

4-Gene Ontology

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Introduction

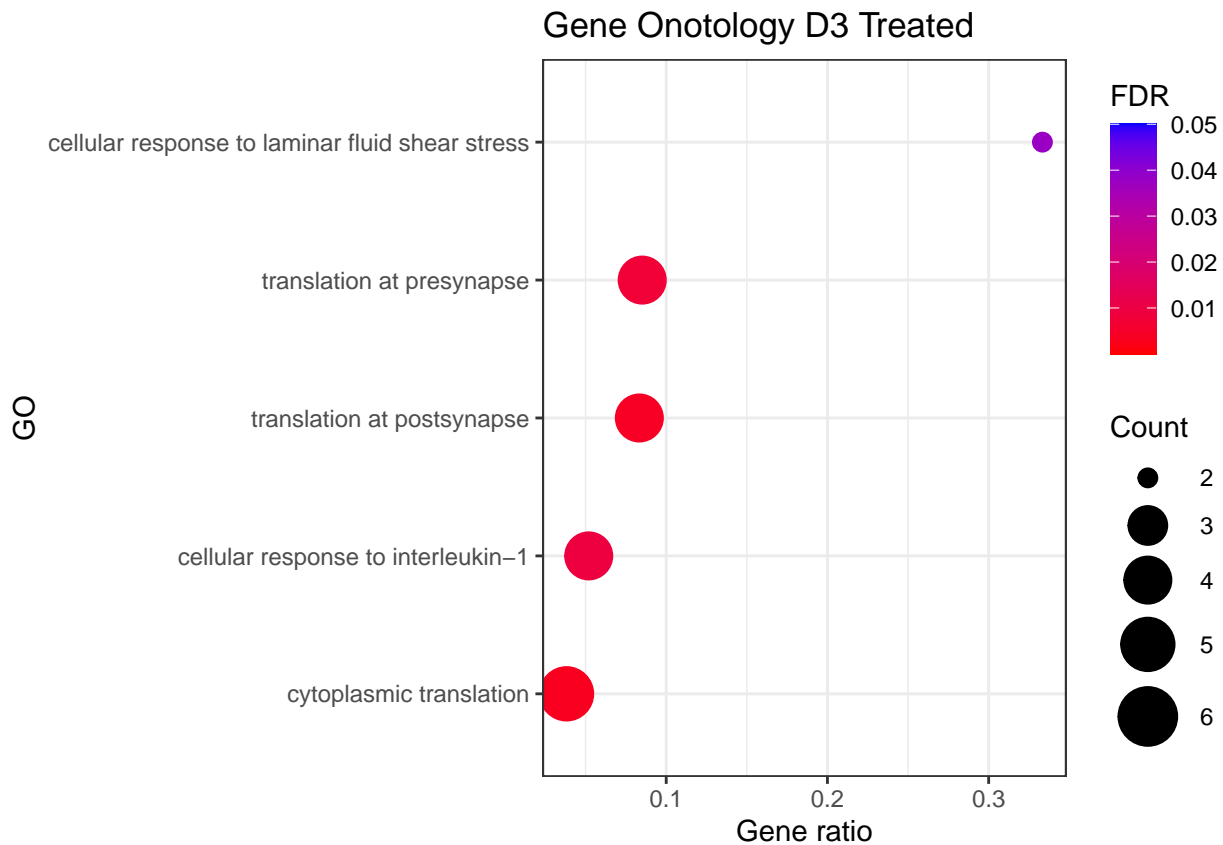
Gene Ontology (GO) enrichment analysis was performed using the web-based tool provided by the Gene Ontology Consortium (<http://geneontology.org/>) on 2025-06-25, using the GO database release from 2025-06-01. For each experimental condition, the top 30 differentially expressed genes ranked by their average log fold change were selected for GO analysis. Enrichment was assessed separately for each gene list, and results were obtained for the “Biological Process” category. Relevant enriched GO terms were visualized using the ggplot2 package. The gene ratio for each GO term was calculated as the number of enriched genes in the gene list divided by the total number of genes in a pathway.

Loading packages

```
suppressMessages({  
  library(dplyr)  
  library(Seurat)  
  library(patchwork)  
  library(ggplot2)  
  library(formatR)  
  library(xlsx)  
  library(tidyverse)  
})
```

Gene ontology at Day 3

```
# Loading gene geneontology.org  
GO_D3 <- read.table("GO_treated_D3.txt", skip = 11, header = T, sep = "\t")  
GO_D3 <- GO_D3[c(2, 4, 5, 7, 9), c(1:3, 6, 8)]  
colnames(GO_D3) <- c("GO", "REF", "Count", "FoldEnrichment", "FDR")  
GO_D3$`Gene ratio` <- GO_D3$Count/GO_D3$REF  
GO_D3$GO <- str_sub(GO_D3$GO, 1, str_length(GO_D3$GO) - 13)  
GO_D3$GO <- fct_rev(factor(GO_D3$GO, levels = GO_D3$GO))  
  
ggplot(GO_D3, aes(x = `Gene ratio`, y = GO, size = Count, color = FDR)) +  
  geom_point() + ggtitle("Gene Ontology D3 Treated") + scale_color_gradient(limit = c(9.03e-09,  
0.05), low = "red", high = "blue") + theme_bw() + scale_size(limit = c(2,  
6), range = c(3, 10))
```

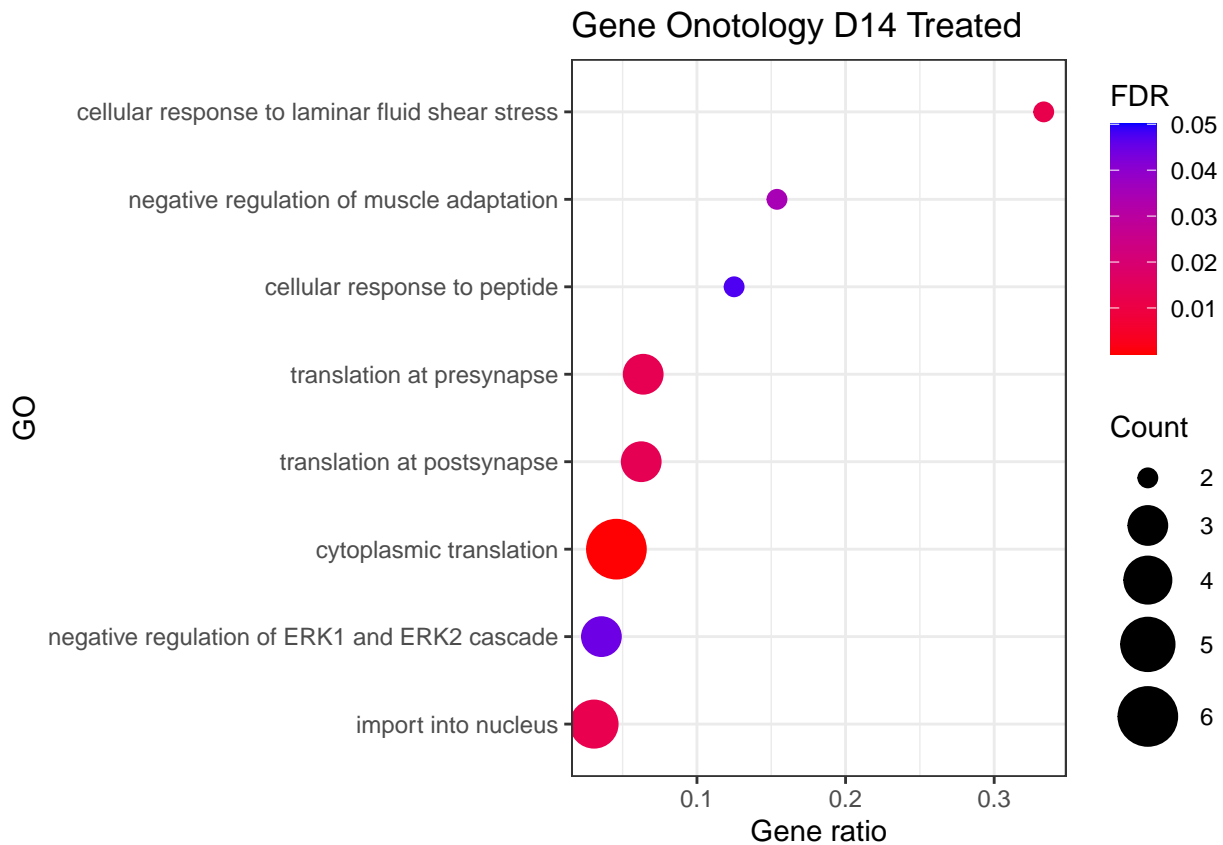


```
# ggsave('GO_J3_Treated.pdf', width = 8, height = 5)
```

Gene ontology at day 14

```
GO_D14 <- read.table("GO_treated_D14.txt", skip = 11, header = T,
  sep = "\t")
GO_D14 <- GO_D14[c(2, 5, 6, 8, 9, 11, 13, 16), c(1:3, 6, 8)]
colnames(GO_D14) <- c("GO", "REF", "Count", "FoldEnrichment", "FDR")
GO_D14$`Gene ratio` <- GO_D14$Count/GO_D14$REF
GO_D14$GO <- str_sub(GO_D14$GO, 1, str_length(GO_D14$GO) - 13)
GO_D14$GO <- fct_rev(factor(GO_D14$GO, levels = GO_D14$GO))

ggplot(GO_D14, aes(x = `Gene ratio`, y = GO, size = Count, color = FDR)) +
  geom_point() + ggtitle("Gene Ontology D14 Treated") + scale_color_gradient(limit = c(9.03e-09,
    0.05), low = "red", high = "blue") + theme_bw() + scale_size(limit = c(2,
    6), range = c(3, 10))
```

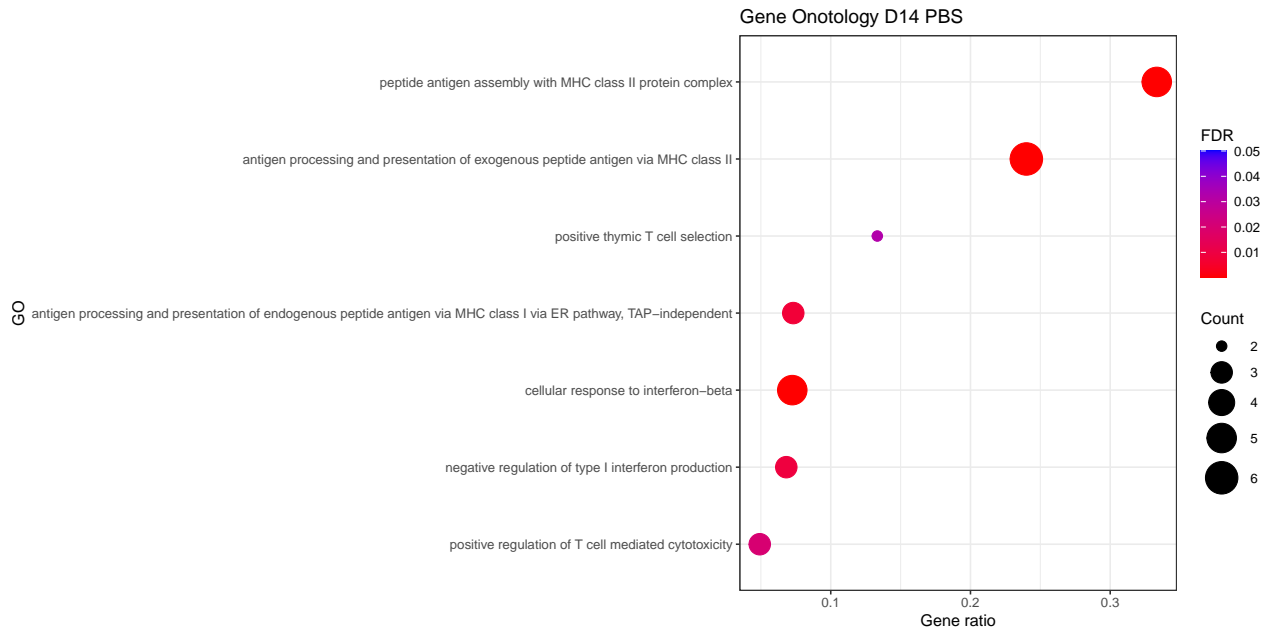


```
# ggsave('GO_J14_Treated.pdf', width = 8, height = 5)
```

Gene ontology for PBS cells

```
GO_PBS <- read.table("GO_treated_D14_PBS.txt", skip = 11, header = T,
  sep = "\t")
GO_PBS <- GO_PBS[c(4, 9, 16, 27, 28, 32, 39), c(1:3, 6, 8)]
colnames(GO_PBS) <- c("GO", "REF", "Count", "FoldEnrichment", "FDR")
GO_PBS$`Gene ratio` <- GO_PBS$Count/GO_PBS$REF
GO_PBS$GO <- str_sub(GO_PBS$GO, 1, str_length(GO_PBS$GO) - 13)
GO_PBS$GO <- fct_rev(factor(GO_PBS$GO, levels = GO_PBS$GO))

ggplot(GO_PBS, aes(x = `Gene ratio`, y = GO, size = Count, color = FDR)) +
  geom_point() + ggtitle("Gene Ontology D14 PBS") + scale_color_gradient(limit = c(1.03e-09,
  0.05), low = "red", high = "blue") + theme_bw() + scale_size(limit = c(2,
  6), range = c(3, 10))
```



```
# ggsave('GO_D14_PBS.pdf', width = 8, height = 5)
```

```
sessionInfo()
```

```
## R version 4.3.3 (2024-02-29)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 24.04.2 LTS
##
## Matrix products: default
## BLAS:   /usr/lib/x86_64-linux-gnu/openblas-pthread/libblas.so.3
## LAPACK: /usr/lib/x86_64-linux-gnu/openblas-pthread/libopenblas-p-r0.3.26.so; LAPACK version 3.12.0
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8      LC_NUMERIC=C
##  [3] LC_TIME=fr_BE.UTF-8      LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=fr_BE.UTF-8  LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=fr_BE.UTF-8     LC_NAME=fr_BE.UTF-8
##  [9] LC_ADDRESS=fr_BE.UTF-8   LC_TELEPHONE=fr_BE.UTF-8
## [11] LC_MEASUREMENT=fr_BE.UTF-8 LC_IDENTIFICATION=fr_BE.UTF-8
##
## time zone: Europe/Brussels
## tzcode source: system (glibc)
##
## attached base packages:
## [1] stats      graphics  grDevices  utils      datasets  methods    base
##
## other attached packages:
##  [1] lubridate_1.9.2   forcats_1.0.0   stringr_1.5.0   purrr_1.0.1
##  [5] readr_2.1.4      tidyr_1.3.0     tibble_3.2.1    tidyverse_2.0.0
##  [9] xlsx_0.6.5       formatR_1.14    ggplot2_3.4.2   patchwork_1.1.2
## [13] SeuratObject_4.1.3 Seurat_4.3.0    dplyr_1.1.2
##
## loaded via a namespace (and not attached):
##  [1] RColorBrewer_1.1-3  rstudioapi_0.14  jsonlite_1.8.7
```

```

## [4] magrittr_2.0.3      spatstat.utils_3.0-3  farver_2.1.1
## [7] rmarkdown_2.23      vctrs_0.6.3          ROCR_1.0-11
## [10] spatstat.explore_3.2-1  htmltools_0.5.5      sctransform_0.3.5
## [13] parallelly_1.36.0     KernSmooth_2.23-22   htmlwidgets_1.6.2
## [16] ica_1.0-3            plyr_1.8.8           plotly_4.10.2
## [19] zoo_1.8-12           igraph_1.5.0.1       mime_0.12
## [22] lifecycle_1.0.3      pkgconfig_2.0.3      Matrix_1.6-1
## [25] R6_2.5.1             fastmap_1.1.1        fitdistrplus_1.1-11
## [28] future_1.33.0        shiny_1.7.4.1        digest_0.6.33
## [31] colorspace_2.1-0     tensor_1.5           irlba_2.3.5.1
## [34] labeling_0.4.2       progressr_0.13.0     timechange_0.2.0
## [37] fansi_1.0.4          spatstat.sparse_3.0-2 httr_1.4.6
## [40] polyclip_1.10-4      abind_1.4-5          compiler_4.3.3
## [43] withr_2.5.0          highr_0.10           MASS_7.3-60.0.1
## [46] tools_4.3.3          lmtest_0.9-40        httpuv_1.6.11
## [49] future.apply_1.11.0  goftest_1.2-3        glue_1.6.2
## [52] nlme_3.1-164         promises_1.2.0.1     grid_4.3.3
## [55] Rtsne_0.16           cluster_2.1.6        reshape2_1.4.4
## [58] generics_0.1.3       gtable_0.3.3         spatstat.data_3.0-1
## [61] tzdb_0.4.0           data.table_1.14.8    hms_1.1.3
## [64] sp_2.2-0             utf8_1.2.3           spatstat.geom_3.2-4
## [67] RcppAnnoy_0.0.21     ggrepel_0.9.3        RANN_2.6.1
## [70] pillar_1.9.0         spam_2.9-1           later_1.3.1
## [73] rJava_1.0-6          splines_4.3.3        lattice_0.22-5
## [76] survival_3.5-8       deldir_1.0-9         tidysselect_1.2.0
## [79] miniUI_0.1.1.1       pbapply_1.7-2        knitr_1.43
## [82] gridExtra_2.3        scattermore_1.2      xfun_0.39
## [85] matrixStats_1.0.0    stringi_1.8.4        lazyeval_0.2.2
## [88] yaml_2.3.7           evaluate_0.21        codetools_0.2-19
## [91] xlsxjars_0.6.1       cli_3.6.1            uwot_0.1.16
## [94] xtable_1.8-4         reticulate_1.30      munsell_0.5.0
## [97] Rcpp_1.0.11          globals_0.16.2       spatstat.random_3.1-5
## [100] png_0.1-8            parallel_4.3.3       ellipsis_0.3.2
## [103] dotCall64_1.0-2     listenv_0.9.0        viridisLite_0.4.2
## [106] scales_1.2.1         gggridges_0.5.4     leiden_0.4.3
## [109] rlang_1.1.1          cowplot_1.1.1

```