Dysregulated processes in NAFLD (BB103X)

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02/03/2022

Introduction

One common way for searching shared functions among genes is to incorporate the biological knowledge provided by biological ontologies. For instance, 1)Gene Ontology (GO) (Ashburner et al., 2000) annotates genes to biological processes, molecular functions, and cellular components in a directed acyclic graph structure, 2)Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa et al., 2010) annotates genes to pathways, 3) Disease Ontology (DO) annotates genes with human disease association (Osborne et al., 2009).

The clusterProfiler package is implemented for gene cluster comparison. clusterProfiler applies biological term classification and enrichment analyses to gene cluster comparison, helping to better understand higher order functions of biological system.

Read paper Yu, Guangchuang, et al. Omics: a journal of integrative biology 16.5 (2012): 284-287.

3.1 Load necessary packages

- Rstudio

```
library(DESeq2)
library(pheatmap)
library(tidyverse)
library(xlsx)
library(readxl)
library(gplots)
library(ggbiplot)
library(piano)
library(venn)
library(ggpubr)
library(clusterProfiler)
library(GEOquery)
library(MAGeCKFlute)
library(openxlsx)
library(GEOquery)
library(ggrepel)
library(DOSE)
library(org.Hs.eg.db)
library(VennDiagram)
```

3.2 Load data from differential analysis

```
### Set 1: Deseq results between NAFL/NASH and control
deseq_data1 = read.xlsx('data/DEseq_results_NAFLvscontrol.xlsx')
deseq_data2 = read.xlsx('data/DEseq_results_NASH_F0-F1vscontrol.xlsx')
```

```r
deseq_data3 = read.xlsx('data/DEseq_results_NASH_F2vscontrol.xlsx')
deseq_data4 = read.xlsx('data/DEseq_results_NASH_F3vscontrol.xlsx')
deseq_data5 = read.xlsx('data/DEseq_results_NASH_F4vscontrol.xlsx')

deseq_data1[1:6,c(2,5:7)]
```

```
##   log2FoldChange      pvalue       padj Gene.name
## 1    -0.01490257 0.986911009 0.99227207      TNMD
## 2     0.03269755 0.811729476 0.88807235      DPM1
## 3    -0.07917556 0.351573381 0.49338748     SCYL3
## 4    -0.48628837 0.007193552 0.02032398  C1orf112
## 5    -0.16389423 0.366075834 0.50859810       FGR
## 6     0.39738106 0.009943955 0.02670424       CFH
```

3.3 Basic statistic - differential expressed gene

- padj < 0.05
- log2FoldChange > 1 or log2FoldChange <-1

```r
deseq_data_sig1 = deseq_data1 %>%
  mutate(sig = case_when((padj < 0.05 & log2FoldChange < -1) ~ "Down",
                         (padj < 0.05 & log2FoldChange > 1) ~ "Up",
                         TRUE ~ "No")) %>%
  filter(!(sig == 'No'))

deseq_data_sig2 = deseq_data2 %>%
  mutate(sig = case_when((padj < 0.05 & log2FoldChange < -1) ~ "Down",
                         (padj < 0.05 & log2FoldChange > 1) ~ "Up",
                         TRUE ~ "No")) %>%
  filter(!(sig == 'No'))

deseq_data_sig3 = deseq_data3 %>%
  mutate(sig = case_when((padj < 0.05 & log2FoldChange < -1) ~ "Down",
                         (padj < 0.05 & log2FoldChange > 1) ~ "Up",
                         TRUE ~ "No")) %>%
  filter(!(sig == 'No'))

deseq_data_sig4 = deseq_data4 %>%
  mutate(sig = case_when((padj < 0.05 & log2FoldChange < -1) ~ "Down",
                         (padj < 0.05 & log2FoldChange > 1) ~ "Up",
                         TRUE ~ "No")) %>%
  filter(!(sig == 'No'))

deseq_data_sig5 = deseq_data5 %>%
  mutate(sig = case_when((padj < 0.05 & log2FoldChange < -1) ~ "Down",
                         (padj < 0.05 & log2FoldChange > 1) ~ "Up",
                         TRUE ~ "No")) %>%
  filter(!(sig == 'No'))

deseq_data_sig1[1:6,c(2,5:8)]
```

```
##   log2FoldChange       pvalue         padj Gene.name  sig
## 1       4.333222 6.167344e-16 4.353204e-14   CYP51A1   Up
## 2       1.090650 9.741048e-08 1.009119e-06    MAD1L1   Up
## 3       1.182610 1.102249e-05 6.851220e-05    DBNDD1   Up
```

```
## 4      -1.656390 3.269275e-12 9.655954e-11      PDK4 Down
## 5      -1.715800 1.827097e-03 6.217729e-03     CALCR Down
## 6      -1.240884 3.480854e-07 3.171995e-06      MCUB Down
```

3.4 summary - differential expressed gene

```
table(deseq_data_sig1$sig)
```

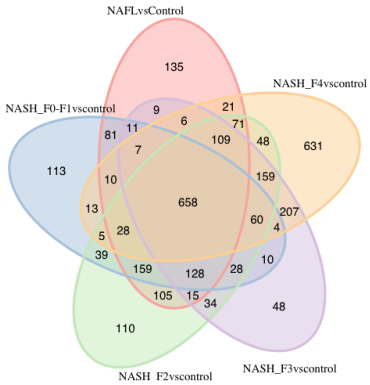
```
##
## Down   Up
##  827  726
```

Venn digram of differentially expressed genes.

- VennDiagram

```
DEG_list = list()
DEG_list[[1]] = deseq_data_sig1$Gene.name
DEG_list[[2]] = deseq_data_sig2$Gene.name
DEG_list[[3]] = deseq_data_sig3$Gene.name
DEG_list[[4]] = deseq_data_sig4$Gene.name
DEG_list[[5]] = deseq_data_sig5$Gene.name

venn.diagram(DEG_list, category.names = c("NAFLvsControl",
                                          "NASH_F0-F1vscontrol",
                                          "NASH_F2vscontrol",
                                          "NASH_F3vscontrol",
                                          "NASH_F4vscontrol"),
             filename = 'Figures/01_venn_diagramm_DEGs.png',
             output = TRUE ,
             imagetype="png" ,height = 480 , width = 480 , resolution = 300,compression = "lzw",lwd = 1
             col=c("#fbb4ae", '#b3cde3', '#ccebc5', '#decbe4','#fed9a6'),
             fill = c("#fbb4ae", '#b3cde3', '#ccebc5', '#decbe4','#fed9a6'),
             cex = 0.3,fontfamily = "sans",cat.cex = 0.3,
             cat.default.pos = "outer",cat.pos = c(-27, 4, 140, 170,6))
```

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



3.4 Bar plot shows the number of DEGs at each comparison

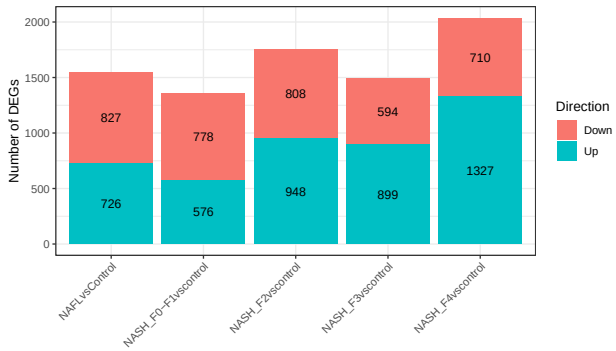
- basic summary

```
all_DEGs = rbind(as.data.frame(table(deseq_data_sig1$sig)),
                 as.data.frame(table(deseq_data_sig2$sig)),
                 as.data.frame(table(deseq_data_sig3$sig)),
                 as.data.frame(table(deseq_data_sig4$sig)),
                 as.data.frame(table(deseq_data_sig5$sig)))
all_DEGs = all_DEGs %>% mutate(comp = c(rep('NAFLvsControl',2),
                                        rep('NASH_F0-F1vscontrol',2),
                                        rep('NASH_F2vscontrol',2),
                                        rep('NASH_F3vscontrol',2),
                                        rep('NASH_F4vscontrol',2)))

all_DEGs
```

```
##    Var1 Freq                comp
## 1  Down  827       NAFLvsControl
## 2    Up  726       NAFLvsControl
## 3  Down  778 NASH_F0-F1vscontrol
## 4    Up  576 NASH_F0-F1vscontrol
## 5  Down  808    NASH_F2vscontrol
## 6    Up  948    NASH_F2vscontrol
## 7  Down  594    NASH_F3vscontrol
## 8    Up  899    NASH_F3vscontrol
## 9  Down  710    NASH_F4vscontrol
## 10   Up 1327    NASH_F4vscontrol
```

- Visualization

```
DEGs_bar = ggplot(all_DEGs, aes(comp, Freq, fill = Var1)) + geom_bar(stat="identity") +
  geom_text(colour = "black", size = 3.5,aes(label = Freq),position=position_stack(vjust=0.5)) +
  theme_bw() + labs(fill = 'Direction', y = 'Number of DEGs') +
  theme(axis.text.x = element_text(angle = 45, hjust = 1),
        axis.title.x = element_blank())
ggsave(DEGs_bar, filename = 'Figures/01-barplot_DEGs.pdf', width = 7, height = 4)
```



3.4 GO enrichment - differential expressed gene

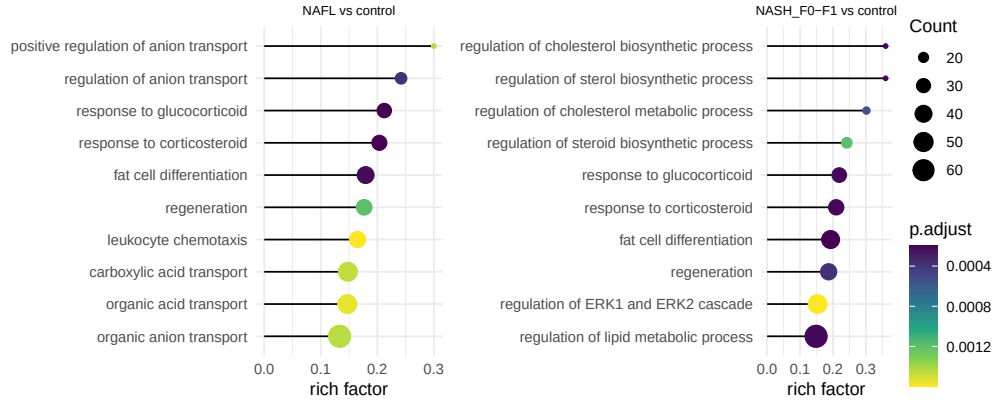
- GO ontology enrichment

```
ego_bp1 <- enrichGO(gene          = deseq_data_sig1$Gene.name,
                    OrgDb         = org.Hs.eg.db,
                    keyType       = 'SYMBOL',
                    ont           = "BP",
                    pAdjustMethod = "BH",
```

```
                pvalueCutoff  = 0.01,
                qvalueCutoff  = 0.05)

ego_bp2 <- enrichGO(gene         = deseq_data_sig2$Gene.name,
                OrgDb         = org.Hs.eg.db,
                keyType       = 'SYMBOL',
                ont           = "BP",
                pAdjustMethod = "BH",
                pvalueCutoff  = 0.01,
                qvalueCutoff  = 0.05)


ego_bp3 <- enrichGO(gene         = deseq_data_sig3$Gene.name,
                OrgDb         = org.Hs.eg.db,
                keyType       = 'SYMBOL',
                ont           = "BP",
                pAdjustMethod = "BH",
                pvalueCutoff  = 0.01,
                qvalueCutoff  = 0.05)



ego_bp4 <- enrichGO(gene         = deseq_data_sig4$Gene.name,
                OrgDb         = org.Hs.eg.db,
                keyType       = 'SYMBOL',
                ont           = "BP",
                pAdjustMethod = "BH",
                pvalueCutoff  = 0.01,
                qvalueCutoff  = 0.05)


ego_bp5 <- enrichGO(gene         = deseq_data_sig5$Gene.name,
                OrgDb         = org.Hs.eg.db,
                keyType       = 'SYMBOL',
                ont           = "BP",
                pAdjustMethod = "BH",
                pvalueCutoff  = 0.01,
                qvalueCutoff  = 0.05)

list_ego = list('NAFLvsControl' = ego_bp1, 'NASH_F0-F1vscontrol' = ego_bp2,
                'NASH_F2vscontrol' = ego_bp3, 'NASH_F3vscontrol' = ego_bp4, 'NASH_F4vscontrol' = ego_bp5

write.xlsx(list_ego, file = 'data/GO_BP_enrichment.NAFLorNASHvsControl.xlsx')
```

```
y1 <- mutate(ego_bp1, richFactor = Count / as.numeric(sub("/\\d+", "", BgRatio)))

y1_p = ggplot(y1, showCategory = 10,
  aes(richFactor, fct_reorder(Description, richFactor))) +
  geom_segment(aes(xend=0, yend = Description)) +
  geom_point(aes(color=p.adjust, size = Count)) +
  scale_color_viridis_c(guide=guide_colorbar(reverse=TRUE)) +
  scale_size_continuous(range=c(1, 6)) +
  theme_minimal() +
  xlab("rich factor") +
```

```
  ylab(NULL) + ggtitle('NAFL vs control') + ggeasy::easy_center_title() + ggeasy::easy_plot_title_size(8

y2 <- mutate(ego_bp2, richFactor = Count / as.numeric(sub("/\\d+", "", BgRatio)))

y2_p = ggplot(y2, showCategory = 10,
  aes(richFactor, fct_reorder(Description, richFactor))) +
  geom_segment(aes(xend=0, yend = Description)) +
  geom_point(aes(color=p.adjust, size = Count)) +
  scale_color_viridis_c(guide=guide_colorbar(reverse=TRUE)) +
  scale_size_continuous(range=c(1, 6)) +
  theme_minimal() +
  xlab("rich factor") +
  ylab(NULL)  + ggtitle('NASH_F0-F1 vs control') + ggeasy::easy_center_title() + ggeasy::easy_plot_title

y1andy2 = ggarrange(y1_p, y2_p, ncol = 2, common.legend = TRUE, legend = 'right')

ggsave(y1andy2, filename = 'Figures/02-goEnrichr-NAFL&NASHf0f1vsControl.pdf', width = 8.5, height = 3.5)
```



3.4 KEGG enrichment - differential expressed gene

- KEGG pathway enrichment

```
### NAFL vs Control

entrezID <- TransGeneID(deseq_data_sig1$Gene.name,
                        fromType = "Symbol",
                        toType = "Entrez",
                        organism = "hsa")

entrezID = as.data.frame(entrezID) %>% mutate(Gene.name = rownames(.))
rownames(deseq_data_sig1)  = deseq_data_sig1$Gene.name
deseq_data_sig_withID = merge(deseq_data_sig1, entrezID, by = 'Gene.name')

geneList = deseq_data_sig_withID$log2FoldChange
names(geneList) = as.character(deseq_data_sig_withID$entrezID)
geneList = sort(geneList, decreasing = TRUE)
gene <- names(geneList)[abs(geneList) > 2]

kk1 <- enrichKEGG(gene     = gene,
                  organism = 'hsa',
```

```
                pvalueCutoff = 0.05)
```

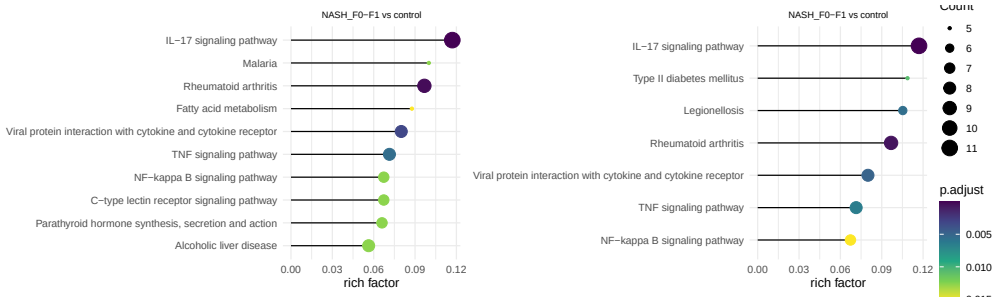
```
## Reading KEGG annotation online:
##
## Reading KEGG annotation online:
```

```
rm(entrezID, deseq_data_sig_withID, geneList, gene)

### NASH_F0-F1 vs Control
entrezID <- TransGeneID(deseq_data_sig2$Gene.name,
                        fromType = "Symbol",
                        toType = "Entrez",
                        organism = "hsa")

entrezID = as.data.frame(entrezID) %>% mutate(Gene.name = rownames(.))
rownames(deseq_data_sig2)  = deseq_data_sig2$Gene.name
deseq_data_sig_withID = merge(deseq_data_sig2, entrezID, by = 'Gene.name')

geneList = deseq_data_sig_withID$log2FoldChange
names(geneList) = as.character(deseq_data_sig_withID$entrezID)
geneList = sort(geneList, decreasing = TRUE)
gene <- names(geneList)[abs(geneList) > 2]

kk2 <- enrichKEGG(gene     = gene,
                  organism = 'hsa',
                  pvalueCutoff = 0.05)

rm(entrezID, deseq_data_sig_withID, geneList, gene)

### NASH_F2 vs Control
entrezID <- TransGeneID(deseq_data_sig3$Gene.name,
                        fromType = "Symbol",
                        toType = "Entrez",
                        organism = "hsa")

entrezID = as.data.frame(entrezID) %>% mutate(Gene.name = rownames(.))
rownames(deseq_data_sig3)  = deseq_data_sig3$Gene.name
deseq_data_sig_withID = merge(deseq_data_sig3, entrezID, by = 'Gene.name')

geneList = deseq_data_sig_withID$log2FoldChange
names(geneList) = as.character(deseq_data_sig_withID$entrezID)
geneList = sort(geneList, decreasing = TRUE)
gene <- names(geneList)[abs(geneList) > 2]

kk3 <- enrichKEGG(gene     = gene,
                  organism = 'hsa',
                  pvalueCutoff = 0.05)

rm(entrezID, deseq_data_sig_withID, geneList, gene)

### NASH_F3 vs Control
entrezID <- TransGeneID(deseq_data_sig4$Gene.name,
                        fromType = "Symbol",
                        toType = "Entrez",
```

```
                        organism = "hsa")

entrezID = as.data.frame(entrezID) %>% mutate(Gene.name = rownames(.))
rownames(deseq_data_sig4)  = deseq_data_sig4$Gene.name
deseq_data_sig_withID = merge(deseq_data_sig4, entrezID, by = 'Gene.name')

geneList = deseq_data_sig_withID$log2FoldChange
names(geneList) = as.character(deseq_data_sig_withID$entrezID)
geneList = sort(geneList, decreasing = TRUE)
gene <- names(geneList)[abs(geneList) > 2]

kk4 <- enrichKEGG(gene     = gene,
                  organism = 'hsa',
                  pvalueCutoff = 0.05)
rm(entrezID, deseq_data_sig_withID, geneList, gene)

### NASH_F4 vs Control
entrezID <- TransGeneID(deseq_data_sig5$Gene.name,
                        fromType = "Symbol",
                        toType = "Entrez",
                        organism = "hsa")

entrezID = as.data.frame(entrezID) %>% mutate(Gene.name = rownames(.))
rownames(deseq_data_sig5)  = deseq_data_sig5$Gene.name
deseq_data_sig_withID = merge(deseq_data_sig5, entrezID, by = 'Gene.name')

geneList = deseq_data_sig_withID$log2FoldChange
names(geneList) = as.character(deseq_data_sig_withID$entrezID)
geneList = sort(geneList, decreasing = TRUE)
gene <- names(geneList)[abs(geneList) > 2]

kk5<- enrichKEGG(gene     = gene,
                 organism = 'hsa',
                 pvalueCutoff = 0.05)

list_kk = list('NAFLvsControl' = kk1, 'NASH_F0-F1vscontrol' = kk2,
               'NASH_F2vscontrol' = kk3, 'NASH_F3vscontrol' = kk4, 'NASH_F4vscontrol' = kk5)

write.xlsx(list_kk, file = 'data/KEGG_pathway_enrichment.NAFLorNASHvsControl.xlsx')
```

```
y1 <- mutate(kk1, richFactor = Count / as.numeric(sub("/\\d+", "", BgRatio)))

y1_p = ggplot(y1, showCategory = 10,
  aes(richFactor, fct_reorder(Description, richFactor))) +
  geom_segment(aes(xend=0, yend = Description)) +
  geom_point(aes(color=p.adjust, size = Count)) +
  scale_color_viridis_c(guide=guide_colorbar(reverse=TRUE)) +
  scale_size_continuous(range=c(1, 6)) +
  theme_minimal() +
  xlab("rich factor") +
  ylab(NULL) + ggtitle('NASH_F0-F1 vs control') + ggeasy::easy_center_title() + ggeasy::easy_plot_title

y2 <- mutate(kk2, richFactor = Count / as.numeric(sub("/\\d+", "", BgRatio)))
```

```
y2_p = ggplot(y2, showCategory = 10,
  aes(richFactor, fct_reorder(Description, richFactor))) +
  geom_segment(aes(xend=0, yend = Description)) +
  geom_point(aes(color=p.adjust, size = Count)) +
  scale_color_viridis_c(guide=guide_colorbar(reverse=TRUE)) +
  scale_size_continuous(range=c(1, 6)) +
  theme_minimal() +
  xlab("rich factor") +
  ylab(NULL) + ggtitle('NASH_F0-F1 vs control') + ggeasy::easy_center_title() + ggeasy::easy_plot_title_

y1andy2 = ggarrange(y1_p, y2_p, ncol = 2, common.legend = TRUE, legend = 'right')

ggsave(y1andy2, filename = 'Figures/02-KEGGEnrichr-NAFL&NASHf0f1vsControl.pdf', width = 12, height = 3.5
```



3.4 disease enrichment - differential expressed gene

- disease enrichment

```
entrezID <- TransGeneID(deseq_data_sig1$Gene.name,
                        fromType = "Symbol",
                        toType = "Entrez",
                        organism = "hsa")

entrezID = as.data.frame(entrezID) %>% mutate(Gene.name = rownames(.))
rownames(deseq_data_sig1)  = deseq_data_sig1$Gene.name
deseq_data_sig_withID = merge(deseq_data_sig1, entrezID, by = 'Gene.name')

geneList = deseq_data_sig_withID$log2FoldChange
names(geneList) = as.character(deseq_data_sig_withID$entrezID)
geneList = sort(geneList, decreasing = TRUE)
gene <- names(geneList)[abs(geneList) > 2]

dd1 <- enrichDO(gene          = gene,
                ont           = "DO",
                pvalueCutoff  = 0.05,
                pAdjustMethod = "BH",
                universe      = names(geneList),
                minGSSize     = 5,
                maxGSSize     = 500,
                qvalueCutoff  = 0.05,
                readable      = FALSE)

rm(entrezID, deseq_data_sig_withID, geneList, gene)
```

```
### NASH_F0-F1 vs Control
entrezID <- TransGeneID(deseq_data_sig2$Gene.name,
                        fromType = "Symbol",
                        toType = "Entrez",
                        organism = "hsa")

entrezID = as.data.frame(entrezID) %>% mutate(Gene.name = rownames(.))
rownames(deseq_data_sig2)  = deseq_data_sig2$Gene.name
deseq_data_sig_withID = merge(deseq_data_sig2, entrezID, by = 'Gene.name')

geneList = deseq_data_sig_withID$log2FoldChange
names(geneList) = as.character(deseq_data_sig_withID$entrezID)
geneList = sort(geneList, decreasing = TRUE)
gene <- names(geneList)[abs(geneList) > 2]

dd2 <- enrichDO(gene          = gene,
              ont           = "DO",
              pvalueCutoff  = 0.05,
              pAdjustMethod = "BH",
              universe      = names(geneList),
              minGSSize     = 5,
              maxGSSize     = 500,
              qvalueCutoff  = 0.05,
              readable      = FALSE)
rm(entrezID, deseq_data_sig_withID, geneList, gene)

### NASH_F2 vs Control
entrezID <- TransGeneID(deseq_data_sig3$Gene.name,
                        fromType = "Symbol",
                        toType = "Entrez",
                        organism = "hsa")

entrezID = as.data.frame(entrezID) %>% mutate(Gene.name = rownames(.))
rownames(deseq_data_sig3)  = deseq_data_sig3$Gene.name
deseq_data_sig_withID = merge(deseq_data_sig3, entrezID, by = 'Gene.name')

geneList = deseq_data_sig_withID$log2FoldChange
names(geneList) = as.character(deseq_data_sig_withID$entrezID)
geneList = sort(geneList, decreasing = TRUE)
gene <- names(geneList)[abs(geneList) > 2]

dd3 <- enrichDO(gene          = gene,
              ont           = "DO",
              pvalueCutoff  = 0.05,
              pAdjustMethod = "BH",
              universe      = names(geneList),
              minGSSize     = 5,
              maxGSSize     = 500,
              qvalueCutoff  = 0.05,
              readable      = FALSE)
rm(entrezID, deseq_data_sig_withID, geneList, gene)

### NASH_F3 vs Control
```

```
entrezID <- TransGeneID(deseq_data_sig4$Gene.name,
                        fromType = "Symbol",
                        toType = "Entrez",
                        organism = "hsa")

entrezID = as.data.frame(entrezID) %>% mutate(Gene.name = rownames(.))
rownames(deseq_data_sig4)  = deseq_data_sig4$Gene.name
deseq_data_sig_withID = merge(deseq_data_sig4, entrezID, by = 'Gene.name')

geneList = deseq_data_sig_withID$log2FoldChange
names(geneList) = as.character(deseq_data_sig_withID$entrezID)
geneList = sort(geneList, decreasing = TRUE)
gene <- names(geneList)[abs(geneList) > 2]

dd4 <- enrichDO(gene          = gene,
              ont           = "DO",
              pvalueCutoff  = 0.05,
              pAdjustMethod = "BH",
              universe      = names(geneList),
              minGSSize     = 5,
              maxGSSize     = 500,
              qvalueCutoff  = 0.05,
              readable      = FALSE)
rm(entrezID, deseq_data_sig_withID, geneList, gene)

### NASH_F4 vs Control
entrezID <- TransGeneID(deseq_data_sig5$Gene.name,
                        fromType = "Symbol",
                        toType = "Entrez",
                        organism = "hsa")

entrezID = as.data.frame(entrezID) %>% mutate(Gene.name = rownames(.))
rownames(deseq_data_sig5)  = deseq_data_sig5$Gene.name
deseq_data_sig_withID = merge(deseq_data_sig5, entrezID, by = 'Gene.name')

geneList = deseq_data_sig_withID$log2FoldChange
names(geneList) = as.character(deseq_data_sig_withID$entrezID)
geneList = sort(geneList, decreasing = TRUE)
gene <- names(geneList)[abs(geneList) > 2]

dd5 <- enrichDO(gene          = gene,
              ont           = "DO",
              pvalueCutoff  = 0.05,
              pAdjustMethod = "BH",
              universe      = names(geneList),
              minGSSize     = 5,
              maxGSSize     = 500,
              qvalueCutoff  = 0.05,
              readable      = FALSE)
rm(entrezID, deseq_data_sig_withID, geneList, gene)

list_dd= list('NAFLvsControl' = dd1, 'NASH_F0-F1vscontrol' = dd2,
               'NASH_F2vscontrol' = dd3, 'NASH_F3vscontrol' = dd4, 'NASH_F4vscontrol' = dd5)
```

```r
write.xlsx(list_dd, file = 'data/Disease_enrichment.NAFLorNASHvsControl.xlsx')
```

```r
y <- mutate(dd1, richFactor = Count / as.numeric(sub("/\\d+", "", BgRatio)))

ggplot(y, showCategory = 20,
  aes(richFactor, fct_reorder(Description, richFactor))) +
  geom_segment(aes(xend=0, yend = Description)) +
  geom_point(aes(color=p.adjust, size = Count)) +
  scale_color_viridis_c(guide=guide_colorbar(reverse=TRUE)) +
  scale_size_continuous(range=c(1, 6)) +
  theme_minimal() +
  xlab("rich factor") +
  ylab(NULL) +
  ggtitle("Enriched Disease Ontology")
```