Package ‘safe’
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Title Significance Analysis of Function and Expression
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Author William T. Barry

Description
SAFE is a resampling-based method for testing functional categories in gene expression experiments. SAFE can be applied to 2-sample and multi-class comparisons, or simple linear regressions. Other experimental designs can also be accommodated through user-defined functions.

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License GPL (>= 2)

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Description

SAFE is a resampling-based method for testing functional categories in gene expression experiments. SAFE can be applied to 2-sample and multi-class comparisons, or simple linear regressions. Other experimental designs can also be accommodated through user-defined functions.

Details

```
Package: safe
Type: Package
Version: 3.0
Date: 2012-09-14
License: GPL (>= 2)
LazyLoad: yes
```

For an overview of how to use the package, including the most important functions, please see the included vignette.

Author(s)

William T. Barry

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References


See Also

`safe`, `safe-package`
gene.results

Gene-specific results from SAFE

Description

Provides gene-specific local statistics and resampling-based p-values for every feature in the category of interest. Features are ordered by the degree and direction of differential expression.

Usage

gene.results(object, cat.name = NULL, error = "none",
              print.it = TRUE, gene.names = NULL)

Arguments

- **object**: Object of class SAFE.
- **cat.name**: Name of the category to be displayed. If omitted, the most significant category is displayed.
- **error**: Specifies a non-resampling based method for adjusting the empirical p-values. A Bonferroni, ("FWER.Bonf"), Holm’s step-up ("FWER.Holm"), and Benjamini-Hochberg step down ("FDR.BH") adjustment can be selected. By default ("none") no error rates are computed.
- **print.it**: Logical determining whether results are printed to screen or returned as a list of data.frames for up- and down-regulated genes.
- **gene.names**: Optional character vector of gene names to append to the SAFE output.

Author(s)

William T. Barry: <bbarry@jimmy.harvard.edu>

References


See also the vignette included with this package.

See Also

safe.
**getCmatrix**

*Generation of a C matrix*

**Description**

This function will construct a matrix of indicator variables for category membership from keyword or gene-indexed lists. Size constraints, the option to prune identical categories, and a vector of present genes can be defined to filter categories and order genes. New to version 3.0.0, annotation can be provided so that each gene, instead of each feature, has equal weight in a category.

**Usage**

```r
cmatrix(keyword.list = NULL, gene.list = NULL,
         present.genes = NULL, min.size = 2, max.size = Inf,
         by.gene = FALSE, gene.names = NULL, prefix = "",
         prune = FALSE,
         as.matrix = FALSE, GO.ont = NULL, ...)
```

**Arguments**

- `keyword.list`: A list containing character vectors for each keyword that specify the gene members.
- `gene.list`: A list containing character vectors for each gene that specify the annotated functional categories.
- `present.genes`: An optional vector used to filter genes in the C matrix. Can be provided as an unordered character vector of gene names that match `names(list)`, or as an ordered vector of presence (1) and absence (0) calls.
- `min.size`: Optional minimum category size to be considered.
- `max.size`: Optional maximum category size to be considered.
- `by.gene`: Optional logical to build 'soft' categories at the gene level, instead of the feature level.
- `gene.names`: Optional character vector of gene names for 'soft' categories.
- `prefix`: Optional character string to precede category names.
- `prune`: Optional logical to remove duplicate categories.
- `as.matrix`: Optional argument to specify a matrix is returned rather than a matrix.csr.
- `GO.ont`: "CC", "BP", or "MF" specify which Gene Ontology.
- `...`: Any extra arguments will be forwarded to the `read.table` function when category assignments are given as a file.

**Details**

Typical usages are

```r
cmatrix(keyword.list, present.genes)
cmatrix(gene.list, present.genes)
```
Value

- **C.mat.csr**: If `as.matrix=F` a sparse matrix is returned with the rows corresponding to the genes and columns are categories.
- **row.names**: Character vector of gene names.
- **col.names**: Character vector of category names.
- **col.synonym**: Pipe-delimited Character vector of matching categories when `prune=T`.

Author(s)

William T. Barry: <bbarry@jimmy.harvard.edu>

References


See also the vignette included with this package.

See Also

- `safe`, `safeplot`, `getPImatrix`.

Examples

```r
if(interactive()){
  require(hgu133a.db)
  genes <- unlist(as.list(hgu133aSYMBOL))
  RS.list <- list(Genes21 = c("ACTB","RPLP0","MYBL2","BIRC5","BAG1",
      "GUSB","CD68","BCL2","MMP11","AURKA",
      "GSTM1","ESR1","TFRC","PGR","CTSL2",
      "GRB7","ERBB2","MKI67","GAPDH","CCNB1",
      "SCUBE2"),
      Genes16 = c("MYBL2","BIRC5","BAG1","CD68","BCL2",
      "MMP11","AURKA","GSTM1","ESR1","PGR","CTSL2",
      "GRB7","ERBB2","MKI67","GAPDH","CCNB1","SCUBE2"))
  RS.list <- lapply(RS.list,function(x) return(names(genes[which( match(genes, x, nomatch = 0) > 0)])))
  C1 <- getCmatrix(keyword.list = RS.list)
}
```

---

**p53.stat**

**p53 Mutation Status**

Description

This data set is included for use in the vignette and provides the p53 mutation status (p53+ = 1 and p53- = 0) for each of 251 samples in the Miller et al. breast cancer study data.
Usage

data(p53.stat)

Format

A data.frame with 2 columns, "samplename" and "p53", and 251 rows.

Source

NCBI's Gene Expression Omnibus, accession GSE3494

References


Description

Performs a significance analysis of function and expression (SAFE) for a gene expression experiment and a set of functional categories specified by the user. SAFE is a two-stage resampling-based method that can be applied to a 2-sample, paired, multi-class, simple linear and right-censored linear regression models. Other experimental designs can also be accommodated through user-defined functions.

Usage

```r
safe(X.mat, y.vec, C.mat = NULL, Z.mat = NULL,
method = "permutation", platform = NULL,
annotate = NULL, min.size = 2, max.size = Inf,
by.gene = FALSE, local = "default", global = "default",
args.local = NULL, args.global = list(one.sided = FALSE),
Pi.mat = NULL, error = "FDR.BH", parallel=FALSE, alpha = NA,
epsilon = 10^(-10), print.it = TRUE, ...)
```

Arguments

- **X.mat**: A matrix or data.frame of expression data of size m by n where each row corresponds to a gene feature and each column to a sample. Data should be properly normalized and cannot contain missing values.
- **y.vec**: A numeric, integer or character vector of length n containing the response of interest. For examples of the acceptable forms `y.vec` can take, see the vignette.
C.mat
A matrix containing the gene category assignments. Each column represents a category and should be named accordingly. For each column, values of 1 (TRUE) and 0 (FALSE) indicate whether the features in the corresponding rows of X.mat are contained in the category. This can also be a list containing a sparse matrix and names as created by getCmatrix.

Z.mat
A data.frame of size n by p, with p covariates as numeric or factors.

method
Type of hypothesis test can be specified as "permutation", "bootstrap.t", and "bootstrap.q". "express" calls the dependent package safeExpress. See vignette for details.

platform
If C.mat is unspecified, a character string of a Bioconductor annotation package can be used to build gene categories. See vignette for details and examples.

annotate
If C.mat is unspecified, a character string to specify the type of gene categories to build from annotation packages. "GO.MF", "GO.BP", "GO.CC", and "GO.ALL" (default) specify one or all Gene Ontologies. "KEGG" specifies pathways, and "PFAM" homologous families from the respective sources.

min.size
Optional minimum category size in building C.mat.

max.size
Optional maximum category size in building C.mat.

by.gene
Logical argument (default = FALSE) specifying whether multiple features to a single gene should be down-weighted.

local
Specifies the gene-specific statistic from the following options: "t.Student", "t.Welch", and "t.paired", for 2-sample designs, "f.ANOVA" for 1-way ANOVAs, and "t.LM" for simple linear regressions. "default" will choose between "t.Student", "f.ANOVA", and "t.LM" based on the form of y.vec. User-defined local statistics can also be used; details are provided in the vignette.

global
Specifies the global statistic for a gene categories. By default, the Wilcoxon rank sum ("Wilcoxon") is used. Else, a Fisher's Exact test statistic ("Fisher"), a Pearson's chi-squared type statistic ("Pearson") or t-statistic for average difference ("AveDiff") is available. User-defined global statistics can also be implemented.

args.local
An optional list to be passed to user-defined local statistics that require additional arguments. By default args.local = NULL.

args.global
An optional list to be passed to global statistics that require additional arguments. For two-sided local statistics, args.global = list(one.sided=F) allows bi-directional differential expression to be considered.

Pi.mat
Either an integer, or a matrix or data.frame containing the permutations. See getPImatrix for the acceptable form of a matrix or data.frame. If Pi.mat is an integer, B, then safe will generate B resamples of X.mat.

error
Specifies the method for computing error rate estimates. By default, Benjamini-Hochberg step down ("FDR.BH") FDR estimates are computed. A Bonferroni ("FWER.Bonf") and Holm's step-up ("FWER.Holm") adjustment can also be specified. Under permutation, "FDR.YB" computes the Yekutieli-Benjamini FDR estimate, and "FWER.WY" computes the Westfall-Young FWER estimate. The user can also specify "none" if no error rates are desired.

parallel
Logical argument (default = FALSE) specifying whether hypothesis test of method should be conducted with parallel processing. Only compatible with error = "none", "FWER.Bonf", or FDR.BH. See vignette for details.
The threshold for significant results to return. By default, alpha will be 0.05 for nominal p-values (error = "none"), and 0.1 for adjusted p-values.

Numeric argument sets the minimum difference for ranking local and global statistics, correcting a numerical precision issue when computing empirical p-values in small data sets (n < 15). The default value is 10^(-10).

Logical argument (default = TRUE) specifying whether to print progress updates to the log for permutation and bootstrap calculations.

Details

safe utilizes a general framework for testing differential expression across gene categories that allows it to be used in various experimental designs. Through structured resampling of the data, safe accounts for the unknown correlation among genes, and enables proper estimation of error rates when testing multiple categories. safe also provides statistics and empirical p-values for the gene-specific differential expression.

Value

The function returns an object of class SAFE. See help for SAFE-class for more details.

Author(s)

William T. Barry: <bbarry@jimmy.harvard.edu>

References


See also the vignette included with this package.

See Also

safeplot, safe.toptable, gene.results, getCMatrix, getPImatrix.

Examples

## Simulate a dataset with 1000 genes and 20 arrays in a 2-sample design.
## The top 100 genes will be differentially expressed at varying levels

g.alt <- 100
g.null <- 900
n <- 20

data <- matrix(rnorm(n*(g.alt+g.null)),g.alt+g.null,n)
data[1:g.alt,1:(n/2)] <- data[1:g.alt,1:(n/2)] +
  seq(2,2/g.alt,length=g.alt)
dimnames(data) <- list(c(paste("Alt",1:g.alt),
paste("Null",1:g.null)),
paste("Array",1:n))
## A treatment vector

```r
trt <- rep(c("Trt","Ctr"),each=n/2)
```

## 2 alt. categories and 18 null categories of size 50

```r
C.matrix <- kronecker(diag(20),rep(1,50))
dimnames(C.matrix) <- list(dimnames(data)[[1]],
    c(paste("TrueCat",1:2),paste("NullCat",1:18)))
dim(C.matrix)

results <- safe(data,trt,C.mat = C.matrix,Pi.mat = 100)
results
```

## SAFE-plot made for the first category

```r
if (interactive()) {
safeplot(results,"TrueCat 1")
}
```

---

### SAFE-class

#### Class SAFE

**Description**

The class SAFE is the output from the function `safe`. It is also the input to the plotting function `safeplot`.

**Slots**

- `local`: Object of class "character" indicating the local statistic used.
- `local.stat`: Object of class "numeric" containing the (unsorted) observed local statistics for genes.
- `local.pval`: Object of class "numeric" containing the (unsorted) empirical p-values for genes.
- `global`: Object of class "character" indicating the global statistic used.
- `global.stat`: Object of class "numeric" containing the (unsorted) observed global statistics for categories.
- `global.pval`: Object of class "numeric" containing the (unsorted) empirical p-values for categories.
- `error`: Object of class "character" indicating the method used to estimate error rates across multiple comparisons.
- `global.error`: Object of class "numeric" containing the (unsorted) error rates (adjusted p-values) for categories. If not computed, it will be set to NA.
- `C.mat`: Object of class "SparseM" containing the category assignments.
- `alpha`: Object of class "numeric" containing the alpha level used in returning significant results.
- `method`: Object of class "character" indicating the resampling method used in `safe`.

---
Methods

- **show** (safe.results): Summarizes the test results of significant categories.
- [ (safe.results): Returns a SAFE object for categories indicated by integer or character strings.
- **safeplot** (safe.results): **safeplot** produces CDFs of the association of expression to phenotype in a category relative to its complement.

Author(s)

William T Barry: <bbarry@jimmy.harvard.edu>

See Also

- safe, safeplot.

---

**safe-toptable**

**Category-specific results from SAFE**

Description

Prints annotated results from SAFE as a data.frame for categories with the strongest association to response/phenotype. This includes (a) category name; (b) category size; (c) global statistic; (d) nominal empirical p-values; (e) adjusted p-values; and (f) descriptions if available.

Usage

```r
safe.toptable(safe, number = 10,
               pretty = TRUE, description = TRUE)
```
safedag

Arguments

safe Object of class SAFE.
number Number of categories to be printed. If omitted, the first 10 categories are reported.
pretty Logical determining whether p-values smaller than 10^-4 are output in scientific notation, rather than as decimals. By default pretty = TRUE.
description Logical determining whether category descriptions are appended to printed output. By default description = TRUE.

Author(s)

William T. Barry: <bbarry@jimmy.harvard.edu>

References


See also the vignette included with this package.

See Also

safe.

Description

SAFE results displayed in Gene Ontology

Usage

safedag(object = NULL, ontology = NULL, top = NULL, color.cutoffs = c(0.1, 0.01, 0.001), filter = 0, max.GOnames = 200)

Arguments

object Object of class SAFE
ontology Gene Ontology of interest. Character strings of "GO.CC", "GO.BP", and "GO.MF" accepted.
top Optional character string giving the top category name from which to draw a subgraph of the tree
color.cutoffs  Numeric vector of length 3 for the cutoffs for coloring significant nodes. Nodes with unadjusted p-values less than color.cutoff[3] are drawn in blue; less than color.cutoff[2] are drawn in green; less than color.cutoff[1] are drawn in red.

filter  Optional integer (1,2,3) to only include branches that contain at least one node as significant as the respective color.cutoff.

max.GO.names  Maximum size of DAG to include category names as labels.

Details

DAG-plots are suggested as a means for visualizing the extent of differential expression in Gene Ontology categories. The relatedness of significant categories suggests whether similar or disparate biological findings are identified.

Author(s)

William T. Barry: <bbarry@jimmy.harvard.edu>

References


See also the vignette included with this package.

See Also

safe.

Description

A SAFE plot for a given category displays the empirical distribution function for the ranked (or unranked) local statistics of a given category.

Usage

safeplot(safe = NULL, cat.name = "", limits = NULL, c.vec = NULL, local.stats = NULL, gene.names = NULL, rank = TRUE, x.limits = NULL, c.thresh = 0, colors = NULL, x.ticks = NULL, t.cex = 1, p.val = NULL, cat.desc = NULL, title = "", ...)
safeplot

Arguments

- **safe**: Object of class SAFE
- **cat.name**: Name of the category to be plotted. If omitted, the most significant category is plotted.
- **limits**: Limits of the shaded region in the plot on the unranked scale
- **c.vec**: Logical vector specifying membership to a gene category
- **local.stats**: Numeric vector of local statistics
- **gene.names**: Optional character vector to replace `names(local.stats)` in labels
- **rank**: Logical to plotted raned (TRUE) or unranked (FALSE) local statistics on the x-axis
- **x.limits**: Optional limits of the x-axis. By default will be range(local.stats)
- **c.thresh**: Optional threshold for plotting tickmarks for a weighted (“soft”) gene category
- **colors**: Optional vector specificy colors for gene labels
- **x.ticks**: Optional location of x-axis tick marks on the ranked scale
- **t.cex**: Text size for gene labels
- **p.val**: Optional numeric value of the category’s empirical p-value
- **cat.desc**: Optional character string as a sub-title beneath the category name
- **title**: Optional title to the plot
- **...**: Allows arguments from version 2.0 to be ignored

Details

SAFE-plots display the differential expression in a given category relative to the complementary set of genes. The empirical cumulative distribution is plotted for local statistics in the category, on either a ranked or unranked scale. Tick marks are drawn along the top of the graph to indicate each gene’s positions, and labeled when sufficient space permits. In this manner, genes with the most extreme local statistics can be identified as contributing to the category’s significance.

Typical usages are

```r
safeplot(safe)
safeplot(safe, cat.name)
safeplot(c.vec, local.stats, p.val, limits)
```

Author(s)

William T. Barry: <bbarry@jimmy.harvard.edu>

References


See also the vignette included with this package.
See Also

safe.

Examples

```r
## Simulate a dataset with 1000 genes and 20 arrays in a 2-sample design.
## The top 100 genes will be differentially expressed at varying levels

g.alt <- 100
g.null <- 900
n <- 20

data<-matrix(rnorm(n*(g.alt+g.null)),g.alt+g.null,n)
data[1:g.alt,1:(n/2)] <- data[1:g.alt,1:(n/2)] +
    seq(2,2/g.alt,length=g.alt)
dimnames(data) <- list(c(paste("Alt",1:g.alt),
                        paste("Null",1:g.null)),
                        paste("Array",1:n))

## A treatment vector
trt <- rep(c("Trt","Ctr"),each=n/2)

## 2 alt. categories and 18 null categories of size 50
C.matrix <- kronecker(diag(20),rep(1,50))
dimnames(C.matrix) <- list(dimnames(data)[[1]],
                            c(paste("TrueCat",1:2),paste("NullCat",1:18)))
dim(C.matrix)

results <- safe(data,trt,C.mat = C.matrix,Pi.mat = 100)
results

## SAFE-plot made for the first category
if (interactive()) {
    safeplot(results,"TrueCat 1")
}
```
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