

PulsePAT-Nanostring

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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 GSE98022. 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)
```

```
## Warning: package 'lattice' was built under R version 3.3.2
```

```
library(ggplot2)
```

```
## Warning: package 'ggplot2' was built under R version 3.3.2
```

```
library(dplyr)
library(RColorBrewer)
library(stats)
library(ggplot2)
library(pheatmap)
library(RColorBrewer)
library(org.Mm.eg.db)
```

```
## Warning: package 'AnnotationDbi' was built under R version 3.3.2
```

```
## Warning: package 'S4Vectors' was built under R version 3.3.2
```

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

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

Compare Control vs. PAT1 vs. PAT3 at day 52 of life

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

## Performing Significance Tests...
```

Figure 2a. Heatmap of significant genes between 3 groups

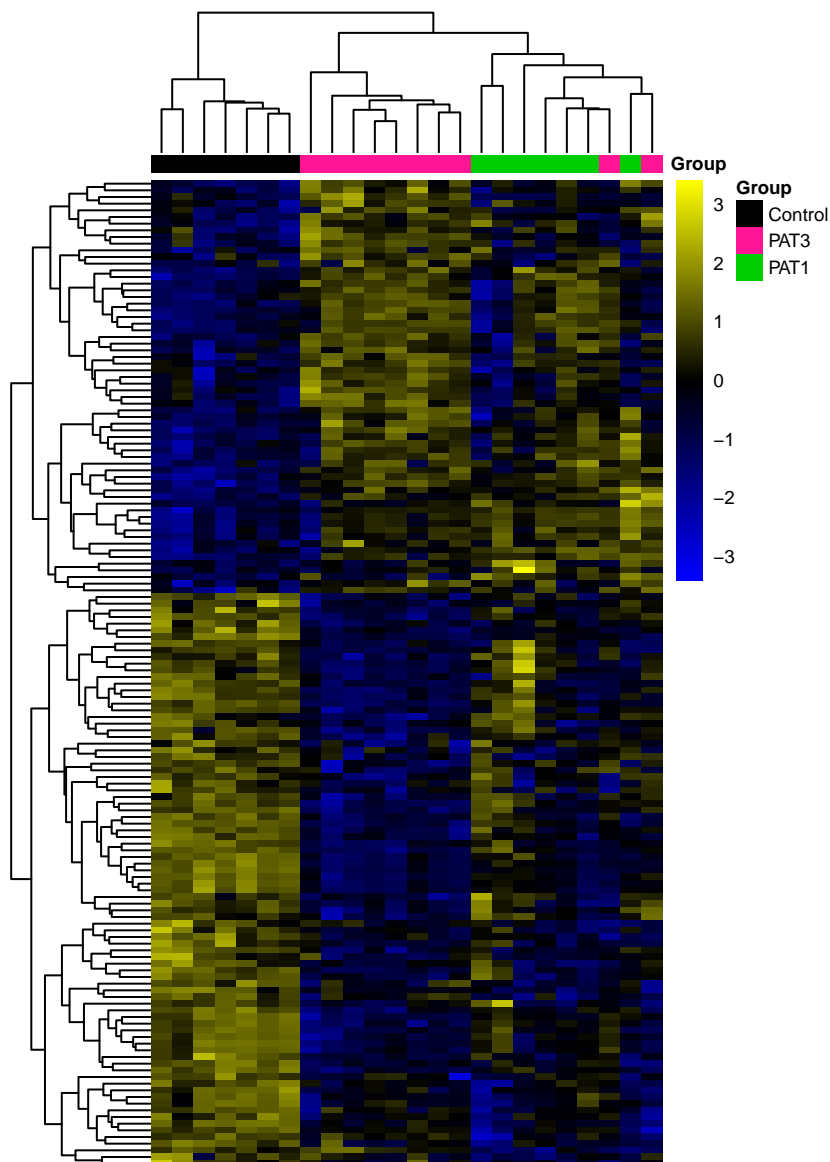
```
# Subset genes by custom significance and gather gene names
pulsePAT_results_filtered = subset(pulsePAT_results$sig_results,
                                   PAT1_Control_padj < 0.05 | PAT3_Control_padj < 0.05) %>%
  row.names()

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

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

# Specify colors you want to annotate the columns by.
ann_colors = list(
  Group = c(Control = "black", PAT3 = "deeppink", PAT1 = "green3")
)

# Plot heatmap with annotations
pheatmap(mat = mat,
          color = colorRampPalette(c("#0000ff", "#000000", "#ffff00"))(n = 255),
          scale = "row",
          show_rownames = F,
          annotation_col = annotation_col,
          annotation_colors = ann_colors,
          fontsize = 6.5,
          cellwidth = 8,
          cellheight = 2.5,
          show_colnames = F)
```



```
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      parallel    stats       graphics    grDevices   utils       datasets
## [8] 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        RColorBrewer_1.1-2
## [7] dplyr_0.5.0            ggplot2_2.2.1         DESeq_1.26.0
```

```

## [10] lattice_0.20-35      locfit_1.5-9.1      Biobase_2.34.0
## [13] BiocGenerics_0.20.0
##
## loaded via a namespace (and not attached):
## [1] Rcpp_0.12.10      plyr_1.8.4      bitops_1.0-6
## [4] tools_3.3.1      digest_0.6.12   tibble_1.3.0
## [7] annotate_1.52.1   evaluate_0.10   RSQLite_1.1-2
## [10] memoise_1.1.0    gtable_0.2.0    Matrix_1.2-8
## [13] DBI_0.6-1        yaml_2.1.14     genefilter_1.56.0
## [16] stringr_1.2.0    knitr_1.15.1    rprojroot_1.2
## [19] grid_3.3.1       R6_2.2.0        XML_3.98-1.6
## [22] survival_2.41-3  rmarkdown_1.5   geneplotter_1.52.0
## [25] magrittr_1.5     codetools_0.2-15 backports_1.0.5
## [28] scales_0.4.1     htmltools_0.3.5 splines_3.3.1
## [31] assertthat_0.2.0 colorspace_1.3-2 xtable_1.8-2
## [34] stringi_1.1.5    lazyeval_0.2.0  RCurl_1.95-4.8
## [37] munsell_0.4.3

```