**Reading in data and initial processing**

1. Installing and loading necessary package

A close-up of a code

Description automatically generated

\*\*If you get an error installing Seurat, use the following line of code instead of devtools::install\_github(“satijalab/seurat”):

install.packages("https://cran.r-project.org/src/contrib/Archive/devtools/devtools\_2.4.3.tar.gz", repos = NULL, type="source")

1. Set working directory



1. Load in files from the “filtered\_feature\_bc\_matrix” directory outputted from cellranger and use these to create a Seurat object

A computer code with text

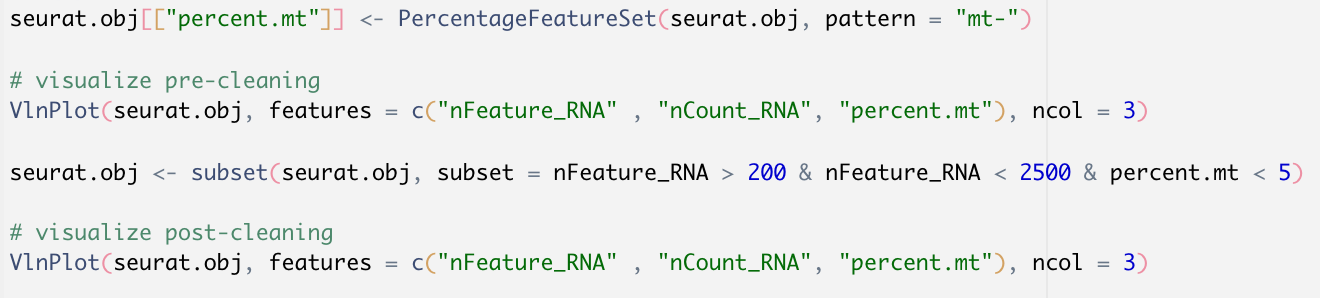
Description automatically generated

1. Add metadata columns to Seurat objects

A picture containing text, font, white, handwriting

Description automatically generated

1. Quality control
   * Remove doublets (nFeature\_RNA < 2500)
   * Remove debris (nFeature\_RNA > 200)
   * Remove mitochondrial contamination (cells/nuclei with percent.mt < 5)



\*\*Note: this step must be done for each Seurat object, substituting obj.7dpi.seurat and obj.4dpi.seurat for searat.obj in each case.

**Merging/Integration**

1. Merging Seurat objects (this has to be done prior to “integration” steps below)

A picture containing text, screenshot, font

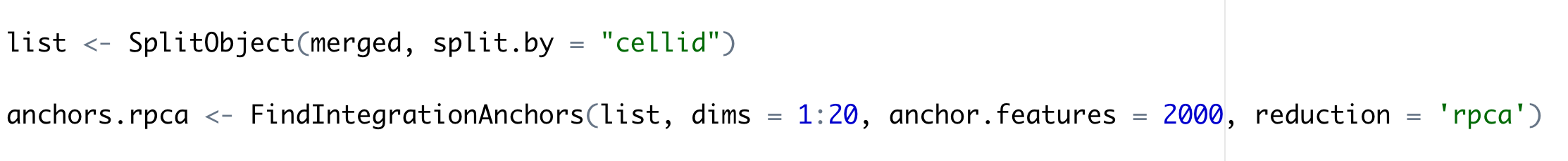
Description automatically generated

1. Processing merged object

A picture containing text, screenshot, font

Description automatically generated

1. Integration using the rpca algorithim



**Clustering and visualization**

1. Read in myogenic subset object

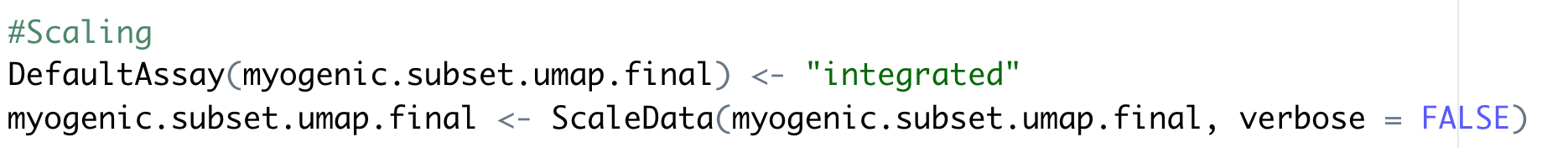


1. Setting parameters for clustering and UMAP

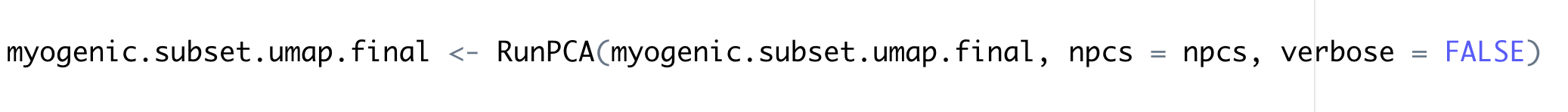
A picture containing text, font, screenshot, white

Description automatically generated

1. Scaling data



1. Conducting PCA reduction



1. A black text on a white background

   Description automatically generatedConducting UMAP reduction (see website for more information <https://pair-code.github.io/understanding-umap/>)
2. A black text on a white background

   Description automatically generatedUsing the louvain algorithim to identify clusters from UMAP processed object
3. A close-up of a label

   Description automatically generatedPlotting and visualizing clusters