Package ‘multiWGCNA’

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**Description**

An R package for deepening mining gene co-expression networks in multi-trait expression data. Provides functions for analyzing, comparing, and visualizing WGCNA networks across conditions. multiWGCNA was designed to handle the common case where there are multiple biologically meaningful sample traits, such as disease vs wildtype across development or anatomical region.
**bidirectionalBestMatches**

**Author(s)**

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Authors:

- Brent Fogel [contributor]

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**bidirectionalBestMatches**

*Best matching modules*

---

**Description**

Find all the modules from dataset1 that have a best match to a module in dataset2 if that module in dataset2 is also a best match to the module in dataset1

**Usage**

```r
bidirectionalBestMatches(comparisonList, plot = TRUE)
```

**Arguments**

- `comparisonList` a list with an element "overlap", which is a data.frame resulting from a call to `computeOverlapsFromWGCNA`
- `plot` whether to generate a heatmap; default is TRUE

**Value**

A ggplot object

**Author(s)**

Dario Tommasini

**Examples**

```r
library(ExperimentHub)
eh = ExperimentHub()
eh_query = query(eh, c("multiWGCNAdata"))
astrocyte_networks = eh_query["EH8222"]
comparisonList = list()
comparisonList$overlaps = computeOverlapsFromWGCNA(astrocyte_networks$EAE, astrocyte_networks$WT)
bidirectionalBestMatches(comparisonList)
```
**cleanDatExpr**

**Description**
A function that converts a data.frame where row 1 is gene symbols to a numeric matrix where columns are genes and rows are samples for compatibility with most WGCNA functions.

**Usage**
cleanDatExpr(datExpr, checkGenesSamples = FALSE)

**Arguments**
datExpr  
a data.frame were columns are samples and rows are samples and the gene symbols are in the first row
cHECKGenesSamples  
call the WGCNA function checkGenesSamples?

**Value**
Returns a datExpr with rows as samples and columns as genes

**Author(s)**
Dario Tommasini

**Examples**
library(ExperimentHub)
eh = ExperimentHub()
eh_query = query(eh, c("multiWGCNAdata"))
astrocyte_se = eh_query[["EH8223"]]
datExpr = data.frame(X = rownames(assays(astrocyte_se)[[1]]), assays(astrocyte_se)[[1]])
cleanDatExpr(datExpr)

---

**coexpressionLineGraph**

**Description**
Plots a line graph showing the co-expression of selected genes across samples

**Usage**
coexpressionLineGraph(datExpr, splitBy = 1, fontSize = 2.15, colors = NULL)
computeOverlapsFromWGCNA

Arguments

- **datExpr**: a data.frame with genes as rows and samples as columns
- **splitBy**: how much to split genes by on line graph
- **fontSize**: the font size of the gene labels
- **colors**: a vector of colors; default is random colors generated by colors function

Value

- a ggplot object

Author(s)

Dario Tommasini

Examples

```r
library(ExperimentHub)
eh = ExperimentHub()
eh_query = query(eh, c("multiWGCNAdata"))
astrocyte_networks = eh_query[["EH8222"]]
datExpr = GetDatExpr(astrocyte_networks[[1]],
    genes = topNGenes(astrocyte_networks$EAE, "EAE_015", 20))
coexpressionLineGraph(datExpr) +
    geom_vline(xintercept = 20.5, linetype='dashed')
```

Description

Computes overlap between the modules of two objects of class WGCNA

Usage

```r
computeOverlapsFromWGCNA(dataset1, dataset2)
```

Arguments

- **dataset1**: an object of class WGCNA to compare with dataset2
- **dataset2**: an object of class WGCNA to compare with dataset1

Value

Returns a data.frame showing the overlap results for modules from dataset1 with dataset2
Author(s)

Dario Tommasini

Examples

```r
library(ExperimentHub)
eh = ExperimentHub()
eh_query = query(eh, c("multiWGCNAdataset"))
astrocyte_networks = eh_query[["EH8222"]]
computeOverlapsFromWGCNA(astrocyte_networks$EAE, astrocyte_networks$WT)
```

---

**constructNetworks**

*constructNetworks: Construct all the weighted gene correlation networks*

Description

A high level function that returns all networks possible for a given experimental design

Usage

```r
constructNetworks(
datExpr,
sampleTable,
conditions1,
conditions2,
write = FALSE,
alphaLevel = 0.05,
plot = FALSE,
...)
```

Arguments

- **datExpr**: either a SummarizedExperiment object or data.frame with genes are rows and samples as columns
- **sampleTable**: data.frame with sample names in first column and sample traits in the second and third column. First column should be called "Sample"
- **conditions1**: first design conditions, ie healthy/disease
- **conditions2**: second design conditions, ie frontal lobe/temporal lobe
- **write**: write results out to files?
- **alphaLevel**: significance value passed to findBestTrait function, default is 0.05
- **plot**: plot modules? Default is false
- **...**: Arguments to pass to blockwiseModules function
**diffCoexpression**

**Value**

A list of WGCNA objects, ie level one, two, and three networks.

**Author(s)**

Dario Tommasini

**Examples**

```r
library(ExperimentHub)
eh = ExperimentHub()
eh_query = query(eh, c("multiWGCNAdata"))
autism_se = eh_query["EH8219"]
set.seed(1)
autism_se = autism_se[sample(rownames(autism_se), 500),]
sampleTable = colData(autism_se)
conditions1 = unique(sampleTable[,2])
conditions2 = unique(sampleTable[,3])
autism_networks = constructNetworks(autism_se, sampleTable, conditions1[[1]], conditions2[[1]],
networkType = "signed", TOMType = "unsigned",
power = 10, minModuleSize = 100, maxBlockSize = 25000,
reassignThreshold = 0, minKMEtoStay = 0, mergeCutHeight = 0,
numericLabels = TRUE, pamRespectsDendro = FALSE,
deepSplit = 4, verbose = 3)
autism_networks["combined"]
```

---

**diffCoexpression**

**Differential co-expression analysis**

**Description**

Performs a differential co-expression analysis given an expression data.frame and a conditions vector.

**Usage**

```r
diffCoexpression(
datExpr,
conditions,
geneList = NULL,
plot = FALSE,
method = c("pearson", "spearman"),
removeFreeNodes = TRUE,
labelSize = 0.5,
labelDist = 0,
shape = "circle",
degreeForSize = FALSE,
```

label = FALSE,
onlyPositive = FALSE,
z.threshold = NULL,
FDR.threshold = 0.05,
nodeSize = 3
)

Arguments

datExpr a data.frame containing expression values
conditions a vector containing conditions for the samples
geneList vector of genes, will use all genes if NULL (default)
plot plot a network?
method either "pearson" or "spearman"
removeFreeNodes remove free nodes from network?
labelSize label size
labelDist distance from labels to nodes
shape shape of nodes
degreeForSize should node size correspond to degree?
label label nodes?
onlyPositive only draw positive correlations?
z.threshold z-score threshold
FDR.threshold FDR threshold
nodeSize size of node

Value
A list including a matrix of z-scores, a matrix of raw p-values, a matrix of adjusted p-values, and a summary data.frame

Author(s)
Dario Tommasini

Examples
library(ExperimentHub)
eh = ExperimentHub()
eh_query = query(eh, c("multiWGCNAdata"))
astrocyte_se = eh_query[["EH8223"]]
datExpr = assays(astrocyte_se)[[1]]
)

**diffModuleExpression**  
*Differential module expression*

**Description**  
Runs (and plots) the differential module expression analysis

**Usage**  

diffModuleExpression(
  WGCNAobject,
  geneList,
  design,
  plotTitle = NULL,
  mode = c("PC1", "Zscore"),
  testColumn = 2,
  refColumn = 3,
  test = c("ANOVA", "PERMANOVA"),
  plot = TRUE
)

**Arguments**  
- **WGCNAobject**  
  WGCNA object  
- **geneList**  
  vector of genes in WGCNAobject  
- **design**  
  the sampleTable  
- **plotTitle**  
  title for the plot  
- **mode**  
  either PC1 or Zscore, default is PC1  
- **testColumn**  
  the column of the sampleTable to be resolved  
- **refColumn**  
  the column of the sampleTable to be used as biological variation  
- **test**  
  statistical test to perform, either "ANOVA" or "PERMANOVA"  
- **plot**  
  generate a plot?

**Value**  
a data.frame with the resulting p-values

**Examples**  
library(ExperimentHub)
eh = ExperimentHub()
eh_query = query(eh, c("multiWGCNAdata"))
astrocyte_se = eh_query[["EH8223"]]
sampleTable = colData(astrocyte_se)
astrocyte_networks = eh_query[["EH8222"]]
diffModuleExpression(astrocyte_networks[["combined"]],
topNGenes(astrocyte_networks$combined, "combined_013"),
sampleTable,
test = "ANOVA",
plotTitle = "combined_013",
plot = TRUE)

drawMultiWGCNAnetwork  

**Description**

Draw a network where nodes are modules and edges represent significant gene overlap. Modules are sorted by levels 1, 2, and 3.

**Usage**

drawMultiWGCNAnetwork(
  WGCNAlist,
  comparisonList,
  moduleOfInterest,
  design,
  overlapCutoff = 0,
  padjCutoff = 1,
  removeOutliers = TRUE,
  alpha = 1e-50,
  layout = NULL,
  hjust = 0.4,
  vjust = 0.3,
  width = 0.5,
  colors = NULL
)

**Arguments**

- **WGCNAlist** list of WGCNA objects
- **comparisonList** the list of overlap comparisons ie from iterate(myNetworks, overlapComparisons, ...)
- **moduleOfInterest** module of interest, ie "combined_001"
- **design** the sampleTable design matrix
- **overlapCutoff** cutoff to remove module correspondences with less than this number of genes
- **padjCutoff** cutoff to remove module correspondences above this significance value
- **removeOutliers** remove outlier modules?
- **alpha** alpha level of significance
GetDatExpr

Description

Returns the expression data frame a WGCNA object as a data.frame

Usage

GetDatExpr(object, genes = NULL)

Arguments

object An object of class WGCNA
genes a list of genes to subset to; default is NULL
Value

a data.frame

Author(s)

Dario Tommasini

Examples

library(ExperimentHub)
eh = ExperimentHub()
eh_query = query(eh, c("multiWGCNAdata"))
astrocyte_networks = eh_query[["EH8222"]]
datExpr = GetDatExpr(astrocyte_networks[[1]],
genes = topNGenes(astrocyte_networks$EAE, "EAE_015", 20))
coexpressionLineGraph(datExpr) +
geom_vline(xintercept = 20.5, linetype='dashed')

getPreservation

Description

Performs a network preservation analysis

Usage

getPreservation(reference, test, nPermutations = 100, write = FALSE)

Arguments

reference reference network of class WGCNA
test test network of class WGCNA
nPermutations number of permutations to perform
write write to file?

Value

a data.frame summarizing results of preservation analysis

Author(s)

Dario Tommasini
iterate

iterate: Iterate function across networks

Description
A high level function that iterates functions across a list of WGCNA objects

Usage
iterate(WGCNAlsit, FUN, ...)

Arguments
WGCNAlsit a vector of objects of type WGCNAobject
FUN function to iterate, either overlapComparisons or preservationComparisons
... arguments to be passed on to overlapComparisons or preservationComparisons

Value
a comparison list from overlapComparisons or preservationComparisons

Author(s)
Dario Tommasini

Examples
library(ExperimentHub)
eh = ExperimentHub()
eh_query = query(eh, c("multiWGCNAdata"))
astrocyte_networks = eh_query["EH8222"]
results = list()
iterate(astrocyte_networks, overlapComparisons, plot=FALSE)

makeTraitTable
Generate a trait table from a sample table

Description
Generates a WGCNA-compatible trait table from a sampleTable dataframe

Usage
makeTraitTable(inputTable, column, detectNumbers = FALSE)
moduleComparisonPlot

**Arguments**

- `inputTable` the sampleTable data.frame
- `column` the column from the sampleTable to use as traits
- `detectNumbers` whether to consider traits with numbers as numerical rather than categorical variables

**Value**

a data.frame with integer values denoting the categorical sample traits

**Examples**

```r
sampleTable = data.frame(Sample = c(paste0("EAE", 1:10), paste0("WT", 1:10)),
                         Disease = c(rep("EAE", 10), rep("WT", 10)),
                         Region = c(rep(c("Cbl", "Sc"), 5)))
makeTraitTable(sampleTable, 2)
```

moduleComparisonPlot  Module comparison plot

**Description**

A plotting function that returns a heatmap and barplot for a module

**Usage**

```r
moduleComparisonPlot(overlapDf, dataset1, dataset2)
```

**Arguments**

- `overlapDf` a data.frame resulting from a call to computeOverlapsFromWGCNA
- `dataset1` an object of class WGCNA to compare with dataset2
- `dataset2` an object of class WGCNA to compare with dataset1

**Value**

Returns a ggplot object (flowplot and heatmap) showing the module correspondence between two objects of class WGCNA

**Author(s)**

Dario Tommasini
moduleExpressionPlot

Examples

```r
library(ExperimentHub)
eh = ExperimentHub()
eh_query = query(eh, c("multiWGCNAdata"))
astrocyte_networks = eh_query[["EH8222"]]
overlapDf = computeOverlapsFromWGCNA(astrocyte_networks$EAE, astrocyte_networks$WT)
moduleComparisonPlot(overlapDf, astrocyte_networks$EAE, astrocyte_networks$WT)
```

---

**moduleExpressionPlot**  
*Plots an expression profile for a module*

**Description**

A plotting function that returns a heatmap and barplot for a module.

**Usage**

```r
moduleExpressionPlot(
  WGCNAobject,  
  geneList,  
  mode = c("PC1", "averageZscore"),  
  legend = FALSE,  
  title = NULL,  
  clusterGenes = FALSE  
)
```

**Arguments**

- **WGCNAobject**: an object of class WGCNAobject
- **geneList**: a vector of gene names to be extracted from WGCNAobject
- **mode**: use first principal component or averageZscore?
- **legend**: plot legend?
- **title**: title of the plot
- **clusterGenes**: cluster heatmap genes by hierarchical clustering?

**Value**

- a patchworked ggplot object

**Author(s)**

Dario Tommasini
Examples

```r
library(ExperimentHub)
eh = ExperimentHub()
eh_query = query(eh, c("multiWGCNAdata"))
astrocyte_networks = eh_query[["EH8222"]]
moduleExpressionPlot(astrocyte_networks[["combined"]],
    geneList = topNGenes(astrocyte_networks$combined, "combined_013"))
```

---

**moduleToModuleHeatmap**  
**Module to module heatmap**

**Description**

Returns a heatmap where color corresponds to FDR-adjusted overlap (hypergeometric test) and the label corresponds to the number of overlapping genes.

**Usage**

```r
moduleToModuleHeatmap(
    comparisonDf,
    dataset1 = NULL,
    dataset2 = NULL,
    trait1 = NULL,
    trait2 = NULL,
    list1 = NULL,
    list2 = NULL,
    filterByTrait = FALSE,
    alphaLevel = 0.05
)
```

**Arguments**

- `comparisonDf`: the data.frame output of `computeOverlapFromWGCNA`
- `dataset1`: optional; WGCNA object for dataset 1
- `dataset2`: optional; WGCNA object for dataset 2
- `trait1`: optional; subset to modules correlated to this trait for dataset 1
- `trait2`: optional; subset to modules correlated to this trait for dataset 2
- `list1`: subset to this list of modules for dataset 1
- `list2`: subset to this list of modules for dataset 2
- `filterByTrait`: only plot for modules that correlate with some trait?
- `alphaLevel`: the alpha level of significance for module-trait correlation, defaults to 0.05

**Value**

A ggplot object
name

Examples

```r
library(ExperimentHub)
eh = ExperimentHub()
eh_query = query(eh, c("multiWGCNAdata"))
astrocyte_networks = eh_query[["EH8222"]]
overlapDf = computeOverlapsFromWGCNA(astrocyte_networks$EAE, astrocyte_networks$WT)
moduleToModuleHeatmap(overlapDf)
```

name

name: Name of WGCNA object

Description

Returns the name of a WGCNA object.

Usage

`name(WGCNAobject)`

Arguments

WGCNAobject an object of class WGCNA

Value

Returns the name of the WGCNA object, i.e, "EAE" for astrocyte_networks$EAE.

Examples

```r
library(ExperimentHub)
eh = ExperimentHub()
eh_query = query(eh, c("multiWGCNAdata"))
astrocyte_networks = eh_query[["EH8222"]]
name(astrocyte_networks$EAE)
```

overlapComparisons

Overlap comparisons

Description

Compares modules between two objects of type WGCNA objects within a WGCNA object list given the indices. Recommended to be used in conjunction with the iterate function.
overlapComparisons

Usage

overlapComparisons(
  comparisonList,
  WGCNAlist,
  first,
  second,
  element,
  plot = TRUE,
  write = FALSE
)

Arguments

comparisonList a list passed by the iterate function
WGCNAlist list of objects of class WGCNA
first index of first WGCNA object
second index of second WGCNA object
element element position in the comparison list (passed by iterate function)
plot generate plots?
write write results to file?

Value

A list, in which the first element is a data.frame showing the overlap results and the second element is a data.frame showing the best matching modules between the two WGCNA objects.

Author(s)

Dario Tommasini

Examples

library(ExperimentHub)
eh = ExperimentHub()
eh_query = query(eh, c("multiWGCNAdata"))
astrocyte_networks = eh_query[["EH8222"]]
results = list()
results$overlaps = iterate(astrocyte_networks, overlapComparisons, plot=FALSE)
performANOVa

**Description**
Test association between module expression to traits using ANOVA

**Usage**

```r
performANOVa(datExpr, design, testCondition, refCondition, alphaLevel = 0.05)
```

**Arguments**

- `datExpr`: expression data.frame
- `design`: the sampleTable
- `testCondition`: test column in sampleTable
- `refCondition`: reference column in sampleTable
- `alphaLevel`: the significance level

**Value**

a data.frame with p-values for each association

---

preservationComparisonPlot

**Preservation Comparison Scatterplot**

**Description**
A plotting function that draws a scatterplot of preservation scores between two WGCNA objects

**Usage**

```r
preservationComparisonPlot(
  preservationList,
  dataset1, dataset2,
  alphaLevel = 0.05,
  outliers = FALSE
)
```
preservationComparisons

Arguments

- preservationList: a list resulting from a call to preservationComparisons
- dataset1: an object of class WGCNAObject to compare with dataset2
- dataset2: an object of class WGCNAObject to compare with dataset1
- alphaLevel: alpha level of significance, default is 0.05
- outliers: leave outlier modules? By default these are removed

Value

- a ggplot object

Author(s)

- Dario Tommasini

Examples

```r
library(ExperimentHub)
eh = ExperimentHub()
eh_query = query(eh, c("multiWGCNAdata"))
astrocyte_networks = eh_query[["EH8222"]]
results = list()
results$preservation=iterate(astrocyte_networks[c("EAE", "WT")],
preservationComparisons,
write=FALSE,
plot=FALSE,
nPermutations=2)
preservationComparisonPlot(results$preservation$EAE_vs_WT,
astrocyte_networks$EAE,
astrocyte_networks$WT)
```

Description

A high-level function that performs a preservation comparison between two WGCNAObjects in a WGCNAList, usually supplied by iterate function.
Usage

```r
preservationComparisons(
  comparisonList,
  WGCNAlist,
  first,
  second,
  element,
  plot = FALSE,
  write = FALSE,
  alphaLevel = 0.05,
  nPermutations = 100
)
```

Arguments

- `comparisonList` a list passed by the iterate function
- `WGCNAlist` list of objects of type WGCNAobject
- `first` index of first WGCNAobject
- `second` index of second WGCNAobject
- `element` element position in the comparison list (passed by iterate function)
- `plot` generate plots?
- `write` write results to file?
- `alphaLevel` alpha level of significance for module-trait correlation
- `nPermutations` number of permutations, defaults to 100

Value

a list of preservation comparisons results across levels 1, 2, 3

Author(s)

Dario Tommasini

Examples

```r
library(ExperimentHub)
eh = ExperimentHub()
eh_query = query(eh, c("multiWGCNAdata"))
astrocyte_networks = eh_query[["EH8222"]]
results = list()
iterate(astrocyte_networks[[c("EAE", "WT")]],
preservationComparisons,
write=FALSE,
plot=FALSE,
nPermutations=2)
```
runDME  
*Run differential module expression*

**Description**
A wrapper to run `diffModuleExpression` on all the modules in a network

**Usage**
```r
runDME(
    WGCNAobject,  
    design,  
    alphaLevel = 0.05,  
    testCondition = NULL,  
    refCondition = NULL,  
    p.adjust = "fdr",  
    plot = FALSE,  
    test = c("ANOVA", "PERMANOVA"),  
    write = FALSE,  
    out = NULL  
)
```

**Arguments**
- `WGCNAobject` object of class WGCNA with the modules to run DME on
- `design` the sampleTable
- `alphaLevel` level of significance
- `testCondition` the column of the sampleTable to be resolved
- `refCondition` the column of the sampleTable to be used as biological variation
- `p.adjust` adjust for multiple comparisons, argument to pass to `p.adjust` function
- `plot` generate a plot?
- `test` statistical test to perform, either "ANOVA" or "PERMANOVA"
- `write` write results to a file?
- `out` file name for DME plots, only used if `write` is TRUE

**Value**
a data.frame summarizing the results of the analysis

**Author(s)**
Dario Tommasini
summarizeResults

Examples

```r
library(ExperimentHub)
eh = ExperimentHub()
eh_query = query(eh, c("multiWGCNAdata"))
astrocyte_se = eh_query["EH8223"]
sampleTable = colData(astrocyte_se)
astrocyte_networks = eh_query["EH8222"]
rundME(astrocyte_networks["combined"],
   design = sampleTable,
   p.adjust = "fdr",
   refCondition = "Region",
   testCondition = "Disease")
```

summarizeResults `summarizeResults`: Summarize results from a results list object

Description

Prints (or writes) a summary of the results from a results list object

Usage

```r
summarizeResults(
   myNetworks,
   results,
   alphaLevel = 0.05,
   write = FALSE,
   outputFile = "results.txt"
)
```

Arguments

- `myNetworks`: a list of WGCNA objects
- `results`: results list
- `alphaLevel`: alpha level of significance
- `write`: write to file?
- `outputFile`: name of output file, defaults to results.txt

Value

Prints a summary of results from the multiWGCNA analysis
Description

Returns the top N number of genes of a module. All genes returned if no number is specified. Genes are in order of intramodular connectivity.

Usage

topNGenes(WGCNAobject, module, nGenes = NULL)

Arguments

WGCNAobject  an object of class WGCNA
module       the name of the module in WGCNAobject
nGenes       an integer from 1 to module size; returns all genes if left NULL

Value

a character vector of the genes/probes in the module

Examples

library(ExperimentHub)
eh = ExperimentHub()
eh_query = query(eh, c("multiWGCNAdataset"))
astrocyte_networks = eh_query[['EH8222']]
topNGenes(astrocyte_networks$EAE, "EAE_015", nGenes = 10)

---

WGCNA-class

The WGCNA Class

Description

The WGCNA class is the main class used in multiWGCNA to store results from a weighted gene co-expression network analysis. These include the original unaltered expression data used as input, connectivity metrics, module assignment, input sample conditions, trait

Value

NA
WGCNA-class

Slots

datExpr  The expression data, connectivity data, and module assignment
conditions  A data.frame with integer conditions for WGCNA
trait  A data.frame showing pearson correlation values to traits
moduleEigengenes  A data.frame of module eigengenes for each module across samples
outlierModules  A vector of modules classified by our algorithm as being driven by sample outliers
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