

Package ‘ToPASeq’

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Title Topology-based pathway analysis of RNA-seq data

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Description Implementation of methods for topology-based pathway analysis of RNA-seq data. This includes Topological Analysis of Pathway Phenotype Association (TAPPA; Gao and Wang, 2007), PathWay Enrichment Analysis (PWEA; Hung et al., 2010), and the Pathway Regulation Score (PRS; Ibrahim et al., 2012).

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CePa

Centrality-based Pathway enrichment (CePa)

Description

The function runs CePa method on microarray or RNA-Seq data. The implementation includes the identification of differentially expressed genes and transformation of pathways' topologies to an appropriate form. Only the ORA version of the CePa method is implemented and covers centralities: equal-weight, in-degree, out-degree, in-reach, out-reach and betweenness.

Usage

```
CePa(x, group, pathways, type, which = "proteins", edgeType = NULL,
     preparePaths = TRUE, norm.method = NULL, test.method = NULL,
     p.th = 0.05, logFC.th = 2, nperm = 1000, both.directions = TRUE,
     maxNodes = 150, minEdges = 0, commonTh = 2, filterSPIA = FALSE,
     convertTo = "none", convertBy = NULL)
```

Arguments

| | |
|--------------|--|
| x | An ExpressionSet object or a gene expression data matrix or count matrix, rows refer to genes, columns to samples |
| group | Name or number of the phenoData column or a character vector or factor that contains required class assignments |
| pathways | A list of pathways in a form from graphite package or created by preparePathways() |
| type | Type of the input data, "MA" for microarray and "RNASeq" for RNA-Seq |
| which | Character, which type of nodes is preserved in a pathway. Possible values are "proteins", "metabolites", "mixed" |
| edgeType | Character, which type of edges is preserved in a pathway. If NULL, all edges are kept. |
| preparePaths | Logical, by default the pathways are transformed with preparePathways(). Use FALSE, if you have done this transformation separately |
| norm.method | Character, the method to normalize RNAseq data. If NULL then vst-normalization is performed. Possible values are: "edgeR", "vst", "rLog", "none" |

| | |
|---|--|
| test.method | Character, the method for differential expression analysis of RNAseq data. If NULL then "voomlimma" is used. Possible values are: "DESeq2", "voomlimma", "vstlimma", "edgeR" |
| p.th | Numeric, threshold for p-values of tests for differential expression of genes. Use 1 if you don't want any threshold to be applied |
| logFC.th | Numeric, threshold for log fold-change of a gene to identify the gene as differentially expressed. Use negative if you don't want any threshold to be applied |
| nperm | Numeric, number of permutations |
| both.directions, maxNodes, minEdges, commonTh, filterSPIA, convertTo, convertBy | Arguments for the preparePathways() |

Value

A list:

| | |
|----------|---|
| res | A matrix, each row refers to one pathway, each column to one centrality and the value is a p-value. |
| topo.sig | A list of weights for genes (nodes) in individual pathways |
| degtest | A numeric vector of gene-level differential expression statistics of all genes in the dataset |

Author(s)

Ivana Ihnatova

References

Gu Z., Liu J., Cao K., Zhang J., Wang J.: Centrality-based pathway enrichment: a systematic approach for finding significant pathways dominated by key genes. *BMC Systems Biology* 2012, 6:56

Examples

```
if (require(breastCancerVDX)) {
  data("vdx")
  pathways<-pathways("hsapiens", "biocarta")[1:3]
  MAdata<-Biobase::exprs(vdx)[,1:10]
  rownames(MAdata)<-Biobase::fData(vdx)[,"Gene.symbol"]
  MAdata<-MAdata[!duplicated(rownames(MAdata)),]

  CePa(MAdata, Biobase::pData(vdx)[,"er"][1:10], pathways, type="MA", convertTo="SYMBOL")
}
```

clipper

clipper

Description

clipper is a method for topological gene set analysis. It implements a two-step empirical approach based on the exploitation of graph decomposition into a junction tree to reconstruct the most relevant signal path. In the first step clipper selects significant pathways according to statistical tests on the means and the concentration matrices of the graphs derived from pathway topologies. Then, it "clips" the whole pathway identifying the signal paths having the greatest association with a specific phenotype.

Usage

```
clipper(x, group, pathways, type, which = "proteins", edgeType = NULL,
        preparePaths = TRUE, norm.method = NULL, test.method = NULL,
        method = "mean", testCliques = FALSE, nperm = 1000,
        alphaV = 0.05, both.directions = TRUE, maxNodes = 150,
        minEdges = 0, commonTh = 2, filterSPIA = FALSE,
        convertTo = "none", convertBy = NULL)
```

Arguments

| | |
|---|---|
| x | An ExpressionSet object or a gene expression data matrix or count matrix, rows refer to genes, columns to samples |
| group | Name or number of the phenoData column or a character vector or factor that contains required class assignments |
| pathways | A list of pathways in a form from graphite package or created by preparePathways() |
| type | Type of the input data, "MA" for microarray and "RNASeq" for RNA-Seq |
| which | Character, which type of nodes is preserved in a pathway. Possible values are "proteins", "metabolites", "mixed" |
| edgeType | Character, which type of edges is preserved in a pathway. If NULL, all edges are kept. |
| preparePaths | Logical, by default the pathways are transformed with preparePathways(). Use FALSE, if you have done this transformation separately |
| norm.method | Character, the method to normalize RNAseq data. If NULL then vst-normalization is performed. Possible values are: "edgeR", "vst", "rLog", "none" |
| test.method | Character, the method for differential expression analysis of RNAseq data. If NULL then "voomlimma" is used. Possible values are: "DESeq2", "voomlimma", "vstlimma", "edgeR". This analysis is needed only for the visualization. |
| method | Character, "mean" or "var", the kind of test to perform on the cliques |
| testCliques | Logical, if TRUE then the test is applied also on the cliques of the each pathway. It is a very time consuming calculation, especially for many or big pathways |
| nperm | Number of permutations, if 0 then asymptotic distribution is used. May not be valid when shrinked estimator is used. |
| alphaV | Numeric, the threshold for variance test. The calculation of mean test depends on the result of variance test. |
| both.directions, maxNodes, minEdges, commonTh, filterSPIA, convertTo, convertBy | Arguments for the preparePathways() |

Value

A list:

res

A list. First slot is a data frame containing p-values and q-values of mean and variance tests on pathways. The second slot is a list containing data.frames of the most affected paths in each pathway. The columns of the data frames contain: 1 - Index of the starting clique 2 - Index of the ending clique 3 - Index of the clique where the maximum value is reached 4 - length of the path 5 - maximum score of the path 6 - average score along the path 7 - percentage of path activation 8 - impact of the path on the entire pathway 9 - clique involved and significant 10 - clique forming the path 11 - genes forming the significant cliques 12 - genes forming the path

topo.sig if testCliques=TRUE, a list where each slot contains the pvalues and a list of cliques in one pathway. NULL otherwise

degtest A data.frame of gene-level differential expression statistics

Author(s)

Ivana Ihnatova

References

Martini P, Sales G, Massa MS, Chiogna M, Romualdi C. Along signal paths: an empirical gene set approach exploiting pathway topology. *Nucleic Acids Res.* 2013 Jan 7;41(1):e19. doi: 10.1093/nar/gks866. Epub 2012 Sep 21. PubMed PMID: 23002139; PubMed Central PMCID: PMC3592432.

Examples

```
## Not run:
if (require(breastCancerVDX)) {
  data("vdx")
  pathways<-pathways("hsapiens","biocarta")[1:3]
  MAdata<-Biobase::exprs(vdx)[,1:10]
  rownames(MAdata)<-Biobase::fData(vdx)[,"Gene.symbol"]
  MAdata<-MAdata[!duplicated(rownames(MAdata)),]

  clipper(MAdata, Biobase::pData(vdx)[,"er"][1:10], pathways, type="MA", convertTo="SYMBOL", nperm=10)
}

## End(Not run)
```

convertIdentifiersByVector

Convert pathway identifiers

Description

This function converts identifiers of nodes in a pathway. It uses the user specified named vector for the conversion.

Usage

```
convertIdentifiersByVector(pathway, conv.table)
```

Arguments

pathway An object of class Pathway

conv.table A data.frame, in which the first column contains the type and the identifiers present in the pathway separated by : and the second column contains the new identifiers and the third columns contains the types of the new identifiers

Value

A Pathway with new identifiers of the nodes

Author(s)

Ivana Ihnatova

See Also[Pathway-class](#)**Examples**

```
g<-pathways("hsapiens", "kegg")
ng<-sapply(g, function(x) length(nodes(x, "mixed")))
g<-g[[which.min(ng)]]
conv<-data.frame(orig=nodes(g, "mixed"), new=LETTERS[seq_len(min(ng))], newtype=rep("LETTERS", min(ng)))
gc<-convertIdentifiersByVector(g, conv.table = conv)@protEdges
```

DEGraph

*Differential Expression of Graph (DEGraph)***Description**

DEGraph implements recent hypothesis testing methods which directly assess whether a particular gene network is differentially expressed between two conditions. It employs Graph Laplacian, Fourier transformation and multivariate T2-statistic

Usage

```
DEGraph(x, group, pathways, type, which = "proteins", edgeType = NULL,
        preparePaths = TRUE, norm.method = NULL, test.method = NULL,
        overall = "biggest", useInteractionSigns = TRUE, EdgeAttrs = NULL,
        both.directions = TRUE, maxNodes = 150, minEdges = 0,
        commonTh = 2, filterSPIA = FALSE, convertTo = "none",
        convertBy = NULL)
```

Arguments

| | |
|--------------|--|
| x | An ExpressionSet object or a gene expression data matrix or count matrix, rows refer to genes, columns to samples |
| group | Name or number of the phenoData column or a character vector or factor that contains required class assignments |
| pathways | A list of pathways in a form from graphite package or created by preparePathways() |
| type | Type of the input data, "MA" for microarray and "RNASeq" for RNA-Seq |
| which | Character, which type of nodes is preserved in a pathway. Possible values are "proteins", "metabolites", "mixed" |
| edgeType | Character, which type of edges is preserved in a pathway. If NULL, all edges are kept. |
| preparePaths | Logical, by default the pathways are transformed with preparePathways(). Use FALSE, if you have done this transformation separately |
| norm.method | Character, the method to normalize RNAseq data. If NULL then vst-normalization is performed. Possible values are: "edgeR", "vst", "rLog", "none" |

| | |
|--|---|
| <code>test.method</code> | Character, the method for differential expression analysis of RNAseq data. If NULL then "voomlimma" is used. Possible values are: "DESeq2", "voomlimma", "vstlimma", "edgeR". This analysis is needed only for the visualization. |
| <code>overall</code> | Character, how should the overall p-value for a pathway be calculated. The possible values are: "mean", "min", "biggest". "biggest" returns the p-value of the biggest connected component. |
| <code>useInteractionSigns</code> | Logical, should types of interaction be included in the analysis? |
| <code>EdgeAttrs</code> | A list containing two data.frames. See <code>edgeData</code> for the details. The interactions are assigned signs according to the beta column of the second data.frame. |
| <code>both.directions</code> , <code>maxNodes</code> , <code>minEdges</code> , <code>commonTh</code> , <code>filterSPIA</code> , <code>convertTo</code> , <code>convertBy</code> | Arguments for the <code>preparePathways()</code> |

Value

A list:

| | |
|-----------------------|---|
| <code>res</code> | Results from analysis of individual pathways. The first column refers to the overall p-value for a pathway. Then groups of four columns follows. One group refers to one connected component and contains a pair of p-values (without and with Fourier transformation), graph and number of Fourier components used in the test. The number of groups is equal to the highest number of components in analysed pathways. Components are sorted in the decreasing order of their nodes number. |
| <code>topo.sig</code> | NULL, present for the compatibility with outputs from other methods |
| <code>degtest</code> | A data.frame of gene-level statistics of all genes in the dataset |

A list:

Author(s)

Ivana Ihnatova

References

L. Jacob, P. Neuvial, and S. Dudoit. Gains in power from structured two-sample tests of means on graphs. Technical Report arXiv:q-bio/1009.5173v1, arXiv, 2010.

Examples

```
if (require(breastCancerVDX)) {
  data("vdx")
  pathways<-pathways("hsapiens", "biocarta")[1:3]
  MAdata<-Biobase::exprs(vdx)[,1:10]
  rownames(MAdata)<-Biobase::fData(vdx)[,"Gene.symbol"]
  MAdata<-MAdata[!duplicated(rownames(MAdata)),]

  DEGraph(MAdata, Biobase::pData(vdx)[,"er"][1:10], pathways, type="MA", convertTo="SYMBOL")
}
```

prs *Pathway regulation score (PRS)*

Description

This function implements the PRS method to analyze pathway enrichment of gene expression data. For PRS, a gene weight correspond to the number of downstream differentially expressed genes.

Usage

```
prs(de, all, pwys, nperm = 1000)
```

```
prsWeights(pwy, de, all)
```

Arguments

| | |
|-------|---|
| de | A named numeric vector containing log2 fold-changes of the differentially expressed genes. Recommended names are Entrez gene IDs. |
| all | A character vector with the gene IDs in the reference set. If the data was obtained from a gene expression experiment, this set will contain all genes measured in the experiment. This vector should contain *all* names of the de argument. |
| pwys | A linkS4class{PathwayList} containing the pathways that should be analyzed for enrichment. |
| nperm | Integer. Number of permutations. |
| pwy | A linkS4class{Pathway} for which the weights should be computed. |

Value

A data.frame with normalized score and p-value for each pathway analyzed.

Author(s)

Ivana Ihnatova

References

Ibrahim et al. (2012) A topology-based score for pathway enrichment. J Comput Biol, 19(5):563-73.

See Also

[pathways](#)

Examples

```
# pathways
library(graphite)
pwys <- pathways("hsapiens", "kegg")[1:10]

# expression data
```



```

all <- nodes(pwys[[1]])
nds <- sample(all, 30)
de <- setNames(rnorm(30), nds)

# executing PRS
prsWeights(pwys[[1]], de, all)
prs(de, all, pwys, nperm=100)

```

PRs_wrapper

*Pathway Regulation Score (PRS)***Description**

A function runs PRS method on a gene expression data matrix or count matrix and vector dividing samples into two groups and a set of pathways from graphite package. The PRS method (please see Reference for the details) was adapted to graphite's graphs where each node is represented only by one gene.

Usage

```

PRs_wrapper(x, group, pathways, type, which = "proteins",
  edgeType = NULL, preparePaths = TRUE, norm.method = NULL,
  test.method = NULL, p.th = 0.05, logFC.th = 2, nperm = 1000,
  both.directions = TRUE, maxNodes = 150, minEdges = 0,
  commonTh = 2, filterSPIA = FALSE, convertTo = "none",
  convertBy = NULL)

```

Arguments

| | |
|--------------|---|
| x | An ExpressionSet object or a gene expression data matrix or count matrix, rows refer to genes, columns to samples |
| group | Name or number of the phenoData column or a character vector or factor that contains required class assignments |
| pathways | A list of pathways in a form from graphite package or created by preparePathways() |
| type | Type of the input data, "MA" for microarray and "RNASeq" for RNA-Seq |
| which | Character, which type of nodes is preserved in a pathway. Possible values are "proteins", "metabolites", "mixed" |
| edgeType | Character, which type of edges is preserved in a pathway. If NULL, all edges are kept. |
| preparePaths | Logical, by default the pathways are transformed with preparePathways(). Use FALSE, if you have done this transformation separately |
| norm.method | Character, the method to normalize RNAseq data. If NULL then vst-normalization is performed. Possible values are: "edgeR", "vst", "rLog", "none" |
| test.method | Character, the method for differential expression analysis of RNAseq data. If NULL then "voomlimma" is used. Possible values are: "DESeq2", "voomlimma", "vstlimma", "edgeR". This analysis is needed only for the visualization. |
| p.th | Numeric, threshold for p-values of tests for differential expression of genes. Use 1 if you don't want any threshold to be applied |

logFC.th Numeric, threshold for log fold-change of a gene to identify the gene as differentially expressed. Use negative if you don't want any threshold to be applied

nperm Numeric, number of permutations

both.directions, maxNodes, minEdges, commonTh, filterSPIA, convertTo, convertBy
Arguments for the preparePathways()

Value

A list:

res A data frame with normalized score, p-value and FDR-adjusted p-value for each pathway

topo.sig A list with log fold-changes and number of downstream differentially expressed nodes for nodes of individual pathways

degtest A named vector of statistics from testing the differential expression of genes

Author(s)

Ivana Ihnatova

References

Maysson Al-Haj Ibrahim, Sabah Jassim, Michael Anthony Cawthorne, and Kenneth Langlands. A Topology-Based Score for Pathway Enrichment, *Journal of Computational Biology*. May 2012, 19(5): 563-573

Examples

```
if (require(breastCancerVDX)) {
  data("vdx")
  pathways<-pathways("hsapiens","biocarta")[1:3]
  MAdata<-Biobase::exprs(vdx)[,1:10]
  rownames(MAdata)<-Biobase::fData(vdx)[,"Gene.symbol"]
  MAdata<-MAdata[!duplicated(rownames(MAdata)),]

  PRS_wrapper(MAdata, Biobase::pData(vdx)[,"er"][1:10], pathways, type="MA", convertTo="SYMBOL", logFC.th=-1, n
}
```

Description

The function runs PWEA method (please see References for the details) on gene expression data matrix, vector specifying to which group a sample belongs and a list of pathway graphs. Briefly, it is a weighted GSEA-like method. The weightes are based on the distance and Pearson's correlation between genes in a pathway.

Usage

```
PWEA(x, group, pathways, type, which = "proteins", edgeType = NULL,
      preparePaths = TRUE, norm.method = NULL, test.method = NULL,
      tif = NULL, alpha = 0.05, nperm = 1000, ncores = 1,
      both.directions = TRUE, maxNodes = 150, minEdges = 0,
      commonTh = 2, filterSPIA = FALSE, convertTo = "none",
      convertBy = NULL)
```

Arguments

| | |
|---|---|
| x | An ExpressionSet object or a gene expression data matrix or count matrix, rows refer to genes, columns to samples |
| group | Name or number of the phenoData column or a character vector or factor that contains required class assignments |
| pathways | A list of pathways in a form from graphite package or created by preparePathways() |
| type | Type of the input data, "MA" for microarray and "RNASeq" for RNA-Seq |
| which | Character, which type of nodes is preserved in a pathway. Possible values are "proteins", "metabolites", "mixed" |
| edgeType | Character, which type of edges is preserved in a pathway. If NULL, all edges are kept. |
| preparePaths | Logical, by default the pathways are transformed with preparePathways(). Use FALSE, if you have done this transformation separately |
| norm.method | Character, the method to normalize RNAseq data. If NULL then vst-normalization is performed. Possible values are: "edgeR", "vst", "rLog", "none" |
| test.method | Character, the method for differentially expression analysis of RNAseq data. If NULL then "voomlimma" is used. Possible values are: "DESeq2", "voomlimma", "vstlimma", "edgeR". This analysis is needed only for the visualization. |
| tif | A list of Topology Influence Factor's. One slot refers to one pathway. Use prepareTIF() to create it. It is required only if type=="DEtable" |
| alpha | Numeric, a threshold value used during TIF calculation |
| nperm | Numeric, number of permutations. Used only if x %in% c("MA", "RNASeq") |
| ncores | Numeric, number of cores. Used only if x %in% c("MA", "RNASeq"). The permutations are calculated in parallel way |
| both.directions, maxNodes, minEdges, commonTh, filterSPIA, convertTo, convertBy | Arguments for the preparePathways() |

Value

A list:

| | |
|----------|---|
| res | A data frame, rows refer to pathways. It contains: Enrichment score for a pathway, p-value and p-value adjusted for multiple hypothesis testing by Benjamini-Hochberg's FDR method. NA's if less than 2 nodes are present in the data |
| topo.sig | A list, topology influence factors for the genes in individual pathways. NULL if less than 2 nodes are present in the data |
| degtest | A named vector of statistics from testing the differential expression |

Author(s)

Ivana Ihnatova

References

Hung, JH., Whitfield, T. W., Yang, TH., Hu, Z., Weng, Z., DeLisi, Ch. (2010) Identification of functional modules that correlate with phenotypic difference: the influence of network topology, *Genome Biology*, 11:R23

Examples

```
## Not run:
if (require(breastCancerVDX)) {
  data("vdx")
  pathways<-pathways("hsapiens","biocarta")[1:3]
  MAdata<-Biobase::exprs(vdx)[,1:10]
  rownames(MAdata)<-Biobase::fData(vdx)[,"Gene.symbol"]
  MAdata<-MAdata[!duplicated(rownames(MAdata)),]

  PWEA(MAdata, Biobase::pData(vdx)[,"er"][1:10], pathways, type="MA", convertTo="SYMBOL", nperm=10)
}

## End(Not run)
```

res

*Functions to extract and display results***Description**

Functions to extract and display results

Usage

```
res(object)

## S3 method for class 'topResult'
res(object)

## S3 method for class 'topResult'
topo.sig(object)

## S3 method for class 'topResult'
degtable(object)
```

Arguments

object an output from one of following function "SPIA", "PRS", "CePA", "PWEA", "TAPPA", "TopologyGSA"
 ... other arguments

Methods (by class)

- `topResult`: Extracts results of topology-based pathway analysis
- `topResult`: Extracts topological significance of genes
- `topResult`: Extracts results of differential expression analysis on genes

SPIA

*Signaling Pathway Impact Analysis (SPIA)***Description**

The function runs SPIA method on microarray or RNA-Seq data. The implementation includes the identification of differentially expressed genes and transformation of pathways' topologies to an appropriate form. The SPIA method combines two independent p-values. One p-value comes from overrepresentation analysis and the other is so called perturbation factor.

Usage

```
SPIA(x, group, pathways, type, which = "proteins", edgeType = NULL,
     preparePaths = TRUE, norm.method = NULL, test.method = NULL,
     p.th = 0.05, logFC.th = 2, nperm = 1000, combine = "fisher",
     both.directions = TRUE, maxNodes = 150, minEdges = 0,
     commonTh = 2, filterSPIA = FALSE, convertTo = "none",
     convertBy = NULL)
```

Arguments

| | |
|---------------------------|--|
| <code>x</code> | An <code>ExpressionSet</code> object or a gene expression data matrix or count matrix, rows refer to genes, columns to samples |
| <code>group</code> | Name or number of the <code>phenoData</code> column or a character vector or factor that contains required class assignments |
| <code>pathways</code> | A list of pathways in a form from <code>graphite</code> package or created by <code>preparePathways()</code> |
| <code>type</code> | Type of the input data, "MA" for microarray and "RNASeq" for RNA-Seq |
| <code>which</code> | Character, which type of nodes is preserved in a pathway. Possible values are "proteins", "metabolites", "mixed" |
| <code>edgeType</code> | Character, which type of edges is preserved in a pathway. If <code>NULL</code> , all edges are kept. |
| <code>preparePaths</code> | Logical, by default the pathways are transformed with <code>preparePathways()</code> . Use <code>FALSE</code> , if you have done this transformation separately |
| <code>norm.method</code> | Character, the method to normalize RNAseq data. If <code>NULL</code> then <code>vst</code> -normalization is performed. Possible values are: "edgeR", "vst", "rLog", "none" |
| <code>test.method</code> | Character, the method for differential expression analysis of RNAseq data. If <code>NULL</code> then "voomlimma" is used. Possible values are: "DESeq2", "voomlimma", "vstlimma", "edgeR". This analysis is needed only for the visualization. |
| <code>p.th</code> | Numeric, threshold for p-values of tests for differential expression of genes. Use 1 if you don't want any threshold to be applied |
| <code>logFC.th</code> | Numeric, threshold for log fold-change of a gene to identify the gene as differentially expressed. Use negative if you don't want any threshold to be applied |

nperm Numeric, number of permutations

combine Character, the method to combine p-values. Defaults to "fisher" for Fisher's method. The other possible value is "norminv" for the normal inversion method.

both.directions, maxNodes, minEdges, commonTh, filterSPIA, convertTo, convertBy
Arguments for the preparePathways()

Value

A list:

res A matrix with columns as described below: pSize - Pathway size, number of genes, NDE - Number of differentially expressed genes, pNDE - P-value of the overrepresentation part of the method, tA - The observed total perturbation accumulation in the pathway, pPERT - P-value of the perturbation part of the method, p - Combined p-value (overrepresentation and perturbation), pFdr - False discovery rate adjusted p, pFWER - FWER adjusted p, Status - If a pathway was identified as Activated or Inhibited

topo.sig A list of accumulated perturbation factors and log fold-changes for genes in individual pathways

degtest A numeric vector of gene-level differential expression statistics of all genes in the dataset

Author(s)

Ivana Ilnatova

References

Tarca AL, Draghici S, Khatri P, Hassan SS, Mittal P, Kim JS, Kim CJ, Kusanovic JP, Romero R. A novel signaling pathway impact analysis. *Bioinformatics*. 2009 Jan 1;25(1):75-82.

Adi L. Tarca, Sorin Draghici, Purvesh Khatri, et. al, A Signaling Pathway Impact Analysis for Microarray Experiments, 2008, *Bioinformatics*, 2009, 25(1):75-82.

Draghici, S., Khatri, P., Tarca, A.L., Amin, K., Done, A., Voichita, C., Georgescu, C., Romero, R.: A systems biology approach for pathway level analysis. *Genome Research*, 17, 2007. Massa MS, Chiogna M, Romualdi C. Gene set analysis exploiting the topology of a pathway. *BMC System Biol*. 2010 Sep 1;4:121.

Examples

```
if (require(breastCancerVDX)) {
  data("vdx")
  pathways<-pathways("hsapiens", "biocarta")[1:3]
  MAdata<-Biobase::exprs(vdx)[,1:10]
  rownames(MAdata)<-Biobase::fData(vdx)[, "Gene.symbol"]
  MAdata<-MAdata[!duplicated(rownames(MAdata)),]

  SPIA(MAdata, Biobase::pData(vdx)[,"er"][1:10], pathways, type="MA", convertTo="SYMBOL", logFC.th=-1)
}
```

Description

The functions analyses the differential expression of pathways via TAPPA method. Expression is compared between two groups of samples by Mann-Whitney test. P-values are later adjusted for multiple hypothesis testing by Benjamini-Hochberg's FDR method.

Usage

```
TAPPA(x, group, pathways, type, which = "proteins", edgeType = NULL,
      preparePaths = TRUE, norm.method = NULL, test.method = NULL,
      test = t.test, normalize = TRUE, verbose = FALSE,
      both.directions = TRUE, maxNodes = 150, minEdges = 0,
      commonTh = 2, filterSPIA = FALSE, convertTo = "none",
      convertBy = NULL)
```

Arguments

| | |
|---|---|
| x | An ExpressionSet object or a gene expression data matrix or count matrix, rows refer to genes, columns to samples |
| group | Name or number of the phenoData column or a character vector or factor that contains required class assignments |
| pathways | A list of pathways in a form from graphite package or created by preparePathways() |
| type | Type of the input data, "MA" for microarray and "RNASeq" for RNA-Seq |
| which | Character, which type of nodes is preserved in a pathway. Possible values are "proteins", "metabolites", "mixed" |
| edgeType | Character, which type of edges is preserved in a pathway. If NULL, all edges are kept. |
| preparePaths | Logical, by default the pathways are transformed with preparePathways(). Use FALSE, if you have done this transformation separately |
| norm.method | Character, the method to normalize RNAseq data. If NULL then vst-normalization is performed. Possible values are: "edgeR", "vst", "rLog", "none" |
| test.method | Character, the method for differentially expression analysis of RNAseq data. If NULL then "voomlimma" is used. Possible values are: "DESeq2", "voomlimma", "vstlimma", "edgeR". This analysis is needed only for the visualization. |
| test | Function implementing a statistical test comparing PCI scores between groups. It is employed as test(PCI~group)\$p.value, where PCI is a numeric vector of the same length as group |
| normalize | Logical, should data be normalized? |
| verbose | Logical, if TRUE names of the pathways are printed as they are analysed |
| both.directions, maxNodes, minEdges, commonTh, filterSPIA, convertTo, convertBy | Arguments for the preparePathways() |

Value

| | |
|----------|--|
| | A list, |
| res | A data frame, rows refer to pathways. Columns contain: number of valid PCI-scores, median, min and max of the PCI scores for each group of samples, p-value of the test (p.val) and adjusted p-value (p.adj). If less than two nodes are present in the data, the function puts NA's in all columns. |
| topo.sig | NULL, it is preserved for the compatibility with other methods implemented in this package |
| degtest | A numeric vector of gene-level differential expression statistics |

Author(s)

Ivana Ilnatova

References

Gao, S. and Wang, X. (2007) TAPPA: topological analysis of pathway phenotype association. *Bioinformatics*, 23, pages 3100-3102

Examples

```

if (require(breastCancerVDX)) {
  data("vdx")
  pathways<-pathways("hsapiens","biocarta")[1:10]
  MAdata<-Biobase::exprs(vdx)[,1:10]
  rownames(MAdata)<-Biobase::fData(vdx)[,"Gene.symbol"]
  MAdata<-MAdata[!duplicated(rownames(MAdata)),]

  TAPPA(MAdata, Biobase::pData(vdx)[,"er"][1:10], pathways, type="MA", convertTo="SYMBOL")
}

```

TopologyGSA

Gene set analysis exploiting the topology of a pathway (TopologyGSA)

Description

TopologyGSA method uses graphical models to test the differential expression of a pathway. It also highlights pathway components involved in the deregulation.

Usage

```

TopologyGSA(x, group, pathways, type, which = "proteins",
  edgeType = NULL, preparePaths = TRUE, norm.method = NULL,
  test.method = NULL, method = "mean", nperm = 1000, alpha = 0.05,
  testCliques = FALSE, both.directions = TRUE, maxNodes = 150,
  minEdges = 0, commonTh = 2, filterSPIA = FALSE,
  convertTo = "none", convertBy = NULL)

```


Arguments

| | |
|---|---|
| x | An ExpressionSet object or a gene expression data matrix or count matrix, rows refer to genes, columns to samples |
| group | Name or number of the phenoData column or a character vector or factor that contains required class assignments |
| pathways | A list of pathways in a form from graphite package or created by preparePathways() |
| type | Type of the input data, "MA" for microarray and "RNASeq" for RNA-Seq |
| which | Character, which type of nodes is preserved in a pathway. Possible values are "proteins", "metabolites", "mixed" |
| edgeType | Character, which type of edges is preserved in a pathway. If NULL, all edges are kept. |
| preparePaths | Logical, by default the pathways are transformed with preparePathways(). Use FALSE, if you have done this transformation separately |
| norm.method | Character, the method to normalize RNAseq data. If NULL then vst-normalization is performed. Possible values are: "edgeR", "vst", "rLog", "none" |
| test.method | Character, the method for differential expression analysis of RNAseq data. If NULL then "voomlimma" is used. Possible values are: "DESeq2", "voomlimma", "vstlimma", "edgeR". This analysis is needed only for the visualization. |
| method | Either "var" and "mean". Determine the type of test used by topologyGSA. |
| nperm | Numeric, number of permutations. |
| alpha | Numeric, threshold for statistical significance of variance test. It influences the method for the mean test |
| testCliques | Logical, if TRUE, then the test is also performed on individual cliques. It can be very computationally complex. |
| both.directions, maxNodes, minEdges, commonTh, filterSPIA, convertTo, convertBy | Arguments for the preparePathways() |

Details

The method requires a Directed Acyclic Graph (DAG). Therefore if a pathway contain also undirected or bidirected edges and error is thrown.

The user can further specify for the mean test:

1. **perms** number of permutations of the test,
2. **paired** logical, if TRUE Hotelling test for paired samples is calculated and the test on the variances is not performed

Or for the variance test:

1. **variance** logical, if TRUE the estimates of the covariance matrices are included in the result.
2. **s1** First group covariance matrix estimation.
3. **s2** Second group covariance matrix estimation.

Value

A list

| | |
|----------|---|
| res | a list with one entry for each successfully analyzed pathway |
| topo.sig | if testCliques=TRUE, a list where each slot contains the pvalues and a list of cliques in one pathway. NULL otherwise |
| degtest | A numeric vector of gene-level differential expression statistics |

Author(s)

Ivana Ihnatova

References

Massa MS, Chiogna M, Romualdi C. Gene set analysis exploiting the topology of a pathway. *BMC System Biol.* 2010 Sep 1;4:121.

Examples

```
## Not run:
if (require(breastCancerVDX)) {
  data("vdx")
  pathways<-pathways("hsapiens","biocarta")[1:3]
  MAdata<-Biobase::exprs(vdx)[,1:10]
  rownames(MAdata)<-Biobase::fData(vdx)[,"Gene.symbol"]
  MAdata<-MAdata[!duplicated(rownames(MAdata)),]

  TopologyGSA(MAdata, Biobase::pData(vdx)[,"er"][1:10], pathways, type="MA", convertTo="SYMBOL", nperm=10)
}

## End(Not run)
```

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