Package ‘eegc’

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Type Package

Title Engineering Evaluation by Gene Categorization (eegc)

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Description This package has been developed to evaluate cellular engineering processes for direct differentiation of stem cells or conversion (transdifferentiation) of somatic cells to primary cells based on high throughput gene expression data screened either by DNA microarray or RNA sequencing. The package takes gene expression profiles as inputs from three types of samples: (i) somatic or stem cells to be (trans)differentiated (input of the engineering process), (ii) induced cells to be evaluated (output of the engineering process) and (iii) target primary cells (reference for the output). The package performs differential gene expression analysis for each pair-wise sample comparison to identify and evaluate the transcriptional differences among the 3 types of samples (input, output, reference). The ideal goal is to have induced and primary reference cell showing overlapping profiles, both very different from the original cells.

VignetteBuilder knitr

Depends R (>= 3.3.0)

Imports R.utils, gplots, sna, wordcloud, igraph, pheatmap, edgeR, DESeq2, clusterProfiler, S4Vectors, ggplot2, org.Hs.eg.db, org.Mm.eg.db, limma, DOSE, AnnotationDbi

Suggests knitr

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```
barplotEnrich Barplot the enrichResult
```

Description

This function is revised from the `barplot.enrichResult` function DOSE package, and used to perform a barplot of the enrichResult object.

Usage

```
barplotEnrich(height, x = "Count", colorBy = "p.adjust", top = 5,
font.size = 12, title = "", color = NULL,...)
```

Arguments

- `height` : enrichResult object. alternatively output from `functionEnrich` function.
- `x` : one of "Count" and "GeneRatio" to specify the x axis.
- `colorBy` : one of `pvalue`, `p.adjust`, `qvalue`.
- `top` : number of top categories to show.
cate.gene

font.size, title
font size and title.
color the color of the bar.
...
see parameters in fortify function.

Value
bar plot of enrichment results

Examples
## Not run:
# plot the "enrichResult" of Inactive category
inactive = goenrichraw[[2]]
barplotEnrich(inactive, top =5, color ="#2c7bb6", title = "Inactive")

## End(Not run)

cate.gene  Categorized genes

Description
A list with five gene categories.

Usage
data(cate.gene)

Format
A list

Value
A list with five gene categories.

cate.ratio  Expression Difference ratios of categorized genes

Description
A list with five data frames of gene ED ratios

Usage
data(cate.ratio)

Format
A list
categorizeGene

Description
This function categorizes differential genes of each pair-wise comparison among e.g. initiating A, derived B and primary C samples during a cellular engineering, into five categories named from Reversed, Inactive, Insufficient, Successful and Over representing the gene reprogrammed states, and calculates the ratio of expression difference (ED) between B and A to the ED between C and A.

Usage
categorizeGene(expr, diffGene, from.sample, to.sample, target.sample)

Arguments
expr a data frame with expression for all genes in diffGene, alternatively output from diffGene function.
diffGene a list of differential genes in three comparisons, alternatively output by diffGene function.
from.sample, to.sample, target.sample character to specify the name of initiating sample, derived sample and primary sample during a cellular engineering, must be consistent with sample names in the expr data frame.

Details
Gene (g) categorization is achieved by considering the pair-wise comparisons (Expression Difference, ED eq. 1) among the three types of samples and the ratio of such differences (EDg ratio, eq. 2). $EDg(B, A) = \frac{\text{average expression of } g \text{ in } B \text{ minus average expression of } g \text{ in } A}{1}$ $EDgratio = \frac{EDg(B, A)}{EDg(C, A)}$ (2)

*Reversed*, $EDg(B, A)$ and $EDg(B, C)$ are significantly differential, while $EDg(C, A)$ is not limited by differential or not, $EDg$ ratio is smaller than 0; *Inactive*, $EDg(C, A)$ and $EDg(B, C)$ are significantly differential, while $EDg(B, A)$ is not differential; *Insufficient*, $EDg(B, A)$, $EDg(C, A)$ and $EDg(B, C)$ are all significantly differential, $EDg$ ratio is between 0 and 1; *Successful*, $EDg(B, A)$ and $EDg(C, A)$ are significantly differential, while $EDg(B, C)$ is not differential; *Over*, $EDg(B, A)$ and $EDg(B, C)$ are significantly differential, while $EDg(C, A)$ is not limited by differential or not, $EDg$ ratio is greater than 1. For the *Inactive* and *Successful* categories, genes with bottom and top 5 percentage ED ratios are removed to avoid the ambiguous categorization with *Reversed, Insufficient* or *Over* categories.

Value
A list with components: a list of the five gene categories a list of the ED ratios for the five gene categories.
densityPlot

Examples

```r
data(expr.filer)
data(diffgene.genes)

category = categorizeGene(expr = expr.filer, diffGene = diffgene.genes,
                         from.sample="DMEC", to.sample="rECHMP", target.sample="CB")
cate.gene = category[[1]]
cate.ratio = category[[2]]
```

densityPlot

Quantify Genes and Corresponding ED ratios in Each Category

Description

Quantify genes in each gene category and their expression difference (ED) ratios in a density plot.

Usage

```r
densityPlot(ratio, color = NULL, main = NA, xlab = NA, ylab = "Density",
legend.labels = NULL, shade = TRUE, transparency = TRUE, proportion = TRUE,
out.file = NULL, ...)
```

Arguments

- `ratio`: a list of ED ratios for five gene categories, alternatively output by `categorizeGene`.
- `color`: vector of colors for the five gene categories.
- `main`, `xlab`, `ylab`: the overall title, tile for x axis, and title for y axis, see `title`.
- `legend.labels`: vector of labels for the legend.
- `shade`: logical to determine if the five categories are filled with shades.
- `transparency`: logical to determine if the density plot is transparent.
- `proportion`: logical to determine whether the proportion of each category genes over the all genes is drawn on the density plot.
- `out.file`: a character string naming the output file with density plot.
- `...`: parameters in `plot`.

Value

a density plot

Examples

```r
data(cate.ratio)
names(cate.ratio)
# make the extreme ED ratios in Reversed and Over categories to the median values
reverse = cate.ratio[[1]]
over = cate.ratio[[5]]
reverse[reverse[,1] <= median(reverse[,1]), 1] = median(reverse[,1])
over[over[,1] >= median(over[,1]),1] = median(over[,1])
cate.ratio[[1]] = reverse
```
diffGene[[5]] = over

# densityPlot(cate.ratio, xlab = "ED ratio", ylab = "Density", proportion = TRUE)

diffGene

Gene Expression Differential Analysis

Description

Identify the differentially expressed genes for each pair-wise comparison of given three types of samples.

Usage

diffGene(expr, array = TRUE, fpkm = FALSE, counts = FALSE, method = c("limma", "DESeq2"),
from.sample, to.sample, target.sample, filter = FALSE, filter.perc = 0.4,
padjust = "fdr", signif = TRUE, pvalue = 0.05)

Arguments

expr a data frame with gene expression data.

array, fpkm, counts logical, specifying the type of input gene expression data.

method differential analysis method, alternatively to "limma" and "DESeq2", default to "limma". "DESeq2" can be chosen only when counts is TRUE.

from.sample, to.sample, target.sample character to specify the name of initiating sample, derived sample and primary sample during a cellular engineering.

filter logical to indicate whether the genes need to be filtered when match the parameter filter.perc, only applied to fpkm and counts data.

filter.perc a 0 to 1 number to specify the gene filter criteria by the percentage of samples with non-zero expression. Only used to fpkm and counts data when filter is TRUE, and filter the genes with non-zero expression in less than filter.perc samples.

padjust indicate the method to do p.value correction, default to "fdr". See p.adjust.

signif logical to indicate whether only the significantly differential genes are output, default to FALSE.

pvalue a cutoff p.value for the significant genes, default to 0.05, only used when signif is TRUE.

Details

This function can be applied on both microarray and RNA-seq data for differential analysis when one of the "array", "fpkm", or "counts" is specified. It does differential analysis to each pair-wise sample comparison among the from.sample, to.sample and target.sample.

Value

A list with components: a list with differential analysis result for each pair-wise comparison; a list with differential gene names for each pair-wise comparison; a data frame with filtered/unfiltered gene expression.
Examples

data(SandlerFPKM)

# differential expression analysis:
diffgene = diffGene(expr = SandlerFPKM, array=FALSE, fpkm=TRUE, counts=FALSE,
from.sample="DMEC", to.sample="rECHMPP", target.sample="CB",
filter=TRUE, filter.perc =0.4, pvalue = 0.05 )

# differential analysis results
diffgene.result = diffgene[[1]]
# differential genes
diffgene.genes = diffgene[[2]]
# filtered expression data
expr.filter = diffgene[[3]]

diffgene.genes  Differential genes for three comparisons

Description
A list of three vectors with significantly differential genes among three comparisons.

Usage
data(diffgene.genes)

Format
A list

Value
A list with differential genes in three comparisons

dotPercentage  Percentage Calculation and Visualization

Description
This function calculate the percentage of genes in each category over given annotated gene sets and plot the percentages.

Usage
dotPercentage(cate.gene, annotated.gene, order.by = NULL, type = "l", lty = 1,
pch = NULL, col = NULL, srt = 50, font = 1, adj = c(1,1), cex = 1, add.line = TRUE,
legend = TRUE, legend.label = NULL, ...)
enrichment

Arguments

cate.gene a list of the five gene categories, alternatively output by categorizeGene.
annotated.gene a list of the annotated gene sets which the cate.gene are proportioned in.
order.by one character out of "Reversed", "Inactive", "Insufficient", "Successful" and "Over" to specify a gene category the percentage is ordered by.
type, lty, pch, col parameters for the plotting, specifying the type of plotting; the line type when type is "l"; the symbol of points on the line; and the color of lines, see graphic parameters in par().
srt, font, cex, adj parameters for the text labeled on x-axis, specifying the string rotation in degrees; the font of text; the text size, see graphic parameters in par().
add.line logical to determine if to add lines on the dots, logical to TRUE.
legend logical to determine whether the legend is added on the figure, default to TRUE.
legend.label labels of the legend, applied only when legend is TRUE.
... other parameters see plot.

Value

a data frame with the percentage of cate.gene in the annotated.gene.

Examples

# load the C/T-specific genes in 16 cells/tissues
data(human.gene)
data(cate.gene)
# perc = dotPercentage(cate.gene = cate.gene, annotated.gene = human.gene,
# # order.by = "Successful")

eegc
eegc

Description

eegc

enrichment Enrichment Analysis by Hypergeometric Distribution Test

Description

Enrichment Analysis by Hypergeometric Distribution Test

Usage

enrichment(cate.gene, annotated.gene, background.gene, padjust.method = "fdr" )
Arguments

cate.gene  a list of the five gene categories, alternatively output by `categorizeGene`.

annotated.gene  a list of annotated gene sets which the `cate.gene` are enriched in.

background.gene  vector of background genes, e.g. all genes screened by microarray or RNA-sequencing.

padjust.method  correction method for enrichment p-values, one of "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none", default to "fdr", see details in `p.adjust`.

Value

A list of enrichment results for the five gene categories.

Examples

# load the cell/tissue-specific genes
data(tissueGenes)
# load the mapping file of cells/tissues to grouped cells/tissues
data(tissueGroup)

# get the background genes
data(expr.filter)
genes = rownames(expr.filter)
# enrichment analysis for the five gene categories
data(cate.gene)
tissueenrich = enrichment(cate.gene = cate.gene, annotated.gene = tissueGenes,
    background.gene = genes, padjust.method = "fdr")
# select a group of cells/tissues
tissueGroup.selec = c("stem cells","B cells","T cells","Myeloid","Endothelial CD105+")
tissues.selec = tissueGroup[tissueGroup[,"Group"] %in% tissueGroup.selec,c(2,3)]
# tissuetable = heatmapPlot(tissueenrich, terms = tissues.selec, GO=FALSE,
#    annotated_row = TRUE,annotation_legend = TRUE,
#    main = "Tissue-specific enrichment")

expr.filter  Filtered expression data

Description

A data frame with filtered RNASeq FPKM data.

Usage

data(expr.filter)

Format

A data frame

Value

A data frame with filtered gene expression
functionEnrich  
*Functional Enrichment Analysis*

**Description**

This function performs Gene Ontology (GO) and KEGG pathways functional enrichment for the five gene categories by calling `clusterProfiler` package.

**Usage**

```r
functionEnrich(cate.gene, organism = "human", convert = TRUE, from = "SYMBOL", ont = "BP", pAdjustMethod = "bonferroni", GO = TRUE, KEGG = FALSE, enrichResult = FALSE)
```

**Arguments**

- **cate.gene**: a list of the five gene categories, alternatively output by `categorizeGene`
- **organism**: a character of organism "human" or "mouse" to indicate the species of background genes.
- **convert**: logical to determine whether the gene ID should be converted to "ENTREZID", default to `TRUE`.
- **from**: the gene id type of input data, see the key types of `org.Hs.eg.db`.
- **ont**: One of "MF", "BP", and "CC" subontologies, see `enrichGO`.
- **pAdjustMethod**: correction method for p-value, one of "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none", default to "fdr", see details in `p.adjust`.
- **GO**: logical to determine whether the functional enrichment is performed on Gene Ontology, default to `TRUE`.
- **KEGG**: logical to determine whether the functional enrichment is performed on KEGG pathways, default to `FALSE`.
- **enrichResult**: logical to determine if the "enrichResult" is output, default to `FALSE` to output a summary of the "enrichResult".

**Value**

Function enrichment analysis results.

**Examples**

```r
data(cate.gene)
# result in "enrichResult" class by specifying TRUE to enrichResult parameter
goenrichraw = functionEnrich(cate.gene, organism = "human", pAdjustMethod = "fdr", GO = TRUE, KEGG = FALSE, enrichResult = TRUE)

# result of the summary of "enrichResult" by specifying FALSE to enrichResult parameter
# GO enrichment
goenrich = functionEnrich(cate.gene, organism = "human", pAdjustMethod = "fdr", GO = TRUE, KEGG = FALSE, enrichResult = FALSE)

# KEGG enrichment
keggenrich = functionEnrich(cate.gene, organism = "human", pAdjustMethod = "fdr", GO = FALSE, KEGG = TRUE, enrichResult = FALSE)
```
goenrich

GO enrichment results

Description
A list of 5 data frames with the Gene ontology enrichment results for the five gene categories.

Usage
data(goenrich)

Format
A list with 5 data frames

Value
A list of GO enrichment results

grnPlot
Gene Regulatory Network Plot

Description
This function plot the a cell/tissue-specific gene regulatory network with genes in the five categories, alternatively reducing the network size by plotting given specific genes as nodes.

Usage
grnPlot(grn.data, cate.gene, filter = TRUE, nodes = NULL, centrality.score, col = NULL, main= NULL, vertex.label =NULL, vertex.label.dist = 0, vertex.label.font = 1, vertex.label.cex = 0.5, vertex.label.color="black", edge.arrow.size = 0.4, edge.color = "grey", layout ="layout_with_lgl", legend.labels = NULL, ...)

Arguments

grn.data a data frame with two columns named "TF" and "TG" to specify the genes as transcription regulators (TF) and target genes being regulated (TG).
cate.gene a list of the five gene categories as nodes in the network, alternatively output by categorizeGene.
filter logical to specify if the network is reduced to less nodes by a filter, logical to TRUE for a clear visualization.
nodes a character vector of genes to kept in the network, only applied when filter is TRUE.
centrality.score a vector or data frame of centrality scores for genes in the network, alternatively calculated by networkAnalyze function.
col colors of gene vertex in each cate.gene.
heatmapPlot

### Heatmap Plot of Enriched Terms

**Description**

This function plots the significantly enriched terms in a heatmap by calling the `pheatmap` package.

**Usage**

```r
heatmapPlot(enrichresult, GO = FALSE, terms = NULL, padjust = TRUE, pvalue = 0.05, 
            top = NA, filter = FALSE, main = NA, annotation = NULL, annotation_col = NULL, 
            annotated_row = FALSE, annotation_row = NULL, 
            annotation_colors = NULL, display_numbers = FALSE, annotation_legend = FALSE,...)
```

**Arguments**

- `enrichresult` a list of data frames with enrichment results, alternatively output by `functionEnrich` or `enrichment`.
- `GO` logical to determine whether the terms are Gene Ontology (GO) terms enriched by `functionEnrich`.
human.gene

A list of 16 human cells/tissues-specific gene sets summarized from the gene regulatory network downloaded from the CellNet website.

**Value**

A list with 16 human cells/tissues-specific gene sets from CellNet.
**human.grn**  
*Human cell/tissue-specific gene-gene regulation*

**Description**
A list of 16 data frames (cells/tissues) with transcription factors (TF) to target genes (TG) regulation information.

**Usage**
data(human.grn)

**Format**
A list with 16 data frames

**Value**
A list of human gene regulatory information.

**human.tf**  
*Gene regulatory network based human cell/tissue-specific transcription factor (TF) regulated gene sets*

**Description**
A list of 1455 human cells/tissues-specific TF regulated gene sets summarized from the gene regulatory network downloaded from the CellNet website.

**Usage**
data(human.tf)

**Format**
A list

**Value**
A list with 1455 human cells/tissues-specific TF regulated gene sets
markers

<table>
<thead>
<tr>
<th>markers</th>
<th>Marker genes</th>
</tr>
</thead>
</table>

**Description**

A vector containing 65 genes specific in endothelial and haematopoietic cells as listed in Sandler’s paper.

**Usage**

data(markers)

**Format**

A vector

**Value**

A vector of marker genes

---

**markerScatter** | Scatter Plot for Gene Expression

**Description**

Generates an expression profile of each gene category in one sample against another, alternatively plot the regression line from linear modeling fitting.

**Usage**

markerScatter(expr, log = FALSE, samples, cate.gene, markers = NULL, pch = 19, cex = 0.5, col = NULL, xlab = NULL, ylab = NULL, main = NULL, add.line = TRUE, text.cex = 1, legend.labels = NULL, ... )

**Arguments**

expr | a data frame with gene expression.
log | logical to determine if the gene expression data is log converted (add a small constant 2), default to FALSE.
samples | a vector of samples to compare on the x axis and y axis.
cate.gene | a list of the gene categories, alternatively output by categorizeGene.
markers | vector of marker genes to be highlighted in the plot. No gene is highlighted when it’s NULL.
pch, cex, col, xlab, ylab, main | plot parameters, see details in par.
add.line | logical to determine if the linear model fitting line is added on the figure.
text.cex | font size for the text on markers, see details in text.
legend.labels | vector of labels for the marker legend.
... | other parameters in plot.
Details
Visualization of gene expression in the five categories under each pair-wised comparison.

Value
plot with gene expression profile.

Examples
# load the marker genes of somatic and primary cells
data(markers)
data(expr.filter)
# scatterplot
col = c("#abd9e9", "#2c7bb6", "#fee090", "#d7191c", "#fdae61")
markerScatter(expr = expr.filter, log = TRUE, samples = c("CB", "DMEC"),
cate.gene = cate.gene[2:4], markers = markers, col = col[2:4],
xlab = expression("log[2]* expression in CB (target)"),
ylab = expression("log[2]* expression in DMEC (input)"),main = "")

mouse.gene
Gene regulatory network based mouse cell/tissue-specific gene sets

Description
A list of 20 mouse cells/tissues-specific gene sets summarized from the gene regulatory network downloaded from the CellNet website.

Usage
data(mouse.gene)

Format
A list

Value
A list of 20 mouse cells/tissues-specific gene sets

mouse.grn
Mouse cell/tissue-specific gene-gene regulation

Description
A list of 20 data frames (cells/tissues) with transcription factors (TF) to target genes (TG) regulation information.

Usage
data(mouse.grn)
**Format**

A list with 20 data frames

**Value**

A list of mouse gene regulatory information.

---

**mouse.tf**

*Gene regulatory network based mouse cell/tissue-specific transcription factor (TF) regulated gene sets*

---

**Description**

A list of 1744 mouse cells/tissues-specific TF regulated gene sets summarized from the gene regulatory network downloaded from the CellNet website.

**Usage**

```r
data(mouse.tf)
```

**Format**

A list

**Value**

A list of 1744 mouse cells/tissues-specific TF regulated gene sets.

---

**networkAnalyze**

*Network Topological Analysis*

---

**Description**

This function analyzes the topological structure of gene regulation network (GRN) by calculating the "degree", "betweenness", "closeness" and "stress" parameters, and output the centrality values for given genes in each gene categories.

**Usage**

```r
networkAnalyze(grn.data, cate.gene, centrality = c("degree", "betweenness", "stress", "closeness"), mode = c("all","in", "out", "total"))
```
Arguments

grn.data  a data frame with two columns named "TF" and "TG" to specify the genes as transcription regulators (TF) and target genes (TG) being regulated.
cate.gene a list of the five gene categories as nodes in the network, alternatively output by categorizeGene.
centrality character string of "degree", "betweenness", "closeness" and "stress" to calculate the centrality of network built from input grn.data, see degree, betweenness, closeness, and stresscent for details.
mode character string of "all", "in", "out" and "total", only used when centrality is "degree" or "closeness", see degree, closeness for details.

Value
data frame with genes and centrality scores.

Examples

# load the CellNet GRN and gene categories
data(human.grn)
data(cate.gene)

# specify a tissue-specific network
tissue = "Hspc"
degree = networkAnalyze(human.grn[[tissue]], cate.gene = cate.gene,
centrality = "degree", mode = "all")

SandlerFPKM  RNA-seq data in FPKM A data frame containing 16692 gene in 7 samples from paper published by Sandler et.al. in 2014., samples are named from somatic dermal microvascular endothelial cell (DMEC) to induced multipotent haematopoietic progenitor (rEChMPP) cells and primary cord blood (CB) cells.

Description

RNA-seq data in FPKM A data frame containing 16692 gene in 7 samples from paper published by Sandler et.al. in 2014., samples are named from somatic dermal microvascular endothelial cell (DMEC) to induced multipotent haematopoietic progenitor (rEChMPP) cells and primary cord blood (CB) cells.

Usage
data(SandlerFPKM)

Format
A data frame with 16692 rows and 7 columns specifying for genes and samples.

Value
A data frame with gene expression data.
tissueGenes

**tissueGenes**  
*Cell/Tissue specific gene sets*

**Description**
A list of 126 cells/tissues-specific gene sets identified by SpeCond algorithm from 126 cells/tissues in Gene Enrichment Profiler database.

**Usage**
data(tissueGenes)

**Format**
A list

**Value**
A list with 126 cells/tissues-specific gene sets

---

tissueGroup

**tissueGroup**  
*Tissue mapping to groups*

**Description**
A data frame with 126 cells/tissues and 30 C/T groups they belong to.

**Usage**
data(tissueGroup)

**Format**
A data frame with 126 rows (tissues) and 3 columns (Tissue, Tissue_abbr, Group)

**Value**
A data frame with 126 cells/tissues and 30 C/T groups they mapped to
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