Package ‘piano’

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Description Piano performs gene set analysis using various statistical methods, from different gene level statistics and a wide range of gene-set collections. Furthermore, the Piano package contains functions for combining the results of multiple runs of gene set analyses.
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piano-package

Description

Run gene set analysis with various statistical methods, from different gene level statistics and a wide range of gene-set collections. Furthermore, the Piano package contains functions for combining the results of multiple runs of gene set analyses.

Details

The Piano package consists of two parts. The major part revolves around gene set analysis (GSA), and the central function for this is `runGSA`. There are some downstream functions (e.g. `GSAsummaryTable` and `geneSetSummary`) that handle the results from the GSA. By running `runGSA` multiple times with different settings it is possible to compute consensus gene set scores. Another set of functions (e.g. `consensusScores` and `consensusHeatmap`) take a list of result objects given by `runGSA` for this step. The second part of the Piano package contains a set of functions devoted for an easy-to-use approach on microarray analysis (wrapped around the `affy` and `limma` packages), which are constructed to integrate nicely with the downstream GSA part. The starting function in this case is `loadMAdata`.

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See Also

`runGSA` and `loadMAdata`
consensusHeatmap

Heatmap of top consensus gene sets

Description

Based on multiple result objects from the runGSA function, this function computes the consensus scores, based on rank aggregation, for each directionality class and produces a heatmap plot of the results.

Usage

consensusHeatmap(resList, method="median", cutoff=5, adjusted=FALSE, plot=TRUE, ncharLabel=25, cellnote="consensusScore", columnnames="full", colorkey=TRUE, colorgrad=NULL, cex=NULL)

Arguments

- **resList**: a list where each element is an object of class GSares, as returned by the runGSA function.
- **method**: a character string selecting the method, either "mean", "median", "Borda" or "Copeland".
- **cutoff**: the maximum consensus score of a gene set, in any of the directionality classes, to be included in the heatmap.
- **adjusted**: a logical, whether to use adjusted p-values or not. Note that if runGSA was run with the argument adjMethod="none", the adjusted p-values will be equal to the original p-values.
- **plot**: whether or not to draw the heatmap. Setting plot=FALSE allows you to save the heatmap as a matrix without plotting it.
- **ncharLabel**: the number of characters to include in the row labels.
- **cellnote**: a character string selecting the information to be printed inside each cell of the heatmap. Either "consensusScore", "medianPvalue", "nGenes" or "none". Note that the actual heatmap will always be based on the consensus scores.
- **columnnames**: either "full" (default) or "abbr" to use full or abbreviated column labels. Will save some space for the heatmap if set to "abbr"
- **colorkey**: a logical (default TRUE), whether or not to display the colorkey. Will save some space for the heatmap if turned off.
- **colorgrad**: a character vector giving the color names to use in the heatmap.
- **cex**: a numeric, to control the text size.

Details

This function computes the consensus gene set scores for each directionality class based on the results (gene set p-values) listed in resList, using the consensusScores function. For each class, only the GSares objects in resList that contain p-values for that class are used as a basis for the rank aggregation. Hence, if not all classes are covered by at least 2 GSares objects in the list, the consensusHeatmap function will not work. The results are displayed in a heatmap showing the consensus scores.
consensusScores

Value
A list, returned invisibly, containing the matrix of consensus scores as represented in the heatmap as well as the matrix of corresponding median p-values and the matrix of number of genes in each gene set (including the subset of up and down regulated genes for the mixed directional classes).

Author(s)
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See Also
piano, runGSA

Examples

# Load some example GSA results:
data(gsa_results)

# Consensus heatmap:
dev.new(width=10,height=10)
consensusHeatmap(resList=gsa_results)

# Store the output:
dev.new(width=10,height=10)
ch <- consensusHeatmap(resList=gsa_results)

# Access the median p-values for gene set s1:
ch$pMat["s1",]

Description
Calculates the consensus scores for the gene sets using multiple gene set analysis methods (with runGSA()). Optionally also produces a boxplot to visualize the results.

Usage

consensusScores(resList, class, direction, n=50, adjusted=FALSE, method="median", plot=TRUE, cexLabel=0.8, cexLegend=1, showLegend=TRUE, rowNames="names", logScale=FALSE, main)

Arguments

resList a list where each element is an object of class GSAres, as returned by the runGSA function.
class a character string determining the p-values of which directionality class that should be used as significance information for the plot. Can be one of "distinct", "mixed", "non".
direction a character string giving the direction of regulation, can be either "up" or "down".
**consensusScores**

- **n**: consensus rank cutoff. All gene sets with consensus rank (see details below) <= n will be included in the plot. Defaults to 50.
- **adjusted**: a logical, whether to use adjusted p-values or not. Note that if runGSA was run with the argument adjMethod="none", the adjusted p-values will be equal to the original p-values.
- **method**: a character string selecting the method, either "mean", "median", "max", "Borda" or "Copeland".
- **plot**: a logical, whether or not to draw the boxplot.
- **cexLabel**: the x- and y-axis label sizes.
- **cexLegend**: the legend text size.
- **showLegend**: a logical, whether or not to show the legend and the individual method ranks as points in the plot.
- **rowNames**: a character string determining which rownames to use, set to either "ranks" for the consensus rank, "names" for the gene set names, or "none" to omit rownames.
- **logScale**: a logical, whether or not to use log-scale for the x-axis.
- **main**: a character vector giving an alternative title of the plot.

**Details**

Based on the results given by the elements of resList, preferably representing similar runs with runGSA but with different methods, this function ranks the gene sets for each GSAres object, based on the selected directionality class. Next, the median rank for each gene set is taken as a score for top-ranking gene sets. The highest scoring gene-sets (with consensus rank, i.e. rank(rankScore,ties.method="min"), smaller or equal to n) are selected and depicted in a boxplot, showing the distribution of individual ranks (shown as colored points), as well as the median rank (shown as a red line). As an alternative of using the median rank as consensus score, it is possible to choose the mean or using the Borda or Copeland method, through the method argument. A more conservative approach can also be taken using the maximum rank as a consensus score, prioritizing gene-sets that are consistently ranked high across all GSA runs. All elements of resList have to be objects containing results for the same number of gene-sets. The ranking procedure handles ties by giving them their minimum rank.

**Value**

A list containing a matrix of the ranks for the top n gene sets, given by each run, as well as the corresponding matrix of p-values, given by each run.

**Author(s)**

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**See Also**

piano, runGSA
### Examples

```r
# Load some example GSA results:
data(gsa_results)

# Consensus scores for the top 50 gene sets (in the non-directional class):
cs <- consensusScores(resList=gsa_results,class="non")

# Access the ranks given to gene set s7 by each individual method:
cs$rankMat["s7",]
```

---

### diffExp

**Perform differential expression analysis**

#### Description

Identifies differentially expressed genes by using the linear model approach of `limma`. Optionally produces a Venn diagram, heatmap, Polar plot and volcano plot.

#### Usage

```r
diffExp(arrayData, contrasts, chromosomeMapping, fitMethod = "ls", adjustMethod = "fdr", significance = 0.001, plot = TRUE, heatmapCutoff = 1e-10, volcanoFC = 2, colors=c("red","green","blue","yellow","orange","purple","tan","cyan","gray60","black"), save = FALSE, verbose = TRUE)
```

#### Arguments

- **arrayData**: an object of class `ArrayData`.
- **contrasts**: a character vector giving the contrasts to be tested for differential expression. Use `extractFactors` to get allowed contrasts.
- **chromosomeMapping**: character string giving the name of the chromosome mapping file, or an object of class `data.frame` or similar containing the chromosome mapping. Required for the Polar plot if the `ArrayData` object lacks annotation information. See details below.
- **fitMethod**: character string giving the fitting method used by `lmFit`. Can be either "ls" for least squares (default) or "robust" for robust regression.
- **adjustMethod**: character string giving the method to use for adjustment of multiple testing. Can be "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr" (default) or "none". See `p.adjust` for details.
- **significance**: number giving the significance cutoff level for the Venn diagram and the horizontal line drawn in the volcano plot. Defaults to 0.001.
- **plot**: should plots be produced? Set either to TRUE (default) or FALSE to control all plots, or to a character vector with any combination of "venn", "heatmap", "polarplot" and "volcano", to control the single plots (e.g. `plot=c("venn","polarplot")` or `plot="heatmap"`).
- **heatmapCutoff**: number giving the significance cutoff level for the heatmap. Defaults to 1e-10.
diffExp

volcanoFC    number giving the x-coordinates of the vertical lines drawn in the volcano plot. Defaults to 2.

colors      character vector of colors to be used by the Venn diagram and Polar plot.

save        should the figures and p-values be saved? Defaults to FALSE.

verbose     verbose? Defaults to TRUE.

Details

This function uses limma to calculate p-values measuring differential expression in the given contrasts. The uniqueFactors given by extractFactors can be used to define a contrast vector, where each element should be a character string on the form "uniqueFactorA - uniqueFactorB", note the space surrounding the -. (See the example below and for extractFactors.)

If appropriate annotation is missing for the ArrayData object the user can supply this as chromosomeMapping. This should be either a data.frame or a tab delimited text file and include the columns chromosome with the chromosome name and chromosome location containing the starting position of each gene. A - sign can be used to denote the antisense strand but this will be disregarded while plotting. The rownames should be probe IDs or, if using a text file, the first column with a column header should contain the probe IDs.

Note that the fitMethod="robust" may need longer time to run.

A Venn diagram can be drawn for up to five contrasts (diffExp() will use vennDiagram).

The heatmap shows normalized expression values of the genes that pass the heatmapCutoff in at least one contrast.

A volcano plot is produced for each contrast showing magnitude of change versus significance.

The Polar plot sorts the genes according to chromosomal location, for each chromosome starting with unknown positions followed by increasing number in the chromosome location column. Genes which do not map to any chromosome are listed as U for unknown. The radial lines in the Polar plot are -log10 scaled p-values, so that a longer line means a smaller p-value. This gives an overview of the magnitude of differential expression for each contrast.

Typical usages are:

```r
# Identify significantly changed genes in 'm1' and 'm2' compared to 'wt':
diffExp(arrayData, contrasts=c("m1 - wt", "m2 - wt"))
```

Value

A list with elements:

- pValues: data.frame containing adjusted p-values (according to argument adjustMethod) for each contrast
- foldChanges: data.frame containing log2 fold changes for each contrast
- resTable: a list with an element for each contrast, each being a data.frame with full result information
- vennMembers: list containing the gene members of each area of the Venn diagram (only returned when a Venn diagram is drawn)

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References

See Also
piano, loadMADatas, extractFactors, polarPlot, runGSA, limma, venn, heatmap.2

Examples

# Get path to example data and setup files:
dataPath <- system.file("extdata", package="piano")

# Load normalized data:
myArrayData <- loadMADatas(datadir=dataPath, dataNorm="norm_data.txt.gz", platform="yeast2")

# Perform differential expression analysis:
pfc <- diffExp(myArrayData, contrasts=c("aerobic_Clim - anaerobic_Clim",
"aerobic_Nlim - anaerobic_Nlim"))

# Order the genes according to p-values, for aerobic_Clim vs anaerobic_Clim:
o <- order(pfc$resTable$'aerobic_Clim - anaerobic_Clim'P.Value)

# Display statistics for the top 10 significant genes:
pfc$resTable$'aerobic_Clim - anaerobic_Clim'[o[1:10],]

extractFactors

Extracts the factors, given by an ArrayData object, that can be used by diffExp

Usage

extractFactors(arrayData)

Arguments

arrayData an ArrayData object.

Value

A list with elements:

factors Assigns one factor to each array
uniqueFactors The unique factors that can be used to form contrasts

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geneSetSummary

See Also

piano, diffExp

Examples

# Get path to example data and setup files:
dataPath <- system.file("extdata", package="piano")

# Load normalized data:
myArrayData <- loadMAdata(datadir=dataPath, dataNorm="norm_data.txt.gz", platform="yeast2")

# Extract the factors that can be used in the call to diffExp:
extractFactors(myArrayData)

geneSetSummary

Gene set summary

Description

Returns a summary of the statistics and gene members of a given gene set in a GSAres object.

Usage

geneSetSummary(gsaRes, geneSet)

Arguments

  gsaRes an object of class GSAres, as returned from runGSA().
  geneSet a character string giving the name of a gene-set.

Details

This function can be used to access information on specific gene sets of interest. The same results are available for all gene sets using GSAsummaryTable.

Value

A list with the elements name, containing the gene-set name, geneLevelStats, containing the gene-level statistics of the member genes, directions, containing the directions of the member genes, and stats, a table of the gene set statistics and p-values.

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See Also

piano, runGSA, GSAsummaryTable
Examples

# Load example input data to GSA:
data("gsa_input")

# Load gene set collection:
gsc <- loadGSC(gsa_input$gsc)

# Run gene set analysis:
gsares <- runGSA(geneLevelStats=gsa_input$pvals, directions=gsa_input$directions,
gsc=gsc, nPerm=500)

# Get info on a specific gene set:
geneSetSummary(gsares,"s1")

---

**GSAheatmap**

**Heatmap of top significant gene sets**

Description

This function selects the top scoring (most significant) gene sets for each directionality class and produces a heatmap plot of the results.

Usage

GSAheatmap(gsaRes, cutoff=5, adjusted=FALSE, ncharLabel=25, cellnote="pvalue", columnnames="full", colorkey=TRUE, colorgrad=NULL, cex=NULL)

Arguments

- **gsaRes**: an object of class GSAres, as returned from runGSA().
- **cutoff**: an integer n, so that the top n gene sets (plus possible ties) in each directionality class will be included in the heatmap.
- **adjusted**: a logical, whether to use adjusted p-values or not. Note that if runGSA was run with the argument adjMethod="none", the adjusted p-values will be equal to the original p-values.
- **ncharLabel**: the number of characters to include in the row labels.
- **cellnote**: a character string selecting the information to be printed inside each cell of the heatmap. Either "pvalue", "rank", "nGenes" or "none". Note that the actual heatmap will always be based on the gene set ranks.
- **columnnames**: either "full" (default) or "abbr" to use full or abbreviated column labels. Will save some space for the heatmap if set to "abbr"
- **colorkey**: a logical (default TRUE), whether or not to display the colorkey. Will save some space for the heatmap if turned off.
- **colorgrad**: a character vector giving the color names to use in the heatmap.
- **cex**: a numeric, to control the text size.
Details
This function selects the top significant gene sets in each directionality class and draws a heatmap of the results. It provides a quick summary alternative to the \texttt{GSAsummaryTable} function or the \texttt{networkPlot}.

Value
A list, returned invisibly, containing the matrix of p-values (adjusted or non-adjusted depending on the settings) as represented in the heatmap as well as the matrix of corresponding ranks and the matrix of number of genes in each gene set (including the subset of up and down regulated genes for the mixed directional classes).

Author(s)
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See Also
\texttt{piano}, \texttt{runGSA}, \texttt{GSAsummaryTable}, \texttt{networkPlot}

Examples

```r
# Load example input data to GSA:
data("gsa_input")

# Load gene set collection:
gsc <- loadGSC(gsa_input$gsc)

# Run gene set analysis:
gsares <- runGSA(geneLevelStats=gsa_input$pvals, directions=gsa_input$directions, gsc=gsc, nPerm=500)

# Make heatmap:
dev.new(width=10,height=10)
GSAheatmap(gsares)
```

\texttt{GSAsummaryTable} \hspace{1cm} \textit{Gene set analysis summary table}

Description
Displays or saves a summary table of the results from \texttt{runGSA}.

Usage
\texttt{GSAsummaryTable(gsaRes, save=FALSE, file=NULL)}

Arguments
\begin{itemize}
\item \texttt{gsaRes} an object of class \texttt{GSAres}, as returned from \texttt{runGSA}.
\item \texttt{save} a logical, whether or not to save the table.
\item \texttt{file} a character string giving the file name to save to.
\end{itemize}
### gsa_input

#### Description

This data set is completely randomly generated and contains p-values for 2000 genes, fold-changes for those genes and a gene set collection giving the connection between genes and 50 gene sets. Only attended to be used as example data for runGSA.

#### Usage

```r
data(gsa_input)
```

#### Format

A list containing 3 elements: `gsa_input$pvals` and `gsa_input$directions` are numeric vectors, `gsa_input$gsc` is a two-column matrix with gene names in the first column and gene set names in the second.
gsa_results

Description

This data set contains gene set analysis results, as returned by the `runGSA` function, that is used as example data for downstream functions. The input data to `runGSA` was randomly generated and is accessible through `data(gsa_input)`.

Usage

data(gsa_results)

Format

A list where each element is an object of class GSAres, as returned by `runGSA`.

loadGSC

Description

Load a gene set collection, to be used in `runGSA`, in GMT, SBML or SIF format, or optionally from a data.frame.

Usage

loadGSC(file, type="auto", addInfo)

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>file</td>
<td>a character string, giving the name of the file containing the gene set collection. Optionally an object that can be coerced into a two-column data.frame, the first column containing genes and the second gene sets, representing all &quot;gene&quot;-to-&quot;gene set&quot; connections.</td>
</tr>
<tr>
<td>type</td>
<td>a character string giving the file type. Can be either of &quot;gmt&quot;, &quot;sbml&quot;, &quot;sif&quot;. If set to &quot;auto&quot; the type will be taken from the file extension. If the gene-set collection is loaded into R from another source and stored in a data.frame, it can be loaded with the setting &quot;data.frame&quot;.</td>
</tr>
<tr>
<td>addInfo</td>
<td>an optional data.frame with two columns, the first containing the gene set names and the second containing additional information for each gene set. Some additional info may load automatically from the different file types.</td>
</tr>
</tbody>
</table>
Details

This function is used to create a gene-set collection object to be used with runGSA.

The "gmt" files available from the Molecular Signatures Database (http://www.broadinstitute.org/gsea/msigdb/) can be loaded using loadGSC. This website is a valuable resource and contains several different collections of gene sets.

By using the functionality of e.g. the biomaRt package, a gene-set collection with custom gene names (matching the statistics used in runGSA) can easily be compiled into a two-column data.frame (column order: genes, gene sets) and loaded with type="data.frame".

If a sif-file is used it is assumed that the first column contains gene sets and the third column contains genes.

A genome-scale metabolic model in SBML format can be used to define gene sets. In this case, metabolites will be the gene sets, containing all the genes that code for enzymes catalyzing reactions in which the metabolite takes part in. In order to load an SBML-file it is required that libSBML and rsbml is installed. Note that the SBML loading is an experimental feature and is highly dependent on the version and format of the SBML file and requires it to contain gene associations for the reactions. By examining the returned GSC object it is easy to see if the correct gene sets were loaded.

Value

A list like object of class GSC containing two elements. The first is gsc, a list of the gene sets, each element a character vector of genes. The second element is addInfo, a data.frame containing the optional additional information.

Author(s)

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See Also

piano, runGSA

Examples

# Randomly generated gene sets:
g <- sort(paste("g",floor(runif(100)*500+1),sep=""))
g <- c(g,sort(paste("g",floor(runif(900)*1000+1),sep="")))
g <- c(g,sort(paste("g",floor(runif(1000)*2000+1),sep="")))
s <- paste("s",floor(rbeta(2000,0.9,1.7)*50+1),sep="")

# Make data.frame:
gsc <- cbind(g,s)

# Load gene set collection from data.frame:
gsc <- loadGSC(gsc)
loadMAdata  

Load and preprocess microarray data

Description

Loads, preprocesses and annotates microarray data to be further used by downstream functions in the piano package.

Usage

loadMAdata(datadir = getwd(), setup = "setup.txt", dataNorm, platform = "NULL", annotation, normalization = "plier", filter = TRUE, verbose = TRUE, ...)

Arguments

datadir character string giving the directory in which to look for the data. Defaults to getwd().

setup character string giving the name of the file containing the experimental setup, or an object of class data.frame or similar containing the experimental setup. Defaults to "setup.txt", see details below for more information.

dataNorm character string giving the name of the normalized data, or an object of class data.frame or similar containing the normalized data. Only to be used if the user wishes to start with normalized data rather then CEL files.

platform character string giving the name of the platform, can be either "yeast2" or NULL. See details below for more information.

annotation character string giving the name of the annotation file, or an object of class data.frame or similar containing the annotation information. The annotation should consist of the columns Gene name, Chromosome and Chromosome location. Not required if platform="yeast2".

normalization character string giving the normalization method, can be either "plier", "rma" or "mas5". Defaults to "PLIER".

filter should the data be filtered? If TRUE then probes not present in the annotation will be discarded. Defaults to TRUE.

verbose verbose? Defaults to TRUE.

... additional arguments to be passed to ReadAffy.

Details

This function requires at least two inputs: (1) data, either CEL files in the directory specified by datadir or normalized data specified by dataNorm, and (2) experimental setup specified by setup.

The setup should be either a tab delimited text file with column headers or a data.frame. The first column should contain the names of the CEL files or the column names used for the normalized data, please be sure to use names valid as column names, e.g. avoid names starting with numbers. Additional columns should assign attributes in some category to each array. (For an example run the example below and look at the object myArrayData$setup.)

The piano package is customized for yeast 2.0 arrays and annotation will work automatically, if the cdfName of the arrays equals Yeast_2. If using normalized yeast 2.0 data as input, the user
needs to set the argument `platform="yeast2"` to tell the function to use yeast annotation. If other platforms than yeast 2.0 is used, set `platform=NULL` (default) and supply appropriate annotation by the argument annotation. Note that the cdfName will override platform, so it can still be set to NULL for yeast 2.0 CEL files. Note also that annotation overrides platform, so if the user wants to use an alternative annotation for yeast, this can be done simply by specifying this in annotation.

The annotation should have the column headers `Gene name`, `Chromosome` and `Chromosome location`. The `Gene name` is used in the heatmap in `diffExp` and the `Chromosome and Chromosome location` is used by the `polarPlot`. The rownames (or first column if using a text file) should contain the `probe IDs`. If using a text file the first column should have the header `probeID` or similar. The filtering step discards all probes not listed in the annotation.

Normalization is performed on all CEL file data using one of the Affymetrix methods: PLIER ("plier") as implemented by `justPlier`, RMA (Robust Multi-Array Average) ("rma") expression measure as implemented by `rma` or MAS 5.0 expression measure "mas5" as implemented by `mas5`.

It is possible to pass additional arguments to `ReadAffy`, e.g. `cdfname` as this might be required for some types of CEL files.

### Value

An `ArrayData` object (which is essentially a `list`) with the following elements:

- `dataRaw` raw data as an `AffyBatch` object
- `dataNorm` data.frame containing normalized expression values
- `setup` data.frame containing experimental setup
- `annotation` data.frame containing annotation

Depending on input arguments the `ArrayData` object may not include `dataRaw` and/or `annotation`.

### Author(s)

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### References


### See Also

- `piano`, `runQC`, `diffExp`, `ReadAffy`, `expresso`, `justPlier`, `yeast2.db`

### Examples

```r
# Get path to example data and setup files:
dataPath <- system.file("extdata", package="piano")

# Load normalized data:
myArrayData <- loadMAdata(datadir=dataPath, dataNorm="norm_data.txt.gz", platform="yeast2")

# Print to look at details:
myArrayData
```
networkPlot

Gene set network plot

Description

Draws a network with gene sets as nodes and the thickness of the edges correlating to the number of shared genes. The gene set significance is visualized as color intensities. Gives an overview of the influence of overlap on significant gene sets.

Usage

networkPlot(gsaRes, class, direction, adjusted=FALSE, significance=0.001, geneSets=NULL, overlap=1, lay=1, label="names", cexLabel=0.9, ncharLabel=25, cexLegend=1, nodeSize=c(10,40), edgeWidth=c(1,15), edgeColor=NULL, scoreColors=NULL, main)

Arguments

gsaRes an object of class GSAres, as returned from runGSA() or an object returned from runGSAhyper().
class a character string determining the p-values of which directionality class that should be used as significance information for the plot. Can be one of "distinct", "mixed", "non". Has to be "non" if the result from runGSAhyper() is used.
direction a character string giving the direction of regulation, can be either "up", "down" or "both" (for pValue="distinct" only).
adjusted a logical, if adjusted p-values should be used, or not. Note that if runGSA was run with the argument adjMethod="none", the adjusted p-values will be equal to the original p-values.
significance the significance cut-off that determines which gene sets are included in the plot. Defaults to 0.001.
geneSets a character vector of gene set names, to be included in the plot. Defaults to NULL, but if given, the argument significance will not be used.
overlap a positive numerical. Determines the smallest number of sharing genes between two gene-sets that is needed in order to draw a line/edge between the gene-sets. Defaults to 1.
lay a numerical between 1-5, or a layout function (see layout in the igraph package). 1-5 sets the layout to one of the five default layout for the network plot.
label a character string, either "names","numbers","numbersAndSizes" or "namesAndSizes", determining the labels used for the nodes. The names are the gene set names, numbers is an arbitrary numbered list of the gene sets used in the plot connected to the named list returned by the function. Sizes are the gene set sizes, e.g. the number of genes.
cexLabel the text size of the node labels.
ncharLabel the number of characters to include in the node labels.
cexLegend the text size of the legend.
nodeSize a numerical vector of length 2 giving the maximum and minimum node sizes. The node size represents the size of the gene set, and all values will be scaled to the given interval.
networkPlot

edgeWidth  a numerical vector of length 2 giving the maximum and minimum edge widths. The edge width represents the number of shared genes between two gene sets, and all values will be scaled to the given interval.

decimalColor  a character vector giving the colors to use for increasing edge width. Can also be set to a single color. Defaults to a gray-scale.

scoreColors  a character vector giving the colors from which the gradient used for node coloring will be created. In the case of pValue="distinct" and direction="both" the first half of the vector will be used for the up-regulated gene sets and the second part will be used for the down-regulated gene sets.

main  an optional character vector setting the title of the plot.

Details

In the case of pValue="distinct" and direction="both", the distinct directional p-values (pDistinctDirUp and pDistinctDirDn, see runGSA) will be used in combination. Using the geneSets and lay arguments, multiple comparative plots (i.e. with the same layout) can be drawn, based for instance on the output gene set list from other network plots with different directionality classes.

Value

Returns a list with two components: geneSets containing the names and numbers of the gene sets in the plot, and layout, containing the saved layout of the plot, which can be passed back to the lay argument in order to draw a subsequent plot with the same layout.

Author(s)

Leif Varemo <piano.rpkg@gmail.com> and Intawat Nookaew <piano.rpkg@gmail.com>

See Also

piano, runGSA, GSAheatmap, layout

Examples

# Load example input data to GSA:
data("gsa_input")

# Load gene set collection:
gsc <- loadGSC(gsa_input$gsc)

# Run gene set analysis:
gsares <- runGSA(geneLevelStats=gsa_input$pvals, directions=gsa_input$directions,
gsc=gsc, nPerm=500)

# Network plot:
networkPlot(gsares,class="non",significance=0.01)

# Use circular layout and save the layout:

# Use the saved layout to overlay the distinct-directional p-values for easy comparison.
# Note that the gene sets are now not selected based on a significance cutoff, but from a list:
networkPlot(gsares,class="distinct",direction="both",lay=nw$layout,geneSets=nw$geneSets)
polarPlot

polarPlot

Description

Produces a Polar plot, mapping p-values to chromosome location. This function is used by `diffExp`.

Usage

```r
polarPlot(pValues, chromosomeMapping, 
    colors = c("red", "green", "blue", "yellow", 
              "orange", "purple", "tan", "cyan", "gray60", "black"), 
    save = FALSE, verbose = TRUE)
```

Arguments

- `pValues`: a data.frame containing p-values for different contrasts in different columns. Column names are used as contrast names. Maximum number of columns allowed are ten.
- `chromosomeMapping`: character string giving the name of the chromosome mapping file, or an object of class data.frame or similar containing the chromosome mapping. See details below.
- `colors`: character vector of colors to be used by the Polar plot.
- `save`: should the figures be saved? Defaults to `FALSE`.
- `verbose`: verbose? Defaults to `TRUE`.

Details

This function is mainly used by `diffExp` but can also be used separately by the user.

The argument `chromosomeMapping` should be either a data.frame or a tab delimited text file and include the columns `chromosome` with the chromosome name and `chromosome location` containing the starting position of each gene. A - sign can be used to denote the antisense strand but this will be disregarded while plotting. The rownames should be `probe IDs` or, if using a text file, the first column with a column header should contain the `probe IDs`. If relying on an ArrayData object (called `arrayData`) and containing an annotation field, the `chromosomeMapping` can be set to `arrayData$annotation[,c(2,3)]` (see the example below).

The Polar plot sorts the genes according to chromosomal location, for each chromosome starting with unknown positions followed by increasing number in the `chromosome location` column. Genes which do not map to any chromosome are listed as `U` for unknown. The radial lines in the Polar plot are -log10 scaled p-values, so that a longer line means a smaller p-value. This gives an overview of the magnitude of differential expression for each contrast.

Author(s)

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See Also

`piano`, `diffExp`, `radial.plot`
Examples

```r
# Get path to example data and setup files:
dataPath <- system.file("extdata", package="piano")

# Load normalized data:
myArrayData <- loadMAdata(datadir=dataPath, dataNorm="norm_data.txt.gz", platform="yeast2")

# Perform differential expression analysis:
pfc <- diffExp(myArrayData, plot=FALSE, contrasts=c("aerobic_Clim - anaerobic_Clim", "aerobic_Nlim - anaerobic_Nlim"))

# Get chromosome mapping from myArrayData:
chrMap <- myArrayData$annotation[,c(2,3)]
# Get p-values from pfc
pval <- pfc$pValues
# Draw the polar plot:
polarPlot(pval, chromosomeMapping=chrMap)
```

runGSA

Gene set analysis

Description

Performs gene set analysis (GSA) based on a given number of gene-level statistics and a gene set collection, using a variety of available methods, returning the gene set statistics and p-values of different directionality classes.

Usage

```r
runGSA(geneLevelStats, directions=NULL, geneSetStat="mean", signifMethod="geneSampling", adjMethod="fdr", gsc, gsSizeLim=c(1,Inf), permStats=NULL, permDirections=NULL, nPerm=1e4, gseaParam=1, ncpus=1, verbose=TRUE)
```

Arguments

geneLevelStats a vector or a one-column data.frame or matrix, containing the gene level statistics. Gene level statistics can be e.g. p-values, t-values or F-values.
directions a vector or a one-column data.frame or matrix, containing fold-change like values for the related gene-level statistics. This is mainly used if statistics are p-values or F-values, but not required. The values should be positive or negative, but only the sign information will be used, so the actual value will not matter.
geneSetStat  the statistical GSA method to use. Can be one of "fisher", "stouffer", "reporter", "tailStrength", "wilcoxon", "mean", "median", "sum", "maxmean", "gsea", "fgsea" or "page". See below for details.

signifMethod  the method for significance assessment of gene sets, i.e. p-value calculation. Can be one of "geneSampling", "samplePermutation" or "nullDist".

adjMethod  the method for adjusting for multiple testing. Can be any of the methods supported by p.adjust, i.e. "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr" or "none". The exception is for geneSetStat="gsea", where only the options "fdr" and "none" can be used.

gsc  a gene set collection given as an object of class GSC as returned by the loadGSC function.

gsSizeLim  a vector of length two, giving the minimum and maximum gene set size (number of member genes) to be kept for the analysis. Defaults to c(1,Inf).

permStats  a matrix with permutated gene-level statistics (columns) for each gene (rows). This should be calculated by the user by randomizing the sample labels in the original data, and recalculating the gene level statistics for each comparison a large number of times, thus generating a vector (rows in the matrix) of background statistics for each gene. This argument is required and only used if signifMethod="samplePermutation".

permDirections  similar to permStats, but should instead contain fold-change like values for the related permuted statistics. This is mainly used if the statistics are p-values or F-values, but not required. The values should be positive or negative, but only the sign information will be used, so the actual value will not matter. This argument is only used if signifMethod="samplePermutation", but not required. Note however, that if directions is give then also permDirections is required, and vice versa.

nPerm  the number of permutations to use for gene sampling, i.e. if signifMethod="geneSampling". The original Reporter features algorithm (geneSetStat="reporter" and signifMethod="nullDist") also uses a permutation step which is controlled by nPerm.

gseaParam  the exponent parameter of the GSEA and FGSEA approach. This defaults to 1, as recommended by the GSEA authors.

ncpus  the number of cpus to use. If larger than 1, the gene permutation part will be run in parallel and thus decrease runtime. Requires R package snowfall to be installed. Should be set so that nPerm/ncpus is a positive integer. (Not used by FGSEA.)

verbose  a logical. Whether or not to display progress messages during the analysis.

Details

The rownames of geneLevelStats and directions should be identical and match the names of the members of the gene sets in gsc. If geneSetStat is set to "fisher", "stouffer", "reporter" or "tailStrength" only p-values are allowed as geneLevelStats. If geneSetStat is set to "maxmean", "gsea", "fgsea" or "page" only t-like geneLevelStats are allowed (e.g. t-values, fold-changes).

For geneSetStat set to "fisher", "stouffer", "reporter", "wilcoxon" or "page", the gene set p-values can be calculated from a theoretical null-distribution, in this case, set signifMethod="nullDist".

For all methods signifMethod="geneSampling" or signifMethod="samplePermutation" can be used, except for "fgsea" where only signifMethod="geneSampling" is allowed. If signifMethod="geneSampling" gene sampling is used, meaning that the gene labels are randomized nPerm times and the gene
set statistics are recalculated so that a background distribution for each original gene set is acquired. The gene set p-values are calculated based on this background distribution. Similarly, if `signifMethod="samplePermutation"` sample permutation is used. In this case the argument `permStats` (and optionally `permDirections`) has to be supplied.

The `runGSA` function returns p-values for each gene set. Depending on the choice of methods and gene statistics up to three classes of p-values can be calculated, describing different aspects of regulation directionality. The three directionality classes are Distinct-directional, Mixed-directional and Non-directional. The non-directional p-values (pNonDirectional) are calculated based on absolute values of the gene statistics (or p-values without sign information), meaning that gene sets containing a high portion of significant genes, independent of direction, will turn up significant. That is, gene-sets with a low pNonDirectional should be interpreted to be significantly affected by gene regulation, but there can be a mix of both up and down regulation involved. The mixed-directional p-values (pMixedDirUp and pMixedDirDn) are calculated using the subset of the gene statistics that are up-regulated and down-regulated, respectively. This means that a gene set with a low pMixedDirUp will have a component of significantly up-regulated genes, regardless of the extent of down-regulated genes, and the reverse for pMixedDirDn. This also means that one can get gene sets that are both significantly affected by down-regulation and significantly affected by up-regulation at the same time. Note that sample permutation cannot be used to calculate pMixedDirUp and pMixedDirDn since the subset sizes will differ. Finally, the distinct-directional p-values (pDistinctDirUp and pDistinctDirDn) are calculated from statistics with sign information (e.g. t-statistics). In this case, if a gene set contains both up- and down-regulated genes, they will cancel out each other. A gene-set with a low pDistinctDirUp will be significantly affected by up-regulation, but not a mix of up- and down-regulation (as in the case of the mixed-directional and non-directional p-values). In order to be able to calculate distinct-directional gene set p-values while using p-values as gene-level statistics, the gene-level p-values are transformed as follows: The up-regulated portion of the p-values are divided by 2 (scaled to range between 0-0.5) and the down-regulated portion of p-values are set to 1-p/2 (scaled to range between 1-0.5). This means that a significantly down-regulated gene will get a p-value close to 1. These new p-values are used as input to the gene-set analysis procedure to get pDistinctDirUp. Similarly, the opposite is done, so that the up-regulated portion is scaled between 1-0.5 and the down-regulated between 0-0.5 to get the pDistinctDirDn.

Value

A list-like object of class `GSAres` containing the following elements:

- `geneStatType`: The interpreted type of gene-level statistics
- `geneSetStat`: The method for gene set statistic calculation
- `signifMethod`: The method for significance estimation
- `adjMethod`: The method of adjustment for multiple testing
- `info`: A list object with detailed info number of genes and gene sets
- `gsSizeLim`: The selected gene set size limits
- `gsStatName`: The name of the gene set statistic type
- `nPerm`: The number of permutations
- `gseaParam`: The GSEA parameter
- `geneLevelStats`: The input gene-level statistics
- `directions`: The input directions
- `gsc`: The input gene set collection
- `nGenesTot`: The total number of genes in each gene set
nGenesUp  The number of up-regulated genes in each gene set
nGenesDn  The number of down-regulated genes in each gene set
statDistinctDir  Gene set statistics of the distinct-directional class
statDistinctDirUp  Gene set statistics of the distinct-directional class
statDistinctDirDn  Gene set statistics of the distinct-directional class
statNonDirectional  Gene set statistics of the non-directional class
statMixedDirUp  Gene set statistics of the mixed-directional class
statMixedDirDn  Gene set statistics of the mixed-directional class
pDistinctDirUp  Gene set p-values of the distinct-directional class
pDistinctDirDn  Gene set p-values of the distinct-directional class
pNonDirectional  Gene set p-values of the non-directional class
pMixedDirUp  Gene set p-values of the mixed-directional class
pMixedDirDn  Gene set p-values of the mixed-directional class
pAdjDistinctDirUp  Adjusted gene set p-values of the distinct-directional class
pAdjDistinctDirDn  Adjusted gene set p-values of the distinct-directional class
pAdjNonDirectional  Adjusted gene set p-values of the non-directional class
pAdjMixedDirUp  Adjusted gene set p-values of the mixed-directional class
pAdjMixedDirDn  Adjusted gene set p-values of the mixed-directional class
runtime  The execution time in seconds

Author(s)
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References
runGSAhyper


See Also
piano, loadGSC, GSAsummaryTable, geneSetSummary, networkPlot, HTSanalyzeR-package, PGSEA, samr, limma, GSA, fgsea

Examples

# Load example input data to GSA:
data("gsa_input")

# Load gene set collection:
gsc <- loadGSC(gsa_input$gsc)

# Run gene set analysis:
gsares <- runGSA(genes=geneLevelStats=gsa_input$pvals , directions=gsa_input$directions, gsc=gsc, nPerm=500)

runGSAhyper

Gene set analysis with Fisher’s exact test

Description
Performs gene set analysis (GSA) based on a list of significant genes and a gene set collection, using Fisher’s exact test, returning the gene set p-values.

Usage
runGSAhyper(genes, pvalues, pcutoff, universe, gsc, gsSizeLim=c(1,Inf), adjMethod="fdr")

Arguments
genes a vector of all genes in your experiment, or a small list of significant genes.
pvalues a vector (or object to be coerced into one) of pvalues for genes or a binary vector with 0 for significant genes. Defaults to rep(0,length(genes)), i.e. genes is a vector of genes of interest.
pcutoff p-value cutoff for significant genes. Defaults to 0 if pvalues are binary. If p-values are spread in [0,1] defaults to 0.05.
universe a vector of genes that represent the universe. Defaults to genes if pvalues are not all 0. If pvalues are all 0, defaults to all unique genes in gsc.
The statistical test performed is a one-tailed Fisher's exact test on the contingency table with columns "In gene set" and "Not in gene set" and rows "Significant" and "Non-significant" (this is equivalent to a hypergeometric test).

Command run for gene set i:

\[
\text{fisher.test(res$contingencyTable[[i]], alternative="greater")},
\]

the res$contingencyTable object is available from the object returned from runGSAhyper.

The main difference between runGSA and runGSAhyper is that runGSA uses the gene-level statistics (numerical values for each gene) to calculate the gene set p-values, whereas runGSAhyper only uses the group membership of each gene (in/not in gene set, significant/non-significant). This means that for runGSAhyper a p-value cut-off for determining significant genes has to be chosen by the user and after this, all significant genes will be seen as equally significant (i.e. the actual p-values are not used). The advantage with runGSAhyper is that you can use it to find enriched gene sets when you only have a list of interesting genes, without any statistics.

### Value

A list-like object containing the following elements:

- **pvalues** a vector of gene set p-values
- **p.adj** a vector of gene set p-values, adjusted for multiple testing
- **resTab** a full result table
- **contingencyTable** a list of the contingency tables used for each gene set
- **gsc** the input gene set collection

### Author(s)

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### See Also

- **piano**
- **loadGSC**
- **runGSA**
- **fisher.test**
- **phyper**
- **networkPlot**

### Examples

```r
# Load example input data (dummy p-values and gene set collection):
data("gsa_input")

# Load gene set collection:
gsc <- loadGSC(gsa_input$gsc)
```
# Randomly select 100 genes of interest (as an example):
genes <- sample(unique(gsa_input$gs[,1]),100)

# Run gene set analysis using Fisher's exact test:
res <- runGSAhyper(genes, gsc=gsc)

# If you have p-values for the genes and want to make a cutoff for significance:
genes <- names(gsa_input$pvals) # All gene names
p <- gsa_input$pvals # p-values for all genes
res <- runGSAhyper(genes, p, pcutoff=0.001, gsc=gsc)

# If the 20 first genes are the interesting/significant ones they can be selected
# with a binary vector:
significant <- c(rep(0,20),rep(1,length(genes)-20))
res <- runGSAhyper(genes, significant, gsc=gsc)

---

runQC

**Run quality control**

**Description**

Performs a set of quality control methods and produces the results as figures.

**Usage**

```r
runQC(arrayData, rnaDeg = TRUE, nuseRle = TRUE, hist = TRUE,
      boxplot = TRUE, pca = TRUE, colorFactor = 1,
      colors = c("red", "green", "blue", "yellow", "orange",
                 "purple", "tan", "cyan", "gray60", "black", "white"),
      save = FALSE, verbose = TRUE)
```

**Arguments**

- `arrayData` - an object of class `ArrayData`.
- `rnaDeg` - should RNA degradation be detected? Defaults to `TRUE`.
- `nuseRle` - should Normalized Unscaled Standard Errors (NUSE) and Relative Log Expressions (RLE) be calculated? Defaults to `TRUE`.
- `hist` - produce histograms of expression values? Defaults to `TRUE`.
- `boxplot` - produce boxplots of expression values? Defaults to `TRUE`.
- `pca` - should PCA be run? Defaults to `TRUE`.
- `colorFactor` - a number specifying which column of the setup (given by the `ArrayData` object) should be used for coloring information for the PCA. Defaults to 1.
- `colors` - a character vector of colors to be used in the PCA plot.
- `save` - should the figures be saved? Defaults to `FALSE`.
- `verbose` - verbose? Defaults to `TRUE`.
Details
This function is essentially a wrapper for various available quality control functions for \texttt{AffyBatch} objects and normalized microarray data. RNA degradation (\texttt{rnaDeg=TRUE}) and NUSE & RLE (\texttt{nuseRle=TRUE}) require raw data (a \texttt{dataRaw} element in the \texttt{ArrayData} object).
The PCA uses \texttt{prcomp} on centralized normalized data.
Typical usages are:
\begin{verbatim}
# Run all quality controls:
runQC(arrayData)
\end{verbatim}

Author(s)
Leif Varemo \texttt{<piano.rpkg@gmail.com>} and Intawat Nookaew \texttt{<piano.rpkg@gmail.com>}

References

See Also
\texttt{piano}, \texttt{loadMAdata}, \texttt{diffExp}, \texttt{AffyRNAdeg}, \texttt{fitPLM}, \texttt{AffyBatch}, \texttt{prcomp}

Examples
\begin{verbatim}
# Get path to example data and setup files:
dataPath <- system.file("extdata", package="piano")

# Load normalized data:
myArrayData <- loadMAdata(datadir=dataPath, dataNorm="norm_data.txt.gz", platform="yeast2")

# Run PCA only:
runQC(myArrayData, rnaDeg=FALSE, nuseRle=FALSE, hist=FALSE, boxplot=FALSE)
\end{verbatim}

writeFilesForKiwi

\texttt{Write files for Kiwi}

Description
Given a single object or a list of objects of class \texttt{GSAres}, extract the information needed for visualization in the external python function \texttt{Kiwi} and write it to files that can be used as input.

Usage
\begin{verbatim}
writeFilesForKiwi(gsaRes, label="", overwrite=FALSE)
\end{verbatim}

Arguments
\begin{itemize}
\item \texttt{gsaRes} \hspace{1cm} either an object of class \texttt{GSAres} or a list where each element is an object of class \texttt{GSAres}, as returned by the \texttt{runGSA} function.
\item \texttt{label} \hspace{1cm} a character string that will be appended to the names of the resulting files.
\item \texttt{overwrite} \hspace{1cm} a logical, whether or not to overwrite existing files with identical names.
\end{itemize}
writeFilesForKiwi

Details
This function takes the result from a gene set analysis as returned by the runGSA function and writes three files that can be directly used as input to Kiwi. Kiwi is an external function in Python that can be used for network-based visualization of the GSA results (http://sysbio.se/kiwi).

Value
Three files are written in the current directory. GSC.txt contains the gene-gene set associations, i.e. the gene set collection. GLS.txt contains the gene-level statistics. GSS.txt contains the gene set statistics.

Author(s)
Leif Varemo <piano.rpkg@gmail.com>

See Also
piano, runGSA, networkPlot

Examples

```r
# Load some example GSA results:
data(gsa_results)

# Write the files:
writeFilesForKiwi(gsa_results, "exp1")
```
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