Dimensionality reduction, differential expression analysis using single-cell RNA-seq data

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Dimensionality reduction

Clustering

Differential expression

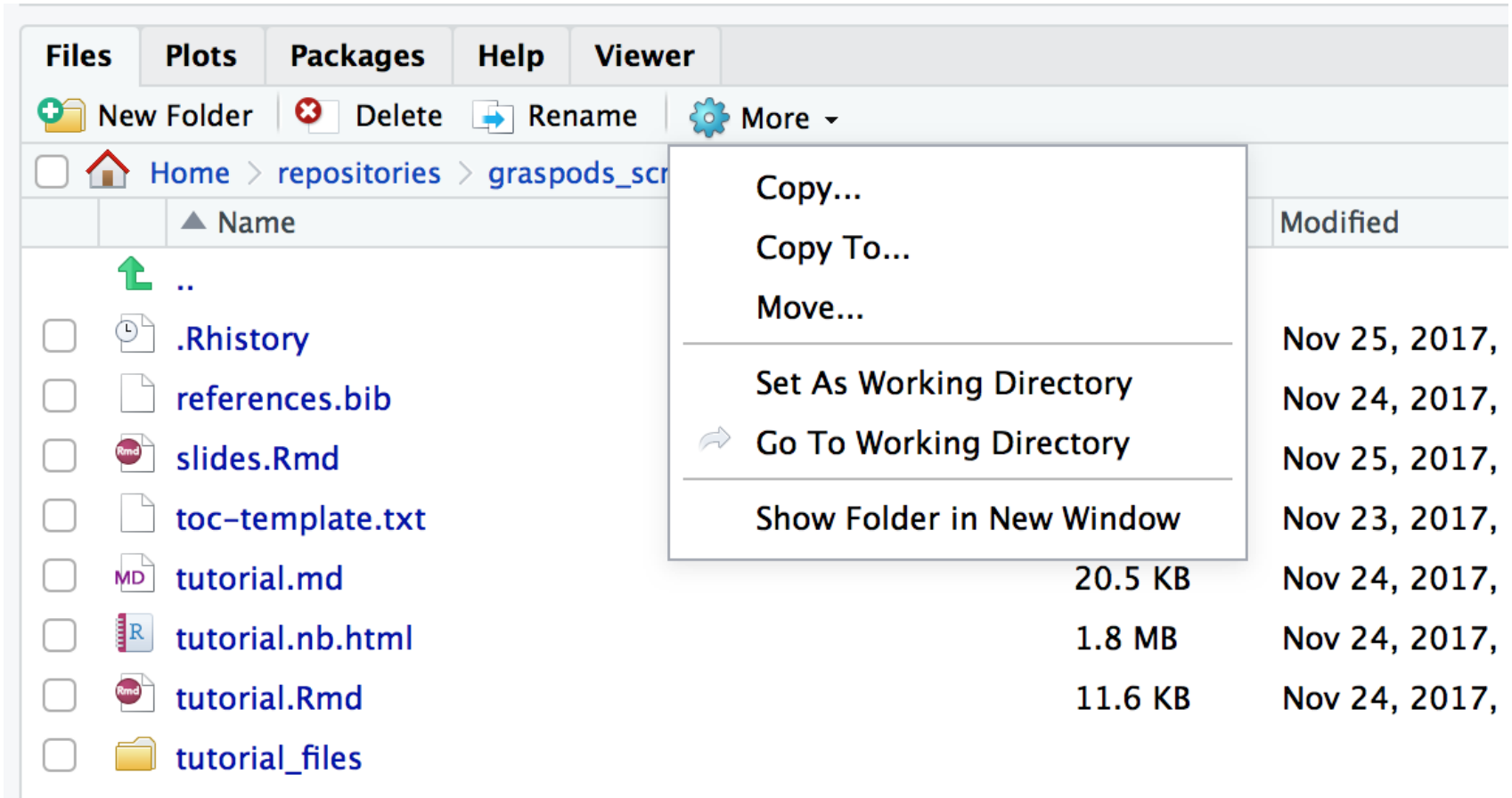
Install required packages

```
if (!require("pacman")) install.packages("pacman")

pacman::p_load(tidyverse, Seurat, stringr,
               Rtsne, cluster, limma, edgeR, statmod)
```

- Make sure you have the latest version of RStudio (https://www.rstudio.com/products/rstudio/download/).

Navigate to working directory



Load the expression data

```
load("../data/pbmc3k_seurat_extracted.rda")
```

- `data_matrix_raw` contains the transcript (technically, UMI) counts for each gene,cell pair
- `data_matrix_scaled` contains the log-normalized and scaled version of the above
- `pbmc_metadata` contains metadata for each cell
- `variable_genes` contains a vector of genes that exhibit substantial expression variability across cells

```
dim(data_matrix_raw)
```

```
## [1] 13714  2700
```

```
dim(pbmc_metadata)
```

```
## [1] 2638    7
```

```
length(variable_genes)
```

```
## [1] 1838
```

```
dim(data_matrix_scaled)
```

```
## [1] 1838 2638
```

```
data_matrix_raw <-
  as.matrix(data_matrix_raw[variable_genes,
                            colnames(data_matrix_scaled)])
```

Dimensionality reduction

Dimensionality reduction

Goal:

- Reduce 1838-dimensional data into lower dimensions
- Why?
  - Visualization
  - Clustering

Dimensionality reduction

How?

- Principal component analysis (PCA)
- t-distributed stochastic neighbour embedding (t-SNE)

Principal component analysis (PCA)

- Expression data for each gene => sum of orthogonal components
- Allows us to write data in terms of these components

```
components <- 20
```

Running PCA

```
pca_results <-
  irlba::irlba(A = t(data_matrix_scaled),
               nv = components)
```

Results

```
dim(pca_results$u)
```

```
## [1] 2638   20
```

- `pca_results$u` is the **factor score** matrix: the values of each PC for each cell

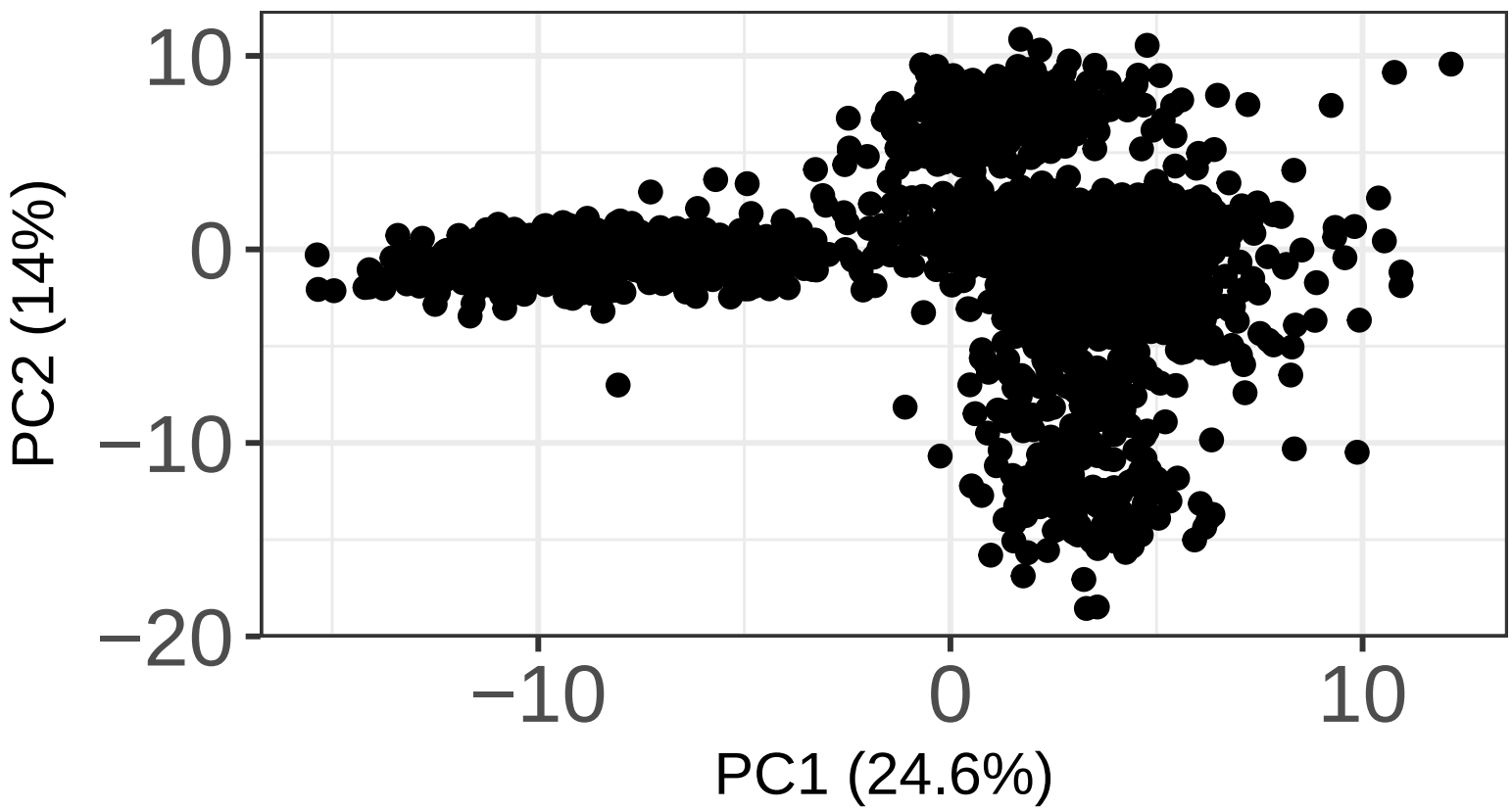
```
dim(pca_results$v)
```

```
## [1] 1838   20
```

- `pca_results$v` is the **loadings** matrix: how to convert PC values back to expression values for each gene

Visualization

```
ggplot(factor_scores, aes(x=PC1, y=PC2)) + geom_point() +
  theme_bw() +
  xlab(paste0("PC1 (", round(frac_vars[1],3)*100, "%)")) +
  ylab(paste0("PC2 (", round(frac_vars[2],2)*100, "%)")) +
  theme(axis.text = element_text(size=15))
```



t-distributed stochastic neighbour embedding (t-SNE)

- Nonlinear dimensionality reduction method

```
dims <- 2
```

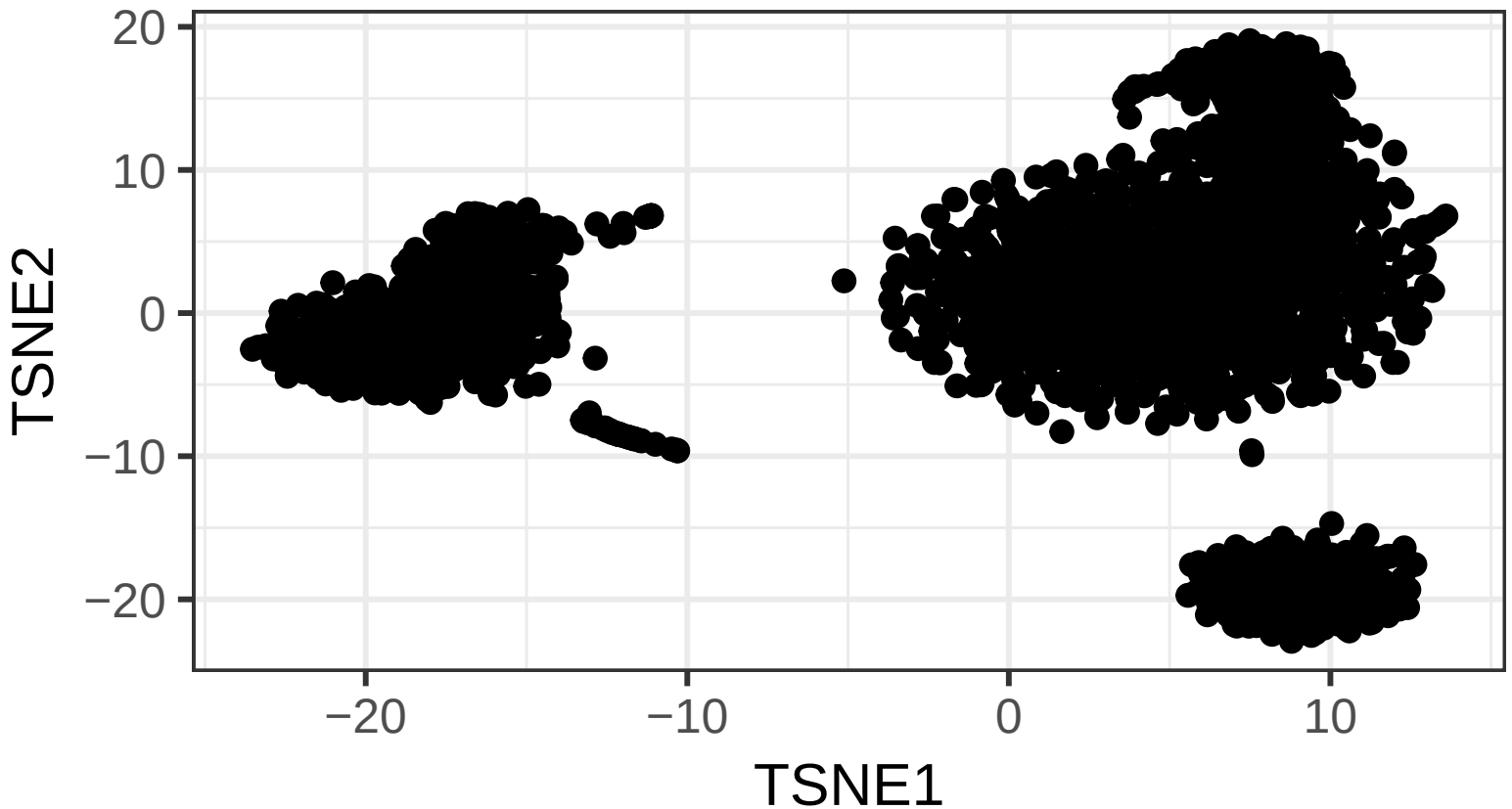
Running t-SNE

```
tsne_results <- Rtsne(X = t(data_matrix_scaled),
                      dims = dims, initial_dims = 50)
```

```
tsne_results <-
  readRDS("../intermediates/tsne_results.rds")
```

Plotting

```
ggplot(tsne_df, aes(x=TSNE1, y=TSNE2)) +
  geom_point() + theme_bw() +
  xlab("TSNE1") + ylab("TSNE2")
```



Clustering

k-medoids

Many ways to cluster, including:

- k-means/k-medoids (k can be selected with silhouette, elbow method, etc.)
- dynamic tree cut method (Langfelder, Zhang, and Horvath 2008)

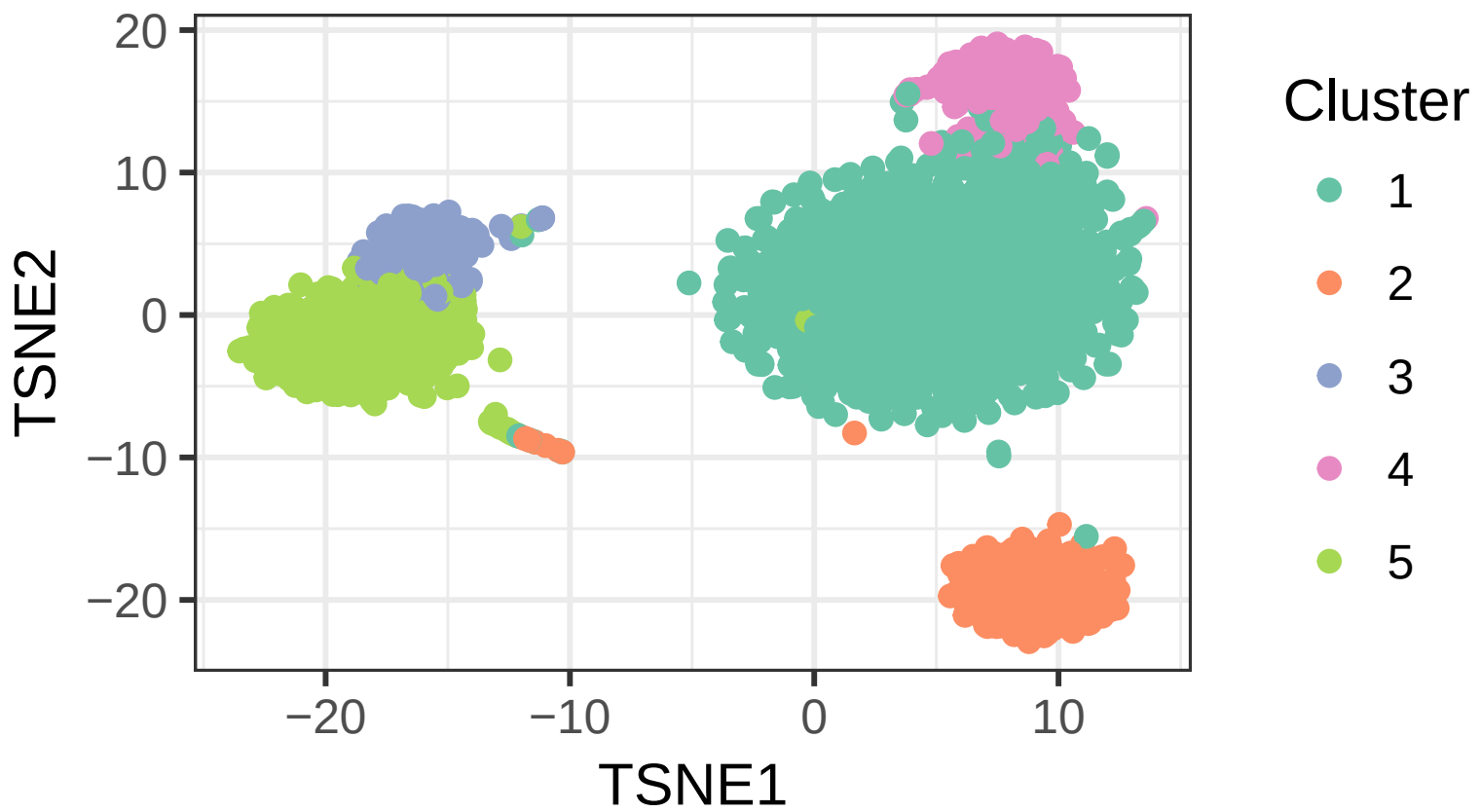
For speed we'll do partitioning around medoids (PAM) with 5 clusters, a greedy implementation of k-medoids.

```
pam_results <- pam(factor_scores, k = 5,
                   metric = "euclidean",
                   diss = FALSE)

tsne_df$cluster <- pam_results$clustering
```

Plotting

```
ggplot(tsne_df, aes(x=TSNE1, y=TSNE2)) +
  geom_point(aes(colour=factor(cluster))) +
  theme_bw() + xlab("TSNE1") + ylab("TSNE2") +
  guides(colour = guide_legend(title = "Cluster")) +
  scale_colour_brewer(palette = "Set2")
```



Differential expression

What methods to use?

- DE methods for bulk RNA-seq data as good as those for single-cell RNA-seq (Soneson and Robinson 2017)

Preparing the design matrix

```
cluster_label <- paste0("C", tsne_df$cluster)

design <- model.matrix(~0 + cluster_label)
colnames(design) <- paste0("C", 1:5)
```

Fitting a GLM

```
d <- DGEList(data_matrix_raw)
d <- calcNormFactors(d)

d <- estimateDisp(d, design)
fit <- glmFit(d, design, robust = TRUE)
```

```
fit <- readRDS("../intermediates/edgeR_glmfit.rds")
```

Comparing clusters 1 and 2

```
contrast.matrix <-
  limma::makeContrasts(C1 - C2, levels = design)
colnames(contrast.matrix) <- c("C1C2")
```

Performing the likelihood test:

```
results <- glmLRT(fit, coef = "C1 - C2",
                  contrast = contrast.matrix)
c1_c2_genes <-
  edgeR::topTags(results, adjust.method = "BH",
                 n = Inf, sort.by = "PValue")
```

Underexpressed in cluster 1 vs. 2

```
c1_c2_genes$table %>%
  subset(logFC < 0, select = c(logFC, FDR)) %>%
  head(5)
```

```
##                logFC             FDR
## CD79A     -4.656100  0.000000e+00
## CD79B     -3.810178  0.000000e+00
## MS4A1     -3.680759  0.000000e+00
## HLA-DRB1 -3.663803  0.000000e+00
## HLA-DPA1 -3.564842 7.195417e-312
```

CD79A

From Wikipedia, the free encyclopedia

Cluster of differentiation CD79A also known as **B-cell antigen receptor complex-associated protein alpha chain** and **MB-1 membrane glycoprotein**, is a protein that in humans is encoded by the CD79A gene.[5]

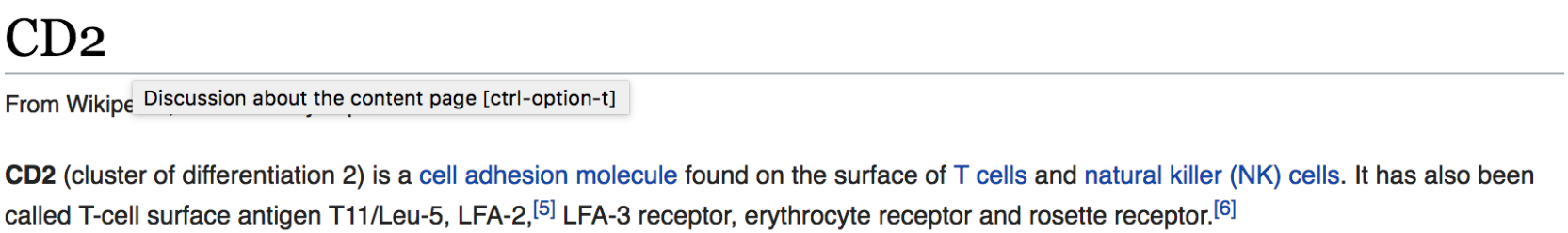
The CD79a protein together with the related CD79b protein, forms a dimer associated with membrane-bound immunoglobulin in B-cells, thus forming the B-cell antigen receptor (BCR).[6] This occurs in a similar manner to the association of CD3 with the T-cell receptor, and enables the cell to respond to the presence of antigens on its surface.[7]

It is associated with agammaglobulinemia-3.[8]

Overexpressed in cluster 1 vs. 2

```
c1_c2_genes$table %>%
  subset(logFC > 0, select = c(logFC, FDR)) %>%
  head(5)
```

```
##              logFC             FDR
## IL32    3.693076 1.582355e-183
## GIMAP7  2.776935  2.128554e-94
## GIMAP4  2.432430  5.059254e-65
## CD2     2.344373  1.014307e-55
## GIMAP5  2.232089  4.864995e-43
```



Comparison to Seurat-annotated clusters

```
tsne_df$annotated_celltype <-
  pbmc_metadata[tsne_df$cell,]$ClusterNames_0.6

with(tsne_df, table(annotated_celltype, cluster))
```

```
##                      cluster
## annotated_celltype     1    2    3    4    5
##   B cells              4  339    0    0    0
##   CD14+ Monocytes      0    0    7    0  471
##   CD4 T cells       1146    0    0    0    3
##   CD8 T cells        228    1    1   78    0
##   Dendritic cells      7   11    0    0   14
##   FCGR3A+ Monocytes    0    0  156    0    2
##   Megakaryocytes       1    0   13    0    1
##   NK cells             2    0    0  153    0
```

Questions?

References

Langfelder, Peter, Bin Zhang, and Steve Horvath. 2008. "Defining clusters from a hierarchical cluster tree: the Dynamic Tree Cut package for R." *Bioinformatics* 24 (5): 719–20. doi:10.1093/bioinformatics/btm563.

Soneson, Charlotte, and Mark D. Robinson. 2017. "Bias, Robustness and Scalability in Differential Expression Analysis of Single-Cell RNA-Seq Data." *Doi.org*, May. Cold Spring Harbor Laboratory, 143289. doi:10.1101/143289.