Single cell RNA sequencing data analysis,
31 March – 3 April, 10 April, 2025

Åsa Björklund, Jennifer Fransson & Susanne Reinsbach

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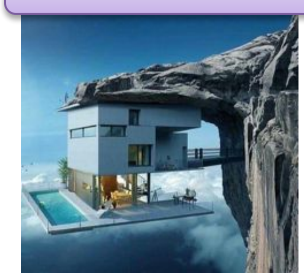
National Bioinformatics Infrastructure Sweden (NBIS)

Future compute infrastructure

Consultations



Compute projects
Software and databases

Data publishing and open science
Secure sharing of sensitive data

Training

Research support

Efficient tools and workflows

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Need help from NBIS?

- Drop-in sessions at some universities
  - online 14.00 Tuesdays
- Consultations
- Courses
- Support:
  - Long-term support (Peer reviewed)
  - Fee-for-service support, hourly fee
  - Partner projects
- Data management
- Advisory program

www.nbis.se

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Who are we?

Course leaders

Jennifer Fransson

Susanne Reinsbach

Åsa Björklund

TAs

Fariba Roshanzamir

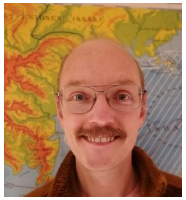
Nima Rafati

Yuan Li

Stefan Ebmeyer

Aditya Singh

Snaevar Sigurdsson

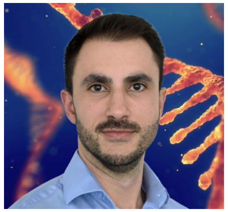


Jason Hill

Teachers

Henrik Gezelius

Nikolay Oskolkov



Paulo Czarnewski

Jakub Westholm

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Practical information - Zoom

- Please keep your cameras on unless you are having severe network issues
  - Both for lectures and exercises.
- Ask questions:
  - Feel free to unmute and interrupt during lectures!
  - Or by raising your hand in Zoom.
  - Or write in the slack channel #lectures.
- We will be recording the lectures for future reference, but only the slides view.
  - Your voice may be included, if you do not want your voice to be public, let us know and we can cut it out.

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Practical information - Zoom

- For exercises we will have 4-5 people in each breakout room.
- We will post a survey on slack where we want to know which toolkit you wish to practise on to create the groups.

Practical information - Slack

- Communication around the course will be handled via slack.
- Please add picture and brief description
- Channels:
    - #general – general information about the course
    - #lectures - questions about lectures
    - #exercises – questions during exercises
    - #precourse – questions about the installations
    - #roomX - one for each breakout room for exercises.

Practical information

- All code via our github page: https://github.com/NBISweden/workshop-scRNAseq

  Use folder compiled/labs!!

- Lunches and coffee breaks - please take a minute to step away from the screen!

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Course credits

NBIS as an organization cannot give out course credits, only the Universities can.

With your course certificate you should contact the person responsible at your department and usually there is no problem getting credits for the course.

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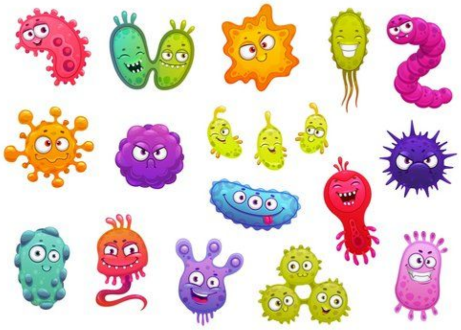
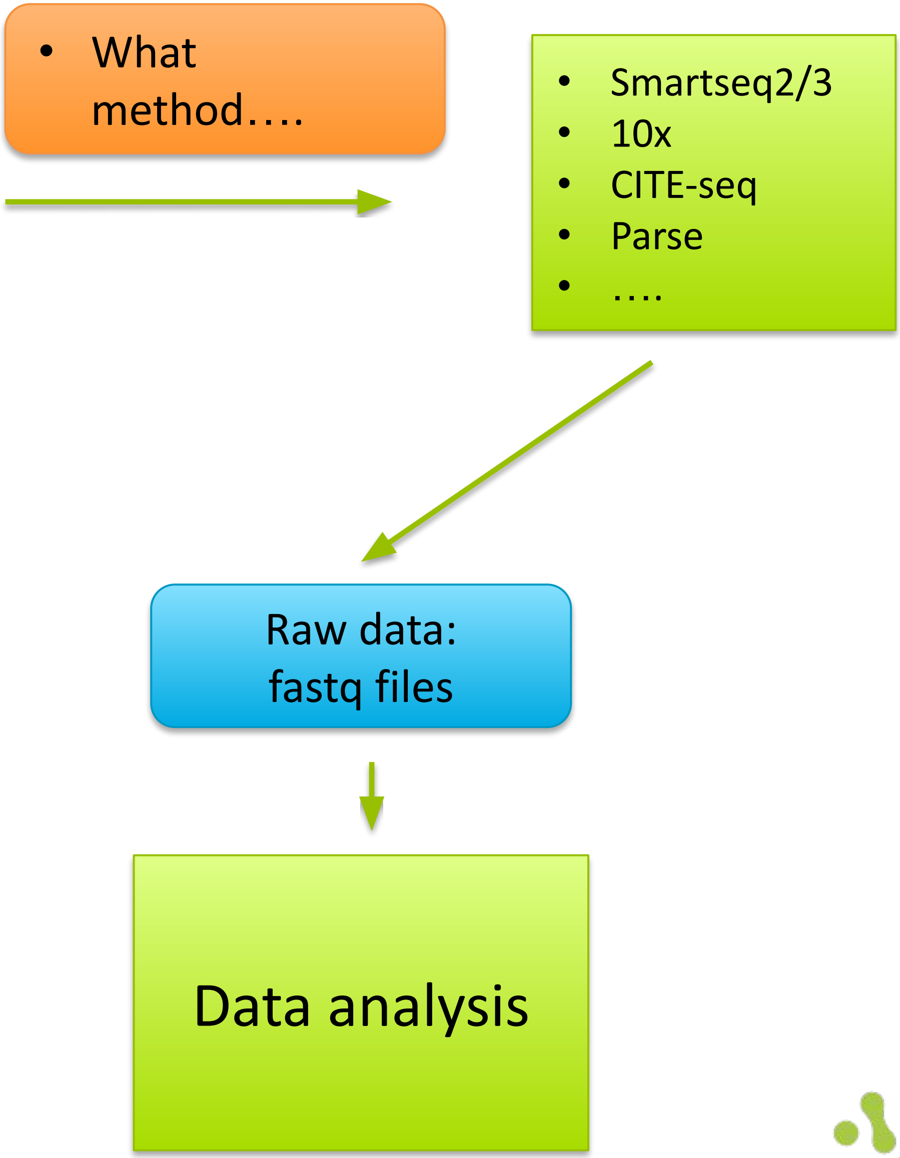
scRNA-seq overview





- What method….

- Smartseq2/3
- 10x
- CITE-seq
- Parse
- ….

Raw data:
fastq files

Data analysis

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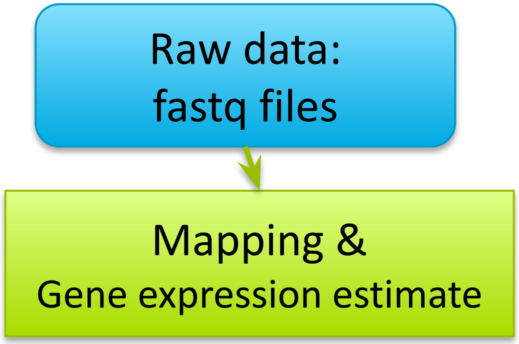
scRNA-seq analysis overview



Raw data:
fastq files

Mapping &
Gene expression estimate

- Cellranger (10x)
- Dropseq - Alevin/Kallisto Bustools (transcriptome only)
- Dropletutils
- nf-core/scrnaseq
- zUMIs
- ….

scRNA-seq analysis overview

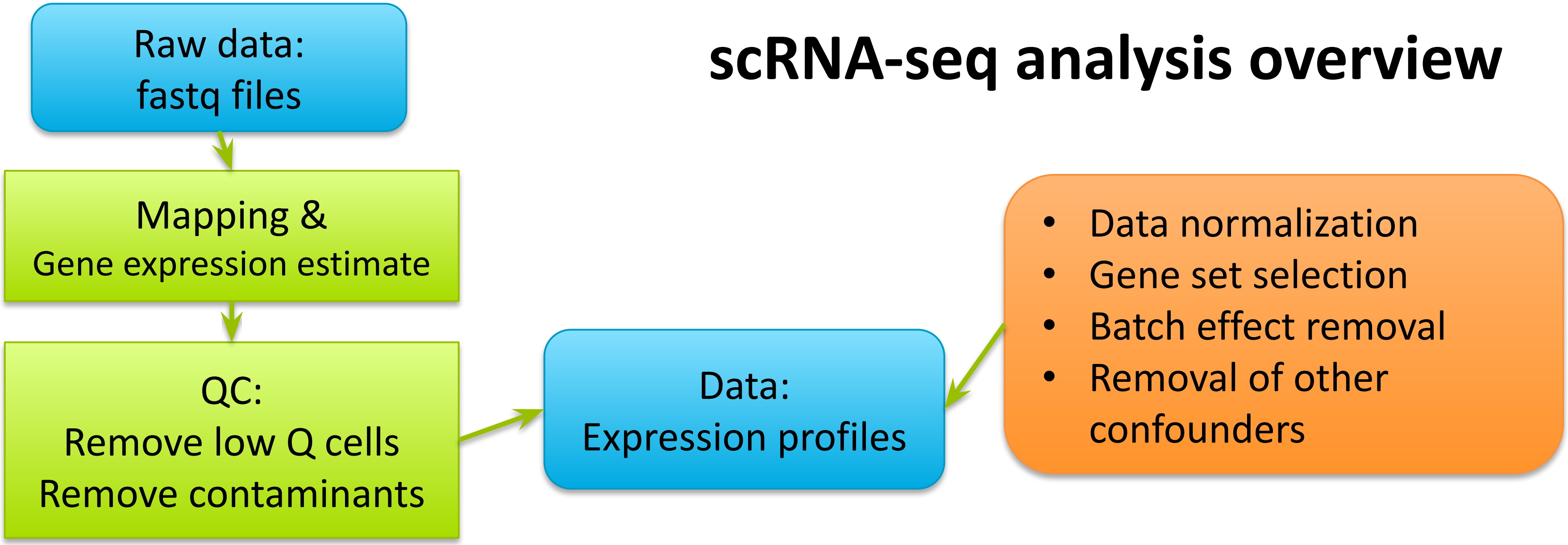
Raw data:
fastq files

Mapping &
Gene expression estimate

QC:
Remove low Q cells
Remove contaminants

- Low/high number of reads
- Low/high number of genes
- Intronic reads
- Mitochondrial content
- Mapping stats
- Filter genes
- …

scRNA-seq analysis overview



Raw data:
fastq files

Mapping &
Gene expression estimate

QC:
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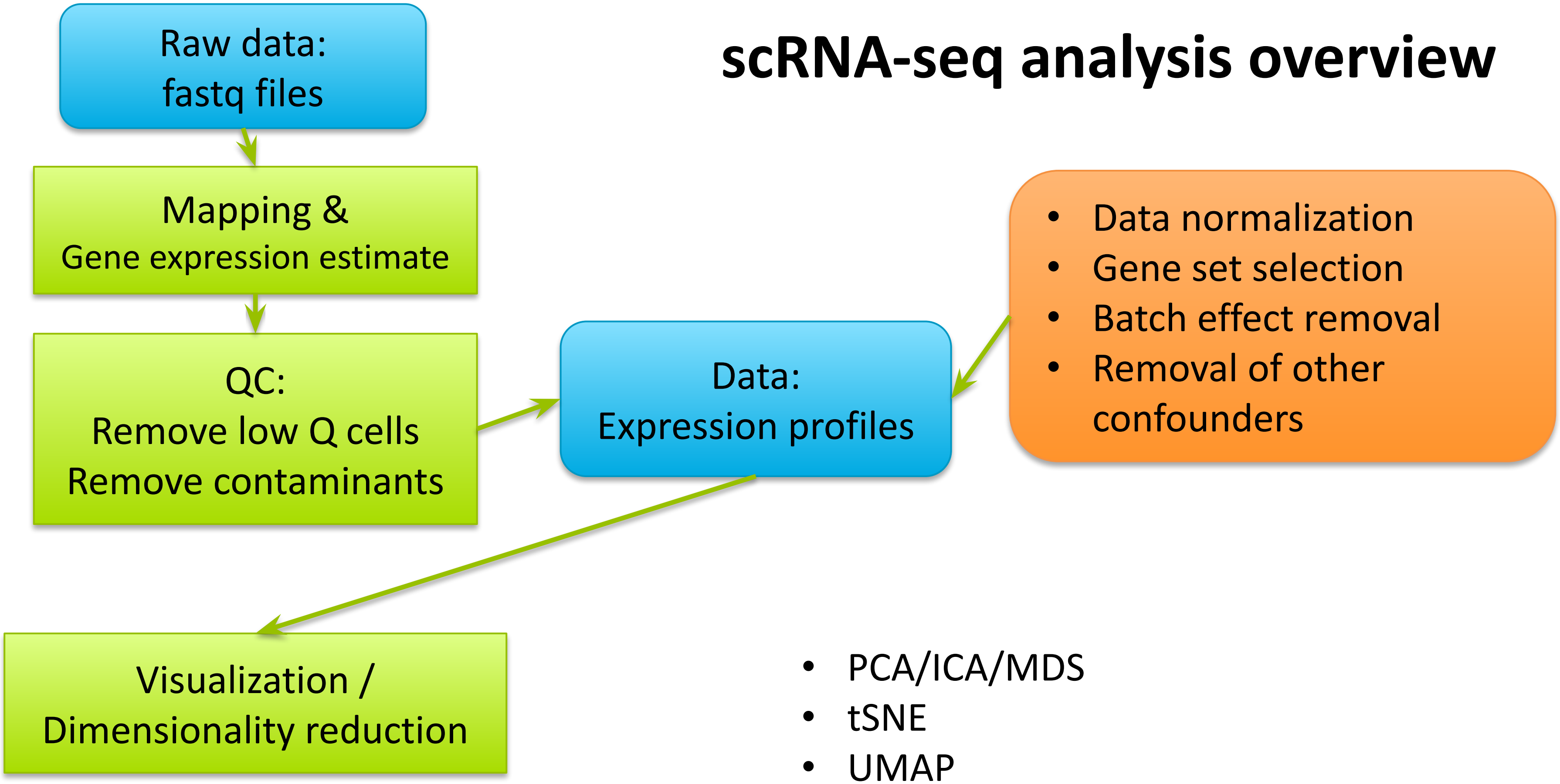
Data:
Expression profiles

- Data normalization
- Gene set selection
- Batch effect removal
- Removal of other confounders

scRNA-seq analysis overview



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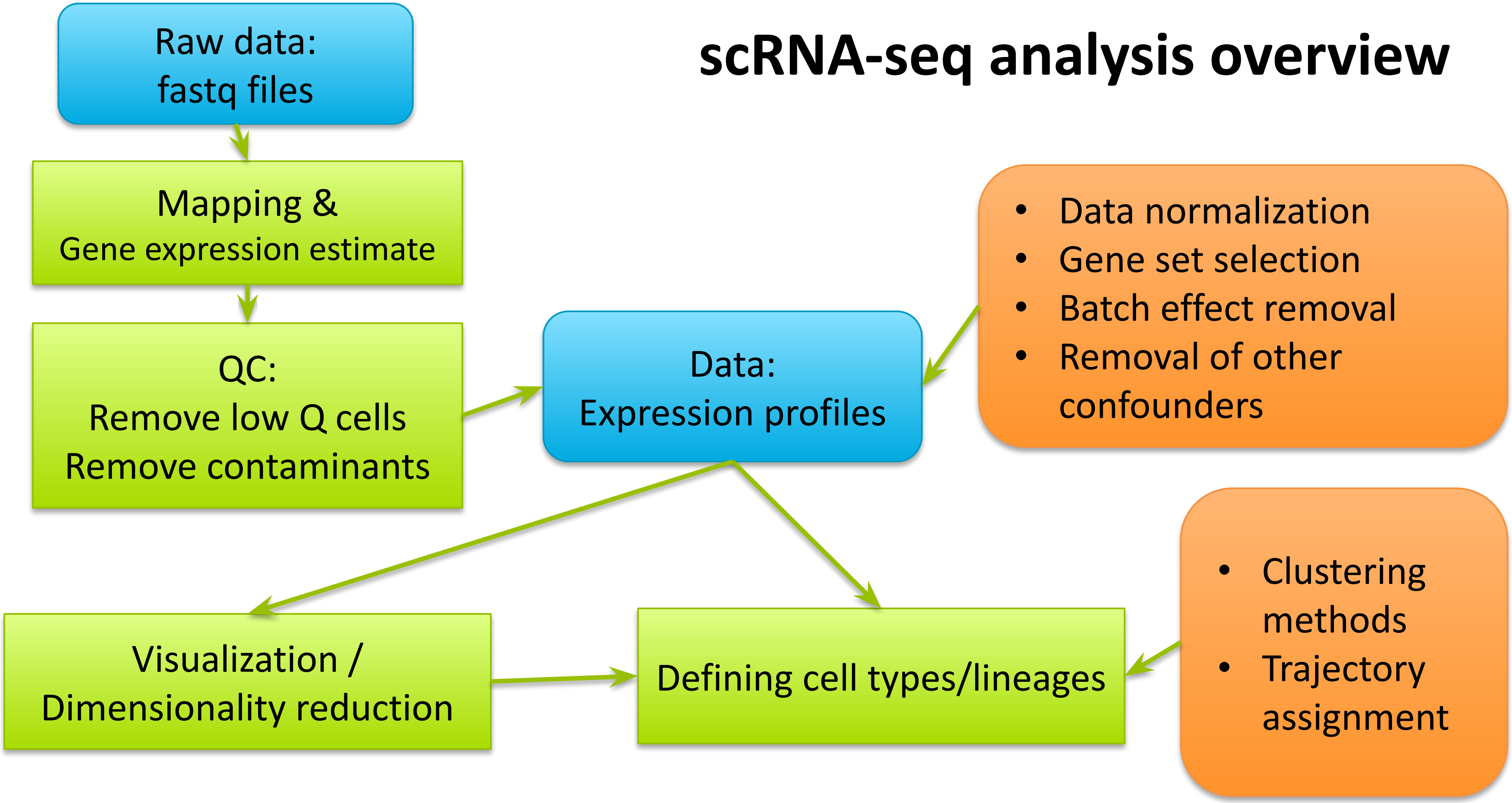
Visualization /
Dimensionality reduction

- PCA/ICA/MDS
- tSNE
- UMAP
- Diffusion maps
- …

scRNA-seq analysis overview



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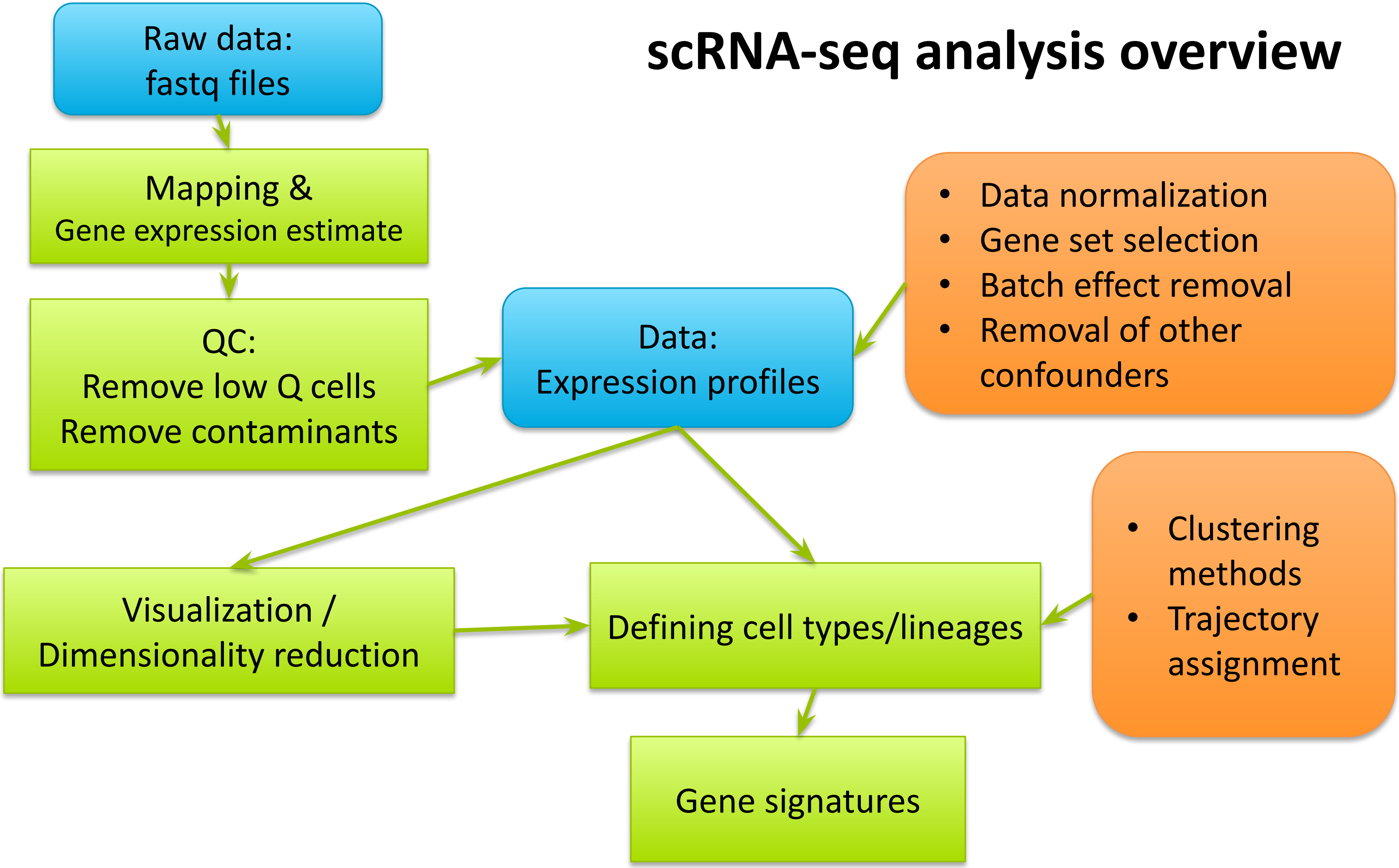
Visualization /
Dimensionality reduction

Defining cell types/lineages

- Clustering methods
- Trajectory assignment

scRNA-seq analysis overview



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Gene signatures

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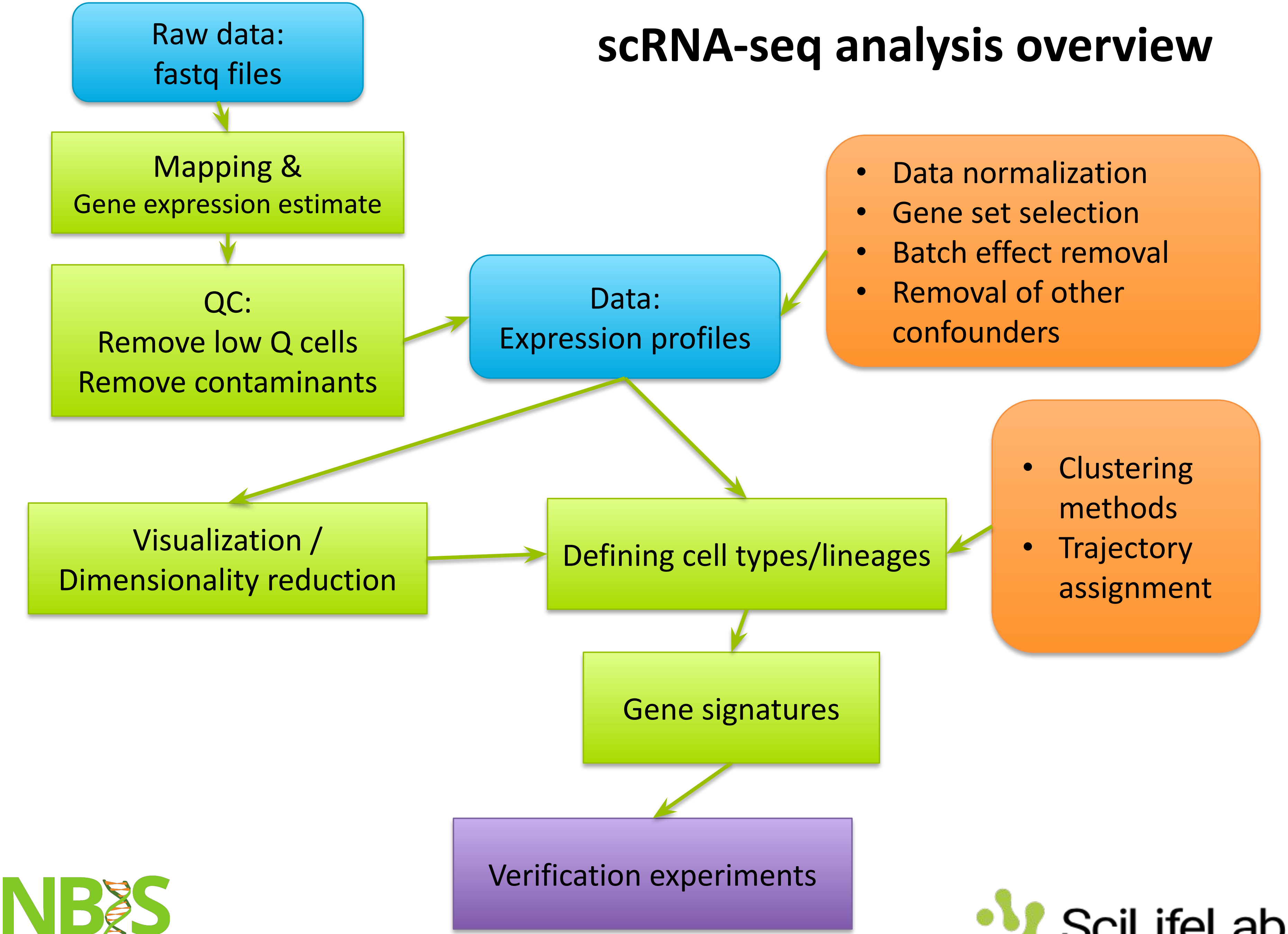
scRNA-seq analysis overview



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Visualization /
Dimensionality reduction

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Gene signatures

Verification experiments

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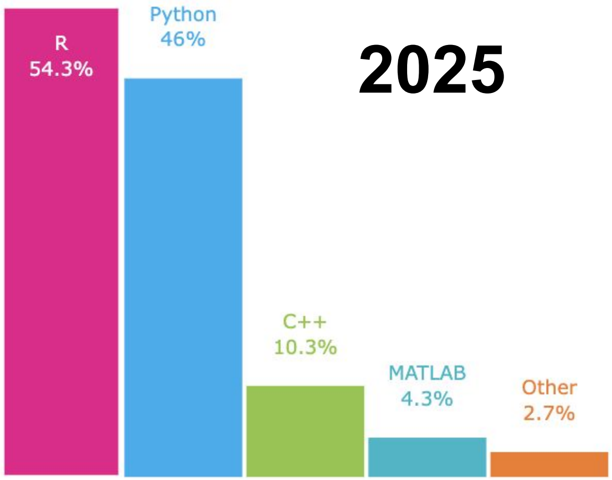
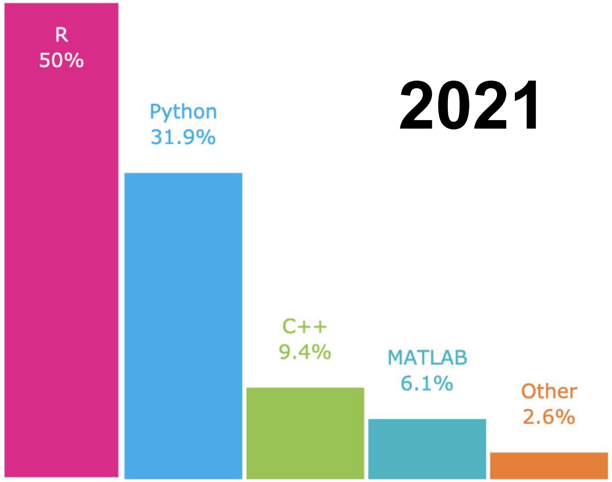
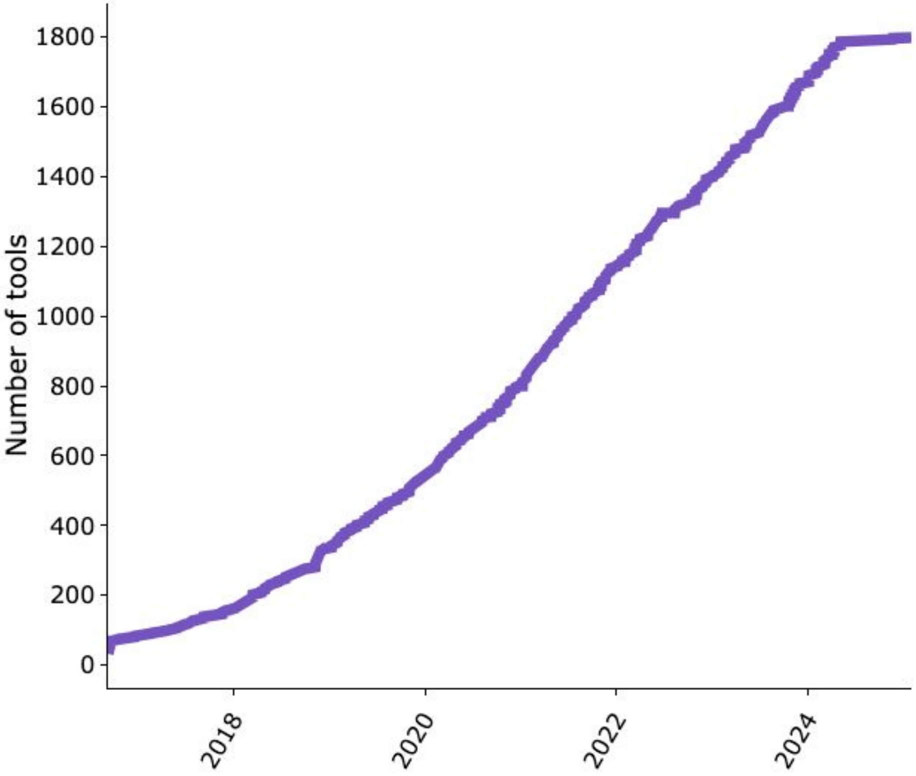
Many available tools for analysis



2025



2021



https://www.scrna-tools.org/

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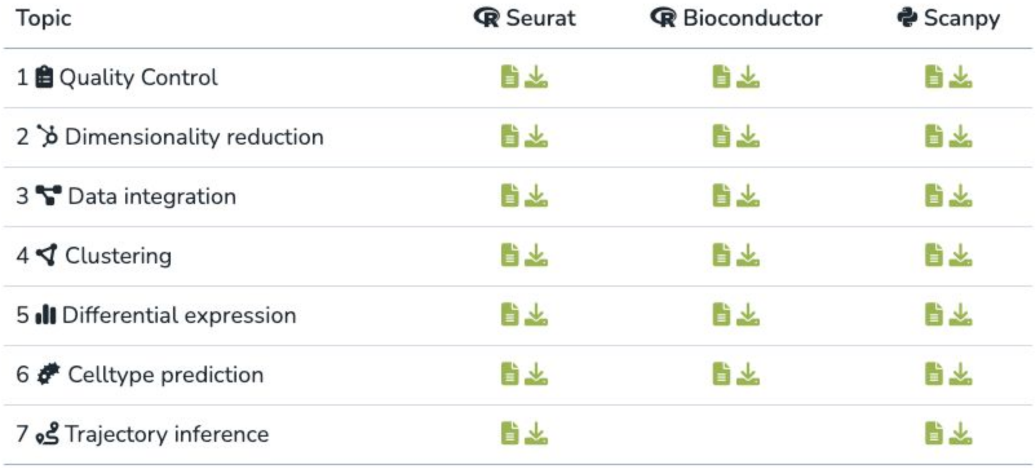
In this course

- We have selected a set of tools/toolkits that we think are relevant for most cases.
- We cannot cover all tools that are out there.
- Use your common sense to select methods for your problem.



Topic	Seurat	Bioconductor	Scanpy
1 Quality Control	📄 ⬇	📄 ⬇	📄 ⬇
2 Dimensionality reduction	📄 ⬇	📄 ⬇	📄 ⬇
3 Data integration	📄 ⬇	📄 ⬇	📄 ⬇
4 Clustering	📄 ⬇	📄 ⬇	📄 ⬇
5 Differential expression	📄 ⬇	📄 ⬇	📄 ⬇
6 Celltype prediction	📄 ⬇	📄 ⬇	📄 ⬇
7 Trajectory inference	📄 ⬇		📄 ⬇

Bring your own data - Thursday April 10th

You can work on any dataset of your choice - your own or a public dataset from a tissue that is relevant to your research.

Try out some of the methods you have worked with in the exercises and we will be there to guide you through.

More detailed information will come on the April 3rd.

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Schedule

https://nbisweden.github.io/workshop-scRNAseq/home_schedule.html

Have links to pdfs with slides, but some are from last year and will be updated as we give the lectures.

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Scilifelab serve account

If you have not requested an account yet, please let us know immediately via slack and we can inform the Serve staff to speed up the process.

Also, Serve is still in beta testing mode, so we want everyone to have Docker installed as an emergency backup solution.

For BYOD the allocated resources may not be enough.

PLEASE ASK QUESTIONS!

slack