ubi-Brk_3h

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2024-04-15

Introduction

This is RNAseq analysis using deseq2. Two conditions: "control" and "ubi-brk", 3 repeats for each condition.

Experimental setup:

SalE/PV-ShineGal4>Brk(x) – dark control or "control" in this analysis;

ubi-ShineGal4>Brk(x) - 3h light induction of UAS-Brk overexpression, called "ubi-brk" here;

SalE/PV-ShineGal4>Brk(x) - 3h ight induction of UAS-Brk overexpression in Sal compartment - not included in this analysis due to low number of def expressed genes.

All conditions were processed at the same time with several rounds of sample collection (different days, several crosses). For each repeat 50 discs were combined in one tube (could use up to 35 next time).

Preparing the data

Loading libraries

```
library(DESeq2)
library(apeglm)
library(dplyr)
library(tibble)
library(tidyverse)
library(data.table)
library(ggplot2)
library(ggfortify)
library(ggrepel)
library(RColorBrewer)
library(pheatmap)
library(plotly)
library(magrittr)
library(AnnotationDbi)
library(org.Dm.eg.db)
library(writexl)
```

Load data

```
counts <- fread(file ="rsem.merged.gene_counts.tsv", header=TRUE)
sample_info <- read.delim(file = "Data info/RN23010-sampleinfo_full.txt")
```

Prepare a matrix for DESeq:

Make first column row names, switch to integers, create a matrix object

```
info<-sample_info %>% column_to_rownames("Sample")
info %>%  filter(Group !='sal-brk') ->ninfo

gcounts <- counts[, -c(2,6:8)]

dt <- as.data.frame(gcounts) %>%
  column_to_rownames("gene_id")

dt[,1:6] <- lapply(dt[,1:6], as.integer)

mcountdata <- as.matrix(dt)

has_rownames(dt)
```

```
## [1] TRUE
```

```
has_rownames(ninfo)
```

```
## [1] TRUE
```

Matching column order between the tables

```
all(rownames(ninfo) == colnames(mcountdata))
```

```
## [1] FALSE
```

```
mcountdata_s <- mcountdata[, rownames(ninfo)]
all(rownames(ninfo) == colnames(mcountdata_s))
```

```
## [1] TRUE
```

Creating levels for samples.

It will read the first level to be compared with the rest.

```
ninfo$Group <- factor(ninfo$Group,levels = c("control", "ubi-brk"))
info$Repeat <- as.factor(info$Repeat)
```

Run deseq.

The object class used by the DESeq2 package to store the read counts and the intermediate estimated quantities during statistical analysis is the **DESeqDataSet**, which will usually be represented in the code here as an object **dds**.

```
dds <- DESeqDataSetFromMatrix(countData = mcountdata_s,
                              colData = ninfo,
                              design = ~Group)
dds <- DESeq(dds)
res <- results(dds)
sum(res$padj < 0.01 & res$log2FoldChange< (-0.3), na.rm=TRUE)
```

```
## [1] 576
```

```
resultsNames(dds)
```

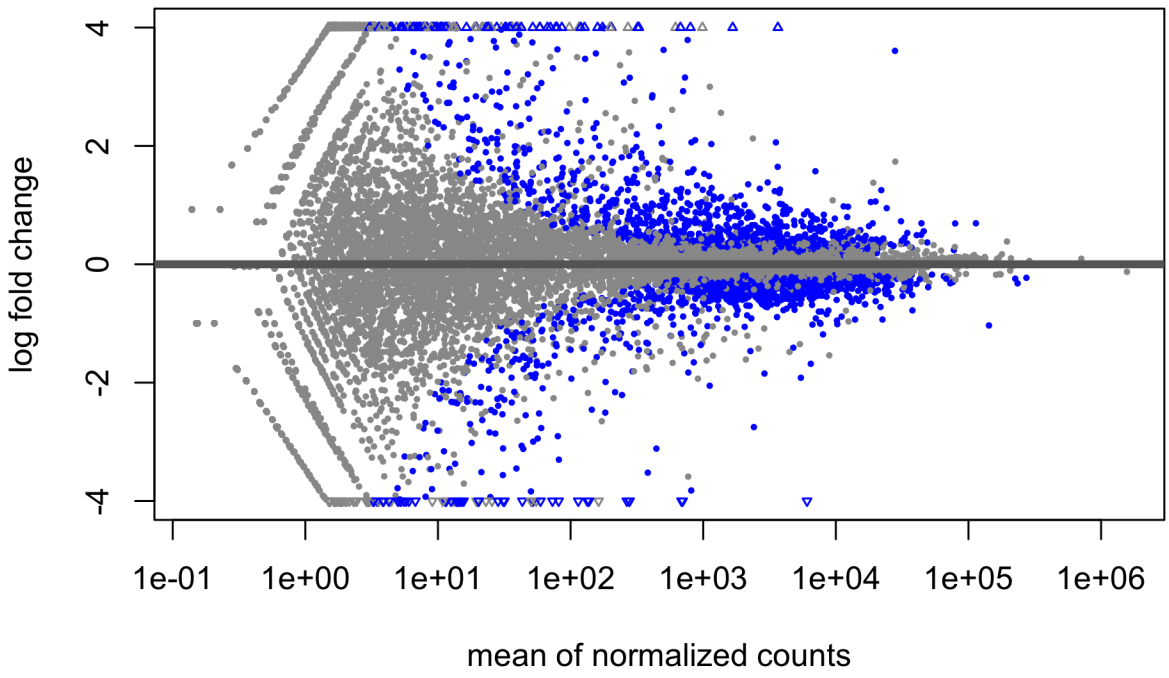
```
## [1] "Intercept"                "Group_ubi.brk_vs_control"
```

Data shrinkage to remove low count data

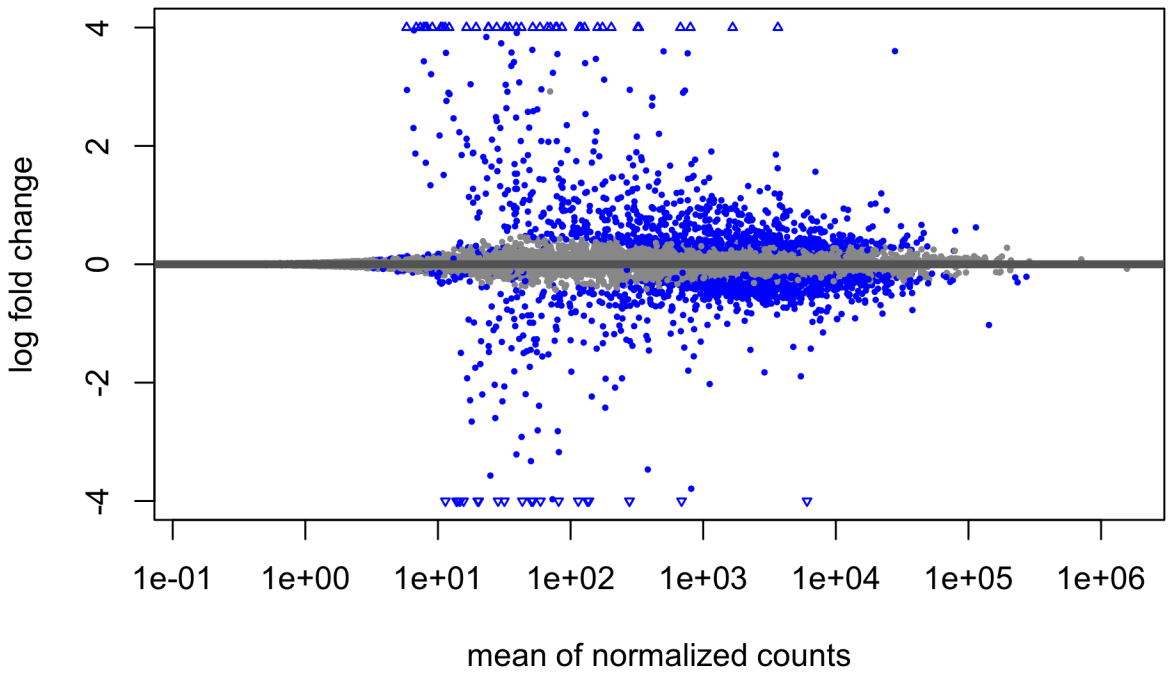
```
ubiLFC <- lfcShrink(dds, coef="Group_ubi.brk_vs_control", type="apeglm")
```

```
## using 'apeglm' for LFC shrinkage. If used in published research, please cite:
##     Zhu, A., Ibrahim, J.G., Love, M.I. (2018) Heavy-tailed prior distributions for
##     sequence count data: removing the noise and preserving large differences.
##     Bioinformatics. https://doi.org/10.1093/bioinformatics/bty895
```

```
plotMA(res, ylim=c(-4,4))
```




```
plotMA(ubiLFC, ylim=c(-4,4))
```



Converting ubiLFC to a table format with gene names added

```
ubiLFC %>%
  as.data.frame() %>%
  mutate(., gene = mapIds(
  org.Dm.eg.db,
  keys = rownames(.),
  column ="SYMBOL",
  keytype = "FLYBASE",
  multiVals = "first"
)) %>%
  rownames_to_column(.) %>%
    mutate(gene = coalesce(gene,rowname)) %>%
  rename(., ID=rowname) -> ubiLFC_genes
```

```
## 'select()' returned 1:1 mapping between keys and columns
```

Filtering significantly diff expressed genes

Padj<001, basemean>100, and arranging according to log2 fold change

```
ubiLFC_genes %>%
  filter((padj<0.01) & (baseMean>100)) %>%
  arrange(log2FoldChange) -> ubiLFC_s
#write_xlsx(ubiLFC_s, "ubi-brk-signif.xlsx")
```

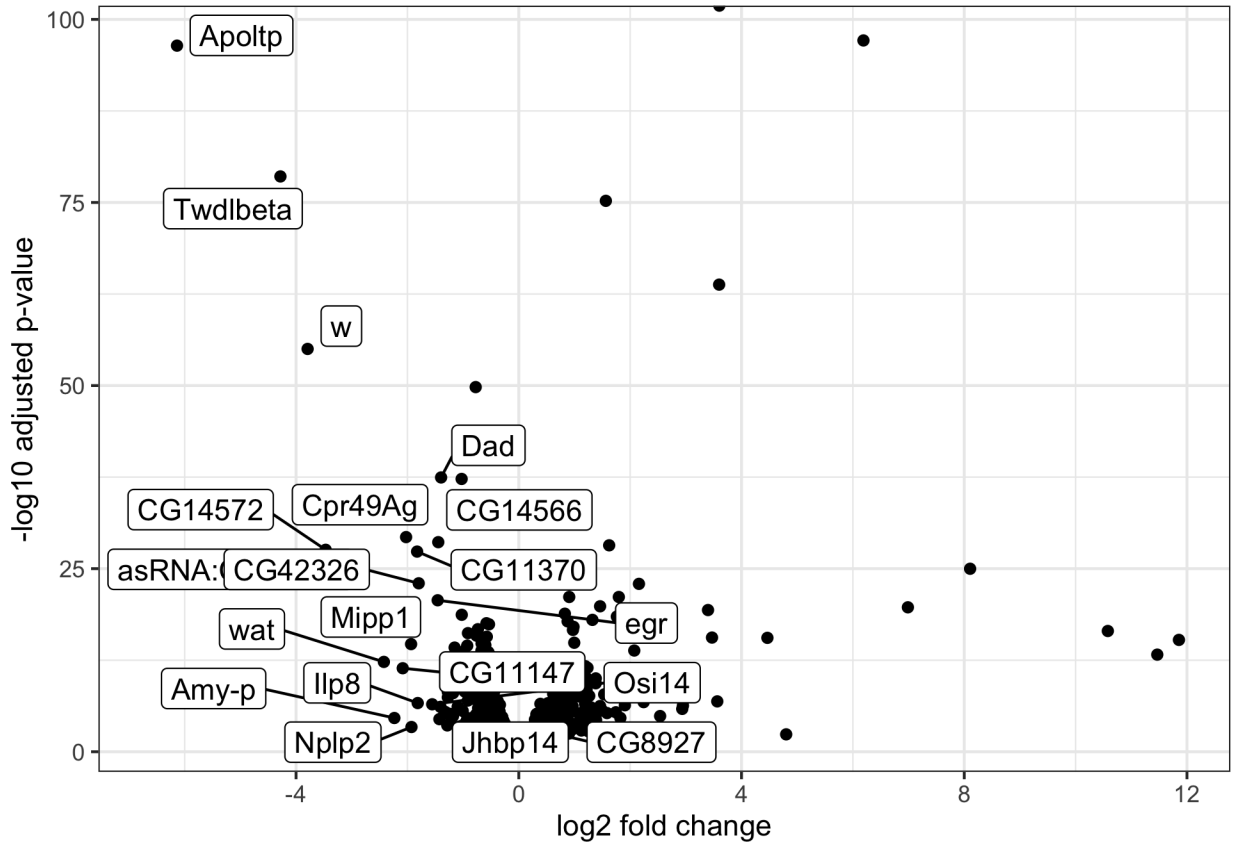
Removing transposon genes

They are present due to differences in cell lines, as I didnt see them in Sal comparison

```
ubiLFC_s %>%
  filter(!stringr::str_detect(ID, "FBti")) ->ubiLFC_s_pel.rm
```

Looking at voclano plot

```
ggplot(ubiLFC_s_pel.rm, aes(log2FoldChange, -log10(padj))) +
  geom_point() +
  xlab("log2 fold change") +
        ylab("-log10 adjusted p-value") +
  theme(legend.position = "none",
              plot.title = element_text(size = rel(1.5), hjust = 0.5),
              axis.title = element_text(size = rel(1.25))) +
  theme_bw() +
  geom_label_repel(data=head(ubiLFC_s_pel.rm, 20), aes(label=gene), max.overlaps=30)
```



Gene set enrichment analysis

```
# we want the log2 fold change
original_gene_list <- ubiLFC_s_pel.rm$log2FoldChange

# name the vector
names(original_gene_list) <- ubiLFC_s_pel.rm$gene
```

```r
# omit any NA values
gene_list<-na.omit(original_gene_list)

# sort the list in decreasing order (required for clusterProfiler)
gene_list = sort(gene_list, decreasing = TRUE)
```

```r
gse<- gseGO (gene_list,
             OrgDb=org.Dm.eg.db,
             ont="BP",
             keyType="SYMBOL",
             pvalueCutoff = 0.05,
             verbose = TRUE,
             minGSSize=3,
             pAdjustMethod = "BH")
```

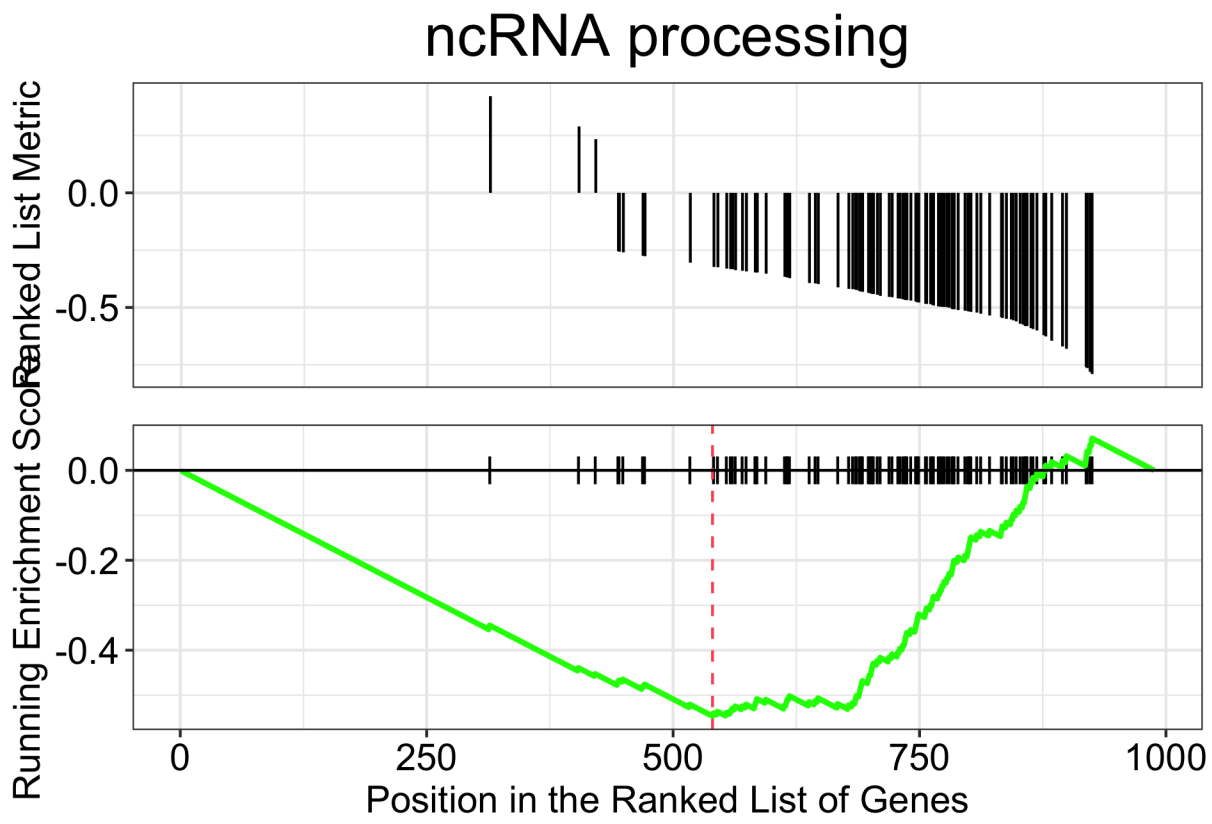
```
## using 'fgsea' for GSEA analysis, please cite Korotkevich et al (2019).

## preparing geneSet collections...

## GSEA analysis...

## 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.

## Warning in fgseaMultilevel(pathways = pathways, stats = stats, minSize =
## minSize, : There were 1 pathways for which P-values were not calculated
## properly due to unbalanced (positive and negative) gene-level statistic values.
## For such pathways pval, padj, NES, log2err are set to NA. You can try to
## increase the value of the argument nPermSimple (for example set it nPermSimple
## = 10000)

## Warning in fgseaMultilevel(pathways = pathways, stats = stats, minSize =
## minSize, : For some of the pathways the P-values were likely overestimated. For
## such pathways log2err is set to NA.

## Warning in fgseaMultilevel(pathways = pathways, stats = stats, minSize =
## minSize, : For some pathways, in reality P-values are less than 1e-10. You can
## set the `eps` argument to zero for better estimation.

## leading edge analysis...

## done...
```

```r
summary(gse) %>% as.tibble() -> gse_sum
```

```
## Warning: `as.tibble()` was deprecated in tibble 2.0.0.
## i Please use `as_tibble()` instead.
## i The signature and semantics have changed, see `?as_tibble`.
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_lifecycle_warnings()` to see where this warning was
## generated.
```

```
## Warning in summary(gse): summary method to convert the object to data.frame is
## deprecated, please use as.data.frame instead.
```

```
gseaplot(gse, by = "all", title = gse$Description[1], geneSetID = 1)
```



slim1-more general, slim2 more specific

```
summary(gse) %>% as.tibble() -> gse_sum
```

```
## Warning in summary(gse): summary method to convert the object to data.frame is
## deprecated, please use as.data.frame instead.
```

```
gse_sum %>% filter(p.adjust<0.05)
```

```
## # A tibble: 15 x 11
##    ID        Description setSize enrichmentScore   NES   pvalue p.adjust  qvalue
##    <chr>     <chr>         <int>           <dbl> <dbl>    <dbl>    <dbl>   <dbl>
##  1 GO:00344~ ncRNA proc~     103          -0.547 -3.04 1   e-10  2.88e-8 2.88e-8
##  2 GO:00422~ ribosome b~     107          -0.535 -2.98 1   e-10  2.88e-8 2.88e-8
##  3 GO:00226~ ribonucleo~     112          -0.521 -2.94 1   e-10  2.88e-8 2.88e-8
##  4 GO:00160~ rRNA metab~      94          -0.533 -2.90 1   e-10  2.88e-8 2.88e-8
##  5 GO:00063~ RNA proces~     117          -0.498 -2.82 1   e-10  2.88e-8 2.88e-8
##  6 GO:00346~ ncRNA meta~     119          -0.485 -2.76 1   e-10  2.88e-8 2.88e-8
##  7 GO:00063~ rRNA proce~      86          -0.537 -2.87 1.77e-10  4.37e-8 4.37e-8
##  8 GO:00346~ cellular n~     348          -0.264 -1.73 4.95e- 7  1.07e-4 1.07e-4
```

```
##  9 GO:00440~ cellular c~     193          -0.333 -2.02 5.77e- 7  1.11e-4 1.11e-4
## 10 GO:00464~ heterocycl~     327          -0.261 -1.71 2.79e- 6  4.83e-4 4.83e-4
## 11 GO:00422~ ribosomal ~      36          -0.595 -2.56 4.19e- 6  6.31e-4 6.31e-4
## 12 GO:00067~ cellular a~     331          -0.257 -1.65 4.37e- 6  6.31e-4 6.31e-4
## 13 GO:19013~ organic cy~     332          -0.255 -1.62 7.59e- 6  1.01e-3 1.01e-3
## 14 GO:00061~ nucleobase~     320          -0.252 -1.67 3.11e- 5  3.85e-3 3.84e-3
## 15 GO:00903~ nucleic ac~     288          -0.252 -1.61 7.75e- 5  8.93e-3 8.93e-3
## # i 3 more variables: rank <dbl>, leading_edge <chr>, core_enrichment <chr>
```

Correct way to do GSEA is to use it on the whole dataset, and then rank according FC*pvalue

```
ubiLFC_genes %>%
  mutate(rank_stats=log2FoldChange*-log10(padj)) %>%
  arrange(desc(rank_stats)) %>%
  filter(!stringr::str_detect(ID, "FBti") & (baseMean>100)) %>%
  na.omit() %>%
  select(gene, rank_stats) %>%
  mutate(rank_stats=ifelse(is.infinite(rank_stats), 1000, rank_stats)) %>%
  deframe()-> ubiLFC_rank
```

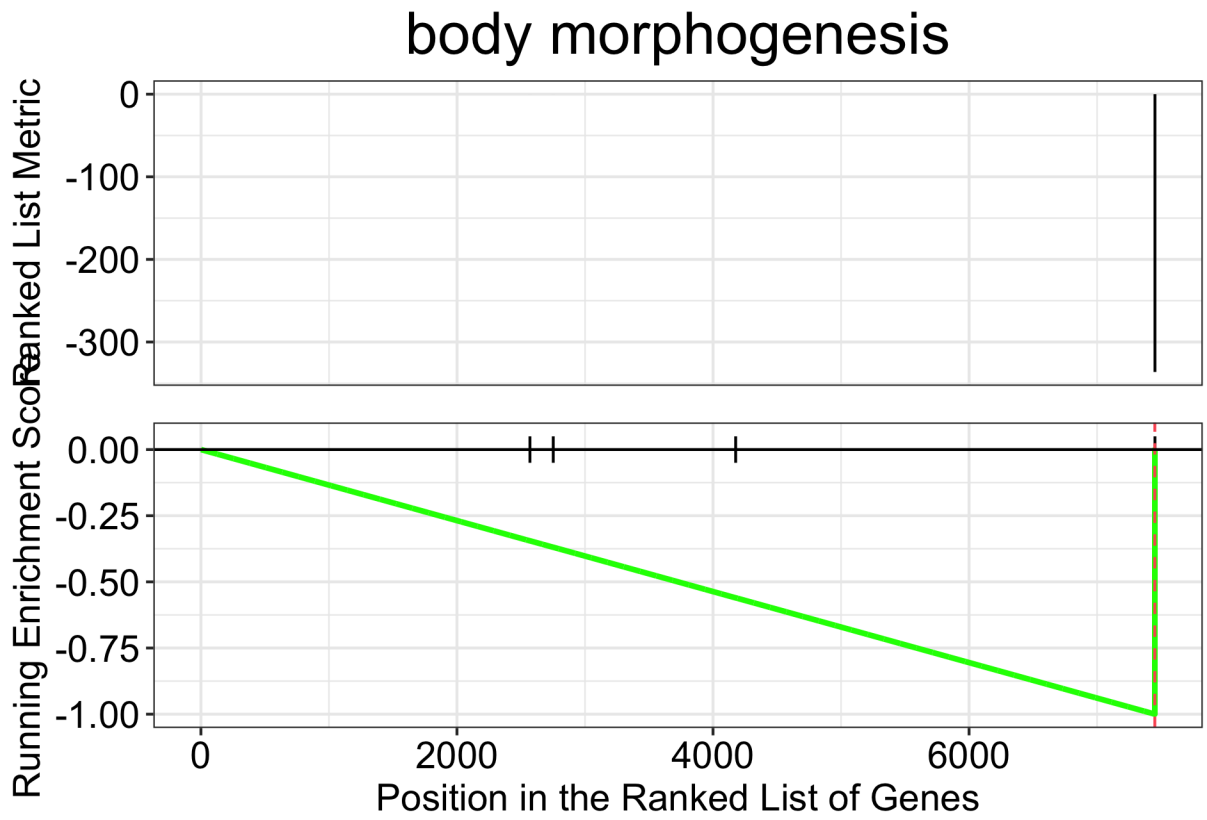
```
gse_cor <- gseGO(ubiLFC_rank,
                 ont="BP",
                 OrgDb=org.Dm.eg.db,
                 keyType="SYMBOL",
                 pvalueCutoff = 0.5,
                 verbose = TRUE,
                 minGSSize=3,
                 pAdjustMethod = "BH")
```

```
## using 'fgsea' for GSEA analysis, please cite Korotkevich et al (2019).

## preparing geneSet collections...

## GSEA analysis...

## 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...
```

```
summary(gse_cor) %>% as.tibble() -> gse_cor.sum
```

```
## Warning in summary(gse_cor): summary method to convert the object to data.frame
## is deprecated, please use as.data.frame instead.
```

```
gseaplot(gse_cor, by = "all", title = gse_cor$Description[1], geneSetID = 1)
```




```
intersect(names(ubiLFC_rank), names(gene_list))
```

```
##   [1] "brk"                       "CG3097"
##   [3] "l(2)03659"                 "Cyp6a17"
##   [5] "CG31601"                   "mthl8"
##   [7] "Gr64a"                     "CG18278"
##   [9] "CG7675"                    "CG32318"
##  [11] "CG30059"                   "CG11594"
##  [13] "CG43333"                   "tinc"
##  [15] "ZnT77C"                    "CG13954"
##  [17] "bab2"                      "CG11498"
##  [19] "CG18853"                   "CG12159"
##  [21] "Cpr49Ac"                   "CG32500"
##  [23] "tun"                       "CG14257"
##  [25] "Cht5"                      "Cpr51A"
##  [27] "MESR3"                     "CG4374"
##  [29] "CG43394"                   "mthl3"
##  [31] "esn"                       "Cyp4e2"
##  [33] "CG13737"                   "hid"
##  [35] "Nepl3"                     "bib"
##  [37] "CAH1"                      "Prx6b"
##  [39] "Ypel"                      "Eip78C"
##  [41] "AhcyL2"                    "Obp99a"
```

```
##  [43] "l(2)k05911"                "Dyro"
##  [45] "Sox102F"                   "Ppn"
##  [47] "TP53INP"                   "ImpE2"
##  [49] "rst"                       "CG14275"
##  [51] "ced-6"                     "CG32486"
##  [53] "ac"                        "Hsp23"
##  [55] "Cht10"                     "glec"
##  [57] "sc"                        "Sox14"
##  [59] "CG2938"                    "Prx6c"
##  [61] "comm"                      "lncRNA:CR33938"
##  [63] "E(spl)m7-HLH"              "CG13024"
##  [65] "CG6770"                    "CG33978"
##  [67] "thw"                       "CG7296"
##  [69] "Spn47C"                    "CG1273"
##  [71] "Phk-3"                     "firl"
##  [73] "tey"                       "whd"
##  [75] "wbl"                       "CG43051"
##  [77] "scyl"                      "CG10936"
##  [79] "nyo"                       "Hsp67Ba"
##  [81] "Appl"                      "p130CAS"
##  [83] "sca"                       "CG14567"
##  [85] "Tl"                        "CG10211"
##  [87] "E(spl)m6-BFM"              "bip1"
##  [89] "Adk3"                      "Obp56d"
##  [91] "NT1"                       "pdm2"
##  [93] "comm2"                     "frm"
##  [95] "trn"                       "smal"
##  [97] "ome"                       "CG11686"
##  [99] "mthl10"                    "obst-B"
## [101] "Trim9"                     "pdgy"
## [103] "tio"                       "asRNA:CR44119"
## [105] "E(spl)m4-BFM"              "unc-13"
## [107] "Eip75B"                    "CadN"
## [109] "asRNA:CR42547"             "lncRNA:CR45232"
## [111] "asRNA:CR45485"             "Ocho"
## [113] "rudhira"                   "REPTOR-BP"
## [115] "Dscam1"                    "br"
## [117] "Cyp310a1"                  "Picot"
## [119] "Ect3"                      "mdy"
## [121] "GV1"                       "sr"
## [123] "E(spl)malpha-BFM"          "CG8249"
## [125] "CG16700"                   "lncRNA:marge"
## [127] "yellow-e2"                 "CG3649"
## [129] "del"                       "mav"
## [131] "CG13676"                   "Ac13E"
## [133] "chrb"                      "rdo"
## [135] "Ugt317A1"                  "Dh31-R"
## [137] "CG11899"                   "GstT3"
## [139] "pip"                       "Cpr66D"
## [141] "Mdr49"                     "CG17754"
## [143] "Rtca"                      "dnr1"
## [145] "l(3)72Dp"                  "CG31937"
## [147] "neo"                       "Glut1"
## [149] "CG9416"                    "Poxn"
```

```
## [151] "lncRNA:CR45473"               "Fatp2"
## [153] "RR48344_transposable_element" "ImpE3"
## [155] "LRP1"                         "otk"
## [157] "CAH2"                         "Brd"
## [159] "CG15628"                      "Mp"
## [161] "Dyrk2"                        "bves"
## [163] "Spn77Ba"                      "FBgn0029082"
## [165] "CG12896"                      "mtgo"
## [167] "fok"                          "ImpE1"
## [169] "CG10311"                      "CG5618"
## [171] "PLCXD"                        "asRNA:CR43944"
## [173] "CG5151"                       "Cad87A"
## [175] "lncRNA:CR42549"               "vri"
## [177] "ldd"                          "CG1265"
## [179] "cer"                          "Mnn1"
## [181] "LManII"                       "teq"
## [183] "al"                           "Sqor"
## [185] "Pvf2"                         "CG46310"
## [187] "magu"                         "Kal1"
## [189] "Pka-C3"                       "hng3"
## [191] "caps"                         "Samuel"
## [193] "CG40160"                      "CG10089"
## [195] "CG12643"                      "mlt"
## [197] "sli"                          "BBS8"
## [199] "CG11170"                      "CG3520"
## [201] "CG18135"                      "tna"
## [203] "rpr"                          "Argl"
## [205] "stx"                          "CG42741"
## [207] "Cirl"                         "Den1"
## [209] "Zmynd10"                      "CG10948"
## [211] "CG16721"                      "Nhe2"
## [213] "Sdr"                          "CG32241"
## [215] "asRNA:CR45141"                "CG5966"
## [217] "tfc"                          "CG8420"
## [219] "stw"                          "CG31997"
## [221] "CG14301"                      "CG6231"
## [223] "CREG"                         "CG42342"
## [225] "fend"                         "CG32809"
## [227] "CG8389"                       "Kr-h1"
## [229] "Rchy1"                        "CG33062"
## [231] "Drat"                         "emp"
## [233] "Ddr"                          "lncRNA:CR42862"
## [235] "CG8034"                       "Tig"
## [237] "CrebA"                        "Tsf3"
## [239] "CG32373"                      "lft"
## [241] "CG6959"                       "Hsp27"
## [243] "CG9003"                       "Pgant9"
## [245] "CG3880"                       "Np"
## [247] "Nep2"                         "MFS18"
## [249] "Non1"                         "BNIP3"
## [251] "MsrA"                         "Lk6"
## [253] "CG10657"                      "side-IV"
## [255] "myd"                          "Cln7"
## [257] "NaCP60E"                      "CG7083"
```

```
## [259] "bbg"                            "LTV1"
## [261] "mrt"                            "Mmp1"
## [263] "E(spl)m8-HLH"                   "asRNA:CR44390"
## [265] "nub"                            "Tina-1"
## [267] "NijA"                           "amd"
## [269] "Ip6k"                           "Svil"
## [271] "lncRNA:CR46450"                 "LanA"
## [273] "Vdup1"                          "CG15544"
## [275] "CG34193"                        "Aldh-III"
## [277] "Tmc"                            "ttk"
## [279] "CG9689"                         "mura"
## [281] "Osi24"                          "Naglu"
## [283] "CRAT"                           "Inos"
## [285] "CG3328"                         "geko"
## [287] "CG17834"                        "wg"
## [289] "RR48810_transposable_element" "CG34056"
## [291] "lbk"                            "kn"
## [293] "Dll"                            "atos"
## [295] "CG30460"                        "CG6966"
## [297] "CG3603"                         "side-V"
## [299] "CG12268"                        "CG7530"
## [301] "tkv"                            "CG12299"
## [303] "CG34125"                        "CG6163"
## [305] "trh"                            "Notum"
## [307] "Nubpl"                          "Nadk1a"
## [309] "CG2698"                         "CG14598"
## [311] "CG17230"                        "CG4364"
## [313] "CG1486"                         "Alh"
## [315] "gpp"                            "Abp1"
## [317] "RhoGAP15B"                      "CG9886"
## [319] "luna"                           "eg"
## [321] "Invadolysin"                    "RhoU"
## [323] "CG17032"                        "Sod3"
## [325] "cnc"                            "CG34398"
## [327] "RanBPM"                         "CG8818"
## [329] "CG6845"                         "Atg6"
## [331] "CG16786"                        "Cda4"
## [333] "Mnt"                            "bnb"
## [335] "CG4313"                         "Sema2b"
## [337] "InR"                            "asRNA:CR44690"
## [339] "CG11275"                        "Aptx"
## [341] "Jheh2"                          "CG4766"
## [343] "strat"                          "norpA"
## [345] "CG17574"                        "Delta"
## [347] "mth"                            "cbt"
## [349] "chas"                           "CG1607"
## [351] "CG3857"                         "Snx21"
## [353] "Tom"                            "CG42837"
## [355] "CG11961"                        "Sirup"
## [357] "Bx"                             "LanB1"
## [359] "CG11550"                        "CG3376"
## [361] "noc"                            "Fkbp14"
## [363] "IP3K2"                          "in"
## [365] "CG31075"                        "CG11883"
```

```
## [367] "Pif1B"                    "Acox57D-p"
## [369] "CG42699"                  "Atg4a"
## [371] "RapGAP1"                  "CG46306"
## [373] "Rcd5"                     "Sema1b"
## [375] "CG10283"                  "peg"
## [377] "ewg"                      "CG30089"
## [379] "CG1316"                   "FBgn0025683"
## [381] "l(2)k09913"               "Sema1a"
## [383] "Cerk"                     "CG14478"
## [385] "red"                      "vg"
## [387] "pix"                      "CG5397"
## [389] "CG42806"                  "sei"
## [391] "CG2812"                   "Toll-7"
## [393] "MED16"                    "Gnptab"
## [395] "klar"                     "Myo95E"
## [397] "CG11247"                  "GEFmeso"
## [399] "RhoGAP5A"                 "CG9902"
## [401] "CG1965"                   "CG12075"
## [403] "phr6-4"                   "svr"
## [405] "Ip259"                    "Tet"
## [407] "Ten-m"                    "for"
## [409] "Oseg1"                    "Rfx"
## [411] "CG1233"                   "CG5674"
## [413] "Taf6"                     "Ptp69D"
## [415] "CG42663"                  "mxt"
## [417] "Rab4"                     "olf413"
## [419] "Ist1"                     "FBgn0026749"
## [421] "Rm62"                     "raskol"
## [423] "Orai"                     "Idh"
## [425] "Rpn10"                    "caz"
## [427] "Nasp"                     "Prosalpha3"
## [429] "spoon"                    "Hrb98DE"
## [431] "Rpn2"                     "alphaTub84B"
## [433] "CG8635"                   "Scsalpha1"
## [435] "Trx2"                     "SdhD"
## [437] "Rbf"                      "mEFTs"
## [439] "Pfdn6"                    "fzy"
## [441] "Aos1"                     "CG9300"
## [443] "CG9630"                   "AsnRS"
## [445] "CG1463"                   "Pdhb"
## [447] "wuho"                     "nesd"
## [449] "Rat1"                     "Cchl"
## [451] "psidin"                   "lncRNA:CR44334"
## [453] "RIOK2"                    "sofe"
## [455] "Polr2E"                   "Ntan1"
## [457] "Prx6a"                    "Gar1"
## [459] "HIP"                      "PolA1"
## [461] "tum"                      "Sps1"
## [463] "CG8149"                   "CG5757"
## [465] "CysRS"                    "roh"
## [467] "COX5B"                    "CG4069"
## [469] "CG3847"                   "CCT3"
## [471] "Caf1-180"                 "ttm50"
## [473] "mRpL12"                   "CG32075"
```

```
## [475] "bonsai"                  "CG3817"
## [477] "CG8097"                  "alpha-PheRS"
## [479] "msk"                     "eIF3j"
## [481] "ATPsynCF6"               "Rpt4"
## [483] "sob"                     "CG12128"
## [485] "Cbs"                     "Idh3b"
## [487] "Mcm2"                    "CG14174"
## [489] "AIMP2"                   "vig2"
## [491] "Cox10"                   "CG9281"
## [493] "Nxt1"                    "blw"
## [495] "Nost"                    "CG11444"
## [497] "salr"                    "CG14543"
## [499] "CCT4"                    "mRpS10"
## [501] "AIMP3"                   "GC1"
## [503] "Prat"                    "CG3760"
## [505] "Smyd5"                   "ATPsynD"
## [507] "dve"                     "mRpL32"
## [509] "Nup107"                  "Naa15-16"
## [511] "Prp19"                   "Prosalpha1R"
## [513] "Kank"                    "aralar1"
## [515] "Men-b"                   "Pdha"
## [517] "Ogdh"                    "CG13630"
## [519] "CG3527"                  "vito"
## [521] "Nuf2"                    "porin"
## [523] "melt"                    "Rpt5"
## [525] "Prps"                    "SerRS"
## [527] "CG11417"                 "CG9095"
## [529] "Chrac-14"                "muc"
## [531] "RecQ4"                   "RfC38"
## [533] "CG6769"                  "RanGAP"
## [535] "twe"                     "Adk2"
## [537] "CG9004"                  "Cdc6"
## [539] "Pfdn2"                   "Tom20"
## [541] "CG8003"                  "Ote"
## [543] "Gnf1"                    "ND-ACP"
## [545] "pav"                     "Rrp42"
## [547] "Vha26"                   "Idh3a"
## [549] "P32"                     "Lsd-2"
## [551] "RnpS1"                   "CG34132"
## [553] "CG9338"                  "Acat1"
## [555] "sgg"                     "CG11164"
## [557] "beta-PheRS"              "CG12134"
## [559] "CG8326"                  "Fs(2)Ket"
## [561] "Adsl"                    "kra"
## [563] "CG10337"                 "Mtr4"
## [565] "CG14207"                 "Dbp73D"
## [567] "ckd"                     "form3"
## [569] "Ipo9"                    "Prp3"
## [571] "dpa"                     "Dph5"
## [573] "CG10576"                 "mRpL52"
## [575] "drm"                     "UQCR-C2"
## [577] "RnrS"                    "Psc"
## [579] "PolD1"                   "CG8891"
## [581] "borr"                    "CG11583"
```

```
## [583] "CG42455"                  "CG15717"
## [585] "Noc3"                     "yellow-c"
## [587] "CG14683"                  "CG46459"
## [589] "CG9107"                   "SdhB"
## [591] "Hpf1"                     "cwo"
## [593] "Rexo5"                    "Adck1"
## [595] "CG8441"                   "D2hgdh"
## [597] "Cdc45"                    "MFS10"
## [599] "mus301"                   "Nmt"
## [601] "Pop1"                     "mRpS30"
## [603] "CG10565"                  "FBgn0052226"
## [605] "CG9393"                   "CG9286"
## [607] "CG44838"                  "ValRS"
## [609] "RanBP3"                   "CG4806"
## [611] "CG11050"                  "CCT8"
## [613] "Shmt"                     "Tcs1"
## [615] "Polr3K"                   "blow"
## [617] "Sms"                      "Mcm7"
## [619] "aurB"                     "CG34231"
## [621] "RPA1"                     "Phb1"
## [623] "CG11030"                  "Chd3"
## [625] "WRNexo"                   "CCT5"
## [627] "CG7841"                   "CG42588"
## [629] "Csl4"                     "wds"
## [631] "eIF2beta"                 "mge"
## [633] "Nup58"                    "CCT7"
## [635] "RnrL"                     "ko"
## [637] "128up"                    "l(2)05287"
## [639] "Uch-L5"                   "CG8630"
## [641] "Aatf"                     "Mdh2"
## [643] "CG15027"                  "dbe"
## [645] "CG11180"                  "CG45085"
## [647] "feo"                      "Polr1B"
## [649] "l(3)72Dn"                 "Polr1F"
## [651] "pont"                     "bero"
## [653] "CG4593"                   "CG14805"
## [655] "PolD2"                    "CG31875"
## [657] "CycB"                     "CG14230"
## [659] "Rtc1"                     "CG10336"
## [661] "Drep2"                    "CG4975"
## [663] "C15"                      "zuc"
## [665] "CG5214"                   "Ns1"
## [667] "Rrp45"                    "CG7966"
## [669] "Ts"                       "CG33509"
## [671] "Cmpk"                     "BckdhA"
## [673] "Atf3"                     "wun2"
## [675] "Ttd14"                    "MRP"
## [677] "nero"                     "Fen1"
## [679] "DAAM"                     "Pfdn5"
## [681] "Mnr"                      "esc"
## [683] "pnut"                     "Phb2"
## [685] "CG11076"                  "Mcm3"
## [687] "Chchd2"                   "CG9593"
## [689] "PolE1"                    "l(1)G0004"
```

```
## [691] "Ski6"                    "Rs1"
## [693] "HipHop"                  "T3dh"
## [695] "so"                      "Sdb"
## [697] "CG8012"                  "Hsp60A"
## [699] "kz"                      "Polr1C"
## [701] "cutlet"                  "sle"
## [703] "dpn"                     "CG8206"
## [705] "sordd2"                  "CG32267"
## [707] "pths"                    "CG15093"
## [709] "CG5290"                  "Chd64"
## [711] "Dus3"                    "CG32694"
## [713] "CG31808"                 "Phf7"
## [715] "DNAlig1"                 "CG11837"
## [717] "CycB3"                   "Ude"
## [719] "CG11123"                 "rept"
## [721] "Nph"                     "Mgstl"
## [723] "Pus1"                    "CG11267"
## [725] "Mcm6"                    "CG4554"
## [727] "Naa40"                   "CG2691"
## [729] "Sox100B"                 "bys"
## [731] "Nop10"                   "Nup54"
## [733] "bora"                    "Doc3"
## [735] "RfC4"                    "mEFG1"
## [737] "Tg"                      "dysf"
## [739] "CG6739"                  "CG3594"
## [741] "RNaseZ"                  "Mcm5"
## [743] "CG18273"                 "bsf"
## [745] "CG18178"                 "Imp"
## [747] "CG13893"                 "CG1572"
## [749] "Hmbs"                    "Orc2"
## [751] "CG7054"                  "Pus7"
## [753] "l(2)k09022"              "Rrp5"
## [755] "opa"                     "PolE2"
## [757] "RIOK1"                   "ND5"
## [759] "NO66"                    "l(2)34Fd"
## [761] "mod"                     "vig"
## [763] "Nmdmc"                   "CG7884"
## [765] "CCT1"                    "CG1703"
## [767] "Art1"                    "FBgn0032906"
## [769] "Polr1E"                  "Ntf-2"
## [771] "lncRNA:CR43264"          "Tret1-1"
## [773] "CCT2"                    "mei-38"
## [775] "Prim1"                   "Trmt61"
## [777] "ais"                     "Doc1"
## [779] "Roe1"                    "Nt5a"
## [781] "ras"                     "CG15879"
## [783] "CG6712"                  "san"
## [785] "Cadps"                   "MtnA"
## [787] "CG8611"                  "Hex-A"
## [789] "Tsr1"                    "Ctps"
## [791] "Polr1A"                  "Nsun5"
## [793] "CG3430"                  "Rcd6"
## [795] "CG8064"                  "Mcm10"
## [797] "fru"                     "eEF1delta"
```

```
## [799] "Art8"                    "Ctf4"
## [801] "FBgn0030170"             "Art3"
## [803] "Hsp110"                  "CG7246"
## [805] "ATP6"                    "ppan"
## [807] "CG9752"                  "CG7006"
## [809] "RFeSP"                   "Nop60B"
## [811] "Rrp46"                   "Non3"
## [813] "Mat89Ba"                 "CG12325"
## [815] "CG9799"                  "CG6937"
## [817] "CG32512"                 "CG13097"
## [819] "AdamTS-B"                "CG10286"
## [821] "Hs6st"                   "tup"
## [823] "CG17734"                 "Idgf3"
## [825] "CG10903"                 "Mys45A"
## [827] "indra"                   "CG3902"
## [829] "GstE7"                   "l(3)07882"
## [831] "Pdp1"                    "mRpL18"
## [833] "CG18789"                 "CG11089"
## [835] "PCNA"                    "CG10341"
## [837] "mRpL44"                  "eIF6"
## [839] "CG32409"                 "CG8026"
## [841] "Cyt-c1"                  "CG5800"
## [843] "FBgn0034496"             "BthD"
## [845] "pnr"                     "PCB"
## [847] "CG43103"                 "Ak3"
## [849] "CG2972"                  "Adss"
## [851] "CG11980"                 "Ns3"
## [853] "PolA2"                   "Noc1"
## [855] "CG13690"                 "CG14984"
## [857] "CG9932"                  "CG12288"
## [859] "nudC"                    "l(1)G0020"
## [861] "SoYb"                    "CG11563"
## [863] "CG9922"                  "ND1"
## [865] "Nsun2"                   "La"
## [867] "Aldh"                    "nop5"
## [869] "CG11779"                 "CG12499"
## [871] "CG6073"                  "CG7728"
## [873] "wcd"                     "CG2260"
## [875] "Bcat"                    "CG30349"
## [877] "CG32281"                 "upd3"
## [879] "CG32344"                 "CG6724"
## [881] "Rif1"                    "alphaTub84D"
## [883] "mbm"                     "CG3226"
## [885] "Droj2"                   "Hsc70-5"
## [887] "CycE"                    "CG8545"
## [889] "Zpr1"                    "CG1542"
## [891] "U3-55K"                  "CG3071"
## [893] "CG1785"                  "Noc2"
## [895] "CG13067"                 "SCaMC"
## [897] "Cyp4p1"                  "Surf6"
## [899] "Dif"                     "Nle"
## [901] "AsnS"                    "Prim2"
## [903] "CG9815"                  "Nnp-1"
## [905] "Nmd3"                    "Fkbp39"
```

```
## [907] "CG7182"                    "RpLP0-like"
## [909] "Rbm13"                     "CG5114"
## [911] "ITP"                       "bor"
## [913] "Gasp"                      "phr"
## [915] "chinmo"                    "Claspin"
## [917] "peng"                      "Jhbp14"
## [919] "Socs36E"                   "Tsp42El"
## [921] "pit"                       "CG5033"
## [923] "Nplp2"                     "Arc1"
## [925] "upd1"                      "CG46385"
## [927] "Sas10"                     "CG13082"
## [929] "rau"                       "CG17190"
## [931] "DnaJ-1"                    "CG12301"
## [933] "ana2"                      "r-l"
## [935] "kdn"                       "Nplp4"
## [937] "ab"                        "CG3085"
## [939] "CG10863"                   "vn"
## [941] "CG8927"                    "l(2)09851"
## [943] "Nopp140"                   "nclb"
## [945] "CG45071"                   "SpdS"
## [947] "CG8939"                    "CG13096"
## [949] "Cyp4p2"                    "CG34325"
## [951] "CG15629"                   "Nop56"
## [953] "dup"                       "Osi14"
## [955] "NHP2"                      "Amy-p"
## [957] "Jhbp7"                     "spz5"
## [959] "CR40190"                   "Cyt-c-p"
## [961] "Ilp8"                      "CG12909"
## [963] "Uhg1"                      "Mybbp1A"
## [965] "hoip"                      "prage"
## [967] "CG46339"                   "CG13516"
## [969] "stg"                       "CG3961"
## [971] "bi"                        "CG4115"
## [973] "CG11147"                   "Mipp1"
## [975] "wat"                       "egr"
## [977] "Hsp83"                     "FBgn0267727"
## [979] "CG42326"                   "CG14566"
## [981] "CG11370"                   "Dad"
## [983] "Cpr49Ag"                   "CG14572"
## [985] "asRNA:CR45713"             "w"
## [987] "Twdlbeta"                  "Apoltp"
```

Another way to make a ranked list is to take LFC values and sort them only according to FC. Which result willI get then?

```
ubiLFC_genes %>%
  arrange(desc(log2FoldChange)) %>%
  filter(!stringr::str_detect(ID, "FBti")) %>%
  na.omit() %>%
  select(gene, log2FoldChange) %>%
  deframe()-> ubiLFC_fc_rank
```

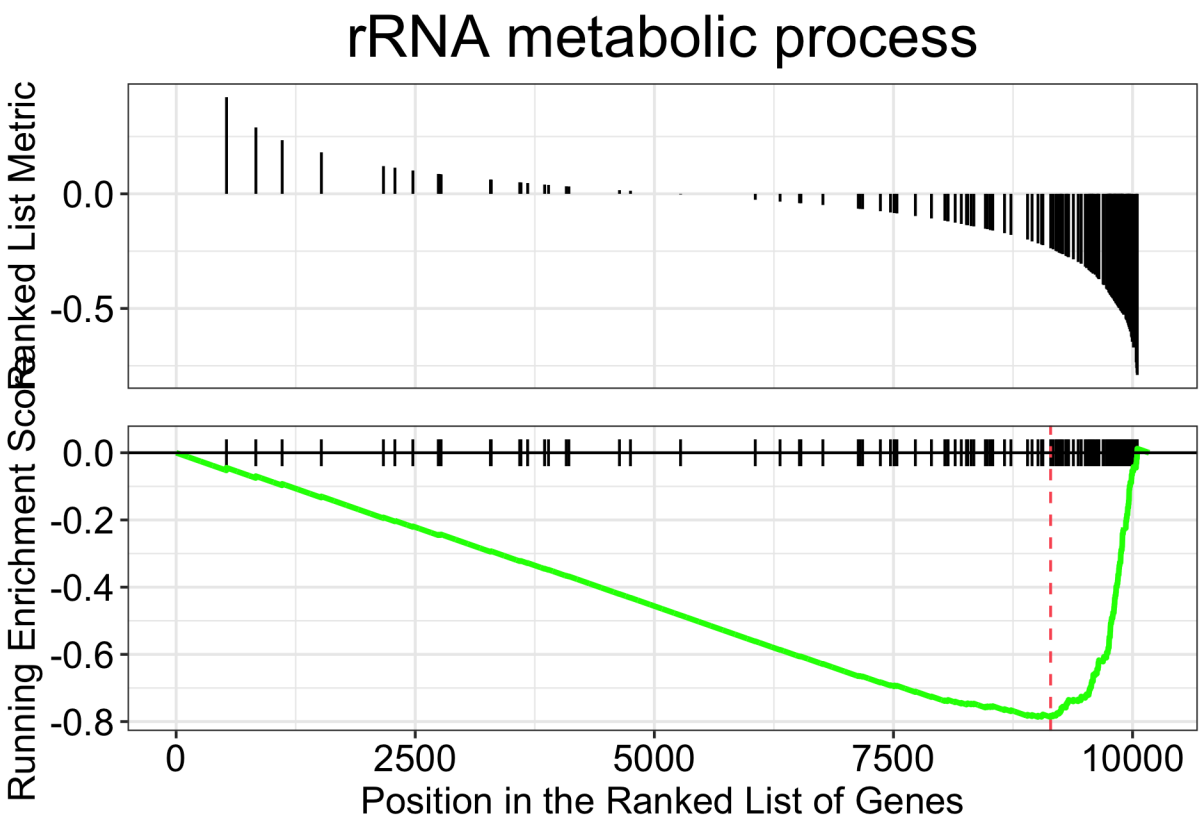
```
gse_fc<- gseGO (ubiLFC_fc_rank,
             ont="BP",
             OrgDb=org.Dm.eg.db,
             keyType="SYMBOL",
             pvalueCutoff = 0.05,
             verbose = TRUE,
             minGSSize=3,
             pAdjustMethod = "BH")
```

```
## using 'fgsea' for GSEA analysis, please cite Korotkevich et al (2019).

## preparing geneSet collections...

## GSEA analysis...

## 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.

## Warning in fgseaMultilevel(pathways = pathways, stats = stats, minSize =
## minSize, : For some pathways, in reality P-values are less than 1e-10. You can
## set the `eps` argument to zero for better estimation.

## leading edge analysis...

## done...
```

```
summary(gse_fc) %>% as.tibble() -> gse_fc_sum
```

```
## Warning in summary(gse_fc): summary method to convert the object to data.frame
## is deprecated, please use as.data.frame instead.
```

```
gseaplot(gse_fc, by = "all", title = gse_fc$Description[1], geneSetID = 1)
```

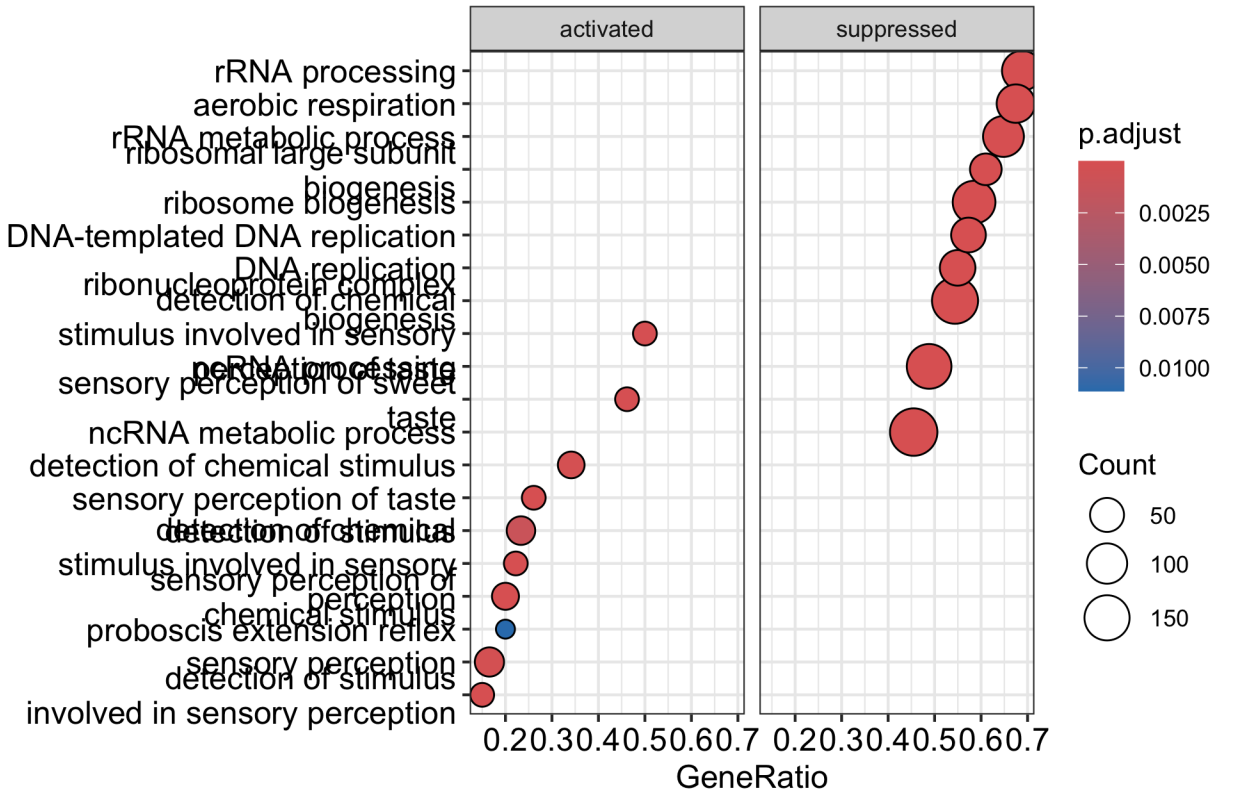



```
#gse_fc_s<-lapply(gse_fc@result$NES, sort, decreasing = TRUE)
#dotplot(gse_fc_s, showCategory=10)
```

```
top20<-dotplot(gse_fc, showCategory=10)
up_down<-dotplot(gse_fc, showCategory=10, split=".sign", orderBy="Counts") + facet_grid(.~.sign)
```

```
## wrong orderBy parameter; set to default ‘orderBy = "x"‘
```

```
up_down
```




```
#cowplot::plot_grid(top20, up_down, ncol=1, labels=LETTERS[1:2])
```

Yet another way is to use Wald statistics, that incorporates the relationship between pvalue and FC

```
wald_stat <- dds@rowRanges@elementMetadata@listData$WaldStatistic_Group_ubi.brk_vs_control
dds_res <- res %>% data.frame()
dds_res %>%
  mutate(., gene = mapIds(
  org.Dm.eg.db,
  keys = rownames(.),
  column ="SYMBOL",
  keytype = "FLYBASE",
  multiVals = "first"
)) %>%
  rownames_to_column(.) %>%
    mutate(gene = coalesce(gene,rowname)) %>%
  rename(., ID=rowname) -> dds_res_genes
```

```
## 'select()' returned 1:1 mapping between keys and columns
```

```
dds_res_genes %>%
  arrange(desc(stat)) %>%
  filter(!stringr::str_detect(ID, "FBti")) %>%
  na.omit() %>%
  select(gene, stat) %>%
  deframe()-> dds_wald_rank
```

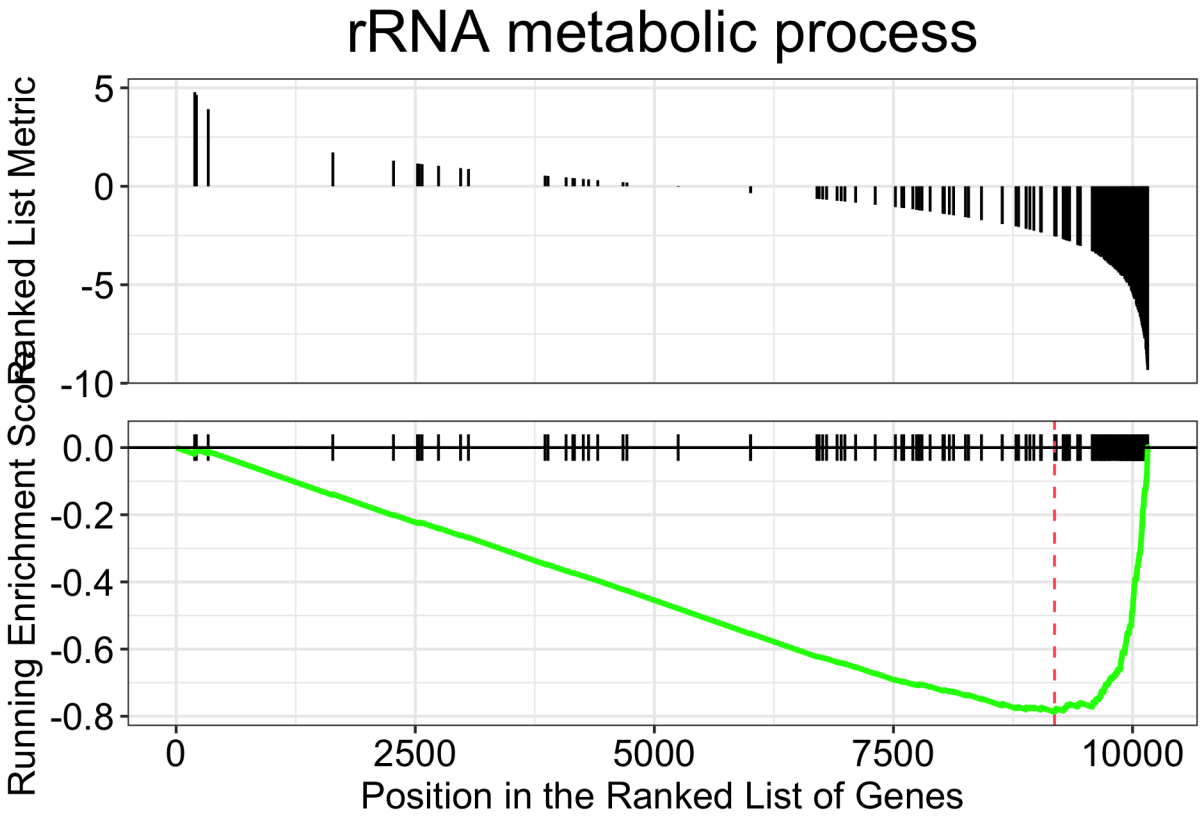
```
gse_wald<- gseGO (dds_wald_rank,
              ont="BP",
              OrgDb=org.Dm.eg.db,
              keyType="SYMBOL",
              pvalueCutoff = 0.05,
              verbose = TRUE,
              minGSSize=3,
              pAdjustMethod = "BH")
```

```
## using 'fgsea' for GSEA analysis, please cite Korotkevich et al (2019).

## preparing geneSet collections...

## GSEA analysis...

## 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.

## Warning in fgseaMultilevel(pathways = pathways, stats = stats, minSize =
## minSize, : For some pathways, in reality P-values are less than 1e-10. You can
## set the `eps` argument to zero for better estimation.

## leading edge analysis...

## done...
```

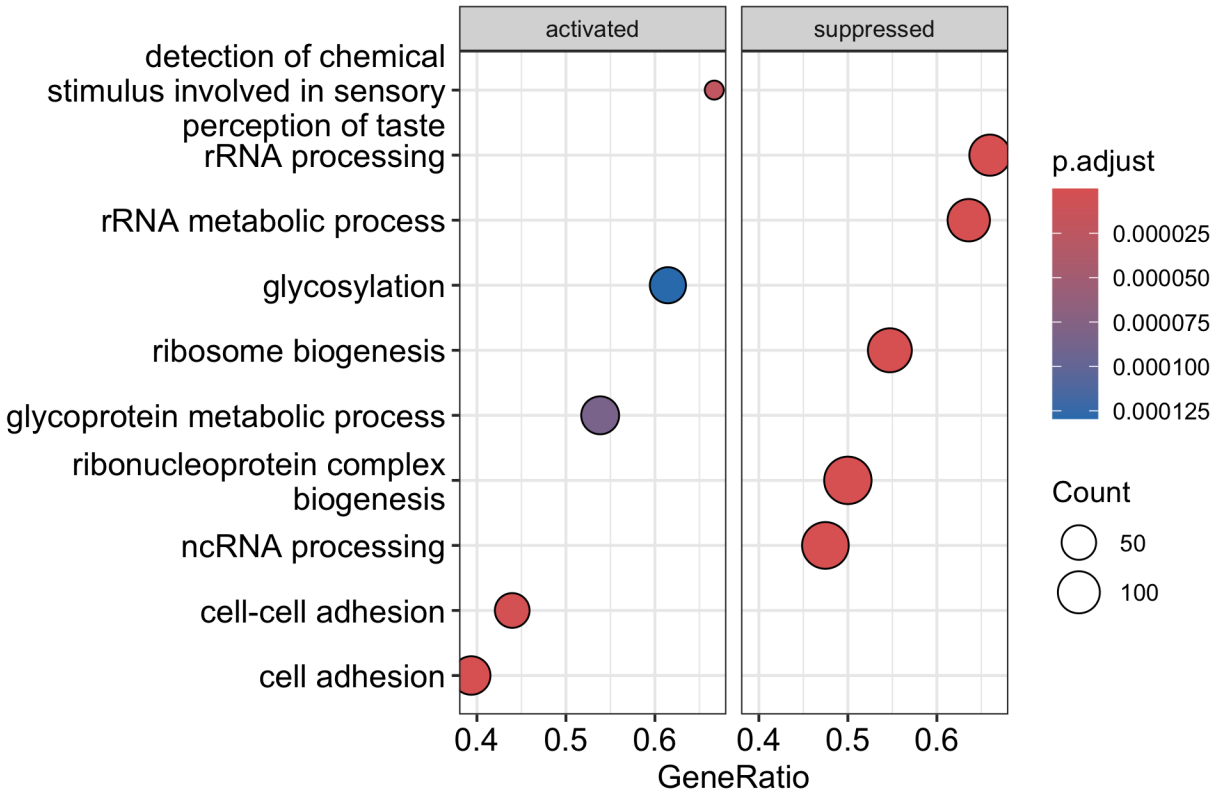
```
summary(gse_wald) %>% as.tibble() -> gse_wald_sum
```

```
## Warning: `as.tibble()` was deprecated in tibble 2.0.0.
## i Please use `as_tibble()` instead.
## i The signature and semantics have changed, see `?as_tibble`.
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_lifecycle_warnings()` to see where this warning was
## generated.

## Warning in summary(gse_wald): summary method to convert the object to
## data.frame is deprecated, please use as.data.frame instead.
```

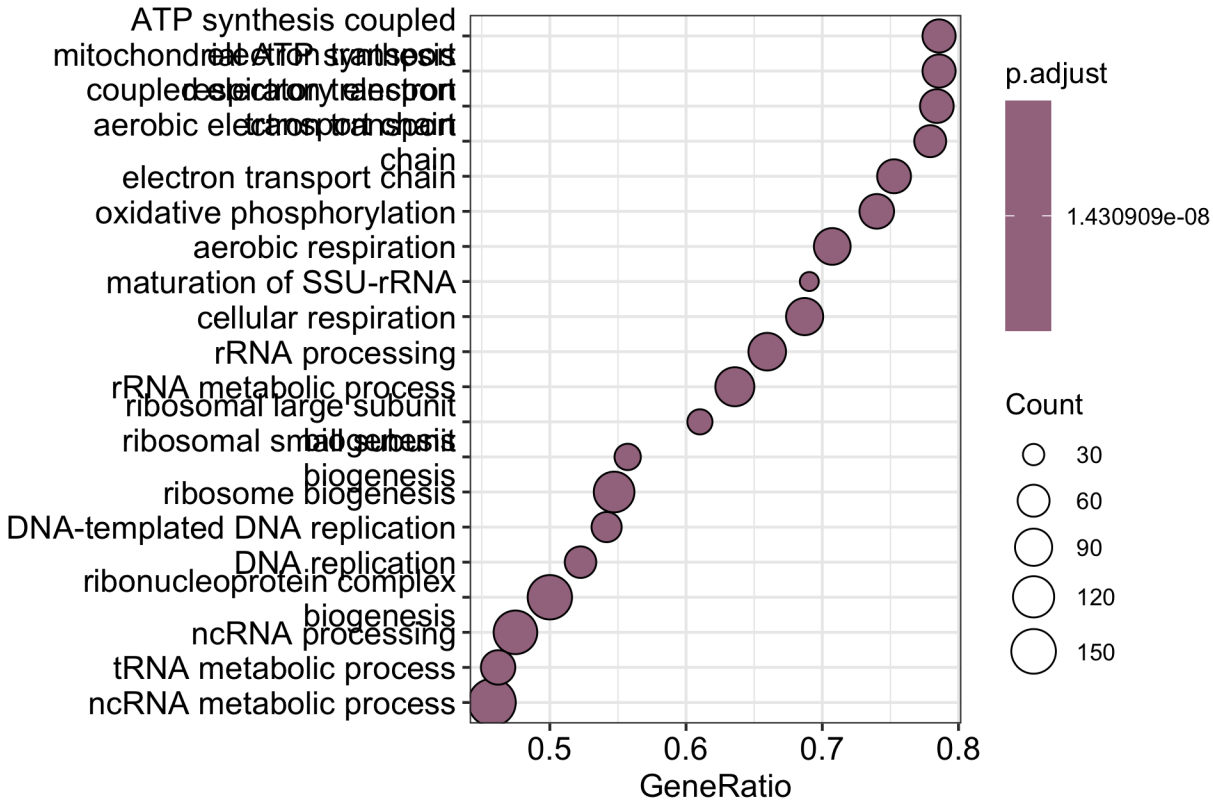
```
gseaplot(gse_wald, by = "all", title = gse_wald$Description[1], geneSetID = 1)
```



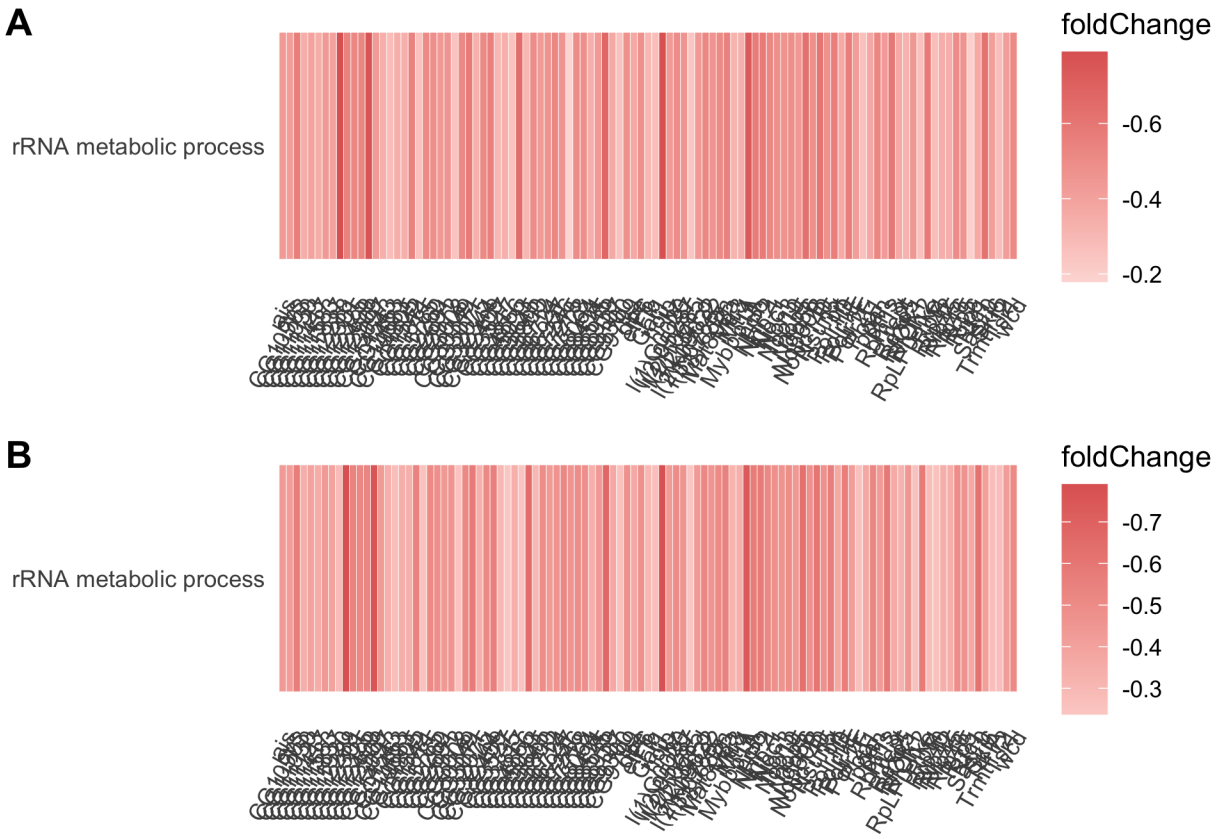

```
dotplot(gse_wald, showCategory=5, split=".sign") + facet_grid(.~.sign)
```



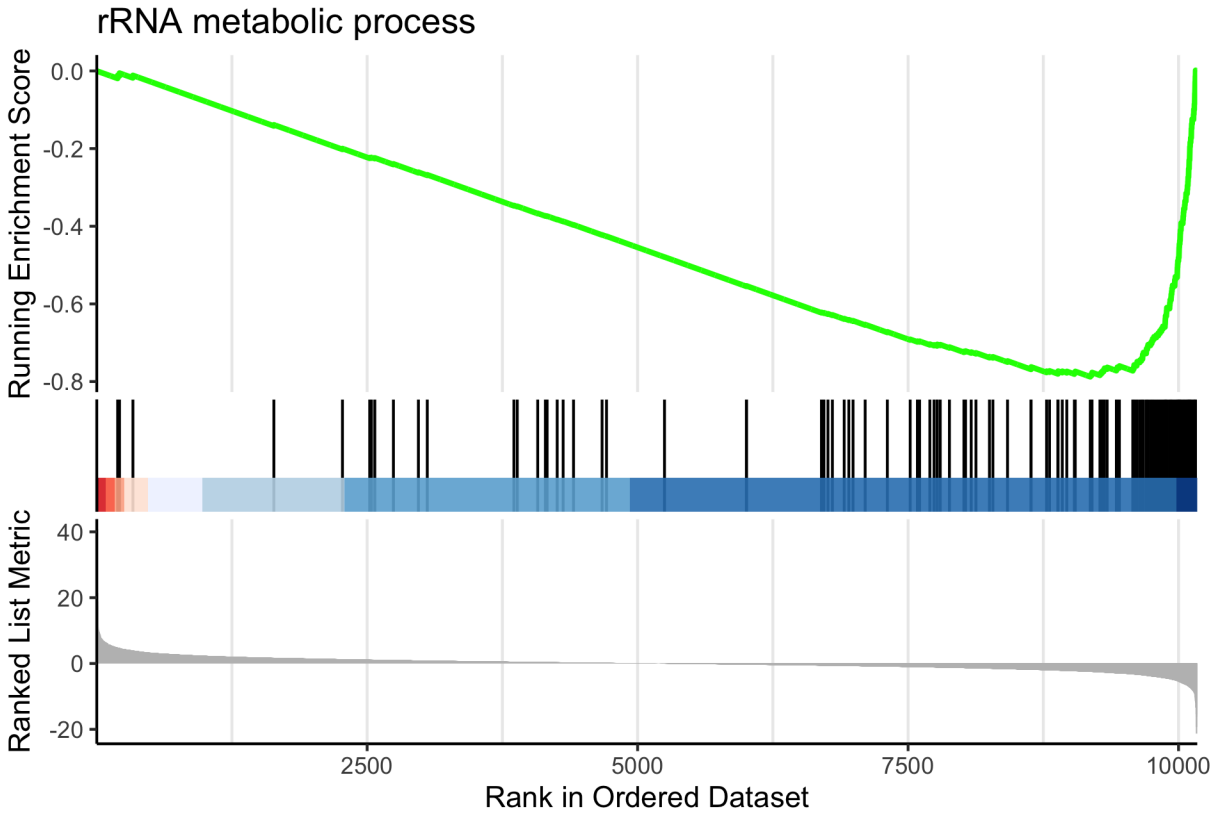

```
dotplot(gse_wald, showCategory=20)
```



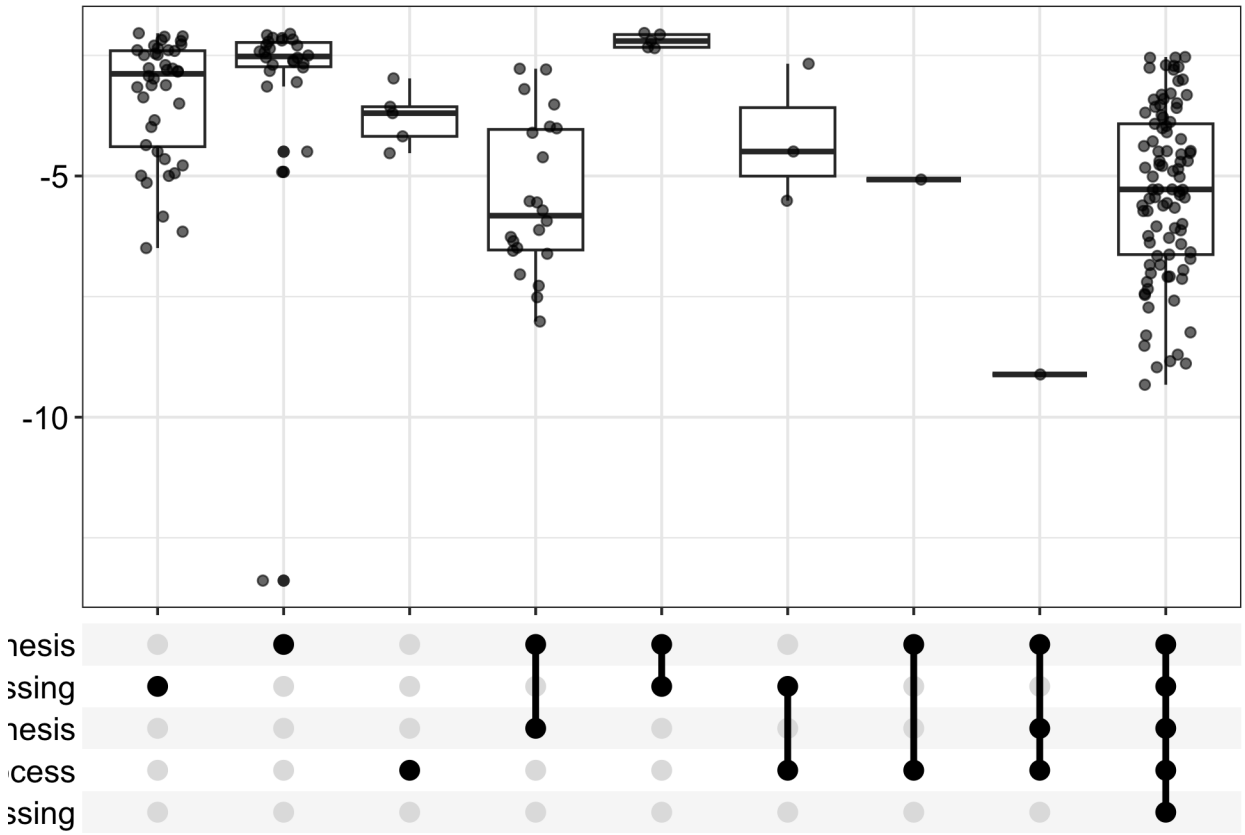

```
wald<-heatplot(gse_wald, showCategory="rRNA metabolic process", foldChange=ubiLFC_fc_rank)
fc<-heatplot(gse_fc, showCategory="rRNA metabolic process", foldChange=ubiLFC_fc_rank)
cowplot::plot_grid(wald, fc, ncol=1, labels=LETTERS[1:2])
```




```
gseaplot2(gse_wald, geneSetID = 1, title = gse_wald$Description[1])
```




```
library(ggupset)
upsetplot(gse_wald, n=5)
```


-5
-10
nesis
ssing
nesis
cess
ssing