



Single cell RNA sequencing data analysis, 27-29 January 2020

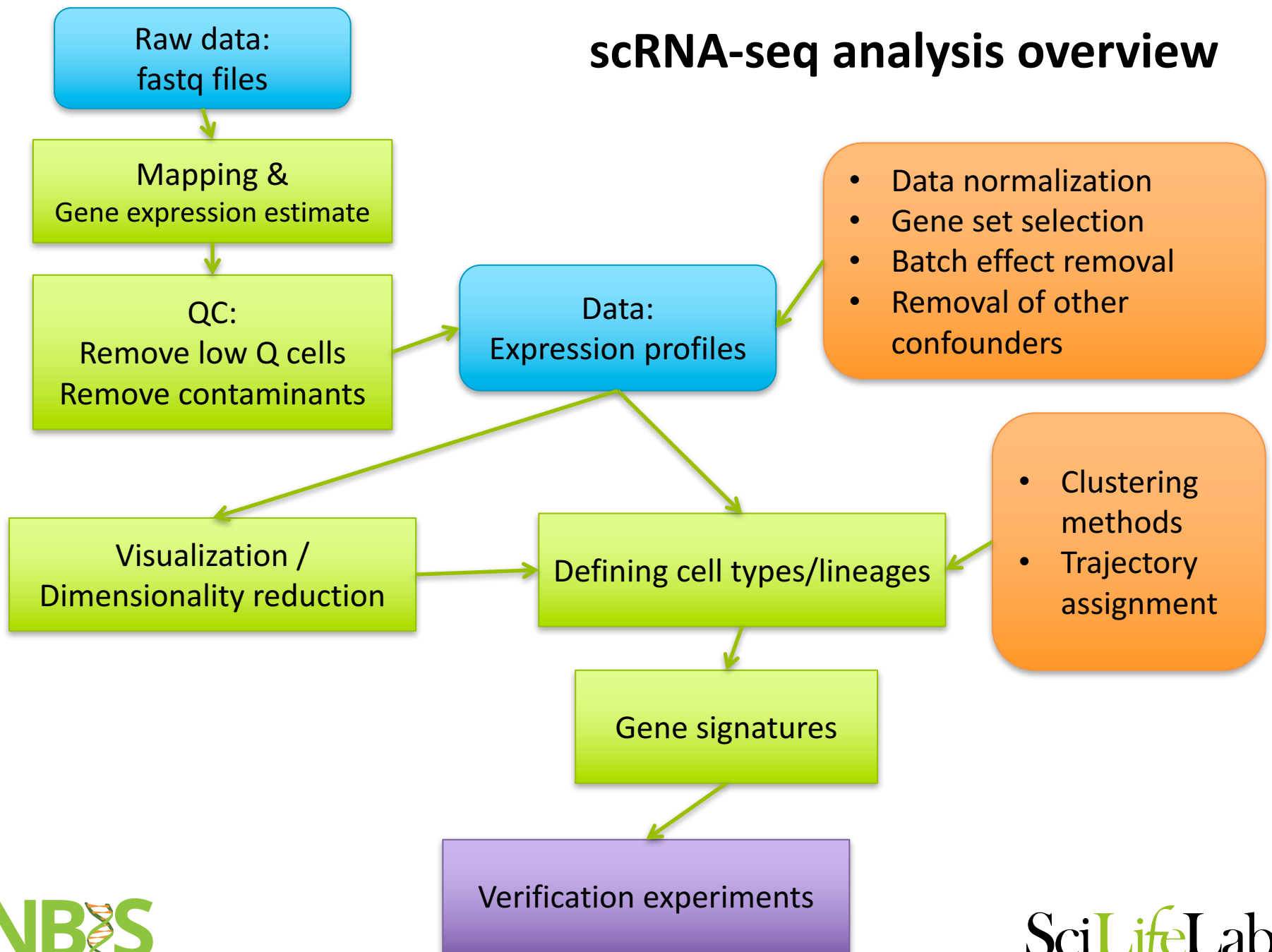
Åsa Björklund

asa.bjorklund@scilifelab.se

Thursday – Bring your own data

- Bring your own data or a publically available dataset from a tissue of interest.
- We will help you import the data into appropriate pipelines.
- During the day we will be around to help you with interpretation of the data.
- At the end we will wrap up and compare experiences.

scRNA-seq analysis overview



Some take-home messages

- Data analysis is very seldom a straight line – one pipeline fits all.
 - Often requires several iterations of filtering data, exploring data, refiltering, exploring again, discovering technical artifacts, normalization, exploring again, etc. etc.

- Get to know your data – what types of variation do you have?
 - PCA is a good tool for exploring data
- Apply appropriate methods to control for problems that you see.

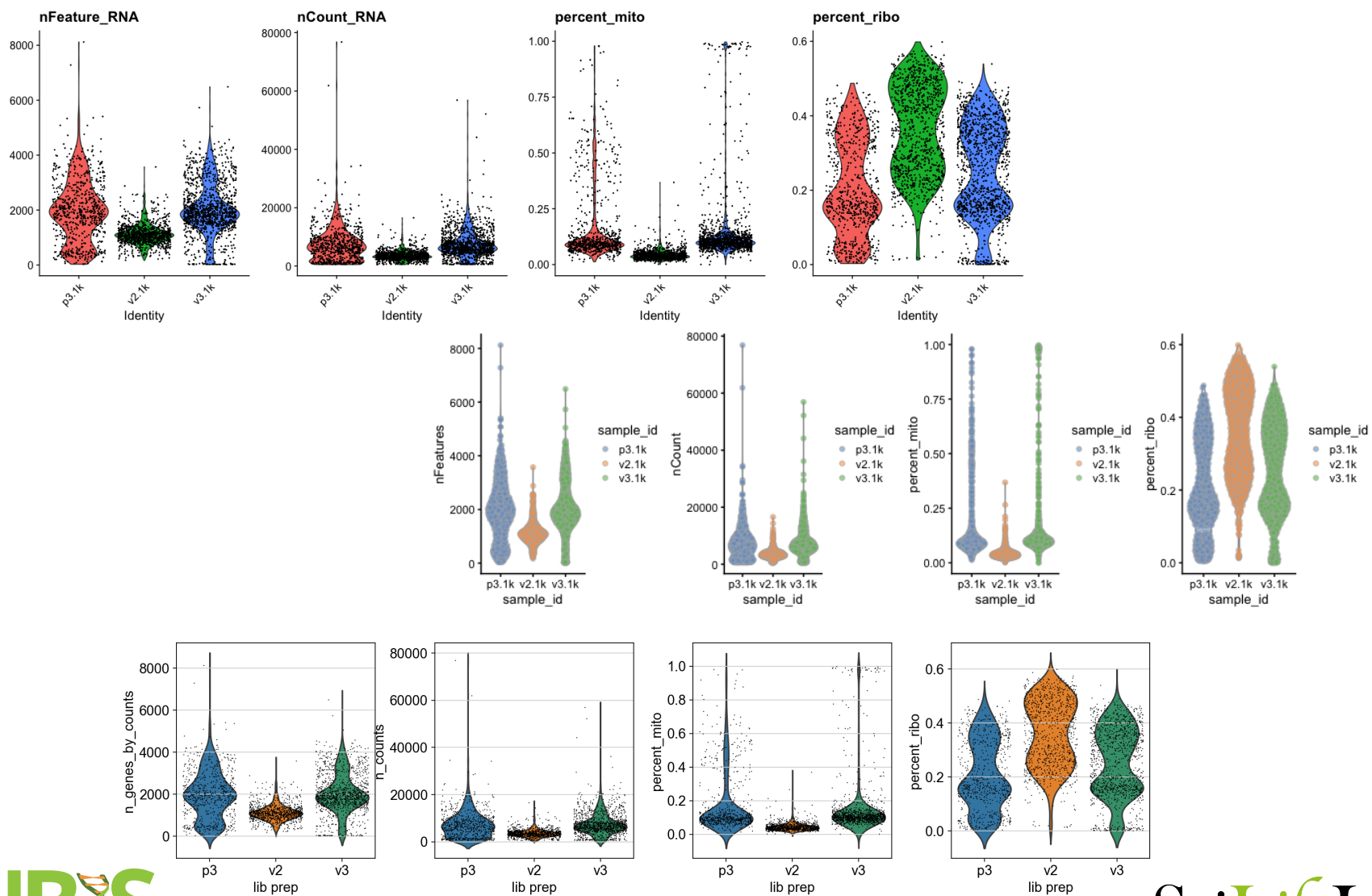
- Always check for:
 - Batch effects – think of all possible batches.
 - Cell cycle effects if appropriate
 - Separation due to nUMI / nGene
- Both at the start of a project and at the end for your final clustering.

- Clustering – try out a few different approaches
 - Consensus of different methods gives confidence
 - If they do not agree – figure out why!

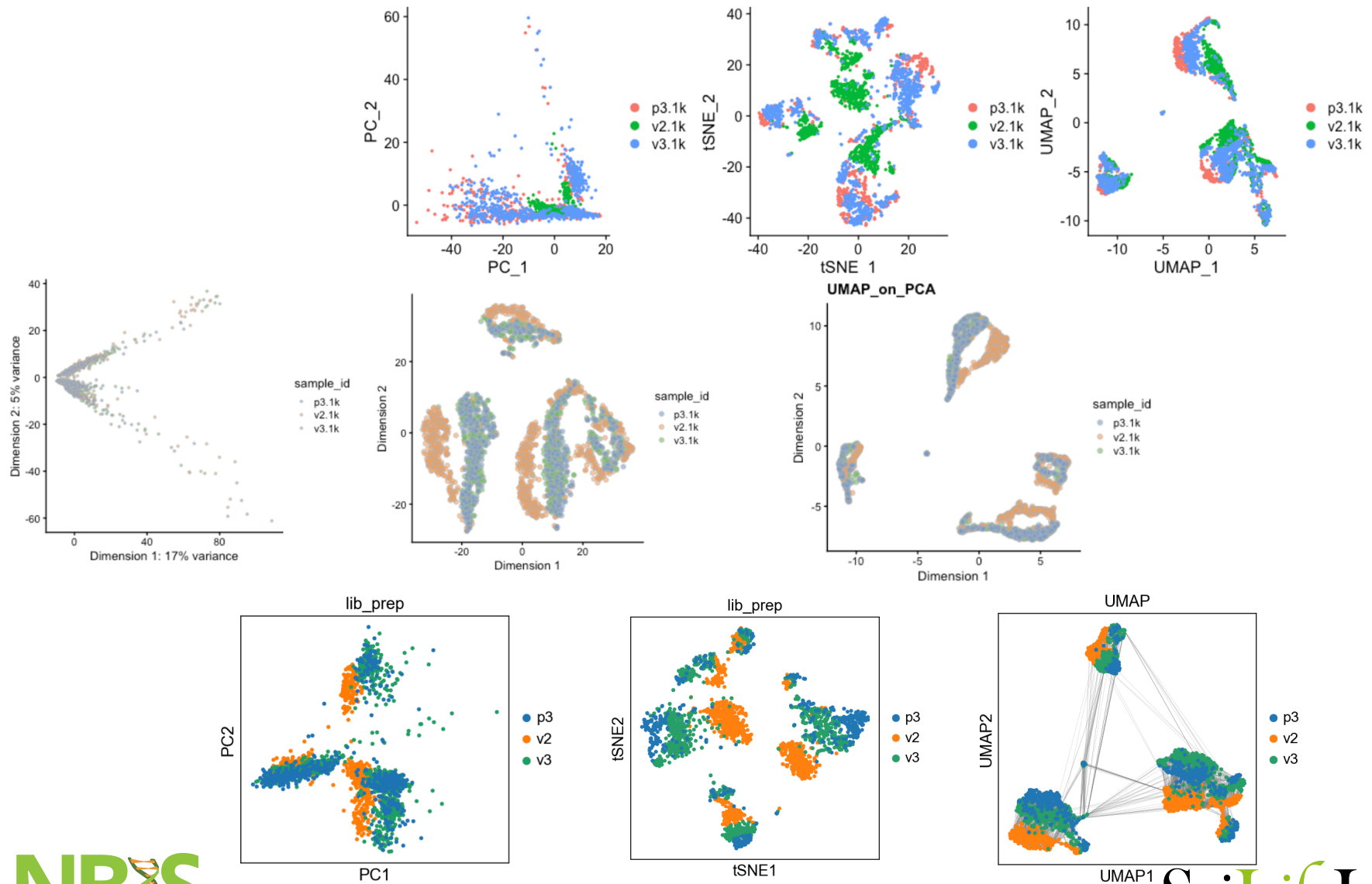
- Use your biological knowledge to evaluate the results
- Warning! Do not overfit your data to fit your initial hypotheses. Keep an open mind ;-)

- scRNAseq analysis is a fast evolving field with new methods being published all the time.
 - Try to keep up with development
 - **BUT!** You cannot test every new method out there!

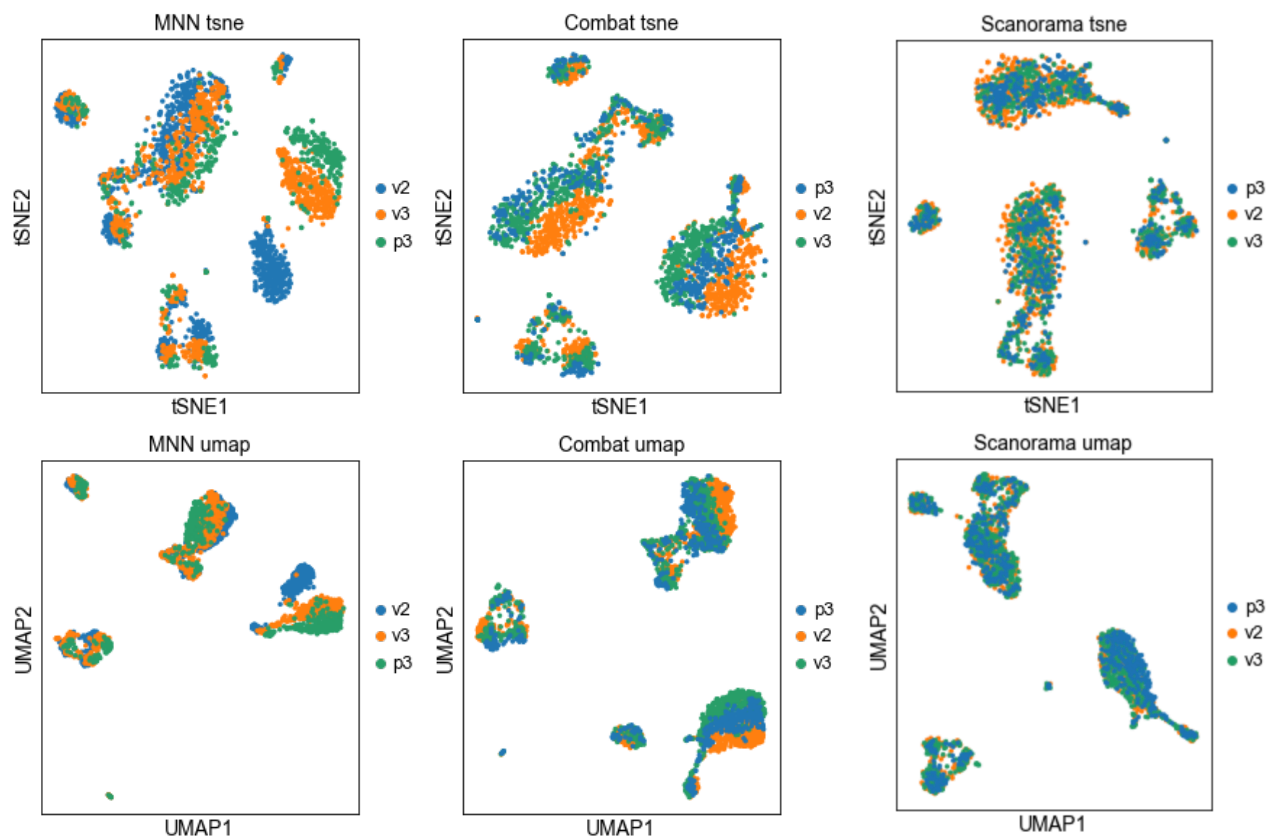
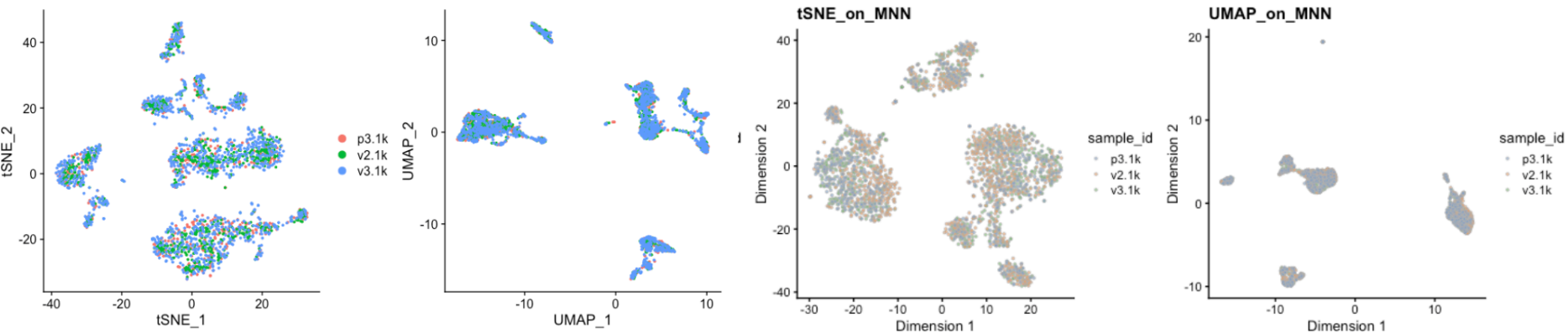
Different pipelines - QC



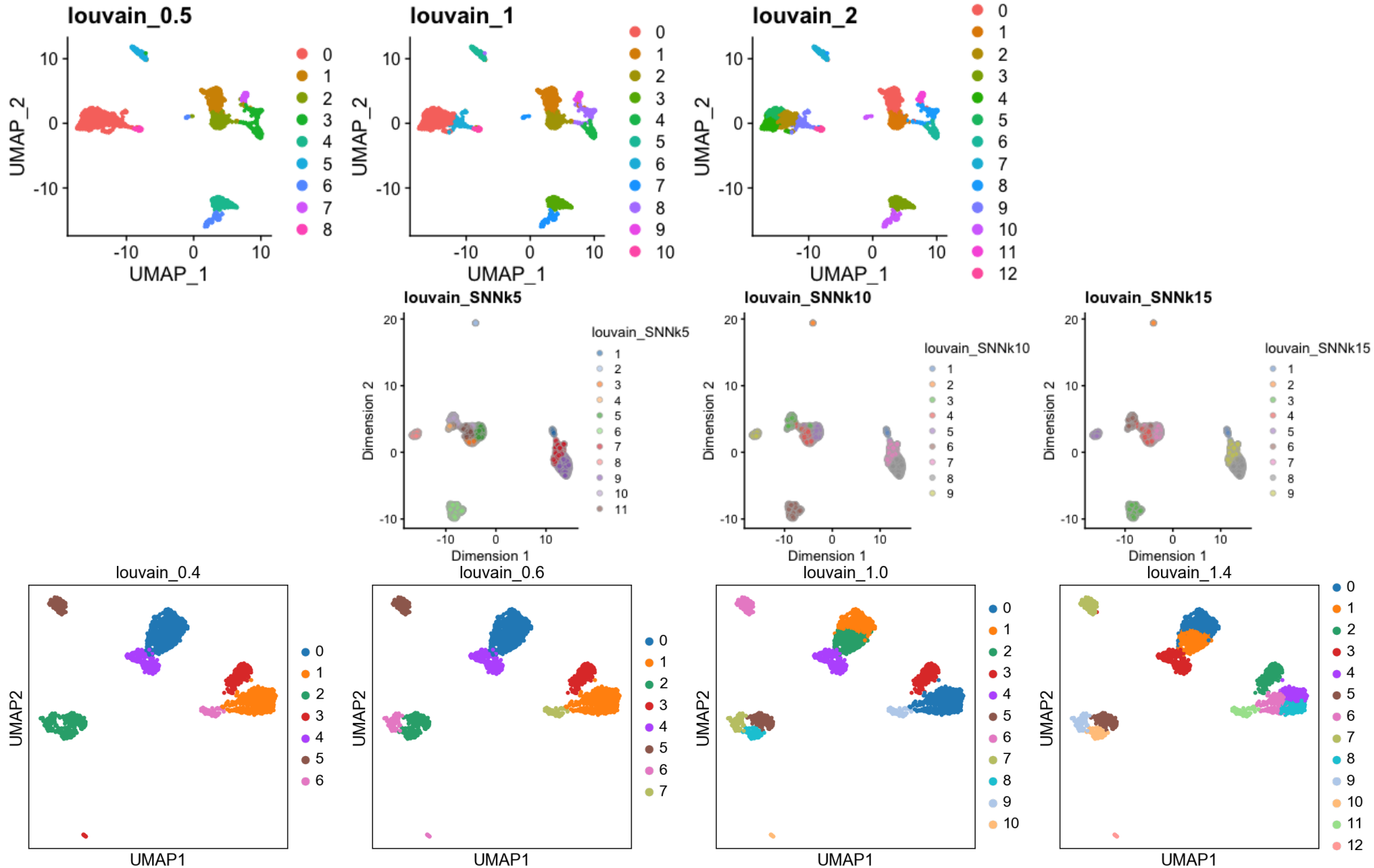
Different pipelines – Dim Reduction



Data pipelines – Data integration

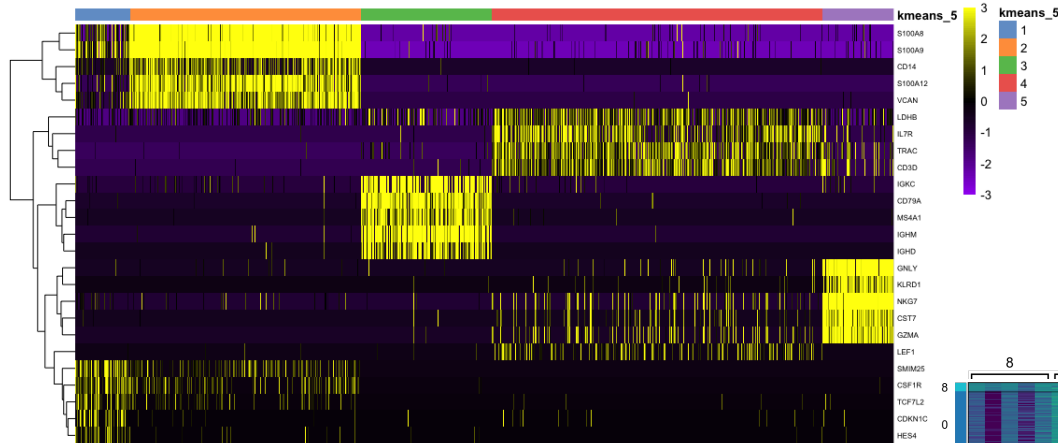
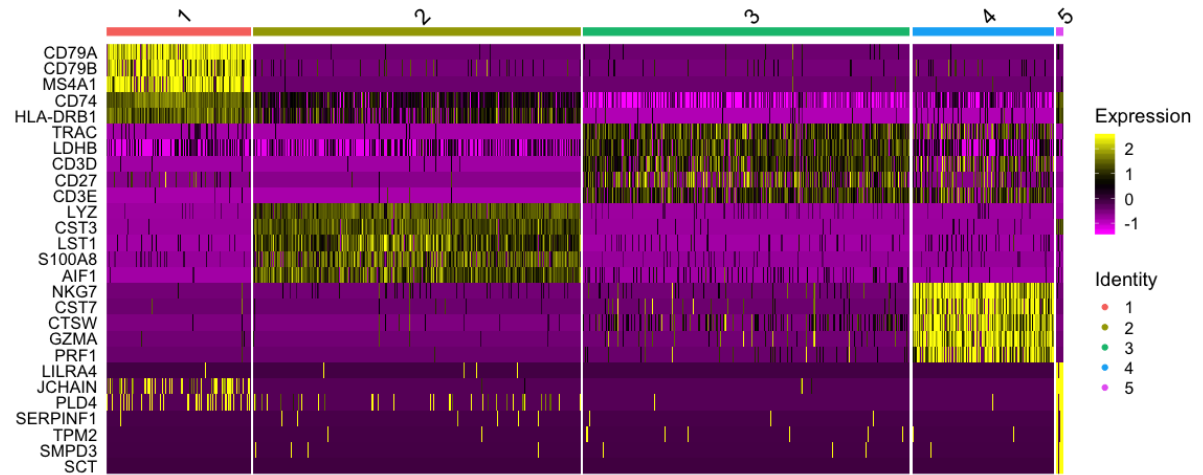


Data pipelines - Clustering



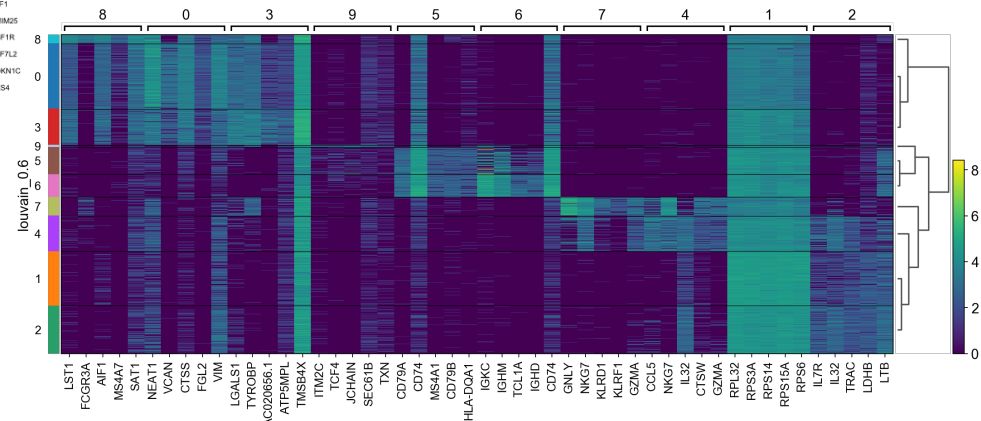
Data pipelines - DE

Seurat wilcox

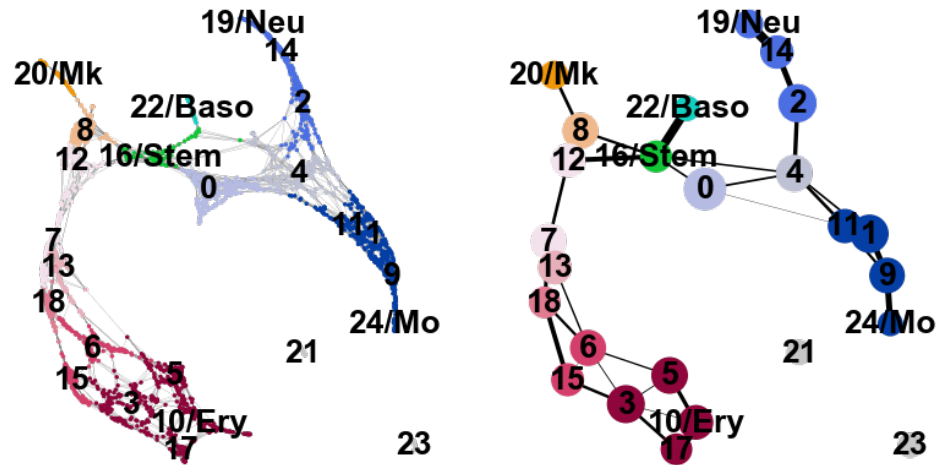
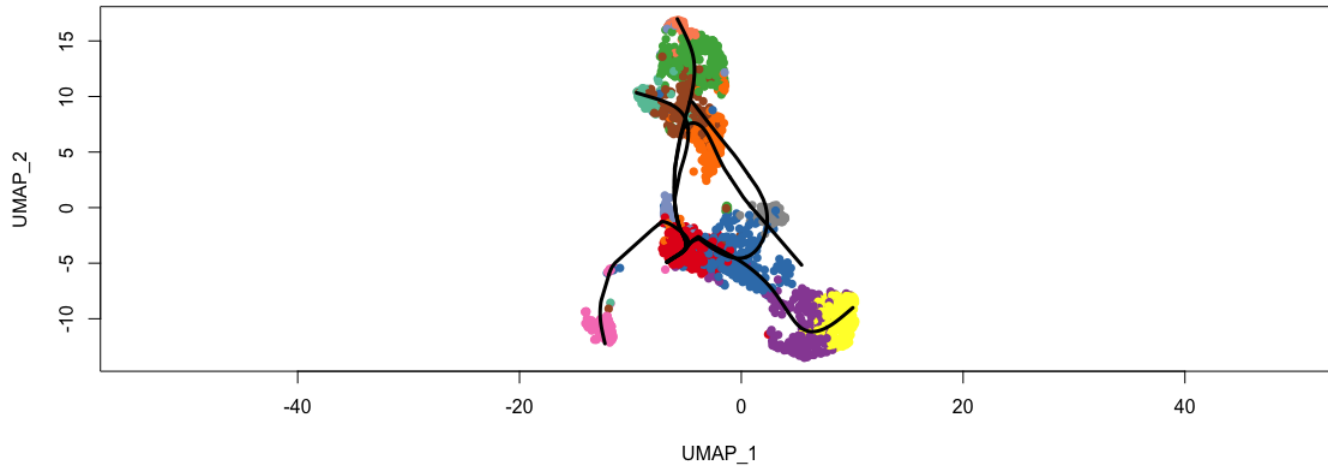


Scanpy wilcox

Scran – pairwiseTtest + combineMarkers



Trajectory – PAGA and Slingshot



Additional comments to the exercises?

Need help?

- NBIS Long term support (aka WABI) – application rounds 3 times a year – 500h for free
- NBIS Short term support – fee for service support. Apply any time.
- Drop-in sessions weekly/bi-weekly at several sites across Sweden
 - SciLifeLab Stockholm – Gamma 2, lunch room, Tuesdays 10.30
 - SciLifeLab Uppsala – Navet floor 3, Thursdays 10.00
 - Umeå, Linköping, Stockholm University, Lund and Göteborg as well.
- More info at: <http://nbis.se/>

Reproducible research in R

- R / Rstudio in Docker containers
 - <https://www.andrewheiss.com/blog/2017/04/27/super-basic-practical-guide-to-docker-and-rstudio/>
 - <https://github.com/rocker-org/rocker>
- OBS! On Uppmax – only Singularity containers are allowed. Most Docker images can be converted.
- Learn more on containers etc:
 - <http://nbis-reproducible-research.readthedocs.io/en/latest/>
- Rstudio package management – Packrat
 - <https://rstudio.github.io/packrat/>
- Conda installations of packages – can use conda on both bianca and rackham – `module load conda`

Please fill in the Evaluation Form

Your feedback is important so that we can help improve the course.

Good luck with your analyses!