

# GF-PAT-Nanostring

*Thomas W. Battaglia*

## 1. Introduction

This is an Rmarkdown document which contains the R code for the Nanostring RNA-array analysis found within the manuscript “Ruiz et al. (2017)”. Details about the tools and steps for processing the ileal transcriptome dataset can be found within the ‘**Methods**’ section of the respective manuscript. The files used to generate this data set is hosted on GEO under the accession number GSE98291. This workflow will use a saved RDA file from the analysis instead of hosting the raw data.

### 1a. Install and load the necessary libraries

```
source("scripts/geneExpress.R")
library(Biobase)
library(DESeq)
library(ggplot2)
library(dplyr)
library(RColorBrewer)
library(stats)
library(ggbiplot)
library(pheatmap)
library(org.Mm.eg.db)
```

### 1b. Import the eSet object containing the counts and metadata

```
load("data/nanostring_eset.rda")
```

---

Compare Control-SPF vs. PAT-SPF vs. Control-GF vs. PAT-GF at day 52 of life

```
# Run significance testing function
## Output is an ExpressionSet, data.frame and significant-genes only data.frame
gfPAT_results <- gene_express(expressionObject = gfpat_eset)

## Performing Significance Tests...
```

Figure 3g. Heatmap of significant genes between 3 groups

```
# Subset genes by custom significance and gather gene names
gf_results_filtered = subset(gfPAT_results$sig_results, PATSPF_ControlSPF_padj < 0.05 | ControlSPF_ControlGF_padj < 0.05)
row.names()

# Subset output expression object by only the significant genes
## make a matrix of values across all samples
mat <- gfPAT_results$sig_expression[gf_results_filtered] %>%
  exprs()
```

```

# Choose which column variables you want to annotate the columns by.
annotation_col = data.frame(
  Group = factor(gfPAT_results$sig_expression$Group),
  row.names = colnames(gfPAT_results$sig_expression)
)

# Specify colors you want to annotate the columns by.
ann_colors = list(
  Group = c(ControlSPF = "black",
    PATSPF = "red",
    ControlGF = "orange",
    PATGF = "grey"))

# Plot heatmap with annotations
pheatmap(mat = mat,
  color = colorRampPalette(c("#0000ff", "#000000", "#ffff00"))(n = 100),
  scale = "row",
  show_rownames = F,
  annotation_col = annotation_col,
  annotation_colors = ann_colors,
  #filename = "Figure3g.pdf",
  fontsize = 6.5,
  cellwidth = 20,
  show_colnames = F)

```

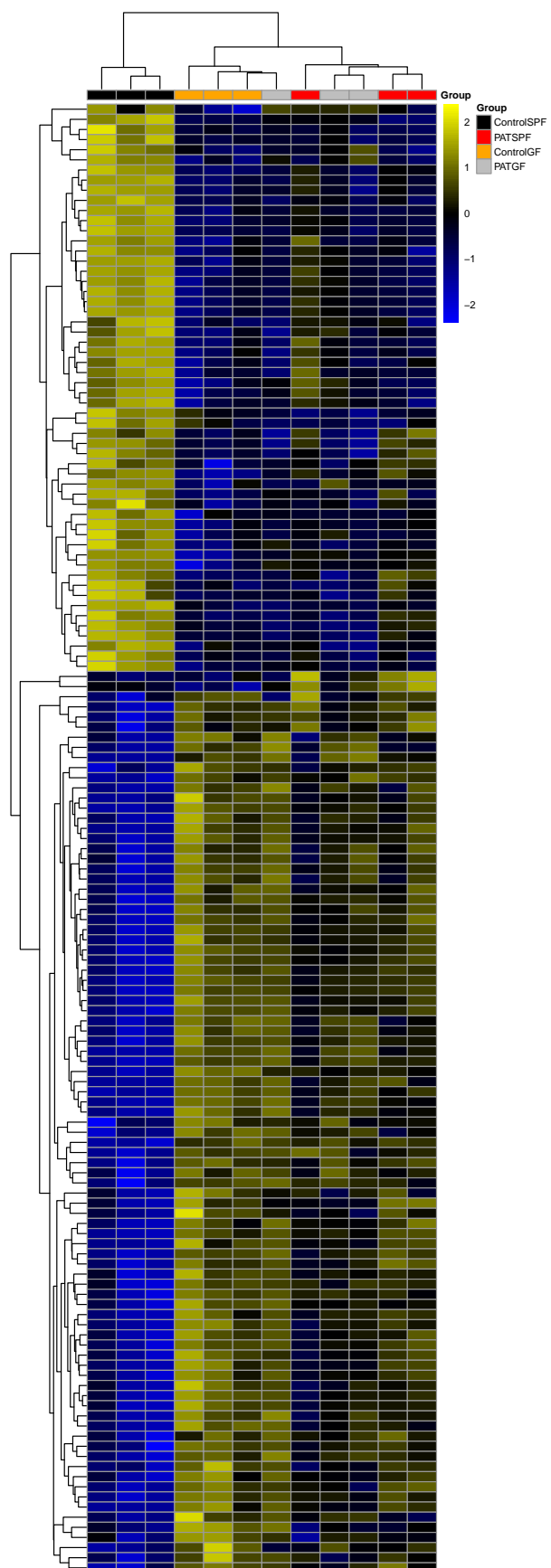
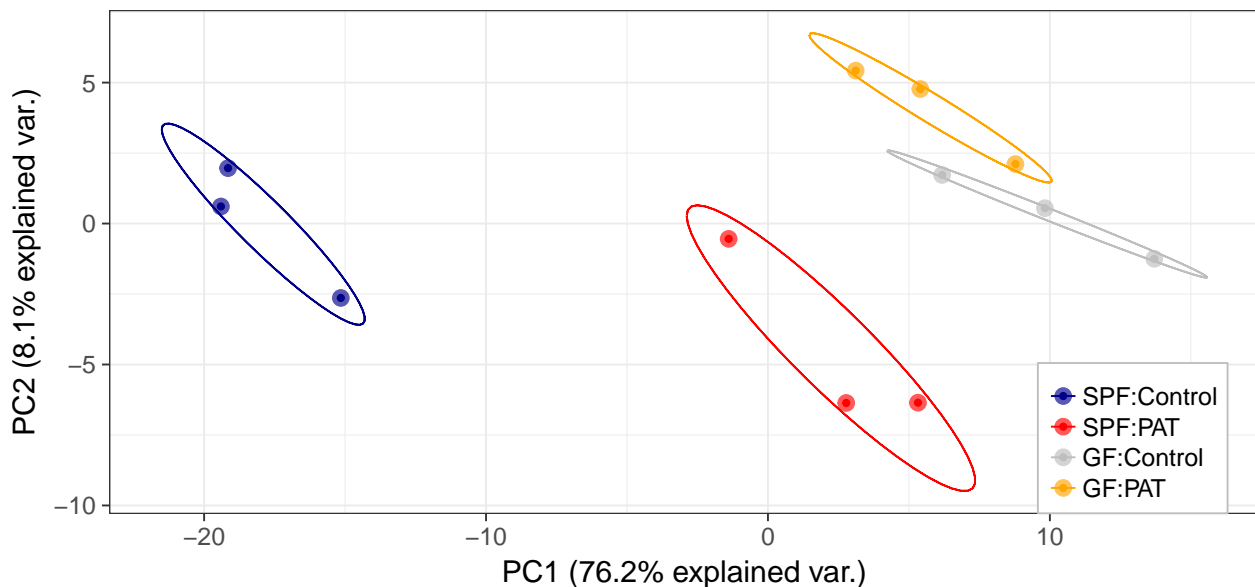


Figure 3f. PCoA Biplot for all 3 groups

```
# Generate Principal Components Analysis data for normalized-log2 values
pca <- gfPAT_results$sig_expression %>%
  exprs() %>%
  t() %>%
  prcomp(scale. = T)

# Use ggbiplot to create a biplot
ggbiplot(pca,
  obs.scale = 1,
  var.scale = 1,
  groups = as.vector(gfPAT_results$sig_expression$Group),
  ellipse = TRUE,
  circle = TRUE,
  var.axes = F) +
  scale_color_manual(breaks = c("ControlSPF", "PATSPF", "ControlGF", "PATGF"),
    labels = c("SPF:Control", "SPF:PAT", "GF:Control", "GF:PAT"),
    values = c("ControlSPF" = "darkblue",
      "PATSPF" = "red",
      "ControlGF" = "grey",
      "PATGF" = "orange")) +
  theme_bw(base_size = 17) +
  geom_point(aes(color = factor(gfPAT_results$sig_expression$Group)), size = 4, alpha = 0.65) +
  theme(legend.title = element_blank(), legend.position = c(0.89, 0.15),
    legend.background = element_rect(size = 0.5, linetype = "solid", colour = "gray"))
```



```
sessionInfo()
```

```
## R version 3.3.1 (2016-06-21)
## Platform: x86_64-apple-darwin13.4.0 (64-bit)
## Running under: OS X 10.10.5 (Yosemite)
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
```

```

##
## attached base packages:
## [1] stats4      grid        parallel    stats      graphics  grDevices  utils
## [8] datasets    methods     base
##
## other attached packages:
## [1] org.Mm.eg.db_3.4.0      AnnotationDbi_1.36.2  IRanges_2.8.1
## [4] S4Vectors_0.12.1       pheatmap_1.0.8        ggbiplot_0.55
## [7] scales_0.4.1           plyr_1.8.4            RColorBrewer_1.1-2
## [10] dplyr_0.5.0            ggplot2_2.2.1         DESeq_1.26.0
## [13] lattice_0.20-35        locfit_1.5-9.1        Biobase_2.34.0
## [16] BiocGenerics_0.20.0
##
## loaded via a namespace (and not attached):
## [1] Rcpp_0.12.10           bitops_1.0-6          tools_3.3.1
## [4] digest_0.6.12          tibble_1.3.0          annotate_1.52.1
## [7] evaluate_0.10          RSQLite_1.1-2         memoise_1.1.0
## [10] gtable_0.2.0           Matrix_1.2-8          DBI_0.6-1
## [13] yaml_2.1.14            genefilter_1.56.0     stringr_1.2.0
## [16] knitr_1.15.1           rprojroot_1.2         R6_2.2.0
## [19] XML_3.98-1.6           survival_2.41-3       rmarkdown_1.5
## [22] geneplotter_1.52.0     magrittr_1.5          codetools_0.2-15
## [25] backports_1.0.5        htmltools_0.3.5       splines_3.3.1
## [28] assertthat_0.2.0       colorspace_1.3-2      xtable_1.8-2
## [31] labeling_0.3           stringi_1.1.5         lazyeval_0.2.0
## [34] RCurl_1.95-4.8         munsell_0.4.3

```