Package: GSRI (via r-universe)

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Description The GSRI package estimates the number of differentially expressed genes in gene sets, utilizing the concept of the Gene Set Regulation Index (GSRI).

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`GSRI-package` *Gene Set Regulation Index (GSRI) package*

Description

The **GSRI** package estimates the number of differentially expressed genes in gene sets, utilizing the concept of the Gene Set Regulation Index (GSRI).

Details

The GSRI approach estimates the number of differentially expressed genes in gene sets. It is independent of the underlying statistical test used for assessing the differential effect of genes and does not require any cut-off values for the distinction between regulated and unregulated genes. The approach is based on the fact that p-values obtained from a statistical test are uniformly distributed under the null hypothesis and are shifted towards zero in case of the alternative hypothesis.

Through non-parametric fitting of the uniform component of the p-value distribution, the fraction of regulated genes r in a gene set is estimated. The GSRI η is then defined as the $\alpha * 100\%$-quantile of the distribution of r, obtained from bootstrapping the samples within the groups. The index indicates that with a probability of $(1 - \alpha)\%$ more than a fraction of η genes in the gene set is differentially expressed. It can also be employed to test the hypothesis whether at least one gene in a gene set is regulated. Further, different gene sets can be compared or ranked according to the estimated amount of regulation.

For details of the method, an application to experimental data, and a comparison with related approaches, see Bartholome et al., 2009.

The package is published under the GPL-3 license.

Author(s)

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References

Kilian Bartholome, Clemens Kreutz, and Jens Timmer: Estimation of gene induction enables a relevance-based ranking of gene sets, Journal of Computational Biology: A Journal of Computational Molecular Cell Biology 16, no. 7 (July 2009): 959-967. http://www.liebertonline.com/doi/abs/10.1089/cmb.2008.0226

The **GSRI** package uses the functionality of the following packages:

Julian Gehring, Clemens Kreutz, Jens Timmer: les: Identifying Loci of Enhanced Significance in Tiling Microarray Data http://bioconductor.org/help/bioc-views/release/bioc/html/les.html

Korbinian Strimmer: fdrtool: Estimation and Control of (Local) False Discovery Rates. http://CRAN.R-project.org/package=fdrtool

Robert Gentleman, Vincent J. Carey, Wolfgang Huber, Florian Hahne: genefilter: methods for filtering genes from microarray experiments. http://bioconductor.org/help/bioc-views/release/bioc/html/genefilter.html

See Also

Class: Gsri

Methods: gsri getGsri getCdf getParms export sortGsri plot show summary readCls readGct

Examples

```
## Simulate expression data for a gene set of
## 100 genes, 20 samples (10 treatment, 10 control)
## and 30 regulated genes
set.seed(1)
exprs <- matrix(rnorm(100*20), 100)
exprs[1:30,1:10] <- rnorm(30*10, mean=2)
rownames(exprs) <- paste("g", 1:nrow(exprs), sep="")
groups <- factor(rep(1:2, each=10))

## Estimate the number of differentially expressed genes
res <- gsri(exprs, groups)
res

## Perform the analysis for different gene set
library(GSEABase)
gs1 <- GeneSet(paste("g", 25:40, sep=""), setName="set1")
gs2 <- GeneSet(paste("g", seq(1, nrow(exprs), by=5), sep=""), setName="set2")
gsc <- GeneSetCollection(gs1, gs2)

res2 <- gsri(exprs, groups, gs1)
res3 <- gsri(exprs, groups, gsc, verbose=TRUE)

summary(res2)
```

---

export *Export results to file*

Description

Export the results of a Gsri object to a file.

Usage

```
export(object, file, ...)
```

Arguments

`object`	An object of class `Gsri` whose results to export.
`file`	Character vector specifying the path of the file to be written.
`...`	Additional arguments, currently not used.

Details

The `export` method writes the results of the GSRI analysis, as obtained with `getGsri`, to a text file. The file is formatted with the standard parameters of the `write.table` function, while "\t" is used as field seperator.

Author(s)

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See Also

Package: GSRI-package

Class: Gsri

Methods: gsri getGsri getCdf getParms export sortGsri plot show summary readCls readGct

Examples

```
## Not run:
export(gsri, file)

## End(Not run)
```

---

get-methods *Get methods for* `Gsri` *class*

Description

Access and extract results from an object of class `Gsri`.

Usage

```
getGsri(object, index, ...)
getCdf(object, index, ...)
getParms(object, ...)
```

Arguments

object	An object of class Gsri whose results to access.
index	Optional argument used to subset the results of individual gene sets. For details, see the plot method.
...	Additional arguments, currently not used.

Details

getGsri returns a data frame with the results of the GSRI analysis, equivalent to the display of the print and show method. For each gene set it contains the estimated fraction and total number of regulated genes, the standard deviation of the estimated fraction, the GSRI, and the total number of genes in the analysis.

getCdf returns a data frame with ECDF of the p-values for a gene set.

getParms returns a list with the parameters used in the analysis, including the weights (weight) for each gene in the gene set, the number (nBoot) of bootstraps for the calculation of the GSRI, the function (test) and its arguments (testArgs) used for assessing the differential effect between the groups, the confidence level for the GSRI, and the application of the Grenander estimatior (grenander) in the calculation of the ECDF.

Value

getGsri and getCdf return data frame with the results from the GSRI analysis and the ECDF, respectively. getParms returns a list with the parameters used in the analysis.

If more than one gene set is accessed, a list with one element per gene set is returned.

Author(s)

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See Also

Package: GSRI-package

Class: Gsri

Methods: gsri getGsri getCdf getParms export sortGsri plot show summary readCls readGct

Examples

```
## Not run:
getGsri(object)
getCdf(object)
getParms(object)

## End(Not run)
```

gsri *Methods for the Gene Set Regulation Index (GSRI)*

Description

Estimate the number of differentially expressed genes in gene sets.

Usage

```
gsri(exprs, groups, geneSet, names=NULL, weight=NULL, nBoot=100,
test=rowt, testArgs=NULL, alpha=0.05, grenander=TRUE, verbose=FALSE,
...)
```

Arguments

exprs	Matrix or object of class ExpressionSet containing the expression intensities of the microarray. If a matrix the rows represent the genes and the columns the samples, respectively.
groups	Factor with the assignments of the microarray samples to the groups, along which the differential effect should be estimated. Must have as many elements as exprs has samples.
geneSet	Optional object of class GeneSet or GeneSetCollection defining the gene set(s) used for the analysis. If missing all genes of exprs are considered to be part of the gene set. If an object of class GeneSet only these genes are considered to be part of the gene set. If an object of class GeneSetCollecton the analysis is performed for each gene set of the collection individually.
names	Optional character vector with the names of the gene set(s). If missing the names are taken from the geneSet argument. Has to have as many unique elements as gene sets in the analysis.
weight	Optional numerical vector of weights specifying the certainty a gene is part of the gene set. If NULL all genes are assumed to have the same weight. Please note that the weights are defined in a relative way and thus any kind of positive weights is feasable. Must have as many elements as eighter the genes defined in geneSet or in exprs.
nBoot	Integer with the number of bootstrap samples to be drawn in the calculation of the GSRI (default: 100).
test	A function defining the statistical test used to assess the differential effect between the groups which are given by the groups argument. In this package, a t-test (rowt) and an F-test (rowF) are already supplied, with rowt being the default. Additionally, a custom test function can be used in order to be able to include any feasible statistical test in the analysis. For details, please see the 'details' section.
testArgs	List of optional arguments used by the test function. For details, please see the 'details' section and the help for test-functions.

`alpha` Single numeric specifying the confidence level for the GSRI. The estimated GSRI is the lower bound of the (1-α)*100% confidence interval obtained from bootstrapping.

`grenander` Logical about whether the modified Grenander estimator for the cumulative density should be used instead of a centered ECDF. By default the modified Grenander estimator is used. For more information, please see the 'details' section.

`verbose` Logical indicating whether the progress of the computation should be printed to the screen (default: FALSE). Most useful if `geneSet` is an object of class `GeneSetCollection`.

`...` Additional arguments, including:

minSize: Integer specifying the minimal number of genes in a gene set in order to perform an analysis. If the gene set has less than `minSize` in `exprs`, the gene set is ignored in the analysis.

nCores: Integer setting the number of cores used for the computation in combination with the **multicore** package for a `GeneSetCollection`. For details, please see the 'details' section.

Details

The `gsri` method estimates the degree of differential expression in gene sets. By assessing the part of the distribution of p-values consistent with the null hypothesis the number of differentially expressed genes is calculated.

Through non-parametric fitting of the uniform component of the p-value distribution, the fraction of regulated genes r in a gene set is estimated. The GSRI η is then defined as the $\alpha * 100\%$-quantile of the distribution of r, obtained from bootstrapping the samples within the groups. The index indicates that with a probability of $(1 - \alpha)\%$ more than a fraction of η genes in the gene set is differentially expressed. It can also be employed to test the hypothesis whether at least one gene in a gene set is regulated. Further, different gene sets can be compared or ranked according to the estimated amount of regulation.

Assessing the differential effect is based on p-values obtained from statistical testing at the level of individual genes between the groups. The GSRI approach is independent of the underlying test and can be chosen according to the experimental design. With the t-test (`rowt`) and F-test (`rowF`) two widely used statistical test are already part of the package. Additional tests can easily used which are passed with the `test` argument to the `gsri` method. For details on how to implement custom test functions, please refer to the help of `rowt` and `rowF` or the vignette of this package.

The GSRI approach further allows weighting the influence of individual genes in the estimation. This can be beneficial including for example the certainty that genes are part of a certain gene set derived from experimental findings or annotations.

Defining gene sets is available through the **GSEABase** package which provides the `GeneSet` and `GeneSetCollection` classes a single or multiple gene sets, respectively. This ensures a powerful approach for obtaining gene sets from data objects, data bases, and other bioconductor packages. For details on how to define or retrieve gene sets, please refer to the documentation of the **GSEABase** package, with a special focus on the `GeneSet` and `GeneSetCollection` classes.

The distribution of the p-values of a gene set is assessed in the cumulative density. In addition to a symmetrical empirical cumulative density function (ECDF), the modified Grenander estimator based on the assumption about the concave shape of the cumulative density is implemented and used

by default. While the modified Grenander estimator reduces the variance and makes the approach more stable especially for small gene set, it underestimates the number of regulated genes and thus leads to conservative estimates.

In the case that the computation is performed for several gene sets in the form of a `GeneSetCollection` object, it can be parallelized with the **multicore** package. Please note that this package is not available on all platforms. Using its capabilities requires attaching **multicore** prior to the calculation and specification of the `nCores` argument. For further details, please refer to the documentation of the **multicore** package. This may be especially relevant in the case that specific seed values for the bootstrapping are of interest.

Value

An object of class `Gsri` with the slots:

`result`**:** Data frame containing the results of the GSRI estimation, with one row for each gene set.

`cdf`**:** List of data frames containing the ECDF of the p-values. Each data frame covers one gene set.

`parms`**:** List containing the parameter values used in the analysis, with the elements.

For details, please see the help for the `Gsri` class.

Methods

Analysis for all genes of `exprs` part of the gene set:

```
signature(exprs="matrix", groups="factor", geneSet="missing")
signature(exprs="ExpressionSet", groups="factor", geneSet="missing")
```

Analysis for one gene set, defined as an object of class `GeneSet`:

```
signature(exprs="matrix", groups="factor", geneSet="GeneSet")
signature(exprs="ExpressionSet", groups="factor", geneSet="GeneSet")
```

Analysis for several gene sets, defined as an object of class `GeneSetCollection`:

```
signature(exprs="matrix", groups="factor", geneSet="GeneSetCollection")
signature(exprs="ExpressionSet", groups="factor", geneSet="GeneSetCollection")
```

In this case parallel computing capabilities provided by the **multicore** package may be available, depending on the platform.

Note

The standard deviation of the estimated number of regulated genes as well as the GSRI are obtained through bootstrapping. Thus, the results for these two parameters may differ slightly for several realizations, especially for small numbers of bootstraps (`nBoot`). Setting the seed of the random number generator avoids this problem and yields exactly the same results for several realizations.

Author(s)

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See Also

Package: GSRI-package

Class: Gsri

Methods: gsri getGsri getCdf getParms export sortGsri plot show summary readCls readGct

Examples

```
## Simulate expression data for a gene set of
## 100 genes, 20 samples (10 treatment, 10 control)
## and 30 regulated genes
set.seed(1)
exprs <- matrix(rnorm(100*20), 100)
exprs[1:30,1:10] <- rnorm(30*10, mean=2)
rownames(exprs) <- paste("g", 1:nrow(exprs), sep="")
groups <- factor(rep(1:2, each=10))

## Estimate the number of differentially expressed genes
res <- gsri(exprs, groups)
res

## Perform the analysis for different gene set
library(GSEABase)
gs1 <- GeneSet(paste("g", 25:40, sep=""), setName="set1")
gs2 <- GeneSet(paste("g", seq(1, nrow(exprs), by=5), sep=""), setName="set2")
gsc <- GeneSetCollection(gs1, gs2)

res2 <- gsri(exprs, groups, gs1)
res3 <- gsri(exprs, groups, gsc, verbose=TRUE)

summary(res2)
```

Gsri-class *Class* Gsri

Description

Objects of the class Gsri contain the results of the GSRI analysis.

Objects from the class

Objects of class Gsri are returned by the gsri methods.

Slots

`result`: Data frame containing the results of the GSRI estimation, with one row for each gene set and the columns:

- `pRegGenes`: Fraction of regulated genes in the gene set
- `pRegGenesSd`: Standard deviation of `pRegGenes` obtained from bootstrapping.
- `nRegGenes`: Total number of regulated genes in the gene set.
- `GSRI('alpha'%)`: Gene Set Regulation Index, corresponding to the 'alpha'% quantile of the bootstrapped distribution.
- `nGenes`: Total number of genes in the gene set.

`cdf`: List of data frames containing the ECDF of the p-values. Each data frame covers one gene set, with the columns:

- `pval`: P-values obtained from the `test` function.
- `cdf`: Empirical cumulative density.

`parms`: List containing the parameter values used in the analysis, with the elements:

- `weight`: Weights for each gene in the gene set
- `nBoot`: Number of bootstraps for the calculation of the GSRI
- `test`: Statistical test function
- `alpha`: Confidence level for the GSRI
- `grenander`: Application of the Grenander estimatior in the calculation of the ECDF
- `testArgs`: Optional arguments for `test` function

Methods

Analysis:

gsri: `signature(exprs="matrix", groups="factor",geneSet="missing")`
`signature(exprs="ExpressionSet", groups="factor",geneSet="missing")`
`signature(exprs="matrix", groups="factor",geneSet="GeneSet")`
`signature(exprs="ExpressionSet", groups="factor",geneSet="GeneSet")`
`signature(exprs="matrix", groups="factor",geneSet="GeneSetCollection")`
`signature(exprs="ExpressionSet", groups="factor",geneSet="GeneSetCollection")`
Assess the degree of differential effect in the expression data.

Visualization:

plot: `signature(x="Gsri", y=ANY)`

Plot the empirical density of p-values and the corresponding estimated effect.

Export to file:

export: `signature(object="Gsri", file="character")`

Get methods:

getGsri: `signature(object="Gsri")`

getCdf: `signature(object="Gsri")`

getParms: `signature(object="Gsri")`

Show:

show: `signature(obejct="Gsri")`

summary: `signature(obejct="Gsri")`

Author(s)

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See Also

Package: GSRI-package

Class: Gsri

Methods: gsri getGsri getCdf getParms export sortGsri plot show summary readCls readGct

Examples

```
showClass("Gsri")
```

`plot-methods` *Visualize GSRI results*

Description

Plot the empirical cumulative density along with the estimated degree of regulation of the p-value distribution for a gene set.

Usage

```
plot(x, y, ...)
```

Arguments

x	An object of class Gsri containing the results to plot.
y	A single integer or character string specifying the results of which gene set to plot. This has to be given if x contains the results of several gene sets. An integer is interpreted as the index of the gene set (i.e. the row number), while a character is matched against the names of the gene sets.
...	Optional arguments used in order to customize the plot. See the 'details' section.

Details

Plotting the p-value distribution and the estimation of the regularized compontent for a gene set allows to insepct the results in detail. The plot illustrates the empirical cumulative density function of the p-values obtained from testing for a differential effect between the groups. Additionally, the fit of the uniformly distributed component along with the estimated fraction of regularized genes and the GSRI is shown.

The `plot` method uses a special system in order to customize the graphical elements of the figure. It allows to refer to the different components with the name of the additional input argument; its value is a list containing named graphical parameters for the underlying plot function. The following list describes the possible names and their contribution.

`plot` Arguments for the axis and the labeling, passed to the `plot` function.

`fit` Arguments for the fit of the linear component of the ECDF, corresponding to the part without differential effect, passed to the `lines` function.

`ecdf` Arguments for the ECDF of the p-values, passed to the `lines` function.

`reg` Arguments for the horizontal line indicating the fraction of regulation, passed to the `lines` function.

`regText` Arguments for the text label of `reg`, passed to the `text` function.

`gsri` Arguments for the horizontal line indicating the GSRI, passed to the `lines` function.

`gsriText` Arguments for the text label of `gsri`, passed to the `text` function.

Thus, changing for example the limit of the y-axis, the plot type and color of the ECDF, and the label of the x-axis, you can use:

```
plot(x, plot=list(ylim=c(0, 0.8), xlab=expression(p)), ecdf=list(type="s", col="darkgreen"))
```

For more details, please see the 'examples' section.

Author(s)

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See Also

Package: `GSRI-package`

Class: `Gsri`

Methods: `gsri` `getGsri` `getCdf` `getParms` `export` `sortGsri` `plot` `show` `summary` `readCls` `readGct`

Examples

```
## Not run:
plot(x)

plot(x, plot=list(main="Example plot"), ecdf=list(pch=21),
fit=list(lty=2, lwd=0.5, col="black"), gsri=list(col="lightblue"))

plot(x2, 2)
```

```
plot(x2, "gs2")

## End(Not run)
```

`read-functions` *Import of '.cls' and '.gct' files*

Description

Import the groups from '.cls' files and the expression data from '.gct' files.

Usage

```
readCls(file, ...)
readGct(file, ...)
```

Arguments

`file`	Character vector specifying the path of the file to be read in.
`...`	Optinal arguments, currently not used.

Details

With these methods the expression data and the assignment of the samples to groups can be read from '.cls' (categorical class) and '.gct' (gene cluster text) files, respectively. Details on the specific formats can be found at http://www.broadinstitute.org/cancer/software/gsea/wiki/index.php/Data_formats.

Please note that the `readCls` method reads only categorical class labels, not continuous ones.

Value

For a '.cls' file, a factor containing the groups.

For a '.gct' file, a matrix containing the expression intensities, with rows corresponding to genes and columns to samples.

Author(s)

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See Also

Package: GSRI-package

Class: Gsri

Methods: gsri getGsri getCdf getParms export sortGsri plot show summary readCls readGct

Examples

```
## Not run:
exprs <- readGct(file)

groups <- readCls(file)

## End(Not run)
```

---

sortGsri *Sort GSRI results*

Description

Sort the results of an Gsri object.

Usage

```
sortGsri(x, names, decreasing=TRUE, na.last=NA, ...)
```

Arguments

x	Object of class Gsri whose results to sort.
names	Columns along which the results of x should be sorted, eighter a character vector with the names of the columns or an integer vector with the index of the columns. If the vector has several elements, sorting is performed along all of them, starting with the first and using subsequent ones to break existing ties. If names is not specified the results are sorted according to pRegGenes.
decreasing	Logical indicating whether the sorting should be in decreasing (default) or ascending order, see sort.
na.last	How NA values in the results should be treated, see sort.
...	Additional arguments, currently not used.

Value

An object of class Gsri, with sorted slots result and cdf.

Methods

signature(x="Gsri", names="ANY")

Author(s)

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See Also

Package: GSRI-package

Class: Gsri

Methods: gsri getGsri getCdf getParms export sortGsri plot show summary readCls readGct

Examples

```
## Not run:
sortGsri(object, c("pRegGenes", "nGenes"))
sortGsri(object, c(1, 5))

## End(Not run)
```

---

test-functions *Statistical test functions*

Description

Assess the differential effect in gene expression between groups of microarray replicates.

Usage

```
rowt(exprs, groups, id, index, testArgs)
rowF(exprs, groups, id, index, testArgs=list(var.equal=TRUE))
limmat(exprs, groups, id, index, testArgs)
```

Arguments

exprs	A matrix of expression values of size n x m, with rows representing the genes and columns representing the samples. The structure is the same as for the exprs argument of the gsri method.
groups	A factor with the length m, specifying the groups of the corresponding samples in exprs. The structure is the same as for the exprs argument of the gsri method.
id	Index vector for the rows of exprs which are part of the current gene set.
index	Index for the columns of exprs, such that exprs[,index] yields the bootstrapped expression matrix. Similar to the index arguments for boot of the **boot** package.
testArgs	Optional list with arguments passed to the test function. If 'NULL' or missing it is not passed to test and any exisiting default value of the function is used instead.
	var.equal: For the rowF function a logical indicating whether equal variances in the groups are assumed for the F-test (default: TRUE). For details, please see rowFtests in the **genefilter** package.

Details

With the t-test and the F-test, two widely used statistical tests are available in this package. To allow a fast computation the implementations from the **genefilter** package is used.

It is also possible to use custom test statistics for assessing the differential effect between groups for each gene. In this case the function is passed as the `test` argument to the `gsri` method, while additional parameters for the function can be passed as a list via the `testArgs` argument. The defined function is required to be called as

`function(exprs, groups, id, index, testArgs)`,

with `exprs` the matrix of expression intensities of the microarray and `groups` the factor of group labels, with the same structure as those passed initially to the `gsri` method. The vector `id` contains the indices of the genes part of the current gene set and is used to subset the expression intensities if necessary. The function has to return one p-value for each gene in the gene set indicating its differential effect. The vector `index` contains the indicies of the samples for the bootstrapping. Applying `index` on the expression matrix in the form of `exprs[ ,index]` generates the bootstrapped data set.

For details on how to define and use your custom test functions, please refer to the 'examples' section or the vignette of this package.

Value

A vector of p-values, indicating the significance of the differential effect between groups for each gene.

Author(s)

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See Also

Package: GSRI-package

Class: Gsri

Methods: gsri getGsri getCdf getParms export sortGsri plot show summary readCls readGct

Statistical tests from the **genefilter** package: rowFtests

Examples

```
## Not run:
## A simple example for a custom test function using a linear model.
## Note that for two groups this is equivalent to a t-test with equal variances.
testFcn <- function(exprs, groups, id, index, testArgs) {

  stat <- function(e, g, f) {
  m <- lm(f)
  pval <- summary(m)$coefficients[2,4]
  }

pvals <- apply(exprs[id,index], 1, stat, groups, testArgs$f)
```

```
return(pvals)
}

## Pass the definition of the linear model through 'testArgs'
f <- formula(e ~ g)

res <- gsri(exprs, groups, test=testFcn, testArgs=list(f=f))

## End(Not run)
```

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