# Package 'eegc'

October 16, 2019

Type Package

Title Engineering Evaluation by Gene Categorization (eegc)

Version 1.10.0

**Date** 2018-12-21

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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 (>= 3.4.0)

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

**biocViews** ImmunoOncology, Microarray, Sequencing, RNASeq, DifferentialExpression, GeneRegulation, GeneSetEnrichment, GeneExpression, GeneTarget

License GPL-2

LazyData TRUE

RoxygenNote 5.0.1

git\_url https://git.bioconductor.org/packages/eegc

git\_branch RELEASE\_3\_9

git\_last\_commit 10f55cc

git\_last\_commit\_date 2019-05-02

Date/Publication 2019-10-15

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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,...)
```

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.
colorBy	one of 'pvalue', 'p.adjust', 'qvalue'.

#### cate.gene

font.size, title	e
	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 catogories.

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

## Value

A list with ED ratios for five gene catogories.

categorizeGene Gene Categorization

#### 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) = average expression of gin Bminus average expression of gin A (1) EDgratio = 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(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

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

```
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

```
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

```
cate.ratio[[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, cou	unts
	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 param- eter 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.

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#### diffgene.genes

#### Examples

data(SandlerFPKM)

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 = "1", lty = 1,
pch = NULL, col = NULL, srt = 50, font = 1, adj = c(1,1), cex = 1, add.line = TRUE,
legend = TRUE, legend.label = NULL, ...)
```

## 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 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 de- grees; 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
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## Description

Enrichment Analysis by Hypergeometric Distribution Test

## Usage

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

#### expr.filter

#### 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", "hom-
mel", "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

## Description

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

#### Usage

```
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 back- ground 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", "bonfer- roni", "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

goenrich

#### 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, alterntively 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, ...)
```

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.	

main	title of the plot.	
vertex.label,	vertex.label.dist, vertex.label.font, vertex.label.cex, vertex.label.color parameters for vertex labels, to specify the labels of vertex, the position of labels on the vertex, font size, cex and color for label, see details in igraph.plotting.	
edge.arrow.size, edge.color		
	parameters for edge to specify the arrow size and color of edge, see details in igraph.plotting.	
layout	the layout of network plot, see details in layout.	
legend.labels	vector of label names for each cate.gene.	
	other parameters used in igraph.plotting.	

## Value

a igraph plot for gene regulatory network.

## Examples

```
## Not run:
    # select genes to shown their regulation with others
    node.genes = c("ZNF641", "BCL6")
    # enlarge the centrality
    centrality.score = degree$centrality*100
    names(centrality.score) = degree$Gene
    par(mar = c(2,2,3,2))
    grnPlot(grn.data = human.grn[[tissue]], cate.gene = cate.gene, filter = TRUE,
        nodes = node.genes, centrality.score = centrality.score,
        main = "Gene regulatory network")
```

## End(Not run)

heatmapPlot

Heatmap Plot of Enriched Terms

#### Description

This function plot the significantly enriched terms in a heatmap by calling **pheatmap** package.

## Usage

```
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 = NA,
display_numbers = FALSE, annotation_legend = FALSE,...)
```

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

terms	a character vector to specify the terms chosen to be listed in the heatmap, se- lected from the enrichment result, or a data frame with terms and correspond- ing term annotations used for annotation_row.	
padjust	logical to determine whether the significantly enriched terms were selected by adjusted p.value rather than the p.value, default to TRUE.	
pvalue	a cutoff value to select the significantly enriched terms.	
top	a number to specify the most significantly enriched terms to be drawn in each category, default to NA without specifying.	
filter	logical to specify whether the enriched terms need to be filered with the ones which are significant among the first four categories.	
main	a character of main title on the heatmap plot.	
annotation, annotation_row, annotation_col, annotation_colors, annotation_legend, see details in pheatmap.		
annotated_row	logical to determine whether the the rows are annotated by annotation_row, default to FALSE. When it's TRUE, annotation_row should be specified or using annotations in a data frame of terms.	
display_numbers		
	logical to determine whether the gene counts number is shown on the heatmap.	

#### Value

heatmap plot and the terms with p.values for the heatmap

## Examples

human.gene

Gene regulatory network based human cell/tissue-specific gene sets

#### Description

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

## Usage

data(human.gene)

## Format

A list

## Value

A list with 16 human cells/tissues-specific gene sets from CellNet.

human.grn

## 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 transcrip-
	tion 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

Marker genes

## 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 catetory 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 ... )

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

```
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)

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#### mouse.tf

## 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 transcrip-
	tion 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

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

```
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	charactor string of "degree", "betweenness", "closeness" and "stress" to calcu- late 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

SandlerFPKM	RNA-seq data in FPKM A data frame containing 16692 gene in 7 sam- ples 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

## 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

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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