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 **GSEA**

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Karina Jhingan¹

¹Fred Hutch



Karina Jhingan

Fred Hutch

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Protocol status: Working

We use this protocol and it's working

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Abstract

This is a GSEA and GSVA analysis on time series data that creates barplots showing how the enrichment score varies over the series of time



Introduction

- 1 This pipeline is based off of <http://yulab-smu.top/biomedical-knowledge-mining-book/universal-api.html>

Load Libraries

- 2 uncomment installs if needed

```
#BiocManager::install(organism, character.only = TRUE)
#install.packages("ggribes") #for ridge plot
#install.packages("ggpubr") # for balloon plots
library(clusterProfiler)
library(msigdb)
library(enrichplot)
library(ggplot2)
library(tidyverse)
library(ggribes)
library(ggpubr)
```

Load & Tidy Data

- 3 reading in log fold change data (this was a excel sheet saved as a csv where column 1 is gene names and column 2 is the log fold change of the naive sample compared to the 24 hour time point sample (+0.1 to avoid dividing by 0)

```
df =
read_csv("/fh/fast/greenberg_p/user/kjhingan/GSEA_GSVA/24hr/24_naive_lfc_data_ranked.csv")
colnames(df)[1] = "gene"
colnames(df)[2] = "lfc"
```

- 4 Assign the log fold change data to a vector as GSEA needs the data as a vector where each value is named by gene.

```
original_gene_list <- df$lfc
```



5 Name the vector

```
names(original_gene_list) <- df$gene
```

Data should now be in this format:

```
IL2RA  IL24  GZMB  IFNG  LIF  NRN1  
  
9.294876 8.206818 8.176261 8.109863 7.973691 7.867302
```

6 Omit any NA values

```
gene_list<-na.omit(original_gene_list)
```

7 If data is not already sorted: sort the list in decreasing order (required for clusterProfiler)

```
gene_list = sort(gene_list, decreasing = TRUE)
```

8 Choose Organism

```
m_df <- msigdbbr(species = "Homo sapiens")
```

Gene set (Term to Gene)

9 in the category option you can change to either: H, C1,C2, C3...C7

```
C7_t2g <- msigdbbr(species = "Homo sapiens", category = "C7") %>%  
  dplyr::select(gs_name, gene_symbol)  
C2_t2g <- msigdbbr(species = "Homo sapiens", category = "C2") %>%  
  dplyr::select(gs_name, gene_symbol)
```

10 This code is if you want to combine gene sets and run analysis on multiple at once



```
gene_set <- rbind(C7_t2g,C2_t2g)
```

11 Custom Gene Sets (Optional):

To run custom gene sets, create a csv where the first column is the gene name and the second column is the gene symbol

Load the Gene set:

```
custom_gs <-  
read_csv("/fh/fast/greenberg_p/user/kjhingan/GSEA_GSVA/custom_gene  
_set.csv")
```

And combine with the rest of the gene sets:

```
gene_set <- rbind(gene_set,custom_gs)
```

Double check the gene sets were combined properly

```
 #(output for these two following lines should be equal)  
 nrow(gene_set)  
 nrow(C7_t2g) + nrow(C5_t2g) + nrow(C2_t2g) + nrow(H_t2g) +  
 nrow(custom_gs)
```

GSEA

12 GSEA

```
gse <- GSEA(gene_list, TERM2GENE = gene_set)
```

13 Filtering Results, adjust p.adjust as necessary

```
gse_filtered <- filter(gse, p.adjust <= 0.05)
```

14 Save dataframe

```
write.csv(gse,  
"Fred_Hutch_R/24_data/log2_24_naive_R_GSEA_C7_custom_results.csv",  
row.names=FALSE)
```

Visualizations (Optional)

15 **Dot Plot**

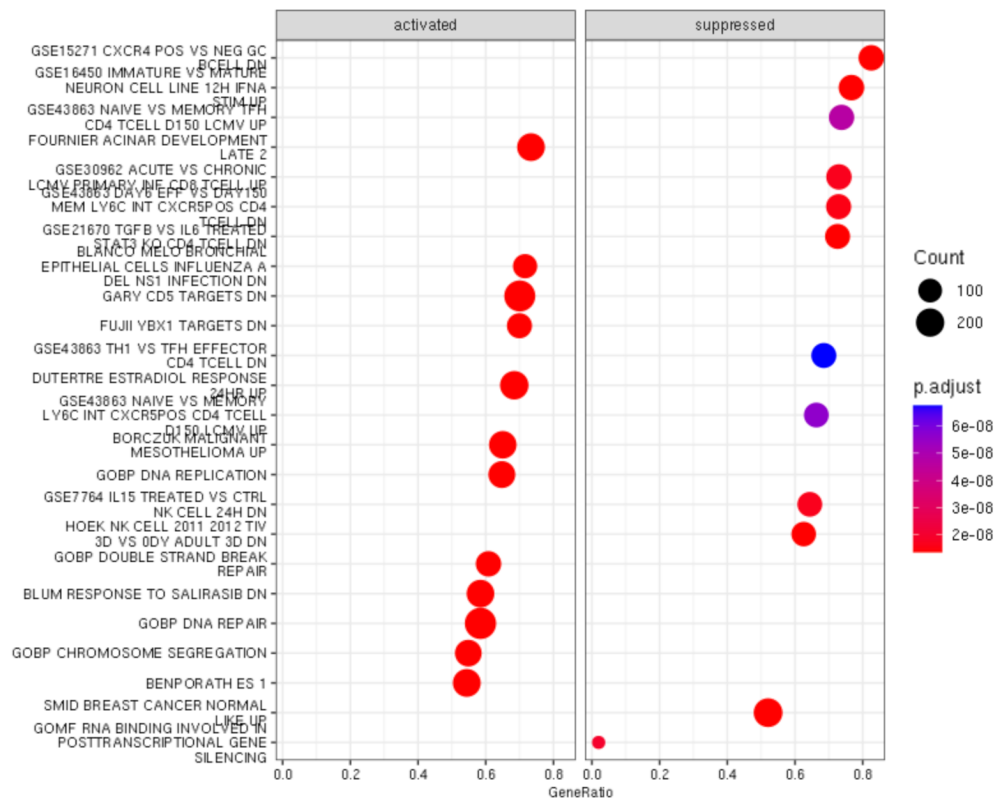
All example visual images are from the 24 hour data.

Documentation:

<https://www.rdocumentation.org/packages/enrichplot/versions/1.13.1.994/topics/dotplot>

adjust showCategory depending on how many categories you want to appear in your dotplot

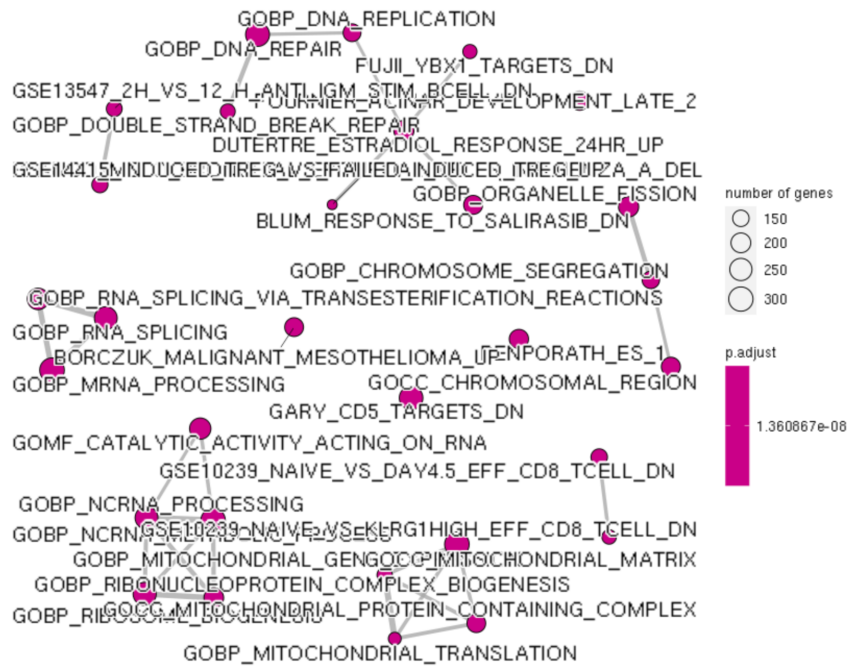
```
require(DOSE)  
dotplot(gse, showCategory=20, split=".sign", font.size = 8) +  
facet_grid(.~.sign)
```



16 Enrichment Map

use pariwise_termsim to quiet errors

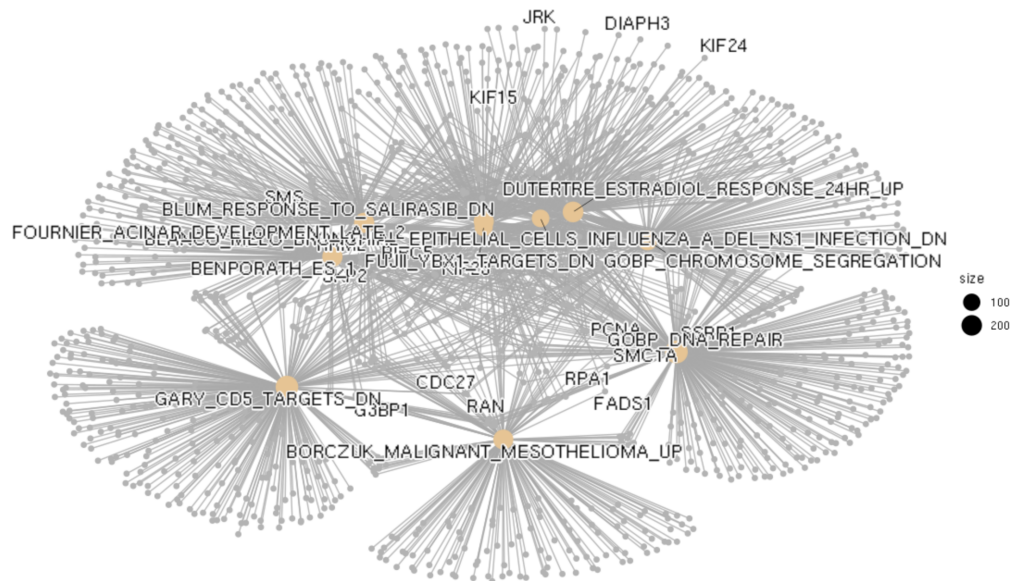
```
gse_emap_data <- pairwise_termsim(gse)
emapplot(gse_emap_data, showCategory = 50, edge.params = list(min = 0.2), cex.params = list(category_label = 0.5))
```



17 Category Netplot

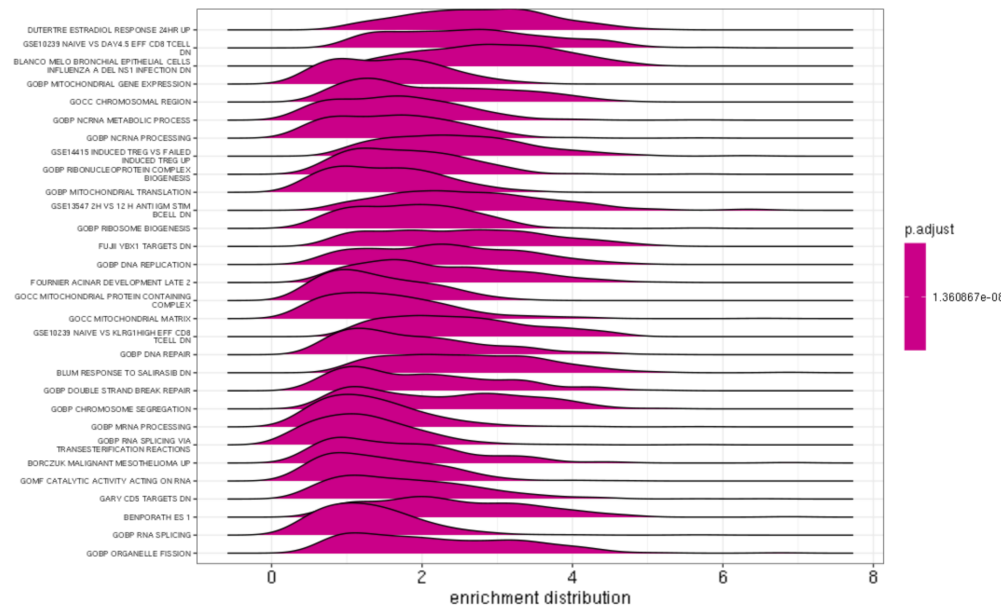
categorySize can be either 'pvalue' or 'geneNum'

```
cnetplot(gse, categorySize="pvalue", color.params =  
list(foldChange = gene_list), showCategory = 10)
```



18 Ridge Plot

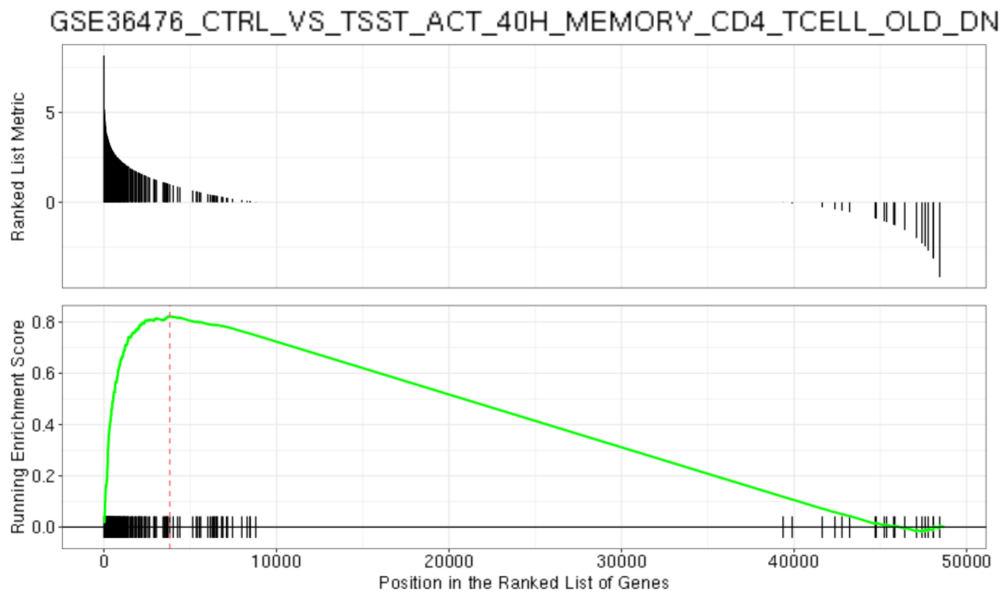
```
ridgeplot(gse, label_format = 40) +  
  labs(x = "enrichment distribution") +  
  theme(axis.text.y = element_text(size = 4))
```



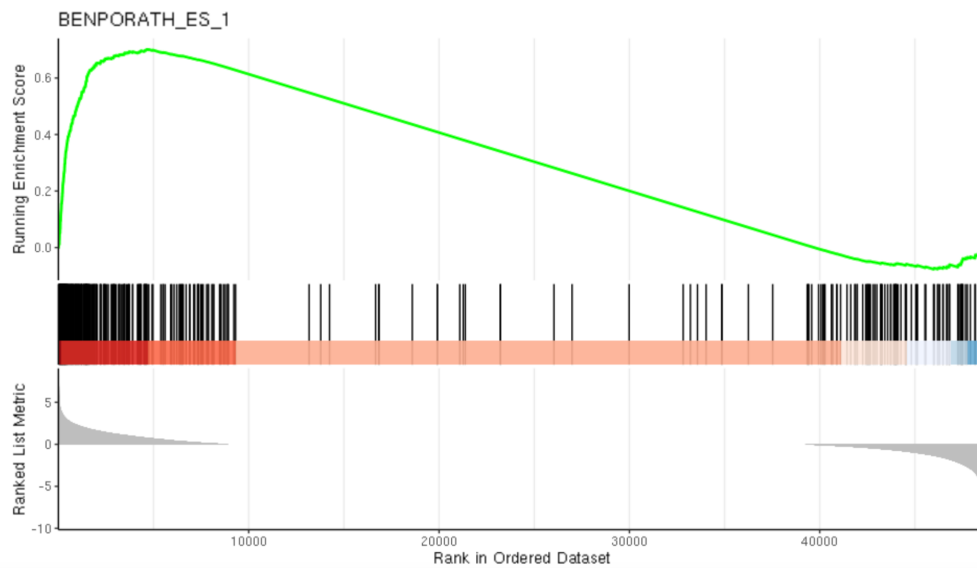
19 GSEA Plot

get geneSetID number from Excel sheet of output of gse (-1 for header) (i.e Hallmark, C7,etc) to input for
index for description and geneSetID

```
gseaplot(gse, by = "all", title = gse$Description[87], geneSetID = 87)
```

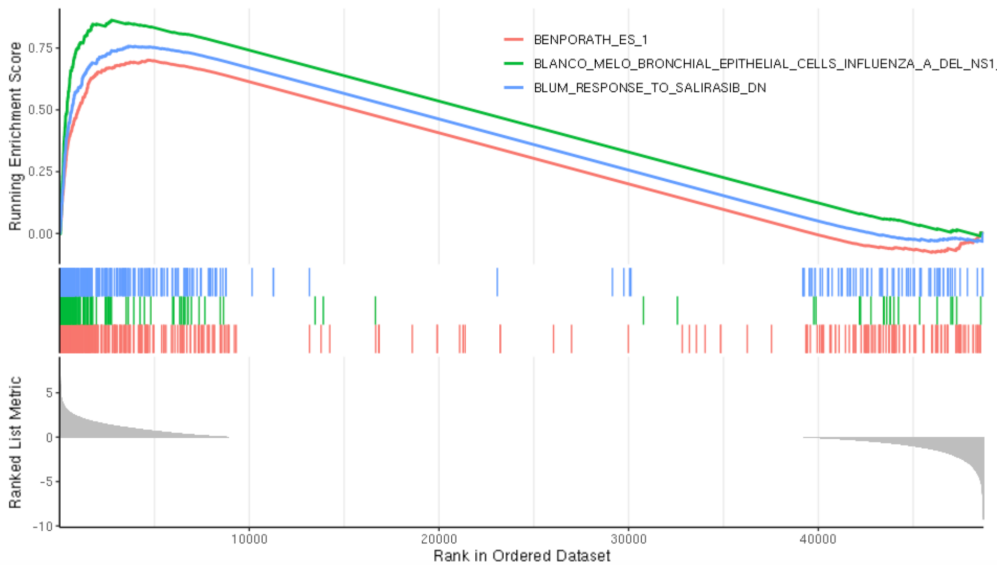


```
gseaplot2(gse, geneSetID = 1, title = gse$Description[1])
```



If you want to plot multiple gsea plots at a time adjust the number in geneSetID.

```
gseaplot2(gse, geneSetID = 1:3)
```



GSVA

- 20 Complete the above steps for all timepoints compared to the control, in our experiment we had naive 0 (naive), 24, 48, and 72 hour as timepoints, and used naive as the control time point so I ran the above section 3 times (naive vs 24, naive vs 48, naive vs 72).

```
results_24 <-  
read_csv("Fred_Hutch_R/24_data/log2_24_naive_R_GSEA_C7_custom_results.csv") %>%  
  #add column for time  
  mutate(time = "24") %>%  
  #select necessary column for visuals  
  select(ID, NES, time)  
  
results_48 <-  
read_csv("Fred_Hutch_R/48_data/log2_48_naive_R_GSEA_C7_custom_results.csv") %>%  
  #add column for time  
  mutate(time = "48") %>%  
  #select necessary column for visuals  
  select(ID, NES, time)  
  
results_72 <-  
read_csv("Fred_Hutch_R/72_data/log2_72_naive_R_GSEA_C7_custom_results.csv") %>%  
  #add column for time  
  mutate(time = "72") %>%  
  #select necessary column for visuals  
  select(ID, NES, time)
```

21 Top 10 genes (up and down regulated)

Sort by ascending order

```
asc_results_24 <- results_24[order(results_24$NES, decreasing =  
FALSE),]  
asc_results_48 <- results_48[order(results_48$NES, decreasing =  
FALSE),]  
asc_results_72 <- results_72[order(results_72$NES, decreasing =  
FALSE),]
```

Get top 10 down-regulated

```
top10down_24 <- slice(asc_results_24, 1:10) %>%  
  #add column for time  
  mutate(time = "24") %>%  
  #select necessary column for visuals  
  select(ID,NES,time)  
top10down_48 <- slice(asc_results_48, 1:10) %>%  
  mutate(time = "48") %>%  
  select(ID,NES,time)  
top10down_72 <- slice(asc_results_72, 1:10) %>%  
  mutate(time = "48") %>%  
  select(ID,NES,time)
```

22 Sort by descending order

```
desc_results_24 <- results_24[order(results_24$NES,decreasing =  
TRUE),]  
desc_results_48 <- results_48[order(results_72$NES,decreasing =  
TRUE),]  
desc_results_72 <- results_72[order(results_72$NES,decreasing =  
TRUE),]
```

Get top 10 up-regulated genes

```
top10up_24 <- slice(desc_results_24, 1:10)  
top10up_48 <- slice(desc_results_48, 1:10)  
top10up_72 <- slice(desc_results_72, 1:10)
```

23 Combine to get top 10 up and down regulated gene in 1 file

```
topdown <- rbind(top10down_24,top10down_48,top10down_72)  
topup <- rbind(top10up_24,top10up_48,top10up_72)  
top <- rbind(topdown,topup)  
head(top)  
#save as csv  
write.csv(top,  
"/fh/fast/greenberg_p/user/kjhingan/GSEA_GSVA/GSVAtop.csv",  
row.names=FALSE)
```



```
ID                                     NES time
<chr>                                <dbl> <chr>
1 GSE21670_TGFB_VS_IL6_TREATED_STAT3_KO_CD4_TCELL_DN -2.01 24
2 GSE15271_CXCR4_POS_VS_NEG_GC_BCELL_DN -2.00 24
3 GSE16450_IMMATURE_VS_MATURE_NEURON_CELL_LINE_12H_IFNA_STIM_UP -1.95 24
4 GOMF_RNA_BINDING_INVOLVED_IN_POSTTRANSCRIPTIONAL_GENE_SILENCING -1.93 24
5 HOEK_NK_CELL_2011_2012_TIV_3D_VS_0DY_ADULT_3D_DN -1.90 24
6 GSE30962_ACUTE_VS_CHRONIC_LCMV_PRIMARY_INF_CD8_TCELL_UP -1.90 24
```

24 Bar plot

Do this for each of the following 20 genes in the top 10 list: (I changed fill color to red brick for the downregulated genes)

Here we are finding the data row for each of the given 20 genes and creating an individual bar plot for each gene

```
gene <- "GSE15930_NAIVE_VS_48H_IN_VITRO_STIM_IL12_CD8_TCELL_DN"
data24_1 <- filter(results_24, ID==gene)
data48_1 <- filter(results_48, ID==gene)
data72_1 <- filter(results_72, ID==gene)
data_1 <- rbind(data24_1, data48_1, data72_1)
head(data_1)
```

```
# A tibble: 3 × 3
ID                                     NES time
<chr>                                <dbl> <chr>
1 GSE15930_NAIVE_VS_48H_IN_VITRO_STIM_IL12_CD8_TCELL_DN 2.11 24
2 GSE15930_NAIVE_VS_48H_IN_VITRO_STIM_IL12_CD8_TCELL_DN 2.11 48
3 GSE15930_NAIVE_VS_48H_IN_VITRO_STIM_IL12_CD8_TCELL_DN 2.07 72
```

```
barplot <- ggplot(data_1,aes(x=time, y=NES))+  
  geom_bar(stat="identity", fill = "forestgreen") +  
  ggtitle(gene)  
barplot  
  
# replace the file name with a name relevant to the gene (DN at  
the end of the file name represents down regulated and UP  
represents up-regulated)  
ggsave(  
  plot = barplot,  
  file.path("/fh/fast/greenberg_p/user/kjhingan/GSEA_GSVA/barplots/G  
SE15930_NAIVE_VS_48H_IN_VITRO_STIM_IL12_CD8_TCELL_DN.png")  
)
```

