MBC
Medical Bioinformatics Centre

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Analysing single-cell data using R

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Outline

- Session 1 (1.5h)
  - Brief introduction to single-cell analysis tools
  - Basic analysis workflow
  - Reading in data and QC
  - Normalization and highly variable features
  - Clustering and visualization
  - Integration

- Session 2 (1h)
  - Automatic annotation
  - Differential expression analysis
  - Trajectory analysis with Totem

- Session website: https://github.com/elolab/Olissipo_hands-on_session2

Single-cell analysis resources in R

- Orchestrating Single-Cell Analysis with Bioconductor (OSCA)
  - http://bioconductor.org/books/release/OSCA/
  - Teaches users some common workflows for the analysis of single-cell RNA-seq data (scRNA-seq)
  - Shows you how to make use of cutting-edge Bioconductor tools to process, analyze, visualize, and explore scRNA-seq data

- Seurat
  - https://satijalab.org/seurat/index.html
  - Seurat is an R package designed for QC, analysis, and exploration of single-cell RNA-seq data
  - Seurat aims to enable users to identify and interpret sources of heterogeneity from single-cell transcriptomic measurements, and to integrate diverse types of single-cell data

Single-cell analysis resources outside R

- Scanpy
  - https://scanpy.readthedocs.io/en/latest/index.html
  - Scanpy is a scalable toolkit for analyzing single-cell gene expression data
  - It includes preprocessing, visualization, clustering, trajectory inference and differential expression testing
  - The Python-based implementation efficiently deals with datasets of more than one million cells
- Single-cell best practices
  - https://www.sc-best-practices.org/preamble.html
  - The goal of this book is to teach newcomers and advanced professionals alike, the best practices of single-cell sequencing analysis
  - This book will teach you the most common analysis steps ranging from preprocessing to visualization to statistical evaluation and beyond
- Cell Ranger
  - Cell Ranger is a set of analysis pipelines that process Chromium single cell data to align reads, generate feature-barcode matrices, perform clustering and other secondary analysis, and more

Public scRNAseq data

- Gene Expression Omnibus (GEO)
  - https://www.ncbi.nlm.nih.gov/geo/
  - Contains processed data
  - FASTQ files usually at SRA
    - https://www.ncbi.nlm.nih.gov/sra/
- Single Cell Expression Atlas
  - https://www.ebi.ac.uk/gxa/sc/home
- Single Cell Portal
  - https://singlecell.broadinstitute.org/single_cell

Our data

- Lee et al: Sci Immunol. 2020 Jul 10;5(49):eabd1554.doi: 10.1126/sciimmunol.abd1554
- GSE149689
- 4 healthy controls, 11 COVID-19 patients, 5 flu patients
- Download the data
  - https://bioinfoshare.utu.fi/DataTransfer/Olissipo_hands-on_session2/data
  - Size: 468M
  - Username: btk
  - Password: WzfwWGUMo7

SCIENCE IMMUNOLOGY | RESEARCH ARTICLE

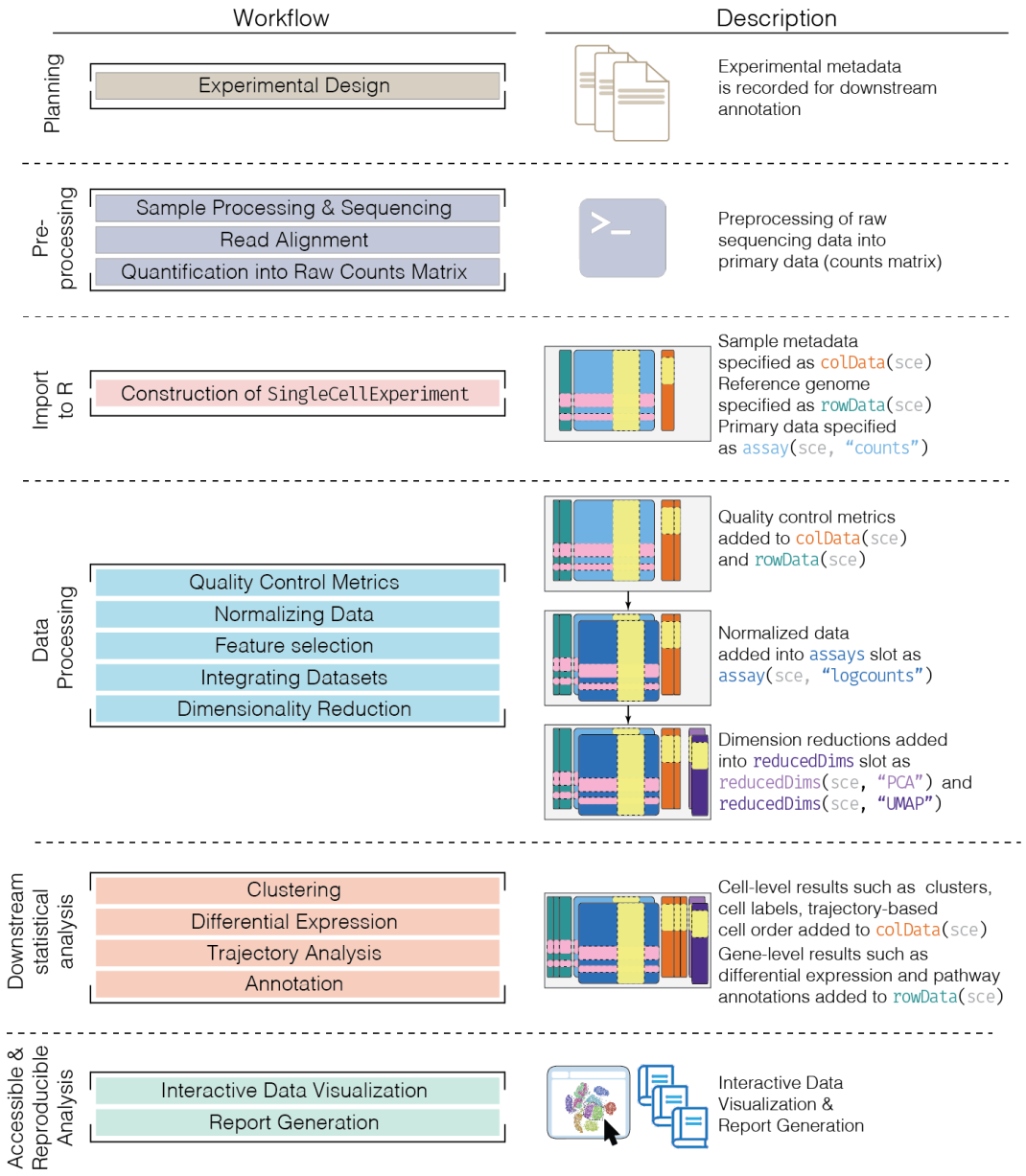
CORONAVIRUS

Immunophenotyping of COVID-19 and influenza highlights the role of type I interferons in development of severe COVID-19

Jeong Seok Lee[1]*, Seongwan Park[2]*, Hye Won Jeong[3]*, Jin Young Ahn[4]*, Seong Jin Choi[1], Hoyoung Lee[1], Baekgyu Choi[2], Su Kyung Nam[2], Moa Sa[1,5], Ji-Soo Kwon[1,6], Su Jin Jeong[4], Heung Kyu Lee[1,5], Sung Ho Park[7], Su-Hyung Park[1,5], Jun Yong Choi[4†], Sung-Han Kim[6†], Inkyung Jung[2†], Eui-Cheol Shin[1,5†]

Basic data analysis workflow

- Script: Lee_data_analysis_with_seurat.R



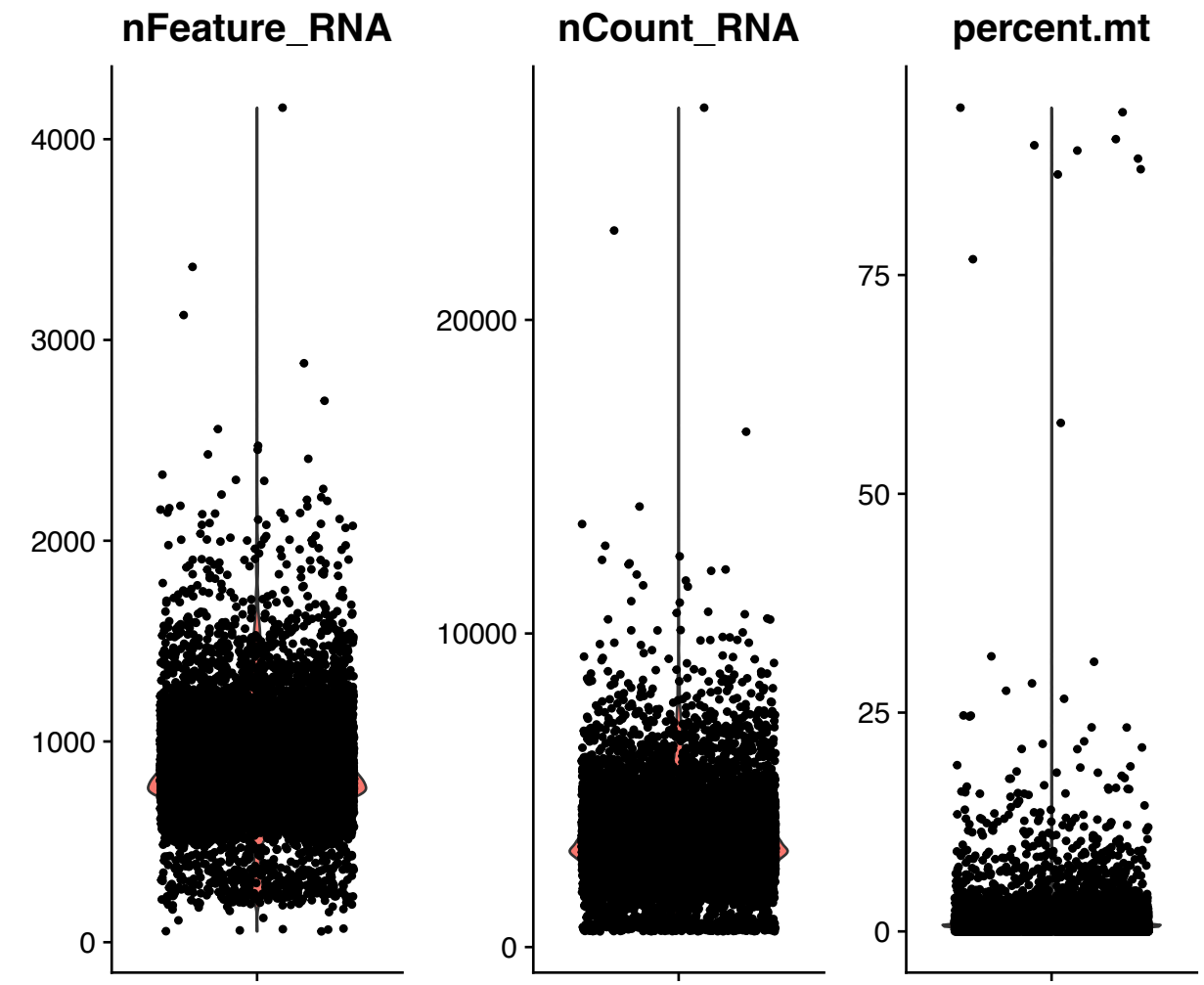
RDatas

- If you have problems running parts of the code, you can download the ready RData objects
- https://bioinfoshare.utu.fi/DataTransfer/Olissipo_hands-on_session2/RData
  - Size: 1.1G
  - Username: btk
  - Password: oKxDvPn9xz

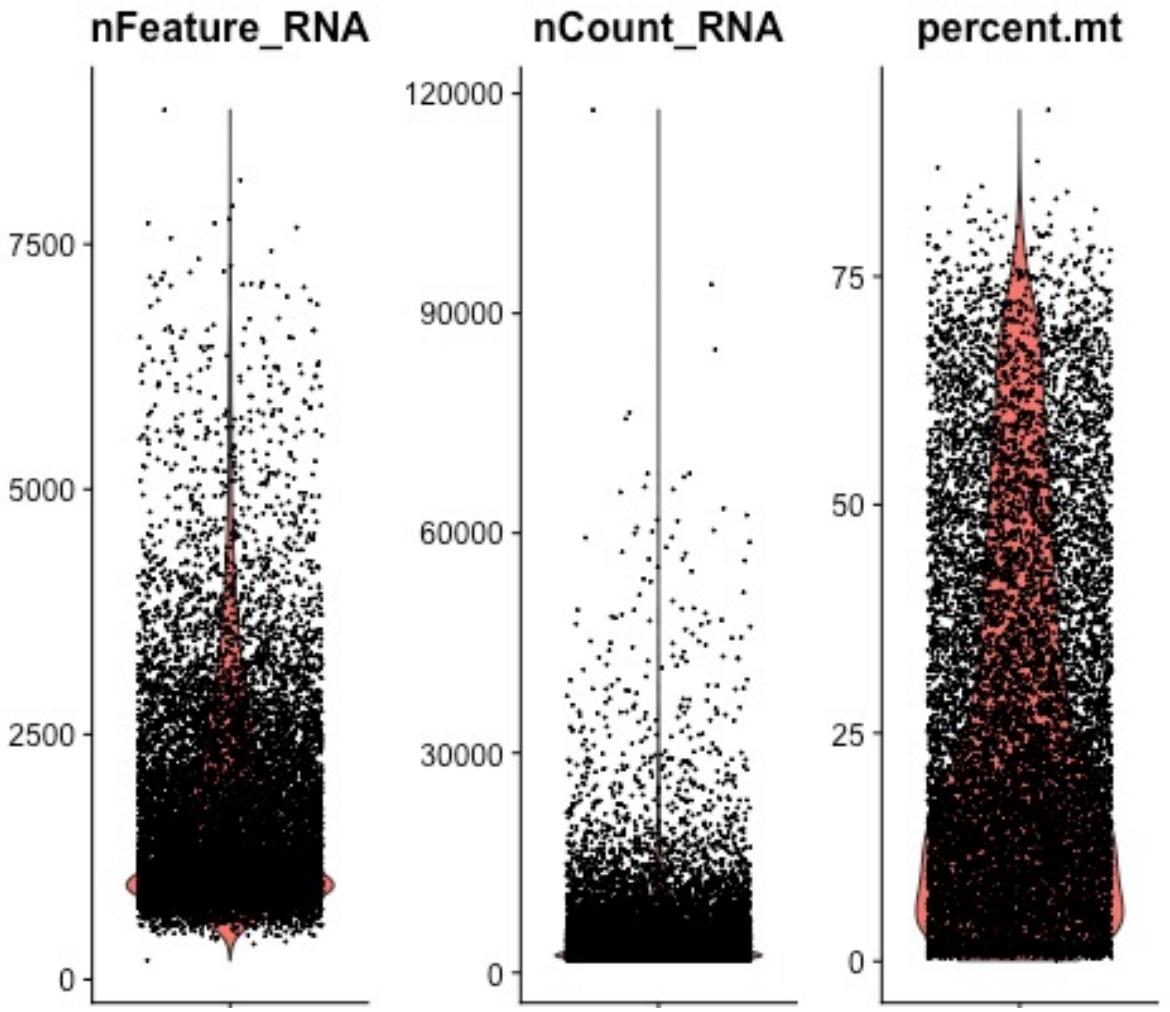
Reading in data and QC

- Usually, raw count data is used as input
- QC is important to filter out low-quality cells
- Percentage of mitochondrial genes, number of genes identified and number of reads/UMI counts is usually checked
- High percentage of mitochondrial genes is indicative of low quality

High-quality data



Not-so-good-quality data



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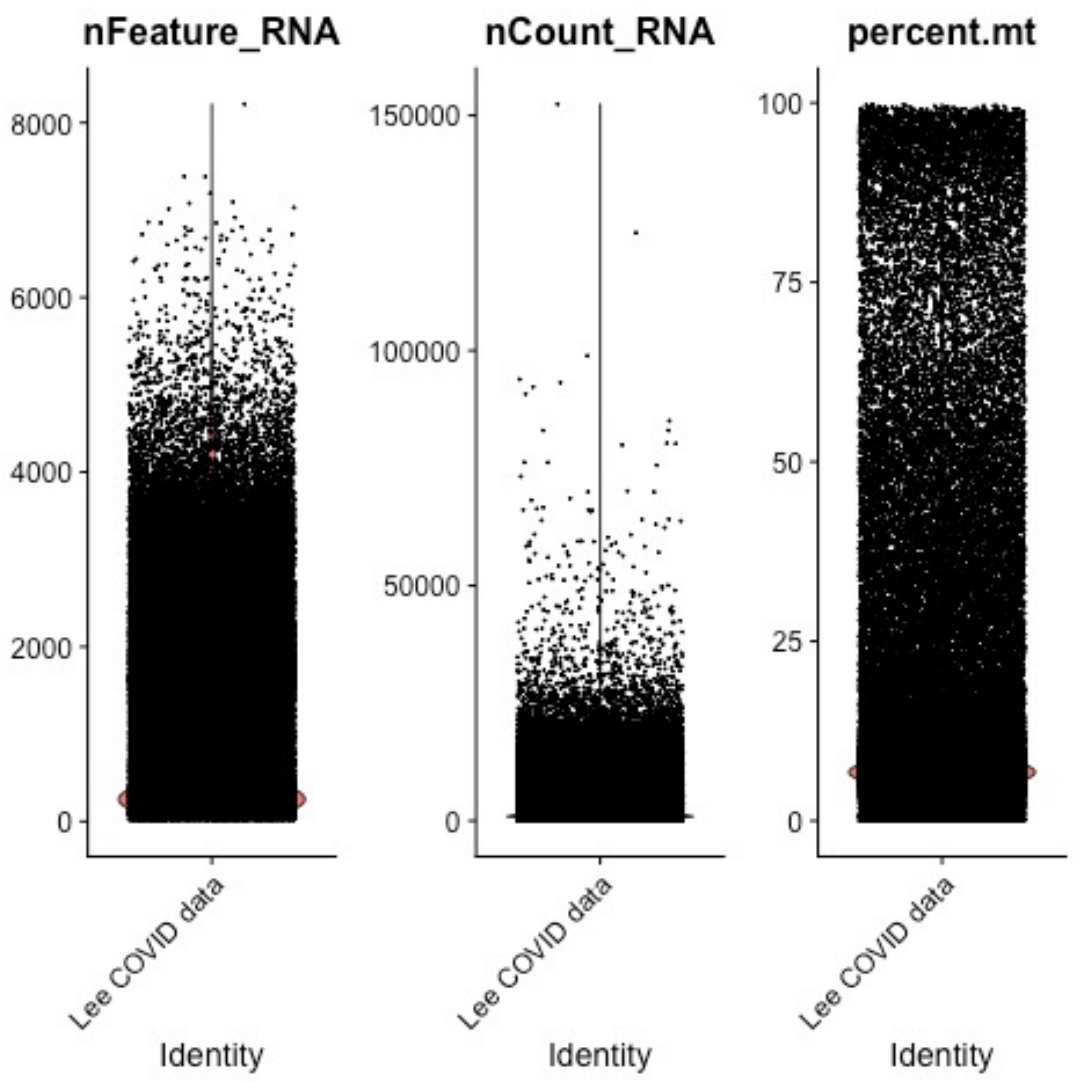
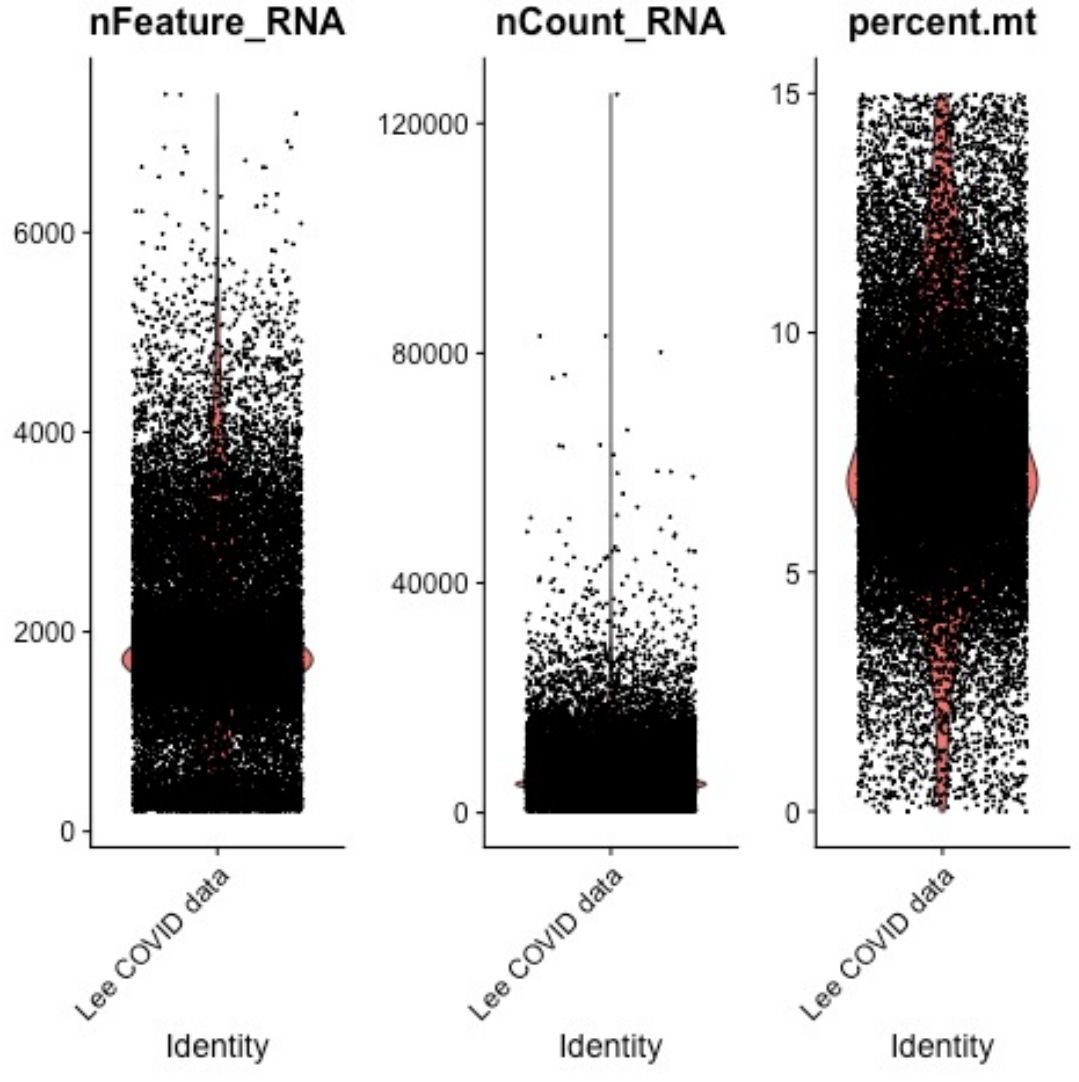
- Run code until line 54
- In the code, the input *.tsv.gz files are in folder called "data"
- All through the code, figures are saved to a folder called "Figures"

- What is your opinion on the data quality?
- How many cells were filtered out?

Reading in data and QC



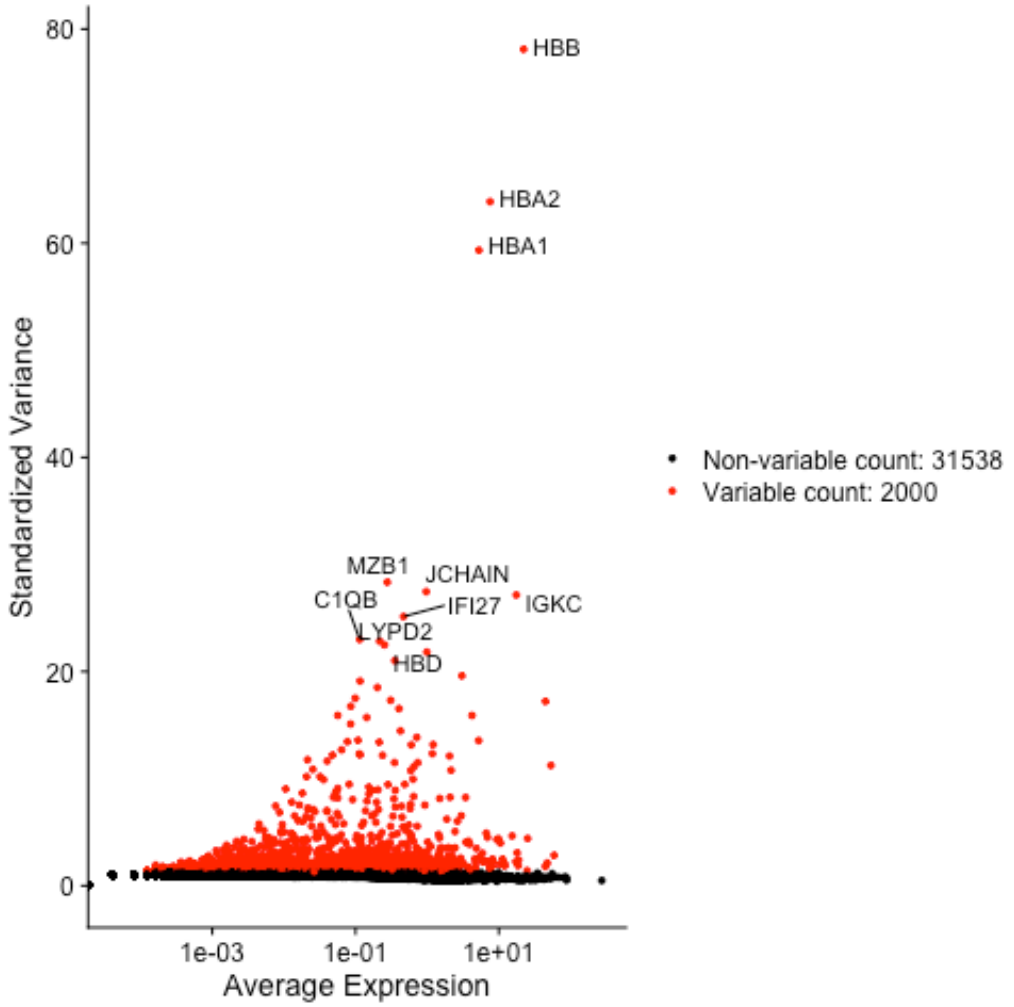


Normalization and highly variable features

- Default normalization method in Seurat is ”LogNormalize”
  - Normalizes the feature expression measurements for each cell by total expression, multiplies this by a scaling factor and performs log transformation
- SCTransform
  - a modeling framework for the normalization and variance stabilization of molecular count data from scRNA-seq experiment
- Identifying highly variable features
  - Find a subset of features that exhibit high cell-to-cell variation

- Run code until line 78
- We’ll keep only four COVID patients and four healthy controls

Normalization and highly variable features



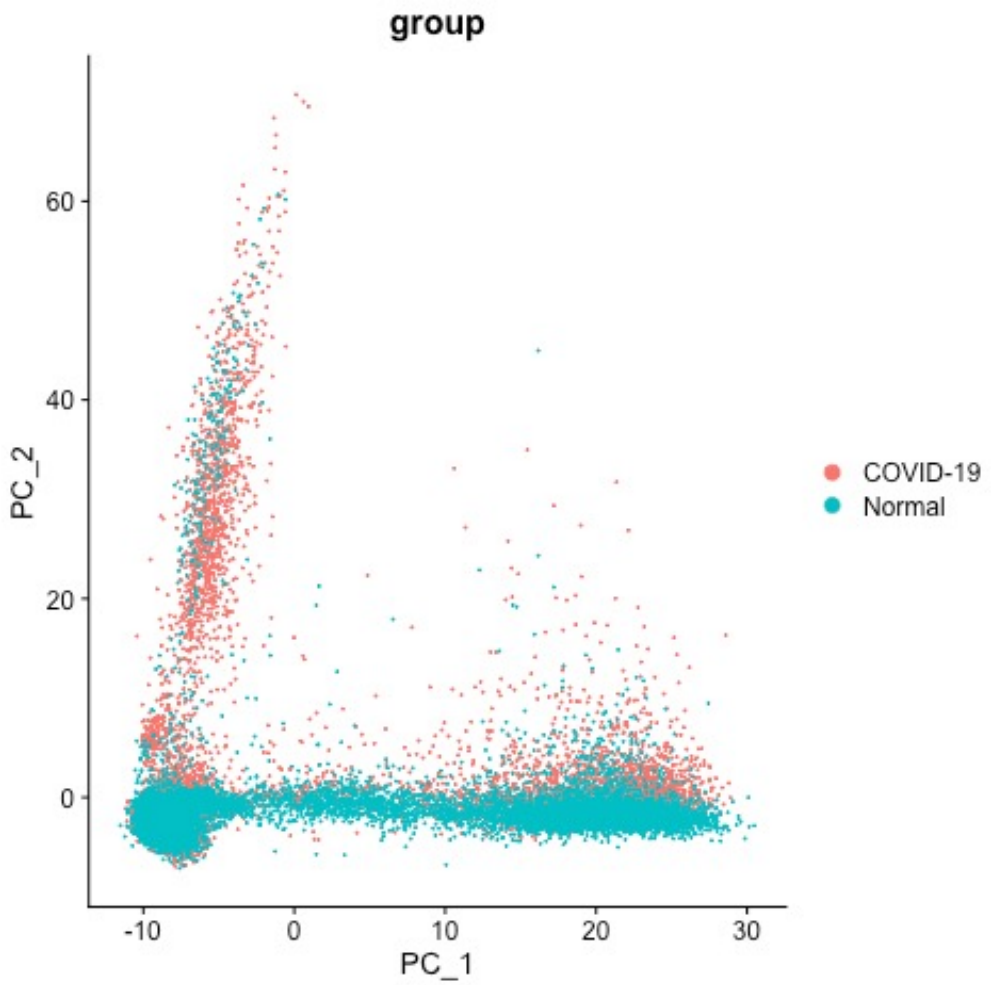
Data scaling and PCA

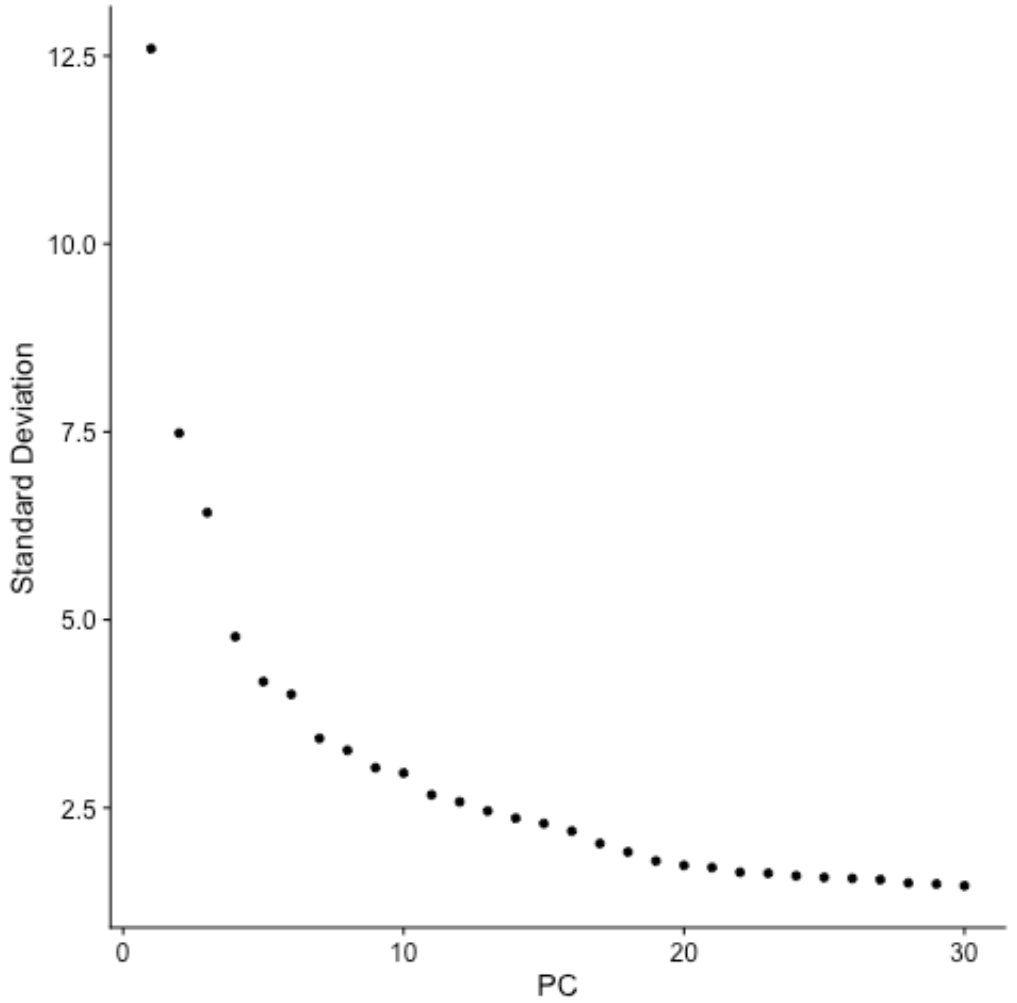
- Scaling applies linear transformation prior to dimensional reduction
- By default, PCA is performed on the variable features
  - Other features can be defined using the features argument

- Top principal components represent a robust compression of the data
- Visualising the "dimensionality" of the data can help to decide how many PCs should be included

- Run code until line 95
- How many PCs would you use?

Data scaling and PCA



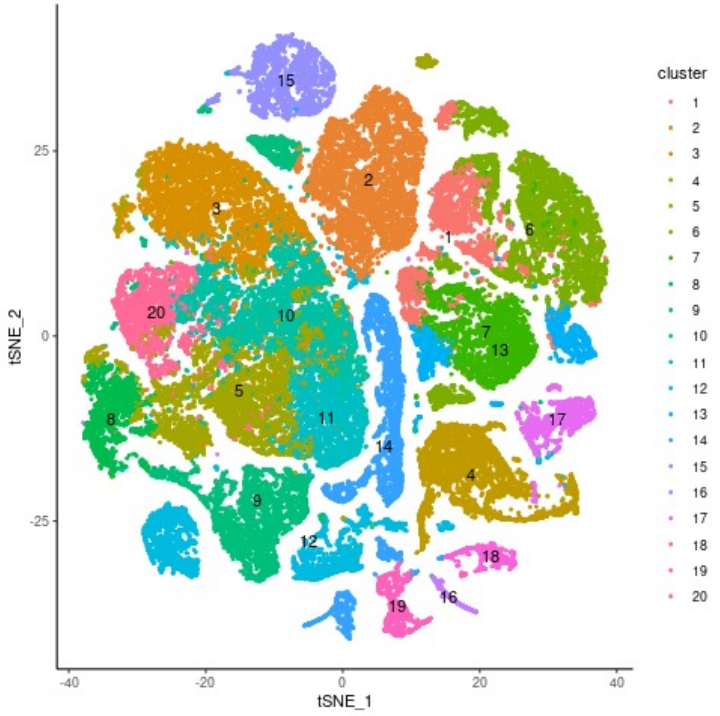


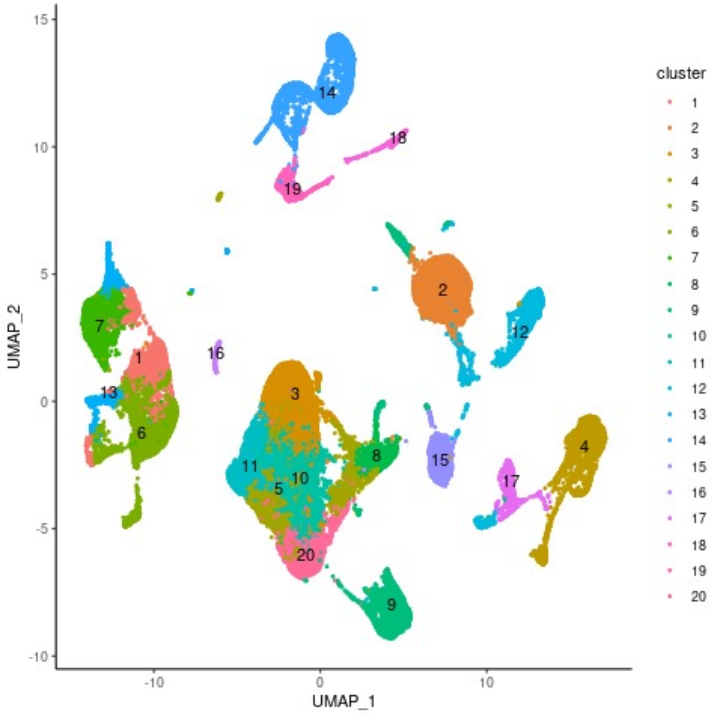
Cell clustering and visualisation

- Clustering is an unsupervised learning procedure that is used to empirically define groups of cells with similar expression profiles
- This allows us to describe population heterogeneity in terms of discrete labels that are easily understood, rather than attempting to comprehend the high-dimensional manifold on which the cells truly reside
- After annotation based on marker genes, the clusters can be treated as proxies for more abstract biological concepts such as cell types or states

Cell clustering and visualisation

- Most popular visualisation methods are t-SNE and UMAP
- t-SNE retains only the local structure
- UMAP attempts to preserve more of the global structure than t-SNE





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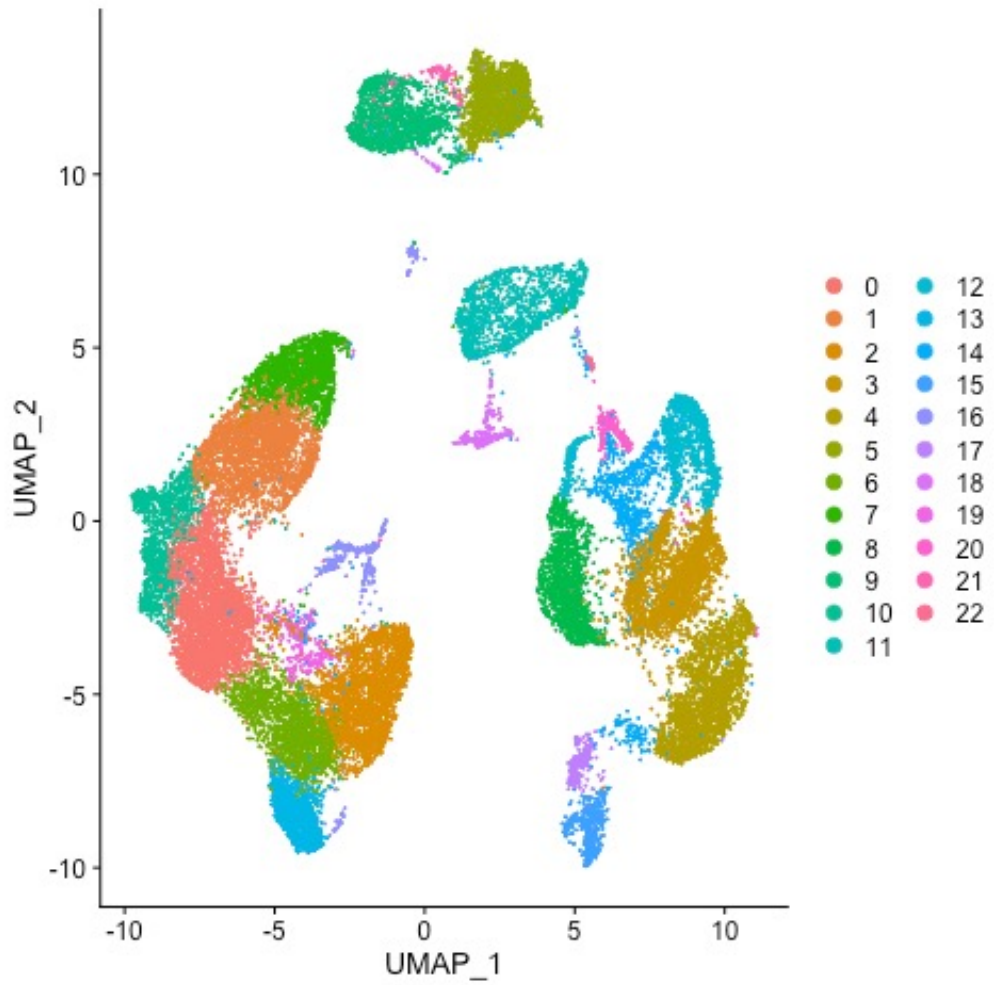
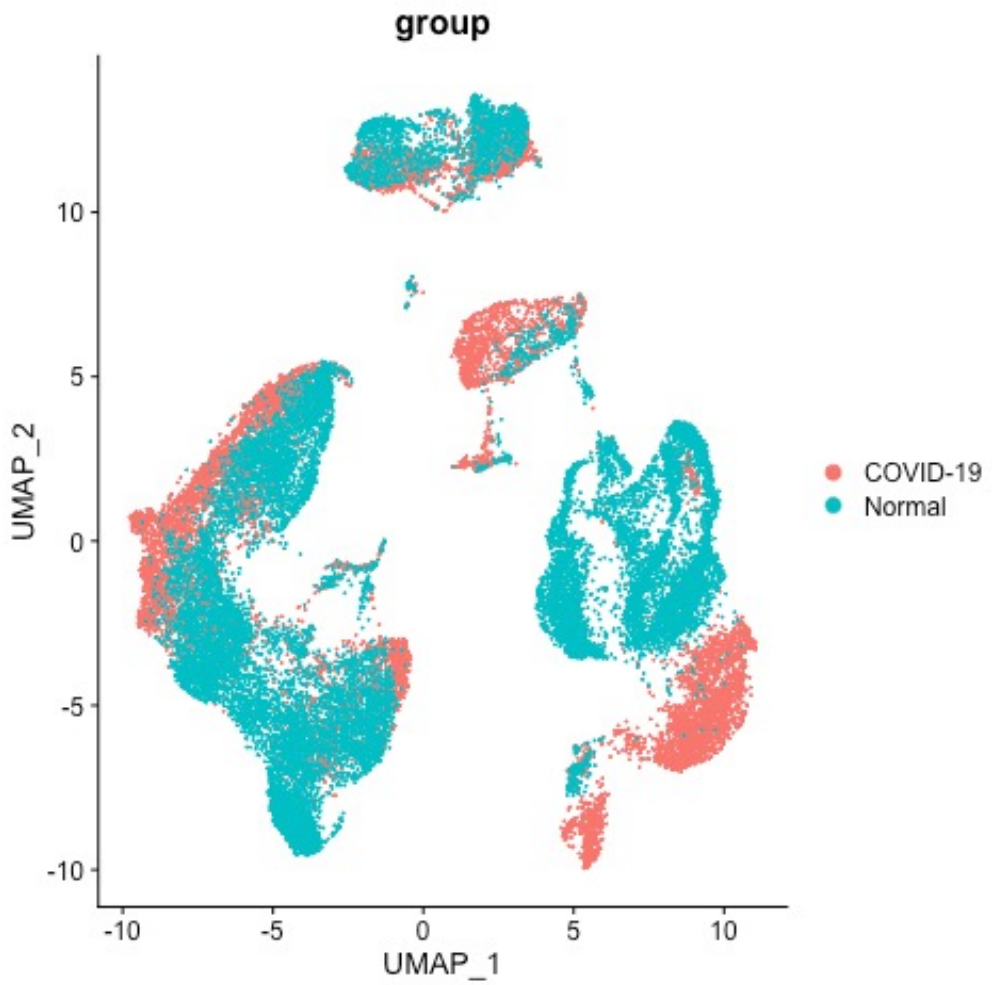
- Run code until 124
- Are there batch effects in these data? Do you think integration is necessary?

Cell clustering and visualisation



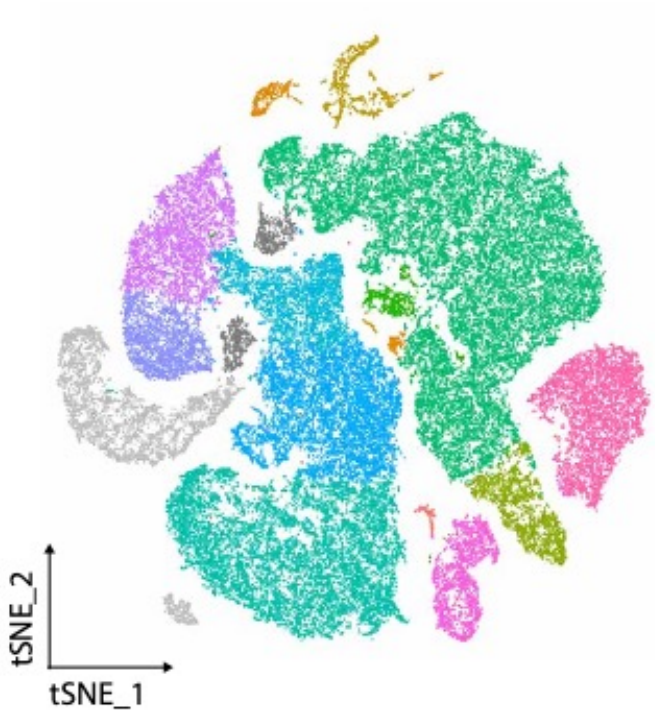


Integrating the data

- When the data contains batch effects (individuals, treatments, library prep…) integration might be needed
- Computational removal of batch-to-batch variation allows us to combine data across multiple batches for a consolidated downstream analysis
- Two integration methods exist in Seurat
  - CCA (Canonical Correlation Analysis)
  - RPCA (Reciprocal PCA)
- Other integration methods are available
  - Harmony, Scanorama, MultiMAP…

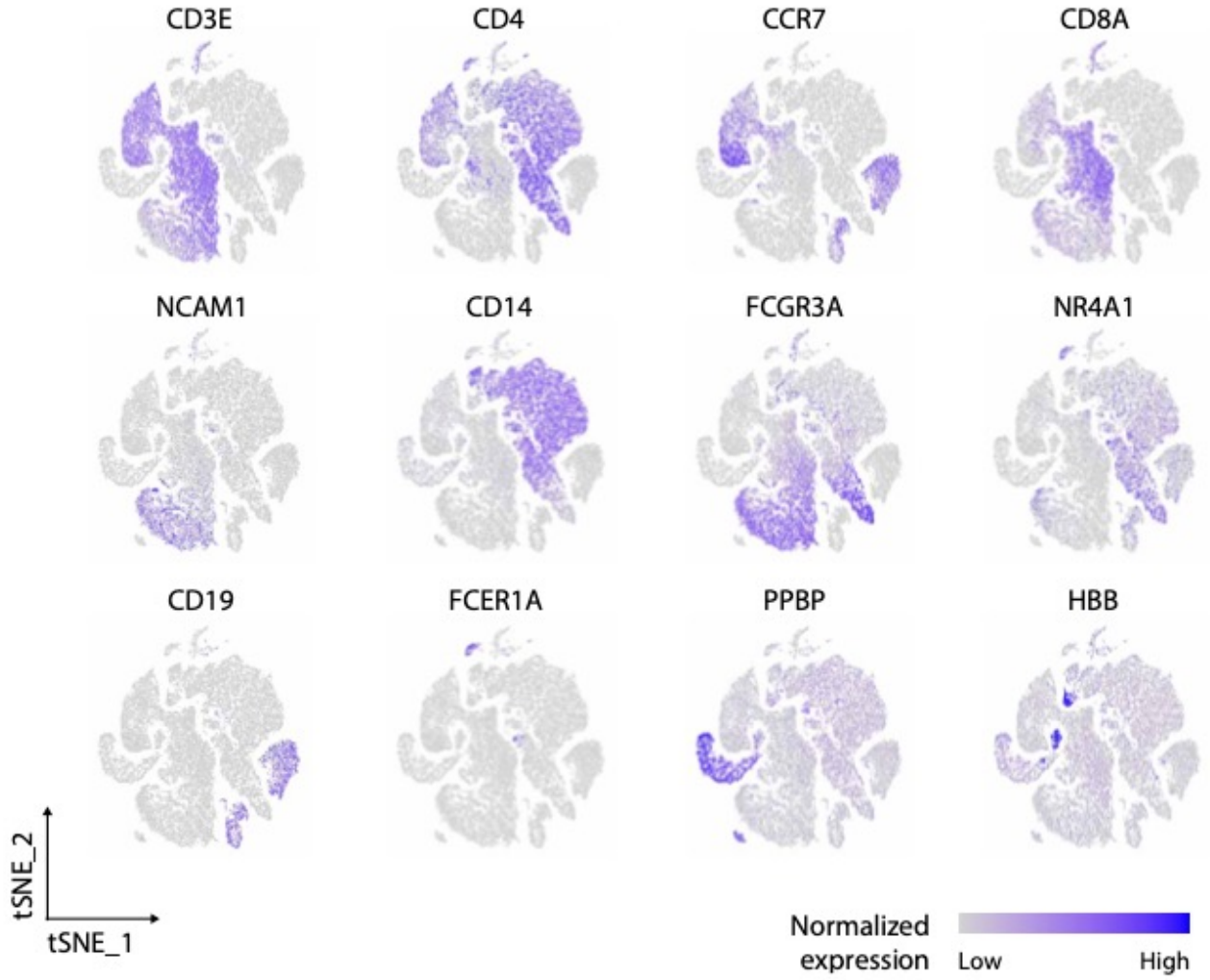
- Run code until line 180
- Do you think the annotation corresponds well with the marker genes?

Marker gene expression in the original publication



Cell type

- B cell, IgG⁻
- B cell, IgG⁺
- CD4⁺ T cell, EM-like
- CD4⁺ T cell, non–EM-like
- CD8⁺ T cell, EM-like
- CD8⁺ T cell, non–EM-like
- NK cell
- Monocyte, classical
- Monocyte, intermediate
- Monocyte, nonclassical
- DC
- Uncategorized 1
- Uncategorized 2
- RBC
- Platelet

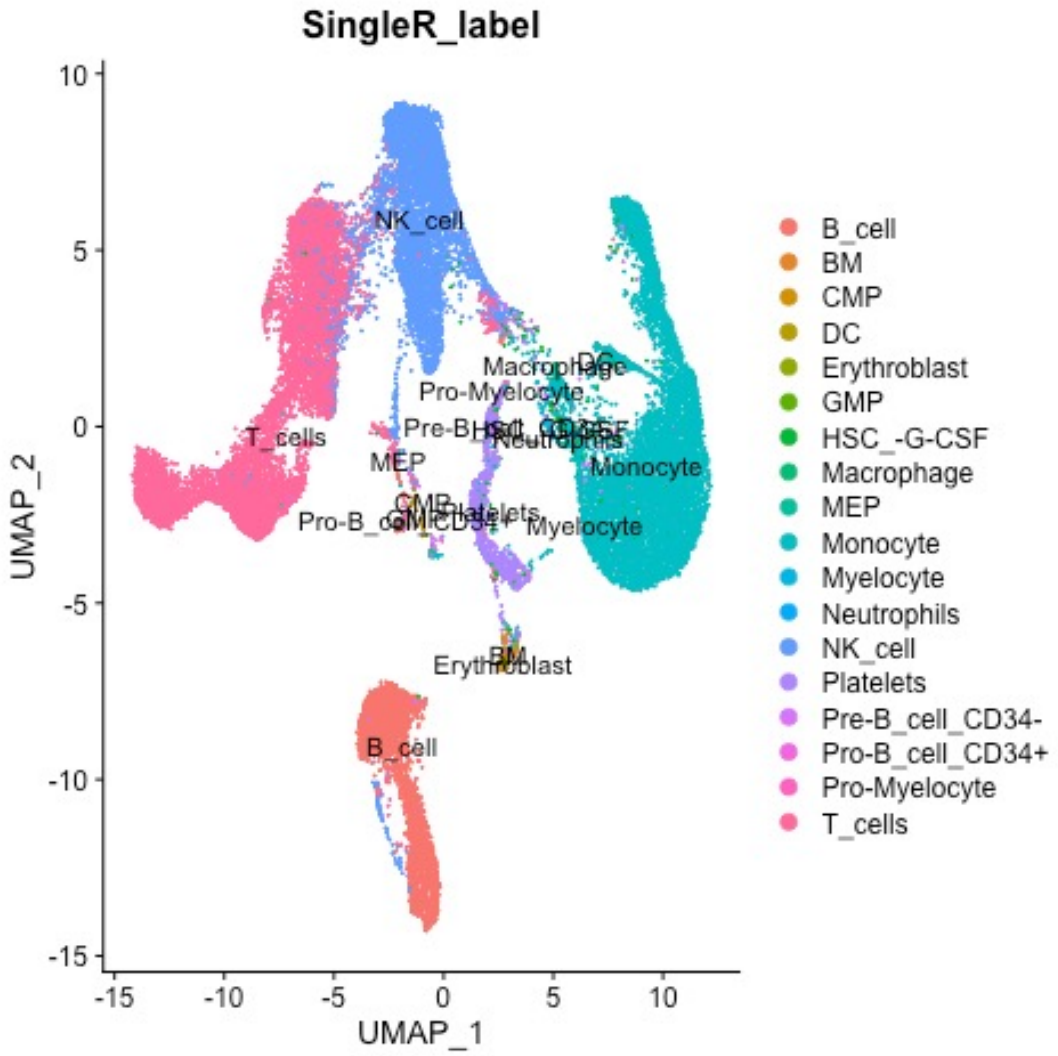


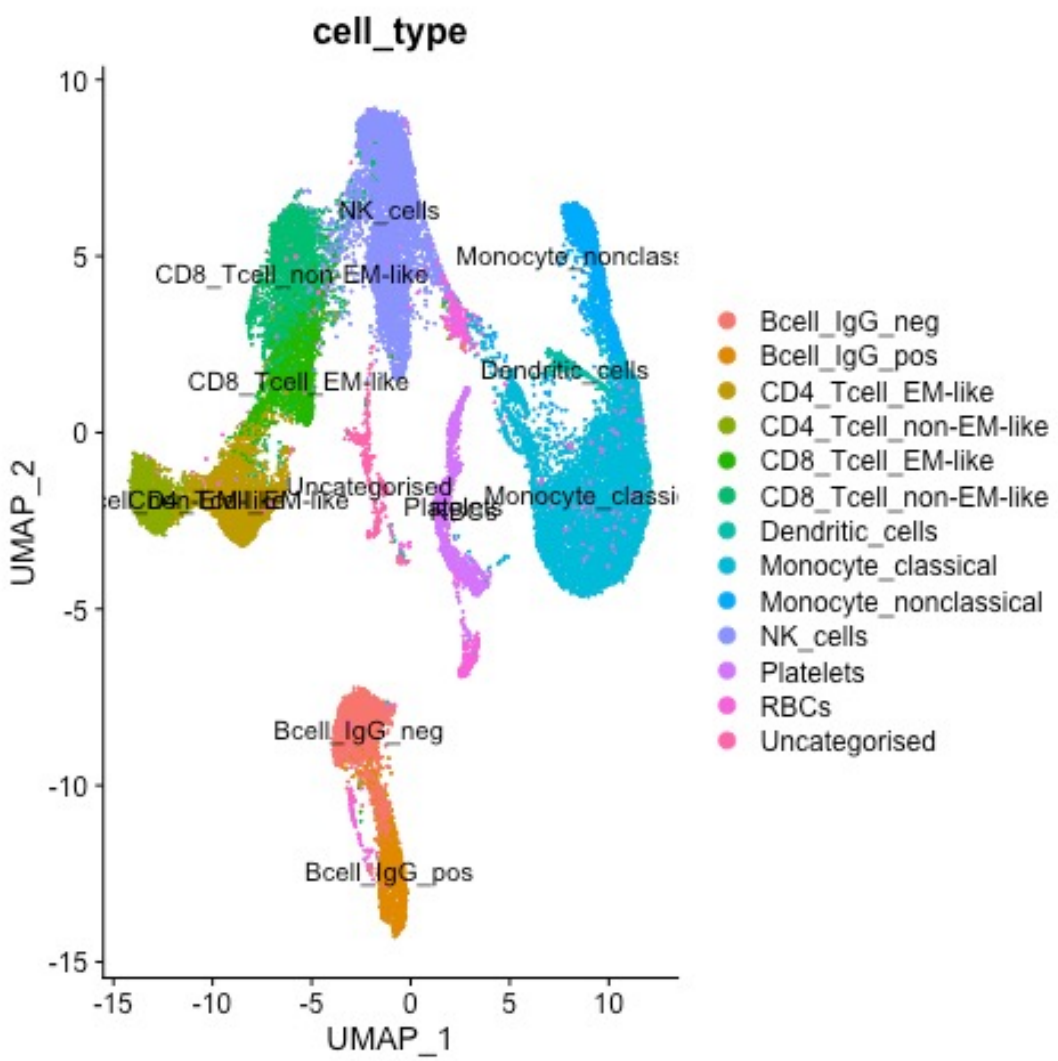
Automatic annotation

- Usually, we don't know the correct cell type for the cells
- Manual annotation is time-consuming and requires expert knowledge
- Automatic annotation can be a fast and easy first step in the annotation process
- Other annotation tools
  - Azimuth (https://azimuth.hubmapconsortium.org)
  - CellTypist (https://www.celltypist.org)

- Run code until line 219
- We'll use SingleR and the Human Primary Cell Atlas for the automatic annotation
- How well did the automatic annotation work?
- What are the main differences to the manual annotation?

Automatic annotation





Differential expression analysis

- Finding marker genes for clusters
  - Compare the gene expression in one cluster to one other cluster or all other clusters

- Identifying differentially expressed genes between conditions
  - Compare the gene expression of samples in one condition to samples in another condition within one cluster
  - Different statistical approaches
    - Naïve methods that use cells as replicates
    - Pseudobulk methods
      - ROTS, limma, DESeq2
    - Mixed models that model the samples as random effect
      - Muscat, MAST_RE

Differential expression analysis

- Run code until line 317
- DE analysis run for dendritic cells and monocytes classical

- How do the DE results differ between the two methods?