Gene set enrichment

2023-11-05

This script is for conducting gene set enrichment analysis (GSEA) on RNA-seq data. It identifies biological processes and pathways enriched among differentially expressed genes in a dataset. Key steps include installing necessary libraries, loading gene expression data, preparing gene lists, and running enrichment analyses using the Gene Ontology (GO). Visualization options, such as dot plots, help to interpret which gene sets are most relevant to the biological questions in the study.

Based on the following script: https://learn.gencore.bio.nyu.edu/rna-seq-analysis/gene-set-enrichment-analysis/

Install packages

```
if (!require("BiocManager", quietly = TRUE))
    install.packages("BiocManager")
```

```
## Bioconductor version '3.16' is out-of-date; the current release version '3.20'
##   is available with R version '4.4'; see https://bioconductor.org/install
```

```
BiocManager::install("clusterProfiler")
```

```
## Bioconductor version 3.16 (BiocManager 1.30.22), R 4.2.2 (2022-10-31)
```

```
## Warning: package(s) not installed when version(s) same as or greater than current; use
##   `force = TRUE` to re-install: 'clusterProfiler'
```

```
## Old packages: 'ape', 'aplot', 'askpass', 'backports', 'BH', 'BiocManager',
##   'bit', 'bit64', 'bitops', 'boot', 'brew', 'brio', 'broom', 'bslib', 'cachem',
##   'callr', 'cli', 'cluster', 'codetools', 'colorspace', 'commonmark',
##   'cowplot', 'cpp11', 'crayon', 'credentials', 'curl', 'data.table', 'DBI',
##   'dbplyr', 'dendextend', 'desc', 'digest', 'downlit', 'evaluate', 'fansi',
##   'farver', 'fastmap', 'foreign', 'fs', 'gert', 'ggforce', 'ggfun', 'ggh4x',
##   'ggnewscale', 'ggplot2', 'ggraph', 'ggrepel', 'gh', 'glue', 'graphlayouts',
##   'gtable', 'haven', 'highr', 'htmltools', 'htmlwidgets', 'httpuv', 'httr2',
##   'igraph', 'jsonlite', 'KernSmooth', 'knitr', 'later', 'lattice', 'markdown',
##   'mgcv', 'munsell', 'nlme', 'openssl', 'patchwork', 'pkgbuild', 'pkgdown',
##   'pkgload', 'plotly', 'polyclip', 'processx', 'profvis', 'progress',
##   'promises', 'ps', 'ragg', 'Rcpp', 'RcppArmadillo', 'RcppEigen', 'RCurl',
##   'readr', 'remotes', 'reprex', 'rlang', 'rmarkdown', 'roxygen2', 'rpart',
##   'RSQLite', 'rstudioapi', 'RUnit', 'rvest', 'sass', 'scales', 'scatterpie',
##   'seriation', 'shadowtext', 'shiny', 'stringi', 'survival', 'sys',
##   'systemfonts', 'testthat', 'textshaping', 'tidygraph', 'tidyr', 'tidyselect',
##   'tidytree', 'timechange', 'tinytex', 'tweenr', 'usethis', 'uuid', 'vctrs',
##   'vegan', 'viridis', 'vroom', 'waldo', 'withr', 'xfun', 'XML', 'xml2',
##   'xopen', 'yaml', 'yulab.utils', 'zip'
```

#install DOSE

```
if (!require("BiocManager", quietly = TRUE))
    install.packages("BiocManager")
BiocManager::install("DOSE")
```

```
## Bioconductor version 3.16 (BiocManager 1.30.22), R 4.2.2 (2022-10-31)

## Warning: package(s) not installed when version(s) same as or greater than current; use
##   `force = TRUE` to re-install: 'DOSE'

## Old packages: 'ape', 'aplot', 'askpass', 'backports', 'BH', 'BiocManager',
##   'bit', 'bit64', 'bitops', 'boot', 'brew', 'brio', 'broom', 'bslib', 'cachem',
##   'callr', 'cli', 'cluster', 'codetools', 'colorspace', 'commonmark',
##   'cowplot', 'cpp11', 'crayon', 'credentials', 'curl', 'data.table', 'DBI',
##   'dbplyr', 'dendextend', 'desc', 'digest', 'downlit', 'evaluate', 'fansi',
##   'farver', 'fastmap', 'foreign', 'fs', 'gert', 'ggforce', 'ggfun', 'ggh4x',
##   'ggnewscale', 'ggplot2', 'ggraph', 'ggrepel', 'gh', 'glue', 'graphlayouts',
##   'gtable', 'haven', 'highr', 'htmltools', 'htmlwidgets', 'httpuv', 'httr2',
##   'igraph', 'jsonlite', 'KernSmooth', 'knitr', 'later', 'lattice', 'markdown',
##   'mgcv', 'munsell', 'nlme', 'openssl', 'patchwork', 'pkgbuild', 'pkgdown',
##   'pkgload', 'plotly', 'polyclip', 'processx', 'profvis', 'progress',
##   'promises', 'ps', 'ragg', 'Rcpp', 'RcppArmadillo', 'RcppEigen', 'RCurl',
##   'readr', 'remotes', 'reprex', 'rlang', 'rmarkdown', 'roxygen2', 'rpart',
##   'RSQLite', 'rstudioapi', 'RUnit', 'rvest', 'sass', 'scales', 'scatterpie',
##   'seriation', 'shadowtext', 'shiny', 'stringi', 'survival', 'sys',
##   'systemfonts', 'testthat', 'textshaping', 'tidygraph', 'tidyr', 'tidyselect',
##   'tidytree', 'timechange', 'tinytex', 'tweenr', 'usethis', 'uuid', 'vctrs',
##   'vegan', 'viridis', 'vroom', 'waldo', 'withr', 'xfun', 'XML', 'xml2',
##   'xopen', 'yaml', 'yulab.utils', 'zip'
```

#If installed all start here

Load Libraries

```
library(clusterProfiler)
```

```
##

## Registered S3 methods overwritten by 'treeio':
##   method              from
##   MRCA.phylo          tidytree
##   MRCA.treedata       tidytree
##   Nnode.treedata      tidytree
##   Ntip.treedata       tidytree
##   ancestor.phylo      tidytree
##   ancestor.treedata   tidytree
##   child.phylo         tidytree
##   child.treedata      tidytree
##   full_join.phylo     tidytree
##   full_join.treedata  tidytree
##   groupClade.phylo    tidytree
##   groupClade.treedata tidytree
##   groupOTU.phylo      tidytree
##   groupOTU.treedata   tidytree
##   is.rooted.treedata  tidytree
##   nodeid.phylo        tidytree
##   nodeid.treedata     tidytree
##   nodelab.phylo       tidytree
##   nodelab.treedata    tidytree
##   offspring.phylo     tidytree
##   offspring.treedata  tidytree
```

```
##   parent.phylo        tidytree
##   parent.treedata     tidytree
##   root.treedata       tidytree
##   rootnode.phylo      tidytree
##   sibling.phylo       tidytree
```

```
## clusterProfiler v4.6.2  For help: https://yulab-smu.top/biomedical-knowledge-mining-book/
##
## If you use clusterProfiler in published research, please cite:
## T Wu, E Hu, S Xu, M Chen, P Guo, Z Dai, T Feng, L Zhou, W Tang, L Zhan, X Fu, S Liu, X Bo, and G Yu.
```

```
##
## Attaching package: 'clusterProfiler'
```

```
## The following object is masked from 'package:stats':
##
##     filter
```

```
library(ggplot2)
```

Load Organism Annotation Data

```
organism = "org.Hs.eg.db"
library(organism, character.only = TRUE)
```

```
## Loading required package: AnnotationDbi
```

```
## Loading required package: stats4
```

```
## Loading required package: BiocGenerics
```

```
##
## Attaching package: 'BiocGenerics'
```

```
## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs
```

```
## The following objects are masked from 'package:base':
##
##     anyDuplicated, aperm, append, as.data.frame, basename, cbind,
##     colnames, dirname, do.call, duplicated, eval, evalq, Filter, Find,
##     get, grep, grepl, intersect, is.unsorted, lapply, Map, mapply,
##     match, mget, order, paste, pmax, pmax.int, pmin, pmin.int,
##     Position, rank, rbind, Reduce, rownames, sapply, setdiff, sort,
##     table, tapply, union, unique, unsplit, which.max, which.min
```

```
## Loading required package: Biobase
```

```
## Welcome to Bioconductor
##
##     Vignettes contain introductory material; view with
##     'browseVignettes()'. To cite Bioconductor, see
##     'citation("Biobase")', and for packages 'citation("pkgname")'.
```

```
## Loading required package: IRanges
```

```
## Loading required package: S4Vectors
```

```
##
## Attaching package: 'S4Vectors'
```

```
## The following object is masked from 'package:clusterProfiler':
##
##     rename

## The following objects are masked from 'package:base':
##
##     expand.grid, I, unname

##
## Attaching package: 'IRanges'

## The following object is masked from 'package:clusterProfiler':
##
##     slice

##
## Attaching package: 'AnnotationDbi'

## The following object is masked from 'package:clusterProfiler':
##
##     select

##
```

Read Differential Expression Data

```
df<-read.csv("/Users/jeffreyreina/Documents/Salk/RNAseq MDA-MB-231 results/03.Result_X202SC23073852-Z01-
```

Prepare Gene List for Enrichment Analysis

```
df$ENSEMBL<-rownames(df) ## Create a new column for ENSEMBL gene IDs
```

Organize data frame

```
# Extract log2 fold change values
original_gene_list <- df$log2FoldChange
names(original_gene_list) <- df$ENSEMBL

# Remove any NA values and sort by decreasing order
gene_list<-na.omit(original_gene_list)
gene_list = sort(gene_list, decreasing = TRUE)
```

Check Available Key Types

```
keytypes(org.Hs.eg.db)
```

```
##  [1] "ACCNUM"       "ALIAS"        "ENSEMBL"      "ENSEMBLPROT"  "ENSEMBLTRANS"
##  [6] "ENTREZID"     "ENZYME"       "EVIDENCE"     "EVIDENCEALL"  "GENENAME"
## [11] "GENETYPE"     "GO"           "GOALL"        "IPI"          "MAP"
## [16] "OMIM"         "ONTOLOGY"     "ONTOLOGYALL"  "PATH"         "PFAM"
## [21] "PMID"         "PROSITE"      "REFSEQ"       "SYMBOL"       "UCSCKG"
## [26] "UNIPROT"
```

Run Gene Set Enrichment Analysis (GSEA) Biological Process

```
gse <- gseGO(geneList=gene_list,
             ont ="BP", # Biological Process ontology
             keyType = "ENSEMBL",
             nPerm = 10000,
             minGSSize = 10,
             maxGSSize = 800,
```

```
              pvalueCutoff = 1,
              verbose = TRUE,
              OrgDb = organism,
              pAdjustMethod = "none")
```

```
## preparing geneSet collections...
```

```
## GSEA analysis...
```

```
## Warning in .GSEA(geneList = geneList, exponent = exponent, minGSSize =
## minGSSize, : We do not recommend using nPerm parameter incurrent and future
## releases
```

```
## Warning in fgsea(pathways = geneSets, stats = geneList, nperm = nPerm, minSize
## = minGSSize, : You are trying to run fgseaSimple. It is recommended to use
## fgseaMultilevel. To run fgseaMultilevel, you need to remove the nperm argument
## in the fgsea function call.
```

```
## Warning in preparePathwaysAndStats(pathways, stats, minSize, maxSize, gseaParam, : There are ties in
## The order of those tied genes will be arbitrary, which may produce unexpected results.
```

```
## leading edge analysis...
```

```
## done...
```

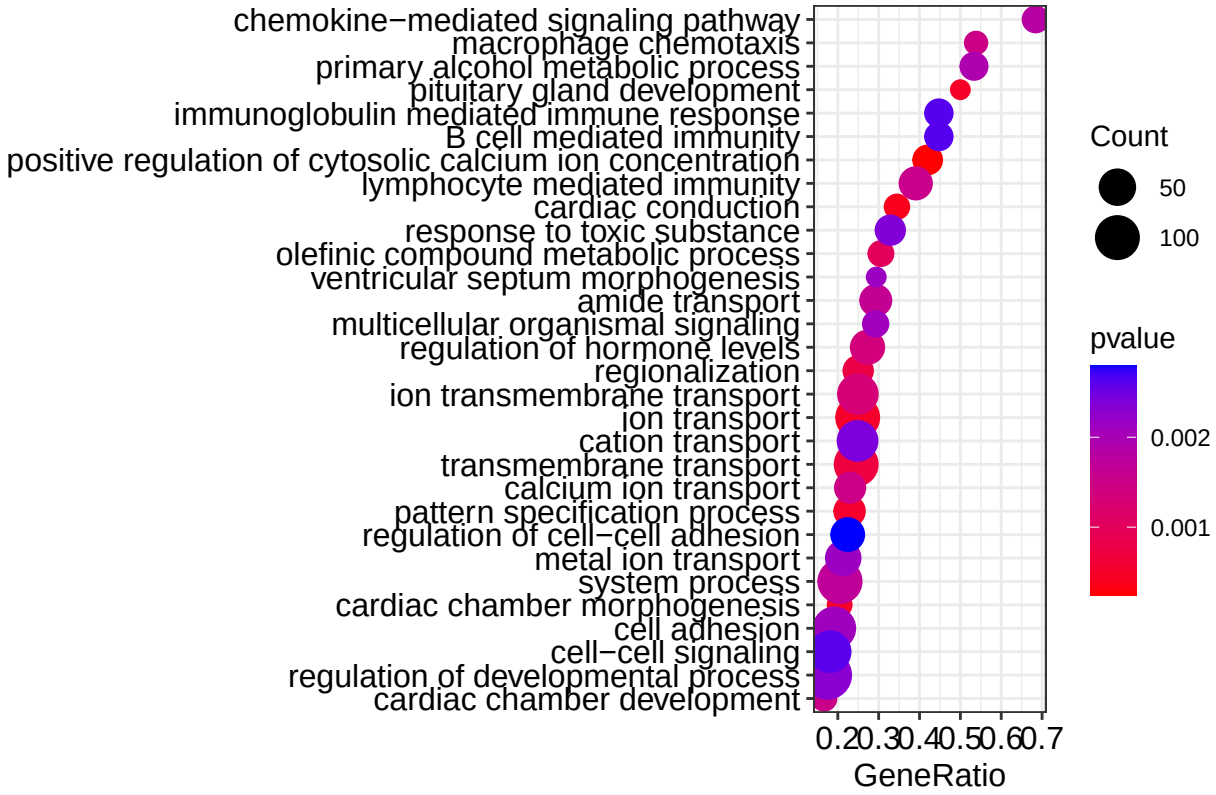
Plotting Enrichment Results

```
require(DOSE)
```

```
## Loading required package: DOSE
```

```
## DOSE v3.24.2  For help: https://yulab-smu.top/biomedical-knowledge-mining-book/
##
## If you use DOSE in published research, please cite:
## Guangchuang Yu, Li-Gen Wang, Guang-Rong Yan, Qing-Yu He. DOSE: an R/Bioconductor package for Disease
```

```
dotplot(gse,
  x = "GeneRatio",
  color = "pvalue",
  showCategory = 30,##how many categories to show
  size = NULL,
  split = NULL,
  font.size = 12,
  title = "",
  orderBy = "x",
  label_format = 60,
  decreasing = TRUE
)
```



Save Plot to PDF

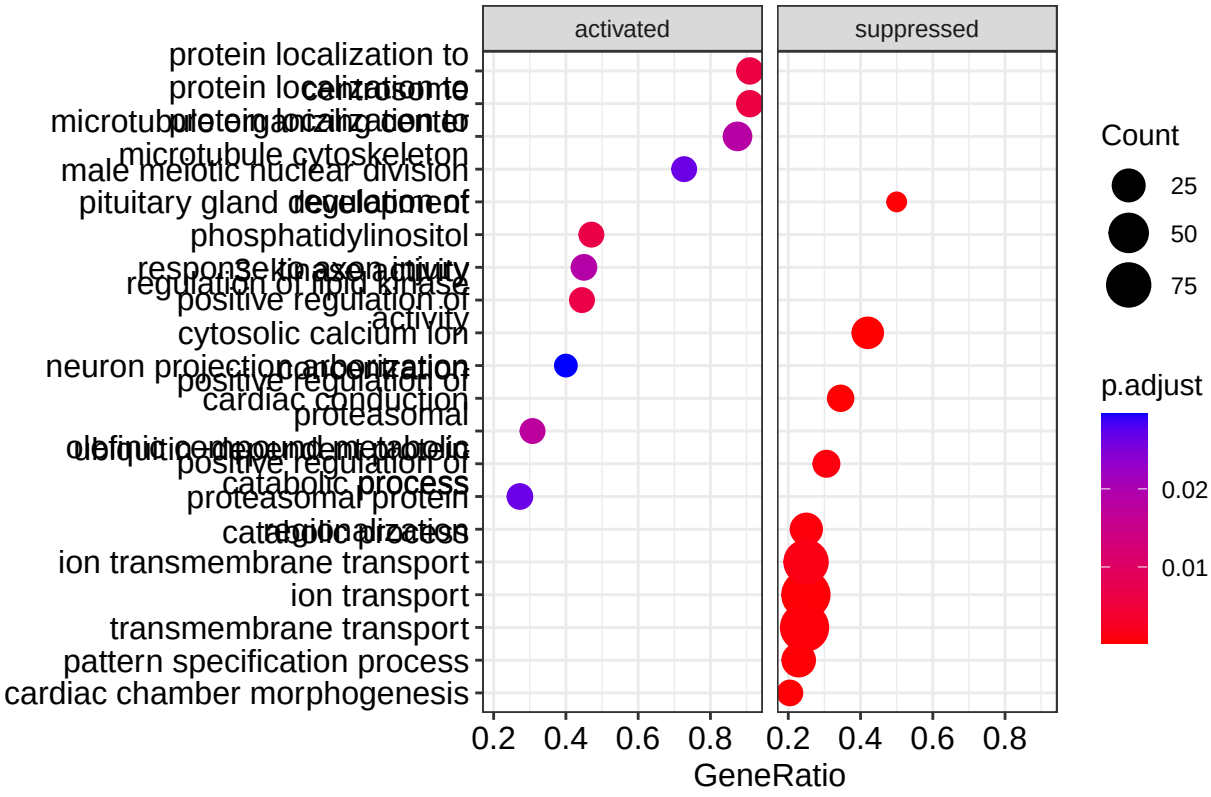
```
pdf("EnrichmentRNAseqKOvsWT.pdf", width=10, height=8)

require(DOSE)
dotplot(gse,
  x = "GeneRatio",
  color = "pvalue",
  showCategory = 30,##how many categories to show
  size = NULL,
  split = NULL,
  font.size = 12,
  title = "",
  label_format = 70,
)
```

Save Results to CSV

```
resultsgseGO<-data.frame(gse@result)
write.csv(resultsgseGO, "resultsgeGOfinal.csv")
```

Alternative Plot: Split by Direction

```
require(DOSE)
dotplot(gse, showCategory=10, split=".sign") + facet_grid(.~.sign)
```


activated
suppressed
protein localization to
protein localization to
microtubule organization
microtubule cytoskeleton
male meiotic nuclear division
pituitary gland development
phosphatidylinositol
regulation of lipid kinase
positive regulation of
cytosolic calcium ion
neuron projection morphogenesis
positive regulation of
cardiac conduction
proteasomal
ubiquitin-dependent
positive regulation of
catabolic process
proteasomal protein
ion transmembrane transport
ion transport
transmembrane transport
pattern specification process
cardiac chamber morphogenesis
0.2
0.4
0.6
0.8
GeneRatio
Count
25
50
75
p.adjust
0.02
0.01