RNA-Seq Exploratory Analysis

2024-08-29

```
suppressPackageStartupMessages({
    library(here)
    library(tidyverse)
    library(biomaRt)
    library(factoextra)
    library(ggrepel)
    library(caret)

library(DESeq2)
    library(enrichR)
    library(xlsx)
    library(circlize)
    library(ComplexHeatmap)
})
```

```
count_paths <- list.files(path = file.path(main_folder,</pre>
    "count_data"), pattern = "quant.genes.sf",
    recursive = T, full.names = T)
count_lists <- lapply(count_paths, function(x) read_tsv(x,</pre>
    col_types = cols()))
names <- lapply(basename(count_paths), function(x) strsplit(x,</pre>
    split = "-")[[1]][1])
names(count_lists) <- names</pre>
counts <- count_lists %>%
    lapply(., function(df) {
            dplyr::select(Name, NumReads)
    }) %>%
    # Map(function(x, n) setNames(x, n))
    \# c(names(x)[1], n)), ., names(.))
Map(function(x, n) setNames(x, c("Ensembl_ID",
    n)), ., names(.)) %>%
    purrr::reduce(inner_join, by = c("Ensembl_ID")) %>%
    as.data.frame(.) %>%
    mutate_if(is.numeric, round)
# write.table(counts,
# file.path(main_folder, 'gene_counts.tsv'),
# quote = F, sep = '\t', row.names = F,
\# col.names = T)
```

```
tpm <- count_lists %>%
    lapply(., function(df) {
        df %>%
            dplyr::select(Name, TPM)
}) %>%
Map(function(x, n) setNames(x, c("Ensembl_ID",
            n)), ., names(.)) %>%
purrr::reduce(inner_join, by = c("Ensembl_ID")) %>%
as.data.frame(.)
```

Convert Ensembl IDs to gene names

```
##
     ensembl gene id version hgnc symbol
                                           gene biotype
## 1
          ENSG0000000457.14
                                   SCYL3 protein_coding
## 2
          ENSG00000000460.17
                                   FIRRM protein_coding
## 3
          ENSG00000000938.13
                                     FGR protein_coding
## 4
          ENSG00000000971.17
                                     CFH protein_coding
## 5
          ENSG0000001460.18
                                   STPG1 protein_coding
## 6
          ENSG0000001461.17
                                  NIPAL3 protein_coding
```

tpm matrix is updated by matching to the retrieved annotation

```
gene_IDs <- gene_IDs %>%
    filter(!hgnc_symbol == "")

tpm <- tpm %>%
    dplyr::rename(ensembl_gene_id_version = "Ensembl_ID") %>%
    right_join(., gene_IDs) %>%
    dplyr::select(-gene_biotype, -ensembl_gene_id_version) %>%
    group_by(hgnc_symbol) %>%
    summarise_all(mean) %>%
    column_to_rownames("hgnc_symbol") %>%
    rownames_to_column("gene_id")
```

Joining with 'by = join_by(ensembl_gene_id_version)'

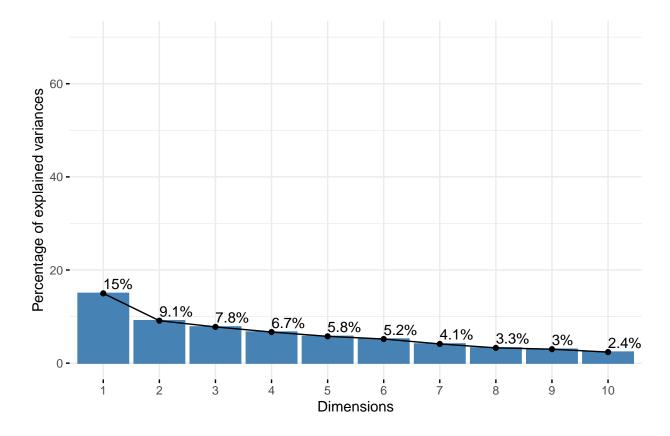
```
head(tpm)
```

```
##
      gene id 10067211 10067219 10067220 10067221 10067222 10067223 10067224
## 1
         A1BG
                 0.000
                         0.051
                                  0.300
                                          23.762
                                                   0.000
                                                            0.000
                                                                     0.035
## 2 A1BG-AS1
                 0.471
                         0.784
                                  0.929
                                          0.251
                                                   0.229
                                                            0.282
                                                                     2.075
          A2M
                31.203
                         6.220 67.024 405.047 45.597
                                                            0.517
                                                                    32.293
## 3
```

```
## 4
       A2M-AS1
                   0.107
                            0.031
                                      0.636
                                               0.764
                                                         0.259
                                                                   0.241
                                                                             1.015
## 5
         A2ML1
                   1.392
                           12.098
                                     27.463
                                                         0.489
                                                                   5.873
                                               0.295
                                                                             1.010
                            0.000
                                      0.000
## 6 A2ML1-AS1
                   0.000
                                                0.000
                                                         0.000
                                                                   0.000
                                                                             0.275
     10067225 10067226 10067227 10067228 10067229 10067231 10067233 10067235
##
## 1
        0.009
                  0.000
                           0.052
                                     0.031
                                               0.063
                                                        5.252
                                                                  0.106
                                                                           0.014
## 2
        0.296
                  0.138
                           0.414
                                     0.194
                                               0.234
                                                        0.079
                                                                  0.603
                                                                           0.584
## 3
                          38.728
                                    41.527
                                             24.947
                                                     316.493
                                                                 10.872
       41.735
                 16.630
                                                                          63.976
## 4
        0.180
                  0.213
                           0.323
                                     0.522
                                               0.260
                                                        0.509
                                                                  0.071
                                                                           0.200
## 5
        0.293
                  8.003
                          65.214
                                    22.106
                                               2.679
                                                        4.249
                                                                187.241
                                                                         126.497
## 6
        0.000
                  0.000
                           0.000
                                     0.000
                                               0.000
                                                        0.000
                                                                  0.000
                                                                           0.000
##
     10067236 10067238 10067241 10067243 10067245 10067246 10067247 10067248
## 1
        0.000
                  0.016
                           0.079
                                     0.014
                                               0.126
                                                        0.073
                                                                  0.000
                                                                           0.128
## 2
        0.000
                  0.212
                           0.393
                                     0.412
                                               1.389
                                                        0.505
                                                                  0.009
                                                                           1.161
## 3
       31.622
                                    34.882 231.743
               118.599
                         154.566
                                                       84.548
                                                                  3.288
                                                                           5.764
## 4
        0.359
                  0.620
                           0.803
                                     0.172
                                               3.606
                                                        0.484
                                                                  0.083
                                                                           0.100
## 5
      102.149
                 87.757
                           1.626
                                    37.808
                                               0.000
                                                        1.299
                                                                  3.621
                                                                          66.894
## 6
        0.000
                  0.000
                           0.000
                                     0.000
                                               0.000
                                                        0.000
                                                                  0.000
                                                                           0.000
##
     10067251 10067261 10067268 10067270 10067273 10067275 10067276 10067488
## 1
                                     0.000
        0.126
                  2.672
                           0.167
                                              0.062
                                                        0.119
                                                                  0.076
                                                                           0.000
## 2
        0.631
                  0.204
                           2.109
                                     0.029
                                               0.128
                                                        0.602
                                                                  0.859
                                                                           0.254
## 3
       20.791
               129.922
                           3.570
                                     3.028
                                              6.225
                                                        7.966
                                                                59.667
                                                                          25.522
## 4
        0.204
                  0.077
                           0.156
                                     0.116
                                               0.073
                                                        0.639
                                                                  0.499
                                                                           0.084
## 5
        0.774
               115.632
                           0.039
                                    20.094
                                             20.266
                                                        0.299
                                                                  0.201
                                                                          57.463
        0.000
                  0.000
                           0.000
                                     0.000
                                               0.000
                                                        0.000
                                                                  0.000
## 6
                                                                           0.000
##
     10067489 10067490 10067492 10067493 10067495 10067496 10067497 10067498
## 1
        0.015
                  0.088
                           0.051
                                     0.048
                                              0.053
                                                        0.214
                                                                  0.000
                                                                           0.200
## 2
        0.829
                  1.531
                           1.321
                                     1.210
                                               0.223
                                                        2.852
                                                                  0.136
                                                                           2.095
## 3
                                             19.376
       21.699
                 12.238
                          46.893
                                    67.824
                                                     290.766
                                                                  3.689
                                                                          31.495
## 4
        0.464
                  0.000
                           0.169
                                     0.295
                                              0.209
                                                                  0.033
                                                        1.271
                                                                           0.080
## 5
        8.295
               127.471
                          91.009
                                     7.284
                                             95.977
                                                        0.225
                                                                  8.516
                                                                          58.071
## 6
        0.000
                  0.000
                           0.000
                                     0.000
                                               0.000
                                                        0.000
                                                                  0.000
                                                                           0.000
##
     10067501 10067502 10067515 10067516 10067517 10067826 10067827 10067829
## 1
        0.014
                  0.000
                           0.055
                                     0.013
                                               0.036
                                                        0.020
                                                                  0.000
                                                                           0.034
## 2
        0.052
                  0.025
                           1.795
                                     0.736
                                               2.559
                                                        0.732
                                                                  0.391
                                                                           1.435
## 3
       21.080
                  1.324
                           8.287
                                    57.370
                                             26.336
                                                       58.414
                                                                 16.430
                                                                         131.753
                           0.044
## 4
        0.245
                  0.024
                                     0.276
                                                        0.080
                                                                  0.094
                                              0.208
                                                                           0.336
## 5
        6.002
                  0.000
                         163.712
                                     0.104
                                             11.507
                                                       55.920
                                                                132.299
                                                                         104.455
## 6
        0.000
                  0.000
                           0.000
                                     0.000
                                              0.000
                                                        0.000
                                                                  0.000
                                                                           0.000
##
     10067830 10067836 10067839
## 1
        0.000
                  0.074
                           0.174
## 2
        0.073
                  0.545
                           1.647
## 3
       22.202
                 90.873
                          80.412
## 4
        0.197
                  0.173
                           0.270
## 5
       16.401
                  2.626
                          10.317
## 6
        0.000
                  0.050
                           0.000
# write.table(tpm,
# file.path(main_folder, 'acc_cibersort_input.tsv'),
# quote = F, sep = '\t', row.names = F,
\# col.names = T)
vst <- read.table(file.path(main_folder,</pre>
    "differential analysis 0824/deseg vst data.txt"))
colnames(vst) <- gsub("^X", "", colnames(vst))</pre>
```

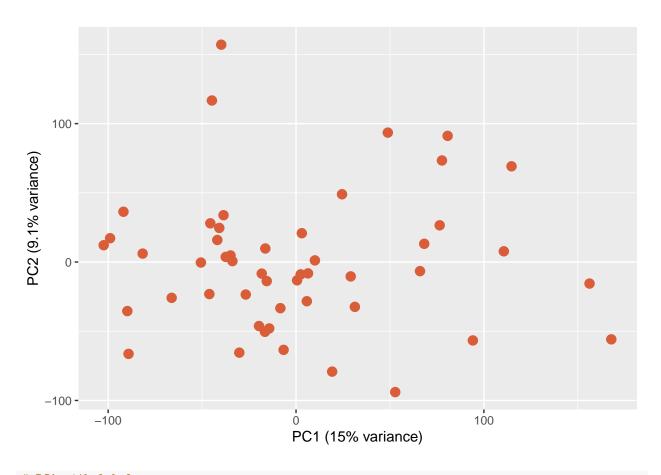
```
pca_data = prcomp(t(vst))
summary(pca_data)
```

```
## Importance of components:
                                                 PC3
                                                          PC4
                                                                   PC5
##
                              PC1
                                        PC2
                                                                             PC6
## Standard deviation
                          63.9867 49.90656 46.10558 42.72332 39.66570 37.63477
## Proportion of Variance
                          0.1499
                                   0.09117
                                            0.07781
                                                      0.06681
                                                               0.05759
## Cumulative Proportion
                           0.1499
                                   0.24103
                                            0.31884
                                                      0.38566
                                                               0.44325
##
                                         PC8
                                                  PC9
                               PC7
                                                          PC10
                                                                   PC11
                          33.58370 29.95445 28.61948 25.48666 24.73158 23.52585
## Standard deviation
## Proportion of Variance
                           0.04128
                                     0.03284
                                              0.02998
                                                       0.02378
                                                                0.02239
                                     0.56922
                                                                0.64536
                                                                         0.66562
## Cumulative Proportion
                           0.53638
                                              0.59920
                                                       0.62298
##
                              PC13
                                        PC14
                                                 PC15
                                                          PC16
                                                                   PC17
                                                                             PC18
## Standard deviation
                          23.34444 22.62875 21.53305 20.93252 20.48017 20.02441
## Proportion of Variance
                          0.01995
                                     0.01874
                                             0.01697
                                                       0.01604
                                                                0.01535
                                                                          0.01468
## Cumulative Proportion
                           0.68557
                                     0.70431
                                              0.72129
                                                       0.73733
                                                                0.75268
                                                                         0.76736
##
                              PC19
                                        PC20
                                                PC21
                                                        PC22
                                                                 PC23
## Standard deviation
                          19.50283 18.97850 18.4789 18.2599 17.54030 17.24837
## Proportion of Variance 0.01392
                                     0.01318
                                             0.0125
                                                     0.0122
                                                             0.01126
                                                                       0.01089
                                                     0.8192
## Cumulative Proportion
                           0.78128
                                     0.79446
                                              0.8070
                                                             0.83043
                                                                       0.84132
##
                              PC25
                                        PC26
                                                 PC27
                                                          PC28
                                                                   PC29
                                                                             PC30
## Standard deviation
                          16.76886 16.48292 16.01702 15.96123 15.61564 15.40634
## Proportion of Variance
                          0.01029
                                    0.00994 0.00939
                                                       0.00933
                                                                0.00893 0.00869
## Cumulative Proportion
                           0.85161
                                    0.86155
                                             0.87095
                                                       0.88027
                                                                0.88920
                                                                         0.89788
##
                              PC31
                                        PC32
                                                 PC33
                                                          PC34
                                                                  PC35
                                                                            PC36
## Standard deviation
                          14.73666 14.48728 14.35203 13.90651 13.6250 13.31766
                                             0.00754
                                                       0.00708
  Proportion of Variance
                          0.00795
                                    0.00768
                                                               0.0068
                                                                       0.00649
  Cumulative Proportion
                                     0.91352
                                              0.92106
                                                       0.92813
                                                                0.9349
                           0.90583
                                                                        0.94142
                                                 PC39
                                                          PC40
##
                              PC37
                                        PC38
                                                                   PC41
                                                                             PC42
## Standard deviation
                          13.00365 12.54243 12.41856 12.28849 12.11249 11.86366
## Proportion of Variance
                                    0.00576
                                             0.00565
                                                      0.00553
                                                                0.00537
                          0.00619
                                                                         0.00515
## Cumulative Proportion
                           0.94761
                                     0.95337
                                              0.95901
                                                       0.96454
                                                                0.96991
                                                                          0.97506
##
                                        PC44
                                                 PC45
                                                          PC46
                                                                   PC47
                                                                           PC48
                              PC43
## Standard deviation
                          11.26985 10.67636 10.36469 10.20151 9.08529 8.84174
## Proportion of Variance
                          0.00465
                                     0.00417
                                              0.00393
                                                      0.00381 0.00302 0.00286
## Cumulative Proportion
                           0.97971
                                     0.98389
                                              0.98782 0.99163 0.99465 0.99751
##
                             PC49
                                        PC50
## Standard deviation
                          8.24825 1.272e-13
## Proportion of Variance 0.00249 0.000e+00
## Cumulative Proportion 1.00000 1.000e+00
p1 <- fviz_eig(pca_data, addlabels = TRUE,</pre>
    ylim = c(0, 70), main = "")
p1
```

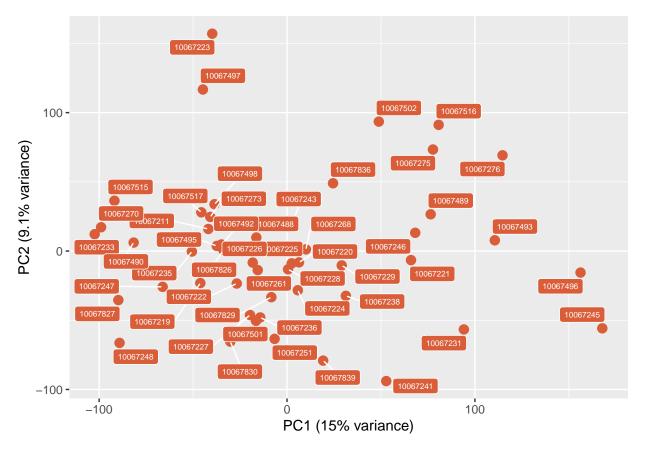


```
png(file.path(main_folder, "pca_screeplot.png"),
    res = 300, units = "px", width = 2500,
    height = 2000)
p1
dev.off()
```

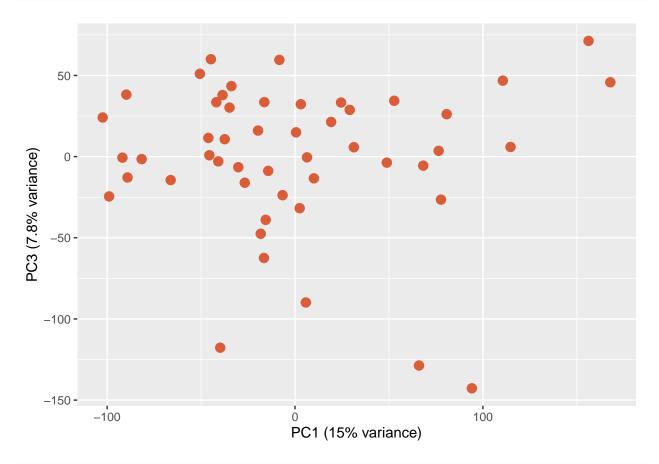
pdf ## 2



```
# PCA with labels
p3 <- ggplot(df_pca_data, aes(PC1, PC2, colour = "#d95d39")) +
    geom_point(size = 3, show.legend = F) +
    scale_color_manual(values = c("#d95d39")) +
    geom_label_repel(aes(label = sample,
        fill = "#d95d39"), color = "white",
        size = 2, max.overlaps = Inf) + scale_fill_manual(values = c("#d95d39")) +
    labs(x = paste0("PC1 (", pca_data_perc[1],
        "% variance)"), y = paste0("PC2 (",
        pca_data_perc[2], "% variance)")) +
    theme(legend.position = "none")
p3</pre>
```



```
png(file.path(main_folder, "PC1_vs_PC2.png"),
    res = 300, units = "px", width = 2500,
    height = 2000)
p2
dev.off()
## pdf
##
     2
png(file.path(main_folder, "PC1_vs_PC2_labels.png"),
    res = 300, units = "px", width = 2500,
   height = 2000)
рЗ
dev.off()
## pdf
##
# PCA for components 183
df_pca_data = data.frame(PC1 = pca_data$x[,
    1], PC3 = pca_data$x[, 3], sample = colnames(vst))
p4 <- ggplot(df_pca_data, aes(PC1, PC3, colour = "#d95d39")) +
    geom_point(size = 3) + scale_color_manual(values = c("#d95d39")) +
```



```
png(file.path(main_folder, "PC1_vs_PC3.png"),
    res = 300, units = "px", width = 2500,
    height = 2000)
p4
dev.off()
```

pdf

[1] 48 6

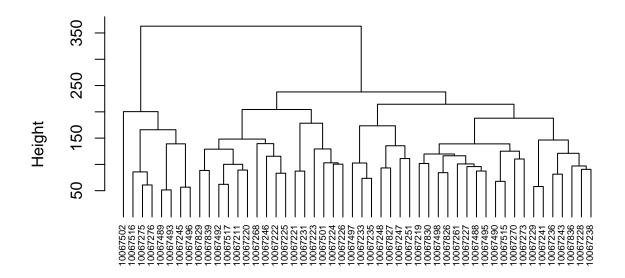
```
# missing samples
samples %>%
   filter(!sample %in% metadata selection$`Tumor/RNA ID`)
##
       sample
## 1 10067275
## 2 10067276
# Tumor/RNA ID entry in metadata file
# for those samples is 10067276/5
metadata selection[nrow(metadata selection) +
    1, ] = c("P-0022", "10067275", "10067150",
    "Cervix", "Cervix", "Baseline")
metadata_selection[nrow(metadata_selection) +
    1, ] = c("P-0022", "10067276", "10067150",
    "Cervix", "Cervix", "Baseline")
dim(metadata_selection)
## [1] 50 6
groups01 <- metadata_selection %>%
    dplyr::select(`Tumor/RNA ID`, `Timepoint of the tumor`) %>%
   dplyr::rename(sample = "Tumor/RNA ID",
        group = "Timepoint of the tumor")
groups01 <- groups01[match(colnames(counts)[2:51],</pre>
   groups01$sample), ]
groups01 %>%
   dplyr::count(group)
           group n
##
## 1
       Baseline 38
## 2
           C3D1 8
## 3 Progression 4
# baseline VS treatment with HDAC
# inhibitors C3D1, Day 1 of
# chemotherapy treatment cycle 3
comparisons01 <- data.frame(treatment = c("C3D1",</pre>
    "Progression"), control = c("Baseline",
    "C3D1"))
counts_hgnc <- counts %>%
   dplyr::rename(ensembl_gene_id_version = "Ensembl_ID") %>%
    right_join(., gene_IDs) %>%
   dplyr::select(-gene_biotype, -ensembl_gene_id_version) %>%
    group_by(hgnc_symbol) %>%
   summarise_all(mean) %>%
    column_to_rownames("hgnc_symbol") %>%
   rownames_to_column("gene_id")
## Joining with 'by = join_by(ensembl_gene_id_version)'
```

AUTOGO

```
files <- list.files(path = file.path(main_folder,
    "auto-go/R"), recursive = T, all.files = T,
    full.names = T)
invisible(sapply(files, source))
autogo_folder <- file.path(main_folder, "")</pre>
deseq_analysis(counts_hgnc, groups01, comparisons01,
   padj_threshold = 0.05, log2FC_threshold = 1,
   pre_filtering = T, save_excel = T, where_results = autogo_folder,
   outfolder = "differential_analysis_0824/")
## converting counts to integer mode
## estimating size factors
## estimating dispersions
## gene-wise dispersion estimates
## mean-dispersion relationship
## final dispersion estimates
## fitting model and testing
## -- replacing outliers and refitting for 2085 genes
## -- DESeq argument 'minReplicatesForReplace' = 7
## -- original counts are preserved in counts(dds)
## estimating dispersions
## fitting model and testing
TPM filtering on baseline expression
baseline <- groups01 %>%
   filter(group == "Baseline") %>%
   pull(sample)
c3d1 <- groups01 %>%
   filter(group == "C3D1") %>%
   pull(sample)
expression_filter_baseline <- tpm %>%
   dplyr::select(any_of(c("gene_id", baseline))) %>%
    column_to_rownames("gene_id")
expression_filter_baseline $\text{smean_baseline} <- apply(expression_filter_baseline,
   1, mean)
```

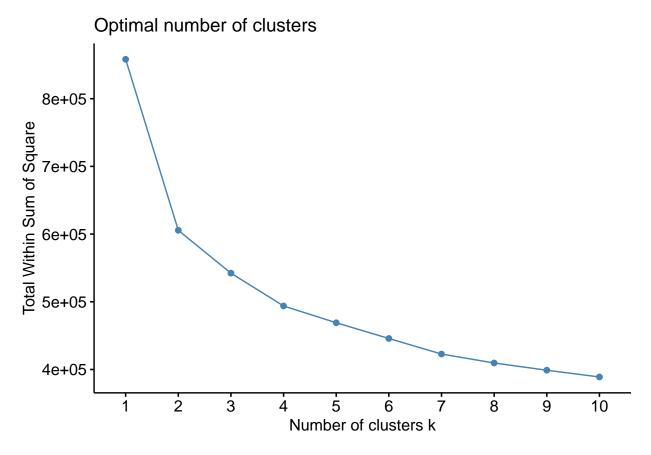
```
expression_filter_baseline <- expression_filter_baseline %>%
    rownames_to_column("gene_id")
expression filter c3d1 <- tpm %>%
    dplyr::select(any_of(c("gene_id", c3d1))) %>%
    column to rownames("gene id")
expression_filter_c3d1$mean_c3d1 <- apply(expression_filter_c3d1,</pre>
    1, mean)
expression_filter_c3d1 <- expression_filter_c3d1 %>%
    rownames to column("gene id")
expression_filter <- inner_join(expression_filter_baseline,</pre>
    expression_filter_c3d1, by = "gene_id") %>%
    dplyr::select(gene_id, mean_baseline,
        mean_c3d1)
filter_out <- expression_filter %>%
    filter(mean_baseline <= 1 | mean_c3d1 <=</pre>
path_res <- file.path(main_folder, "differential_analysis_0824/")</pre>
all_path_res <- list.files(path = path_res,</pre>
    pattern = "_allres.tsv", recursive = T,
    full.names = T)
res_lists <- lapply(all_path_res, function(x) read_tsv(x,
    col_types = cols()))
names(res_lists) <- gsub(paste0(path_res,</pre>
    "/|/DE_.*"), "", all_path_res)
# for (th in c(1)) {
# lapply(names(res_lists), function (i)
# volcanoplot(res_lists[[i]],
# my_comparison = i, log2FC_thresh =
# th, padj_thresh = 0.05, where_results
# = autogo_folder, outfolder =
# 'differential_analysis_0824/')) }
for (th in c(1)) {
    lapply(names(res_lists), function(i) volcanoplot_tpm(res_lists[[i]],
        my comparison = i, log2FC thresh = th,
        padj_thresh = 0.05, where_results = autogo_folder,
        outfolder = "differential analysis 0824/"))
}
# Reading gene lists for enrichment
lista_p005_fc1 <- read_gene_list(where_results = autogo_folder,</pre>
    outfolder = "differential_analysis_0824/",
    log2FC_threshold = 1, padj_threshold = 0.05,
    which_list = "everything")
# Enrichment analysis
lapply(names(lista_p005_fc1), function(i) autoGO(lista_p005_fc1[[i]],
    my_comparison = i, dbs = c("GO_Molecular_Function_2021",
        "GO_Cellular_Component_2021", "GO_Biological_Process_2021",
```

```
"KEGG_2021_Human"), where_results = autogo_folder,
    outfolder = "differential_analysis_0824/",
    excel = T))
tab_p005_fc1 <- read_enrich_tables(where_results = autogo_folder,</pre>
    outfolder = "differential_analysis_0824/",
    log2FC threshold = 1, padj threshold = 0.05,
    which_list = "everything")
invisible(lapply(names(tab_p005_fc1), function(i) barplotGO(tab_p005_fc1[[i]],
    my_comparison = i, where_results = autogo_folder,
    outfolder = "differential_analysis_0824/")))
invisible(lapply(names(tab p005 fc1), function(i) lollig0(tab p005 fc1[[i]],
    my_comparison = i, where_results = autogo_folder,
    outfolder = "differential_analysis_0824/")))
groups02 <- metadata_selection %>%
    dplyr::select(`Tumor/RNA ID`, `Primary Cancer Type`) %>%
    dplyr::rename(sample = "Tumor/RNA ID",
        group = "Primary Cancer Type")
groups02 <- groups02[match(colnames(counts)[2:51],</pre>
    groups02$sample), ]
groups02 %>%
    dplyr::count(group)
##
             group n
## 1
             Anus 14
            Cervix 15
## 3 Head and Neck 6
## 4
          Lung 1
## 5
           Penis 6
## 6
            Vulva 8
## Hierarchical Clustering
# feature selection keep top genes
# based on median absolute deviation
mads <- apply(vst, 1, mad)</pre>
# selecting features
mad2k <- vst[rev(order(mads))[1:2000], ]
# calculate distances (default:
# Eucledian distance)
dist matrix <- dist(t(mad2k))</pre>
# perform hierarchical clustering using
# ? linkage
hc <- hclust(dist_matrix, method = "ward.D2")</pre>
plot(hc, main = "", hang = -1, cex = 0.6,
xlab = "")
```



hclust (*, "ward.D2")

```
fviz_nbclust(mad2k, FUN = hcut, method = "wss")
```



```
prediction <- cutree(hc, k = 4)</pre>
prediction
## 10067211 10067219 10067220 10067221 10067222 10067223 10067224 10067225
## 10067226 10067227 10067228 10067229 10067231 10067233 10067235 10067236
  10067238 10067241 10067243 10067245 10067246 10067247 10067248 10067251
##
  10067261 10067268 10067270 10067273 10067275 10067276 10067488 10067489
##
                             2
                                      2
                                                                  2
                   1
   10067490 10067492 10067493 10067495 10067496 10067497 10067498 10067501
                                      2
                                                        3
   10067502 10067515 10067516 10067517 10067826 10067827 10067829 10067830
##
          4
                            4
                                      1
                                               2
                                                        3
## 10067836 10067839
##
          2
levels <- groups02 %>%
    mutate(level = ifelse(group == "Anus",
        1, "")) %>%
    mutate(level = ifelse(group == "Cervix",
        2, level)) %>%
    mutate(level = ifelse(group == "Head and Neck",
```

3, level)) %>%

```
mutate(level = ifelse(group == "Lung",
       4, level)) %>%
   mutate(level = ifelse(group == "Penis",
       5, level)) %>%
   mutate(level = ifelse(group == "Vulva",
       6, level))
target <- levels$level</pre>
Reference = groups02$group
Prediction <- cutree(hc, k = 4)
table(Prediction, Reference)
##
            Reference
## Prediction Anus Cervix Head and Neck Lung Penis Vulva
          1
                6
                       4
                                    1 1
           2
                       6
                                         0
                                                     4
##
                6
                                               2
                                    1
                                         0
                                                     0
##
           3
              2
                       0
                                               3
##
                0
                       5
                                         0
                                                     0
                                               1
confusionMatrix(as.factor(Prediction), as.factor(target),
   mode = "everything")
## Confusion Matrix and Statistics
##
##
            Reference
## Prediction 1 2 3 4 5 6
           1641104
##
           2661024
##
           3 2 0 2 0 3 0
##
##
           4 0 5 2 0 1 0
##
           5 0 0 0 0 0 0
##
           6000000
##
## Overall Statistics
##
##
                 Accuracy: 0.28
##
                   95% CI: (0.1623, 0.4249)
##
      No Information Rate: 0.3
      P-Value [Acc > NIR] : 0.6721
##
##
##
                    Kappa: 0.0726
##
  Mcnemar's Test P-Value : NA
##
## Statistics by Class:
##
##
                       Class: 1 Class: 2 Class: 3 Class: 4 Class: 5 Class: 6
                                                                       0.00
## Sensitivity
                         0.4286 0.4000 0.3333 0.0000
                                                              0.00
                                                  0.8367
## Specificity
                         0.7222 0.6286
                                         0.8864
                                                              1.00
                                                                       1.00
## Pos Pred Value
                                         0.2857
                                                  0.0000
                                                              {\tt NaN}
                                                                       NaN
                         0.3750 0.3158
## Neg Pred Value
                         0.7647 0.7097 0.9070 0.9762
                                                              0.88
                                                                       0.84
## Precision
                         0.3750 0.3158
                                         0.2857 0.0000
                                                              NA
                                                                        NA
```

##	Recall	0.4286	0.4000	0.3333	0.0000	0.00	0.00
##	F1	0.4000	0.3529	0.3077	NaN	NA	NA
##	Prevalence	0.2800	0.3000	0.1200	0.0200	0.12	0.16
##	Detection Rate	0.1200	0.1200	0.0400	0.0000	0.00	0.00
##	Detection Prevalence	0.3200	0.3800	0.1400	0.1600	0.00	0.00
##	Balanced Accuracy	0.5754	0.5143	0.6098	0.4184	0.50	0.50