Group 2 report: Characterization of Alzheimer's disease

A project for the course Statistics in Bioinformatics

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1 Setup

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

#BiocManager::install("RCy3")
```

```
# Libraries we need
libs <- c(
  "tidyverse", "readxl", "limma", "ggrepel", "magrittr", "kableExtra",
  "patchwork", "DT", "tidymodels", "ggbeeswarm", "gt", "skimr", "GGally",
  "visdat", "corrr", "ggsignif", "vip", "themis", "keras", "xgboost", "kknn",
  "tensorflow", "xlsx", "HDAnalyzeR", "plotly", "umap", "uwot", "ggplotify",
  "cowplot", "ggvenn", "UpSetR", "ComplexUpset", "DESeq2", "pheatmap", "randomForest",
  "caret", "edgeR", "STRINGdb", "igraph", "clusterProfiler", "org.Hs.eg.db", "enrichplot",
  "egg", "RCy3", "RColorBrewer"
)

# Install missing libraries
```

```r
installed_libs <- libs %in% rownames(installed.packages())
if (any(installed_libs == FALSE)) {
  install.packages(libs[!installed_libs])
}

# Load libraries
invisible(lapply(libs, library, character.only = TRUE))
```

2 QC

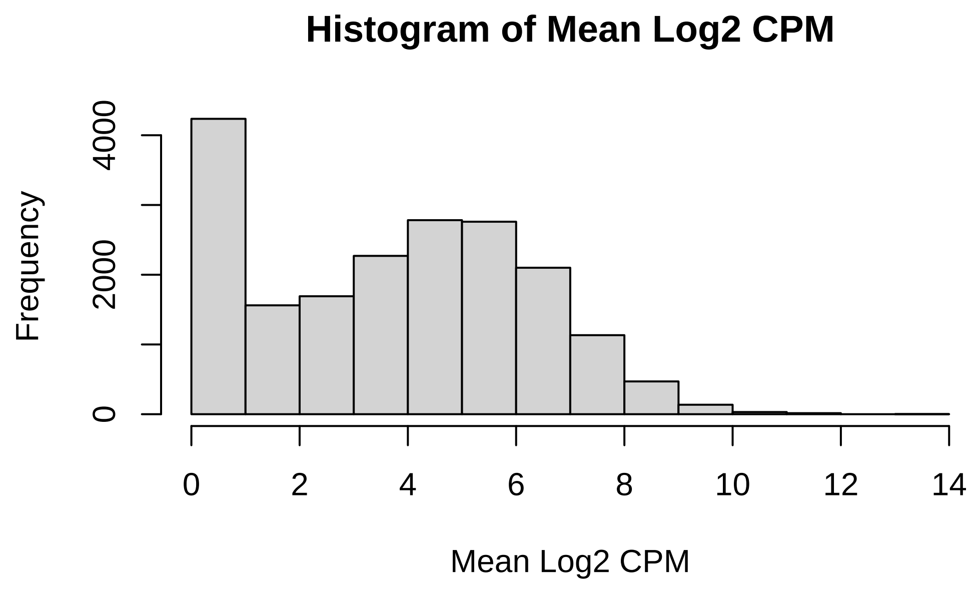
```r
# Load gene expression count data
count_data <- read.table("data/GSE53697_RNAseq_AD.txt", header = TRUE, sep = "\t", row.names
  dplyr::select(matches("_raw$"))
```

```r
# Round counts to integers
count_data_filtered <- count_data %>%
  dplyr::select(starts_with("C"), starts_with("A")) %>%
  mutate_all(~round(.))
```

```r
count_data_filtered <- count_data
genes_before_filtering <- nrow(count_data_filtered)
cat("Number of genes before filtering:", genes_before_filtering, "\n")
```

```
Number of genes before filtering: 19185
```

```r
dge <- DGEList(counts = count_data_filtered)
# dge <- calcNormFactors(dge)
meanLog2CPM <- rowMeans(log2(cpm(dge) + 1))
hist(meanLog2CPM, main = "Histogram of Mean Log2 CPM", xlab = "Mean Log2 CPM")
```

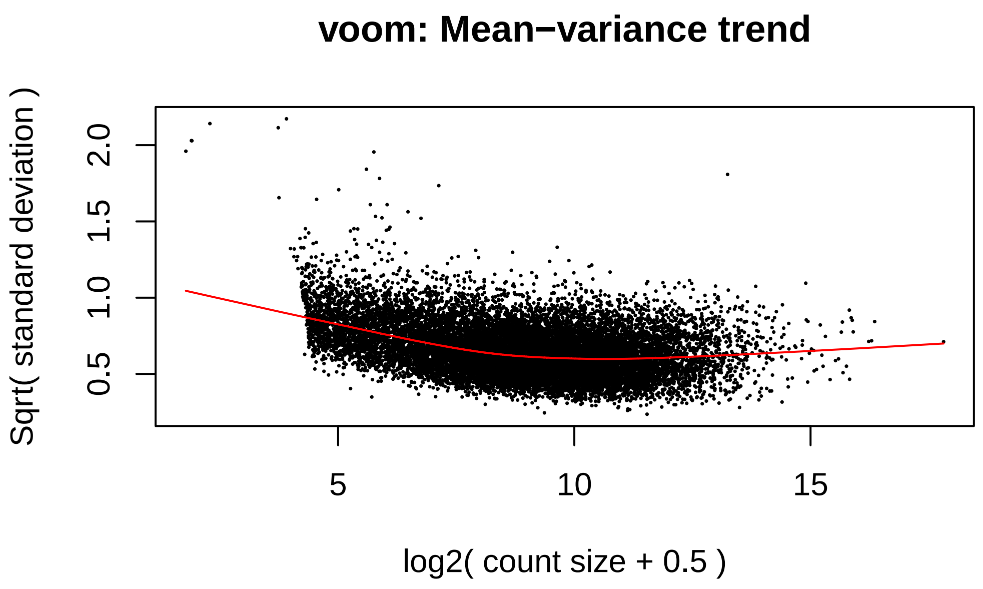


```
count_data_filtered <- count_data_filtered[meanLog2CPM > 1, ]
genes_after_filtering <- nrow(count_data_filtered)
cat("Number of genes after filtering:", genes_after_filtering, "\n")
```

```
Number of genes after filtering: 14950
```

```
sample_conditions <- data.frame(
  sample = colnames(count_data_filtered),
  condition = ifelse(grepl("^C", colnames(count_data_filtered)), "HC", "AD"),
  batch = c(0, 0, 0, 1, 1, 1, 1, 1, 0, 0, 0, 1, 1, 1, 1, 1, 1)  # Manually adding batch info
)
```

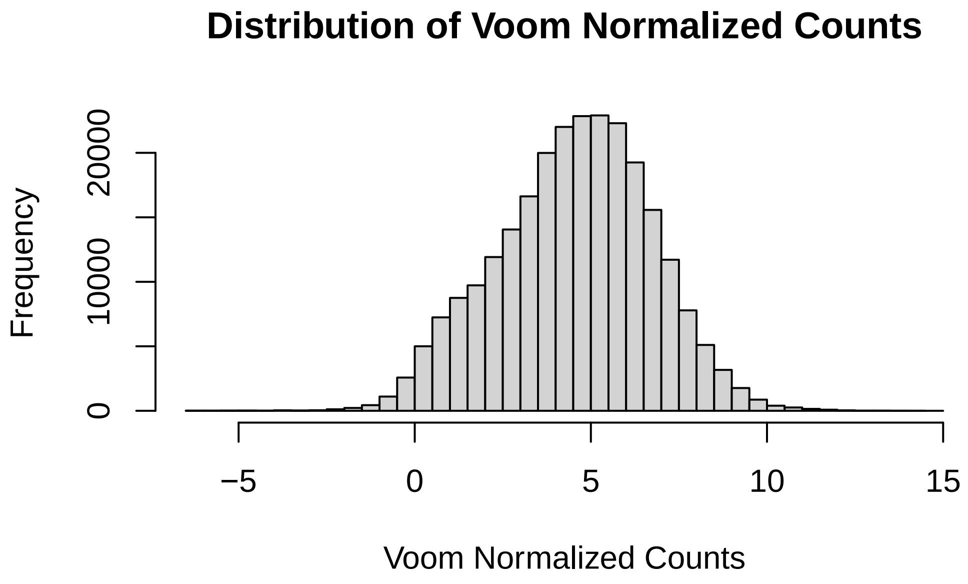
```
dge <- DGEList(counts = count_data_filtered)
dge <- calcNormFactors(dge)
# design <- model.matrix(~ condition + batch, data = sample_conditions)
design <- model.matrix(~ condition + factor(batch), data = sample_conditions)
v <- voom(dge, design, plot = TRUE)
```



```
head(v$E)
```

```
     C1_raw   C2_raw   C3_raw   C4_raw   C5_raw   C6_raw   C7_raw   C8_raw
1  1.666028 3.622681 2.402353 2.242940 2.175301 1.945160 2.220551 2.047241
2  8.171797 7.319737 8.634353 8.026213 9.276020 8.897405 7.848728 7.295732
12 6.987940 2.022997 3.397751 3.657259 6.019456 7.878962 2.560113 3.316176
14 6.421959 6.029584 6.000448 6.510198 5.319996 6.775631 6.584768 6.530001
16 8.762372 8.293814 8.435144 8.724345 7.865274 8.882593 8.895612 8.728100
18 8.144870 7.275008 8.455469 8.478941 8.341384 8.366975 8.453630 8.680521
     A9_raw  A10_raw  A11_raw  A12_raw  A13_raw  A14_raw  A15_raw  A16_raw
1  1.906348 1.627370 2.073706 2.429696 2.914332 2.243341 2.639876 2.290996
2  8.228932 8.609914 7.917652 8.579893 8.510049 8.863482 8.725027 8.051962
12 4.499693 5.975793 3.873325 6.067364 7.633415 7.161695 5.411179 8.052767
14 6.392968 6.333542 6.492932 6.165991 5.714636 6.341330 6.336129 6.563178
16 8.703734 8.418269 8.892265 8.288686 8.648926 8.520732 8.270540 8.844795
18 8.301662 8.294551 8.288104 8.189206 8.289339 8.255371 8.281214 8.574186
    A17_raw
1  2.772543
2  8.536880
12 5.083795
14 6.130582
16 8.484317
18 8.402370
```

```r
hist(v$E, breaks = 50, main = "Distribution of Voom Normalized Counts", xlab = "Voom Normali
```

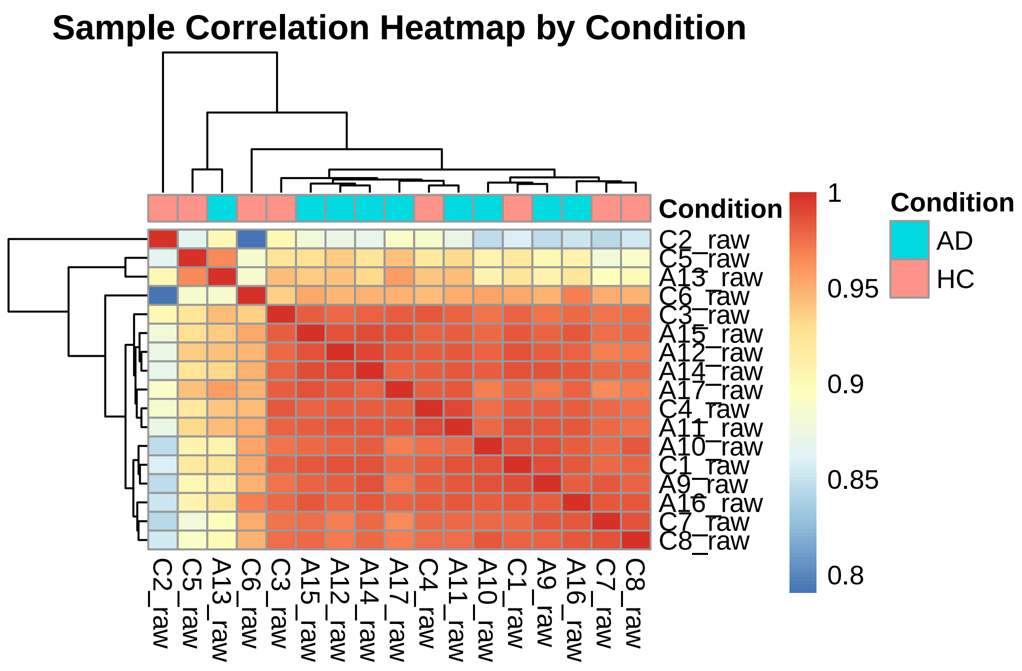


```r
# dds <- DESeqDataSetFromMatrix(countData = count_data_filtered,
#                               colData = sample_conditions,
#                               design = ~ condition + factor(batch))
# Normalize counts using variance stabilizing transformation (vst)
# normCounts <- vst(dds, blind = TRUE)
# assay(normCounts)[1:5, 1:5]
# hist(assay(normCounts), breaks = 50, main = "Distribution of VST Normalized Counts", xlab =
```

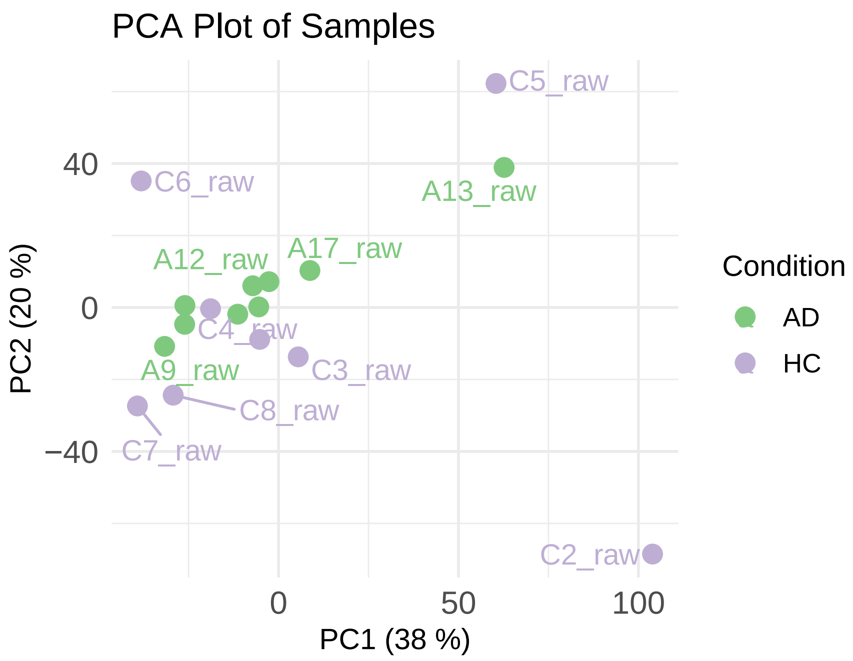
```r
count_data_with_symbols <- read.table("data/GSE53697_RNAseq_AD.txt", header = TRUE, sep = "\
gene_symbols <- count_data_with_symbols[, "GeneSymbol", drop = FALSE]
voom_normalized <- as.data.frame(v$E)  # Convert v$E to a data frame
voom_normalized$GeneID <- rownames(voom_normalized)  # Add GeneID as a column
gene_info_voom <- merge(voom_normalized, gene_symbols, by.x = "GeneID", by.y = "row.names",
gene_info_voom <- gene_info_voom[, c("GeneID", "GeneSymbol", setdiff(names(gene_info_voom),
# write.table(gene_info_voom, file = "voom_normalized.tsv", sep = "\t", quote = FALSE, row.na
```

```r
# sampleDist <- cor(assay(normCounts), method = "spearman")
sampleDist <- cor(v$E, method = "spearman")
sampleColor <- brewer.pal(3, "Accent")[1:2]
```

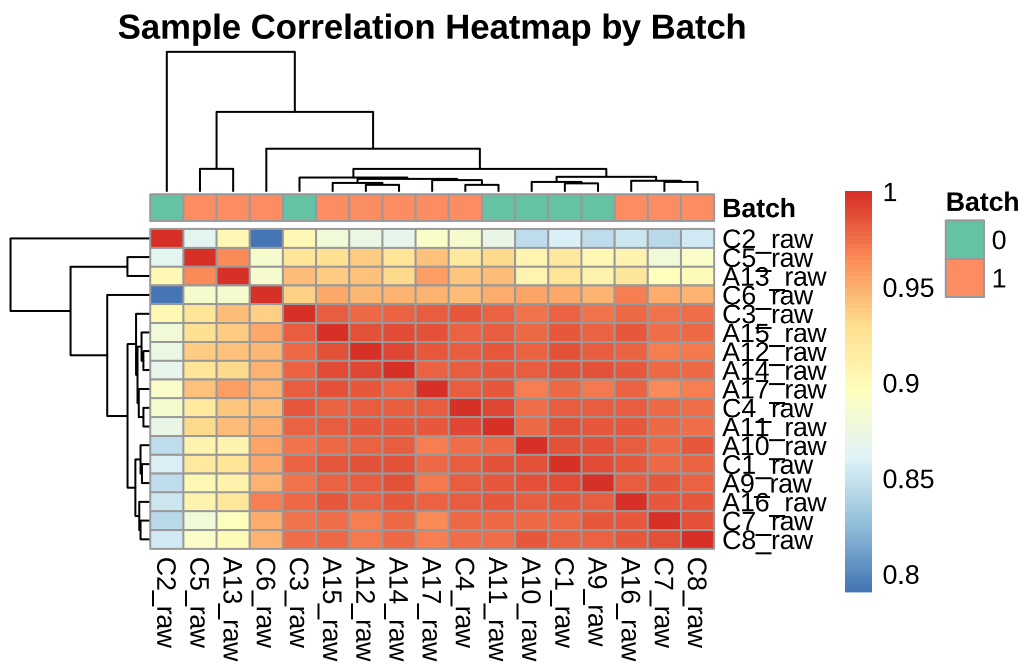
```r
names(sampleColor) <- unique(sample_conditions$condition)

pheatmap(sampleDist,
  clustering_distance_rows = as.dist(1 - sampleDist),
  clustering_distance_cols = as.dist(1 - sampleDist),
  annotation_col = data.frame(Condition = sample_conditions$condition, row.names = sample_co
  annotation_colors = list(Storage = sampleColor),
  main = "Sample Correlation Heatmap by Condition")
```



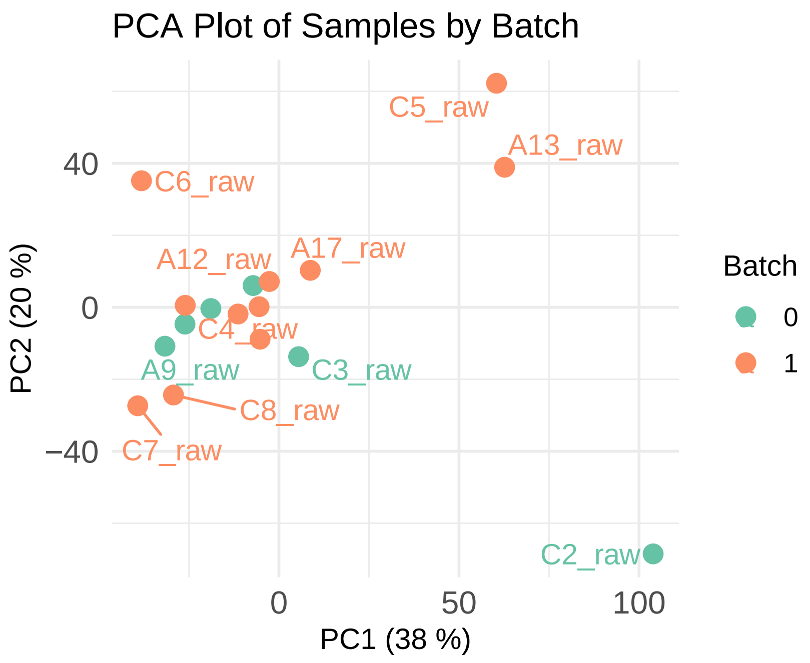
```r
# pcaRes <- prcomp(t(assay(normCounts)))
pcaRes <- prcomp(t(v$E))
varExp <- round(pcaRes$sdev^2 / sum(pcaRes$sdev^2) * 100)
pcaDF <- data.frame(
  PC1 = pcaRes$x[, 1],
  PC2 = pcaRes$x[, 2],
  Condition = sample_conditions$condition,
  Sample = sample_conditions$sample
)
pcaPlot <- ggplot(
  data = pcaDF,
  mapping = aes(x = PC1, y = PC2, color = Condition, label = Sample)
) +
  geom_point(size = 3) +
  geom_text_repel(size = 4) +
  labs(x = paste0("PC1 (", varExp[1], " %)") ,
       y = paste0("PC2 (", varExp[2], " %)") ,
       title = "PCA Plot of Samples") +
  theme_minimal() +
  theme(axis.text = element_text(size = 12), legend.text = element_text(size = 10)) +
  scale_color_manual(values = brewer.pal(3, "Accent")) +
  coord_fixed(ratio = 1)  # Added fixed ratio to ensure equal scaling between PC1 and PC2
print(pcaPlot)
```



```
# Create a heatmap showing Batch 1 and Batch 2 samples separately
sampleDist_batch <- cor(v$E, method = "spearman")
batchColor <- brewer.pal(3, "Set2")[1:2]
names(batchColor) <- unique(sample_conditions$batch)

pheatmap(sampleDist_batch,
  clustering_distance_rows = as.dist(1 - sampleDist_batch),
  clustering_distance_cols = as.dist(1 - sampleDist_batch),
  annotation_col = data.frame(Batch = factor(sample_conditions$batch), row.names = sample_co
  annotation_colors = list(Batch = batchColor),
  main = "Sample Correlation Heatmap by Batch")
```



```r
# PCA Plot by Batch
pcaRes_batch <- prcomp(t(v$E))
varExp_batch <- round(pcaRes_batch$sdev^2 / sum(pcaRes_batch$sdev^2) * 100)
pcaDF_batch <- data.frame(
  PC1 = pcaRes_batch$x[, 1],
  PC2 = pcaRes_batch$x[, 2],
  Batch = factor(sample_conditions$batch),
  Sample = sample_conditions$sample
)
pcaPlot_batch <- ggplot(
  data = pcaDF_batch,
  mapping = aes(x = PC1, y = PC2, color = Batch, label = Sample)
) +
  geom_point(size = 3) +
  geom_text_repel(size = 4) +
  labs(x = paste0("PC1 (", varExp_batch[1], " %)"),
       y = paste0("PC2 (", varExp_batch[2], " %)"),
       title = "PCA Plot of Samples by Batch") +
  theme_minimal() +
  theme(axis.text = element_text(size = 12), legend.text = element_text(size = 10)) +
  scale_color_manual(values = brewer.pal(3, "Set2")) +
  coord_fixed(ratio = 1)
print(pcaPlot_batch)
```



```
# Remove identified outliers
# outliers <- c("C1_raw", "C2_raw", "C4_raw", "C5_raw", "C6_raw")
outliers <- c("C1_raw", "C5_raw", "C6_raw")
remaining_samples <- setdiff(colnames(count_data_filtered), outliers)
count_data_filtered <- count_data_filtered[, remaining_samples]
sample_conditions <- sample_conditions[!sample_conditions$sample %in% outliers, ]
```

3 Differential expression analysis

3.1 Design matrix

```
# Define design matrix including batch effect
# designMatrix <- model.matrix(~ 0 + condition + batch, data = sample_conditions)
designMatrix <- model.matrix(~ 0 + condition + factor(batch), data = sample_conditions)

colnames(designMatrix) <- make.names(colnames(designMatrix))  # Ensure valid column names fo
cat("Design Matrix with Batch Effect:\n")
```

```
Design Matrix with Batch Effect:
```

```
print(head(designMatrix))
```

```
  conditionAD conditionHC factor.batch.1
2           0           1              0
3           0           1              0
4           0           1              1
7           0           1              1
8           0           1              1
9           1           0              0
```

```
# Define contrast matrix
contrastMatrix <- makeContrasts(AD_vs_HC = conditionAD - conditionHC, levels = designMatrix)
cat("Contrast Matrix:\n")
```

```
Contrast Matrix:
```

```
print(contrastMatrix)
```

```
                Contrasts
Levels           AD_vs_HC
  conditionAD           1
  conditionHC          -1
  factor.batch.1        0
```

```
# Prepare DGEList and estimate dispersion
dge <- DGEList(count_data_filtered)
dge <- calcNormFactors(dge)
dge <- estimateDisp(dge, designMatrix, robust = TRUE)

# Fit the model using likelihood ratio test (LRT), now including batch in the design
fit <- glmFit(dge, designMatrix)
lrt <- glmLRT(fit, contrast = contrastMatrix)
```

3.2 Hypothesis correction

```
res <- topTags(lrt, n = nrow(count_data_filtered))
res$table$FDR <- p.adjust(res$table$PValue, method = "BH")
cat("Summary of p-values:\n")
```

```
Summary of p-values:
```

```
print(summary(res$table$PValue))
```

```
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
 0.0000  0.1672  0.4128  0.4423  0.7062  1.0000 
```

```
cat("Summary of FDRs:\n")
```

```
Summary of FDRs:
```

```
print(summary(res$table$FDR))
```

```
     Min.   1st Qu.    Median      Mean   3rd Qu.      Max. 
0.0000056 0.6683192 0.8253958 0.7729789 0.9415187 1.0000000 
```

```
sigRes <- subset(res$table, FDR < 0.05 & abs(logFC) > 1)
cat("Number of significant genes:", nrow(sigRes), "\n")
```

```
Number of significant genes: 63
```

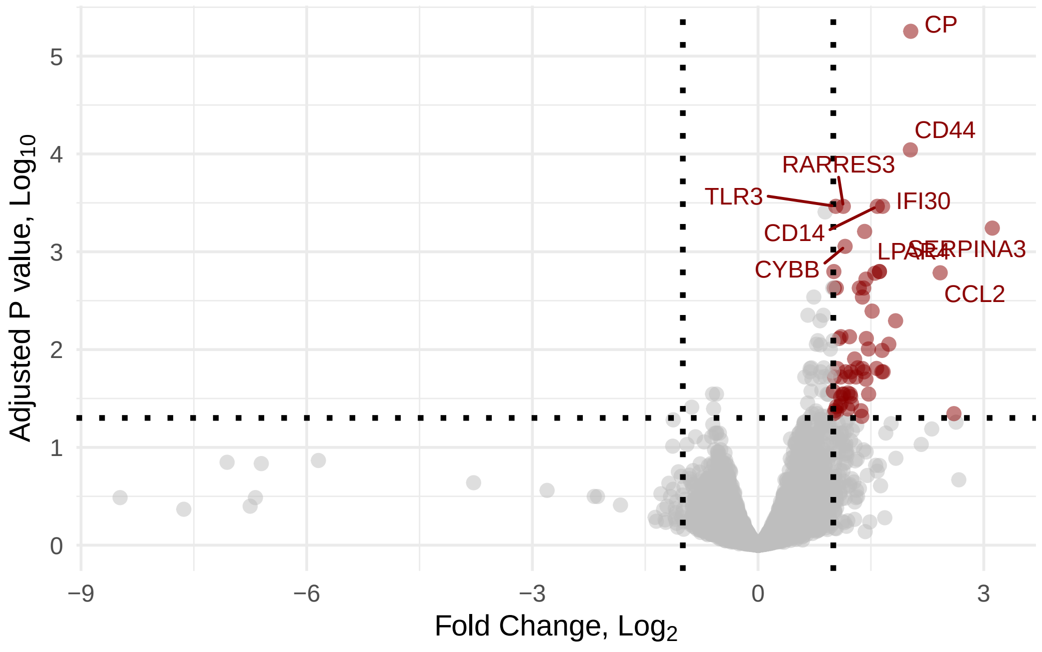
```
knitr::kable(head(sigRes))
```

	logFC	logCPM	LR	PValue	FDR
1356	2.029851	5.348685	39.25040	0e+00	0.0000056
960	2.024781	5.760368	32.46153	0e+00	0.0000909
5920	1.133116	3.334786	28.76463	1e-07	0.0003432
10437	1.655241	2.208464	28.46395	1e-07	0.0003432
7098	1.032912	1.682106	27.89196	1e-07	0.0003432
929	1.585105	3.429787	27.75458	1e-07	0.0003432

```
count_data_with_symbols <- read.table("data/GSE53697_RNAseq_AD.txt", header = TRUE, sep = "\
gene_symbols <- count_data_with_symbols[, "GeneSymbol", drop = FALSE]
sigRes$GeneID <- rownames(sigRes)  # Add GeneID as a column to the results
gene_info <- merge(sigRes, gene_symbols, by.x = "GeneID", by.y = "row.names", all.x = TRUE)
gene_info <- gene_info[, c("GeneID", "GeneSymbol", setdiff(names(gene_info), c("GeneID", "Ge
# write.table(gene_info, file = "sig_genes.tsv", sep = "\t", quote = FALSE, row.names = FALS
```

3.3 Volcano plot

```
# Add gene symbols to the results
count_data_with_symbols <- read.table("data/GSE53697_RNAseq_AD.txt", header = TRUE, sep = "\t
gene_symbols <- count_data_with_symbols[, "GeneSymbol", drop = FALSE]
res$table$GeneID <- rownames(res$table)
gene_info_all <- merge(res$table, gene_symbols, by.x = "GeneID", by.y = "row.names", all.x =

# Volcano plot using gene symbols for all genes
volcanoPlot <- ggplot(gene_info_all, aes(x = logFC, y = -log10(FDR),
                                          color = ifelse(FDR < 0.05 & abs(logFC) > 1, "darkred
                                          label = ifelse(FDR < 0.05 & abs(logFC) > 1, GeneSymb
  geom_point(alpha = 0.5, size = 2) +
  xlab(expression("Fold Change, Log"[2]*"")) +
  ylab(expression("Adjusted P value, Log"[10]*"")) +
  geom_vline(xintercept = c(-1, 1), linetype = "dotted", linewidth = 1) +
  geom_hline(yintercept = -log10(0.05), linetype = "dotted", linewidth = 1) +
  theme_minimal() +
  theme(legend.position = "none") +
  scale_colour_manual(values = c("darkred", "grey")) +
  geom_text_repel(size = 3, max.overlaps = 10)

print(volcanoPlot)
```



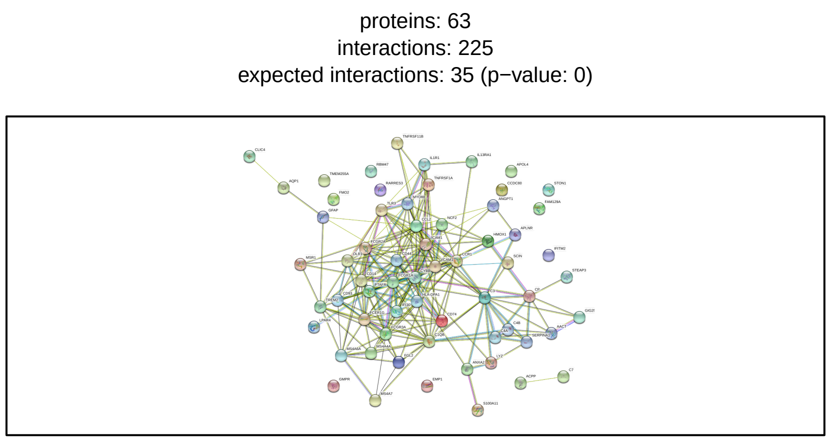
4 Protein-protein interaction analysis

```
# Initialize STRINGdb
string_db <- STRINGdb$new(version = "11", species = 9606, score_threshold = 400, input_direc
gene_symbols <- gene_info$GeneSymbol
gene_symbols <- data.frame(GeneSymbol = gene_symbols, stringsAsFactors = FALSE)
mapped_genes <- string_db$map(gene_symbols, "GeneSymbol", removeUnmappedRows = TRUE)
```

```
Warning:  we couldn't map to STRING 1% of your identifiers
```

```
# Get PPI Network for the mapped genes
ppi_network <- string_db$get_interactions(mapped_genes$STRING_id)
string_db$plot_network(mapped_genes$STRING_id)
```

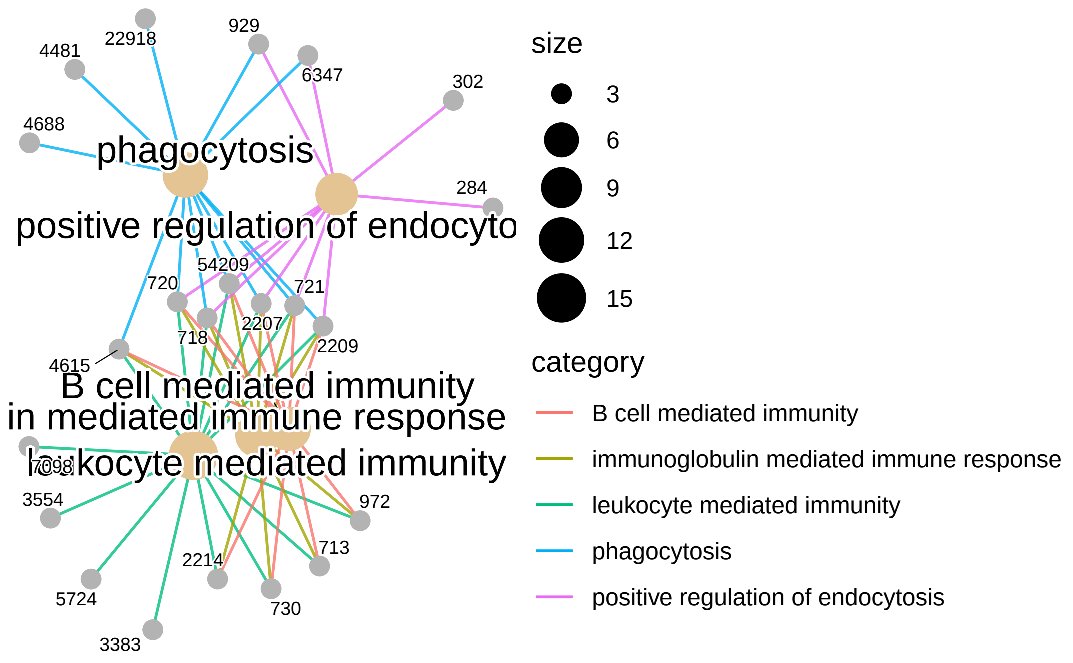
```
[1] "Parameter add_link not available in version 11.0 (please use 11.0b or later)"
```



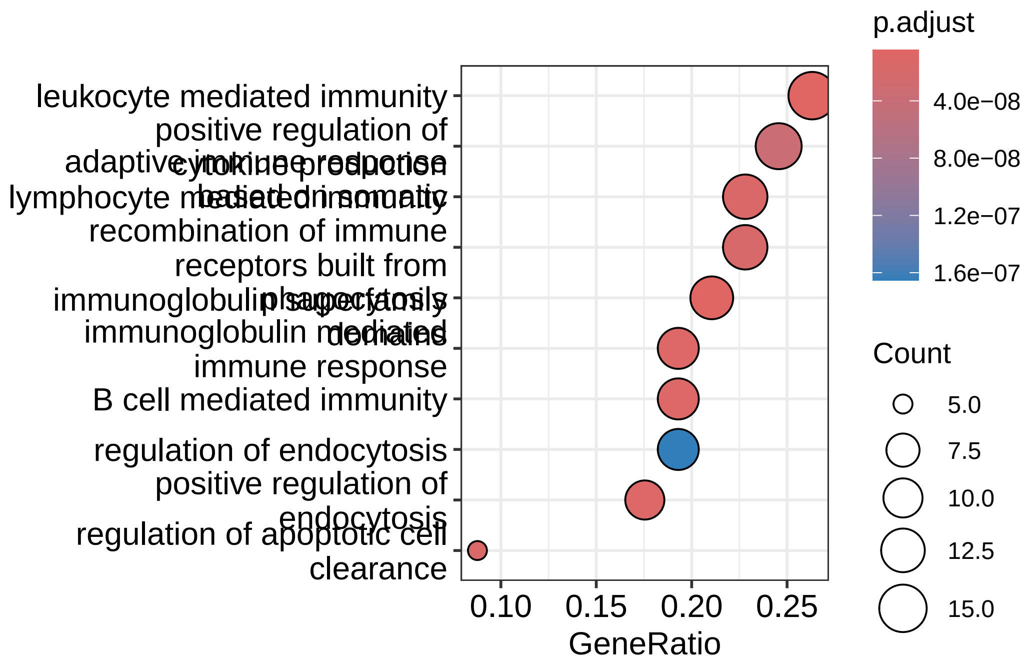
```r
gene_symbols_vector <- gene_symbols$GeneSymbol  # Extract the gene symbols as a vector
mapped_genes_entrez <- bitr(gene_symbols_vector,
                            fromType = "SYMBOL",
                            toType = "ENTREZID",
                            OrgDb = org.Hs.eg.db)

goSEA <- enrichGO(gene = mapped_genes_entrez$ENTREZID,
                  OrgDb = org.Hs.eg.db,
                  keyType = "ENTREZID",
                  ont = "BP",
                  pAdjustMethod = "BH",
                  pvalueCutoff = 0.05,
                  qvalueCutoff = 0.05)
```

5 Enrichment analysis

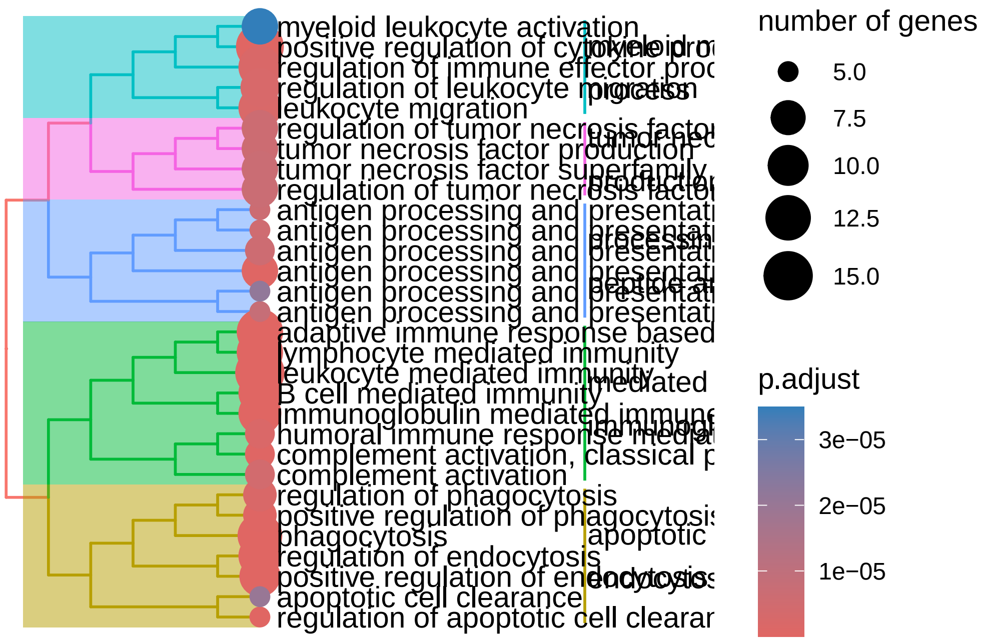
```r
cnetplot(goSEA, colorEdge = TRUE, cex_label_gene = 0.5)
```



```
dotplot(goSEA)
```



```r
goSEA <- pairwise_termsim(goSEA)
treeplot(goSEA)
```



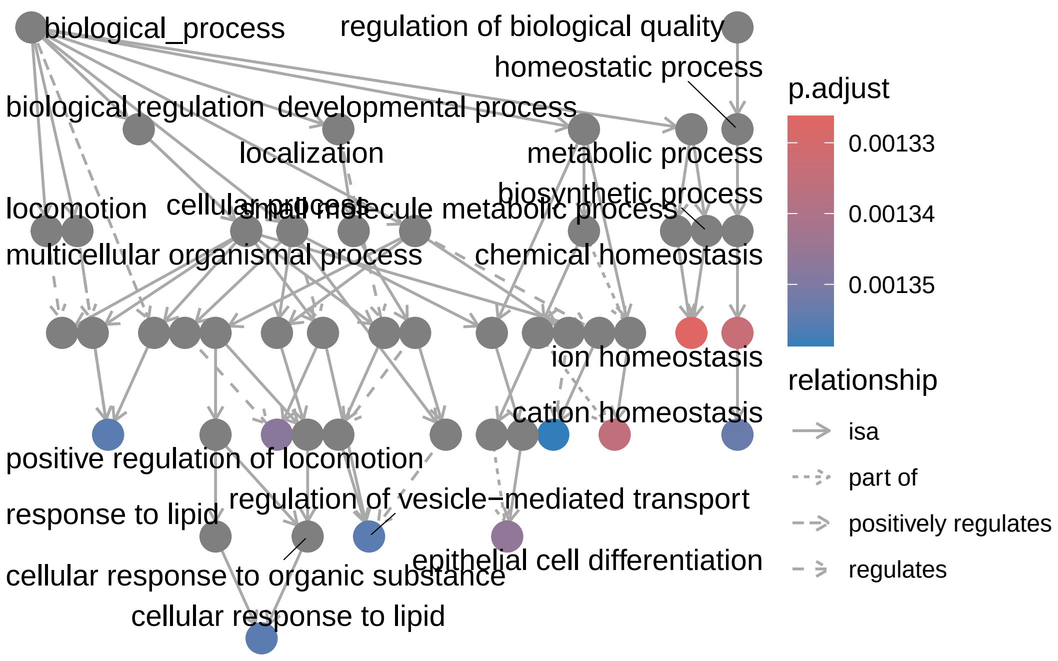
```r
# import data
significant_genes <- read_tsv('data/significant_genes.tsv')
```

6 GSEA Analysis

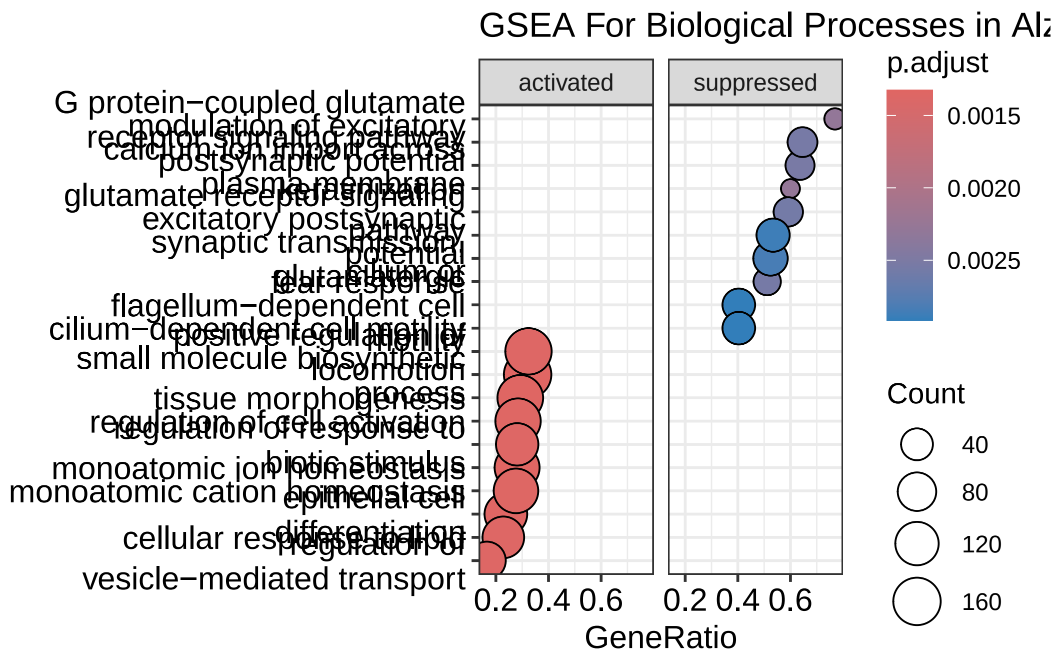
```r
all_genes <- as.data.frame(read_csv('data/all_gene_processed.csv'))
rownames(all_genes) <- all_genes$`...1`
all_genes <- all_genes[,2:6]

processed_genes_for_gse <- as.vector(all_genes$logFC)  # p value vector
names(processed_genes_for_gse) <- as.vector(rownames(all_genes)) # gene ids
processed_genes_for_gse <- processed_genes_for_gse[order(-processed_genes_for_gse)] # order i
#processed_genes_for_gse


ego <- gseGO(geneList      = processed_genes_for_gse,
              OrgDb        = org.Hs.eg.db,
              ont          = "BP", ## Molecular Function (MF), Biological Process (BP), and
              nPerm        = 1000,
              minGSSize    = 3,
              maxGSSize    = 500,
              pvalueCutoff = 0.05,
              verbose      = TRUE,
              by = "fgsea",
              pAdjustMethod = "none")

ego
```

```
#
# Gene Set Enrichment Analysis
#
#...@organism    Homo sapiens
#...@setType     BP
#...@keytype     ENTREZID
#...@geneList    Named num [1:14947] 3.11 2.67 2.63 2.6 2.42 ...
 - attr(*, "names")= chr [1:14947] "12" "4057" "1117" "80832" ...
#...nPerm    1000
#...pvalues adjusted by 'none' with cutoff <0.05
#...2340 enriched terms found
'data.frame':   2340 obs. of  11 variables:
 $ ID             : chr  "GO:0044283" "GO:0050801" "GO:0048729" "GO:0030855" ...
 $ Description    : chr  "small molecule biosynthetic process" "monoatomic ion homeostasis"
 $ setSize        : int  499 481 485 479 489 474 460 460 459 395 ...
 $ enrichmentScore: num  0.377 0.332 0.325 0.341 0.472 ...
 $ NES            : num  1.77 1.56 1.53 1.6 2.22 ...
 $ pvalue         : num  0.00133 0.00133 0.00134 0.00135 0.00135 ...
 $ p.adjust       : num  0.00133 0.00133 0.00134 0.00135 0.00135 ...
 $ qvalue         : num  0.0201 0.0201 0.0201 0.0201 0.0201 ...
 $ rank           : num  3114 3066 3112 2089 2021 ...
 $ leading_edge   : chr  "tags=32%, list=21%, signal=26%" "tags=28%, list=21%, signal=23%" "
 $ core_enrichment: chr  "5724/834/51302/241/972/344/27306/5354/301/341/8942/1109/653/2247/8
#...Citation
 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,
 clusterProfiler 4.0: A universal enrichment tool for interpreting omics data.
 The Innovation. 2021, 2(3):100141
```

```r
goplot(ego)
```



```r
dotplot(ego, showCategory=10, split=".sign") + facet_grid(.~.sign) + labs(title = "GSEA For B
```

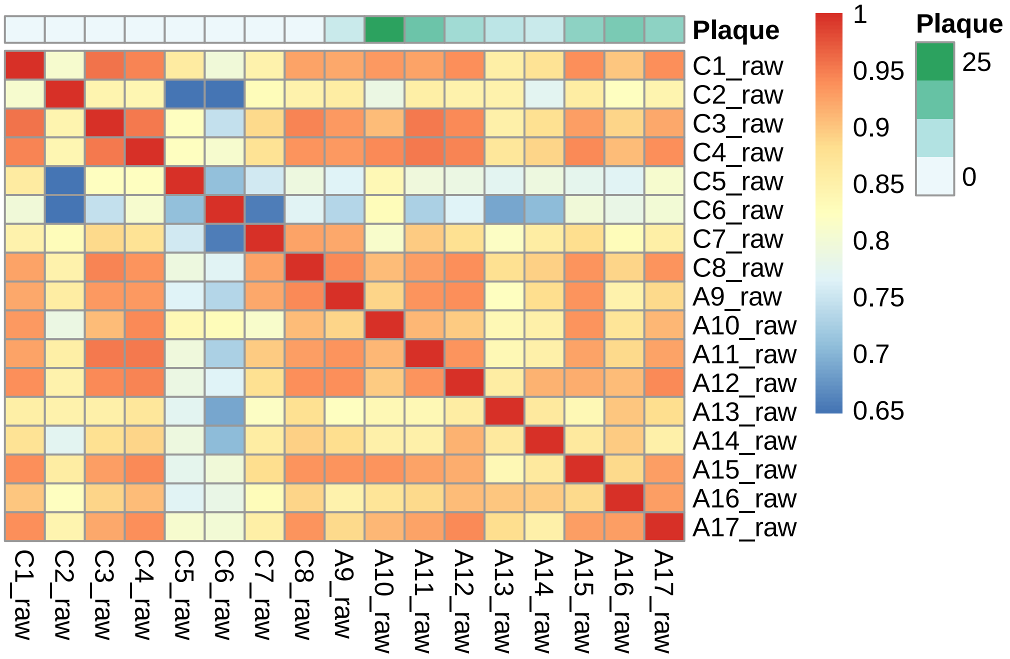


```r
count_data <- read.table("data/GSE53697_RNAseq_AD.txt", header = TRUE, sep = "\t", row.names
  dplyr::select(matches("_raw$"))
subset_count_data <- count_data[rownames(count_data) %in% as.vector(significant_genes$GeneID)
metadata <- read_excel('data/ad_metadata.xlsx')
```

```r
sampleDist <- cor(subset_count_data, method = "spearman")

sample_conditions <- data.frame(
  sample = colnames(subset_count_data),
  condition = ifelse(grepl("^C", colnames(subset_count_data)), "HC", "AD"),
  batch = c(0, 0, 0, 1, 1, 1, 1, 1, 0, 0, 0, 1, 1, 1, 1, 1, 1),  ## Manually adding batch in
  plaque = metadata$BM9_plaque
)
sample_conditions
```

sample	condition	batch	plaque
C1_raw	HC	0	0.0
C2_raw	HC	0	0.0
C3_raw	HC	0	0.0
C4_raw	HC	1	0.0
C5_raw	HC	1	0.0
C6_raw	HC	1	0.0
C7_raw	HC	1	0.0
C8_raw	HC	1	0.0
A9_raw	AD	0	5.6
A10_raw	AD	0	27.6
A11_raw	AD	0	17.6
A12_raw	AD	1	11.2
A13_raw	AD	1	7.6
A14_raw	AD	1	5.6
A15_raw	AD	1	13.6
A16_raw	AD	1	16.0
A17_raw	AD	1	13.7

```r
heat_colors <- list(condition = c("HC" = "#01bfc5", "AD" = "salmon"))


pheatmap(mat = sampleDist,
    cluster_rows = FALSE,
    cluster_cols = FALSE,
    annotation_col = data.frame(Plaque = sample_conditions$plaque, row.names = sample_condit
```



7 Incorrect GSEA Analysis

The below analysis does not follow the expected steps! TO AVOID.

7.1 Prepare data

```
# create input vector for gene enrichment
significant_genes_for_gse <- as.vector(significant_genes$logFC)  # p value vector
names(significant_genes_for_gse) <- as.vector(significant_genes$GeneID) # gene ids
significant_genes_for_gse <- significant_genes_for_gse[order(-significant_genes_for_gse)] # 
```

7.2 Run gseGO

```
# run gseGO for ontology ALL
ego_all <- gseGO(geneList    = significant_genes_for_gse,
                 OrgDb        = org.Hs.eg.db,
                 ont          = "ALL", ## Molecular Function (MF), Biological Process (BP), and
                 nPerm        = 1000,
                 minGSSize    = 10,
                 maxGSSize    = 500,
                 pvalueCutoff = 0.05,
                 verbose      = TRUE,
                 by = "fgsea",
                 pAdjustMethod = "none")
```

```
# run gseGO for ontology MF
ego1 <- gseGO(geneList     = significant_genes_for_gse,
              OrgDb        = org.Hs.eg.db,
              ont          = "MF", ## Molecular Function (MF), Biological Process (BP), and
              nPerm        = 1000,
              minGSSize    = 10,
              maxGSSize    = 500,
              pvalueCutoff = 0.05,
              verbose      = TRUE,
              by = "fgsea",
              pAdjustMethod = "none")

## gave 0 enriched genes
```

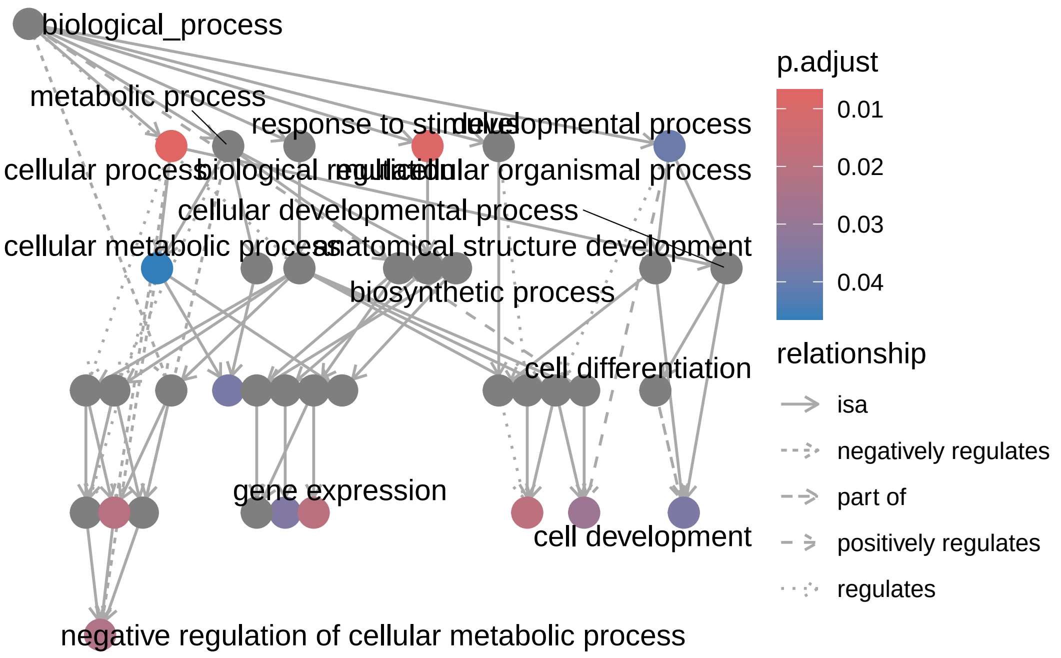
```
# run gseGO for ontology CC
ego2 <- gseGO(geneList     = significant_genes_for_gse,
              OrgDb        = org.Hs.eg.db,
              ont          = "CC", ## Molecular Function (MF), Biological Process (BP), and
              nPerm        = 1000,
              minGSSize    = 10,
              maxGSSize    = 500,
              pvalueCutoff = 0.05,
              verbose      = TRUE,
              by = "fgsea",
              pAdjustMethod = "none")
```

```
# run gseGO for ontology BP
ego3 <- gseGO(geneList     = significant_genes_for_gse,
              OrgDb        = org.Hs.eg.db,
              ont          = "BP", ## Molecular Function (MF), Biological Process (BP), and
              nPerm        = 1000,
              minGSSize    = 10,
              maxGSSize     = 500,
              pvalueCutoff = 0.05,
              verbose       = TRUE,
              by = "fgsea",
              pAdjustMethod = "none") # "holm", "hochberg", "hommel", "bonferroni", "BY
```

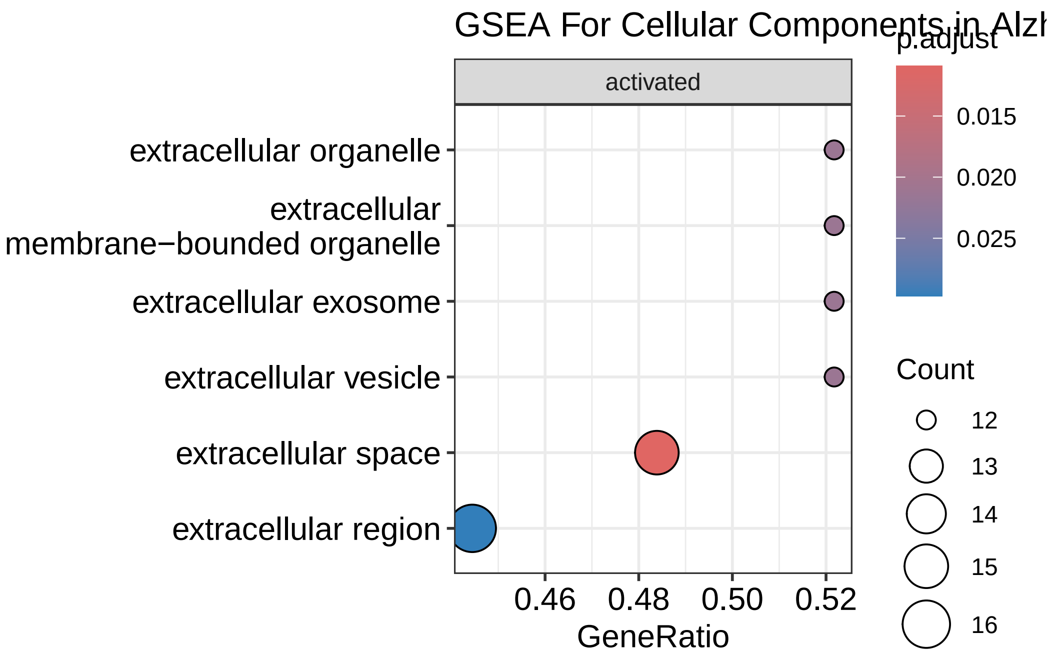
```
# compare the processes and components identified as signifcant between the ontologies
#ego3$Description
#ego2$Description
#ego_all$Description

#setdiff(ego2$Description, ego_all$Description)
#setdiff(ego3$Description, ego_all$Description) # "cellular response to stress" disappears fr
```

7.3 Plots

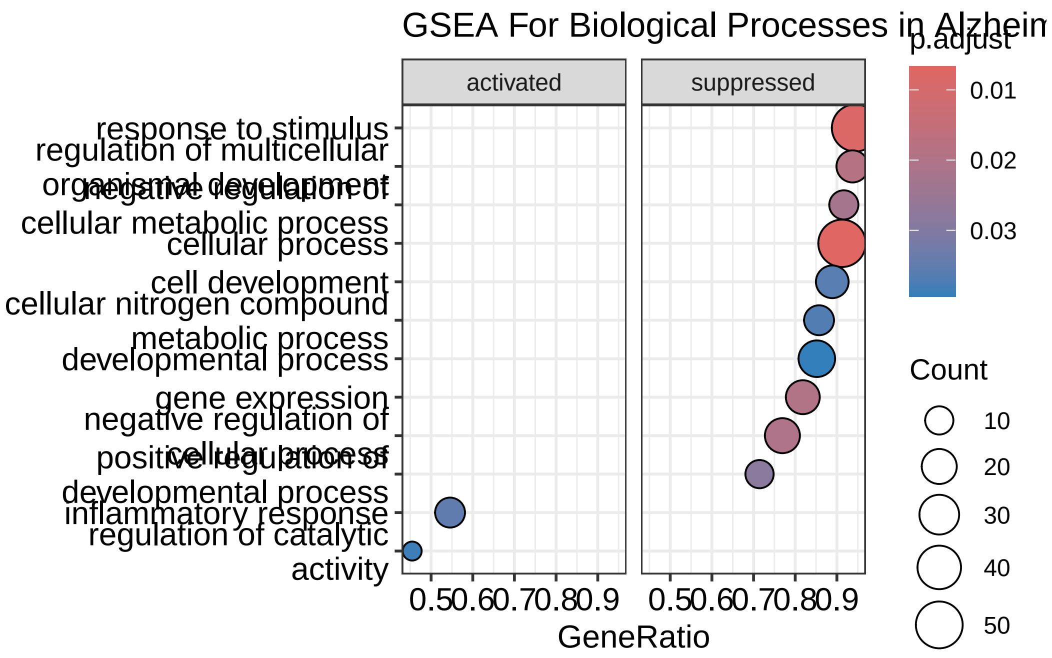
```
goplot(ego3)
```



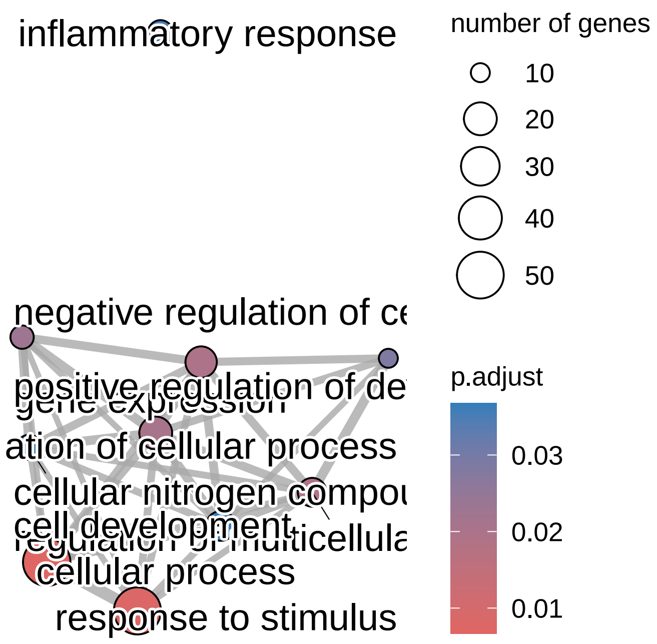
```
dotplot(ego2, showCategory=10, split=".sign") + facet_grid(.~.sign) + labs(title = "GSEA For
```



```
dotplot(ego3, showCategory=10, split=".sign") + facet_grid(.~.sign) + labs(title = "GSEA For
```

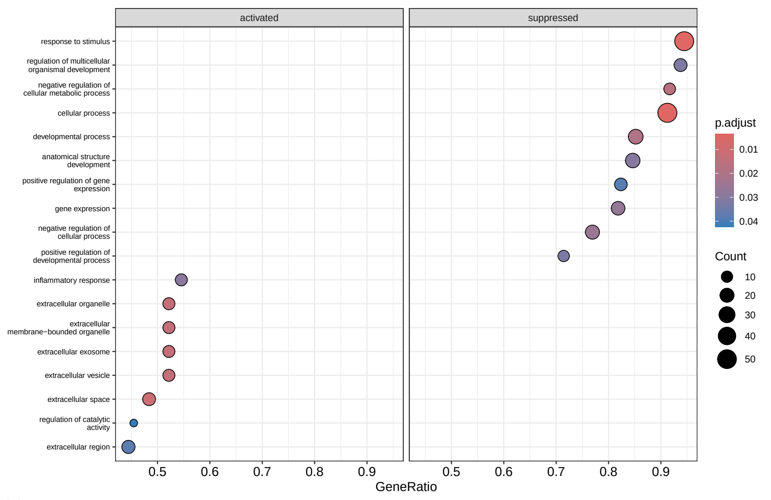


```
emapplot(pairwise_termsim(ego3), showCategory = 10)
```



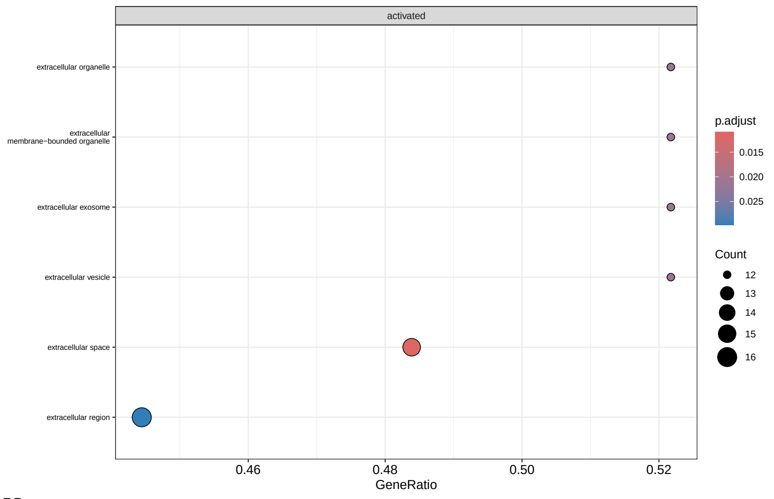
```
## compare the different plots
d_all <- dotplot(ego_all, showCategory=10, split=".sign") + facet_grid(.~.sign) +  theme(axis
d2 <- dotplot(ego2, showCategory=10, split=".sign") + facet_grid(.~.sign)+  theme(axis.text.y
d3 <- dotplot(ego3, showCategory=10, split=".sign") + facet_grid(.~.sign)+  theme(axis.text.y

ggarrange(d_all, d2, d3,
          labels = c("ALL", "CC", "BP"),
          ncol = 1, nrow = 3)
```

ALL



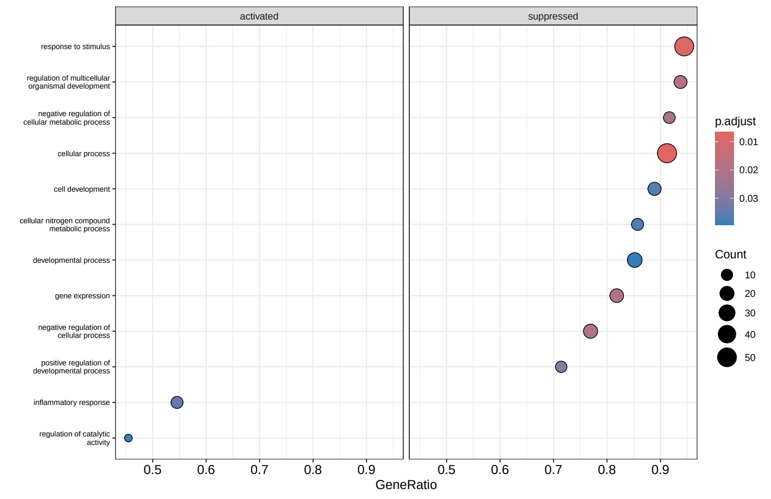
activated
suppressed
response to stimulus
regulation of multicellular organismal development
negative regulation of cellular metabolic process
cellular process
developmental process
anatomical structure development
positive regulation of gene expression
gene expression
negative regulation of cellular process
positive regulation of developmental process
inflammatory response
extracellular organelle
extracellular membrane−bounded organelle
extracellular exosome
extracellular vesicle
extracellular space
regulation of catalytic activity
extracellular region
0.5
0.6
0.7
0.8
0.9
GeneRatio
p.adjust
0.01
0.02
0.03
0.04
Count
10
20
30
40
50

CC



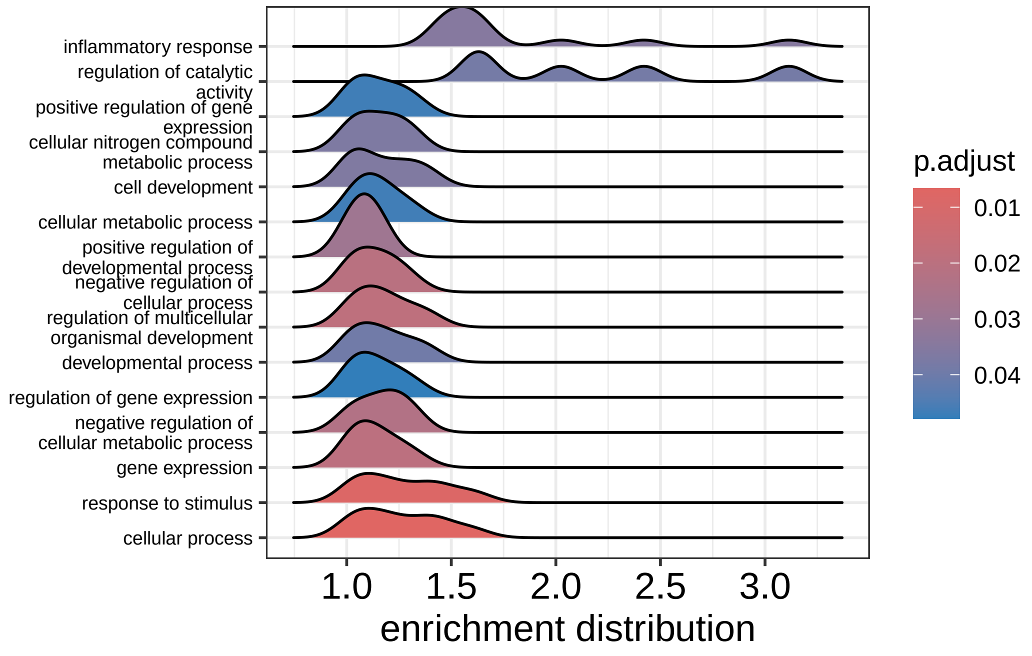
activated
extracellular organelle
extracellular membrane−bounded organelle
extracellular exosome
extracellular vesicle
extracellular space
extracellular region
0.46
0.48
0.50
0.52
GeneRatio
p.adjust
0.015
0.020
0.025
Count
12
13
14
15
16

BP

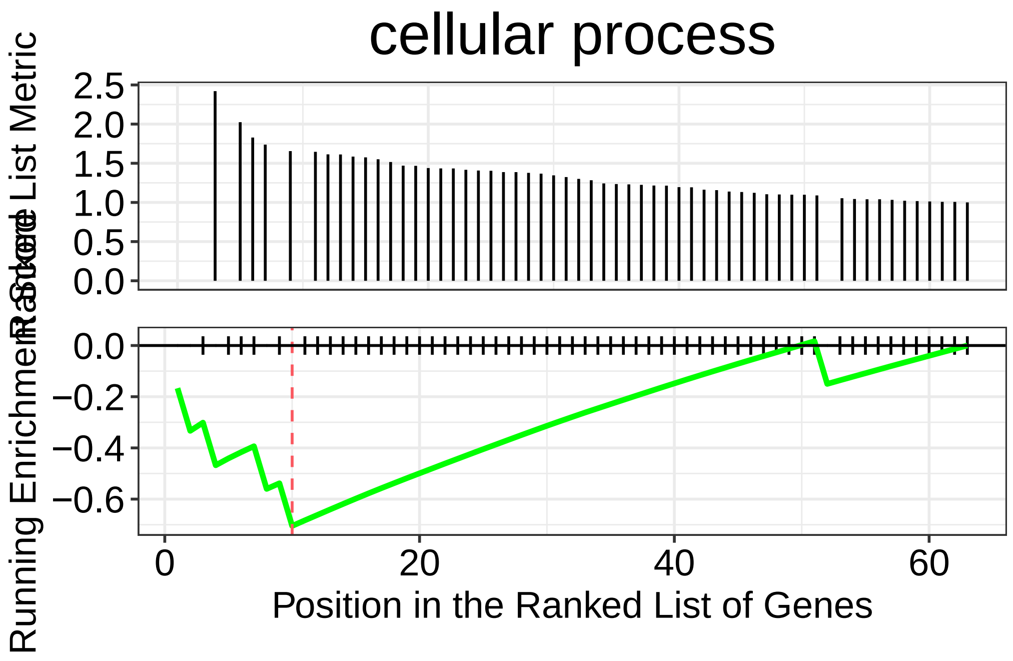


activated
suppressed
response to stimulus
regulation of multicellular organismal development
negative regulation of cellular metabolic process
cellular process
cell development
cellular nitrogen compound metabolic process
developmental process
gene expression
negative regulation of cellular process
positive regulation of developmental process
inflammatory response
regulation of catalytic activity
0.5
0.6
0.7
0.8
0.9
GeneRatio
p.adjust
0.01
0.02
0.03
Count
10
20
30
40
50

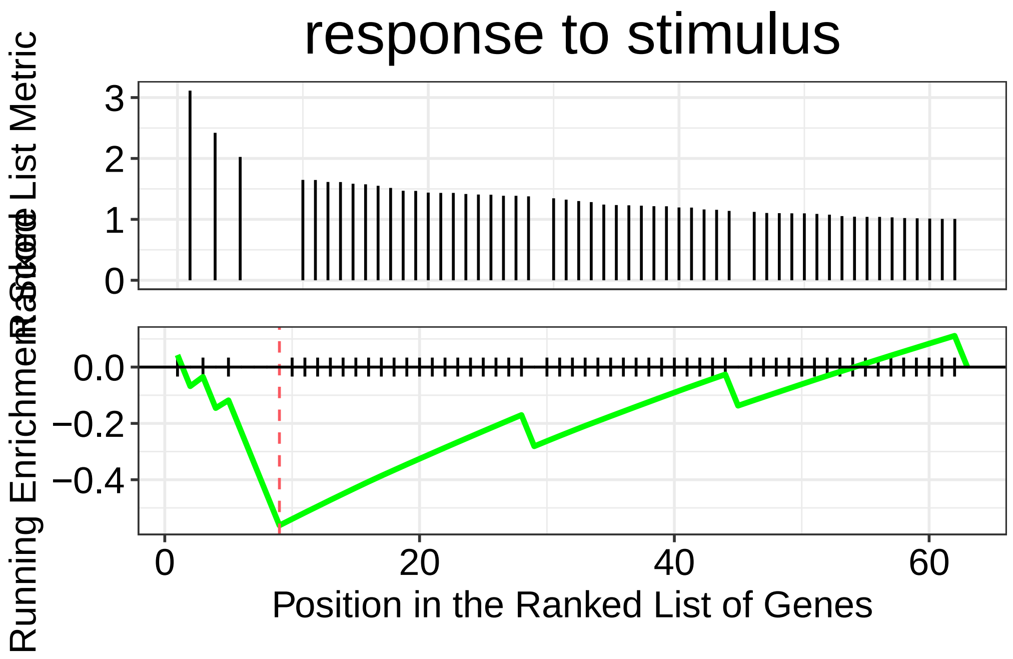
```
ridgeplot(ego3) + labs(x = "enrichment distribution") +   theme(axis.text.y = element_text(h
```



```
gseaplot(ego3, by = "all", title = ego3$Description[1], geneSetID = 1)
```

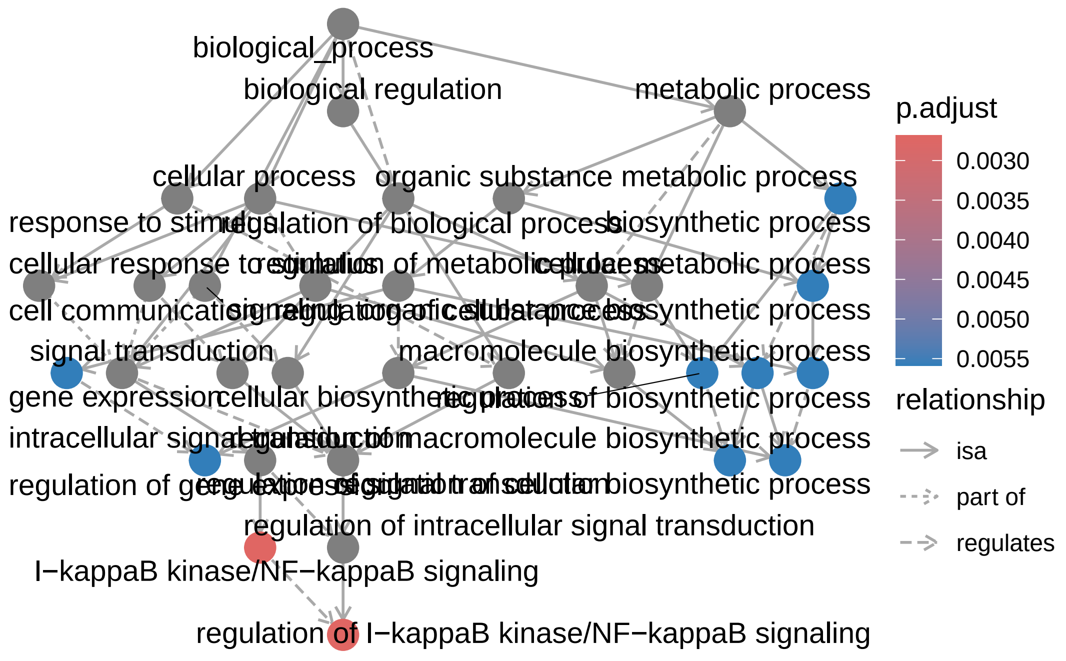


```
gseaplot(ego3, by = "all", title = ego3$Description[2], geneSetID = 2)
```



7.4 GSEA on specific clusters

```
all_genes <- as.data.frame(read_csv('data/all_gene_processed.csv'))
rownames(all_genes) <- all_genes$`...1`
all_genes <- all_genes[,2:6]

voom_normalized <- read_tsv('data/voom_normalized.tsv')

gene_cluster <- read_delim('data/cluster1.txt', delim='\n', col_names = FALSE)
gene_cluster_data <- voom_normalized[voom_normalized$GeneSymbol %in% as.vector(gene_cluster$X
genes_for_gse <- all_genes[rownames(all_genes) %in% gene_cluster_data$GeneID,]

processed_genes_for_gse <- as.vector(genes_for_gse$logFC)  # p value vector
names(processed_genes_for_gse) <- as.vector(rownames(genes_for_gse)) # gene ids
processed_genes_for_gse <- processed_genes_for_gse[order(-processed_genes_for_gse)] # order i

ego <- gseGO(geneList      = processed_genes_for_gse,
              OrgDb         = org.Hs.eg.db,
              ont          = "BP", ## Molecular Function (MF), Biological Process (BP), and C
              nPerm        = 1000,
              minGSSize    = 3,
              maxGSSize    = 500,
              pValueCutoff = 0.05,
              verbose      = TRUE,
              by = "fgsea",
              pAdjustMethod = "none")

goplot(ego)
```



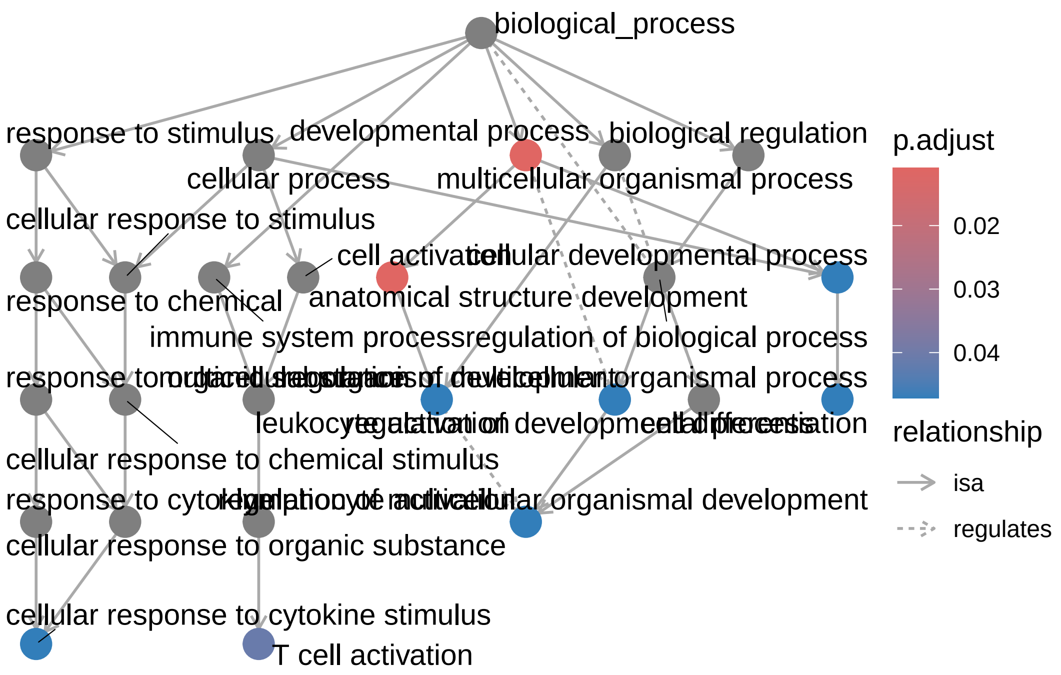
biological_process
biological regulation
metabolic process
cellular process
organic substance metabolic process
response to stimulus
regulation of biological process
biosynthetic process
cellular response to stimulus
regulation of metabolic process
cellular metabolic process
cell communication
signaling
regulation of cellular process
organic substance biosynthetic process
signal transduction
macromolecule biosynthetic process
gene expression
cellular biosynthetic process
regulation of biosynthetic process
intracellular signal transduction
regulation of macromolecule biosynthetic process
regulation of gene expression
regulation of signal transduction
regulation of cellular biosynthetic process
regulation of intracellular signal transduction
I−kappaB kinase/NF−kappaB signaling
regulation of I−kappaB kinase/NF−kappaB signaling
p.adjust
0.0030
0.0035
0.0040
0.0045
0.0050
0.0055
relationship
isa
part of
regulates

```
all_genes <- as.data.frame(read_csv('data/all_gene_processed.csv'))
rownames(all_genes) <- all_genes$`...1`
all_genes <- all_genes[,2:6]

voom_normalized <- read_tsv('data/voom_normalized.tsv')

gene_cluster <- read_delim('data/cluster2.txt', delim='\n', col_names = FALSE)
gene_cluster_data <- voom_normalized[voom_normalized$GeneSymbol %in% as.vector(gene_cluster$X
genes_for_gse <- all_genes[rownames(all_genes) %in% gene_cluster_data$GeneID,]

processed_genes_for_gse <- as.vector(genes_for_gse$logFC)  # p value vector
```

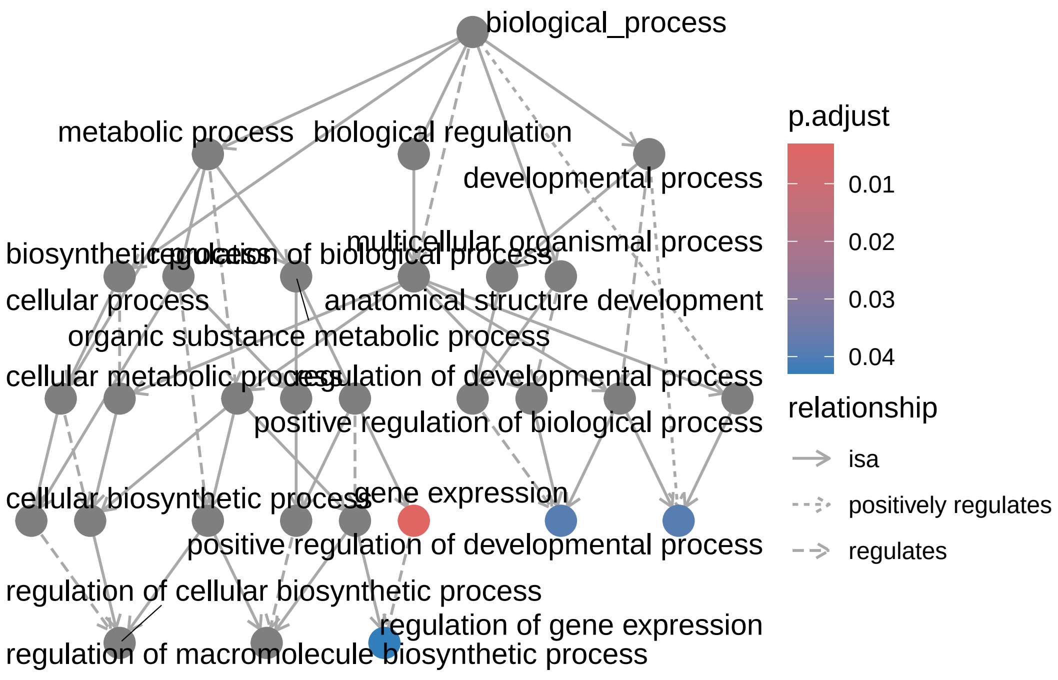
```r
names(processed_genes_for_gse) <- as.vector(rownames(genes_for_gse)) # gene ids
processed_genes_for_gse <- processed_genes_for_gse[order(-processed_genes_for_gse)] # order i
processed_genes_for_gse
```

```
   51338    10437     2214    58475      929     4069      713     5724
1.662620 1.655218 1.646171 1.612562 1.585090 1.469628 1.439121 1.434389
   64231     1230     2207    10581     3113    10875     1536     5920
1.407308 1.404221 1.345758 1.242926 1.234730 1.215954 1.156830 1.133084
    4688      972
1.104455 1.098473
```

```r
ego <- gseGO(geneList     = processed_genes_for_gse,
              OrgDb        = org.Hs.eg.db,
              ont          = "BP", ## Molecular Function (MF), Biological Process (BP), and C
              nPerm        = 1000,
              minGSSize    = 3,
              maxGSSize    = 500,
              pvalueCutoff = 0.05,
              verbose      = TRUE,
              by = "fgsea",
              pAdjustMethod = "none")

goplot(ego)
```


biological_process
response to stimulus
developmental process
biological regulation
cellular process
multicellular organismal process
cellular response to stimulus
cell activation
cellular developmental process
response to chemical
anatomical structure development
immune system process
regulation of biological process
regulation of multicellular organismal process
leukocyte activation
cell differentiation
cellular response to chemical stimulus
regulation of multicellular organismal development
cellular response to organic substance
cellular response to cytokine stimulus
T cell activation
p.adjust
0.02
0.03
0.04
relationship
isa
regulates

```r
all_genes <- as.data.frame(read_csv('data/all_gene_processed.csv'))
rownames(all_genes) <- all_genes$`...1`
all_genes <- all_genes[,2:6]

voom_normalized <- read_tsv('data/voom_normalized.tsv')

gene_cluster <- read_delim('data/cluster5.txt', delim='\n', col_names = FALSE)
gene_cluster_data <- voom_normalized[voom_normalized$GeneSymbol %in% as.vector(gene_cluster$
genes_for_gse <- all_genes[rownames(all_genes) %in% gene_cluster_data$GeneID,]

processed_genes_for_gse <- as.vector(genes_for_gse$logFC)  # p value vector
names(processed_genes_for_gse) <- as.vector(rownames(genes_for_gse)) # gene ids
processed_genes_for_gse <- processed_genes_for_gse[order(-processed_genes_for_gse)] # order

ego <- gseGO(geneList     = processed_genes_for_gse,
              OrgDb        = org.Hs.eg.db,
              ont          = "BP", ## Molecular Function (MF), Biological Process (BP), and
              nPerm        = 1000,
              minGSSize    = 3,
              maxGSSize    = 500,
              pvalueCutoff = 0.05,
              verbose      = TRUE,
              by = "fgsea",
                pAdjustMethod = "none")

goplot(ego)
```



biological_process
metabolic process
biological regulation
developmental process
biosynthetic process
regulation of biological process
multicellular organismal process
cellular process
anatomical structure development
organic substance metabolic process
cellular metabolic process
regulation of developmental process
positive regulation of biological process
cellular biosynthetic process
gene expression
positive regulation of developmental process
regulation of cellular biosynthetic process
regulation of gene expression
regulation of macromolecule biosynthetic process
p.adjust
0.01
0.02
0.03
0.04
relationship
isa
positively regulates
regulates

8 CytoScape

We will investigate protein-protein interactions (PPIs) for a given list of proteins. To do so, we will retreive PPI data from the STRING database and visualize networks in Cytoscape using the R package RCy3. We will further cluster the network to investigate whether subsets of proteins share a particular molecular function.

Requirements: Cytoscape running, with the clusterMaker app installed.

```
# Confirm that Cytoscape is running
cytoscapeVersionInfo ()
```

```
     apiVersion cytoscapeVersion 
           "v1"         "3.10.3" 
```

8.1 Load protein list

```
proteins <- readLines("data/gene_symbols_list.txt")
head(proteins)
```

```
[1] "IFI30"    "IFITM2"   "FGL2"     "STON1"    "FAM129A"  "SERPINA3"
```

8.2 Initialize STRING db

```
# Initialize STRINGdb object
string_db <- STRINGdb$new(version="12.0",
                          species=9606, # Homo sapiens
                          score_threshold=400,
                          input_directory="")
```

8.3 Map gene names to STRING aliases

```
# Create a dataframe with uppercase letters (to match with STRING alias mapping)
data <- data.frame(query.term = toupper(proteins))

# check if file with mapping to STRING aliases exists
# and create it if it doesn't
if (file.exists("data/mapped2STRING.csv")) {

  mapped <- read.csv("data/mapped2STRING.csv")

} else {

mapped <- string_db$map(my_data_frame = data,
                        my_data_frame_id_col_names = "query.term",
                        takeFirst=TRUE,
                        removeUnmappedRows=TRUE,
                        quiet=FALSE)
write.csv(x = mapped, file = "data/mapped2STRING.csv", quote = FALSE, row.names = FALSE)

}

head(mapped)
```

query.term	STRING_id
IFI30	9606.ENSP00000384886
IFITM2	9606.ENSP00000484689
FGL2	9606.ENSP00000248598
STON1	9606.ENSP00000384615
FAM129A	9606.ENSP00000356481
SERPINA3	9606.ENSP00000450540

```
# Check how many proteins were not mapped
# Since some gene IDs can map to several STRING identifiers, we account for duplicates
length(unique(data$query.term)) - length(unique(mapped$query.term))
```

```
[1] 1
```

8.4 Fetch interaction data

```
# Get interactions for the mapped proteins if the file isn't already there

# check if file with interaction data exists
# and create it if it doesn't
if (file.exists("data/interactions.csv")) {

  interactions <- read.csv("data/interactions.csv")

} else {

interactions <- string_db$get_interactions(mapped$STRING_id)
write.csv(x = interactions, file = "data/interactions.csv", quote = FALSE, row.names = FALSE
}

# View the first few interactions
head(interactions)
```

from	to	combined_score
9606.ENSP00000162749	9606.ENSP00000216117	435
9606.ENSP00000162749	9606.ENSP00000216117	435
9606.ENSP00000009530	9606.ENSP00000225831	497
9606.ENSP00000009530	9606.ENSP00000225831	497

from	to	combined_score
9606.ENSP00000162749	9606.ENSP00000225831	768
9606.ENSP00000162749	9606.ENSP00000225831	768

8.5 Create dataframe for PPI network

Define nodes for the network

```
# Get node columns for the Cytoscape network
nodes <- data.frame(id = unique(c(interactions$from, interactions$to)))

# Merge with the mapped protein names to include original protein names as labels
nodes <- merge(nodes, mapped[, c("STRING_id", "query.term")],
               by.x = "id", by.y = "STRING_id",
               all.x = TRUE,
               all.y = TRUE)

head(nodes)
```

id	query.term
9606.ENSP00000009530	CD74
9606.ENSP00000162749	TNFRSF1A
9606.ENSP00000206423	CCDC80
9606.ENSP00000209929	FMO2
9606.ENSP00000216117	HMOX1
9606.ENSP00000225831	CCL2

Define edges for the network

```
edges <- data.frame(source = interactions$from, target = interactions$to)

# Remove directionality of edges (necessary for various clusterings later)
# Combine and sort the columns, then get unique rows
unique_edges <- unique(t(apply(edges, 1, function(x) sort(x))))

# Convert back to a dataframe
edges <- as.data.frame(unique_edges, stringsAsFactors = FALSE)

# Rename the columns if needed
```

```
colnames(edges) <- c("source", "target")

head(edges)
```

source	target
9606.ENSP00000162749	9606.ENSP00000216117
9606.ENSP00000009530	9606.ENSP00000225831
9606.ENSP00000162749	9606.ENSP00000225831
9606.ENSP00000216117	9606.ENSP00000225831
9606.ENSP00000225831	9606.ENSP00000245907
9606.ENSP00000245907	9606.ENSP00000246006

8.6 Make the network in Cytoscape

```
# Connect to Cytoscape
cytoscapePing()

# Create a new Cytoscape network from your data
createNetworkFromDataFrames(nodes = nodes,
                            edges = edges,
                            # title = mytitle,
                            collection = "My Collection")
```

```
networkSUID
       4048
```

```
# Set node labels to the original protein names, and other visual tweaks
setNodeLabelMapping('query.term')
```

```
NULL
```

```
setNodeShapeDefault('ELLIPSE')
setNodeColorDefault('#9fbcda')
```

Now we modify the network layout in the app to reduce overlap of nodes and make it overall more aesthetically pleasing. Parameters will depend on the network

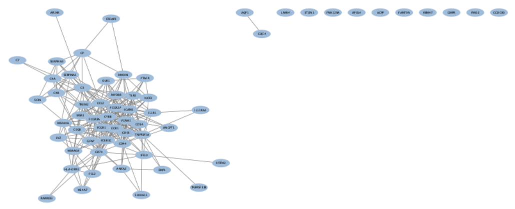
```
# using force-directed layout. Higher coefficients means nodes are closer together.
layoutNetwork('force-directed defaultSpringCoefficient=0.000006 defaultSpringLength=1')
```

8.7 Save and View the network

```
# Make sure network fits in the frame to be saved
fitContent()

#save network image
exportImage(filename = "currentnetwork.png")
```

```
                                                              file
"/Users/emiljohansson/R/MedBioinfo/func-omics/currentnetwork.png"
```

```
# view network image
knitr::include_graphics("currentnetwork.png")
```



8.8 Cluster PPI network

commands specify: restoreEdges: restores edges after clustering, showUI: displays the new network, and undirectedEdges: assumes edges are undirected

8.8.1 GLay clustering

Run GLay community clustering

```
# Run GLay community clustering
RCy3::commandsRun("cluster glay restoreEdges= true showUI = true undirectedEdges = true")
```

```
[1] "Clusters: 14"        "Average size: 4,429" "Maximum size: 18"
[4] "Minimum size: 1"     "Modularity: 0,589"
```

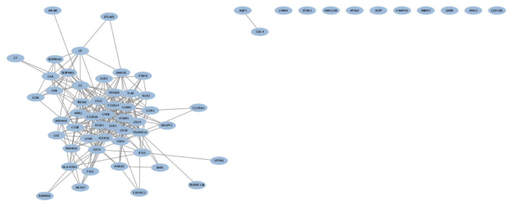
```
# reduce overlaps by making the nodes less inclined to be close to each other
layoutNetwork('force-directed defaultSpringCoefficient=0.000006 defaultSpringLength=1')
```

Visualize network after clustering

```
# Make sure network fits in the frame to be saved
fitContent()

#save network image
exportImage(filename = "currentnetwork_clustered.png")
```

```
                                                                              file
"/Users/emiljohansson/R/MedBioinfo/func-omics/currentnetwork_clustered.png"
```

```
# view network image
knitr::include_graphics("currentnetwork_clustered.png")
```



8.9 Save network info

```
# Get the table with clustering results
network_table <- getTableColumns()
clusterinfo <- table(network_table$`__glayCluster`)
clusterinfo
```

```
 1  2  3  4  5  6  7  8  9 10 11 12 13 14
18 18 14  1  1  2  1  1  1  1  1  1  1  1
```

8.10 Members of each big cluster

```r
# Save the members of clusters with >5 nodes for subsequent analysis
bigclusters <- names(clusterinfo)[(table(network_table$`__glayCluster`) > 5)]
bigclusternames <- paste0("cluster", bigclusters)

# empty list of clusters to cycle through
clusterlist <- list()

for (i in 1:length(bigclusters)){

  # create given clustername
  given_clustername <- bigclusternames[i]

  # make a list of these lists
  clusterlist[[i]] <- network_table$query.term[network_table$`__glayCluster` == bigclusters[
}

names(clusterlist) <- bigclusternames
```

Members of each cluster

```r
clusterlist
```

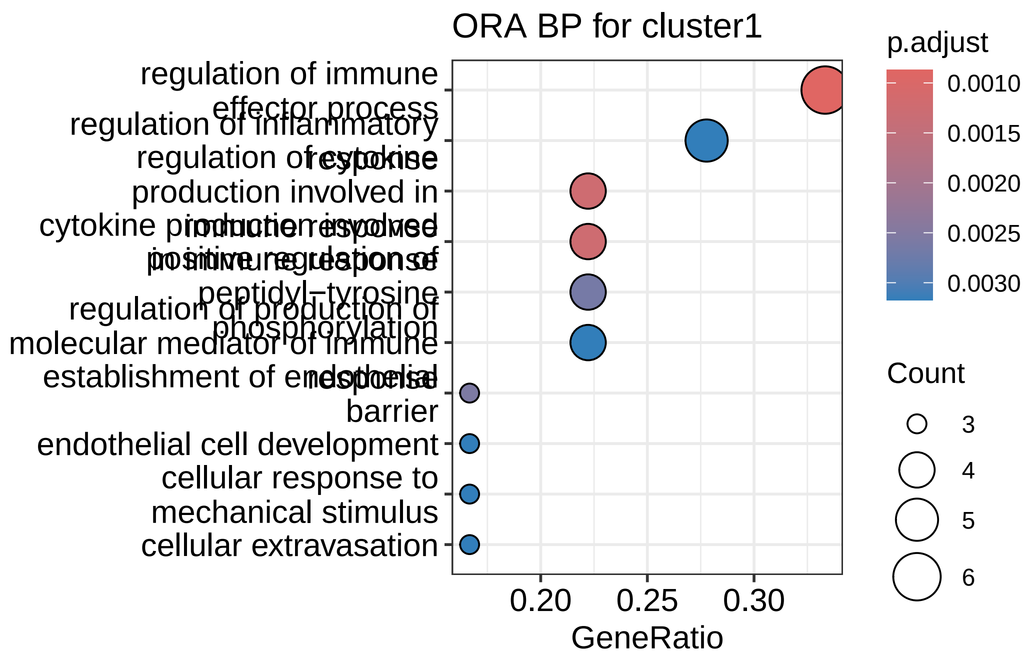
```
$cluster1
 [1] "CD93"      "EMP1"      "ICAM1"     "FCGR2A"    "S100A11"   "VCAM1"
 [7] "TLR3"      "TNFRSF11B" "OLR1"      "ANXA2"     "FCGR1A"    "IL13RA1"
[13] "IL1R1"     "CD44"      "MSR1"      "ANGPT1"    "MYD88"     "TNFRSF1A"

$cluster2
 [1] "FGL2"     "RARRES3"  "LYZ"      "FCER1G"   "CCR1"     "MS4A7"
 [7] "C1QB"     "MS4A4A"   "NCF2"     "FCGR3A"   "CYBB"     "IFI30"
[13] "CD14"     "HLA-DPA1" "MS4A6A"   "PTAFR"    "IFITM2"   "CD74"

$cluster3
 [1] "C3"       "GFAP"     "CP"       "SCIN"     "C7"       "TREM2"
 [7] "STEAP3"   "C4A"      "C4B"      "SERPINA1" "SERPINA3" "APLNR"
[13] "HMOX1"    "CCL2"
```

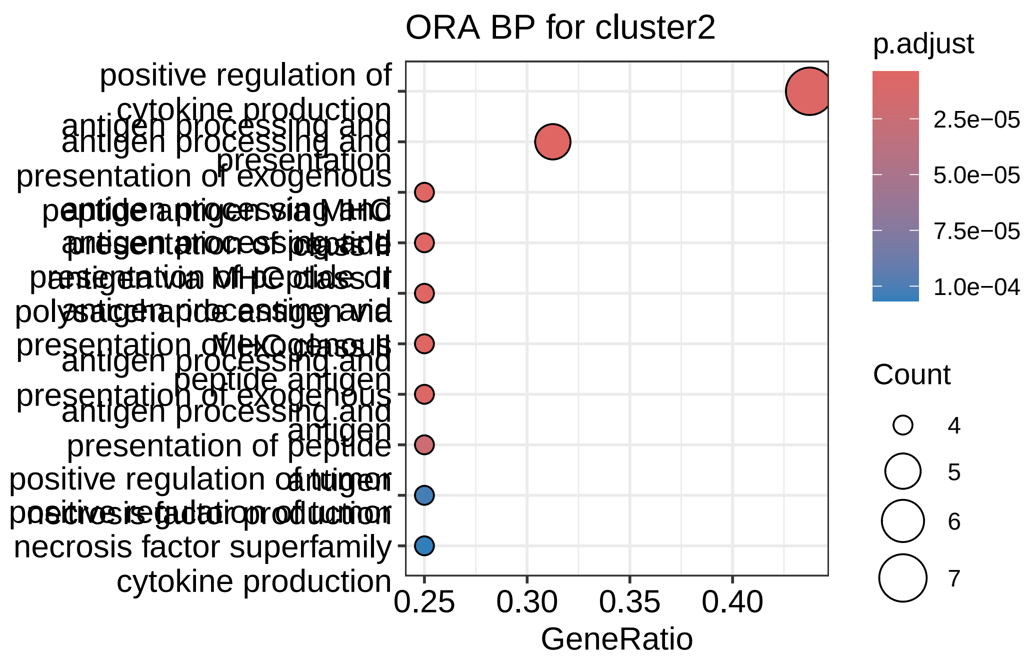
9 Enrichment analysis for each cluster (GO Biological Process)

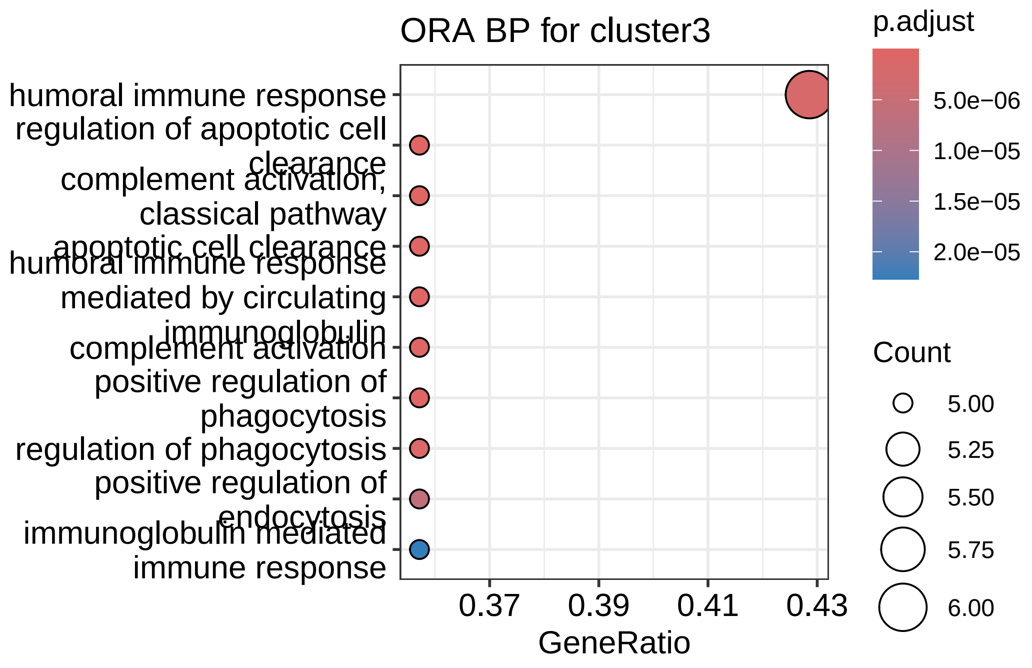
```r
# Run enrichment analysis on each cluster
GO_BP_enrichments <- lapply(clusterlist, function(cluster) {

  enrichGO(gene = cluster,
           OrgDb = org.Hs.eg.db,
           keyType = "SYMBOL",
           ont = "BP",
           pAdjustMethod = "BH",
           qvalueCutoff = 0.01)
})
```

Create dotplots for results

```r
for (x in 1:length(GO_BP_enrichments)){

  # pick out object with enrichments
  enrichresult <- GO_BP_enrichments[[x]]

  # make dotplot object
  p <- dotplot(object = enrichresult) +
    ggtitle(paste0("ORA BP for ", names(clusterlist)[x]))

  print(p)
}
```


ORA BP for cluster1
regulation of immune effector process
regulation of inflammatory response
regulation of cytokine production involved in immune response
cytokine production involved in immune response
positive regulation of peptidyl−tyrosine phosphorylation
regulation of production of molecular mediator of immune response
establishment of endothelial barrier
endothelial cell development
cellular response to mechanical stimulus
cellular extravasation
0.20
0.25
0.30
GeneRatio
p.adjust
0.0010
0.0015
0.0020
0.0025
0.0030
Count
3
4
5
6


ORA BP for cluster2
positive regulation of cytokine production
antigen processing and presentation
antigen processing and presentation of exogenous peptide antigen via MHC class II
antigen processing and presentation of peptide antigen via MHC class II
antigen processing and presentation of peptide or polysaccharide antigen via MHC class II
antigen processing and presentation of exogenous peptide antigen
antigen processing and presentation of exogenous antigen
antigen processing and presentation of peptide antigen
positive regulation of tumor necrosis factor production
positive regulation of tumor necrosis factor superfamily cytokine production
0.25
0.30
0.35
0.40
GeneRatio
p.adjust
2.5e−05
5.0e−05
7.5e−05
1.0e−04
Count
4
5
6
7

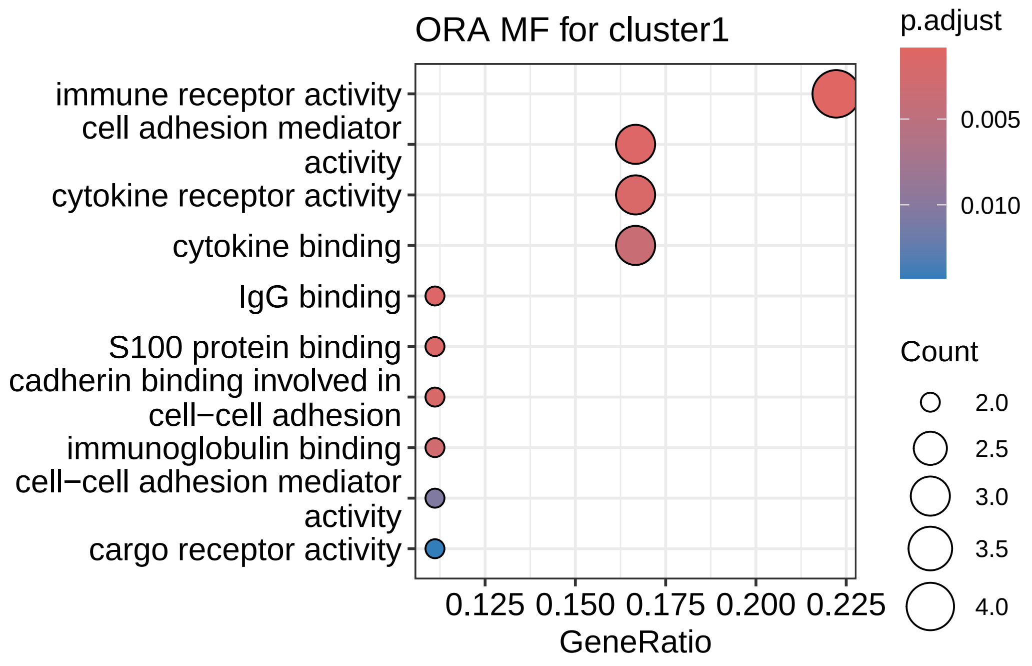


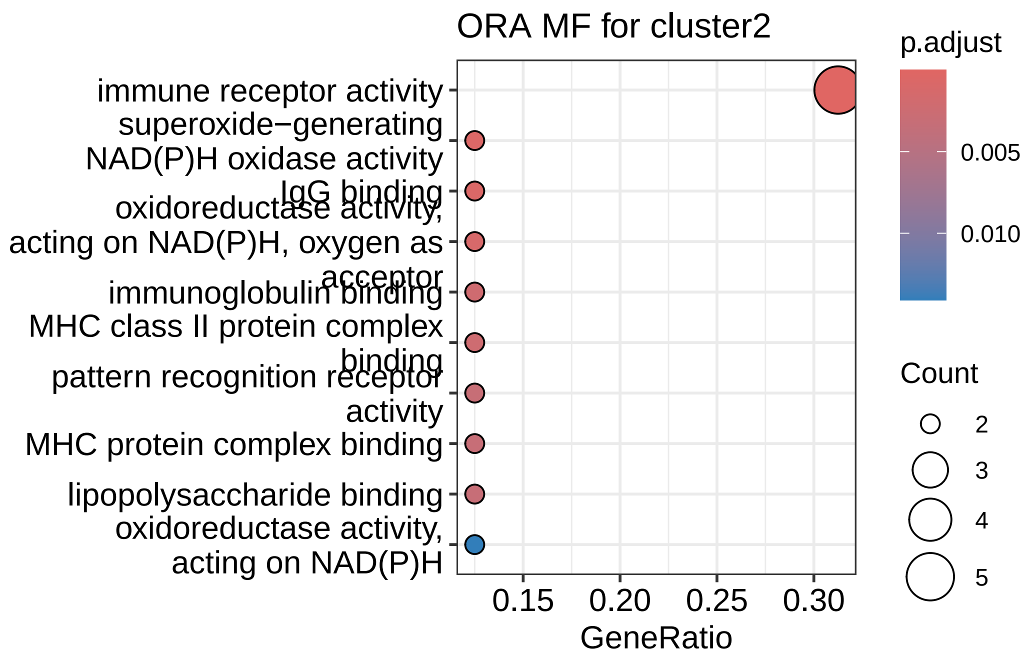
9.1 Enrichment analysis for each cluster (GO Molecular Function)

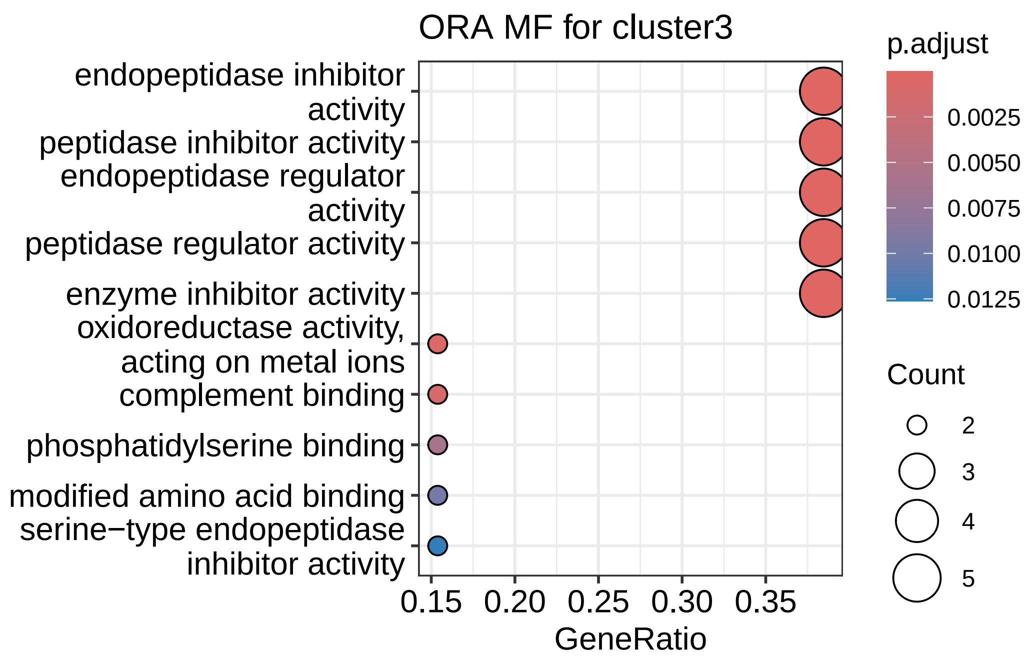
```
# Run enrichment analysis on each cluster
GO_MF_enrichments <- lapply(clusterlist, function(cluster) {

  enrichGO(gene = cluster,
           OrgDb = org.Hs.eg.db,
           keyType = "SYMBOL",
           ont = "MF",
           pAdjustMethod = "BH",
           qvalueCutoff = 0.01)

})
```

Create dotplots for results

```
for (x in 1:length(GO_MF_enrichments)){

  # pick out object with enrichments
  enrichresult <- GO_MF_enrichments[[x]]
  # make dotplot object
  p <- dotplot(object = enrichresult) +
    ggtitle(paste0("ORA MF for ", names(clusterlist)[x]))

  print(p)
}
```




ORA MF for cluster2
immune receptor activity
superoxide−generating NAD(P)H oxidase activity
IgG binding
oxidoreductase activity, acting on NAD(P)H, oxygen as acceptor
immunoglobulin binding
MHC class II protein complex binding
pattern recognition receptor activity
MHC protein complex binding
lipopolysaccharide binding
oxidoreductase activity, acting on NAD(P)H
0.15
0.20
0.25
0.30
GeneRatio
p.adjust
0.005
0.010
Count
2
3
4
5

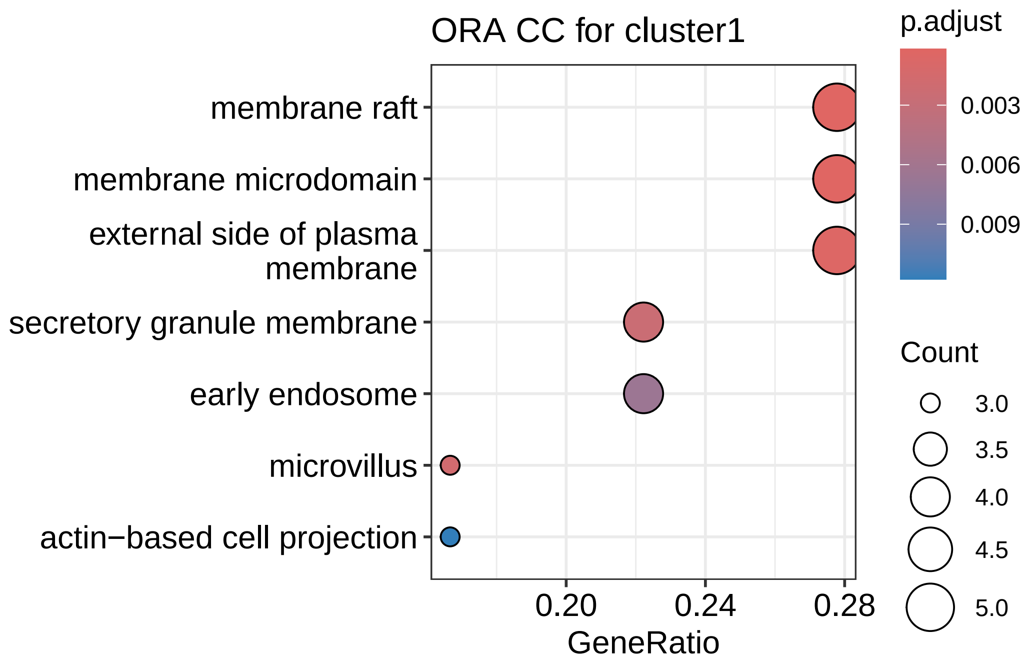

ORA MF for cluster3
endopeptidase inhibitor activity
peptidase inhibitor activity
endopeptidase regulator activity
peptidase regulator activity
enzyme inhibitor activity
oxidoreductase activity, acting on metal ions
complement binding
phosphatidylserine binding
modified amino acid binding
serine−type endopeptidase inhibitor activity
0.15
0.20
0.25
0.30
0.35
GeneRatio
p.adjust
0.0025
0.0050
0.0075
0.0100
0.0125
Count
2
3
4
5

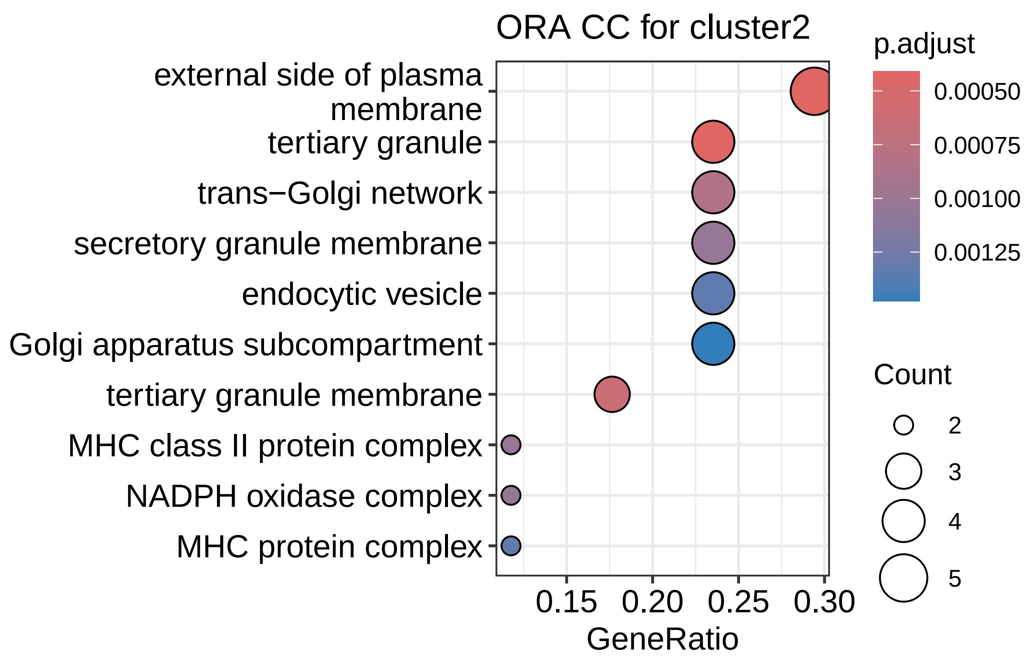
9.2 Enrichment analysis for each cluster (GO Cellular Component)

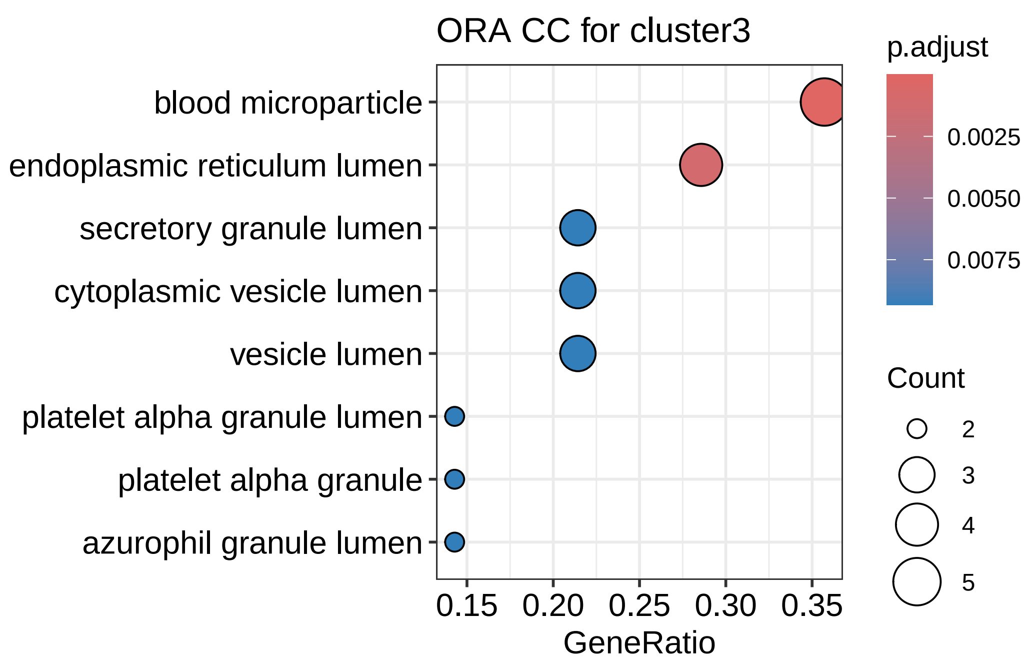
```r
# Run enrichment analysis on each cluster
GO_CC_enrichments <- lapply(clusterlist, function(cluster) {

  enrichGO(gene = cluster,
           OrgDb = org.Hs.eg.db,
           keyType = "SYMBOL",
           ont = "CC",
           pAdjustMethod = "BH",
           qvalueCutoff = 0.01)
})
```

Create dotplots for results

```r
for (x in 1:length(GO_CC_enrichments)){

  # pick out object with enrichments
  enrichresult <- GO_CC_enrichments[[x]]

  # make dotplot object
  p <- dotplot(object = enrichresult) +
    ggtitle(paste0("ORA CC for ", names(clusterlist)[x]))

  print(p)
}
```


ORA CC for cluster1
membrane raft
membrane microdomain
external side of plasma membrane
secretory granule membrane
early endosome
microvillus
actin−based cell projection
0.20
0.24
0.28
GeneRatio
p.adjust
0.003
0.006
0.009
Count
3.0
3.5
4.0
4.5
5.0


ORA CC for cluster2
external side of plasma membrane
tertiary granule
trans−Golgi network
secretory granule membrane
endocytic vesicle
Golgi apparatus subcompartment
tertiary granule membrane
MHC class II protein complex
NADPH oxidase complex
MHC protein complex
0.15
0.20
0.25
0.30
GeneRatio
p.adjust
0.00050
0.00075
0.00100
0.00125
Count
2
3
4
5



10 Random forest classification

10.1 Prepare data

```
# Load and prepare the data
data_raw <- read_tsv("data/voom_normalized.tsv")
data <- data_raw %>%
  distinct(GeneSymbol, .keep_all = TRUE) %>%
  dplyr::select(-GeneID) %>%
  filter(!is.na(GeneSymbol)) %>%
  column_to_rownames(var = "GeneSymbol") %>%
  mutate_all(~ ifelse(. < 0, 0, .)) %>%
  mutate_all(as.integer)

# Prepare metadata
metadata_raw <- read_xlsx("data/ad_metadata.xlsx")
metadata <- metadata_raw %>%
  mutate(Index = row_number()) %>%
  column_to_rownames(var = "Index") %>%
  dplyr::rename(sample = ID, disease = Disease) %>%
  mutate(individual = gsub("_raw", "", sample)) %>%
  dplyr::select(sample, individual, disease) %>%
  mutate(individual = factor(individual), disease = factor(disease))

# Ensure the data is in a matrix form with gene symbols as rownames
countData <- as.matrix(data)

# Prepare DESeqDataSet
dds <- DESeqDataSetFromMatrix(
  countData = countData,
  colData = metadata,
  design = ~ 0 + disease
)

# Estimate size factors
dds <- estimateSizeFactors(dds)

# Estimate gene-wise dispersions
dds <- estimateDispersionsGeneEst(dds)

# Directly use the gene-wise estimates
dispersions(dds) <- mcols(dds)$dispGeneEst

normCounts <- varianceStabilizingTransformation(dds, blind = FALSE)
```

```
#Define model fitting procedure
#NB: you will also need the packages statmod and e1071 for this analysis
#New score function
rfSBF$score <- function(x, y){
sd(x) / mean(x)
}
#New filter function
rfSBF$filter <- function(score, x, y){
meanLog2CPM <- rowMeans(log2(cpm(data) + 1))
selection <- score > quantile(score, 0.9) & meanLog2CPM > 1
names(score) %in% names(score)[selection]
}
```

```
#Create training set
trainingSet <- data.frame(t(assay(normCounts)))
#Train and validate decision tree (C5.0)
dtControl <- sbfControl(
  functions = rfSBF,
  method = "loocv",
  saveDetails = TRUE,
  verbose = FALSE)

dtModel <- sbf(
  trainingSet,
  metadata$disease,
  sbfControl = dtControl)

#Show model
#summary(dtModel$fit)
```

10.2 Performance evaluation

```
#Print confusion matrix from validation
confusionMatrix(dtModel$pred$predictions$pred, metadata$disease)
```

```
Confusion Matrix and Statistics

          Reference
Prediction AD Control
   AD       5       7
   Control  4       1

               Accuracy : 0.3529
                 95% CI : (0.1421, 0.6167)
    No Information Rate : 0.5294
    P-Value [Acc > NIR] : 0.9561

                  Kappa : -0.3262

 Mcnemar's Test P-Value : 0.5465

            Sensitivity : 0.5556
            Specificity : 0.1250
         Pos Pred Value : 0.4167
         Neg Pred Value : 0.2000
             Prevalence : 0.5294
         Detection Rate : 0.2941
   Detection Prevalence : 0.7059
      Balanced Accuracy : 0.3403

       'Positive' Class : AD
```

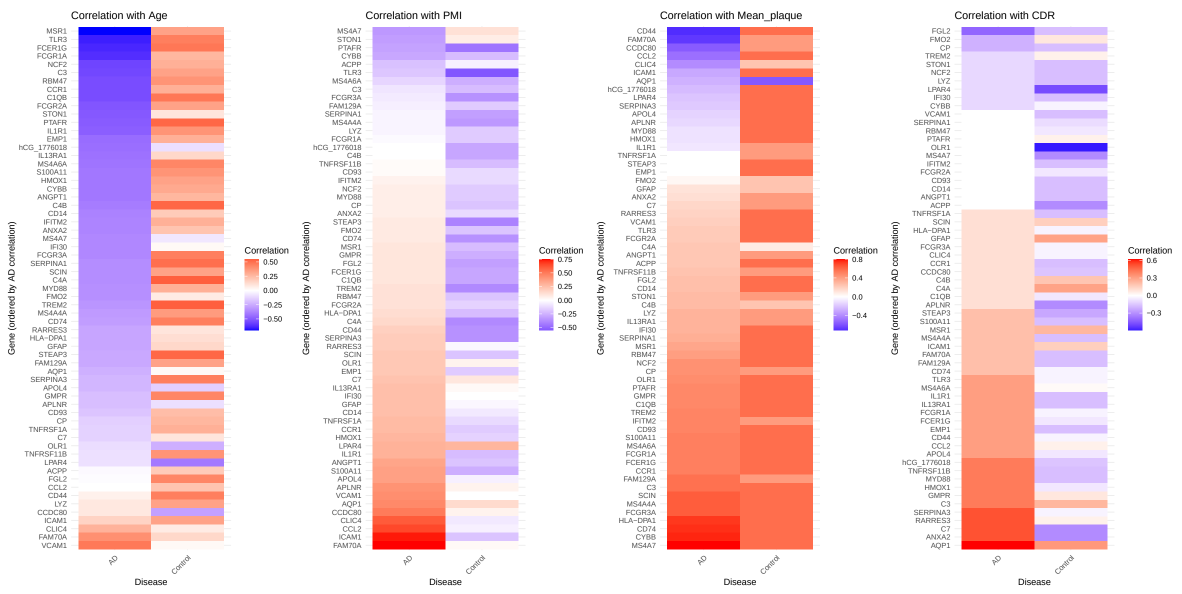
11 Spearman correlation analysis

Loading the data for the correlaton analysis

```
data <- read_tsv("data/voom_normalized.tsv")
de_results <- read_tsv("data/significant_genes.tsv")
metadata <- read_xlsx("data/ad_metadata.xlsx")
```

Performing Spearman correlation of the 63 upregulated genes on selected clinical variables of interest and comparing the correlation between Alzheimer's disease and control samples.

```
# Function to create correlation data and plot
create_correlation_plot <- function(data, metadata, gene_symbols, variable, title) {
  correlation_data <- data %>%
    filter(GeneSymbol %in% gene_symbols) %>%
    pivot_longer(cols = -GeneSymbol, names_to = "ID", values_to = "value") %>%
    right_join(metadata, by = "ID") %>%
    group_by(GeneSymbol, Disease) %>%
    summarise(correlation = cor(value, !!rlang::sym(variable), method = "spearman"), .groups
    ungroup()

  # Extract the order of genes based on their correlation in AD
  ad_correlations <- correlation_data %>%
    filter(Disease == "AD") %>%
    arrange(desc(correlation)) %>%
    pull(GeneSymbol)

  # Ensure that all GeneSymbols are included, even those not present in AD data
  full_gene_list <- unique(correlation_data$GeneSymbol)
  ad_order <- unique(c(ad_correlations, full_gene_list)) # This ensures all genes are includ

  # Set the levels of GeneSymbol according to their order in AD
  correlation_data$GeneSymbol <- factor(correlation_data$GeneSymbol, levels = ad_order)

  # Plot the heatmap with genes ordered by their correlation in AD
  heatmap_plot <- ggplot(correlation_data, aes(x = Disease, y = GeneSymbol, fill = correlatio
    geom_tile() +
    scale_fill_gradient2(low = "blue", high = "red", mid = "white", midpoint = 0) +
    theme_minimal() +
    theme(axis.text.x = element_text(angle = 45, hjust = 1)) +
    labs(fill = "Correlation", y = "Gene (ordered by AD correlation)", x = "Disease", title =

  return(heatmap_plot)
}

# Variables of interest
variables_of_interest <- c("Age", "PMI", "Mean_plaque", "CDR")

# Plot list
plot_list <- list()

# Loop over variables and create plots
for (variable in variables_of_interest) {
  plot_list[[variable]] <- create_correlation_plot(data, metadata, de_results$GeneSymbol, var
}

# Combine plots
combined_plot <- wrap_plots(plot_list, nrow = 1)

# Print the combined plot
print(combined_plot)
```



12 Final reflection (1/2 A4)

Statistical challenges: I think for our project there was a few challanges that we tried to overcome. The first one was when picking a completely new dataset unfamiliar to us. Even though some of the group members had worked on RNA-seq data before, it was a challenge for me to understand all the important steps during the pre-processing and considerations when analyzing. Since my background is in plasma proteomics, I found some similarities in the dataframe and how I could analyze the data. For instance, I was not used to the idea of count data because I normally work with either normalized protein expression (NPX) or medium florecence intensity (MFI).

My largest contributions to this work was the attempt to classify Alzheimer's Disease (AD) using a leave-one-out (LOO) cross-validation (CV). In the short time limit that we had, I chose to apply a similar pipeline that was presented during the course. There was an issue with the vst() transformation funciton, which led me to perform some extra steps before I could apply the spelled out version of the function, which gave me a functional object from which I could proceed with this pipeline. In the end, the random forest classification gave an AUC of 0.41, which is worse than a random guess. There could have been several reasons behind this. On the one hand, since we may have picked the wrong data set or performed unlegitimate pre-processing, the data could have been more noisy.

The other contribution was to perform Spearman correlation analysis of the significant upregulated genes from the differential expression analysis on the AD patients and the controls. Some biological relevancies was found like the higher correlation to CDR. However, it didn't make any sense to me that the top markers would have a negative correlation with age since age is a big factor behind AD, but this could be because of the noisy data.

Final reflection: In light of my own PhD project and what I have learnt from this course, I now feel more confortable applying these concept to my plasma proteomics pipelines and I now have more thoughts on how I could improve my current projects with methods such as canonical correlation analysis (CCA). My goal is to characterize the cardiovascular proteome and I think it will include some more multivariate analysis, therefore many of these concept I learnt during the course will be of great help.

13 Session info

```r
sessionInfo()
```

```
R version 4.3.2 (2023-10-31)
Platform: aarch64-apple-darwin20 (64-bit)
Running under: macOS Sonoma 14.5

Matrix products: default
BLAS:   /Library/Frameworks/R.framework/Versions/4.3-arm64/Resources/lib/libRblas.0.dylib
LAPACK: /Library/Frameworks/R.framework/Versions/4.3-arm64/Resources/lib/libRlapack.dylib;  

locale:
[1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8

time zone: Europe/Stockholm
tzcode source: internal

attached base packages:
[1] stats4    grid      stats     graphics  grDevices utils     datasets 
[8] methods   base     

other attached packages:
 [1] RColorBrewer_1.1-3          RCy3_2.22.1                
 [3] egg_0.4.5                   gridExtra_2.3              
 [5] enrichplot_1.22.0           org.Hs.eg.db_3.18.0        
 [7] AnnotationDbi_1.64.1        clusterProfiler_4.10.1     
 [9] igraph_2.0.3                STRINGdb_2.14.3            
[11] edgeR_4.0.16                caret_6.0-94               
[13] lattice_0.22-6              randomForest_4.7-1.1       
```

```
[15] pheatmap_1.0.12             DESeq2_1.42.1
[17] SummarizedExperiment_1.32.0 Biobase_2.62.0
[19] MatrixGenerics_1.14.0       matrixStats_1.4.1
[21] GenomicRanges_1.54.1        GenomeInfoDb_1.38.8
[23] IRanges_2.36.0              S4Vectors_0.40.2
[25] BiocGenerics_0.48.1         ComplexUpset_1.3.3
[27] UpSetR_1.4.0                ggvenn_0.1.10
[29] cowplot_1.1.3               ggplotify_0.1.2
[31] uwot_0.2.2                  Matrix_1.6-4
[33] umap_0.2.10.0               plotly_4.10.4
[35] HDAnalyzeR_1.0.0            xlsx_0.6.5
[37] tensorflow_2.16.0           kknn_1.3.1
[39] xgboost_1.7.8.1             keras_2.15.0
[41] themis_1.0.2                vip_0.4.1
[43] ggsignif_0.6.4              corrr_0.4.4
[45] visdat_0.6.0                GGally_2.2.1
[47] skimr_2.1.5                 gt_0.11.0
[49] ggbeeswarm_0.7.2            yardstick_1.3.1
[51] workflowsets_1.1.0          workflows_1.1.4
[53] tune_1.2.1                  rsample_1.2.1
[55] recipes_1.1.0               parsnip_1.2.1
[57] modeldata_1.4.0             infer_1.0.7
[59] dials_1.3.0                 scales_1.3.0
[61] broom_1.0.6                 tidymodels_1.2.0
[63] DT_0.33                     patchwork_1.3.0
[65] kableExtra_1.4.0            magrittr_2.0.3
[67] ggrepel_0.9.6               limma_3.58.1
[69] readxl_1.4.3                lubridate_1.9.3
[71] forcats_1.0.0               stringr_1.5.1
[73] dplyr_1.1.4                 purrr_1.0.2
[75] readr_2.1.5                 tidyr_1.3.1
[77] tibble_3.2.1                ggplot2_3.5.1
[79] tidyverse_2.0.0

loaded via a namespace (and not attached):
  [1] vroom_1.6.5            nnet_7.3-19            Biostrings_2.70.3
  [4] vctrs_0.6.5            proxy_0.4-27           digest_0.6.37
  [7] png_0.1-8              tfruns_1.5.3           IRdisplay_1.1
 [10] parallelly_1.38.0      MASS_7.3-60            reshape2_1.4.4
 [13] foreach_1.5.2          qvalue_2.34.0          withr_3.0.1
 [16] xfun_0.49              ggfun_0.1.6            survival_3.7-0
 [19] memoise_2.0.1          gson_0.1.0             systemfonts_1.1.0
 [22] tidytree_0.4.6         gtools_3.9.5           IRkernel_1.3.2
 [25] KEGGREST_1.42.0        httr_1.4.7             globals_0.16.3
 [28] hash_2.2.6.3           rstudioapi_0.16.0      archive_1.1.9
 [31] generics_0.1.3         DOSE_3.28.2            base64enc_0.1-3
 [34] curl_5.2.2             repr_1.1.7             zlibbioc_1.48.2
 [37] ggraph_2.2.1           polyclip_1.10-7        GenomeInfoDbData_1.2.11
 [40] SparseArray_1.2.4      evaluate_1.0.0         S4Arrays_1.2.1
 [43] hms_1.1.3              colorspace_2.1-1       reticulate_1.39.0
 [46] viridis_0.6.5          ggtree_3.10.1          future.apply_1.11.2
 [49] lhs_1.2.0              XML_3.99-0.17          shadowtext_0.1.4
 [52] ROSE_0.0-4             class_7.3-22           pillar_1.9.0
 [55] nlme_3.1-166           iterators_1.0.14       GPfit_1.0-8
 [58] caTools_1.18.3         compiler_4.3.2         RSpectra_0.16-2
 [61] stringi_1.8.4          gower_1.0.1            plyr_1.8.9
 [64] crayon_1.5.3           abind_1.4-8            gridGraphics_0.5-1
 [67] chron_2.3-61           locfit_1.5-9.10        graphlayouts_1.1.1
 [70] bit_4.0.5              fastmatch_1.1-4        whisker_0.4.1
 [73] codetools_0.2-20       openssl_2.2.1          e1071_1.7-16
 [76] splines_4.3.2          Rcpp_1.0.13            DiceDesign_1.10
 [79] HDO.db_0.99.1          cellranger_1.1.0       knitr_1.48
 [82] blob_1.2.4             utf8_1.2.4             pbdZMQ_0.3-13
 [85] RJSONIO_1.3-1.9        fs_1.6.4               listenv_0.9.1
 [88] sqldf_0.4-11           statmod_1.5.0          tzdb_0.4.0
 [91] svglite_2.1.3          tweenr_2.0.3           pkgconfig_2.0.3
 [94] tools_4.3.2            cachem_1.1.0           RSQLite_2.3.7
 [97] viridisLite_0.4.2      DBI_1.2.3              fastmap_1.2.0
[100] rmarkdown_2.28         ggstats_0.6.0          graph_1.80.0
[103] rpart_4.1.23           farver_2.1.2           tidygraph_1.3.1
[106] scatterpie_0.2.4       gsubfn_0.7             yaml_2.3.10
[109] cli_3.6.3              lifecycle_1.0.4        askpass_1.2.0
[112] lava_1.8.0             backports_1.5.0        BiocParallel_1.36.0
[115] timechange_0.3.0       gtable_0.3.5           ggridges_0.5.6
[118] parallel_4.3.2         pROC_1.18.5            ape_5.8
[121] jsonlite_1.8.8         bitops_1.0-8           bit64_4.0.5
[124] yulab.utils_0.1.7      proto_1.0.0            GOSemSim_2.28.1
[127] zeallot_0.1.0          timeDate_4032.109      lazyeval_0.2.2
[130] htmltools_0.5.8.1      rJava_1.0-11           GO.db_3.18.0
[133] glue_1.7.0             XVector_0.42.0         RCurl_1.98-1.16
[136] treeio_1.26.0          R6_2.5.1               gplots_3.1.3.1
[139] labeling_0.4.3         xlsxjars_0.6.1         aplot_0.2.3
[142] ipred_0.9-15           DelayedArray_0.28.0    tidyselect_1.2.1
[145] vipor_0.4.7            plotrix_3.8-4          ggforce_0.4.2
[148] xml2_1.3.6             future_1.34.0          ModelMetrics_1.2.2.2
[151] munsell_0.5.1          KernSmooth_2.23-24     furrr_0.3.1
```

```
[154] data.table_1.16.0       htmlwidgets_1.6.4       fgsea_1.28.0
[157] rlang_1.1.4             uuid_1.2-1              ggnewscale_0.5.0
[160] fansi_1.0.6             base64url_1.4           hardhat_1.4.0
[163] beeswarm_0.4.0          prodlim_2024.06.25
```