Package 'clipper'

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Title Gene set analysis exploiting pathway topology

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Description clipper is a package 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.

Depends R (>= 2.15.0), Matrix, graph

Imports methods, Biobase, Rcpp, igraph, gRbase (>= 1.6.6), qpgraph, KEGGgraph, corpcor, RBGL

Suggests RUnit, BiocGenerics, RCytoscape (>= 1.6.3), graphite, ALL, hgu95av2.db, MASS, BiocStyle

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clipper

Dissect the pathway to find the path with the greatest association with phenotype.

Description

Basing on either variance or mean clique test, this function identifies the paths that are mostly related with the phenotype under study.

Usage

```
clipper(expr, classes, graph, method=c("variance","mean", "both",
    "paired"), nperm=100, alphaV=0.05, b=100, root=NULL, trZero=0.001, signThr=0.05,
    maxGap=1, permute=TRUE)
```

Arguments

expr	an expression matrix or ExpressionSet with colnames for samples and row name for genes.
classes	vector of 1,2 indicating the classes of samples (columns).
graph	a graphNEL object.
method	the kind of test to perform on the cliques. It could be mean, variance, mixed (the best between variance and mean) or paired mean.
nperm	number of permutations. Default = 100 .
alphaV	pvalue threshold for variance test to be used during mean test. Default = 0.05 .
b	number of permutations for mean analysis. Default = 100.
root	nodes by which rip ordering is performed (as far as possible) on the variables using the maximum cardinality search algorithm.
trZero	lowest pvalue detectable. This threshold avoids that -log(p) goes infinite.
signThr	significance threshold for clique pvalues.
maxGap	allow up to maxGap gaps in the best path computation. Default = 1 .
permute	always performs permutations in the concentration matrix test. If FALSE, the test is made using the asymptotic distribution of the log-likelihood ratio. This option should be use only if samples size is $>=40$ per class.

clipper

Details

The both method combines the results obtained from the mean and variance test. In particular it assign to the cliques the minimum of mean and variance p-values.

Value

A matrix with a row for each paths. Columns are organized as follows:

- 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. Cliques involved and significant
- 10. Cliques forming the path
- 11. Genes forming the significant cliques
- 12. Genes forming the path

References

Martini P, Sales G, Massa MS, Chiogna M, Romualdi C. Along signal paths: an empirical gene set approach exploiting pathway topology. NAR. 2012 Sep.

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

See Also

cliqueVarianceTest, cliqueMeanTest, getJunctionTreePaths

```
if (require(graphite) & require(ALL)){
  graph <- pathwayGraph(convertIdentifiers(kegg$Chronic myeloid leukemia, "entrez"))
  genes <- nodes(graph)
  data(ALL)
  all <- ALL[1:length(genes),1:20]
  classes <- c(rep(1,10), rep(2,10))
  featureNames(all@assayData)<- genes
  graph <- subGraph(genes, graph)
  clipped <- clipper(all, classes, graph, "var", trZero=0.01, permute=FALSE)
  clipped[,1:5]
}</pre>
```

clipperAllRoots

Description

Basing on either variance or mean clique test, this function identifies the paths that are mostly related with the phenotype under study.

Usage

```
clipperAllRoots(expr, classes, graph, method=c("variance","mean",
"both", "paired"), nperm=100, alphaV=0.05, b=100, trZero=0.001, signThr=0.05,
maxGap=1, permute=TRUE)
```

Arguments

expr	an expression matrix or ExpressionSet with colnames for samples and row name for genes.
classes	vector of 1,2 indicating the classes of samples (columns).
graph	a graphNEL object.
method	the kind of test to perform on the cliques. It could be mean, variance, mixed (the best between variance and mean) or paired mean.
nperm	number of permutations. $Default = 100$.
alphaV	pvalue threshold for variance test to be used during mean test. Default = 0.05 .
b	number of permutations for mean analysis. Default = 100.
trZero	lowest pvalue detectable. This threshold avoids that -log(p) goes infinite.
signThr	significance threshold for clique pvalues.
maxGap	allow up to maxGap gaps in the best path computation. Default = 1 .
permute	always performs permutations in the concentration matrix test. If FALSE, the test is made using the asymptotic distribution of the log-likelihood ratio. This option should be use only if samples size is $>=40$ per class.

Details

The both method combines the results obtained from the mean and variance test. In particular it assign to the cliques the minimum of mean and variance p-values.

Value

A matrix with a row for each paths. Rownames have the form:

roots-paths.

Columns are organized as follows:

clipperAllRoots

- 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. Cliques involved and significant
- 10. Cliques forming the path
- 11. Genes forming the significant cliques
- 12. Genes forming the path

References

Martini P, Sales G, Massa MS, Chiogna M, Romualdi C. Along signal paths: an empirical gene set approach exploiting pathway topology. NAR. 2012 Sep.

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

See Also

cliqueVarianceTest, cliqueMeanTest, getJunctionTreePaths

```
if (require(graphite) & require(ALL)){
  graph <- pathwayGraph(convertIdentifiers(kegg$Chronic myeloid leukemia, "entrez"))
  genes <- nodes(graph)
  data(ALL)
  all <- ALL[1:length(genes),1:20]
   classes <- c(rep(1,10), rep(2,10))
  featureNames(all@assayData)<- genes
  graph <- subGraph(genes, graph)
   clipped <- clipperAllRoots(all, classes, graph, "var", trZero=0.01, permute=FALSE)
   clipped[,1:5]
}</pre>
```

cliqueMeanTest

Description

It decomposes the graph in cliques and performs the mean test in every one.

Usage

```
cliqueMeanTest(expr, classes, graph, nperm, alphaV=0.05, b=100,
root=NULL, permute=TRUE, alwaysShrink=FALSE)
```

Arguments

expr	an expression matrix or ExpressionSet with colnames for samples and row name for genes.
classes	vector of 1,2 indicating the classes of samples (columns).
graph	a graphNEL object.
nperm	number of permutations.
alphaV	pvalue threshold for variance test to be used during mean test.
b	number of permutations for mean analysis.
root	nodes by which rip ordering is performed (as far as possible) on the variables using the maximum cardinality search algorithm.
permute	always performs permutations in the concentration matrix test. If FALSE, the test is made using the asymptotic distribution of the log-likelihood ratio. This option should be use only if samples size is $>=40$ per class.
alwaysShrink	always perform the shrinkage estimates of variance.

Value

a list with alphas (vector of cliques pvalues based on the mean test) and cliques (list of the cliques and related elements).

References

Martini P, Sales G, Massa MS, Chiogna M, Romualdi C. Along signal paths: an empirical gene set approach exploiting pathway topology. NAR. 2012 Sep.

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

See Also

cliqueVarianceTest.

cliqueMixedTest

Examples

```
if (require(graphite) & require(ALL)){
  graph <- pathwayGraph(convertIdentifiers(kegg$Chronic myeloid leukemia, "entrez"))
  genes <- nodes(graph)
  data(ALL)
  all <- ALL[1:length(genes),1:20]
  classes <- c(rep(1,10), rep(2,10))
  featureNames(all@assayData)<- genes
  graph <- subGraph(genes, graph)
  cliqueMeanTest(all, classes, graph, nperm=100, permute=FALSE)$alpha
}</pre>
```

cliqueMixedTest Mean test for cliques.

Description

It decomposes the graph in cliques and performs the combination of mean e variance test in every one.

Usage

```
cliqueMixedTest(expr, classes, graph, nperm, alphaV=0.05, b=100,
root=NULL, permute=TRUE)
```

Arguments

expr	an expression matrix or ExpressionSet with colnames for samples and row name for genes.
classes	vector of 1,2 indicating the classes of samples (columns).
graph	a graphNEL object.
nperm	number of permutations.
alphaV	pvalue threshold for variance test to be used during mean test.
b	number of permutations for mean analysis.
root	nodes by which rip ordering is performed (as far as possible) on the variables using the maximum cardinality search algorithm.
permute	always performs permutations in the concentration matrix test. If FALSE, the test is made using the asymptotic distribution of the log-likelihood ratio. This option should be use only if samples size is $>=40$ per class.

Details

The method combines the results obtained from the mean and variance test. In particular it assign to the cliques the minimum of mean and variance p-values.

Value

a list with alphas (vector of cliques pvalues based on the variance test) and cliques (list of the cliques and related elements).

References

Martini P, Sales G, Massa MS, Chiogna M, Romualdi C. Along signal paths: an empirical gene set approach exploiting pathway topology. NAR. 2012 Sep.

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

See Also

cliqueVarianceTest.

Examples

```
if (require(graphite) & require(ALL)){
  graph <- pathwayGraph(convertIdentifiers(kegg$Chronic myeloid leukemia, "entrez"))
  genes <- nodes(graph)
  data(ALL)
  all <- ALL[1:length(genes),1:20]
   classes <- c(rep(1,10), rep(2,10))
  featureNames(all@assayData)<- genes
  graph <- subGraph(genes, graph)
   cliqueMeanTest(all, classes, graph, nperm=100, permute=FALSE)$alpha
}</pre>
```

cliquePairedTest *Paired mean test for cliques.*

Description

It decomposes the graph in cliques and performs the paired mean test in every one.

Usage

```
cliquePairedTest(expr, classes, graph, nperm, alphaV=0.05, b=100,
root=NULL, permute=TRUE, alwaysShrink=FALSE)
```

Arguments

expr	an expression matrix or ExpressionSet with colnames for samples and row name for genes.
classes	vector of 1,2 indicating the classes of samples (columns). It is assumed that class labels are ordered so that the first occurrence of class 2 is paired with the first occurrence of class 1 and so on.

cliquePairedTest

graph	a graphNEL object.
nperm	number of permutations.
alphaV	pvalue threshold for variance test to be used during mean test.
b	number of permutations for mean analysis.
root	nodes by which rip ordering is performed (as far as possible) on the variables using the maximum cardinality search algorithm.
permute	always performs permutations in the concentration matrix test. If FALSE, the test is made using the asymptotic distribution of the log-likelihood ratio. This option should be use only if samples size is $>=40$ per class.
alwaysShrink	always perform the shrinkage estimates of variance.

Value

a list with alphas (vector of cliques pvalues based on the variance test) and cliques (list of the cliques and related elements).

References

Martini P, Sales G, Massa MS, Chiogna M, Romualdi C. Along signal paths: an empirical gene set approach exploiting pathway topology. NAR. 2012 Sep.

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

See Also

cliqueVarianceTest.

```
if (require(graphite) & require(ALL)){
  graph <- pathwayGraph(convertIdentifiers(kegg$Chronic myeloid leukemia, "entrez"))
  genes <- nodes(graph)
  data(ALL)
  all <- ALL[1:length(genes),1:20]
   classes <- c(rep(1,10), rep(2,10))
  featureNames(all@assayData)<- genes
  graph <- subGraph(genes, graph)
   cliquePairedTest(all, classes, graph, nperm=100, permute=FALSE)$alpha
}</pre>
```

cliqueVarianceTest Variance test for cliques.

Description

It decomposes the graph in cliques and performs the variance test in every one.

Usage

```
cliqueVarianceTest(expr, classes, graph, nperm, alphaV=0.05,
b=100, root=NULL, permute=TRUE, alwaysShrink=FALSE)
```

Arguments

expr	an expression matrix or ExpressionSet with colnames for samples and row name for genes.
classes	vector of 1,2 indicating the classes of samples (columns).
graph	a graphNEL object.
nperm	number of permutations.
alphaV	pvalue threshold for variance test to be used during mean test.
b	number of permutations for mean analysis.
root	nodes by which rip ordering is performed (as far as possible) on the variables using the maximum cardinality search algorithm.
permute	always performs permutations in the concentration matrix test. If FALSE, the test is made using the asymptotic distribution of the log-likelihood ratio. This option should be use only if samples size is $>=40$ per class.
alwaysShrink	always perform the shrinkage estimates of variance.

Value

a list with alphas (vector of cliques pvalues based on the variance test) and cliques (list of the cliques and related elements).

References

Martini P, Sales G, Massa MS, Chiogna M, Romualdi C. Along signal paths: an empirical gene set approach exploiting pathway topology. NAR. 2012 Sep.

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

See Also

cliqueMeanTest.

deleteEdge

Examples

```
if (require(graphite) & require(ALL)){
  graph <- pathwayGraph(convertIdentifiers(kegg$Chronic myeloid leukemia, "entrez"))
  genes <- nodes(graph)
  data(ALL)
  all <- ALL[1:length(genes),1:20]
  classes <- c(rep(1,10), rep(2,10))
  featureNames(all@assayData)<- genes
  graph <- subGraph(genes, graph)
  cliqueVarianceTest(all, classes, graph, nperm=100, permute=FALSE)$alpha
}</pre>
```

deleteEdge Remove

Remove an edge from graphNel object.

Description

Remove from a graphNEL object the edge specified.

Usage

deleteEdge(graph, from, to)

Arguments

graph	a graphNEL object.
from	a string with the name of the node where the edge start.
to	a string with the name of the node where the edge end.

Value

a graphNEL object.

```
if (require(graphite)) {
  graph <- pathwayGraph(convertIdentifiers(kegg$Chronic myeloid leukemia, "entrez"))
  head(edges(graph))
  ## We are going to remove the edge 1026-1019
  head(edges(deleteEdge(graph, "1026", "1019")))
}</pre>
```

easyClip

Description

Easy clip function allows the full exploitation of Clipper Package features in a unique and easy to use function. Starting from an expression matrix and a pathway, these function extact the most transcriptionally altered portions of the graph.

Usage

```
easyClip(expr, classes, graph, method=c("variance", "mean"), pathThr=0.05, pruneLevel=0.2, nperm=100, a
```

Arguments

expr	an expression matrix or ExpressionSet with colnames for samples and row name for genes.
classes	vector of 1,2 indicating the classes of samples (columns).
graph	a graphNEL object.
method	the kind of test to perform on the cliques. It could be either mean or variance.
pathThr	The significance threshold of the whole pathway test. Deafault = 0.05
pruneLevel	a dissimilarity threshold. NULL means no pruning.
nperm	number of permutations. $Default = 100$.
alphaV	pvalue threshold for variance test to be used during mean test. Default = 0.05 .
b	number of permutations for mean analysis. Default = 100.
root	nodes by which rip ordering is performed (as far as possible) on the variables using the maximum cardinality search algorithm.
trZero	lowest pvalue detectable. This threshold avoids that -log(p) goes infinite.
signThr	significance threshold for clique pvalues.
maxGap	allow up to maxGap gaps in the best path computation. Default = 1 .
permute	always performs permutations in the concentration matrix test. If FALSE, the test is made using the asymptotic distribution of the log-likelihood ratio. This option should be use only if samples size is $>=40$ per class.

Value

a matrix with row as the different paths. Columns are organized as follwes: 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)

easyLook

References

Martini P, Sales G, Massa MS, Chiogna M, Romualdi C. Along signal paths: an empirical gene set approach exploiting pathway topology. NAR. 2012 Sep.

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

See Also

cliqueVarianceTest, cliqueMeanTest, getJunctionTreePaths

Examples

```
if (require(graphite) & require(ALL)){
  graph <- pathwayGraph(convertIdentifiers(kegg$Chronic myeloid leukemia, "entrez"))
  genes <- nodes(graph)
  data(ALL)
  all <- ALL[1:length(genes),1:20]
  classes <- c(rep(1,10), rep(2,10))
  featureNames(all@assayData)<- genes
  graph <- subGraph(genes, graph)
  clipped <- easyClip(all, classes, graph, nperm=10)
  clipped[,1:5]
}</pre>
```

easyLook

Summarize clipper output.

Description

Summarization of the result for a quick look of clipper function.

Usage

easyLook(clipped)

Arguments

clipped the output of either clipper o easyClip.

Value

Nice formatted output of clipper function.

References

Martini P, Sales G, Massa MS, Chiogna M, Romualdi C. Along signal paths: an empirical gene set approach exploiting pathway topology. NAR. 2012 Sep.

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

getGraphEntryGenes *Extract all the possible entry point (genes with no entering edges) from graph.*

Description

It extracts the possible entry point of the graph. Entry points are defined as nodes with no entering edges.

Usage

```
getGraphEntryGenes(graph, byCliques=FALSE, root=NULL)
```

Arguments

graph	a graphNEL object.
byCliques	when TRUE it returns a list where entry point are organized by cliques.
root	nodes by which rip ordering is performed (as far as possible) on the variables using the maximum cardinality search algorithm.

Value

a vector of gene names representing the entry point of graph.

References

Martini P, Sales G, Massa MS, Chiogna M, Romualdi C. Along signal paths: an empirical gene set approach exploiting pathway topology. NAR. 2012 Sep.

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

```
if (require(graphite)) {
   graph <- pathwayGraph(convertIdentifiers(kegg$Chronic myeloid leukemia, "entrez"))
   getGraphEntryGenes(graph)
}</pre>
```

getJunctionTreePaths Extract the shortest paths along the junction tree of the graph.

Description

Find the shortest paths in the Junction tree designed with the cliques of the graph.

Usage

```
getJunctionTreePaths(graph, root=NULL)
```

Arguments

graph	a graphNEL object.
root	nodes by which rip ordering is performed (as far as possible) on the variables
	using the maximum cardinality search algorithm.

Value

list of clique indices representing the shortest paths of the graph.

References

Martini P, Sales G, Massa MS, Chiogna M, Romualdi C. Along signal paths: an empirical gene set approach exploiting pathway topology. NAR. 2012 Sep.

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(graphite)) {
   graph <- pathwayGraph(convertIdentifiers(kegg$Chronic myeloid leukemia, "entrez"))
   getJunctionTreePaths(graph)
}</pre>
```

Description

Starting from the sorted elements of each clique of the list, this function generates names fusing in a string the element names.

Usage

nameCliques(cliques)

Arguments

cliques a list where each element is a clique.

Value

vector of strings

Examples

```
toyCliques <- list(c(45,36,90), c(36,1000,35))
nameCliques(toyCliques)</pre>
```

pathwayTest Whole pathway test using qpipf.

Description

Performs variance and mean test using qpipf on the whole pathway.

Usage

```
pathQ(expr, classes, graph, nperm=100, alphaV=0.05, b=100,
permute=TRUE, paired=FALSE, alwaysShrink=FALSE)
```

Arguments

expr	an expression matrix or ExpressionSet with colnames for samples and rownames for expression features.
classes	vector of 1,2 indicating the classes of the samples (columns).
graph	a graphNEL object.
nperm	number of permutations. Default = 100 .
alphaV	pvalue significance threshold for variance test to be used during mean test. Default = 0.05 .
b	number of permutations for mean analysis. Default = 100.
permute	always performs permutations in the concentration matrix test. If FALSE, the test is made using the asymptotic distribution of the log-likelihood ratio. This option should be use only if samples size is $>=40$ per class.
paired	perform the test for paired sample. It assumes that class labels are ordered so that the first occurrence of class 2 is paired with the first occurrence of class 1 and so on.
alwaysShrink	always perform the shrinkage estimates of variance.

Value

a list with alphaVar (pvalue for the variance test) and alphaMean (pvalue for mean test).

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plotInCytoscape

Note

This function is based on the Gaussian Graphical Models and to use it in a proper way it is necessary that the graph is an Direct Acyclic Graph. Please check any graph in input using isAcyclic from ggm package.

References

Martini P, Sales G, Massa MS, Chiogna M, Romualdi C. Along signal paths: an empirical gene set approach exploiting pathway topology. NAR. 2012 Sep.

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(graphite) & require(ALL)){
  graph <- pathwayGraph(convertIdentifiers(kegg$Chronic myeloid leukemia, "entrez"))
  genes <- nodes(graph)
  data(ALL)
  all <- ALL[1:length(genes),1:20]
   classes <- c(rep(1,10), rep(2,10))
  featureNames(all@assayData)<- genes
  graph <- subGraph(genes, graph)
  pathQ(all, classes, graph, nperm=100, permute=FALSE)
}</pre>
```

plotInCytoscape	Plot a pathway graph in C	Cytoscape highlighting the relevant p	oath.
-----------------	---------------------------	---------------------------------------	-------

Description

Renders the topology of a pathway as a Cytoscape graph and marks the genes of the selected path.

Usage

```
plotInCytoscape(graph, path, color="#6699FF", main="graph", layout="jgraph-spring")
```

Arguments

graph	a graphNEL object.
path	vector summarizing a path (a rows of clipper output matrix).
color	color code string: genes of the most involved fragment will be colored using color. Deafult = "#6699FF"
main	a graph name to be used in Cytoscape. Default = 'graph'
layout	a 'string' of choice among the values returned by 'getLayoutNames', default = 'jgraph-spring'

prunePaths

Details

Requires the RCytoscape package.

See Also

clipper

Examples

```
## Not run: if (require(graphite)) {
    graph <- pathwayGraph(convertIdentifiers(kegg$Chronic myeloid leukemia, "entrez"))
}
path <- c(3,17,5,9,13.04,2.60,0.209,0.321,"6,7,8,9,10","3,5,6,7,8,9,10,14,17","1029;4193;7157","1019;1021;1026;
plotInCytoscape(graph,path)
## End(Not run)</pre>
```

```
prunePaths
```

Summarize the paths obtained by clipper according to their similarity.

Description

This function allows the user to chose only one representant of those paths that have more than 1-thr similarity. The best scoring path is choosen.

Usage

```
prunePaths(pathSummary, thr=NULL, clust=NULL, sep=";")
```

Arguments

pathSummary	a matrix resulting from clipper function.
thr	a dissimilarity threshold. NULL means no pruning.
clust	filename where path-cluster is saved. NULL means no cluster saved.
sep	the separator to split genes for similarity computation. Default = ;

Value

a matrix

See Also

clipper

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prunePaths

```
toyEx <- matrix(c(1,1,5,3,5,2,5,3,8.2,3,2,1,0.3,0.1,2,1,"1;2;3;4;5","1;2;3","1;2;3;4;5","1;2;3","1;2;3;4;5","1;
row.names(toyEx) <- c("1;5","1;3")
toyEx
prunePaths(toyEx, thr=0.1)
```

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