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PLATFORM

Single Cell RNAseq Analysis : from the Monash Bioinformatics Platform

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<https://platforms.monash.edu/bioinformatics>



Today's Agenda

- What to do?
- Where to start?
 - Common downstream analysis
 - What tools to use?
- How exactly?
 - Seurat tutorial walkthrough

What is the biological question?

Characterising
a cell type in
tissue.

Developmental
trajectories of
differentiating cells?

Find marker
genes?

**Biological
question**

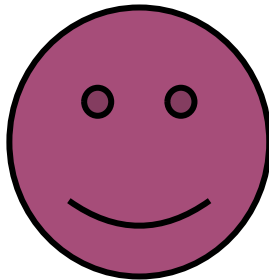
Changes in
cell type
composition?

Differential
expression

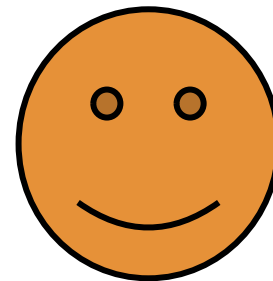
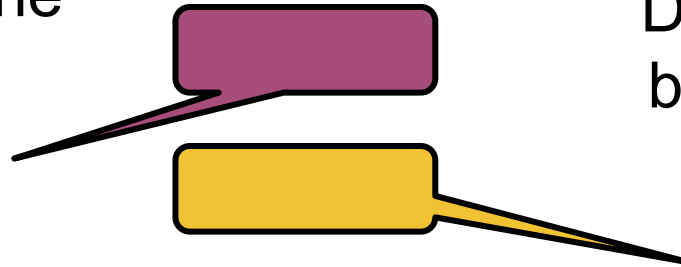
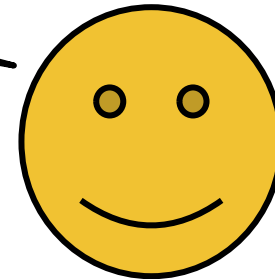
Sequence a
rare sorted cell
type?

Important to have a dialogue going

Discuss with the
biologist



Discuss with the
bioinformatician

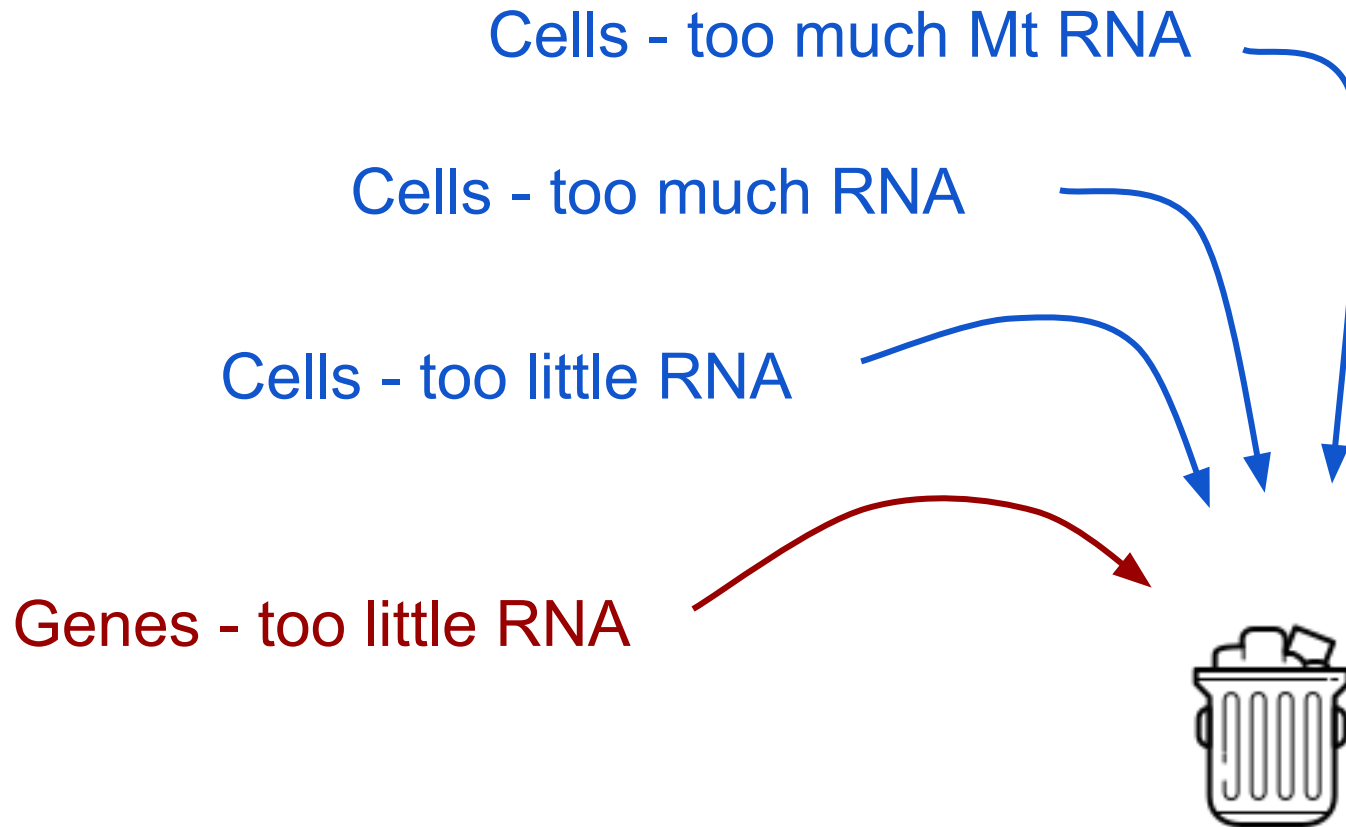


Or be both

The fun starts after the counts matrix ...

	AAACATACAACCAC	AAACATTGAGCTAC	AAACATTGATCAGC	AAACCGTGCTCCG	AAACCGTGATGCG	AAACGCACTGGTAC	AAACGCTGACCAGT	AAACGCTGGTCTT	AAACGCTGTAGCCA	AAACGCTGTTCTG	AAACTTGAAAAACG	AAACTTGATCCAGA	AAAGAGACGAGATA	AAAGAGACGCGAGA	AAAGAGACGGACTT	AAAGAGACGGCATT	AAAGCAGATATCGG	AAAGCCTGTATGCG	AAAGGCCTGTCTAG	AAAGTTTGATCAGC
AL627309.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AP006222.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RP11-206L10.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RP11-206L10.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LINC00115	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NOC2L	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
KLHL17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PLEKHN1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RP11-5407.17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HES4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
RP11-5407.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ISG15	0	0	1	9	0	1	0	0	0	3	0	0	1	5	0	2	1	0	0	0
AGRN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C1orf159	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TNFRSF18	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TNFRSF4	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	12	0	0
SDF4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
B3GALT6	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
FAM132A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UBE2J2	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	1	0	0

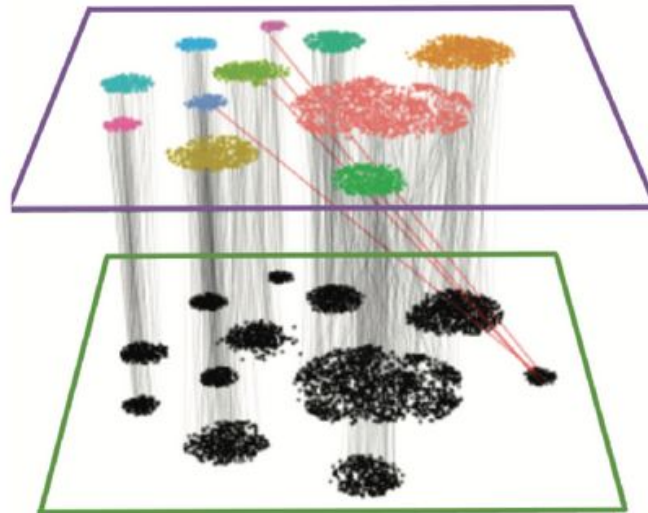
Gene counts > QC > Norm/Trans/Batch-correct > Dim Reduction > Clustering > Identification > Diff Expr



Gene counts > QC > Norm/Trans/Batch-correct > Dim Reduction > Clustering > Identification > Diff Expr

Transformation, Normalisation, batch correction

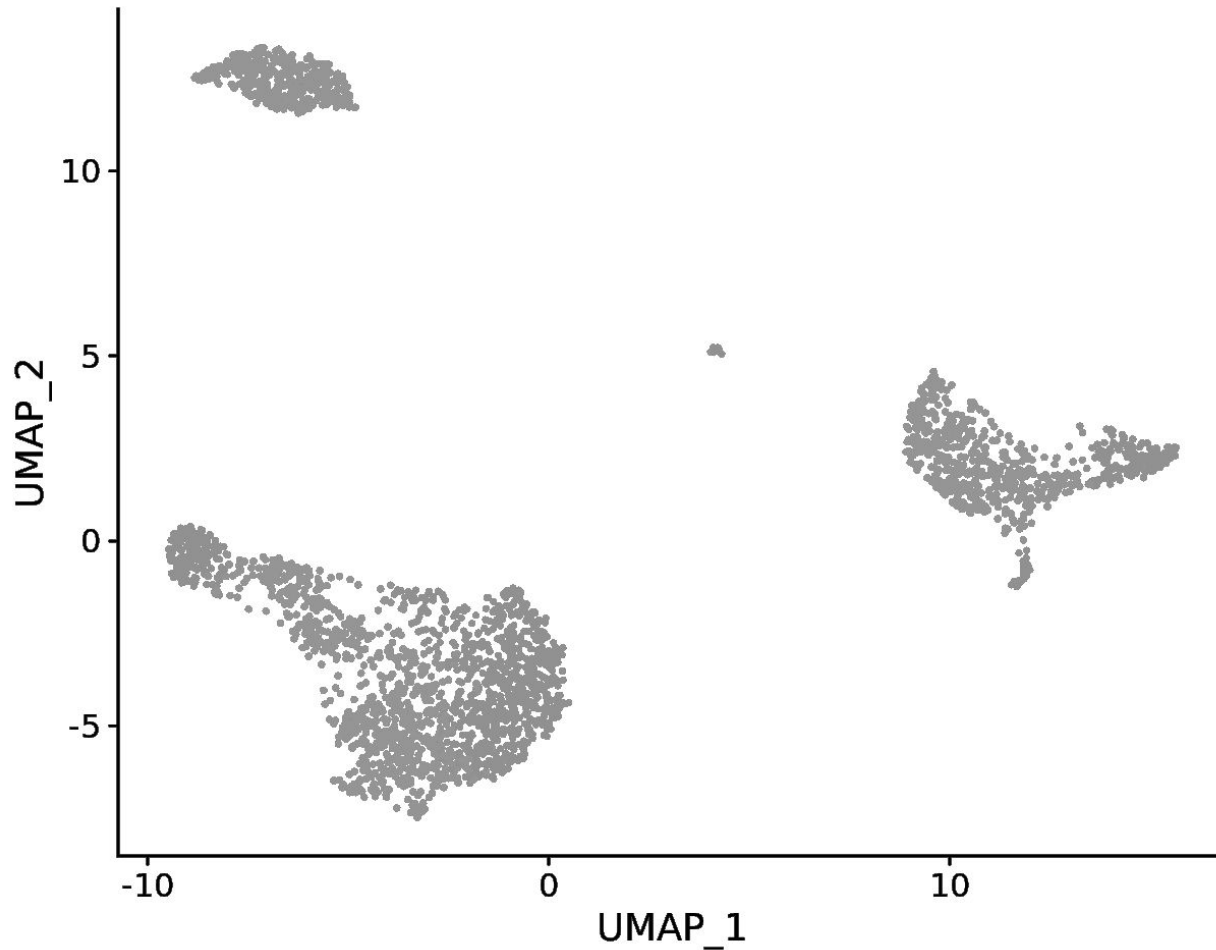
- Log-scaled, normalised expression
- Batch effects



Seurat batch integration

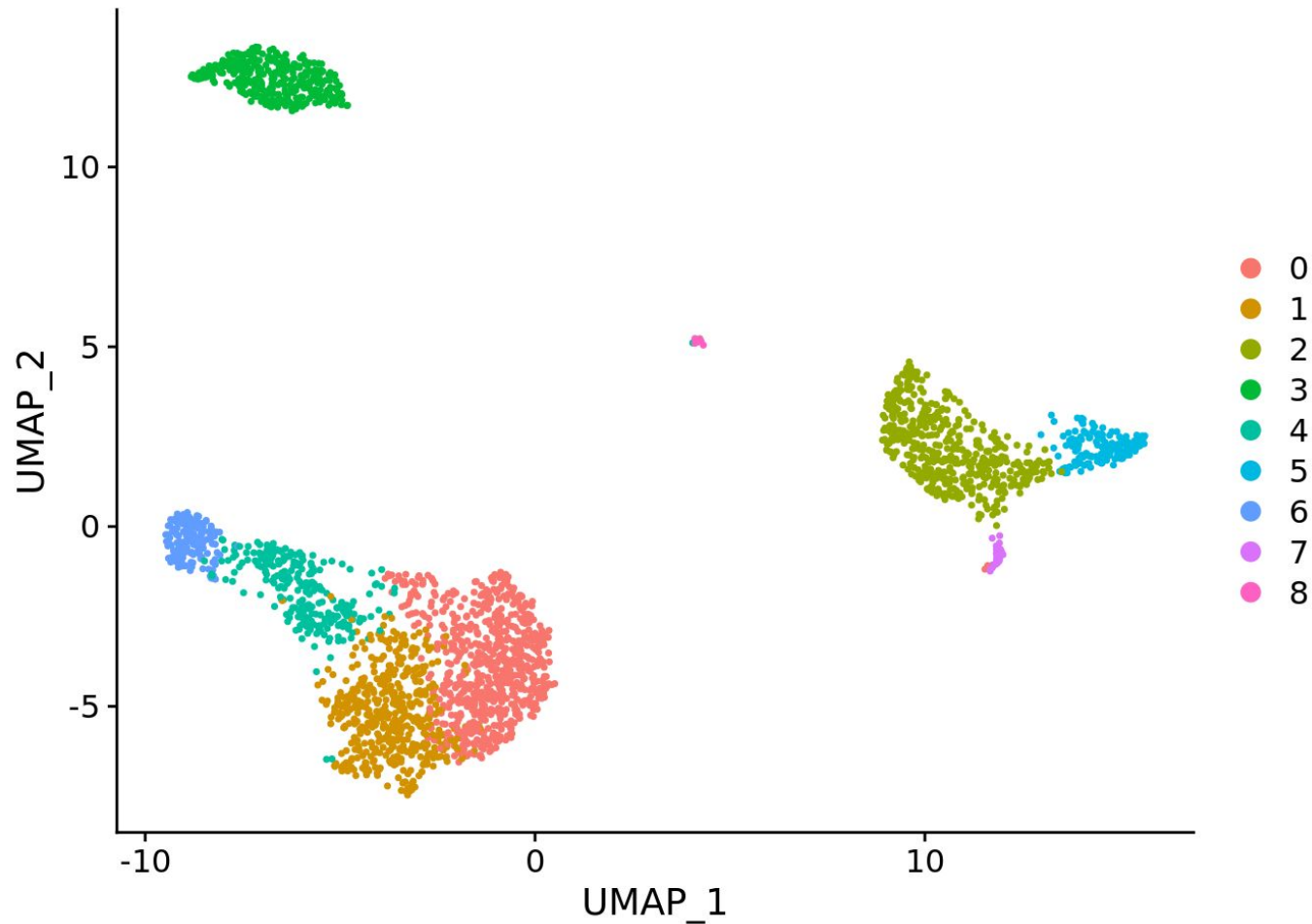
Gene counts > QC > **Norm/Trans/Batch-correct** > Dim Reduction > Clustering > Identification > Diff Expr

Dimensional Reduction - TSNE or UMAP



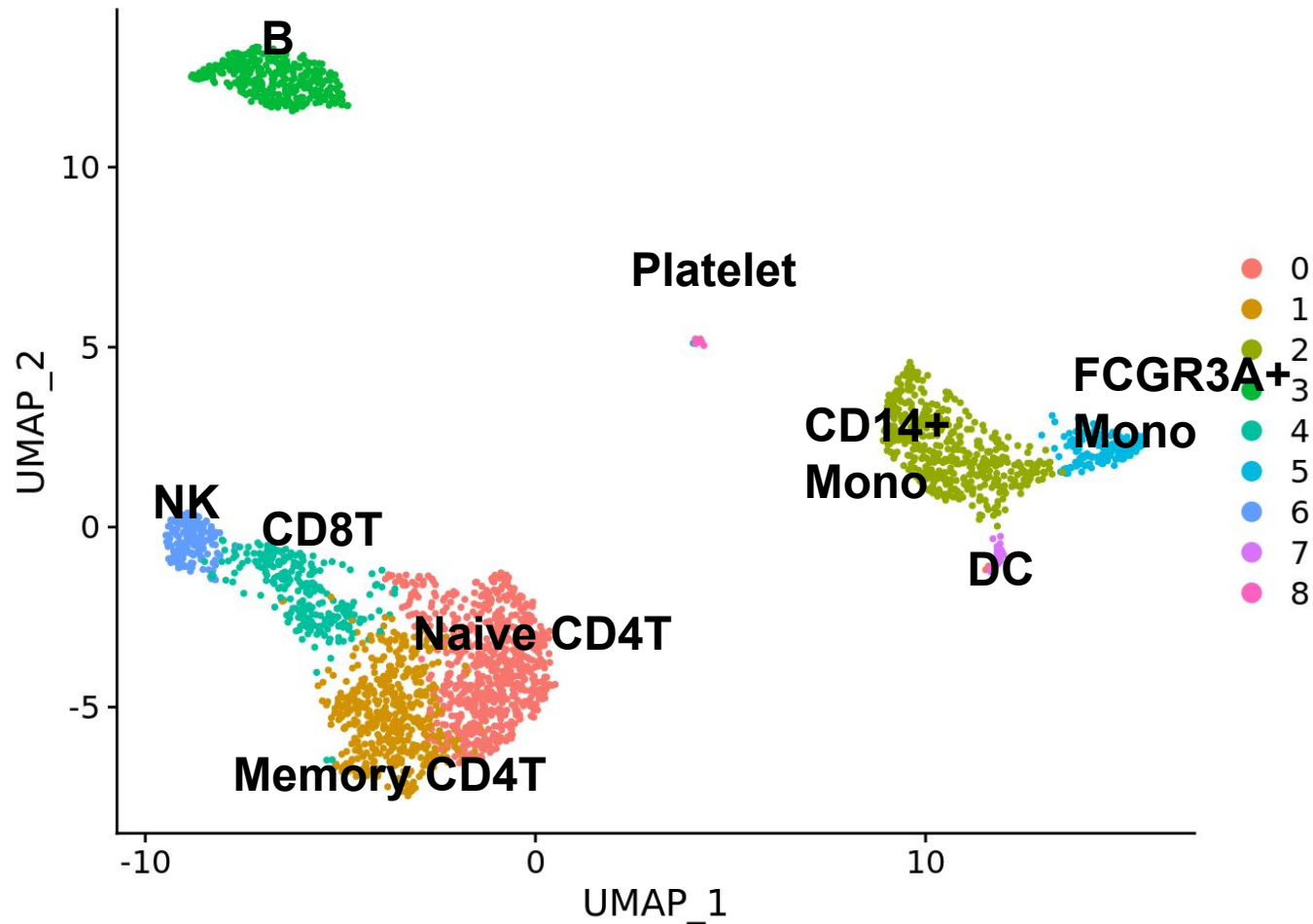
Gene counts > QC > Norm/Trans/Batch-correct > **Dim Reduction** > Clustering > Identification > Diff Expr

Clustering



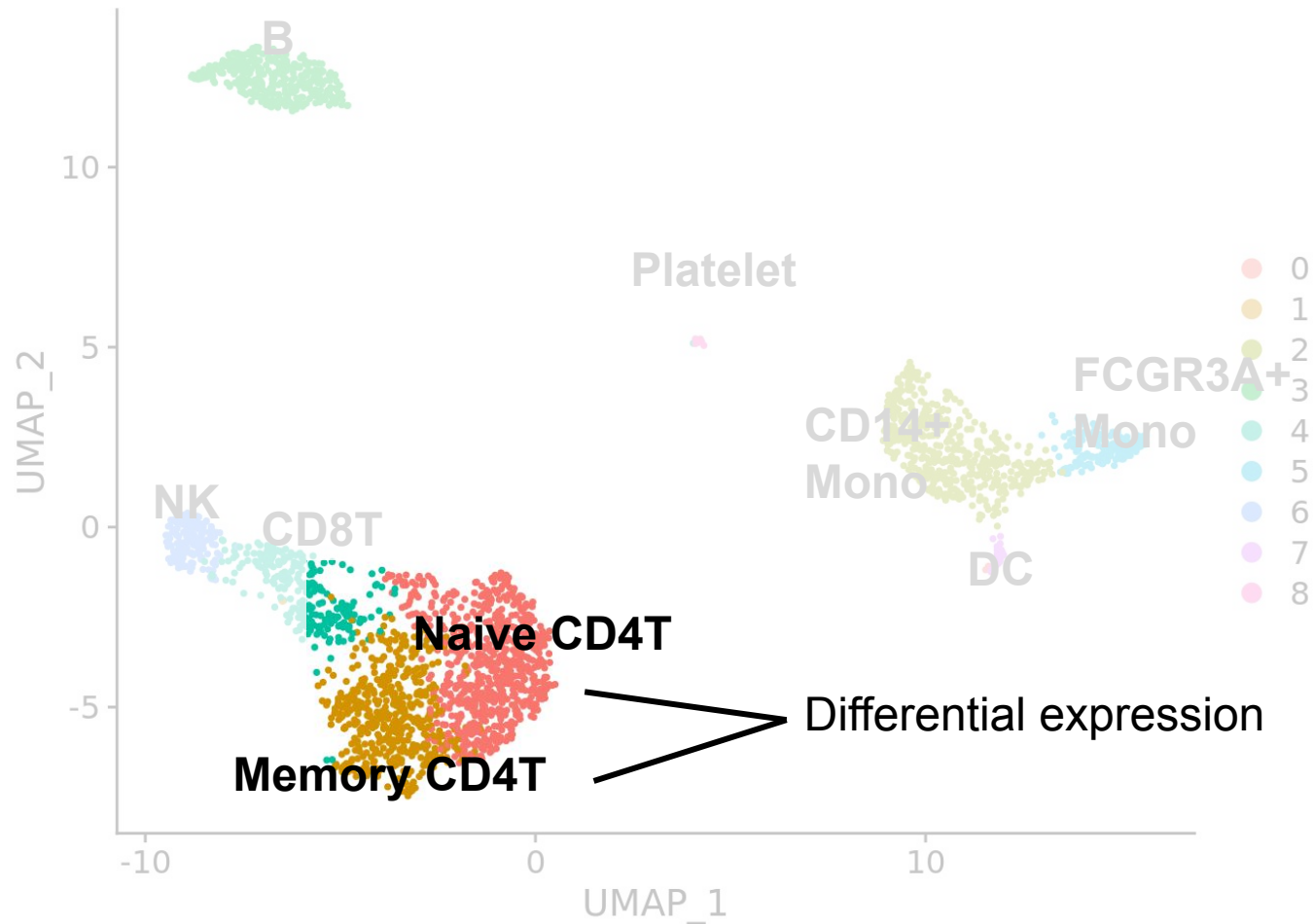
Gene counts > QC > Norm/Trans/Batch-correct > Dim Reduction > **Clustering** > Identification > Diff Expr

Cluster Identification



Gene counts > QC > Norm/Trans/Batch-correct > Dim Reduction > Clustering > **Identification** > Diff Expr

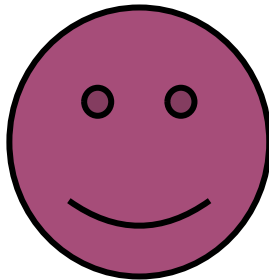
Differential Expression



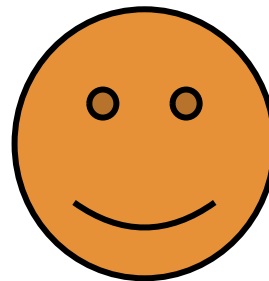
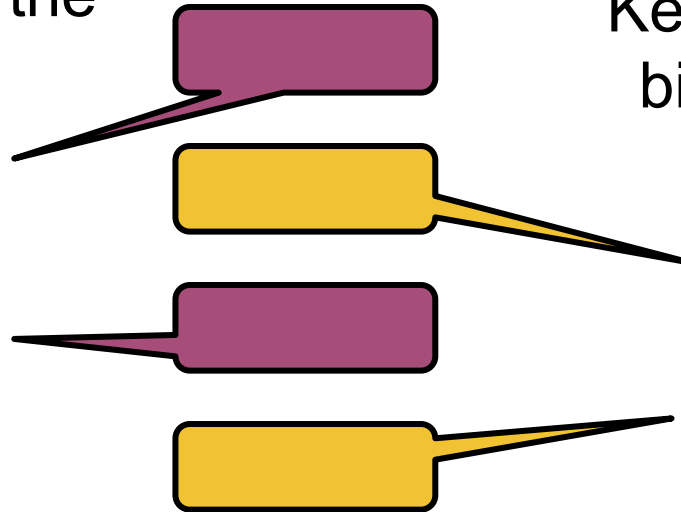
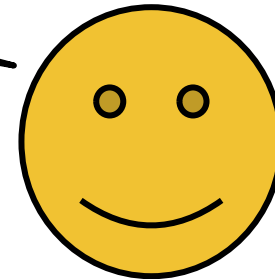
Gene counts > QC > Norm/Trans/Batch-correct > Dim Reduction > Clustering > Identification > **Diff Expr**

Keep the dialogue going

Keep talking to the
biologist!



Keep talking to the
bioinformatician!

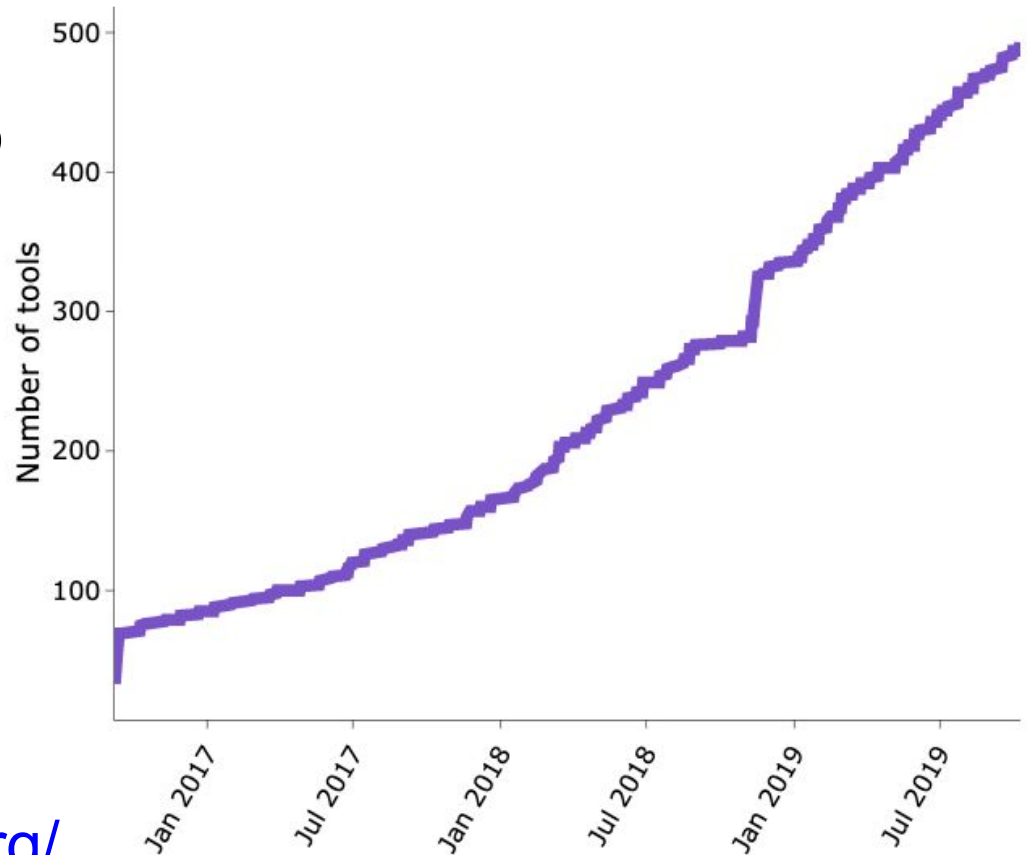


Or be both

So. Many. Tools

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

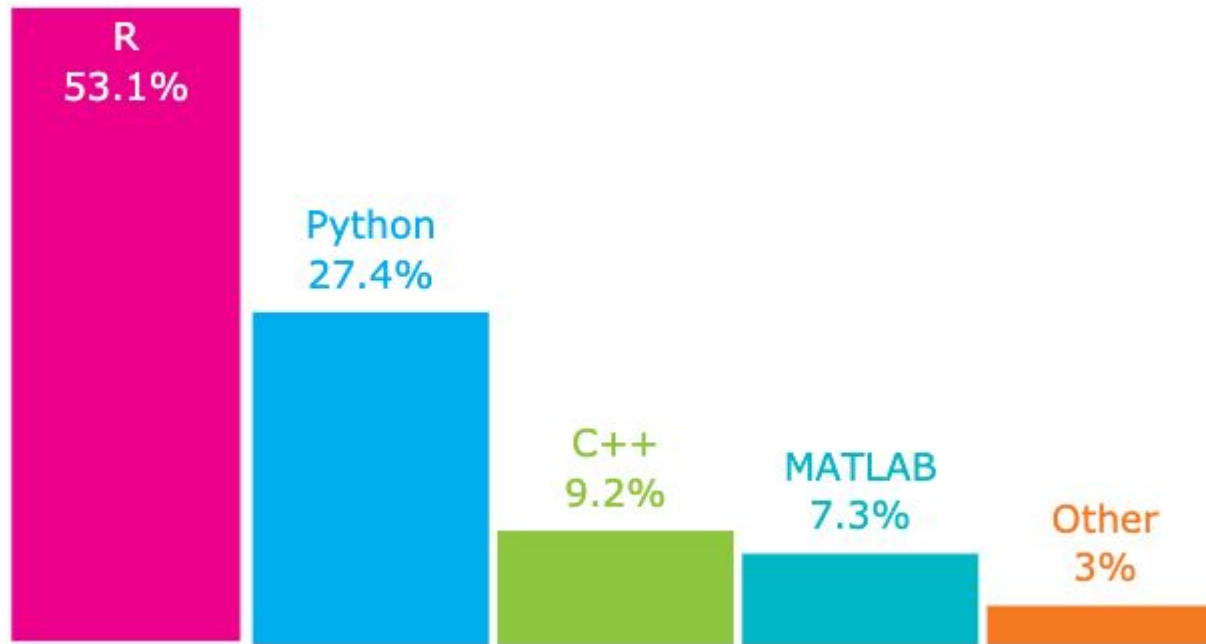
With so many tools, how do you then pick which one to use?



<http://guidelines.dynverse.org/>

So. Many. Tools

<https://www.scrna-tools.org/analysis>



R is popular, python too

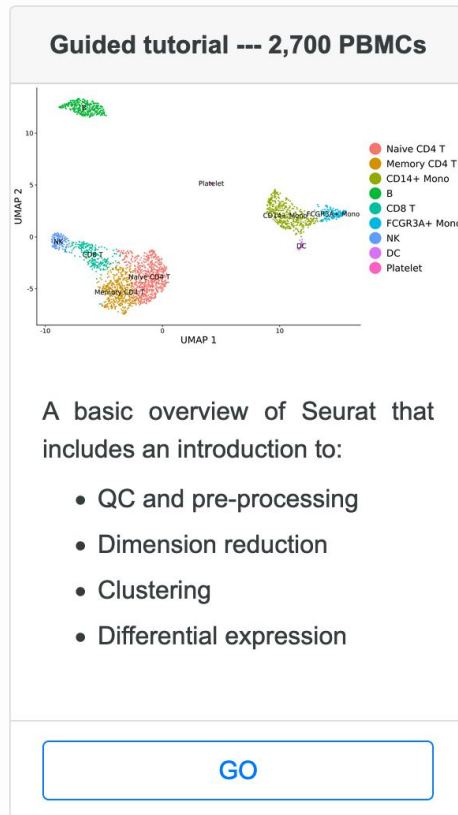
Considerations When Picking A Tool

- Is it appropriate for my type of data? (tools and techniques for 10x data are different for data from fluidigm C1, etc)
- How easy it to use?
- How easy is it to install?
- Does it have good documentation?
- Can it scale to my dataset size? (Also, can my computer handle the dataset?)
- Is it still supported/being actively developed?

100

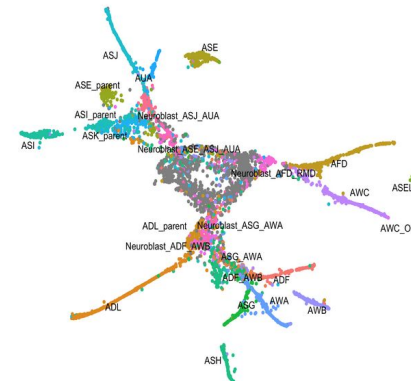
Seurat - scRNA analysis

<https://satijalab.org/seurat/vignettes.html>



Monocle 3 - Trajectory analysis

<https://cole-trapnell-lab.github.io/monocle3/>



Trajectories

Build single-cell trajectories with the software that introduced pseudotime. Find cell fate decisions and the genes regulated as they're made.

[View details »](#)

Example Workflows

Typically, tools will have an example workflow to follow and adapt to your dataset.

Seurat (R): <https://satijalab.org/seurat/vignettes.html>

Monocle3 (R): <https://cole-trapnell-lab.github.io/monocle3/>

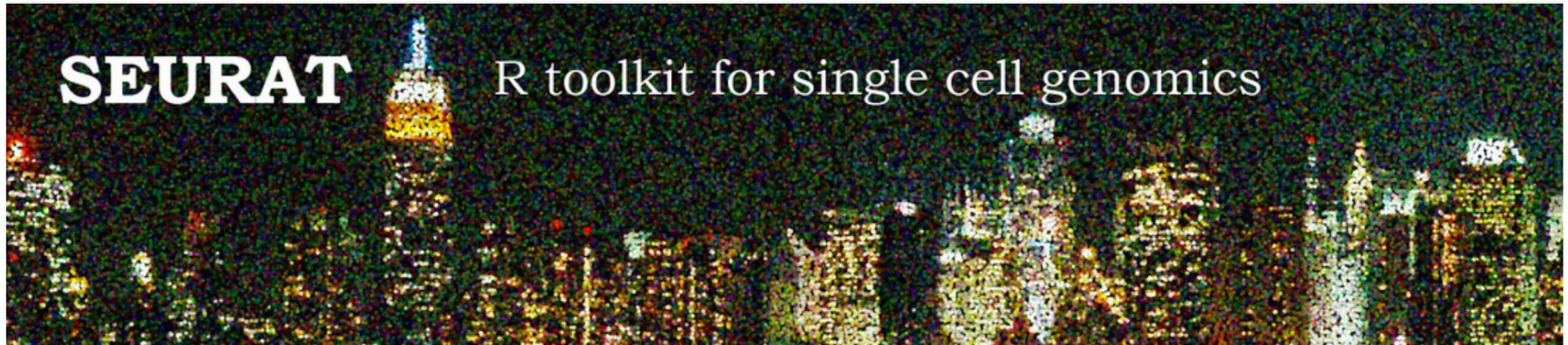
Scran (R):

[https://bioconductor.org/packages/release/bioc/vignettes/scr
an/inst/doc/scrان.html](https://bioconductor.org/packages/release/bioc/vignettes/scr
an/inst/doc/scrان.html)

Scanpy (Python):

<https://icb-scanpy.readthedocs-hosted.com/en/stable/>

- Bioconductor: <https://osca.bioconductor.org/>
- University of Cambridge bioinformatics training unit: <https://scrnaseq-course.cog.sanger.ac.uk/website/index.html>
- Broad Institute sc 2019 workshop: https://broadinstitute.github.io/2019_scWorkshop/
- UC Davis Bioinformatics Core Workshop: https://ucdavis-bioinformatics-training.github.io/2017_2018-single-cell-RNA-sequencing-Workshop-UCD_UCB_UCSF/



About

Install

Vignettes

Extensions

FAQs

Contact

Search

Seurat - Guided Clustering Tutorial

Compiled: October 08, 2019

https://satijalab.org/seurat/v3.1/pbm3k_tutorial.html