**Theory and Practice in gene expression analysis**

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**Dates**:

* 14.11.2023
* 15.11.2023
* 17.11.2023

**NOTE**: the exercises in this part will use R. Please [install R](https://cran.r-project.org/) version 4.3.1 and [RStudio](https://posit.co/download/rstudio-desktop/), if you have not installed it already. 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** bioinformaticsexperiment
* **Cell type classification and annotation**
* **Combining datasets and correcting for batch effects**

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# 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](https://satijalab.org/seurat/articles/pbmc3k_tutorial) as well as for more [advanced topics](https://satijalab.org/seurat/articles/get_started.html). 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.

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**Before**

**After**

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.](https://pubmed.ncbi.nlm.nih.gov/31217225/)

# 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 of .RData

By default, RStudio has an undiserable 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 behaviour off, go to:

*Tools -> Global options … ->* and set the settings like in the figure below:

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## 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:

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* + 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 “1\_Course\_scRNA-seq\_Exercise.Rproj”
  + If there is no script file open in RStudio yet, navigate to “Files” and open the R markdown file “1.1\_Exercise1\_Rscript.Rmd” containing the script for this exercise:

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* + The R markdown file is intended to be viewed in the “Visual” mode within RStudio, so make sure to select it in the top-left corner:

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