

# 3-Dimensional t-SNE Analysis of Type 2 diabetic Single Cell Ensemble Transcriptomes

## Introduction

This file will detail the steps used to perform unsupervised t-SNE analysis on the Type 2 diabetic single cell transcriptomes. The data was reduced to three dimensions in order to produce a 3D gif animation.

## t-SNE Analysis

```
suppressPackageStartupMessages(library(Biobase))
suppressPackageStartupMessages(library(edgeR))
suppressPackageStartupMessages(library(Rtsne))
suppressPackageStartupMessages(library(scatterplot3d))
suppressPackageStartupMessages(library(rgl))
suppressPackageStartupMessages(library(pca3d))
suppressPackageStartupMessages(library(RColorBrewer))
library(Biobase)
library(edgeR)
library(Rtsne)
library(scatterplot3d)
library(rgl)
library(pca3d)
library(RColorBrewer)
rm(list = ls())
set.seed(125342)
# Load in Single Cell RNA-seq data
setwd("/Users/lawlon/Documents/Final_RNA_Seq_3/Data/")
load("T2D.rdata")
# File name
name = "T2D.log2.cpm"
# Probe annotation data
p.anns <- as(featureData(cnts.eset), "data.frame")
# Sample annotation data
s.anns <- pData(cnts.eset)
# Remove multiples and keep all other groups
s.anns.sel <- s.anns[s.anns$cell.type %in% c("INS", "PPY", "GCG", "SST",
      "COL1A1", "KRT19", "PRSS1", "none"),]
# Expression data
counts <- exprs(cnts.eset)
# Calculate cpm of data
cpm <- cpm(x = counts)
cpm.vals <- log2(cpm+1)
cpm.vals <- cpm.vals[,rownames(s.anns.sel)]
r.max <- apply(cpm.vals,1,max)
# Use highly expressed genes
cpm.sel <- cpm.vals[r.max > 10.5,]
# Transpose the matrix
cpm1 <- t(cpm.sel)
# Remove groups that are all zeros
```

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df <- cpm1[,apply(cpm1, 2, var, na.rm=TRUE) != 0]
#Run tsne with defaults
rtsne_out <- Rtsne(as.matrix(df), dims = 3)
# Set rownames of matrix to tsne matrix
rownames(rtsne_out$Y) <- rownames(cpm1)
# Write tsne matrix to file
write.csv(rtsne_out$Y, file = paste(name, "tsne.matrix.data.3D.csv", sep="."))
# Color Schema
grey <- brewer.pal(n=9, name="Greys")
colorCodes <- c(INS="#e41a1c", GCG = "#377eb8", SST = "#4daf4a", PPY = "#984ea3",
  COL1A1 = grey[9], PRSS1 = grey[7], KRT19 = grey[5],
  none = grey[3])

namelist <- c("Beta", "Alpha", "Delta", "Gamma",
  "Stellate", "Acinar", "Ductal", "none")
# Make variable of endocrine vs exocrine for 3D plot
type = NULL
for (i in 1:length(s.anns.sel$cell.type)){
  if ((s.anns.sel$cell.type[i] %in% c("INS", "GCG", "SST", "PPY")) == TRUE) {
    idx = "sphere"
    type = c(type, idx)
  } else {
    idx = "tetrahedron"
    type = c(type, idx)
  }
}
# Match up cell type name with hormone type
cellnames = NULL
for (i in 1:length(s.anns.sel$cell.type)) {
  if (s.anns.sel$cell.type[i] %in% names(namelist) == TRUE) {
    cellnames = c(cellnames, as.character(namelist[s.anns.sel$cell.type[i]]))
  }
}
# Match up colors and hormone labels
cols = NULL
for (i in 1:length(s.anns.sel$cell.type)) {
  if ((s.anns.sel$cell.type[i] %in% names(colorCodes)) == TRUE) {
    cols <- c(cols, colorCodes[s.anns.sel$cell.type[i]])
  }
}
# Match up cell name with hormone name
# Have cell type name and color
for (i in 1:length(cols)) {
  if (names(cols)[i] %in% names(namelist) == TRUE) {
    names(cols)[i] <- namelist[names(cols)[i]]
  }
}
# Make movie
pca3d(rtsne_out$Y, components = c(1:3), col = cols, title=name,
  legend = TRUE, new = TRUE, shape = type,
  axe.titles = c("t-SNE 1", "t-SNE 2", "t-SNE 3"))
legend3d("topright", c("Beta (INS)", "Alpha (GCG)",
  "Delta (SST)", "Gamma (PPY)",

```

```

        "Stellate (COL1A1)", "Acinar (PRSS1)", "Ductal (KRT19)", "None"),
        text.col = colorCodes, pch = c(20,20,20,20,17,17,17,17), col = colorCodes)

#Take picture of the 3d ploot
rgl.snapshot(filename = paste(name,"3D.TSNE.plot.png", sep = "."), fmt = "png")

#Take pictures of multiple angles of the plot to create a gif
for (i in 1:360) {
  rgl.viewpoint(i, 20)
  filename <- paste(name, formatC(i, digits = 1, width = 3, flag = "0"), ".png", sep = "")
  rgl.snapshot(filename)
}

# Make a gif animation using ImageMagick
system(command = "convert -delay 10 *.png -loop 0 pic.gif")

```

## Session Information

```

suppressPackageStartupMessages(library(Biobase))
suppressPackageStartupMessages(library(edgeR))

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

suppressPackageStartupMessages(library(Rtsne))
suppressPackageStartupMessages(library(scatterplot3d))
suppressPackageStartupMessages(library(rgl))
suppressPackageStartupMessages(library(pca3d))
suppressPackageStartupMessages(library(RColorBrewer))
library(Biobase)
library(edgeR)
library(Rtsne)
library(scatterplot3d)
library(rgl)
library(pca3d)
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] RColorBrewer_1.1-2   pca3d_0.8           rgl_0.96.0
##  [4] scatterplot3d_0.3-37 Rtsne_0.11          edgeR_3.14.0
##  [7] limma_3.28.21        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    ellipse_0.3-8
## [5] xtable_1.8-2      R6_2.2.0        stringr_1.1.0   tools_3.3.0
## [9] htmltools_0.3.5  yaml_2.1.13     assertthat_0.1  digest_0.6.10
## [13] tibble_1.2        shiny_0.14.1    formatR_1.4     htmlwidgets_0.7
## [17] evaluate_0.10     mime_0.5        rmarkdown_1.1   stringi_1.1.2
## [21] jsonlite_1.1      httpuv_1.3.3
```