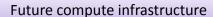
Single cell RNA sequencing data analysis, 31 March – 3 April, 10 April, 2025

Åsa Björklund, Jennifer Fransson & Susanne Reinsbach





National Bioinformatics Infrastructure Sweden (NBIS)











Compute projects
Software and databases



Data publishing and open science Secure sharing of sensitive data



Training



Efficient tools and workflows



Research support





Need help from NBIS?

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





Who are we?

Course leaders



Jennifer Fransson



Susanne Reinsbach



Åsa Björklund

TAs



Fariba Roshanzamir



Nima Rafati



Yuan Li



Teachers

Henrik Gezelius



Nikolay Oskolkov



Stefan Ebmeyer



Aditya Singh



Snaevar Sigurdsson



Paulo Czarnewski Jakub Westholm







Jason Hill



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, your voice may be included.
 - If you do not want your voice to be public, let us know and we can cut it out.





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
 https://github.com/NBISweden/workshop-scRNAseq
 Use folder compiled/labs!!
- Lunches and coffee breaks please take a minute to step away from the screen!





Course credits

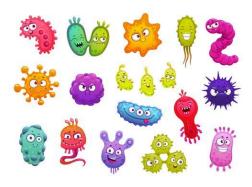
NBIS as an organization cannot give out course credits, only the Universities can.

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





scRNA-seq overview



What method....

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

Raw data: fastq files Data analysis







scRNA-seq analysis overview

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





Raw data: fastq files Mapping & Gene expression estimate QC: Remove low Q cells Remove contaminants

scRNA-seq analysis overview

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





Raw data: fastq files

scRNA-seq analysis overview

Mapping & Gene expression estimate

QC:

Remove low Q cells
Remove contaminants

Data: Expression profiles

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





Raw data: fastq files

scRNA-seq analysis overview

Mapping & Gene expression estimate

QC:

Remove low Q cells

Remove contaminants

Data: Expression profiles

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

Visualization /

Dimensionality reduction

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





Raw data: scRNA-seq analysis overview fastq files Mapping & Data normalization Gene expression estimate Gene set selection Batch effect removal Removal of other Data: QC: confounders **Expression profiles** Remove low Q cells Remove contaminants Clustering methods Visualization / Trajectory Defining cell types/lineages Dimensionality reduction assignment





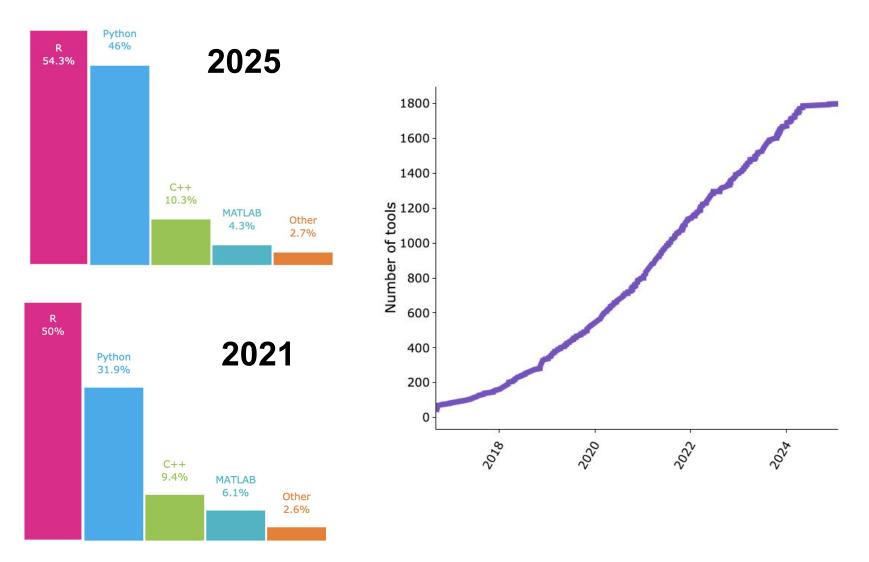
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Raw data: scRNA-seq analysis overview fastq files Mapping & Data normalization Gene expression estimate Gene set selection Batch effect removal Removal of other Data: QC: confounders **Expression profiles** Remove low Q cells Remove contaminants Clustering methods Visualization / Trajectory Defining cell types/lineages Dimensionality reduction assignment Gene signatures Verification experiments

Many available tools for analysis







In this course

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





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.





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.





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!





