Project Bioinformatic Resources

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Introduction

The following analysis focus on RNA-seq count data extracted from different cancer datasets from the Cancer Genome Atlas (TCGA). From the original TCGA data 50 cases (tumor samples) and 50 controls (normal samples) were randomly selected.

Analysis

The packages needed for the analysis are the following:

```
library(sessioninfo)
library(tidyverse)
library(biomaRt)
library(MotifDb)
                 # large collection of motifs across different species
library(seqLogo)
library(PWMEnrich)
library(PWMEnrich.Hsapiens.background)
library(GEOquery)
library(oligo)
library(pd.hg.u133.plus.2)
library(hgu133plus2.db)
library(genefilter)
library(limma)
library(pheatmap)
library(stringr)
library(GenomicFeatures) # to build object with specific information such as genomic coordinates
library(ggplot2)
library(edgeR) # designed to perform Differential Expression Analysis from RNA-Seq data
library(fgsea) # computation of enrichment scores and other statistics
library(org.Hs.eg.db) # database annotation human specific transcript from Ensembl
library(clusterProfiler) # uses Fisher test, explore gene list of interest against a reference
library(enrichplot) # build enrich map
library(ggnewscale)
library(DOSE)
library(pathview) # contains cartoons of pathways and we project on them our genes of interest
```

Task 1: Load data

In particular, we consider data coming from Lung adenocarcinoma.

```
load("./RData/Lung_adenocarcinoma.RData")
```

After loading the data, the following three data-frames are available:

- raw_counts_df which contains the raw RNA-seq counts;
- c_anno_df, which contains sample name and condition (case and control);
- r_anno_df, which contains the ENSEMBL genes ids, the length of the genes and the genes symbols.

Task 2: Filter protein coding genes

To extract only protein coding genes from raw_counts_df and r_anno_df, we use the biomaRt package.

First, we retrieve the information about the protein coding genes from Ensembl.

```
database <- useMart("ensembl")
datasetHuman <- useDataset("hsapiens_gene_ensembl", mart = database)
query <- getBM(attributes = c("ensembl_gene_id", "external_gene_name", "gene_biotype"),
    filters = c("ensembl_gene_id"), values = r_anno_df$ensembl_gene_id, mart = datasetHuman)
query_protein_coding <- query[which(query$gene_biotype == "protein_coding"), ]</pre>
```

Then, we filter the data frames containing the raw counts and the annotation to keep only the protein coding genes.

```
indexes_r_anno_df <- which(r_anno_df\ensembl_gene_id %in% query_protein_coding\ensembl_gene_id)
r_anno_df_protein_coding <- r_anno_df[indexes_r_anno_df, ]

indexes_raw_counts_df <- which(rownames(raw_counts_df) %in% query_protein_coding\ensembl_gene_id)
raw_counts_df_protein_coding <- raw_counts_df[indexes_raw_counts_df, ]</pre>
```

Task 3: Differential expression analysis

To perform the differential expression analysis, we will use the edgeR package.

It is important to remove genes with low signal that will have low statistical power. Since, we want to focus on the transcripts we can use to perform the analysis, we can filter raw counts data using a threshold of raw count > 20 in at least 5 replicates.

```
# count threshold
count_thr <- 20

# number of replicates with more counts than the count threshold
repl_thr <- 5

filter_vec <- apply(raw_counts_df_protein_coding,1, # go through all count matrices by rows
    function(y) max(by(y, c_anno_df$condition, function(x) sum(x>=count_thr))))
    # groups the values on each condition and sum all the values above the count
```

Then, we filter the previously updated data frames.

```
filter_counts_df <- raw_counts_df_protein_coding[filter_vec >= repl_thr, ]
dim(filter_counts_df) # 17289

## [1] 17289 100

filter_anno_df <- r_anno_df_protein_coding[rownames(filter_counts_df), ]
dim(filter_anno_df) # 17289

## [1] 17289 3</pre>
```

Now we check the library size of each sample (how many reads we have sequenced for each experiment)

```
size_df <- data.frame(sample = colnames(filter_counts_df), read_millions = colSums(filter_counts_df)/1e

ggplot(data = size_df, aes(sample, read_millions)) + geom_bar(stat = "identity",
    fill = "indianred", colour = "indianred", width = 0.7, alpha = 0.7) + coord_flip() +
    theme_bw()</pre>
```

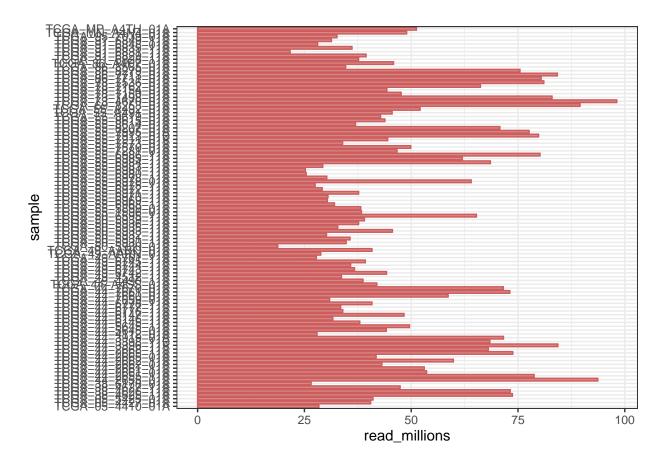


Figure 1: Library size of each sample.

Then, we visualize a boxplot of gene counts.

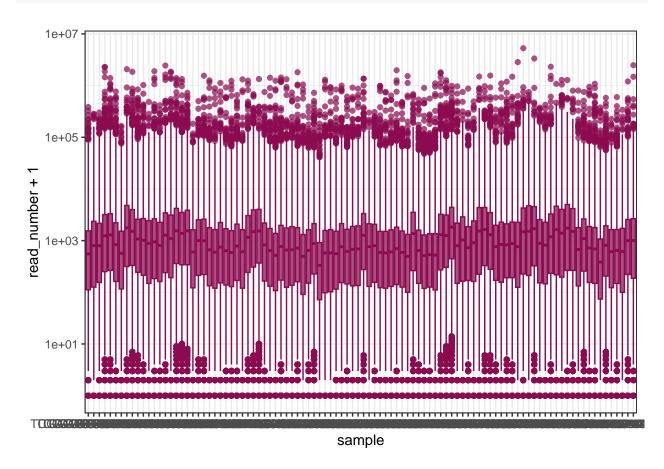


Figure 2: Boxplot of gene counts before normalization.

As we can see from the plots, there is a significant variability across samples in term of library sizes (reads per million). We have to take into account this aspect because the expected size of each count is the product of the relative abundance of that gene in that sample but also of the library size.

As we can see from the boxplot, we need to normalize our data before testing for differential expression. Normalization can be obtained using different methodologies. Among them, TMM (the default method) is a method that consider in the normalization also variables related to the library size.

To perform the DEG analysis, we first need to create a DGRList object containing information about counts, annotation, samples and genes. To do that, we use the function DGEList to create the input for the following normalization step. This object contains information about counts, samples and genes.

The normalization intra- and inter-sample is done using the function calcNormFactors and specifying method = "TMM", which is the weighted trimmed mean of M-values approach.

Then, we create a cpm table containing the normalized expression values for each transcript expressed as counts per million (CPM).

```
# create a cpm table (normalized expression values)
cpm_table <- as.data.frame(round(cpm(edge_n), 2))</pre>
```

To see the effect of the normalization, we look at the boxplot distribution of gene expression signals after normalization.

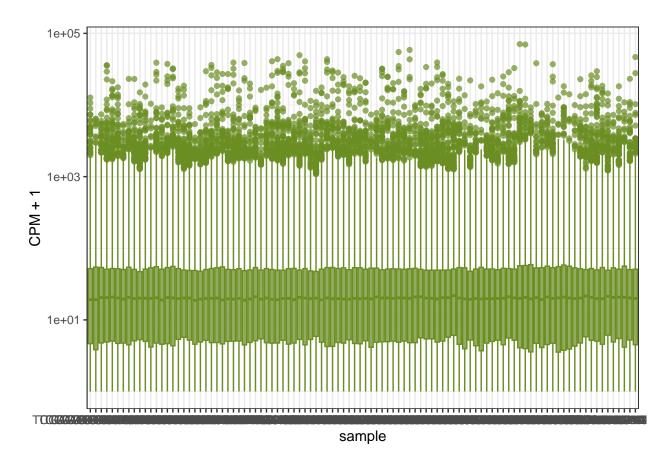


Figure 3: Boxplot distribution of gene expression signals after normalization.

We notice that, with respect to the previous boxplot, after the normalization, the distributions are comparable. That means that now our data is ready to be tested for DE analysis.

We define the experimental design matrix, later needed to calculate the dispersion and fit. This matrix is based on the experimental design, so we define the conditions we want to test (case VS control).

```
design <- model.matrix(~0 + group, data = edge_n$samples)
colnames(design) <- levels(edge_n$samples$group)
rownames(design) <- edge_n$samples$sample</pre>
```

Once we normalized the data and the design, we proceed by calculating the dispersion fit.

```
# calculate dispersion and fit with edgeR (necessary for differential
# expression analysis)
edge_d <- estimateDisp(edge_n, design)
edge_f <- glmQLFit(edge_d, design)</pre>
```

The estimateDisp function tries to estimate the variability at different levels: in the data, inter sample and intra sample. The over dispersion of counts across the samples can be modeled as a Poisson distribution, which can be approximated using a negative binomial distribution by using glmQLFit function.

We define the contrasts (conditions to be compared).

```
contro <- makeContrasts("control-case", levels = design)</pre>
```

We performed a test through function glmQLFTest to determine which genes are differentially expressed. Then, we selected genes based on a 0.01 p-value cutoff and ordered them based on the log2 fold change.

```
# fit the model with generalized linear models
edge_t <- glmQLFTest(edge_f, contrast = contro)
DEGs <- as.data.frame(topTags(edge_t, n = 20000, p.value = 0.01, sort.by = "logFC"))</pre>
```

Then, we improve the selection, considering not only the p-value, but also the average expression of the genes (logCPM). We used the logFC value to assign genes to different classes:

• up-regulated genes if logFC > 1.5

head(DEGs)

- down-regulated genes if logFC < -1.5
- not significant genes (unchanged) otherwise.

```
DEGs$class <- "="

DEGs$class[which(DEGs$logCPM > 1 & DEGs$logFC > 1.5 & DEGs$FDR < 0.25)] = "+" # upregulated genes

DEGs$class[which(DEGs$logCPM > 1 & DEGs$logFC < (-1.5) & DEGs$FDR < 0.25)] = "-" # downregulated genes

DEGs <- DEGs[order(DEGs$logFC, decreasing = T), ]
```

```
##
                   ensembl_gene_id external_gene_name length
                                                                logFC
                                                                          logCPM
## ENSG00000179914 ENSG00000179914
                                                ITLN1
                                                        8640 6.684901 5.8661078
## ENSG00000108576 ENSG00000108576
                                               SLC6A4
                                                       41683 6.084705 6.6338451
## ENSG00000180440 ENSG00000180440
                                               SERTM1
                                                       23819 5.663190 3.2093449
## ENSG00000108342 ENSG00000108342
                                                 CSF3
                                                        2452 5.588520 5.2512424
## ENSG00000034971 ENSG00000034971
                                                 MYOC 17271 5.397462 1.5811486
## ENSG00000178084 ENSG00000178084
                                                HTR3C
                                                        7626 5.325742 0.5517057
##
                           F
                                   PValue
                                                   FDR class
## ENSG00000179914 75.57703 6.388727e-14 4.459213e-13
## ENSG00000108576 122.16543 3.992071e-19 7.518401e-18
```

From the results, we saw that 1193 genes are down-regulated, 877 genes are up-regulated and 14949 genes have no changes in their regulation.

We then create the volcano plot.

We also report the heatmap of only up and down-regulated genes.

To simplify the later analysis, we save the list of differentially expressed genes in a text file.

Task 4: Gene set enrichment analysis

To perform the gene set enrichment analysis, we will use the clusterProfiler package.

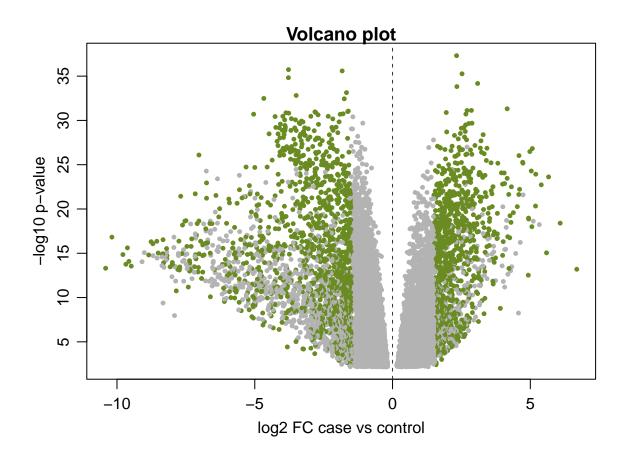


Figure 4: Volcano plot

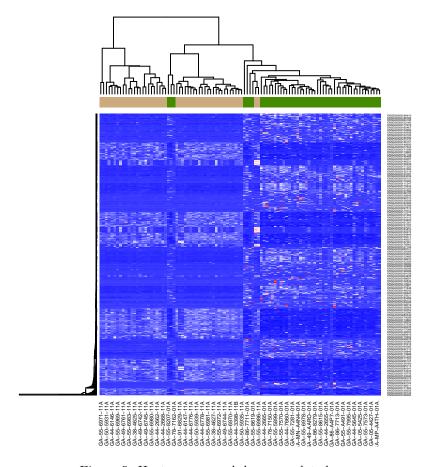


Figure 5: Heatmap up and down-regulated genes.

```
DEGs <- read.table("DEGs.txt", header = T, sep = "\t", as.is = T)
table(DEGs$class)
##
## - + =
## 1199 884 9258</pre>
```

We use the biomaRt package to retrieve the entrezgene_id for all the genes in the DEGs dataset.

```
ensembl <- useEnsembl(biomart = "ensembl", dataset = "hsapiens_gene_ensembl")
convert <- getBM(attributes = c("ensembl_gene_id", "entrezgene_id"), filters = c("ensembl_gene_id"),
    values = DEGs$ensembl_gene_id, mart = ensembl)

DEGs <- merge(DEGs, convert, by.x = "ensembl_gene_id", by.y = "ensembl_gene_id") # include the new inf
DEGs <- DEGs[which(!is.na(DEGs$entrezgene_id)), ]
DEGs <- DEGs[-which(duplicated(DEGs$entrezgene_id)), ]</pre>
```

We removed all the NA and duplicates in the dataset DEGs.

We created new lists for the down and up-regulated genes.

```
# list of up-regulated genes
upDEGs <- DEGs %>%
    filter(class == "+")
# list of down-regulated genes
downDEGs <- DEGs %>%
    filter(class == "-")
```

Performing enrichment analysis for GO biological process

```
# biological process up regulated genes
ego_BP_up <- enrichGO(gene = upDEGs$external_gene_name, OrgDb = org.Hs.eg.db, keyType = "SYMBOL",
    ont = "BP", pAdjustMethod = "BH", pvalueCutoff = 0.05, qvalueCutoff = 0.05)

# biological process down regulated genes
ego_BP_down <- enrichGO(gene = downDEGs$external_gene_name, OrgDb = org.Hs.eg.db,
    keyType = "SYMBOL", ont = "BP", pAdjustMethod = "BH", pvalueCutoff = 0.05, qvalueCutoff = 0.05)
barplot(ego_BP_up, showCategory = 10)</pre>
```

Both the first two biological processes are related to the microtubules, which might be explained by the fact that tumour cells have a high growth rate thus the cytoskeleton is assembled over and over again. We also notice that angiogenesis is among the most enriched biological processes as we expected. In fact, angiogenesis is particularly important in tumorigenesis to allow the growth of the tumour tissue, providing nutrients to cells.

```
barplot(ego_BP_down, showCategory = 10)
```

We would expect most of these biological processes to be enriched in the up-regulated genes since tumour cells tend to replicate more than normal cells. A possible explaination is that tumour cells genes accumulate numerous mutations usually ending up in loss of function or downregulation. These mutations might occur on transcription factors binding sites or RNA binding sites, reducing their expression.

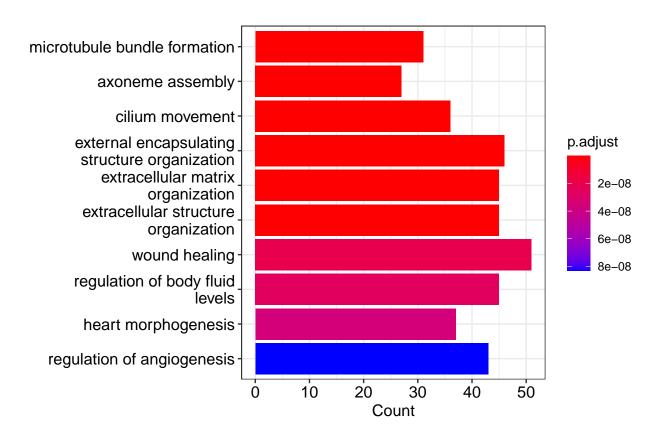


Figure 6: Biological process up regulated genes

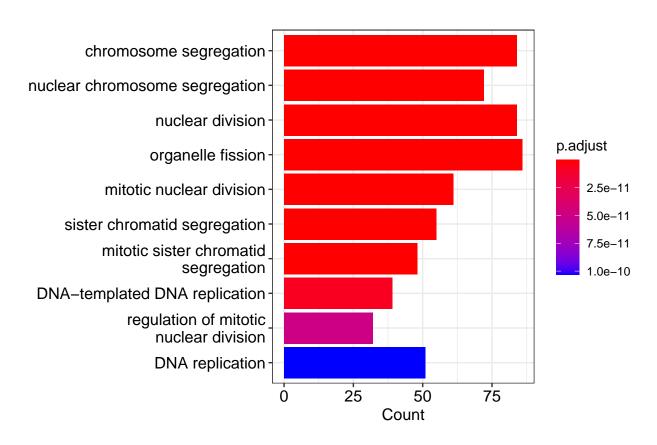


Figure 7: Biological process down regulated genes

Performing enrichment analysis for GO molecular function

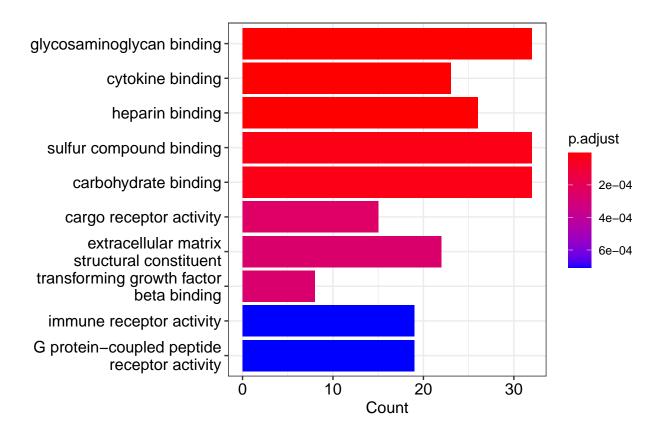


Figure 8: Molecular function up regulated genes

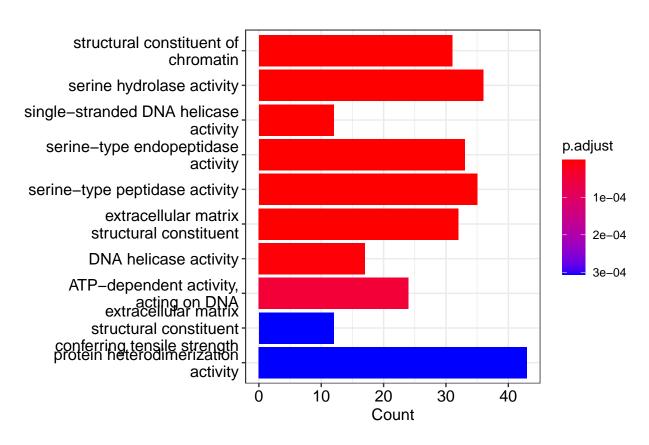


Figure 9: Molecular function down regulated genes

```
## WP2806 WP2806
                                                         Complement system
## WP545
           WP545
                                                     Complement activation
## WP558
           WP558
                                       Complement and coagulation cascades
## WP2431 WP2431
                                                        Spinal cord injury
## WP5094 WP5094
                                                   Orexin receptor pathway
## WP5090 WP5090 Complement system in neuronal development and plasticity
                                               p.adjust
          GeneRatio BgRatio
                                   pvalue
             19/436 97/8442 3.960197e-07 0.0001892974 0.0001725812
## WP2806
## WP545
              8/436
                     23/8442 1.171095e-05 0.0027989174 0.0025517547
             12/436 58/8442 3.152788e-05 0.0050234421 0.0045798393
## WP558
## WP2431
             18/436 121/8442 4.427616e-05 0.0052910008 0.0048237708
             24/436 201/8442 1.015995e-04 0.0087342052 0.0079629178
## WP5094
             16/436 107/8442 1.096344e-04 0.0087342052 0.0079629178
## WP5090
##
## WP2806
                                  6401/729/2219/2160/7448/6404/730/653509/22918/5199/5648/11326/5806/235
## WP545
                                                                                                  732/729
## WP558
                                                                              729/1361/7450/730/5648/713/
## WP2431
                                             2920/4842/57556/6347/1958/3164/7538/3569/7099/9353/2353/361
## WP5094 6401/862/2920/4616/1839/8013/1958/1959/3164/2354/3949/5468/3569/9314/2890/1346/2353/5581/3400
                                                         732/729/2219/7448/730/5199/5648/8547/81035/1524
##
          Count
## WP2806
             19
## WP545
## WP558
## WP2431
             18
## WP5094
             24
## WP5090
             16
eWP_down <- enrichWP(gene = downDEGs$entrezgene_id, organism = "Homo sapiens", pvalueCutoff = 0.05,
    qvalueCutoff = 0.1)
head(eWP_down, n = 10)
              ID
## WP2446 WP2446
## WP2361 WP2361
## WP179
           WP179
## WP1604 WP1604
## WP45
            WP45
## WP466
           WP466
## WP4240 WP4240
## WP698
           WP698
## WP4016 WP4016
## WP2525 WP2525
##
                                                                               Description
## WP2446
                                                            Retinoblastoma gene in cancer
## WP2361
                                                                 Gastric cancer network 1
## WP179
                                                                                Cell cycle
## WP1604
                                                          Codeine and morphine metabolism
## WP45
                                                               G1 to S cell cycle control
## WP466
                                                                           DNA replication
## WP4240 Regulation of sister chromatid separation at the metaphase-anaphase transition
                                                                           Glucuronidation
## WP4016
                                              DNA IR-damage and cellular response via ATR
```

```
## WP2525
                           Trans-sulfuration, one-carbon metabolism and related pathways
##
                                   pvalue
          GeneRatio BgRatio
                                              p.adjust
                                                             qvalue
## WP2446
            26/618 90/8442 5.976882e-10 3.107978e-07 2.850029e-07
## WP2361
             13/618 28/8442 2.054960e-08 4.361461e-06 3.999477e-06
## WP179
             28/618 120/8442 2.516227e-08 4.361461e-06 3.999477e-06
## WP1604
             9/618 16/8442 4.091055e-07 4.391632e-05 4.027145e-05
            18/618 64/8442 4.222724e-07 4.391632e-05 4.027145e-05
## WP45
## WP466
             13/618 42/8442 5.366172e-06 4.650682e-04 4.264694e-04
## WP4240
             7/618 15/8442 4.168136e-05 3.096330e-03 2.839347e-03
              9/618 26/8442 5.735318e-05 3.541417e-03 3.247494e-03
## WP698
## WP4016
             17/618 81/8442 6.129375e-05 3.541417e-03 3.247494e-03
## WP2525
             15/618 68/8442 9.059661e-05 4.711024e-03 4.320028e-03
##
             1870/54443/4175/4998/24137/8318/5427/1869/4173/898/1871/7272/7153/891/10733/890/1111/9133/
## WP2446
## WP2361
                                                                       6790/22974/4605/4173/1894/1063/71
## WP179 1870/4171/4175/4998/23594/8318/990/1869/4173/898/1871/7272/991/9088/891/9700/890/1029/1111/91
## WP1604
                                                                                             1244/1565/8
## WP45
                                                     1870/4171/4175/4998/23594/8318/5427/1869/4173/898/
## WP466
                                                                           55388/4171/4175/4998/23594/83
## WP4240
## WP698
                                                                                           7358/7363/545
## WP4016
                                                         672/5888/4171/63967/8318/1869/2305/83990/55215
## WP2525
                                                            2729/2730/10797/1789/2571/23743/29968/570/5
##
          Count
## WP2446
             26
## WP2361
             13
## WP179
             28
## WP1604
              9
## WP45
             18
## WP466
             13
## WP4240
             7
## WP698
              9
## WP4016
             17
```

Chiedere a Romanel per pathway.id (KEGG o WP)?

15

WP2525

Task 5: Visualization of the enriched pathway

```
## hsa04270 hsa04270
                                                Vascular smooth muscle contraction
## hsa04061 hsa04061 Viral protein interaction with cytokine and cytokine receptor
                                                          Renin-angiotensin system
## hsa04614 hsa04614
## hsa03320 hsa03320
                                                            PPAR signaling pathway
## hsa04380 hsa04380
                                                        Osteoclast differentiation
## hsa04514 hsa04514
                                                           Cell adhesion molecules
## hsa04060 hsa04060
                                           Cytokine-cytokine receptor interaction
## hsa04924 hsa04924
                                                                   Renin secretion
## hsa04080 hsa04080
                                           Neuroactive ligand-receptor interaction
## hsa04925 hsa04925
                                               Aldosterone synthesis and secretion
## hsa04976 hsa04976
                                                                    Bile secretion
            GeneRatio BgRatio
                                     pvalue
                                                p.adjust
                                                               qvalue
              13/382 50/8461 2.045971e-07 3.077765e-05 2.686624e-05
## hsa05144
## hsa04610
             17/382 86/8461 2.144784e-07 3.077765e-05 2.686624e-05
            17/382 134/8461 1.038943e-04 9.939223e-03 8.676086e-03
## hsa04270
              14/382 100/8461 1.469697e-04 1.054508e-02 9.204945e-03
## hsa04061
## hsa04614
              6/382 23/8461 4.269912e-04 2.382406e-02 2.079635e-02
## hsa03320
            11/382 75/8461 4.980639e-04 2.382406e-02 2.079635e-02
## hsa04380
            15/382 128/8461 6.235812e-04 2.501803e-02 2.183859e-02
            17/382 157/8461 6.973666e-04 2.501803e-02 2.183859e-02
## hsa04514
## hsa04060
            26/382 295/8461 8.073811e-04 2.574649e-02 2.247447e-02
## hsa04924
            10/382 69/8461 9.796110e-04 2.811484e-02 2.454183e-02
## hsa04080
            30/382 367/8461 1.126599e-03 2.939399e-02 2.565843e-02
            12/382 98/8461 1.460139e-03 3.492166e-02 3.048360e-02
## hsa04925
## hsa04976
            11/382 89/8461 2.111646e-03 4.661864e-02 4.069406e-02
##
## hsa05144
                                                                                                6401/14
## hsa04610
                                                                                   732/729/1361/2160/74
## hsa04270
                                                                    10203/1906/50487/10268/196883/10266
## hsa04061
                                                                                        53832/2920/6369
## hsa04614
## hsa03320
## hsa04380
                                                                                 7305/6688/10326/54/110
## hsa04514
                                                                   6401/51208/8516/64115/6404/90952/584
## hsa04060
                              53832/2920/3568/6369/1440/6347/2690/3977/9173/8809/650/51554/3569/90865/9
## hsa04924
## hsa04080 153/10203/1906/1511/5023/2690/7433/1268/5737/8698/2922/187/1910/6751/185/2900/2890/5745/112
## hsa04925
## hsa04976
##
            Count
## hsa05144
              13
## hsa04610
              17
## hsa04270
## hsa04061
              14
## hsa04614
## hsa03320
              11
## hsa04380
              15
## hsa04514
              17
## hsa04060
              26
## hsa04924
              10
## hsa04080
              30
## hsa04925
              12
## hsa04976
```

Task 6: Enrichment score transcription factors

```
promoter_regions <- getSequence(id = downDEGs\ensembl_gene_id, type = "ensembl_gene_id",
    seqType = "gene_flank", upstream = 500, mart = ensembl)
data(PWMLogn.hg19.MotifDb.Hsap)
seq <- lapply(promoter_regions$gene_flank, function(x) DNAString(x))</pre>
enriched_TFs = motifEnrichment(seq, PWMLogn.hg19.MotifDb.Hsap, score = "affinity")
## Calculating motif enrichment scores ...
report = groupReport(enriched_TFs)
report
## An object of class 'MotifEnrichmentReport':
##
          rank target
                                              raw.score
                                                                     p.value
## 1
             1 PGAM2
                                PGAM2 7.47058707343782 2.41159784654048e-86
               TFAP4
## 2
             2
                           M2944_1.02 3.42391273544219 3.36836197404242e-84
## 3
             3
                  SP2
                                  SP2 119.54842162525 6.25197615973972e-84
## 4
             4 CEBPB
                           M4556_1.02 2.30767167521828 1.7054813305699e-83
                 JUND
                           M4506_1.02 6.8634137704217 1.50332672791467e-81
## 5
             5
## 6
             6
                 MAFK
                           M4573_1.02 2.41935358645508 4.83938302985492e-81
## 7
             7
               ZMAT2
                                ZMAT2 2.16420212862343 9.19698805317131e-81
## 8
             8
                  JUN
                           M4591 1.02 2.76520576103839 2.36897498529839e-80
## 9
             9
                 KLF5
                                 KLF5 30.1023912345585 2.91297830264159e-79
                                  NNT 1.87741003326598 6.17885793377267e-79
## 10
            10
                  NNT
## ...
## 2287 2001.5 Oct-1 NBT06/Oct-1.pwm 1.72351938773035
                                                                           1
##
            top.motif.prop
         0.177364864864865
## 1
## 2
         0.178209459459459
## 3
         0.185810810810811
## 4
         0.174831081081081
## 5
        0.185810810810811
## 6
        0.171452702702703
## 7
         0.163851351351351
## 8
         0.168074324324324
## 9
         0.158783783783784
## 10
         0.173986486486486
## 2287 0.0278716216216216
```

Task 7

Select one among the top enriched TFs, compute the empirical distributions of scores for all PWMs that you find in MotifDB for the selected TF and determine for all of them the distribution (log2) threshold cutoff at 99.75%.

```
tfs <- report$target[1]
tfs_motifs = subset(MotifDb, organism == "Hsapiens" & geneSymbol == tfs)
# tfs_motifs
PWM = toPWM(as.list(tfs_motifs))
PWM
## $`Hsapiens-hPDI-PGAM2`
## An object of class 'PWM'
## ID: Hsapiens-hPDI-PGAM2
## Target name: Hsapiens-hPDI-PGAM2
## Frequency matrix:
## $pfm
##
     1
        2 3 4 5 6 7 8
     0 0 0 25
## A
                 0 12 12
## C 0 12 12 25 0 0 0
## G 50 38 38 0 50 38 38 50
## T O O O O O O O
## Position weight matrix (PWM):
## $pwm
##
            1
                       2
                                  3
## A -5.672425 -5.6724253 -5.6724253 0.9857861 -5.672425 -0.0577155 -0.0577155
## C -5.672425 -0.0577155 -0.0577155 0.9857861 -5.672425 -5.6724253 -5.6724253
## G 1.978626 1.5849625 1.5849625 -5.6724253 1.978626 1.5849625 1.5849625
## T -5.672425 -5.6724253 -5.6724253 -5.6724253 -5.6724253 -5.6724253 -5.6724253
##
## A -5.672425
## C -5.672425
## G 1.978626
## T -5.672425
## With background nucleotide frequencies which also serve as pseudo-count:
## $prior.params
##
     Α
          С
## 0.25 0.25 0.25 0.25
ecdf = motifEcdf(PWM, organism = "hg19", quick = TRUE) # calculate for each motif of my gene the empir
# ecdf
thresholds = lapply(ecdf, function(x) log2(quantile(x, 0.9975))) # for each of the distribution, take
# thresholds
```

Task 8

Identify which up-regulated (or down-regulated depending on the choice you made at point 7) genes have a region in their promoter (defined as previously) with binding scores above the computed thresholds for any of the previously selected PWMs. Use pattern matching as done during the course

```
scores = motifScores(seq, PWM, raw.scores = FALSE, cutoff = unlist(thresholds)) # in my promoters, i w
head(scores)
```

```
Hsapiens-hPDI-PGAM2
##
## [1,]
                          4
##
  [2,]
                          4
## [3,]
                          1
## [4,]
                          2
## [5,]
                          7
## [6,]
fractions_{99_{75}} = c()
fractions_99_75 = c(fractions_99_75, length(which(apply(scores, 1, sum) > 0))/100)
fractions_99_75
## [1] 10.07
highscore_seq <- which(apply(scores, 1, sum) > 0)
down_DEGs[highscore_seq, ] # keeping only sequences which have higher score than threshold
                   ensembl_gene_id external_gene_name
                                                       length
                                                                    logFC
                                                                             logCPM
## ENSG00000130234 ENSG00000130234
                                                        112670 -1.500042
                                                                           2.537010
                                                  ACE2
## ENSG00000139998 ENSG00000139998
                                                 RAB15
                                                         27412 -1.500270
                                                                           5.323017
## ENSG00000088826 ENSG00000088826
                                                  SMOX
                                                         66767 -1.503458
                                                                           3.277682
## ENSG00000240972 ENSG00000240972
                                                           844 -1.503544
                                                   MTF
                                                                          7.546681
## ENSG00000085465 ENSG00000085465
                                                 OVGP1
                                                         13416 -1.504675
                                                                           1.551978
## ENSG00000183196 ENSG00000183196
                                                 CHST6
                                                         23393 -1.506132
                                                                          3.263537
## ENSG00000121380 ENSG00000121380
                                               BCL2L14
                                                        161240 -1.509102
                                                                          1.183644
## ENSG00000112312 ENSG00000112312
                                                  GMNN
                                                         11168 -1.509589
                                                                           3.686536
## ENSG00000064300 ENSG00000064300
                                                  NGFR
                                                         19715 -1.509766
                                                                           2.645172
## ENSG00000170779 ENSG00000170779
                                                 CDCA4
                                                         11510 -1.512081
                                                                           3.782223
## ENSG00000139835 ENSG00000139835
                                                 GRTP1
                                                         39985 -1.514213
                                                                           3.059998
## ENSG00000164171 ENSG00000164171
                                                 ITGA2
                                                        105439 -1.514961
                                                                           6.213954
## ENSG00000178852 ENSG00000178852
                                               EFCAB13
                                                        118022 -1.516200
                                                                           1.732368
## ENSG00000137699 ENSG00000137699
                                                TRIM29
                                                         74243 -1.516640
                                                                          5.146690
## ENSG00000100167 ENSG00000100167
                                                         28778 -1.519337
                                               SEPTIN3
                                                                           2.424322
## ENSG00000137747 ENSG00000137747
                                              TMPRSS13
                                                         28818 -1.520302
                                                                          4.713471
## ENSG00000185101 ENSG00000185101
                                                  ANO9
                                                         24078 -1.520674
                                                                           4.414709
## ENSG00000265690 ENSG00000265690
                                                          2430 -1.522722
                                                                          2.289592
## ENSG00000251357 ENSG00000251357
                                                         33035 -1.522779
                                                                           6.898722
## ENSG00000248485 ENSG00000248485
                                                PCP4L1
                                                         26705 -1.522794
                                                                          3.897882
## ENSG00000212916 ENSG00000212916
                                                 MAP10
                                                          4513 -1.524873
                                                                          1.849379
## ENSG00000182199 ENSG00000182199
                                                 SHMT2
                                                          5362 -1.526742
                                                                         6.142538
## ENSG00000136295 ENSG00000136295
                                                 TTYH3
                                                         32841 -1.527712
                                                                           6.694196
## ENSG00000262660 ENSG00000262660
                                                         17168 -1.530243
                                                                           5.628142
## ENSG00000145908 ENSG00000145908
                                                ZNF300
                                                         10591 -1.531791
                                                                           2.317176
## ENSG00000141682 ENSG00000141682
                                                PMAIP1
                                                          4309 -1.531812
                                                                           3.680478
## ENSG00000104368 ENSG00000104368
                                                  PLAT
                                                         32991 -1.533091
                                                                           6.679663
## ENSG00000057294 ENSG00000057294
                                                  PKP2
                                                        106032 -1.535496
                                                                           3.836982
## ENSG00000183605 ENSG00000183605
                                                 SFXN4
                                                         24947 -1.536623
                                                                          4.086809
## ENSG00000112242 ENSG00000112242
                                                  E2F3
                                                         91835 -1.539108
                                                                           4.420271
## ENSG00000143578 ENSG00000143578
                                                                           4.257557
                                               CREB3L4
                                                          6827 -1.540545
## ENSG00000160200 ENSG00000160200
                                                   CBS
                                                         23752 -1.541278
                                                                           4.078383
## ENSG00000161692 ENSG00000161692
                                                 DBF4B
                                                         43656 -1.541794
                                                                           2.704834
## ENSG00000198648 ENSG00000198648
                                                 STK39
                                                        293575 -1.541851
                                                                           5.356302
```

CDK5R1

4260 -1.544475

1.888809

ENSG00000176749 ENSG00000176749

```
## ENSG00000167759 ENSG00000167759
                                                  KLK13
                                                           9488 -1.545001 1.376170
  ENSG00000163689 ENSG00000163689
                                              CFAP20DC
                                                        332719 -1.546260
                                                                           1.290553
  ENSG00000259207 ENSG00000259207
                                                  ITGB3
                                                          59916 -1.547201
                                                                           3.544269
  ENSG00000159958 ENSG00000159958
                                             TNFRSF13C
                                                           4774 -1.548716
                                                                           1.919789
   ENSG00000078269 ENSG00000078269
                                                 SYNJ2
                                                         117313 -1.549474
                                                                           4.320316
  ENSG00000205336 ENSG00000205336
                                                          54928 -1.550081
                                                 ADGRG1
                                                                           7.077630
   ENSG00000065911 ENSG00000065911
                                                MTHFD2
                                                          31393 -1.550408
                                                                           5.930603
  ENSG00000131941 ENSG00000131941
                                                 RHPN2
                                                          86296 -1.552602
                                                                           4.994363
  ENSG00000198743 ENSG00000198743
                                                SLC5A3
                                                          32682 -1.556584
                                                                            5.729588
   ENSG00000112559 ENSG00000112559
                                                   MDFI
                                                          17362 -1.557440
                                                                           4.164127
   ENSG00000109654 ENSG00000109654
                                                  TRIM2
                                                         187156 -1.557838
                                                                           6.443849
  ENSG00000164877 ENSG00000164877
                                               MICALL2
                                                          31005 -1.558882
                                                                           5.008547
   ENSG00000155974 ENSG00000155974
                                                  GRIP1
                                                         721731 -1.561022
                                                                           1.417162
   ENSG00000112699 ENSG00000112699
                                                   GMDS
                                                         621799 -1.561800
                                                                           4.391701
   ENSG00000159753 ENSG00000159753
                                                CARMIL2
                                                          12581 -1.561935
                                                                            2.336659
   ENSG00000204410 ENSG00000204410
                                                   MSH5
                                                          22999 -1.563964
                                                                            3.241788
   ENSG00000143850 ENSG00000143850
                                               PLEKHA6
                                                         158812 -1.565081
                                                                           5.170103
   ENSG00000128039 ENSG00000128039
                                                SRD5A3
                                                          26887 -1.565301
                                                                            5.658470
  ENSG00000178343 ENSG00000178343
                                                SHISA3
                                                           4999 -1.566492
                                                                           3.644319
  ENSG00000113569 ENSG00000113569
                                                NUP155
                                                          82969 -1.568674
                                                                           5.361621
  ENSG00000153044 ENSG00000153044
                                                 CENPH
                                                          20783 -1.568678
                                                                           2.533422
   ENSG00000086991 ENSG00000086991
                                                   NOX4
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                                                                            2.046545
  ENSG00000136883 ENSG00000136883
                                                 KIF12
                                                          13166 -1.571670
                                                                            2.767915
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                                               FAM151A
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                                                                            1.436993
                                               SELENOI
  ENSG00000138018 ENSG00000138018
                                                          87338 -1.573746
                                                                           5.508538
   ENSG00000203668 ENSG00000203668
                                                   CHML
                                                          11518 -1.573916
                                                                           5.398477
  ENSG00000232434 ENSG00000232434
                                                           6323 -1.576989
                                                   AJM1
                                                                           1.888029
   ENSG00000185033 ENSG00000185033
                                                SEMA4B
                                                          69075 -1.577951
                                                                           7.030599
   ENSG00000069764 ENSG00000069764
                                                PLA2G10
                                                          21760 -1.578783
                                                                           3.531953
   ENSG00000123213 ENSG00000123213
                                                    NLN
                                                         149520 -1.583338
                                                                           4.702906
   ENSG00000261115 ENSG00000261115
                                              TMEM178B
                                                         406316 -1.585155
                                                                            1.977196
   ENSG00000170417 ENSG00000170417
                                               TMEM182
                                                         282995 -1.585317
                                                                            2.481047
   ENSG00000132436 ENSG00000132436
                                                FIGNL1
                                                          98407 -1.585702
                                                                            3.523938
  ENSG00000124813 ENSG00000124813
                                                         336192 -1.587013
                                                  RUNX2
                                                                           3.536683
   ENSG00000139880 ENSG00000139880
                                                  CDH24
                                                          10476 -1.587873
                                                                            3.140076
                                                          83006 -1.587874
  ENSG00000198088 ENSG00000198088
                                               NUP62CL
                                                                           1.244016
  ENSG00000104892 ENSG00000104892
                                                   KLC3
                                                          18086 -1.588140
                                                                           2.533676
  ENSG00000173917 ENSG00000173917
                                                 HOXB2
                                                           4215 -1.588560
                                                                           3.049117
  ENSG00000143845 ENSG00000143845
                                                          20982 -1.589736
                                                 ETNK2
                                                                            3.314283
  ENSG00000076003 ENSG00000076003
                                                          36817 -1.589848
                                                   MCM6
                                                                           5.216820
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                                                          22135 -1.589873
                                                 PRDX4
                                                                           6.456921
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                                                   UCK2
                                                          84004 -1.590596
                                                                           4.567539
  ENSG00000132329 ENSG00000132329
                                                 RAMP1
                                                          53213 -1.591557
                                                                            3.900210
   ENSG00000243509 ENSG00000243509
                                              TNFRSF6B
                                                           2032 -1.594019
                                                                           3.100374
   ENSG00000197506 ENSG00000197506
                                                SLC28A3
                                                          65301 -1.594022
                                                                            2.327855
  ENSG00000133110 ENSG00000133110
                                                          36261 -1.597107
                                                                            7.452977
                                                  POSTN
   ENSG00000259753 ENSG00000259753
                                                          90395 -1.597434
                                                                            2.867014
   ENSG00000130649 ENSG00000130649
                                                CYP2E1
                                                          40814 -1.597748
                                                                            1.547884
  ENSG00000120334 ENSG00000120334
                                                 CENPL
                                                          25333 -1.598123
                                                                            2.789177
  ENSG00000138182 ENSG00000138182
                                                KIF20B
                                                          73349 -1.599807
                                                                            3.505943
  ENSG00000140263 ENSG00000140263
                                                          54038 -1.600171
                                                   SORD
                                                                           4.543541
  ENSG00000167103 ENSG00000167103
                                               PIP5KL1
                                                           9904 -1.601125
                                                                           2.582204
  ENSG00000164466 ENSG00000164466
                                                  SFXN1
                                                          52680 -1.604628
                                                                           4.933393
## ENSG00000173137 ENSG00000173137
                                                  ADCK5
                                                          20141 -1.605181
                                                                           3.059048
```

```
## ENSG00000155792 ENSG00000155792
                                                DEPTOR 177201 -1.606566
                                                                          4.300060
  ENSG00000161267 ENSG00000161267
                                                          63540 -1.607779
                                                  BDH1
                                                                           4.019304
  ENSG00000242110 ENSG00000242110
                                                 AMACR
                                                          21939 -1.608449
                                                                           5.313321
  ENSG00000196136 ENSG00000196136
                                              SERPINA3
                                                          11671 -1.609010
                                                                           6.684014
  ENSG00000162073 ENSG00000162073
                                                 PAQR4
                                                           4214 -1.610444
                                                                           4.422533
  ENSG00000259075 ENSG00000259075
                                          POC1B-GALNT4
                                                           6854 -1.610847
                                                                           5.101249
   ENSG00000243449 ENSG00000243449
                                               C4orf48
                                                           1969 -1.610905
                                                                           1.247054
                                               CEACAM6
  ENSG00000086548 ENSG00000086548
                                                          21234 -1.612033 10.345139
  ENSG00000109881 ENSG00000109881
                                                CCDC34
                                                          32407 -1.613544
                                                                           3.125278
  ENSG00000258839 ENSG00000258839
                                                  MC1R
                                                           8854 -1.614936
                                                                           3.114489
   ENSG00000075213 ENSG00000075213
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                                                                           3.790847
  ENSG00000145545 ENSG00000145545
                                                          40959 -1.617653
                                                SRD5A1
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   ENSG00000273259 ENSG00000273259
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                                                                           6.669098
   ENSG00000198937 ENSG00000198937
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                                                                           3.486574
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                                                                           4.117135
   ENSG00000174938 ENSG00000174938
                                                SEZ6L2
                                                          28391 -1.622972
                                                                           5.860033
   ENSG00000077264 ENSG00000077264
                                                         283076 -1.624828
                                                  PAK3
                                                                           1.734596
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                                                 MY019
                                                          47799 -1.624985
                                                                           5.214996
                                                           7340 -1.630031
  ENSG00000144120 ENSG00000144120
                                               TMEM177
                                                                           2.828744
  ENSG00000166428 ENSG00000166428
                                                  PLD4
                                                          13048 -1.631748
                                                                           3.899638
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                                                   MF.T
                                                         126181 -1.636003
                                                                           7.934410
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                                                 NRCAM
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                                                 POC1A
                                                                           2.599371
  ENSG00000166508 ENSG00000166508
                                                  MCM7
                                                           9212 -1.640390
                                                                           6.635150
  ENSG00000117122 ENSG00000117122
                                                 MFAP2
                                                           6130 -1.644966
                                                                           4.511989
  ENSG00000113296 ENSG00000113296
                                                 THBS4
                                                          91976 -1.648073
                                                                           2.946677
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                                               RACGAP1
                                                          56213 -1.648103
                                                                           4.610426
   ENSG00000257594 ENSG00000257594
                                                           5384 -1.650028
                                                GALNT4
                                                                           5.106323
   ENSG00000114455 ENSG00000114455
                                                 HHLA2
                                                          81756 -1.652739
                                                                           3.694455
  ENSG00000136881 ENSG00000136881
                                                  BAAT
                                                          31218 -1.653082
                                                                           1.843507
   ENSG00000133710 ENSG00000133710
                                                SPINK5
                                                         111699 -1.653368
                                                                           4.425006
   ENSG00000133302 ENSG00000133302
                                                  SLF1
                                                         120767 -1.653608
                                                                           3.579773
  ENSG00000156738 ENSG00000156738
                                                          14906 -1.653857
                                                 MS4A1
                                                                           3.194135
   ENSG00000048462 ENSG00000048462
                                              TNFRSF17
                                                           2858 -1.655170
                                                                           1.054242
  ENSG00000152669 ENSG00000152669
                                                  CCNO
                                                           2456 -1.655353
                                                                           2.159665
  ENSG00000136928 ENSG00000136928
                                                GABBR2
                                                         420826 -1.657768
                                                                           1.369475
  ENSG00000183486 ENSG00000183486
                                                          47394 -1.658764
                                                   MX2
                                                                           5.407462
  ENSG00000117215 ENSG00000117215
                                               PLA2G2D
                                                           7627 -1.660125
                                                                           2.538527
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                                                          51681 -1.661573
                                                 IL2RA
                                                                           2.683285
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                                                          13890 -1.661625
                                          LYNX1-SLURP2
                                                                           1.556809
  ENSG00000049249 ENSG00000049249
                                               TNFRSF9
                                                          27294 -1.662267
                                                                           2.397052
  ENSG00000133962 ENSG00000133962
                                              CATSPERB
                                                         200011 -1.664955
                                                                           2.204809
  ENSG00000050344 ENSG00000050344
                                                          34939 -1.666688
                                                NFE2L3
                                                                           5.087323
   ENSG00000204681 ENSG00000204681
                                                GABBR1
                                                          78347 -1.670810
                                                                           6.053908
  ENSG00000180198 ENSG00000180198
                                                          33357 -1.671017
                                                  RCC1
                                                                           5.347353
   ENSG00000173207 ENSG00000173207
                                                 CKS1B
                                                           4598 -1.674507
                                                                           3.873388
   ENSG00000134285 ENSG00000134285
                                                FKBP11
                                                           4956 -1.674800
                                                                           4.994151
  ENSG00000261236 ENSG00000261236
                                                  BOP1
                                                          29393 -1.674908
                                                                           5 190988
  ENSG00000143630 ENSG00000143630
                                                  HCN3
                                                          12385 -1.675806
                                                                           1.997056
  ENSG00000203814 ENSG00000203814
                                                          29684 -1.677643
                                                H2BC18
                                                                           2.206070
  ENSG00000160072 ENSG00000160072
                                                ATAD3B
                                                          26083 -1.678884
                                                                           4.510528
  ENSG00000265817 ENSG00000265817
                                                  FSBP
                                                          58575 -1.683251
                                                                           2.020709
## ENSG00000152256 ENSG00000152256
                                                  PDK1
                                                          53296 -1.683360 4.362333
```

```
## ENSG00000164379 ENSG00000164379
                                                  FOXQ1
                                                           2660 -1.684285
                                                                           4.667092
  ENSG00000101412 ENSG00000101412
                                                  E2F1
                                                          10908 -1.684535
                                                                           3.975675
  ENSG00000164237 ENSG00000164237
                                                  CMBL
                                                          32027 -1.690178
                                                                           5.482579
  ENSG00000203760 ENSG00000203760
                                                  CENPW
                                                           8760 -1.690218
                                                                           2.252028
   ENSG00000013573 ENSG00000013573
                                                 DDX11
                                                          30939 -1.690692
                                                                           4.421889
  ENSG00000022556 ENSG00000022556
                                                          48012 -1.691743
                                                 NLRP2
                                                                           4.583769
   ENSG00000115758 ENSG00000115758
                                                   ODC1
                                                           8359 -1.691854
                                                                           8.045807
  ENSG00000049768 ENSG00000049768
                                                 FOXP3
                                                          14362 -1.691996
                                                                           1.881087
  ENSG00000101463 ENSG00000101463
                                               SYNDIG1
                                                         196987 -1.694317
                                                                            1.071526
  ENSG00000101825 ENSG00000101825
                                                 MXRA5
                                                          38087 -1.695109
                                                                            6.500404
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                                                 AKR1E2
                                                          61433 -1.698689
                                                                           1.394481
                                                         102087 -1.700917
  ENSG00000137261 ENSG00000137261
                                              KIAA0319
                                                                            2.922126
   ENSG00000104833 ENSG00000104833
                                                TUBB4A
                                                           8529 -1.701652
                                                                           2.163941
   ENSG00000277363 ENSG00000277363
                                                SRCIN1
                                                          75921 -1.701875
                                                                           3.073811
   ENSG00000273604 ENSG00000273604
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Task 9

Use STRING database to find PPI interactions among differentially expressed genes and export the network in TSV format.

Task 10

Import the network in R and using igraph package and identify and plot the largest connected component.