Theory and Practice in gene expression analysis

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Dates:

- 12.11.2024
- 13.11.2024
- 15.11.2024

NOTE: the exercises in this part will use R. Please install R version 4.4.x and RStudio.

If your operating system is Windows, you also need to install RTools 4.4.

If your operating syste is MacOS, please make sure to read the installation instructions carefully. You also need XQuartz, Xcode and GNU Fortran compiler (see installation instructions in this link)

If you need help with the installation, we can support you.

NOTE: For experienced R users, as well as R newcomers, there are some heavily recommended tips & tricks at the end of this word file. Giving a whole tutorial on R and RStudio is out-of-scope for this course. However, for R newcomers we added some links to beginner guides.

Overall goals

- **Using existing packages for single-cell omics analysis** which make your life easier and speed up analyses
- Creating a **reproducible** bioinformatics experiment
- **Cell type classification and annotation**
- **Combining datasets and correcting for batch effects**

Table of Contents

Introduction

Figure 1 shows an overview of the general single-cell RNA-seq (scRNA-seq) data analysis workflow. In this exercise, you will learn about **Seurat**, the most popular R package for scRNA-seq, and how you can use it for:

- Quality control (QC) & pre-processing
- Data exploration & visualization
- Downstream analysis (clustering, differential gene expression, etc.)

If you want to know more about Seurat, then there are great tutorials available on their website for a basic introduction as well as for more advanced topics. Seurat can also be used for spatial transcriptomics and integrative multimodal analysis, e.g. combining scRNA-seq and scATAC-seq. If you prefer working in Python, there is the *Scanpy* package which reproduces many but not all functions of Seurat.

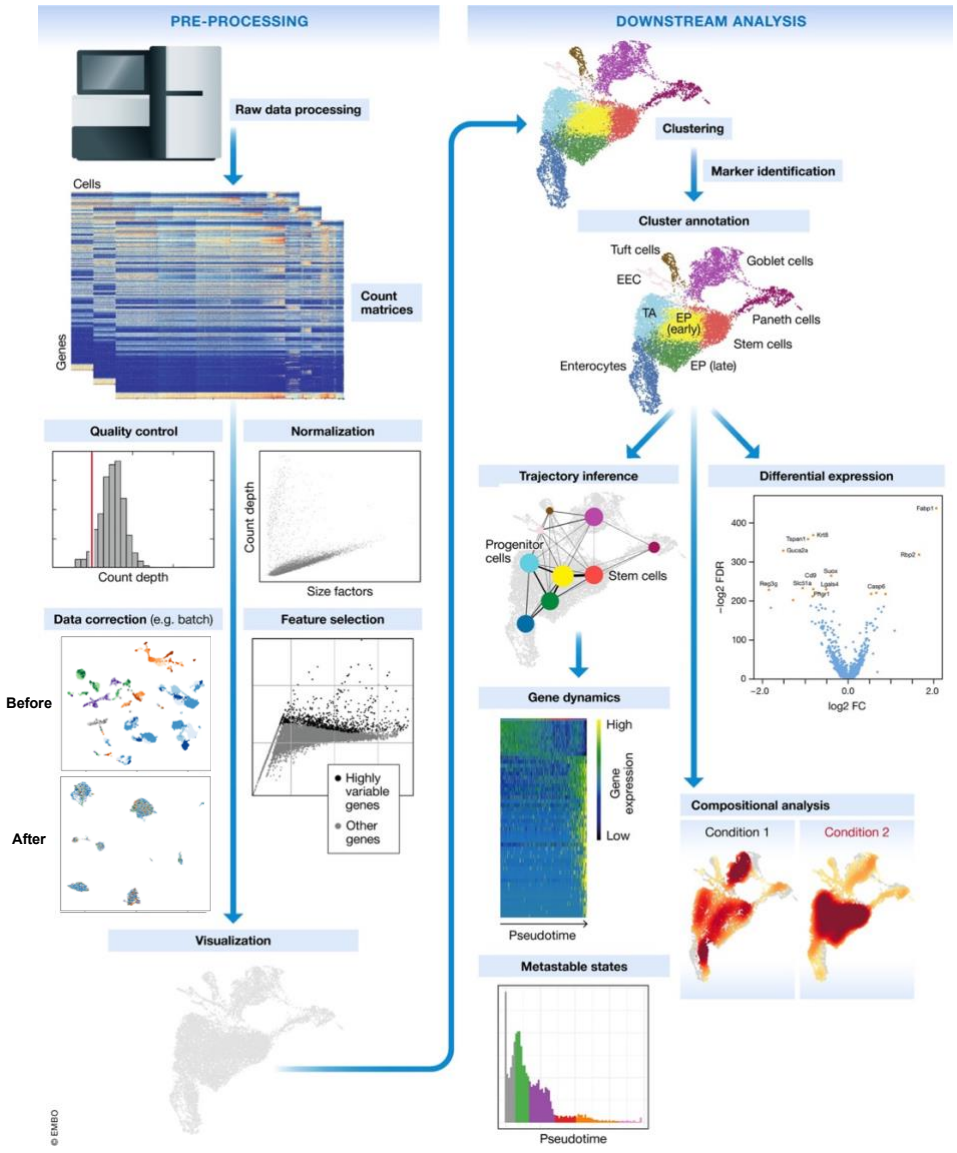


Figure 1: Overview of a standard scRNA-seq processing workflow. Modified from: Luecken MD, Theis FJ. Current best practices in single-cell RNA-seq analysis: a tutorial. Mol Syst Biol. 2019 Jun 19;15(6):e8746.

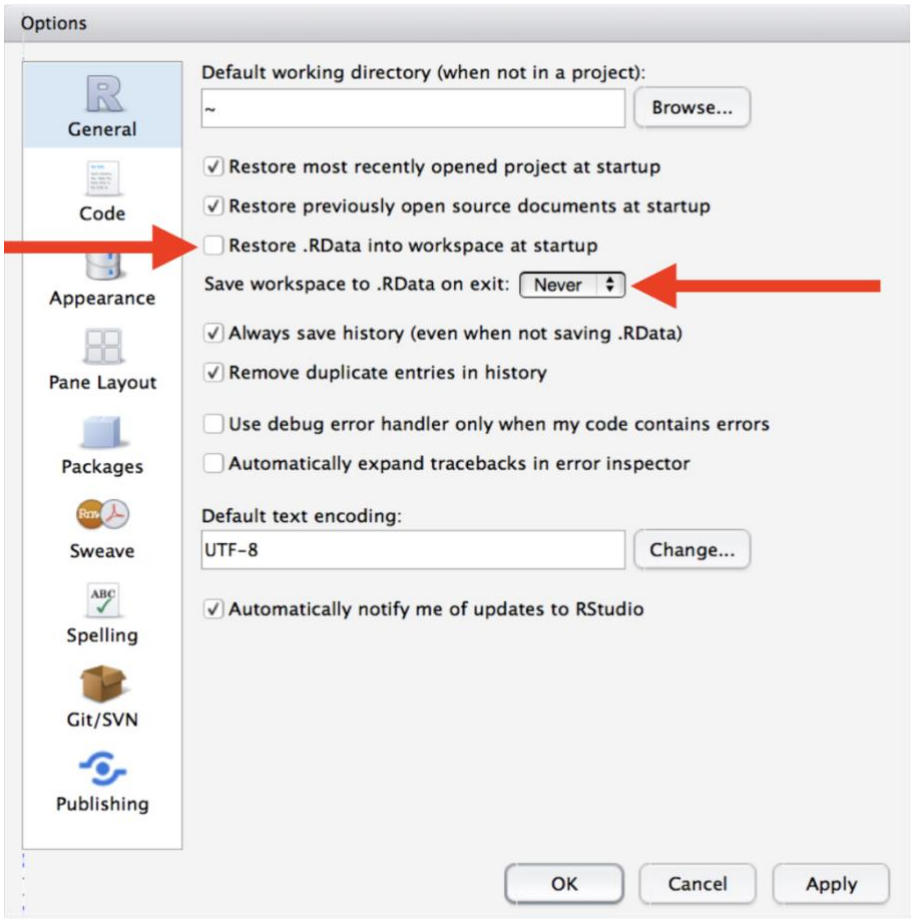
Links to R + RStudio beginner guides

https://www.datacamp.com/tutorial/r-studio-tutorial
https://moderndive.netlify.app/1-getting-started.html
https://education.rstudio.com/learn/beginner/
https://r4ds.had.co.nz/index.html

RStudio basic tips & tricks

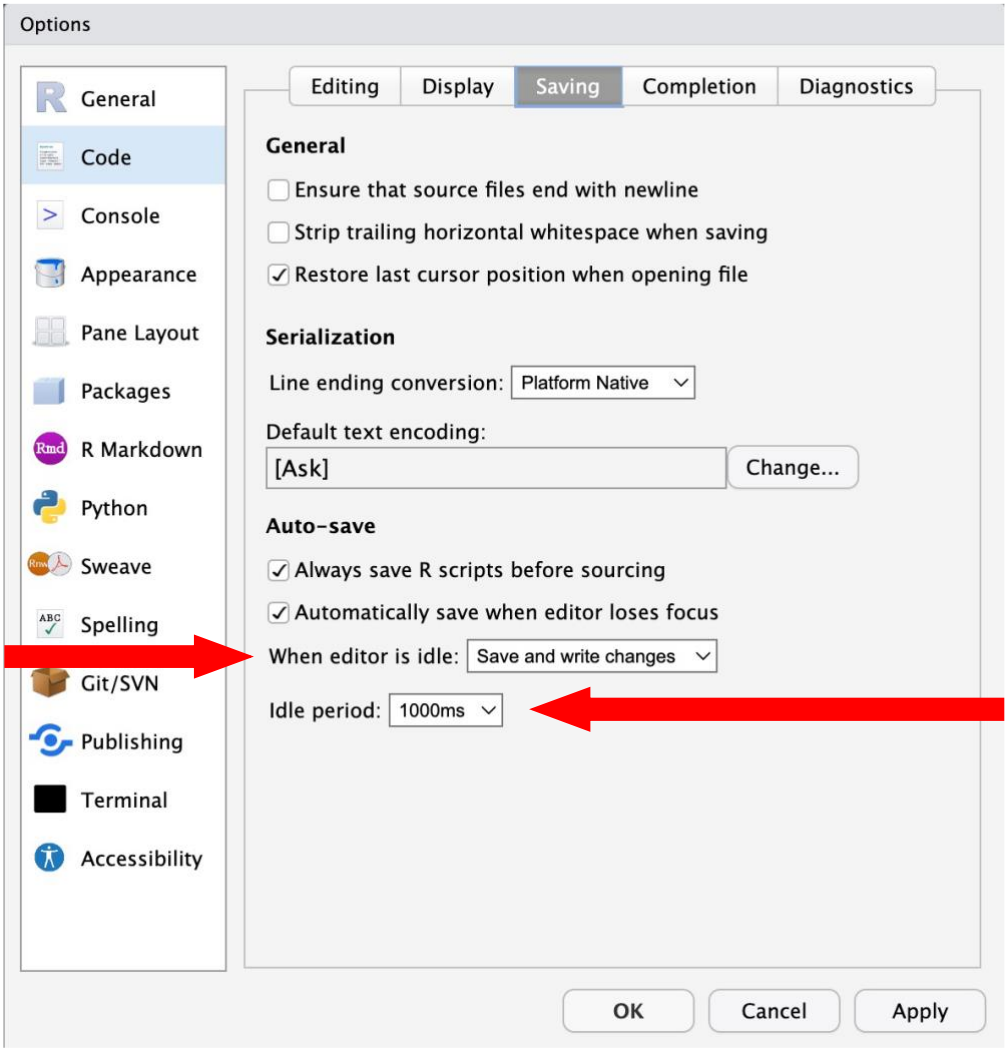
Turn off .RData

By default, RStudio has an undesirable functionality: it creates an .RData file, where it stores the workspace, such as variables you created during your work on an R script or project. When you close and re-open RStudio it will load this workspace from the .RData. However, **it can be very misleading to re-load some variable, rather than re-creating it from your script**, for example if you manually assigned a variable or it was created before you made some change to the source code and did not re-run the code to create the newly updated variable. To turn this behavior off, go to:
Tools -> Global options ... -> and set the settings like in the figure below:



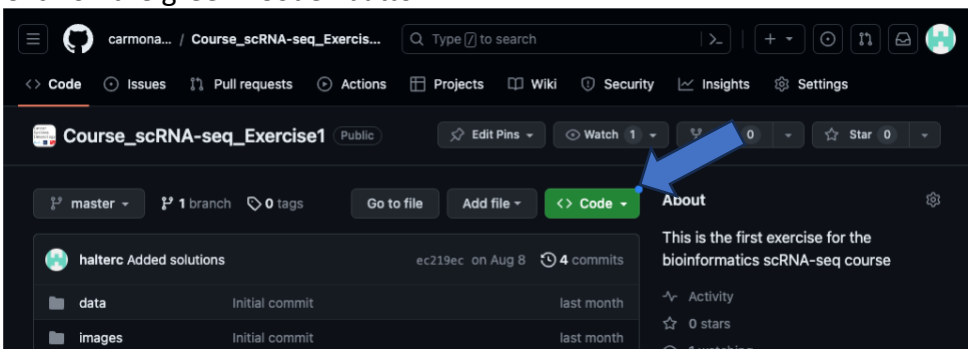
Auto-save

Another very useful function to prevent data loss in case R hangs itself up (e.g. due to out-of-memory or some other freezing error) is located here:

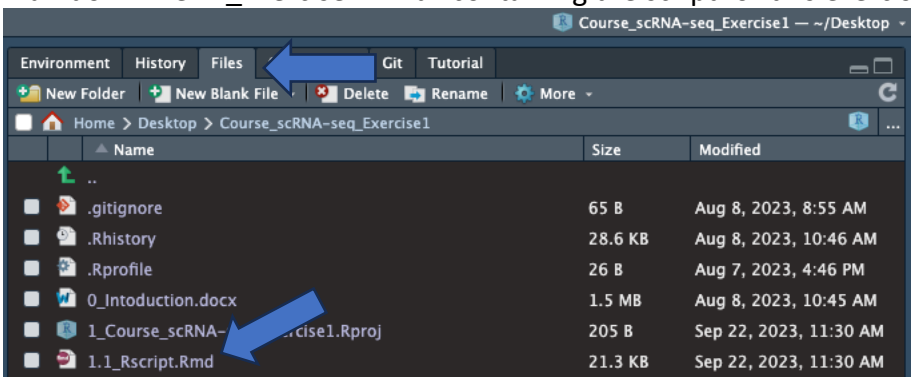


Exercise 1

- Clone the GitHub repository for exercise 1 to your computer
  - Open this link: https://github.com/carmonalab/Course_scRNA-seq_Exercise1
  - Click on the green “Code” button:



  - Select *Download ZIP*
  - Open the downloaded ZIP file containing the exercise repo and extract it anywhere you like
  - Open the folder and open the file “0_Course_scRNA-seq_Exercise.Rproj”
  - If there is no script file open in RStudio yet, navigate to “Files” and open the R markdown file “1_Exercise1.Rmd” containing the script for this exercise:



  - The R markdown file can be viewed in the “Source” or “Visual” mode within RStudio. Markdown can help you create nice reports in PDF format. You can select the view mode in the top-left corner: