# Single cell RNA sequencing data analysis, 4-6 February 2019

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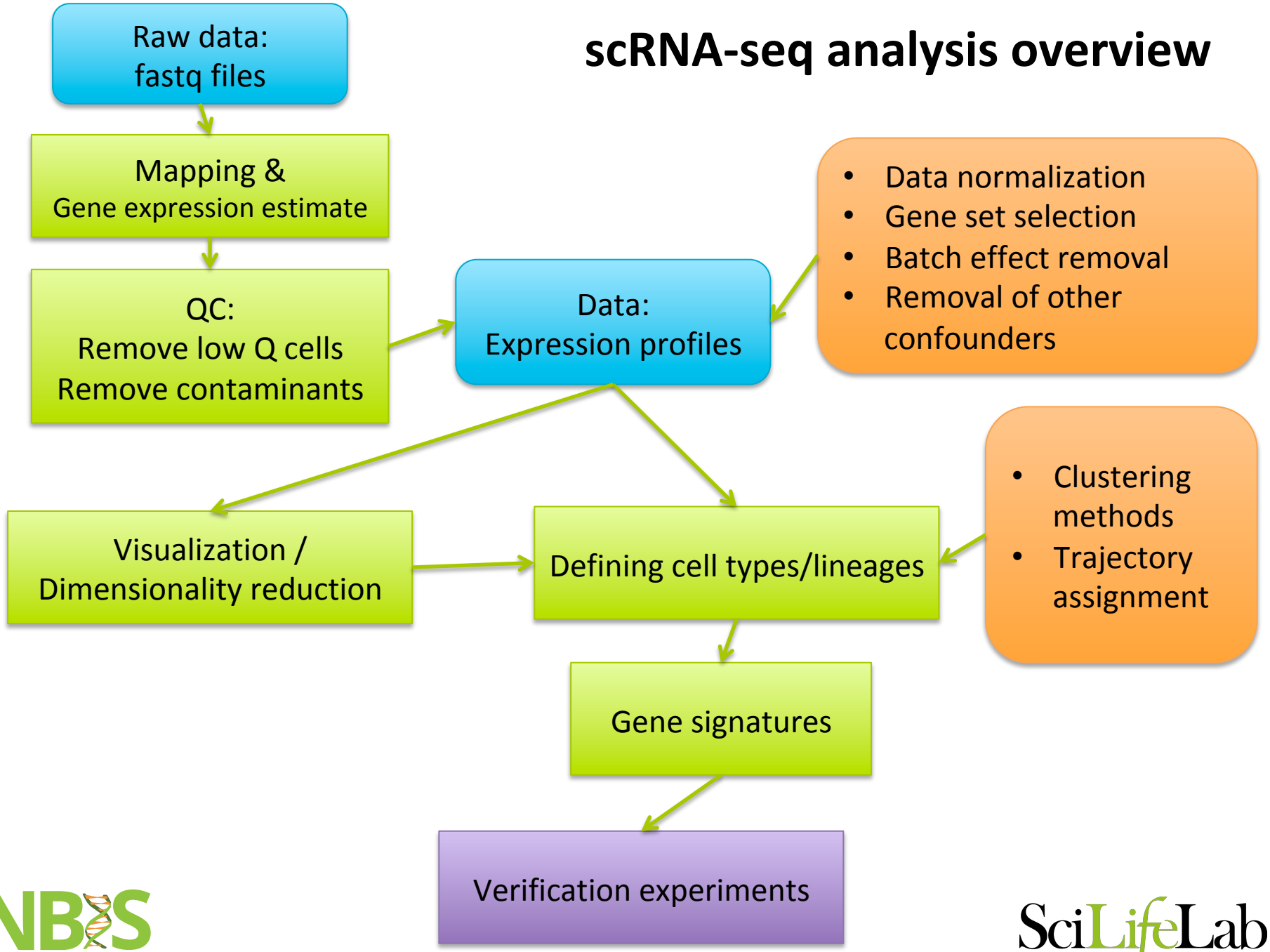
# scRNA-seq analysis overview



# 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!

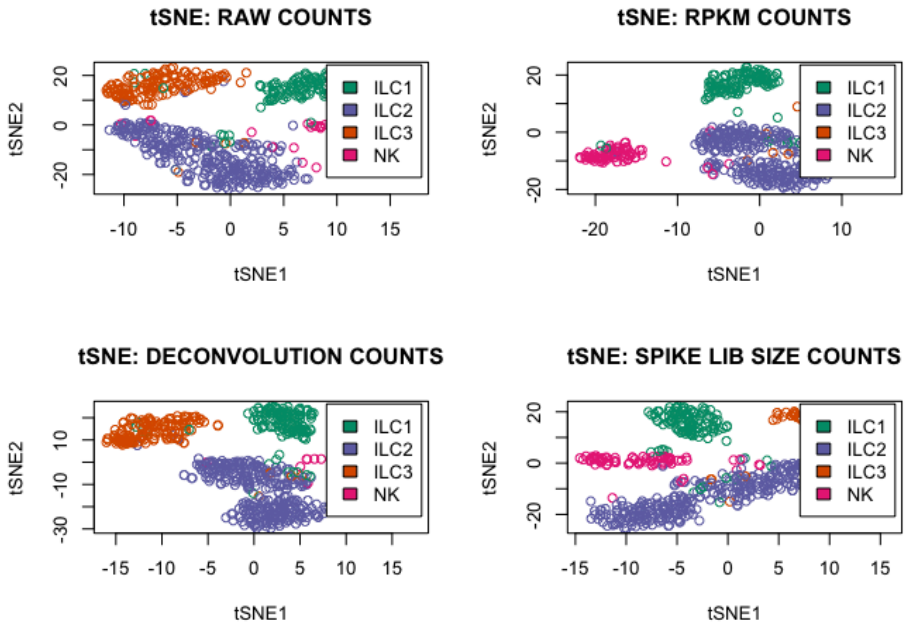
# Practical exercises:

- Pipeline – SmartSeq2
- PCA, tSNE, clustering – own code
- KNN-graphs – igraph package
- QC with Scater package
- Batch effects
- Normalization methods
- SC3 clustering
- Pagoda

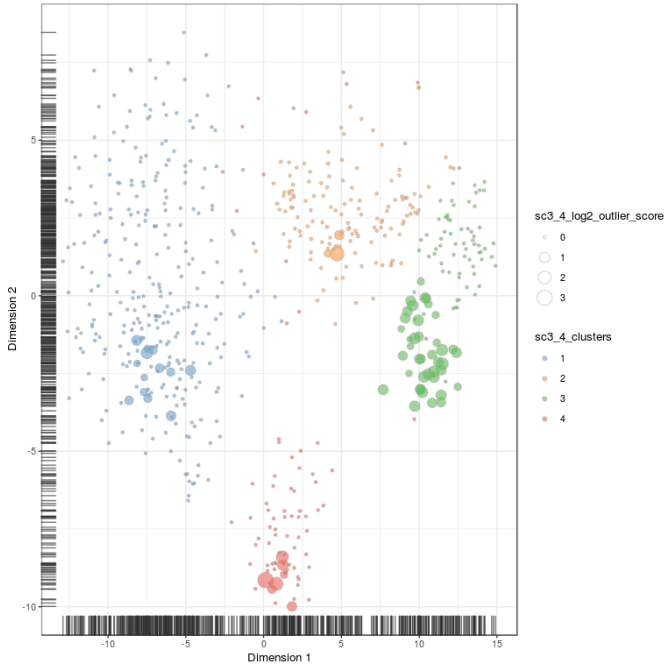
- Seurat package
- Differential expression
- Monocle pseudtime

- Biomart
- Example Sbatch script

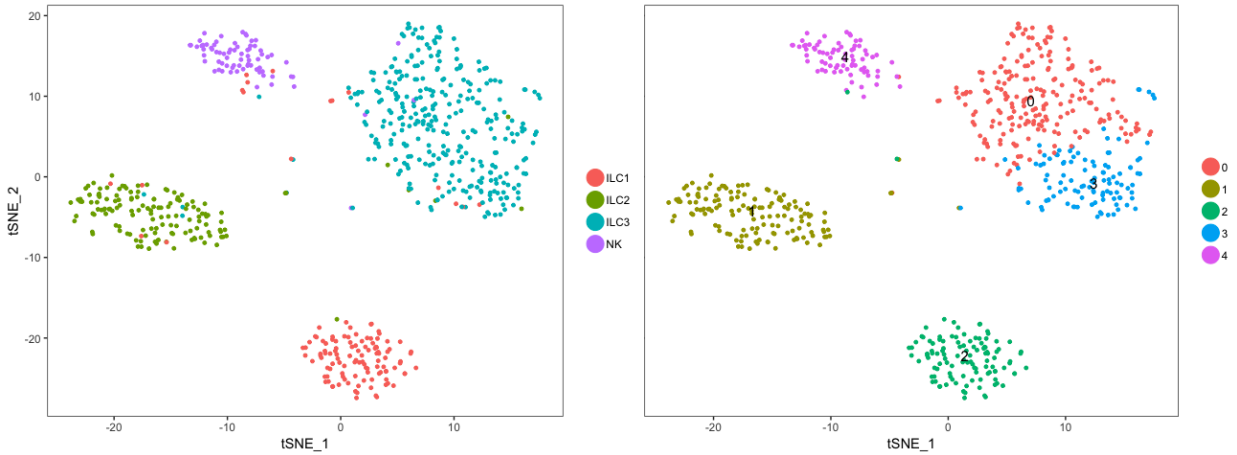
# Different normalizations




## SC3




## Seurat

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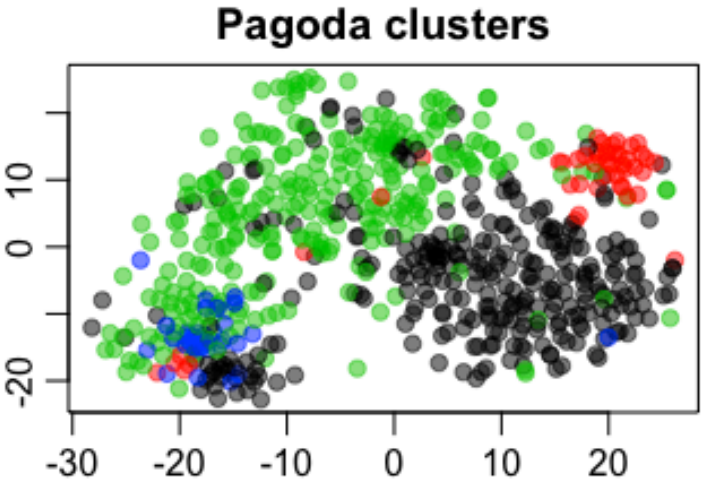
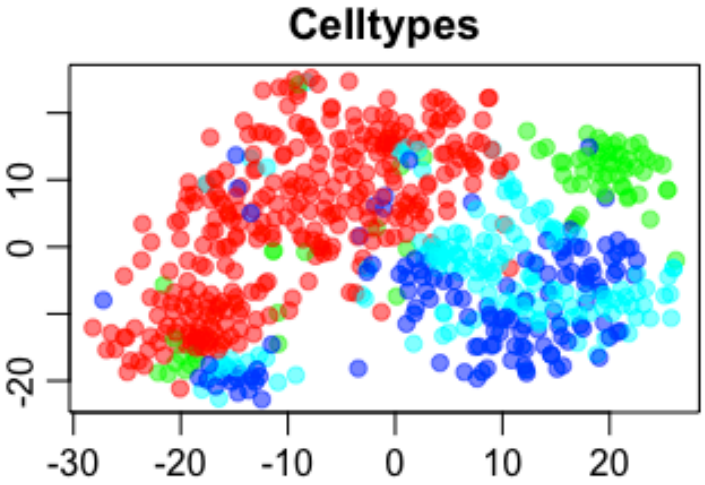
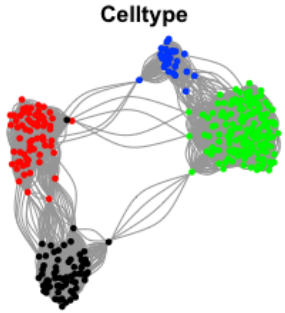
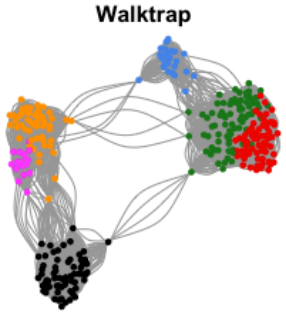
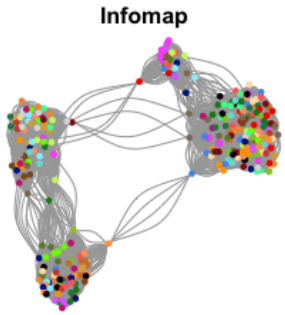


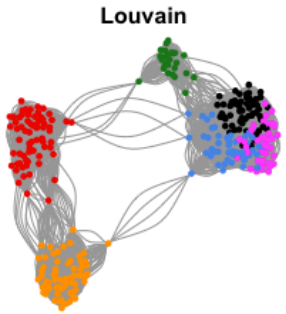




Igraph

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# 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 Götebort as well.
- More info at: http://nbis.se/

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# 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/

# Please fill in the Evaluation Form

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

## Good luck with your analyses!