



Single cell RNA sequencing data analysis

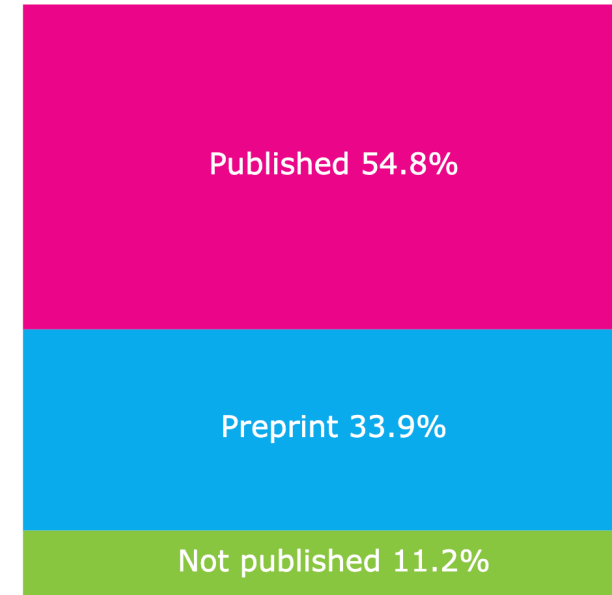
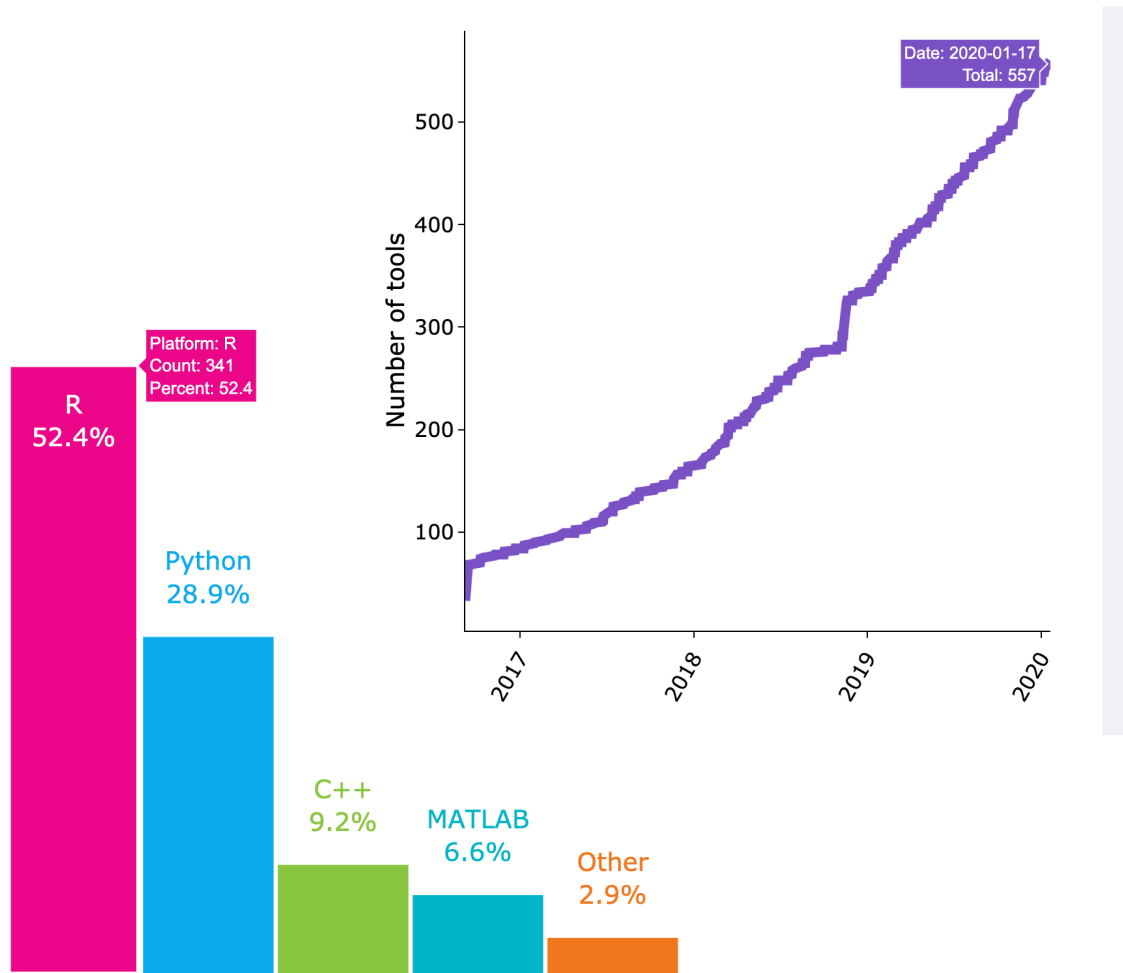
Practical exercises

4-6 February 2019

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Many available tools for analysis



Three main pipelines for analysing single cell data:

- **Seurat:**
 - R based, centered around Seurat objects.
 - Mainly developed for droplet based data
 - Easy to use, recommended for R beginners
- **Scran:**
 - R based, centered around SingleCellExperiment objects
 - Has more different statistical methods
- **Scanpy:**
 - Python based
 - Does not yet have all the functionality of the R based tools
 - Handles large datasets better.

The exercises

Tutorial



Quality Control

Seurat_qc

Scater_qc

ScanPY_qc



Dimensionality reduction

Seurat_dr

Scater_dr

Scanpy_dr



Data integration

Seurat_integr

Scater_integr

Scanpy_integr



Clustering

Seurat_clust

Scater_clust

Scanpy_clust



Differential expression

Seurat_dge

Scater_dge

Scanpy_dge



Trajectory inference

Slingshot_ti

PAGA_ti

The datasets

- Peripheral blood mononucleated cells (PBMC) with 10x Genomics
 - One dataset with version2 chemistry
 - One dataset with version3 chemistry
 - One dataset with version3 chemistry combined with protein detection (we will only analyse the expression data)
- Should contain the main immune celltypes: T-cells, B-cells, NK-cells, monocytes and dendritic cells

Installation of all packages

- We have created a conda environment for the course that should contain all packages you need for the exercises
- However, for slingshot trajectory inference lab, there is an additional conda environment that needs to be installed.
- If you chose to instead work with standard R installations, you can use the list of required packages in the environment file and install them on your own.

The code:

- All code for the exercises is available as R-markdown documents, or jupyter notebooks in the folder:
workshop-scRNAseq/labs/compiled/
- Please report to us if you find any errors in the code!

Practical exercises

- Everyone works in very different pace. Focus on one of the pipelines first. If you have time left over, you can also try out the other ones.
- Hopefully everyone will have time to finish the labs in the allocated time, if not, there is extra time on Thursday to also finish labs and get help with that.

Troubleshooting

- We have put up a FAQ page where we will try to put up common problems & questions
- It is important that you learn how to troubleshoot yourselves.
 - Look at your error messages, perhaps the answer is there?
 - If not – Google is your best friend! Forums like Seqanswers, Stackexchange, Bioconductor support forum and some specific forums for each package may have the answer.
- TAs are there to answer any questions and give suggestions, but we may not always have the answer.