of pData(eSet). The original ExpressionSet will be

left unchanged.

biomart\_name The Ensembl BioMart database to which connect to.

biomart\_dataset

The Ensembl BioMart dataset identifier corresponding to the species studied. If not specified and no custom annotations were provided, the method will attempt to automatically identify the adequate dataset from the first feature identifier in the dataset. Use data(prefix2dataset) to access a table listing valid choices.

microarray The identifier in the Ensembl BioMart corresponding to the microarray platform

used. If not specified and no custom annotations were provided, the method will attempt to automatically identify the platform used from the first feature identifier in the dataset. Use data(microarray2dataset) to access a table

listing valid choices.

method The statistical framework to score genes and gene ontologies. Either "random-Forest" or "rf" to use the random forest algorithm, or alternatively either of

"anova" or "a" to use the one-way ANOVA model. Default is "randomForest".

rank.by Either of "rank" or "score" to chose the metric used to order the gene and GO

term result tables. Default to 'rank'.

do.trace Only used if method="randomForest". If set to TRUE, gives a more verbose output as randomForest is run. If set to some integer, then running output is

printed for every do.trace trees. Default is 100.

ntree Only used if method="randomForest". Number of trees to grow. This should be set to a number large enough to ensure that every input row gets predicted at

least a few times

mtry Only used if method="randomForest". Number of features randomly sampled as candidates at each split. Default value is 2\*sqrt(gene count) which is approx-

as candidates at each spirt. Default value is 2 sqrt(gene\_count) which is app

imately 220 genes for a dataset of 12,000 genes.

GO\_genes Custom annotations associating features present in the expression dataset to gene ontology identifiers. This must be provided as a data-frame of two columns, named gene\_id and go\_id. If provided, no call to the Ensembl BioMart server will be done, and arguments all\_GO and all\_genes should be provided as well, to enable all downstream features of GOexpress. An example is provided in

AlvMac\_GOgenes.

all\_GO Custom annotations used to annotate each GO identifier present in GO\_genes

with the ontology name (e.g. "apoptotic process") and namespace (i.e. "biological\_process", "molecular\_function", or "cellular\_component"). This must be provided as a data-frame containing at least one column named go\_id, and preferably two more columns named name\_1006 and namespace\_1003 for consistency with the Ensembl BioMart. Supported alternative column headers are name and namespace. Respectively, name should be used to provide the description of the GO term, and namespace should contain one of "biological\_process", "molecular\_function" and "cellular\_component". name is used to generate the title of ontology-based figured, and namespace is important to enable subsequent filtering of results by their corresponding value. An example is provided

in data(AlvMac\_allGO).

all\_genes

Custom annotations used to annotate each feature identifier in the expression dataset with the gene name or symbol (e.g. "TNF"), and an optional description. This must be provided as a data-frame containing at least a column named gene\_id and preferably two more columns named external\_gene\_name and description for consistency with the Ensembl BioMart. A supported alternative header is name. While external\_gene\_name is important to enable subsequent visualisation of results by gene symbol, description is only displayed for readability of result tables. An example is provided in data(AlvMac\_allgenes).

FUN.GO Function to summarise the score and rank of all feature associated with each

gene ontology. Default is mean function. If using "lambda-like" (anonymous) functions, these must take a list of numeric values as an input, and return a single

numeric value as an output.

... Additional arguments passed on to the randomForest and useMart methods, if

applicable.

#### **Details**

The default scoring functions strongly favor GO terms associated with fewer genes at the top of the ranking. This bias may actually be seen as a valuable feature which enables the user to browse through GO terms of increasing "granularity", i.e. associated with increasingly large sets of genes, although consequently being increasingly vague and blurry (e.g. "protein binding" molecular function associated with over 6,000 genes).

It is suggested to use the subset\_scores() function to subsequently filter out GO terms with fewer than 5+ genes associated with it. Indeed, those GO terms are more sensitive to outlier genes as they were scored on the average of a handful of genes.

Additionally, the pValue\_GO function may be used to generate a permutation-based P-value indicating the chance of seeing each GO term reaching an equal or higher rank – or score – by chance.

#### Value

A list containing the results of the analysis. Some elements are specific to the output of each analysis method.

Core elements:

GO A table ranking all GO terms related to genes in the expression dataset based on

the average ability of their related genes to cluster the samples according to the

predefined grouping factor.

mapping The table mapping genes present in the dataset to GO terms.

genes A table ranking all genes present in the expression dataset based on their ability

to cluster the samples according to the predefined grouping factor.

factor The predefined grouping factor.
method The statistical framework used.

subset The filters used to run the analysis only on a subet of the samples. NULL if no

filter was applied.

rank.by The metric used to rank order the genes and gene ontologies.

FUN. GO The function used to summarise the score and rank of all gene features associ-

ated with each gene ontology.

Random Forest additional elements:

ntree Number of trees grown.

mtry Number of variables randomly sampled as candidates at each split.

One-way ANOVA does not have additional arguments.

## Warning

Make sure that the factor f is an actual factor in the R language meaning. This is important for the underlying statistical framework to identify the groups of samples defined by their level of this factor.

If the column defining the factor (e.g. "Treatment") in phenodata is not an R factor, use pData(targets)\$Treatment = factor(pData(targets)\$Treatment) to convert the character values into an actual R factor with appropriate levels.

## Author(s)

Kevin Rue-Albrecht

#### See Also

Methods subset\_scores, pValue\_GO, getBM, randomForest, and oneway.test.

#### **Examples**

```
# Load example data subset
data(AlvMac)
# Load a local copy of annotations obtained from the Ensembl Biomart server
data(AlvMac_GOgenes)
data(AlvMac_allgenes)
data(AlvMac_allGO)
# Run the analysis on factor "Treatment",
# considering only treatments "MB" and "TB" at time-point "48H"
# using a local copy of annotations obtained from the Ensembl BioMart server
AlvMac_results <- GO_analyse(
    eSet=AlvMac, f="Treatment",
    subset=list(Time=c("48H"), Treatment=c("MB", "TB")),
    GO_genes=AlvMac_GOgenes, all_genes=AlvMac_allgenes, all_GO=AlvMac_allGO
    )
# Valid Ensembl BioMart datasets are listed in the following variable
data(prefix2dataset)
# Valid microarray= values are listed in the following variable
data(microarray2dataset)
## Not run:
# Other valid but time-consuming examples:
# Run the analysis on factor "Treatment" including all samples
GO_analyse(eSet=AlvMac, f="Treatment")
# Run the analysis on factor "Treatment" using ANOVA method
GO_analyse(eSet=AlvMac, f="Treatment", method="anova")
```

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```
# Use alternative GO scoring/summarisation functions (Default is: average)
# Named functions
GO_analyse(eSet=AlvMac, f="Treatment", FUN.GO = median)
# Anonymous functions (simple example without scientific value)
GO_analyse(eSet=AlvMac, f="Treatment", FUN.GO = function(x){median(x)/100})
# Syntax examples without actual data:
# To force the use of the Ensembl BioMart for the human species, use:
GO_analyse(eSet, f, biomart_dataset = "hsapiens_gene_ensembl")
# To force use of the bovine affy_bovine microarray annotations use:
GO_analyse(eSet, f, microarray = "affy_bovine")
## End(Not run)
```

heatmap\_GO

Generates a heatmap and hierarchical clustering of the samples and the genes

#### **Description**

Clusters the samples and the genes associated with a GO term using the expression levels of genes related to a given ontology. Represents expression levels of those genes in a heatmap.

#### Usage

```
heatmap_GO(
    go_id, result, eSet, f=result$factor, subset=NULL, gene_names=TRUE,
    NA.names=FALSE, margins=c(7 ,5),
    scale="none", cexCol=1.2, cexRow=0.5,
    labRow=NULL,
    cex.main=1, trace="none", expr.col=bluered(75),
    row.col.palette="Accent",
    row.col=c(),
    main=paste(
        go_id, result$GO[result$GO$go_id == go_id,"name_1006"]
        ),
    main.Lsplit=NULL,
    ...)
```

## **Arguments**

go\_id A Gene Ontology (GO) identifier.

result The output of GO\_analyse() or a subset of it obtained from subset\_scores().

eSet ExpressionSet of the Biobase package including a gene-by-sample expression matrix in the assayData slot, and a phenotypic information data-frame in the phenodata slot. In the expression matrix, row names are Ensembl gene identifiers or probeset identifiers, and column names are sample identifiers. In

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the phentypic data-frame, row names are sample idenfifiers, column names are grouping factors and phenotypic traits usable for the one-way ANOVA.

f The grouping factor in phenodata to label the samples by.

subset A named list to subset eSet. Names must be column names existing in col-

names(pData(eSet)). Values must be vectors of values existing in the corre-

sponding column of pData(eSet).

gene\_names A boolean value. Default is TRUE, to label genes by their associated gene name.

If FALSE, labels the genes by their feature identifier in the expression dataset

(i.e. Ensembl gene identifier or microarray probeset).

NA. names A boolean value. Default is TRUE. If labelling genes by their associated gene

name (see argument gene\_names), whether to display the gene feature identifier

for gene features without associated gene name.

margins A numeric vector of length 2 specifying the number of lines of margins to apply

for the bottom and right margins, respectively. Defaults are 7 (bottom) and 5 (right). For Ensembl gene identifiers, we suggest setting manually a bottom

margin of 13. See heatmap. 2().

scale Character indicating if the values should be centered and scaled in either the row

direction or the column direction, or none. Default is "none". See heatmap.2().

cexCol, cexRow Positive numbers, used as cex.axis in for the row or column axis labeling. De-

faults are 1.2 and 1, respectively. See heatmap. 2().

labRow A character vector of names to re-label the rows (samples). If vector of length 1,

it is assumed to be a column name in the phenoData slot. Default are the values

of the factor f. See heatmap. 2().

cex.main Scaling factor of the main title font size. Default is 1. We suggest to use it in

combination with the argument main.Lsplit for GO terms with long names.

trace Character string indicating whether a solid "trace" line should be drawn across

each 'row' or down each 'column', both' or 'none'. The distance of the line from the center of each color-cell is proportional to the size of the measurement.

Defaults to 'none'.

expr.col Character vector indicating the colors to represent the different levels of gene

expression. Defaults to a colormap of 75 shades ranging from blue (low) to red (high) and centered around white. If using differential expression data, you

should probably use greenred (75) instead.

row.col.palette

A valid RColorBrewer palette name to fetch the colormap from, to color-code

the groups of samples.

row.col A vector of color names or codes. The number of colors provided must match

the number of levels of the grouping factor. Default to an palette of up to 9 colors marking the different levels of the predefined grouping factor on the left

side of the heatmap.

main Main title of the figure. Default is paste(go\_id, go\_name).

main.Lsplit Number of characters after which a new-line character will be inserted in the

main title. If this would occur within a word, the new-line character will be inserted before this word. Default is NULL, leaving the title on a single line.

... Additional arguments passed on to heatmap.2().

#### Value

Returns the output of the heatmap. 2() function.

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#### Author(s)

Kevin Rue-Albrecht

#### See Also

Method heatmap. 2, GO\_analyse, and brewer.pal.info.

#### **Examples**

```
# load the sample output data
data(AlvMac_results)

# Heatmap the top-ranked GO term (toll-like receptor 4 signaling pathway) as
# example
heatmap_GO(go_id="GO:0034142", result=AlvMac_results, eSet=AlvMac)

# Same with larger sample labels on the right hand side.
heatmap_GO(go_id="GO:0034142", result=AlvMac_results, eSet=AlvMac, cexRow=1)

# Change the color-coding to green-black-red gradient (more appropriate for
# differential expression values)
library(gplots)
heatmap_GO(
    go_id="GO:0034142", result=AlvMac_results, eSet=AlvMac,
    expr.col=greenred(75)
    )
```

hist\_scores

Plots the distribution of scores following an GOexpress analysis.

## **Description**

Plots the an histogram representing the frequencies of scores in the output variable of the GO\_analyse() function.

This function can also be used on the output of subset\_scores() function as it returns a value formatted identically to the output of the GO\_analyse() function.

## Usage

## Arguments

```
result The output of the GO_analyse() function.

main, xlab These arguments to title have useful defaults here.

... Additional arguments passed on to hist().
```

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#### Value

Returns the output of the hist() function.

#### Author(s)

Kevin Rue-Albrecht

#### See Also

Method hist, and GO\_analyse.

#### **Examples**

```
# load the sample output data with p.values computed
data(AlvMac_results.pVal)

# Histogram of scores (labelled with counts)
hist_scores(result=AlvMac_results, breaks=20, labels=TRUE)

# filter for Biological Processes associated with 5+ genes and <=0.05 P-value
filtered_results <- subset_scores(
    result=AlvMac_results.pVal, total_count=5, p.val=0.05,
    namespace="BP")

# Histogram of scores (labelled with counts)
hist_scores(result=filtered_results, breaks=20, labels=TRUE)</pre>
```

list\_genes

Returns the genes associated with a Gene Ontology

## **Description**

Given a Gene Ontology (GO) identifier represented in the dataset, returns a character vector listing the feature identifiers annotated to it.

## Usage

```
list_genes(go_id, result, data.only=TRUE)
```

## Arguments

go\_id A Gene Ontology (GO) identifier represented in the dataset.

result The output of GO\_analyse() or a subset of it obtained from subset\_scores().

data.only Whether to return only the feature identifiers present in the given dataset or alternatively returns all feature identifiers associated with the GO term in the Ensembl BioMart. Default is TRUE.

## Value

A character vector listing the feature identifiers of the genes associated with the GO term.

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#### Author(s)

Kevin Rue-Albrecht

#### See Also

Method GO\_analyse.

#### **Examples**

```
# load the sample output data
data(AlvMac_results)

# List of genes associated with the GO term "toll-like receptor 4 signaling
# pathway"
list_genes(result=AlvMac_results, go_id="GO:0034142")
```

microarray2dataset

Table mapping probeset identifier prefixes to datasets in the Ensembl BioMart.

#### **Description**

The species corresponding to an probeset identifier can often be identified from the prefix of the identifier (e.g. Bt.457.1.S1\_at corresponds to *Bos taurus* which is only associated with a single microarray platform in the Ensembl BioMart). This table maps some manually curated unique patterns to the corresponding species and microarray platform.

## Usage

```
data(microarray2dataset)
```

## **Details**

All Agilent microarray share the same prefix pattern, making it very difficult to differentiate. Many Affymetrix microarrays also share the same prefix "AFFX" for several probesets.

The dataset and microarray arguments of the GO\_analyse method are the best way to specify which BioMart information should be used to annotate the features in your expression dataset.

#### Value

A data frame with 158 rows and the 6 columns. Rows are sorted alphabetically by species name and each refer to a unique combination of dataset and microarray identifier in the Ensembl BioMart. The columns are described below:

- dataset contains species-specific biomaRt dataset names.
- microarray contains the microarray identifier in the Ensembl BioMart dataset above.
- sample contains a sample probeset from this combination of Ensembl BioMart dataset and microarray.
- species contains an example probeset sampled from the corresponding Ensembl BioMart dataset and microarray.

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• pattern contains the corresponding manually curated pattern representative of probesets for this microarray platform. This pattern may or may not be unique to the microarray. Therefore we encourage users to use the 'microarray' argument of the GO\_analyse method to specify the Ensembl BioMart dataset to use.

• unique contains a boolean value stating whether the pattern was found unique to the microarray or not.

#### Source

The microarray2dataset.build method stored in the toolkit.R script was used to query the Ensembl BioMart server and build this table.

## **Examples**

```
data(microarray2dataset)
microarray2dataset
```

plot\_design

Plot Univariate Effects for genes associated with a Gene Ontology

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### **Description**

Successively plots univariate effects of one or more factors, typically for a designed experiment as analyzed by aov().

## Usage

```
plot_design(
    go_id, result, eSet, subset=NULL,
    factors=colnames(pData(eSet)), main="", main.Lsplit=NULL, ...)
```

## **Arguments**

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go_id	A Gene Ontology (GO) identifier represented by at least one gene in the dataset.
result	The output of GO_analyse() or a subset of it obtained from subset_scores().
eSet	ExpressionSet of the Biobase package including a gene-by-sample expression matrix in the AssayData slot, and a phenotypic information data-frame in the phenodata slot. In the expression matrix, row names are Ensembl gene identifiers or probeset identifiers, and column names are sample identifiers. In the phentypic data-frame, row names are sample idenfifiers, column names are grouping factors and phenotypic traits usable for the one-way ANOVA.
subset	A named list to subset eSet. Names must be column names existing in colnames(pData(eSet)). Values must be vectors of values existing in the corresponding column of pData(eSet).
factors	A set of column names from phenodata. Each of these values will be represented on the X-axis to investigate its effect on the average expression of a given genes for each level of that factor.
main	Changes the main title of the plots.
main.Lsplit	Number of characters after which a new-line character will be inserted in the main title. If this would occur within a word, the new-line character will be inserted before this word. Default is NULL, leaving the title on a single line. Additional arguments which will be passed on to the plot.design() function.

32 prefix2dataset

#### Value

The output of the plot.design() function.

#### Author(s)

Kevin Rue-Albrecht

#### See Also

Method plot.design.

## **Examples**

```
# load the sample output data
data(AlvMac_results)

# Univariate plot
plot_design(go_id="G0:0034142", eSet=AlvMac, result=AlvMac_results)
```

prefix2dataset

Table mapping Ensembl gene identifier prefixes to BioMart datasets.

## **Description**

The species corresponding to an Ensembl gene identifier can typically be identified from the prefix of the identifier (e.g. ENSBTAG corresponds to *Bos taurus*). This table maps each known unique prefix to the corresponding species.

#### Usage

```
data(prefix2dataset)
```

## **Details**

C. elegans, D. melanogaster, and S. cerevisiae have atypical identifier pattern and prefixes in their Ensembl gene identifiers. However, the automatically extracted prefix for C. elegans and D. melanogaster – respectively "WBgene" and "FBgn" – can be used as such to identify datasets from those species. On the oher hand, prefixes used for the S. cerevisiae include "YHR", "YAL", and many others. Consequently, expression data from S. cerevisiae species is identified without referring to the "prefix2dataset"" table; instead, such datasets are identified if the first gene identifier in the dataset starts with "Y".

## Value

A data frame with 69 rows and 4 columns. Each row refers to one dataset in the Ensembl BioMart. The columns are described below:

- dataset contains the biomaRt dataset name.
- species contains the corresponding species name.
- prefix contains the corresponding unique prefix.
- sample contains a sample Ensembl gene identifier from this dataset.

pValue\_GO 33

#### **Source**

The prefix2dataset.build method stored in the toolkit.R script was used to query the Ensembl BioMart server and build this table.

## **Examples**

```
data(prefix2dataset)
str(prefix2dataset)
prefix2dataset
```

pValue\_G0

Compute p-values for ontologies by randomising gene labels.

## Description

Permutes the rank of genes processed by GO\_analyse() to compute a P-value for each ontology

#### Usage

```
pValue_GO(
    result, N=1000, ranked.by=result$rank.by, rank.by='P',
    FUN.GO=result$FUN.GO)
```

#### **Arguments**

result	The output of GO_analyse() or a subset of it obtained from subset_scores().
N	The number of permutation desired. Default to 1000.
ranked.by	Either of 'rank' or 'score'. The metric used to compare whether GO terms in the randomised data are lative to their original value. Default to the current ordering method of the result object.
rank.by	Either of 'rank', 'score', or 'p.val'. The metric used to order the GO terms after computing of the P-value. Default to 'p.val'.
FUN.GO	Function to summarise the score and rank of all feature associated with each gene ontology. Logically, default is the function used in the call to GO_analyse. If using "lambda-like" (anonymous) functions, these must take a list of numeric values as an input, and return a single numeric value as an output.

#### **Details**

This function is relatively lengthy. Its procedure can be divided in several steps: (1) assemble a copy of all genes used in the original scoring step, then for each of the N permutations, (2) permute the gene labels, (3) aggregate the rank – or score – of all genes belonging to each GO term, (4) decide whether each GO term is ranked – or scored –better or worst than originally, (5) return a p-value based on the number of permutations where each GO term was better ranked – or scored – than originally.

## Value

A list formatted identically to the results of the analysis, with an added 'p.val' column in the GO slot, and re-ordered by the chosen metric.

34 quantiles\_scores

#### Author(s)

Kevin Rue-Albrecht

#### See Also

Method GO\_analyse.

#### **Examples**

```
## Not run:
# Time-consuming examples:
# Limited here to N=1 for time constraint
# Recommended N=1000 (or at least 100).

# Run the analysis on factor "Treatment" including all samples
AlvMac.Pvals <- pValue_GO(result, N=1, ranked.by=result$rank.by, rank.by='P')
## End(Not run)</pre>
```

quantiles\_scores

Returns the quantiles of scores following an GOexpress analysis.

## **Description**

Returns a set of quantiles in indicating the scores reached by given proportions of the GO terms.

This function can also be used on the output of subset\_scores() function as it returns a value formatted identically to the output of the GO\_analyse() function.

#### Usage

```
quantiles_scores(result, probs=c(0.9, 0.95, 0.99, 0.999, 0.9999), quartiles=FALSE)
```

## **Arguments**

result The output of GO\_analyse() or a subset of it obtained from subset\_scores().

Probs Numeric vector of probabilities with values in [0,1]. (Values up to 2e-14 outside that range are accepted and moved to the nearby endpoint.) See quantile quartiles A numeric vector of the percentiles for which the scores are desired.

#### Value

A named vector of percentiles and corresponding scores.

#### Author(s)

Kevin Rue-Albrecht

#### See Also

Method quantile.

rerank 35

#### **Examples**

```
# load the sample output data with p.values computed
data(AlvMac_results.pVal)
# filter for Biological Processes associated with 5+ genes and <=0.05 P-value
filtered_results <- subset_scores(</pre>
    result=AlvMac_results.pVal, total_count=5, p.val=0.05,
    namespace="BP")
# Quantiles of scores
quantiles_scores(result=filtered_results)
```

rerank

Reorder the result variable by alternative metrics.

## **Description**

Reorder the ranked tables of GO terms and genes either by increasing (average) rank or decreasing (average) score.

#### Usage

```
rerank(result, rank.by = 'rank')
```

#### **Arguments**

rank.by

result The output of GO\_analyse() or a subset of it obtained from subset\_scores(). Either of 'rank', 'score' or 'p.val'; the metric to rank the GO terms and genes.

Note that 'pval' is only applicable on the output of the pValue\_GO() function.

See details for breaking ties.

## **Details**

Taking an example, to rank GO terms by P-value and break ties by average rank, rerank first by 'rank', and then rerank the resulting object by 'p.val'.

#### Value

A list formatted identically to the results of the analysis, but ordered by the chosen metric.

## Note

The name reorder() was not used to avoid conflict with package stats.

## Author(s)

Kevin Rue-Albrecht

#### See Also

Method GO\_analyse.

36 subEset

#### **Examples**

```
# load the sample output data
data(AlvMac_results)

# Re-rank the GO terms and genes based on the actual score instead of the rank
reranked.byScore <- rerank(result=AlvMac_results, rank.by="score")

# load the sample output data with p.values computed
data(AlvMac_results.pVal)

# To rank by P-value, while breaking the ties by ave_rank,
# rank first by ave_rank
reranked.byRank <- rerank(result=AlvMac_results.pVal, rank.by="rank")
# rank the result by P-value
reranked.pVal_rank <- rerank(result=reranked.byRank, rank.by="p.val")</pre>
```

subEset

Subset an ExpressionSet to values of phenotypic data columns.

## Description

Given a list of column names and corresponding values present in the phenoData slot of an ExpressionSet object, this method returns the subset of the ExpressionSet restricted to samples associated with the given values in the given columns.

## Usage

```
subEset(eSet, subset = list())
```

## **Arguments**

eSet

ExpressionSet of the Biobase package including a gene-by-sample expression matrix in the AssayData slot, and a phenotypic information data-frame in the phenodate slot. In the expression matrix, row names are Ensembl gene identifiers or probeset identifiers, and column names are sample identifiers. In the phentypic data-frame, row names are sample idenfifiers, column names are grouping factors and phenotypic traits usable for the one-way ANOVA.

subset

A named list to subset eSet. Names must be column names existing in colnames(pData(eSet)). Values must be vectors of values existing in the corresponding column of pData(eSet).

#### Value

A subset of the given ExpressionSet restricted to samples associated with the given values in the given columns

## Author(s)

Kevin Rue-Albrecht

subset\_scores 37

#### See Also

Class ExpressionSet.

#### **Examples**

```
# Load example data set
data(AlvMac)

# Subset it to only samples of "CN" and "MB" treatments, and also only "2H",
# "6H", and "24H" time-points
sub.AlvMac <- subEset(
    eSet=AlvMac,
    subset=list(
        Treatment=c("CN","MB"),
        Time=c("2H","6H")
    )
)</pre>
```

subset\_scores

Returns a filtered list from GO\_analyse results.

#### **Description**

Given an output variable from a GO\_analyse analysis and a set of valid filters and thresholds, returns an identically formatted list keeping only the rows of the score table passing all the filters.

#### Usage

```
subset_scores(result, ...)

# Suggested use:
# on the output of GO_analyse()
# subset_scores(result, total=5, namespace="BP")
# or after application of pValue_GO()
# subset_scores(result, total=5, namespace="BP", p.val=0.05)
```

#### **Arguments**

result The output of the GO\_analyse() function.

... Additional pairs of filter and threshold values in the format "filter=threshold".

Filters must be valid names from colnames(result\$GO).

#### **Details**

It is highly recommended to filter out GO terms with very few genes (e.g. less than 5 genes), as the scoring function is biased for those GO terms (see UsersGuide).

Suggested filters:

total\_count, total: Filter keeping only GO terms associated with at least the given count of genes in the annupous p.val, p, P: Filter keeping only the GO terms with P-value lower or equal to the given cutoff (This filter keeping only the GO terms of a given type. Valid values are "biological\_process", The power of the given count of genes in the annupous power or equal to the given cutoff (This filter keeping only the GO terms of a given type. Valid values are "biological\_process", The power of the given count of genes in the annupous power or equal to the given cutoff (This filter keeping only the GO terms of a given type. Valid values are "biological\_process", The power of the given count of genes in the annupous power or equal to the given cutoff (This filter keeping only the GO terms of a given type. Valid values are "biological\_process", The power of the given count of genes in the annupous power or equal to the given cutoff (This filter keeping only the GO terms of a given type. Valid values are "biological\_process", The power of the given cutoff (This filter keeping only the GO terms of a given type. Valid values are "biological\_process", The power of the given cutoff (This filter keeping only the GO terms of a given type. Valid values are "biological\_process", The power of the given cutoff (This filter keeping only the GO terms of a given type. Valid values are "biological\_process", The power of the given cutoff (This filter keeping only the GO terms of a given type. The power of the given cutoff (This filter keeping only the GO terms of a given type.)

38 subset\_scores

#### Additional filters:

data\_count, data: Filter keeping only GO terms associated with at least the given count of genes, present in the Express ave\_rank, rank: Filter keeping only GO terms with an ave\_rank value equal or lower than the given cutoff (average of ave\_score, score: Filter keeping only GO terms with an ave\_score value equal or higher than the given cutoff (average of ave\_score).

#### Value

A list formatted identically to the result object provided, with an added or updated filters.GO slot stating the filters and cutoffs applied, and restricted to:

- the gene ontologies passing the given filters and cutoff values.
- the genes mapped to those remaining ontologies (i.e. all genes not associated with an ontology are discarded in the output object).
- gene-mapping annotations related to the remaining gene ontologies.

#### Note

It is possible to further filter a filter result object. Some warnings may appear if the new filter cutoff conflict with the ones previously applied (stored in the filters.GO slot of the filtered object). Example of conflicting filter values are:

- Filtering for 'biological\_process' a result object that was previously filtered for 'molecular\_function' (no more 'BP' terms are present in the first filtered object).
- filtering for a total\_count of 5 or more a result object previously filtered for a total\_count
  of 10 or more.

#### Author(s)

Kevin Rue-Albrecht

#### See Also

Method GO\_analyse.

## **Examples**

```
# load the sample output data
data(AlvMac_results.pVal)

# have an overview of the result variable
str(AlvMac_results.pVal)

# filter for Biological Processes associated with 5+ genes and <=0.05 P-value
filtered_results <- subset_scores(
    result=AlvMac_results.pVal, total_count=5, p.val=0.05,
    namespace="BP")

# have an overview of the filtered result variable
str(filtered_results)</pre>
```

table\_genes 39

table_genes	Returns a table listing the genes associated with a given Gene Ontol-
	ogy

## **Description**

Given a Gene Ontology (GO) identifier represented in the dataset and the output variable of a GO\_analyse() function, table\_genes() returns a table listing the genes associated with that go\_id, their associated gene name, and description.

## Usage

```
table_genes(go_id, result, data.only=FALSE, order.by='rank')
```

## Arguments

_	
go_id	A Gene Ontology (GO) identifier.
result	The output of the GO_analyse() function.
data.only	Whether to return only the feature identifiers present in the given dataset or alternatively returns all feature identifiers associated with the GO term in the Ensembl BioMart.
order.by	The metric or name to order the output table. Default is increasing 'rank', which corresponds to decreasing score. Valid values are 'rank' (increasing) equivalent to 'score' (decreasing), 'gene_id', 'name', equivalent to 'external_gene_name' and 'external_gene_id'. Text ranking in alphabetical order.

#### Value

A data frame listing the statistics and annotations for the genes present in the expression dataset and associated with the GO term.

## Author(s)

Kevin Rue-Albrecht

## See Also

Method GO\_analyse.

## **Examples**

```
# load the sample output data
data(AlvMac_results)

# Table of result for genes associated with the GO term
# "toll-like receptor 4 signaling pathway"
table_genes(result=AlvMac_results, go_id="GO:0034142")

# ordered by gene name
table_genes(result=AlvMac_results, go_id="GO:0034142", order.by='name')
```

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