PulsePAT-Nanostring

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Contents

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

```r
# 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 expresion 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)
```
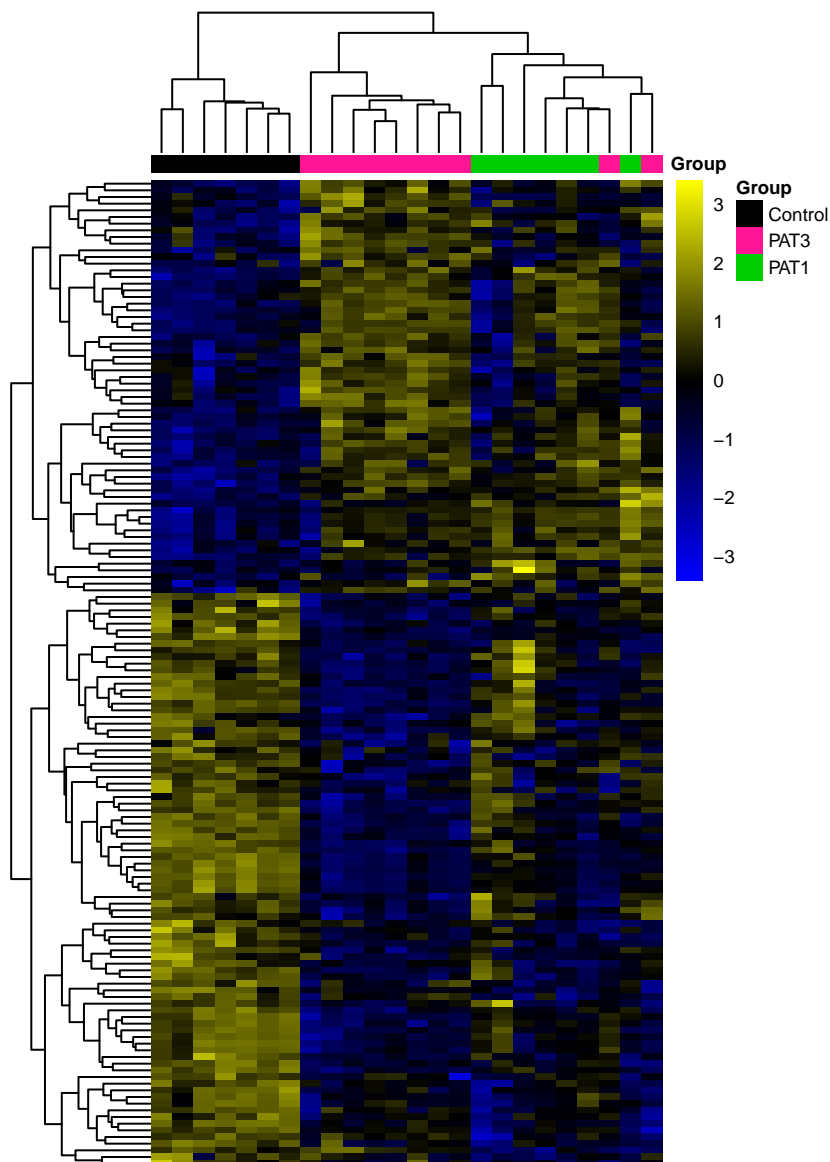
```r
sessionInfo()
```

```
## 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
```