Computational single-cell biology course

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Contents

1 Dimensionality reduction and clustering on scRNA-seq data (hands-on)

> We will work on scRNA-seq data of mouse gastrulation and early organogenesis from Pijuan-Sala et al., 2019. This Shiny application provides an interactive interface that allows users to validate their own analysis on this data. You can reach the original data and related scripts from the Github page.

Gastrulation is a phase early in the embryonic development of most animals, during which the single-layered blastula is reorganized into a multilayered structure known as the gastrula (Wikipedia, 2020-06-02).

1.1 Getting familiar with the pre-processed data

Let's start with including required libraries.

```
  suppressPackageStartupMessages({
  library(Seurat)
  library(ggplot2)
  library(dplyr)
  library(data.table)
  library(cowplot)
  set.seed(1)
})
```

In the previous practical, we have learned the essential preprocessing steps for working with scRNA-seq. Here we will start working with the preprocessed version of the mouse gastrulation scRNA-seq data. We will load the pre-saved `Seurat` object of this data.

Warning: The pre-processed mouse gastrulation scRNA-seq Seurat object is large (~2GBs). In today's practical, we will subset the data for fast computation. You can, however, work on the original data for additional practices. The meta-data is provided seperately.

```
    mgsc <- readRDS('data/gastrulation/mgsc.rds')
    mgsc
## An object of class Seurat
## 29452 features across 116312 samples within 1 assay
## Active assay: RNA (29452 features, 0 variable features)
```

Reminder:

- number of rows = genes = features,
- number of columns = cells = samples

```
    # let's see the available slots
    slotNames(mgsc)
##  [1] "assays"       "meta.data"    "active.assay" "active.ident" "graphs"
##  [6] "neighbors"    "reductions"   "project.name" "misc"         "version"
## [11] "commands"     "tools"
```

Now let's look at to the metadata table of the dataset that contains an overview of the samples.

```
    # see it is empty for now
    head(mgsc@meta.data, 5)
## data frame with 0 columns and 5 rows

    # now let's load the metadata
    metadata <- fread('data/gastrulation/sample_metadata.txt.gz') %>% .[stripped==FALSE & doublet==FALSE]

    # and add  the metadata  to our seurat object
    mgsc <- AddMetaData(mgsc, metadata = data.frame(metadata, row.names = metadata$cell))

    # now let's see once more
    head(mgsc@meta.data, 5)
##          cell        barcode sample stage sequencing.batch doublet stripped
## cell_1 cell_1 AAAGGCCTCCACAA      1  E6.5                1   FALSE    FALSE
## cell_2 cell_2 AACAAACTCGCCTT      1  E6.5                1   FALSE    FALSE
## cell_5 cell_5 AACAGAGAATCAGC      1  E6.5                1   FALSE    FALSE
## cell_6 cell_6 AACATATGAATCGC      1  E6.5                1   FALSE    FALSE
## cell_8 cell_8 AACCGATGGCTTCC      1  E6.5                1   FALSE    FALSE
##                celltype      umapX      umapY        celltype2    celltype3
## cell_1         Epiblast -10.227546 -2.8816875         Epiblast  Epiblast-PS
## cell_2 Primitive_Streak  -6.625458  0.1089605 Primitive_Streak  Epiblast-PS
## cell_5     ExE_ectoderm  10.061009 -0.0293132     ExE_ectoderm ExE_ectoderm
## cell_6         Epiblast -10.454418 -0.2694517         Epiblast  Epiblast-PS
## cell_8         Epiblast -11.047206 -2.2052687         Epiblast  Epiblast-PS
```

Now we are able to see the annotations (e.g. cell type) for each cell. There is also a column named `stage` which shows the embryonic day the cells were sequenced. To speed up our experiments, we will work on a subset of cells that belong to stage `E6.75`.

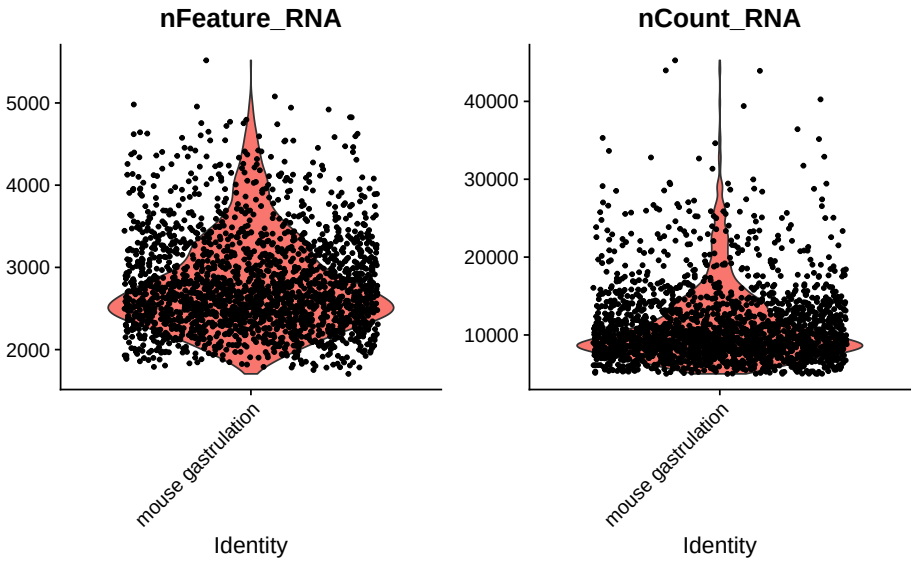
```
    mgsc_subset <- mgsc[ , mgsc@meta.data$stage=='E6.75']
    mgsc_subset
## An object of class Seurat
## 29452 features across 2075 samples within 1 assay
## Active assay: RNA (29452 features, 0 variable features)
    # now lets save this subset
    saveRDS(mgsc_subset, file = "data/gastrulation/mgsc_e675.rds")

    mgsc <- mgsc_subset
```

This is where you are starting from! :) Make sure to download `mgsc_e675.rds` if you haven't already.

2 TASK 1: Pre-processing

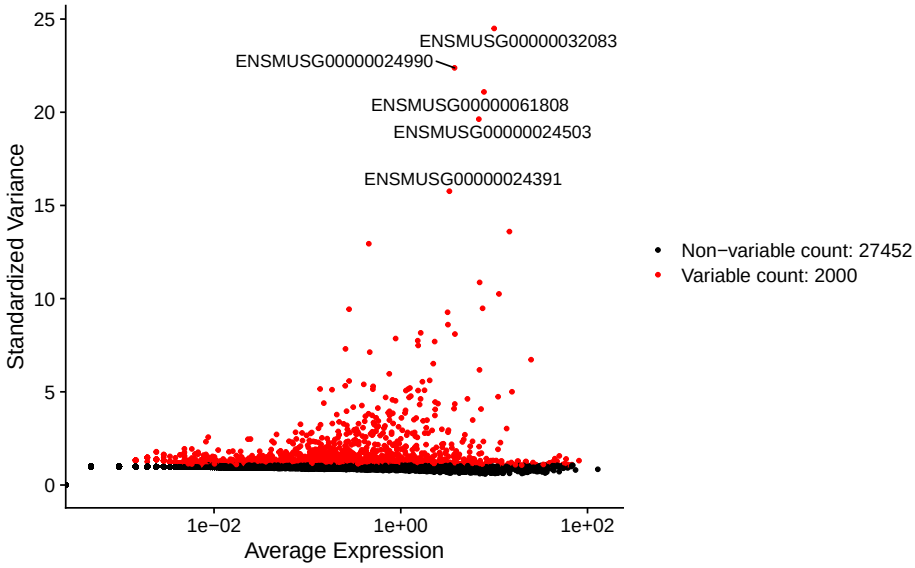
1.1: Let's load the mgsc_e675 data and take look at the distribution of the features to observe whether there are remaining outliers.

```
## An object of class Seurat
## 29452 features across 2075 samples within 1 assay
## Active assay: RNA (29452 features, 0 variable features)
```



1.2: Now let's plot the most variable features and annotate top5 most variable genes. Scale the data aftwerwards.

```
## Warning: Using `as.character()` on a quosure is deprecated as of rlang 0.3.0.
## Please use `as_label()` or `as_name()` instead.
## This warning is displayed once per session.
## Warning: Transformation introduced infinite values in continuous x-axis
```




```
##                       mean variance variance.standardized
## ENSMUSG00000032083 10.008193 971.0756              24.49617
## ENSMUSG00000024990  3.765301 210.4092              22.38176
## ENSMUSG00000061808  7.784578 546.4217              21.09222
## ENSMUSG00000024503  6.853976 419.2819              19.63029
## ENSMUSG00000024391  3.319036 112.9888              15.76248
```

```
## Centering and scaling data matrix
```

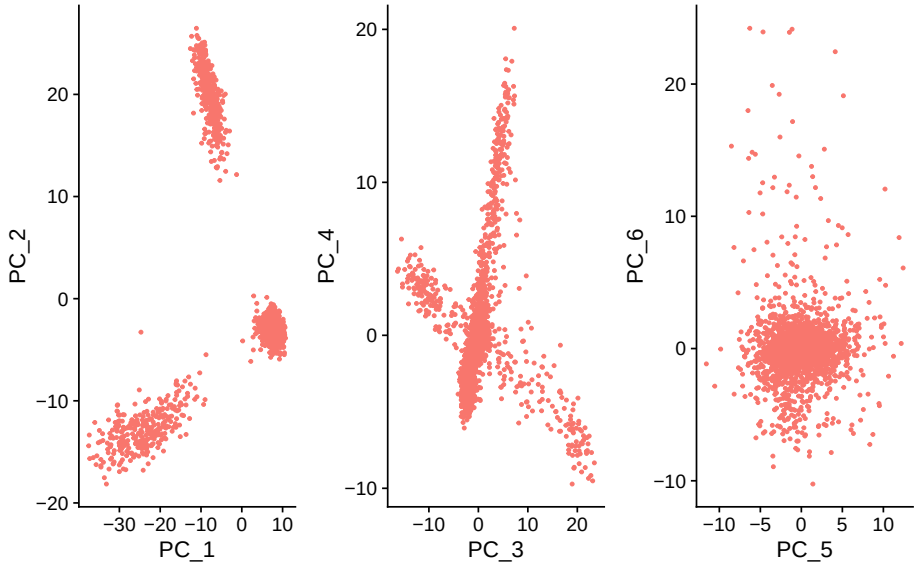
It might be possible that instead of gene symbols, we get our genes with Ensemble GeneIDs. In that case we will need to map to gene symbols first.

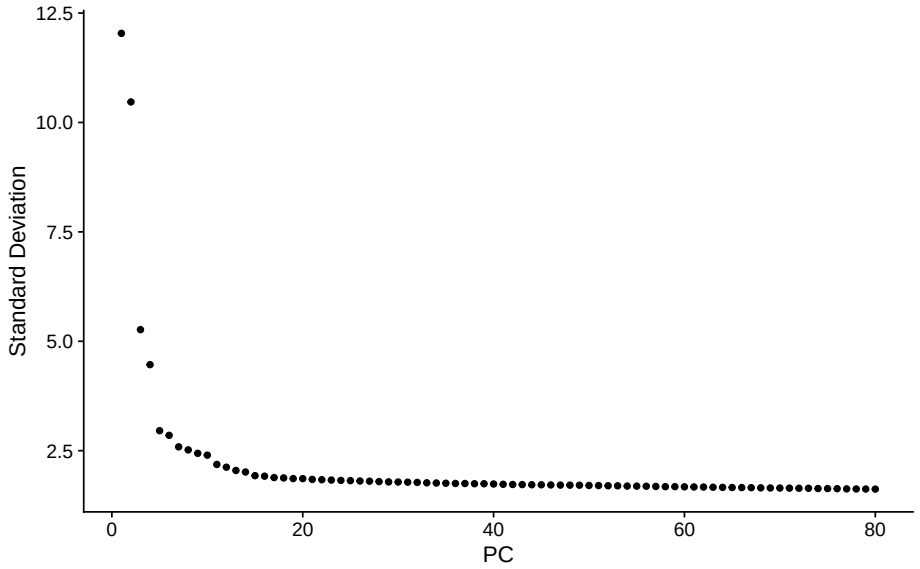
```
library(biomaRt)
ensembl <- useMart("ensembl", dataset="mmusculus_gene_ensembl")
annot<-getBM(c("ensembl_gene_id", "mgi_symbol", "chromosome_name", "strand", "start_position", "end_position"
## Warning: `select_()` is deprecated as of dplyr 0.7.0.
## Please use `select()` instead.
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_warnings()` to see where this warning was generated.
## Warning: `filter_()` is deprecated as of dplyr 0.7.0.
## Please use `filter()` instead.
## See vignette('programming') for more help
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_warnings()` to see where this warning was generated.
## Cache found
```

1.3: Print out the gene names of the top10 variable genes. (`Hint:` you can make use of `match()` function.)

```
##  [1] "Apoa1"  "Rbp4"   "Ttr"    "Spink1" "Apom"   "Apoe"   "Dkk1"   "Ctsl"
##  [9] "Rhox5"  "Gsto1"
```

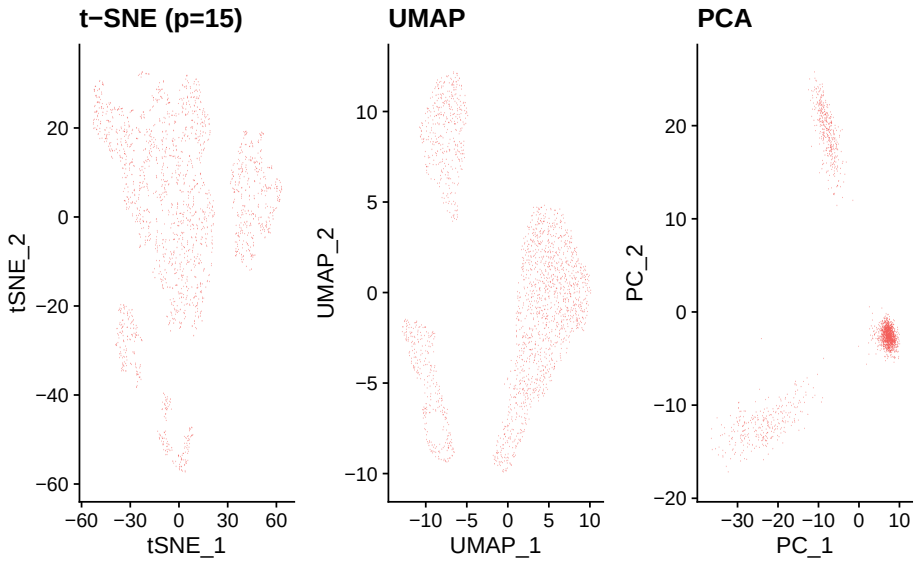
1.4: Apply PCA and determine the dimensionality. Generate the following output: three 2D plots with the first six PCs and print them side by side. i.e. PC1-PC2, PC3-PC4, PC5-PC6.





1.5. Plot the projections of the dataset with three of the dimensionality reduction techniques printed side by side. Use UMAP with n.neighbors=20, min.dist=7 and tSNE with perplexity=15. Use first 10 PCs.

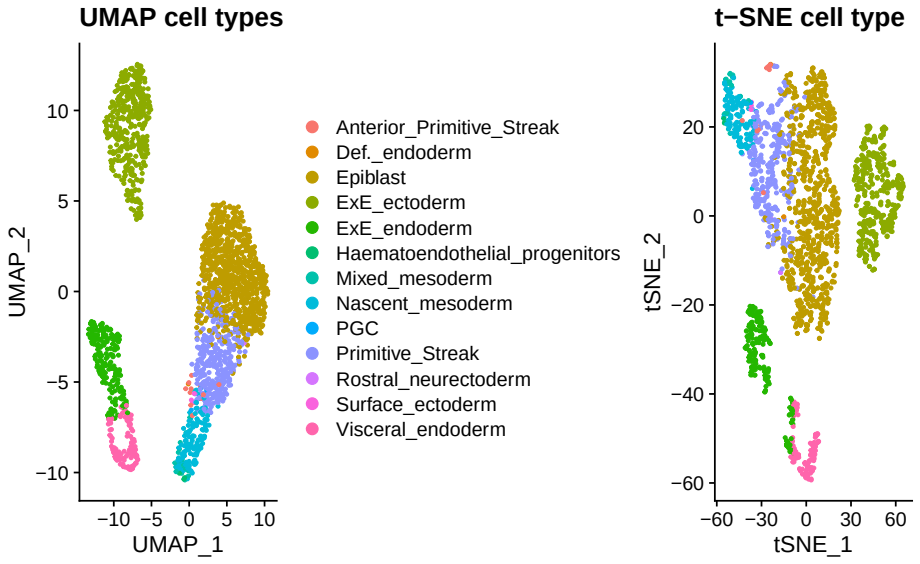
```
## Warning: The default method for RunUMAP has changed from calling Python UMAP via reticulate to the R-nativ
## To use Python UMAP via reticulate, set umap.method to 'umap-learn' and metric to 'correlation'
## This message will be shown once per session
## 11:13:39 UMAP embedding parameters a = 0.3208 b = 1.563
## 11:13:39 Read 2075 rows and found 10 numeric columns
## 11:13:39 Using Annoy for neighbor search, n_neighbors = 20
## 11:13:39 Building Annoy index with metric = cosine, n_trees = 50
## 0%   10   20   30   40   50   60   70   80   90   100%
## [----|----|----|----|----|----|----|----|----|----|
## **************************************************|
## 11:13:39 Writing NN index file to temp file /var/folders/l5/1nfd7mk14835td6hgk_71p880000gs/T//RtmpCWq5Ph/f
## 11:13:39 Searching Annoy index using 1 thread, search_k = 2000
## 11:13:40 Annoy recall = 100%
## 11:13:40 Commencing smooth kNN distance calibration using 1 thread
## 11:13:41 Found 2 connected components, falling back to 'spca' initialization with init_sdev = 1
## 11:13:41 Initializing from PCA
## 11:13:41 PCA: 2 components explained 74.03% variance
## 11:13:41 Commencing optimization for 500 epochs, with 53554 positive edges
## 11:13:43 Optimization finished
```



3 TASK 2: Cluster cells

For our subset of time point `E6.75`, the original meta-data stores the assigned cell type annotation information.

2.1: How many clusters did the original study identify? Reproduce the following plots colored by annotated cell types. (`Hint:` You can make use `group.by` argument in `DimPlot` to extract stored cluster IDs.)



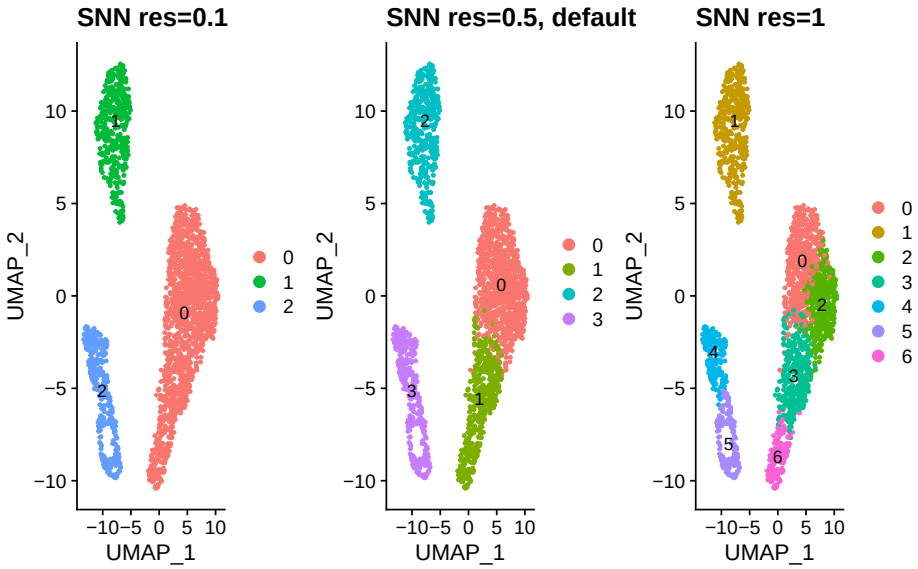

```
##  [1] "Epiblast"                "Primitive_Streak"
##  [3] "ExE_endoderm"            "Visceral_endoderm"
##  [5] "ExE_ectoderm"            "Nascent_mesoderm"
##  [7] "Anterior_Primitive_Streak" "PGC"
##  [9] "Mixed_mesoderm"          "Rostral_neurectoderm"
## [11] "Surface_ectoderm"        "Def._endoderm"
```

```
## [13] "Haematoendothelial_progenitors"
```

2.2: Now let's use Seurat's graph-based clustering to identify the clusters and observe whether we can reproduce the above conclusion. Use first 30 PCs and 10 nearest neighbors for resolutions r=0.1, 0.5, 1.

Hint: The output of FindClusters is saved in `mgsc meta.data$seurat_clusters`. This resets each time clustering is performed. You can use `Idents` function of Seurat to save cluster ids.

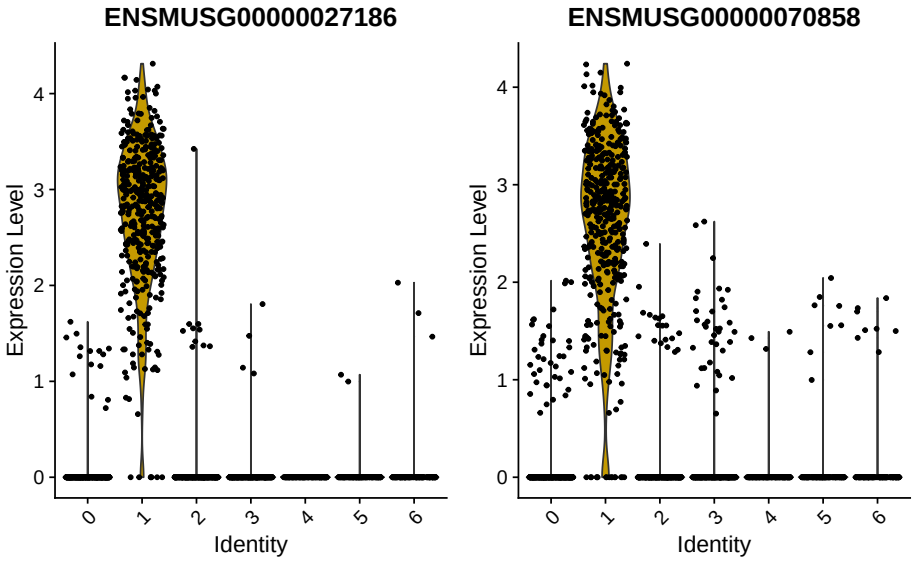
```
mgsc <- FindNeighbors(mgsc,  k.param = 20, dims = 1:50, reduction = "pca")
mgsc <- FindClusters(mgsc, resolution = 0.5)
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
##
## Number of nodes: 2075
## Number of edges: 143303
##
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.8227
## Number of communities: 4
## Elapsed time: 0 seconds
mgsc[["snn_05"]] <- Idents(object = mgsc)
mgsc <- FindClusters(mgsc, resolution = 0.1)
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
##
## Number of nodes: 2075
## Number of edges: 143303
##
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.9544
## Number of communities: 3
## Elapsed time: 0 seconds
mgsc[["snn_01"]] <- Idents(object = mgsc)
mgsc <- FindClusters(mgsc,  resolution = 1)
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
##
## Number of nodes: 2075
## Number of edges: 143303
##
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.7164
## Number of communities: 7
## Elapsed time: 0 seconds
mgsc[["snn_1"]] <- Idents(object = mgsc)


plot_grid(nrow=1, ncol = 3,
  DimPlot(mgsc, reduction = "UMAP_n20", group.by = "snn_01", label=TRUE)+ggtitle("SNN res=0.1"),
  DimPlot(mgsc, reduction = "UMAP_n20", group.by = "snn_05", label=TRUE)+ggtitle("SNN res=0.5, default"),
  DimPlot(mgsc, reduction = "UMAP_n20", group.by = "snn_1", label=TRUE)+ggtitle("SNN res=1")
  )
```



2.3: Let's find the markers of the cluster 1. Investigate the first two markers and find out gene names of the top 10.

```
## For a more efficient implementation of the Wilcoxon Rank Sum Test,
## (default method for FindMarkers) please install the limma package
## --------------------------------------------
## install.packages('BiocManager')
## BiocManager::install('limma')
## --------------------------------------------
## After installation of limma, Seurat will automatically use the more
## efficient implementation (no further action necessary).
## This message will be shown once per session
```

Note that the original study does not employ `Seurat` but `scran` and `Scanpy` packages, therefore it's expected that we might have slightly different results.

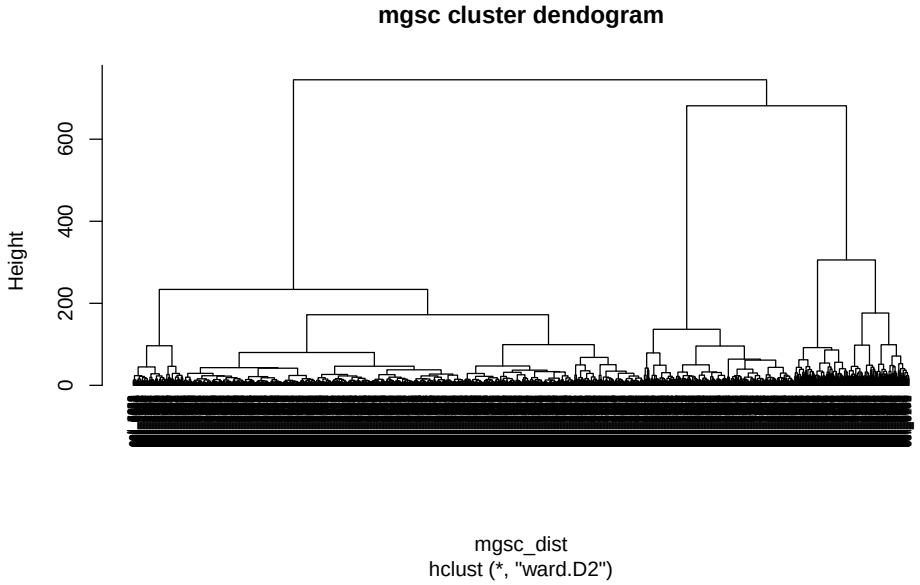


```
##  [1] "Elf5"    "Gm1673"  "Nup62cl" "Tex19.1" "S100a6"  "Gjb3"    "Cldn3"
##  [8] "Anxa5"   "Dppa4"   "Zfp42"
```

4 Homework (Hierarchical Clustering)

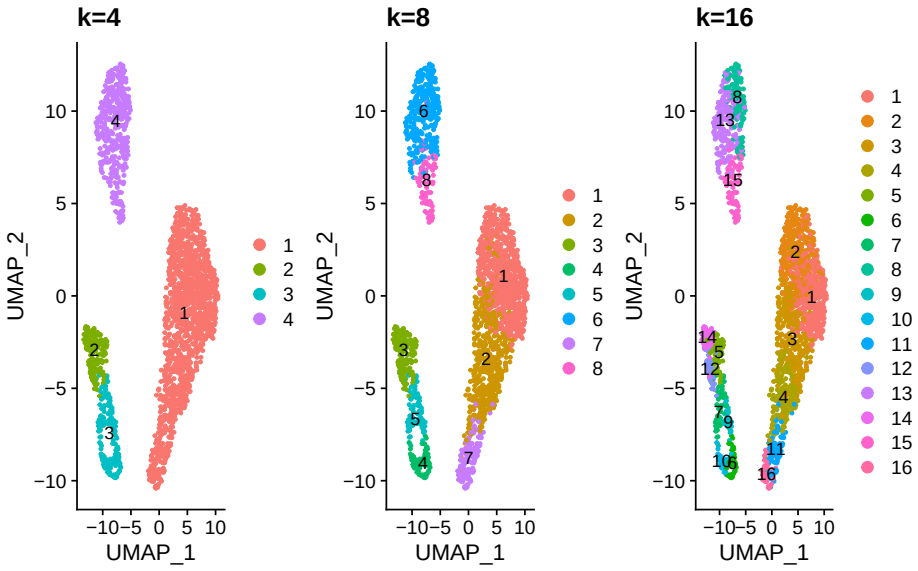
Seurat does not support hierarchical clustering too? But we can do it!!

H1: Which elements do we need to perform hierarchical clustering? Try to reproduce the following dendogram. Use `Euclidian` as distance metric and `ward.D2` as linkage method.



Looking at the dendogram, we can investigate different numbers of clusters using `cutree` function that cuts the hierarchical clustering tree into given number of clusters.

H2: How about we print different clustering outcomes with $k = 4, 8, 16$ with UMAP?


k=4
k=8
k=16
UMAP_2
UMAP_1
10
5
0
−5
−10
−10 −5 0 5 10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16