

# Delta Cell Receptor Heatmap

## Introduction

This report will explain the steps used to make a heatmap produce of average  $\log_2(\text{CPM})$  expression of selected delta cell receptor genes across all non-diabetic single cell samples. All non-diabetic samples were included in the heat map excluding “none” and “multiple” classified samples.

```
# Load in libraries
suppressPackageStartupMessages(library(Biobase))
suppressPackageStartupMessages(library(pheatmap))
suppressPackageStartupMessages(library(RColorBrewer))
suppressPackageStartupMessages(library(edgeR))

## Warning: package 'limma' was built under R version 3.3.1

suppressPackageStartupMessages(library(gplots))
suppressPackageStartupMessages(library(ggplot2))
library(Biobase)
library(pheatmap)
library(gplots)
library(ggplot2)
library(edgeR)
library(RColorBrewer)
rm(list=ls())

# Load in data
setwd("/Users/lawlon/Documents/Final_RNA_Seq_3/Data/")
load("nonT2D.rdata")
s.anns <- pData(cnts.eset)
p.anns <- as(featureData(cnts.eset), "data.frame")
counts <- exprs(cnts.eset)
# Calculate the cpm of the data
cpms <- cpm(x = counts)
data <- log2(cpms+1)

# Load in bulk data
load("/Users/lawlon/Documents/Final_RNA_Seq/islet_bulk_uniq_data.rdata")
bulk.cnts <- exprs(bulk.cnts)
bulk.anns <- pData(bulk.cnts)
# Get bulk intact, ND samples
intact <- which(bulk.anns$Type == "Intact" & bulk.anns$Phenotype == "ND")
# Calculate cpm
bulk.cpm <- cpm(x = bulk.cnts)
bulk.log <- log2(bulk.cpm+1)
bulk.sel <- bulk.log[, intact]
bulk.avg <- rowMeans(bulk.sel)

# Get cell types in order
s.1 <- s.anns[s.anns$cell.type %in% c("INS"),]
s.2 <- s.anns[s.anns$cell.type %in% c("GCG"),]
s.3 <- s.anns[s.anns$cell.type %in% c("SST"),]
s.4 <- s.anns[s.anns$cell.type %in% c("PPY"),]
s.5 <- s.anns[s.anns$cell.type %in% c("PRSS1"),]
```



```

s.6 <- s.anns[s.anns$cell.type %in% c("KRT19"),]
s.7 <- s.anns[s.anns$cell.type %in% c("COL1A1"),]

# Get Expression matrices and average mean expression
f.1 <- data[, rownames(s.1)]
avg1 <- rowMeans(f.1)
f.2 <- data[, rownames(s.2)]
avg2 <- rowMeans(f.2)
f.3 <- data[, rownames(s.3)]
avg3 <- rowMeans(f.3)
f.4 <- data[, rownames(s.4)]
avg4 <- rowMeans(f.4)
f.5 <- data[, rownames(s.5)]
avg5 <- rowMeans(f.5)
f.6 <- data[, rownames(s.6)]
avg6 <- rowMeans(f.6)
f.7 <- data[, rownames(s.7)]
avg7 <- rowMeans(f.7)

# Match up cell type with hormone marker
namelist <- c(INS="Beta", GCG="Alpha", SST="Delta", PPY="Gamma",
              GHRL="Epsilon", COL1A1="Stellate", PRSS1="Acinar", KRT19="Ductal", none="None")

# Combine all cell expression data into one matrix
mat.orig <- cbind(f.1, f.2, f.3, f.4, f.5, f.6, f.7)
mat.avg <- cbind(avg1, avg2, avg3, avg4, avg5, avg6, avg7, bulk.avg)
colnames(mat.avg) <- c("Beta", "Alpha", "Delta", "Gamma", "Acinar", "Ductal", "Stellate", "Bulk")
# Transpose avg matrix
trans.mat <- t(mat.avg)
# Change colnames of mat to gene symbol
colnames(trans.mat) <- p.anns$Associated.Gene.Name

# Specify genes of interest
genes.sel <- c("LEPR", "GHSR", "DRD2", "HHEX")

genes.ids <- NULL
for (i in 1:length(genes.sel)) {
  idx <- which(colnames(trans.mat) == genes.sel[i])
  genes.ids <- c(genes.ids, idx)
}

# Extract genes of interest
mat.sel <- trans.mat[, genes.ids]

# Mean center the data
# Mean center by column (gene)
center_apply <- function(x) {
  apply(x, 2, function(y) y - mean(y))
}

mat.center <- center_apply(mat.sel)

# Scale the data between -1 and 1

```



```

nor.min.max <- function(x) {
  if (is.numeric(x) == FALSE) {
    stop("Please input numeric for x")
  }
  x.min <- min(x)
  x.max <- max(x)
  x <- 2*((x - x.min) / (x.max - x.min)) - 1
  return (x)
}

# Only use if want to scale data
mat.scale <- t(apply(mat.center, 1, nor.min.max))

# Annotation matrix
annotation_col = data.frame(Cell_Type = colnames(mat.scale))
rownames(annotation_col) <- colnames(mat.scale)

# Specify cell type colors
grey <- brewer.pal(n=9, name="Greys")

# change column names
rownames(mat.sel) <- c("Beta", "Alpha", "Delta",
                      "Gamma", "Acinar", "Ductal", "Stellate", "Bulk")

# transpose again
mat.sel <- t(mat.sel)

```



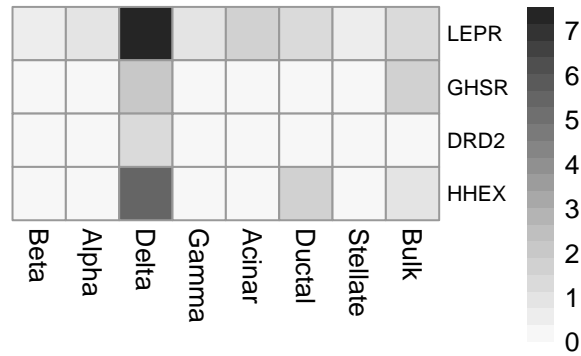


Figure 1: Heat map of average log<sub>2</sub>(CPM) expression of delta cell receptor genes across non-diabetic endocrine single cell samples.

```
# Make heatmap
pheatmap(mat = mat.sel, cluster_rows = FALSE, cluster_cols = FALSE,
          color = colorRampPalette(brewer.pal(n = 7, name = "Greys"))(20),
          clustering_distance_rows = NULL,
          clustering_method=NULL, show_rownames=TRUE,
          show_colnames = TRUE, annotation_names_row = TRUE,
          annotation_names_col = FALSE, trace = "none", fontsize_row = 8,
          cellwidth = 20, cellheight = 20, annotation_legend = FALSE)
```

## Session Information

```
# Load libraries
suppressPackageStartupMessages(library(Biobase))
suppressPackageStartupMessages(library(pheatmap))
suppressPackageStartupMessages(library(RColorBrewer))
suppressPackageStartupMessages(library(edgeR))
suppressPackageStartupMessages(library(gplots))
suppressPackageStartupMessages(library(ggplot2))
library(Biobase)
library(pheatmap)
library(gplots)
library(ggplot2)
library(edgeR)
library(RColorBrewer)
sessionInfo()

## R version 3.3.0 (2016-05-03)
## Platform: x86_64-apple-darwin13.4.0 (64-bit)
## Running under: OS X 10.11.6 (El Capitan)
##
## 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] parallel stats graphics grDevices utils datasets methods
## [8] base
##
```



```

## other attached packages:
## [1] ggplot2_2.1.0      gplots_3.0.1      edgeR_3.14.0
## [4] limma_3.28.21      RColorBrewer_1.1-2  pheatmap_1.0.8
## [7] Biobase_2.32.0      BiocGenerics_0.18.0
##
## loaded via a namespace (and not attached):
## [1] Rcpp_0.12.7      knitr_1.14      magrittr_1.5
## [4] munsell_0.4.3    colorspace_1.2-7  stringr_1.1.0
## [7] plyr_1.8.4      caTools_1.17.1   tools_3.3.0
## [10] grid_3.3.0      gtable_0.2.0     KernSmooth_2.23-15
## [13] htmltools_0.3.5  gtools_3.5.0     yaml_2.1.13
## [16] assertthat_0.1   digest_0.6.10    tibble_1.2
## [19] formatR_1.4      bitops_1.0-6     evaluate_0.10
## [22] rmarkdown_1.1    gdata_2.17.0     stringi_1.1.2
## [25] scales_0.4.0

```