More methods

Hardware, protocols and software

Thomas D. Otto

Overview

This lecture is to complement teaching material with current technologies. We will introduce different (many) key technologies so that you can place the different technologies and complement your knowledge

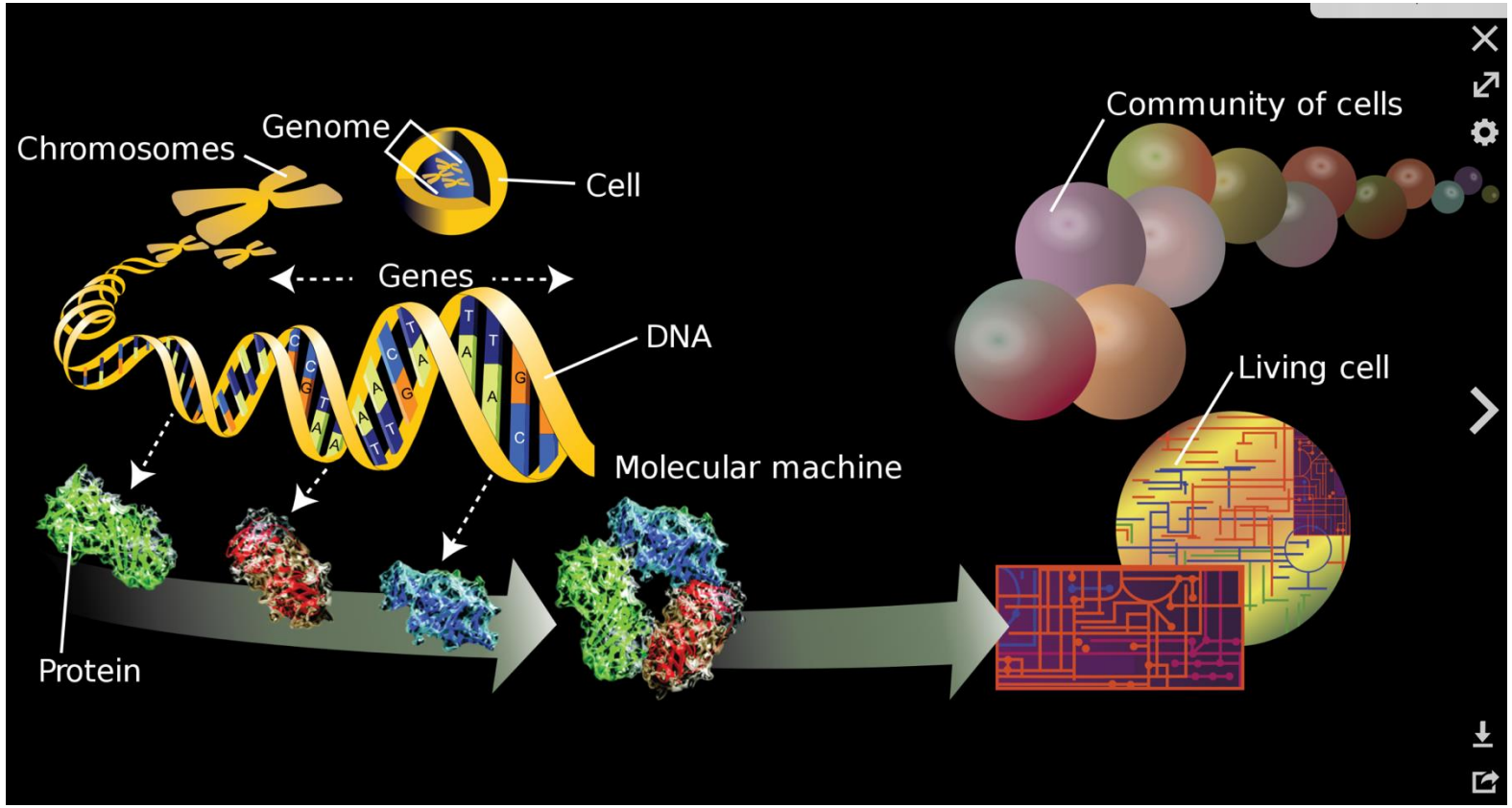
- More about single-cell!
- other OMICS methods
- Machine learning basic thoughts

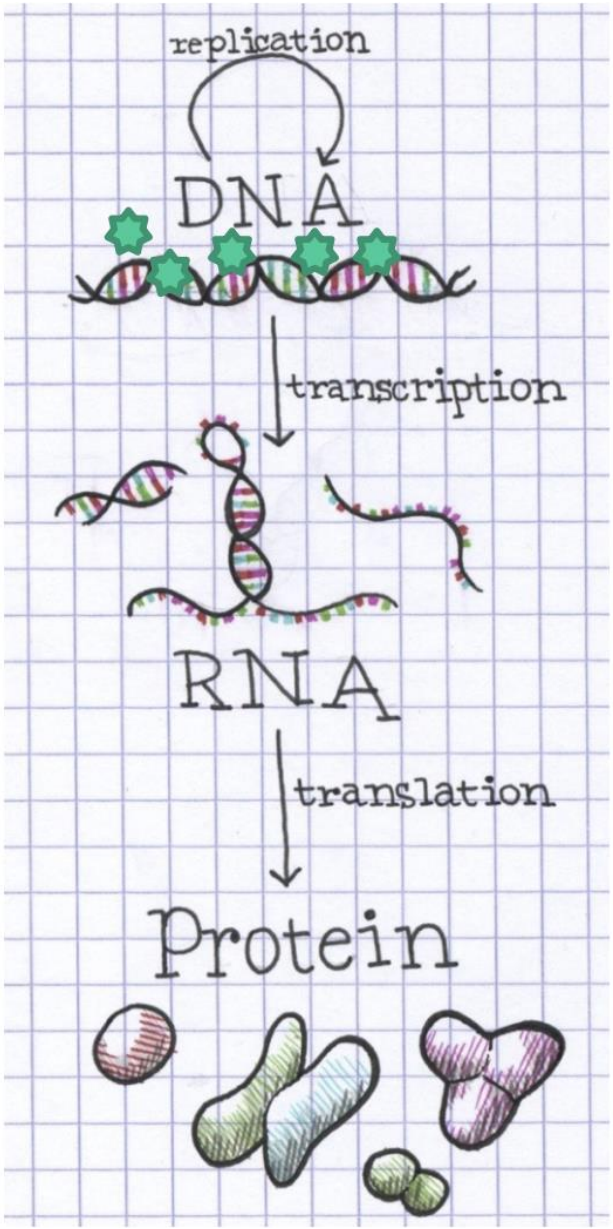
Indented learning outcome

- Grasp opportunities of different OMICS methods
- Reflect which methods can be used in which contect

- Explore the idea of machine learning (ML)
- Reflect on the application of ML in medical sciences

Where are we?





R. Burchmore Lecture

More scRNA-Seq methods

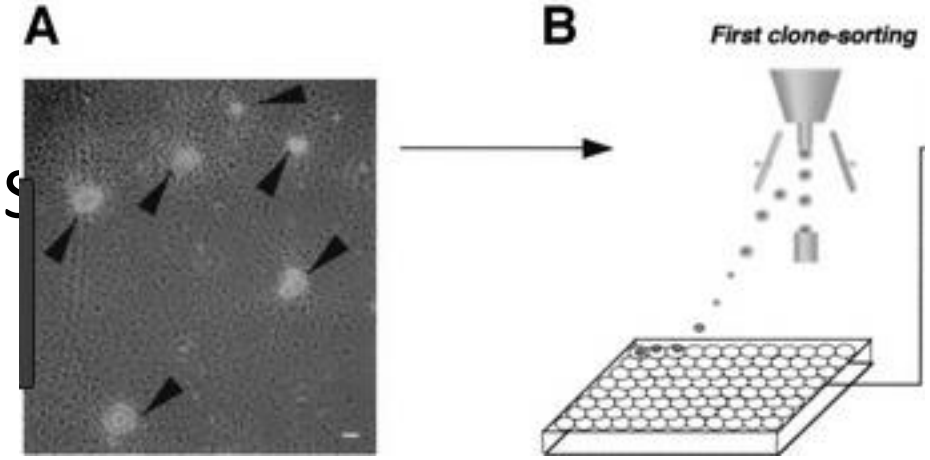
- Reference based mapping
- cell-cell interaction
- spatial transcriptomics

- Epigenetics / ATAC-Seq
- B/T cell repertoire sequencing
- Other sequencing methods: SMART-Seq2 & BDRhapsody

- Mix of computational and wetlab methods

Other sequencing methods

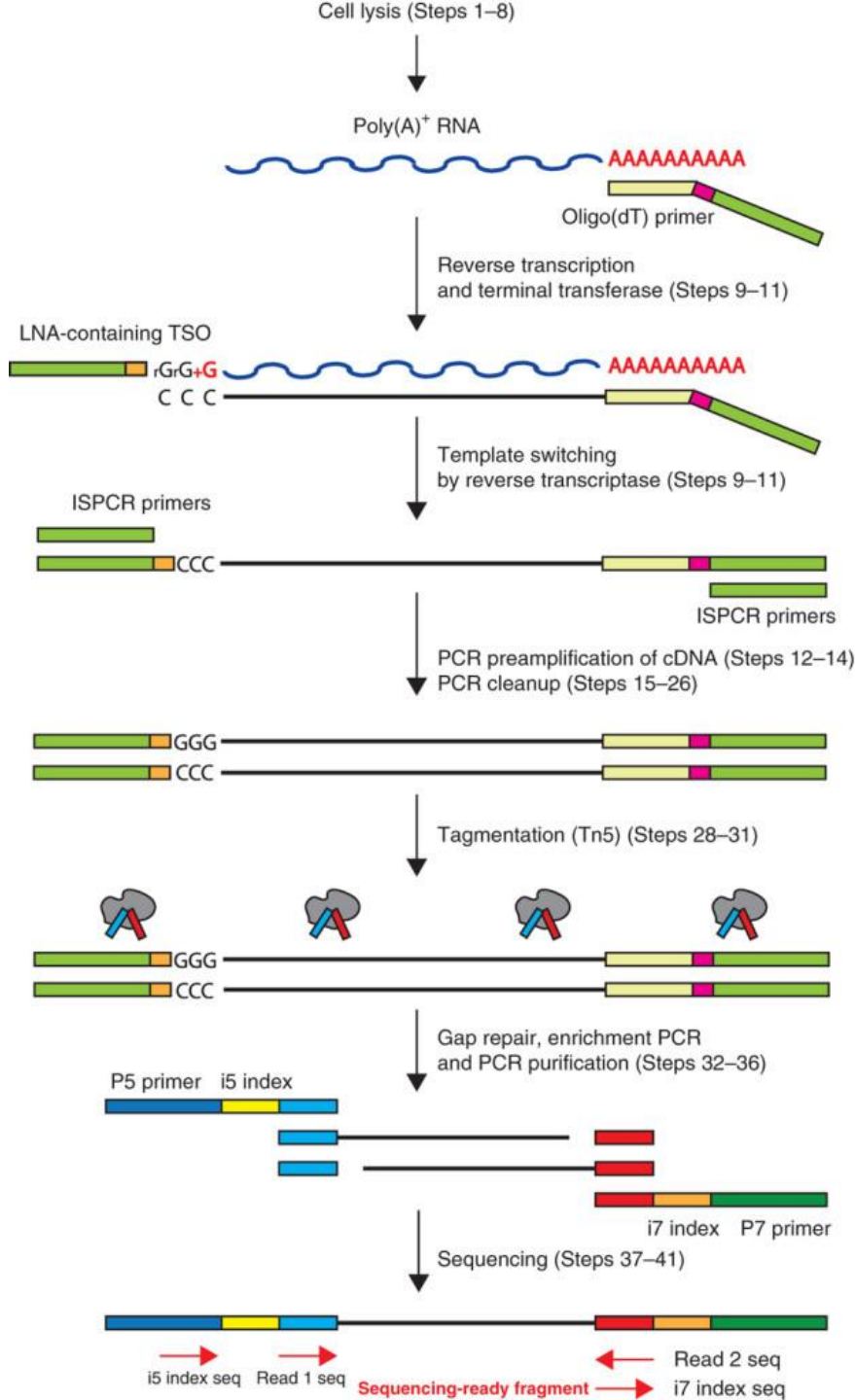
- Plate based, like Fluidigm or SMART-Seq2 / S



SMART-Seq2 method

- allows the generation of full-length cDNA and sequencing libraries by using standard reagents
- protocol takes ~2 d from cell picking to having a final library ready for sequencing
- limitations are the lack of strand specificity and the inability to detect nonpolyadenylated (polyA-) RNA.

- Low throughput, but better capturing of transcripts
- **Also full length (1million read per cell), looking alternative splicing**
- SMART-Seq3 does 3' sequencing & UMI



Picelli *Nature Methods* **volume 10**, pages1096–1098(2013)

Different analysis pipeline: Scater

- A little bit different to Seurat
- pre-processing
- QC
- normalization
- Visualization

- DE with S3C
- VERY similar to use – you could do it!

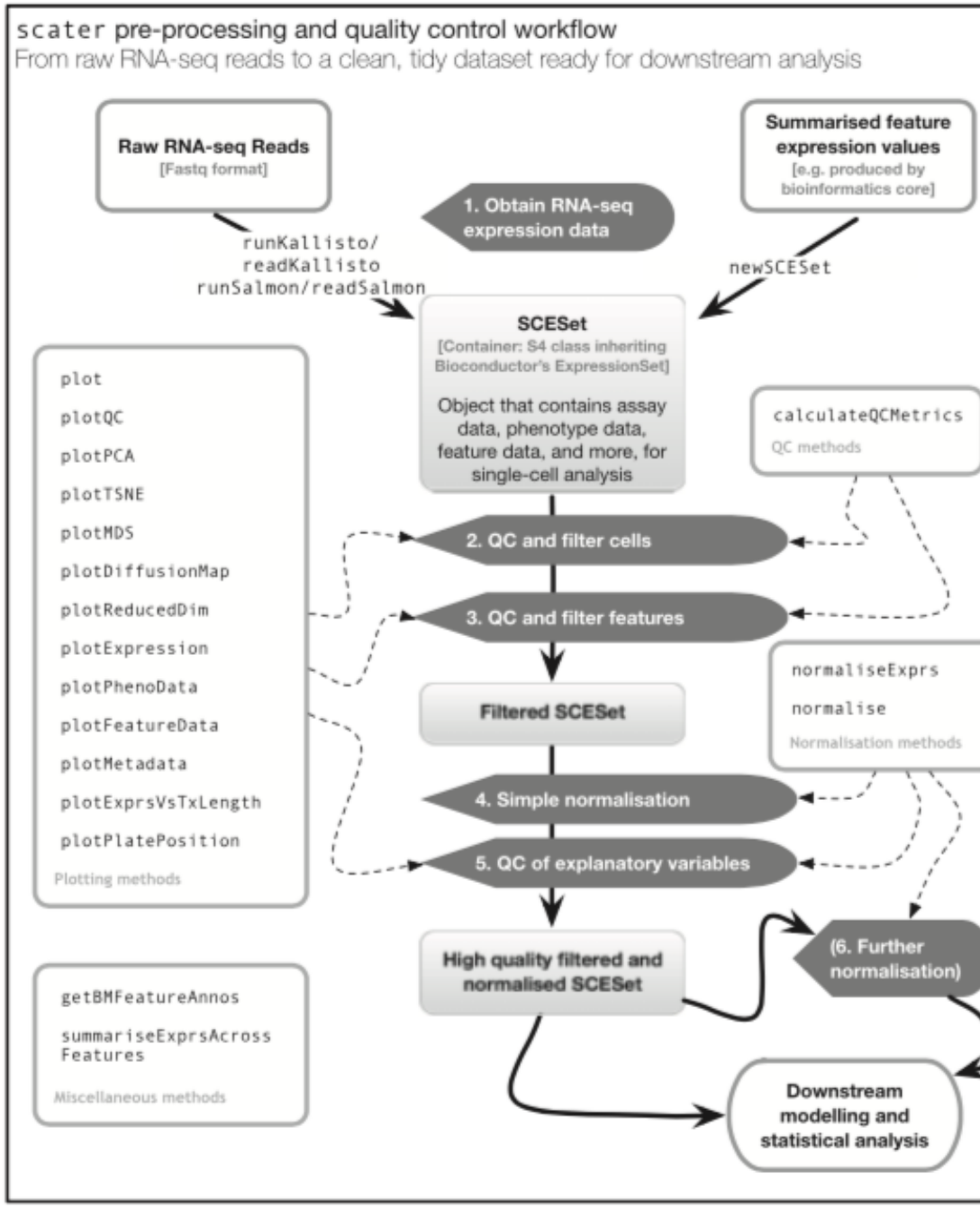


Fig. 1. An overview of the *scater* workflow, from raw sequenced reads

BD Rhapsody

- Combination of scRNA-Seq & antibody sequencing (later),
- Gene panel sequencing (~500 genes)
- Cheaper

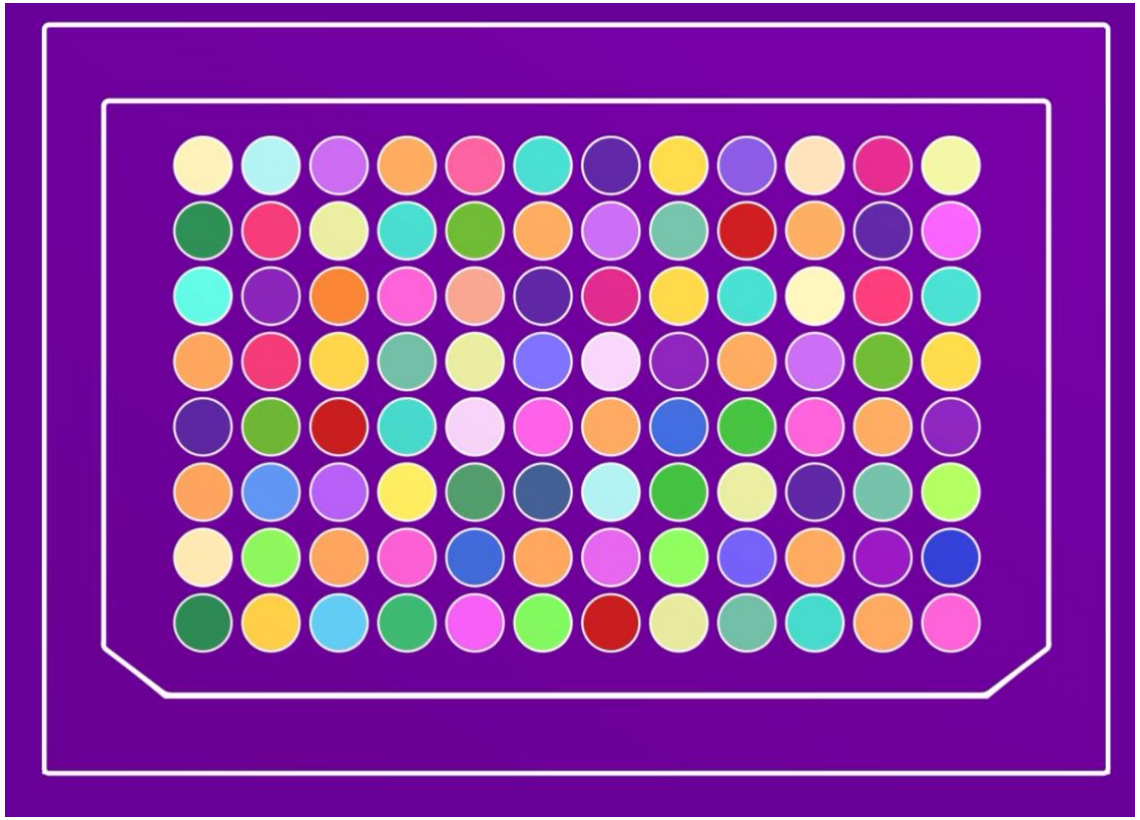
- Also 2D matrix
- Can use Seurat

https://www.bdbiosciences.com/en-gb/products/instruments/single-cell-multiomics-systems/rhapsody

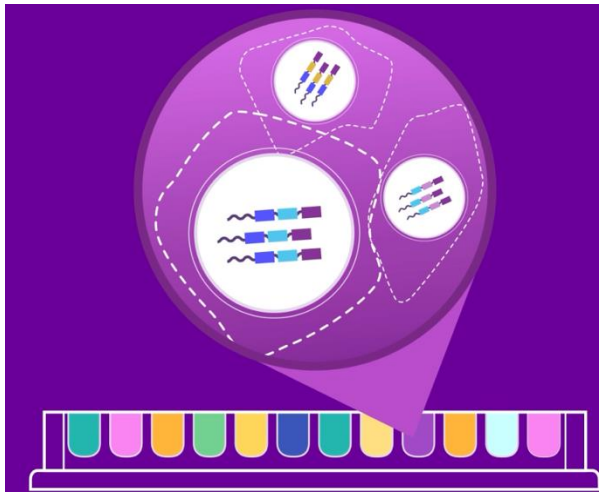
New: HIVE – good, not as good as 10X?

https://honeycomb.bio/the-hive/

Parse-Seq (version2)



Fix cells (dead)
Put them on plate
Barcode all the mRNA of each cells (tricky **the trick – in cell reverse transcription**)
Then pool cells, split them on plate and barcode mRNA again and again



No instrument required

Unmatched data quality

Fixation of cells and nuclei

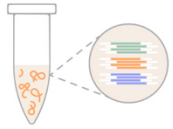
https://www.parsebiosciences.com/

Illumina: PipSeq – they bought it

Simple and scalable workflow

Sample prep

Create templated emulsions using a vortex mixer to capture and barcode single-cell mRNA.



Library prep

Generate cDNA and prepare single-cell libraries for sequencing.

Sequencing

Sequence on an NGS system to match the scale of your study.

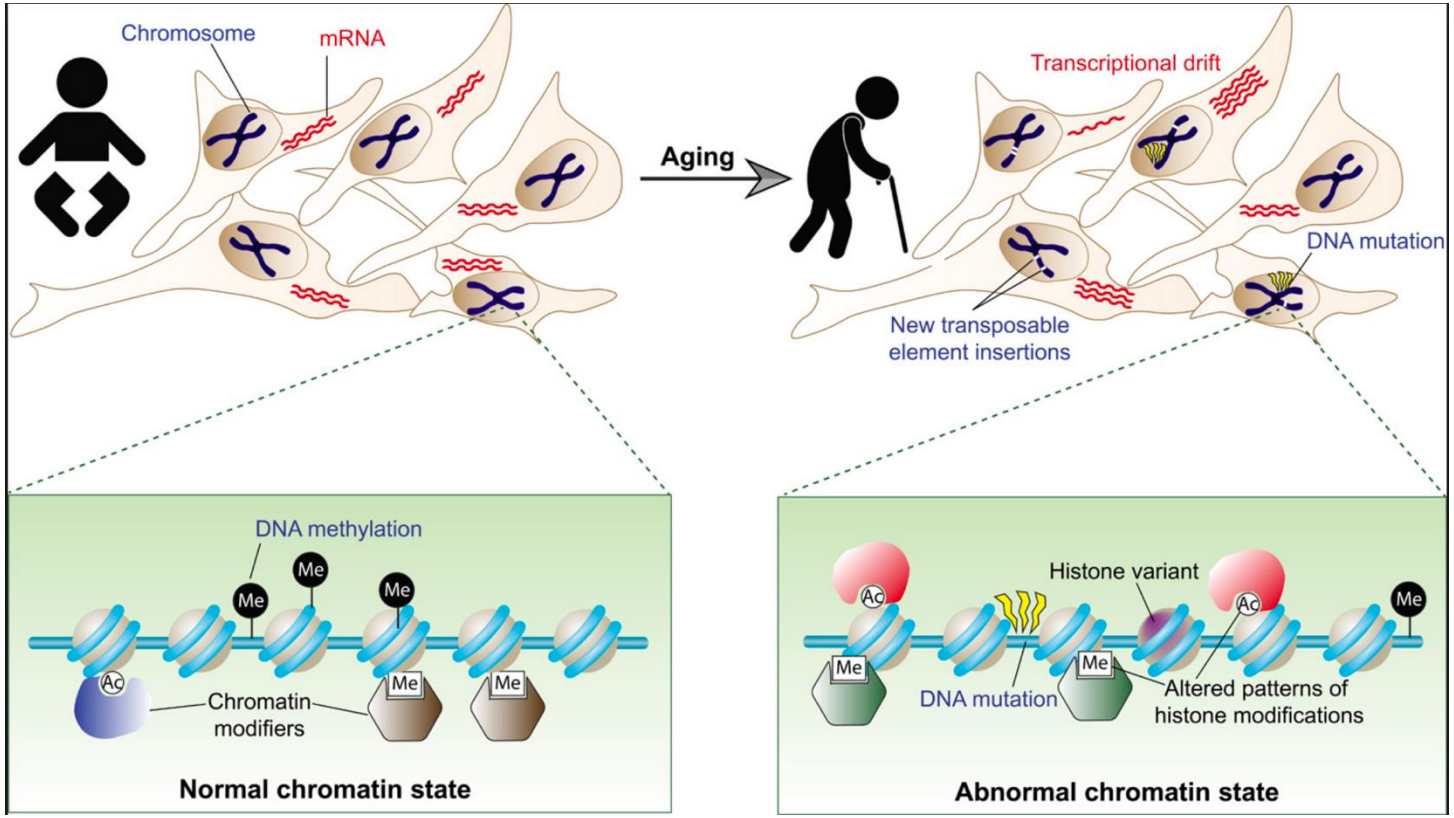
Analysis

Analyze and visualize single-cell data using DRAGEN and Partek software.

Sorry, but all company drive!

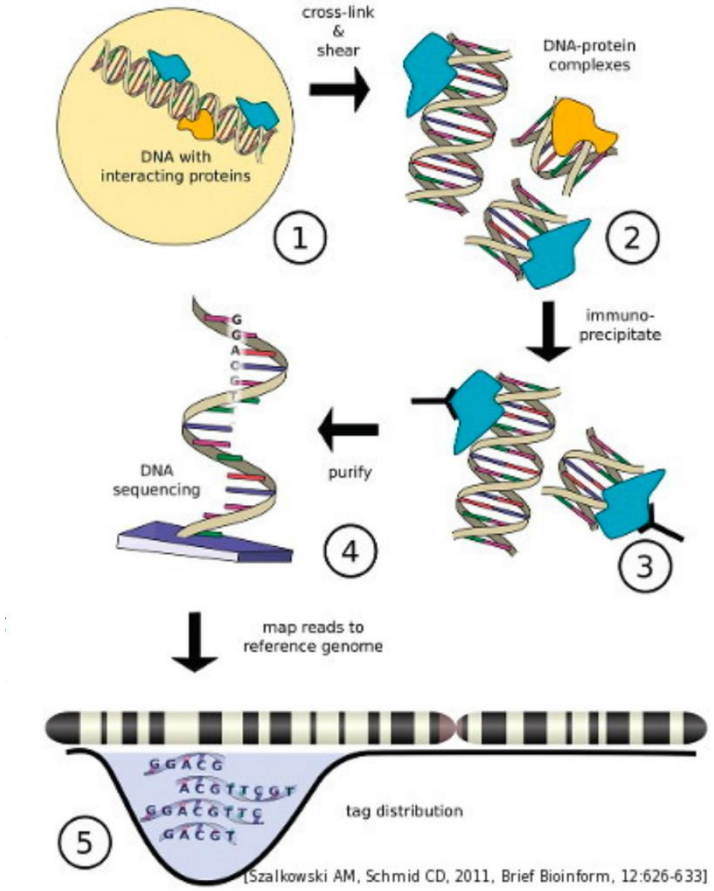
- They make money…
- … so more technologies to come!
- More to come, like

Epigenetics…



http://advances.sciencemag.org/content/2/7/e1600584.full

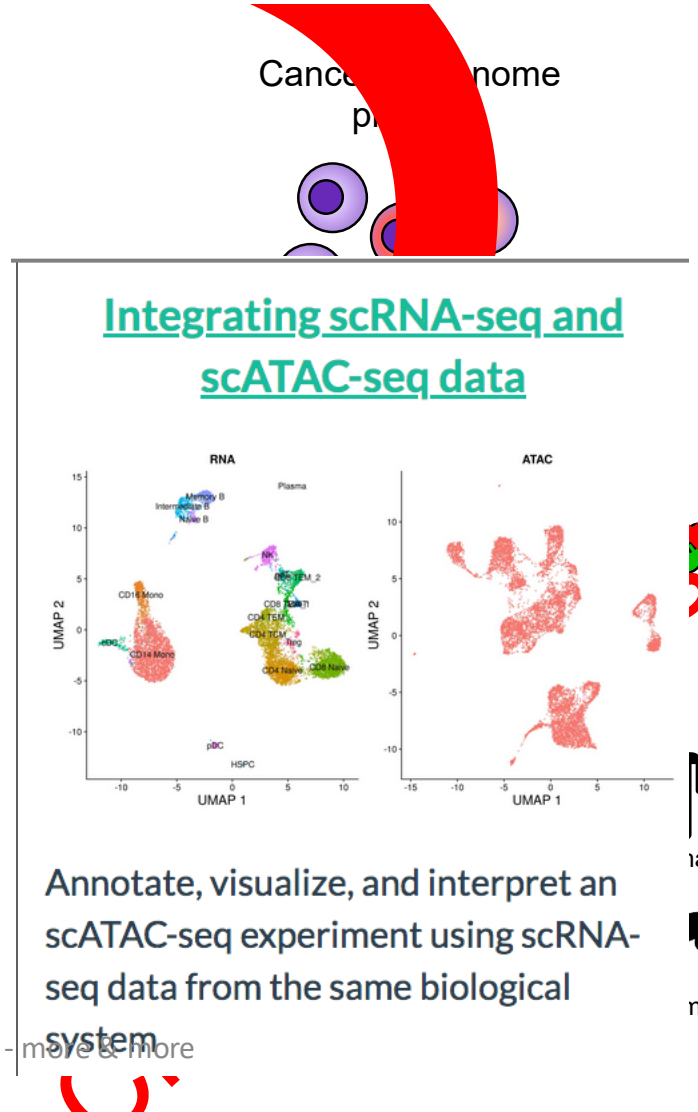
chromatin immunoprecipitation sequencing (ChIP-Seq)

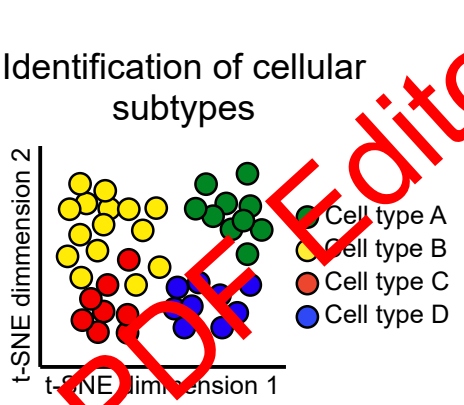


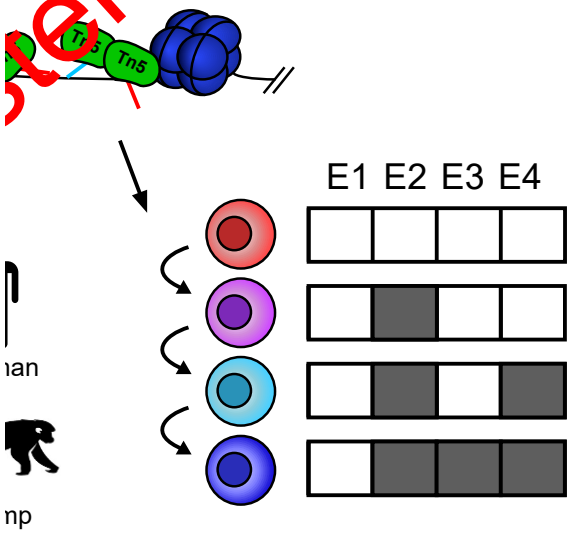
- You must test that for the different antibody
- Noisy methods (immunoprecipitation) so replicates are crucial

Assay for **T**ransposase-**A**ccessible **C**hromatin using **seq**uencing - ATAQ-seq

- assess genome-wide chromatin accessibility.
- Tn5 transposase cleaves and tags double-stranded DNA with sequencing adaptors (tagmentation)
- Single cell methods exists! Possible to do scATAQ-Seq and scRNA-Seq from the same cell (10X multiome method)







Enhancer usage analysis during cell differentiation

wikepedia

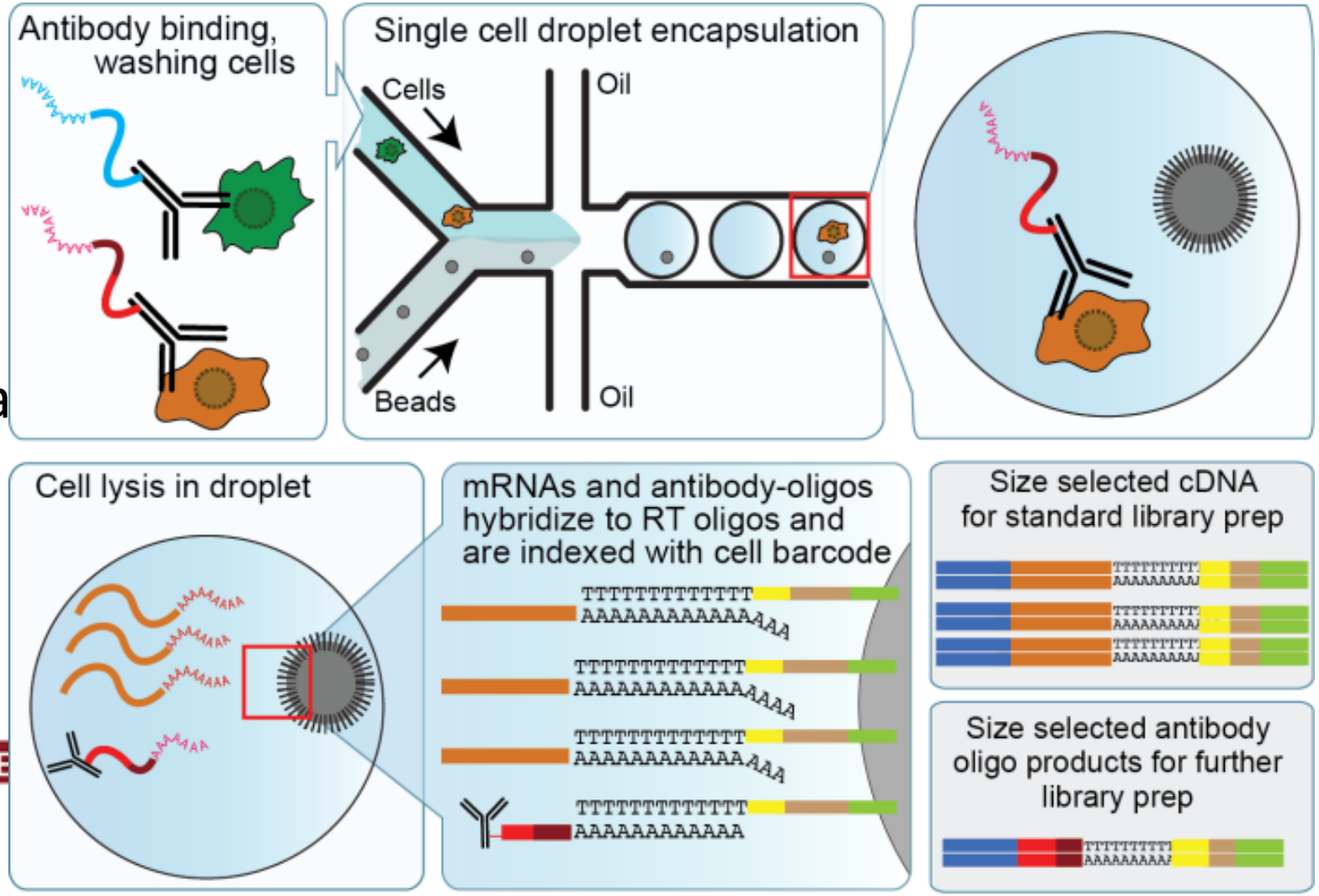
Repertoire sequencing!

- Video: https://www.10xgenomics.com/products/single-cell-immune-profiling

- Capturing the repertoire of T or B cells through PCR.
- Possible to look for expansion

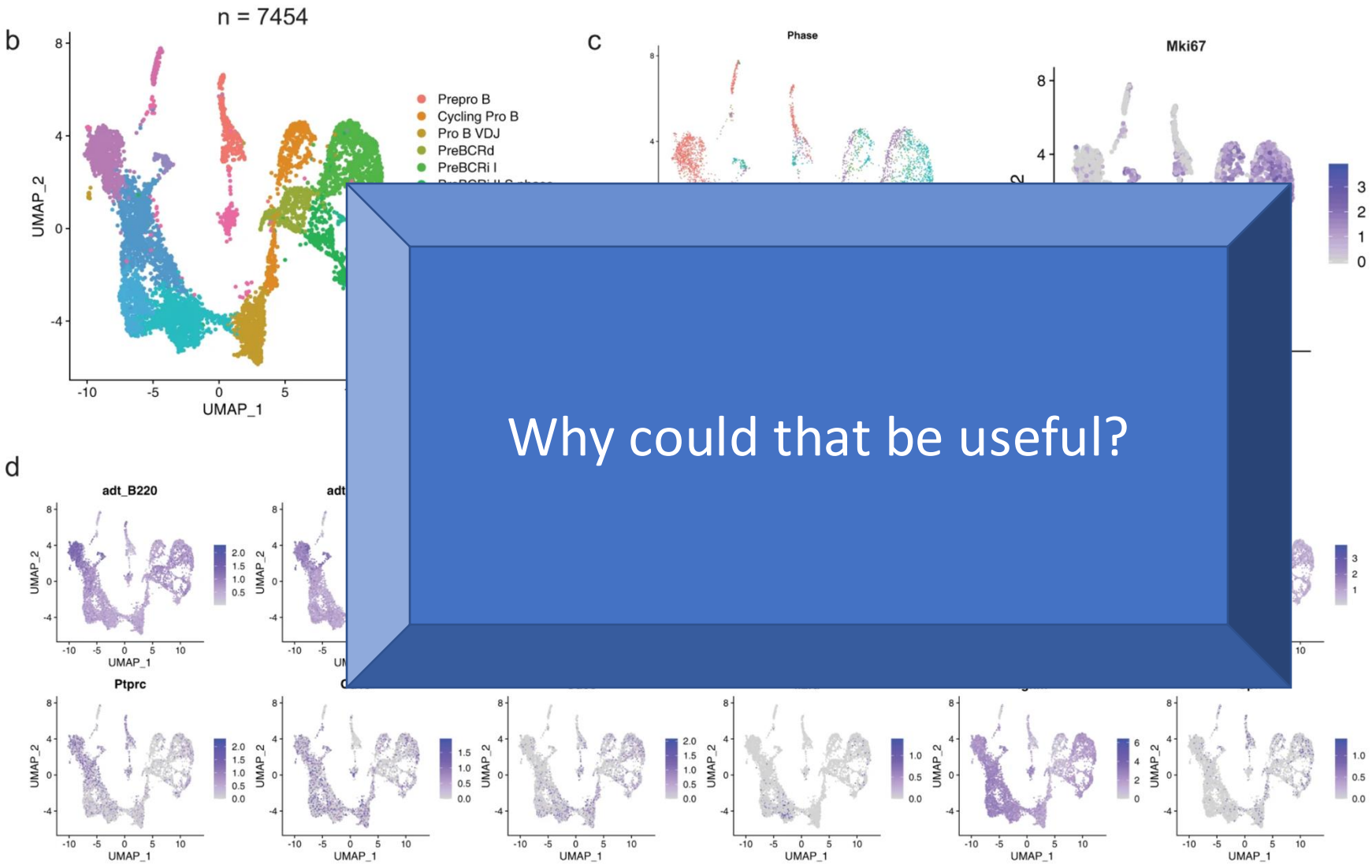
Cellular Indexing of Transcriptomes and Epitopes by Sequencing (CITE-seq)

- Capture scRNA-Seq and protein on surface
- Need for each proteins a construct $$
- Extra sequencing



https://cite-seq.com/ // wikipedia

Example of several antibodies



https://www.nature.com/articles/s41467-021-27232-5

- Very good for the ADT 150 antibody panel

Single cell sequencing is not cheap

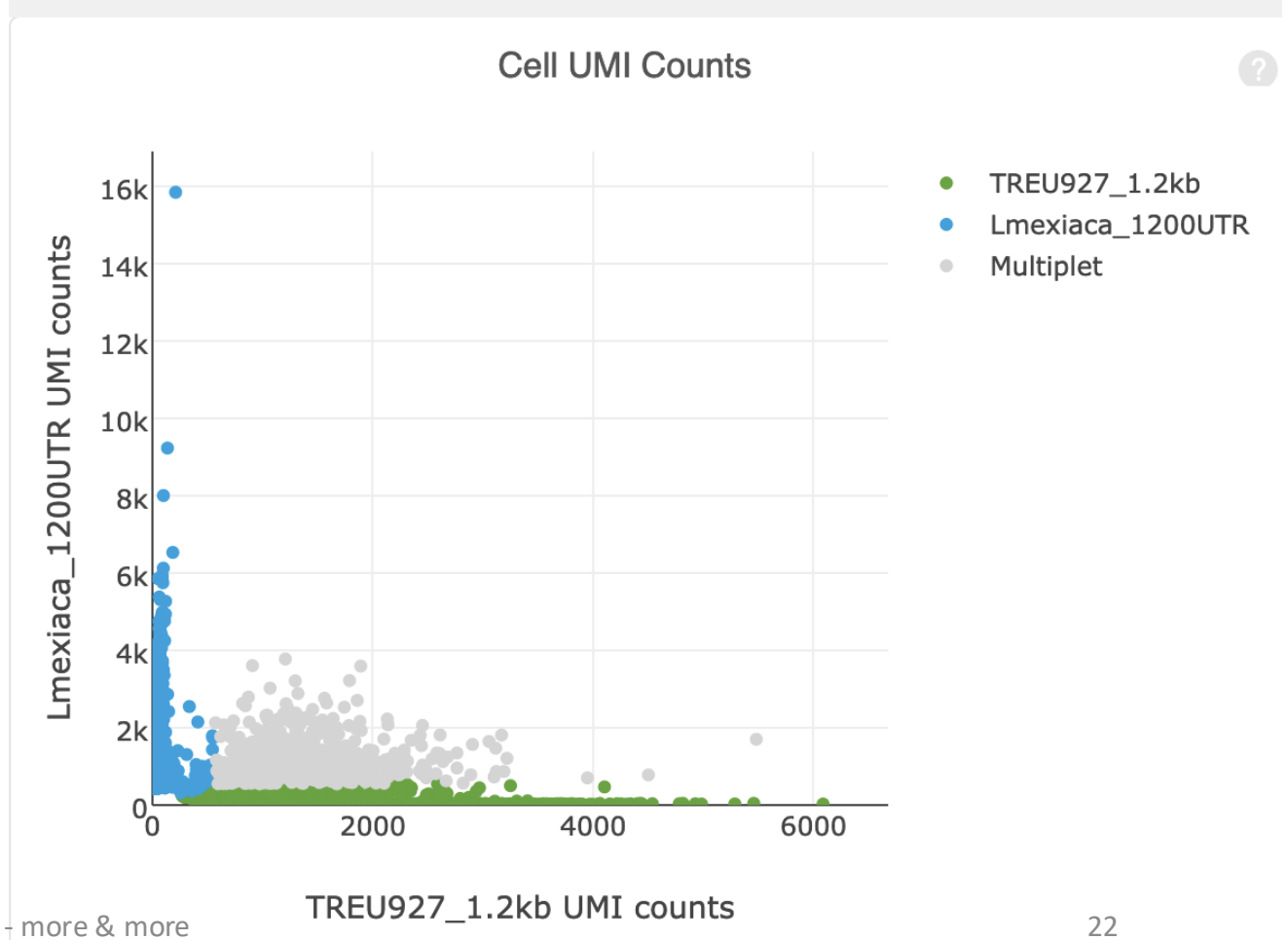
- ~ £1500 10X kit, + ~ £1500 for sequencing 6000 cells to 50k
- ~ £1500 10X kit, + ~ £500 for sequencing 2000 cells to 50k
- ~ £1500 10X kit, + ~ £3000 for sequencing 12000 cells to 50k
- Let’s pool samples to get the 10X cost down!

Mix 2 parasites

- cheaper (+)
- Need to be all ready at the exact time (-)
- Need to publish all data at once? (-)
- competitive mapping
- We can even use double, when between parasites! (+)

Estimated Number of Cells
14,563

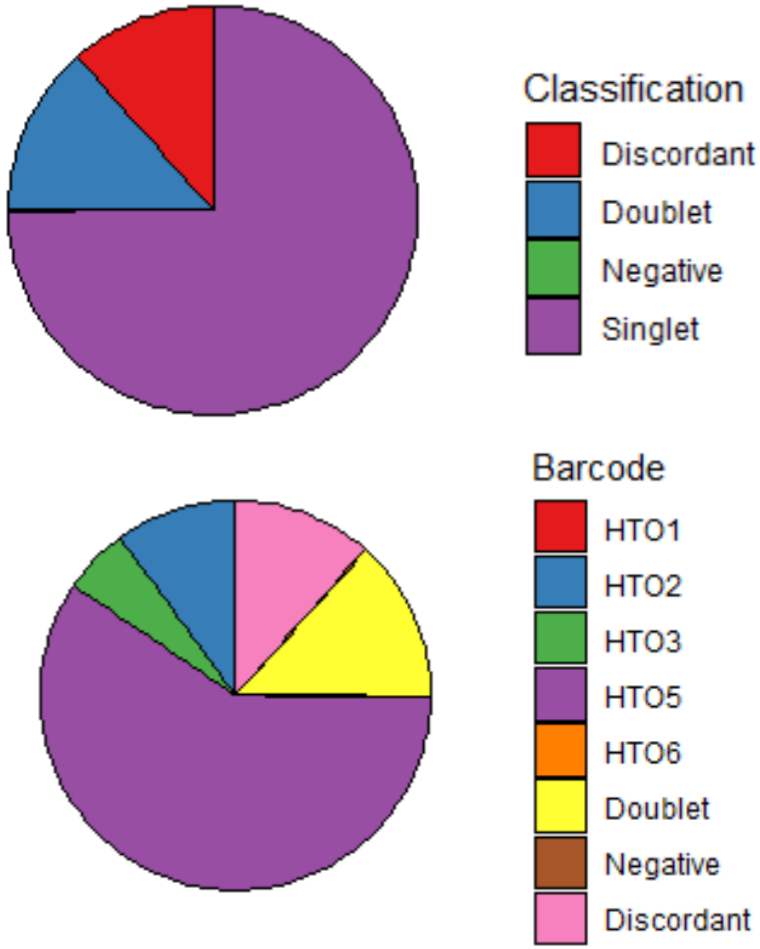
Mean Reads per Cell
32,577



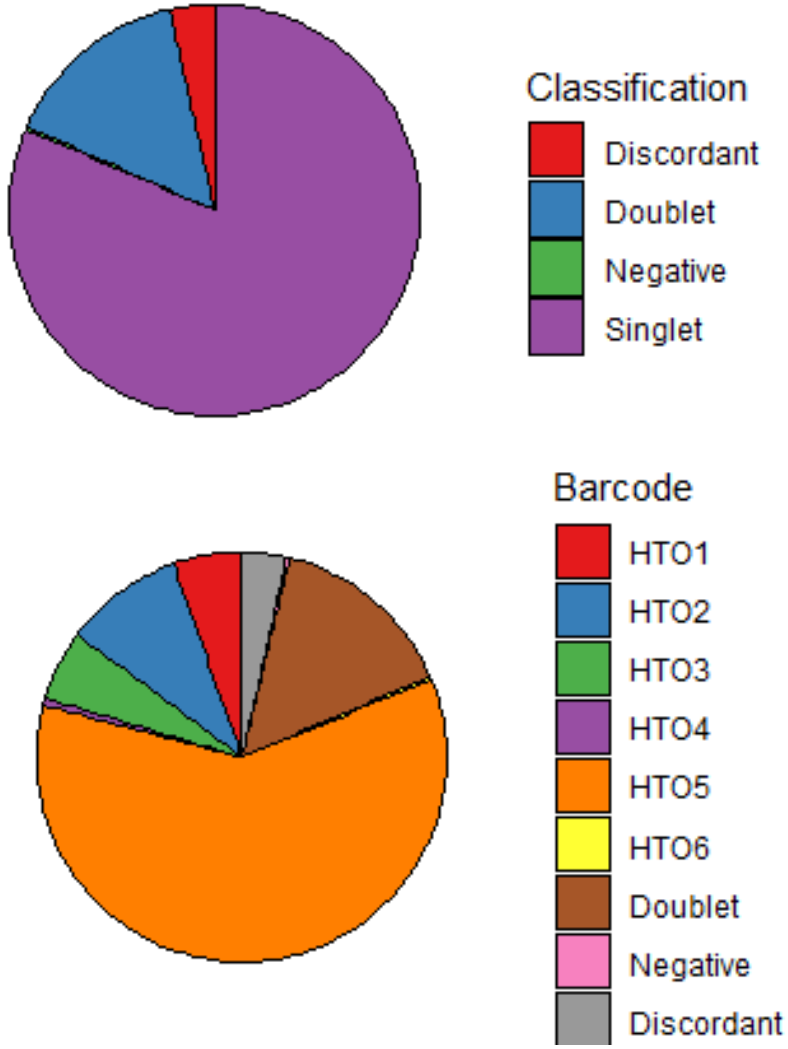
Emma Briggs / Felix Warren

HTO Cite Seq - Hashtag – readcounts/cell increased

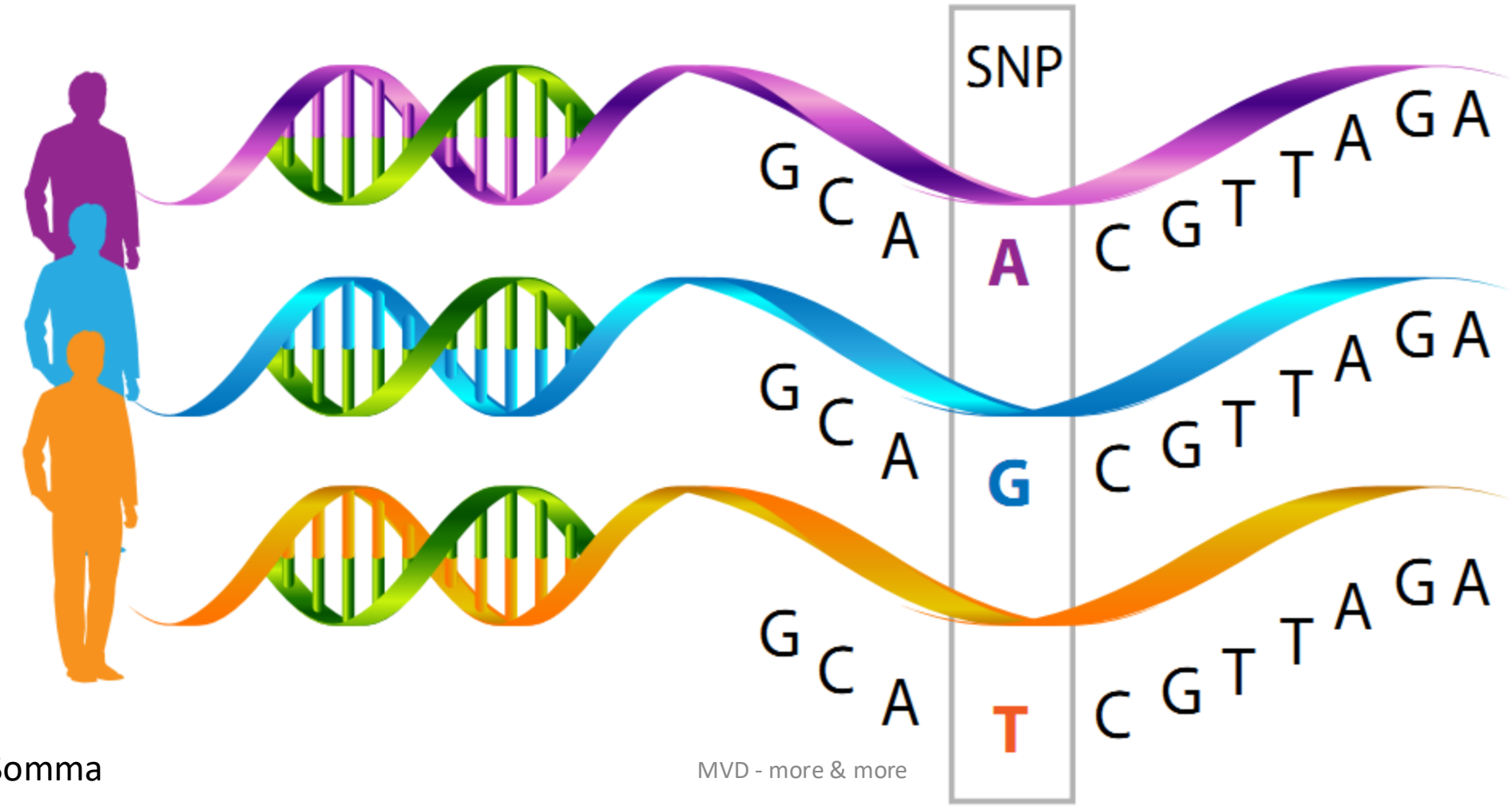
500 reads/cell:



5000 reads/cell:



Use SNPs to demultiplex



nature methods
ARTICLES
https://doi.org/10.1038/s41592-020-0820-1
Check for updates

Souporcell: robust clustering of single-cell RNA-seq data by genotype without reference genotypes

Haynes Heaton[1]✉, Arthur M. Talman[2], Andrew Knights[1], Maria Imaz[1,3], Daniel J. Gaffney[1], Richard Durbin[4], Martin Hemberg[1]✉ and Mara K. N. Lawniczak[1]✉

Demuxlet is the control with classic genotype identification

Vireo and scSplit are two alternative software

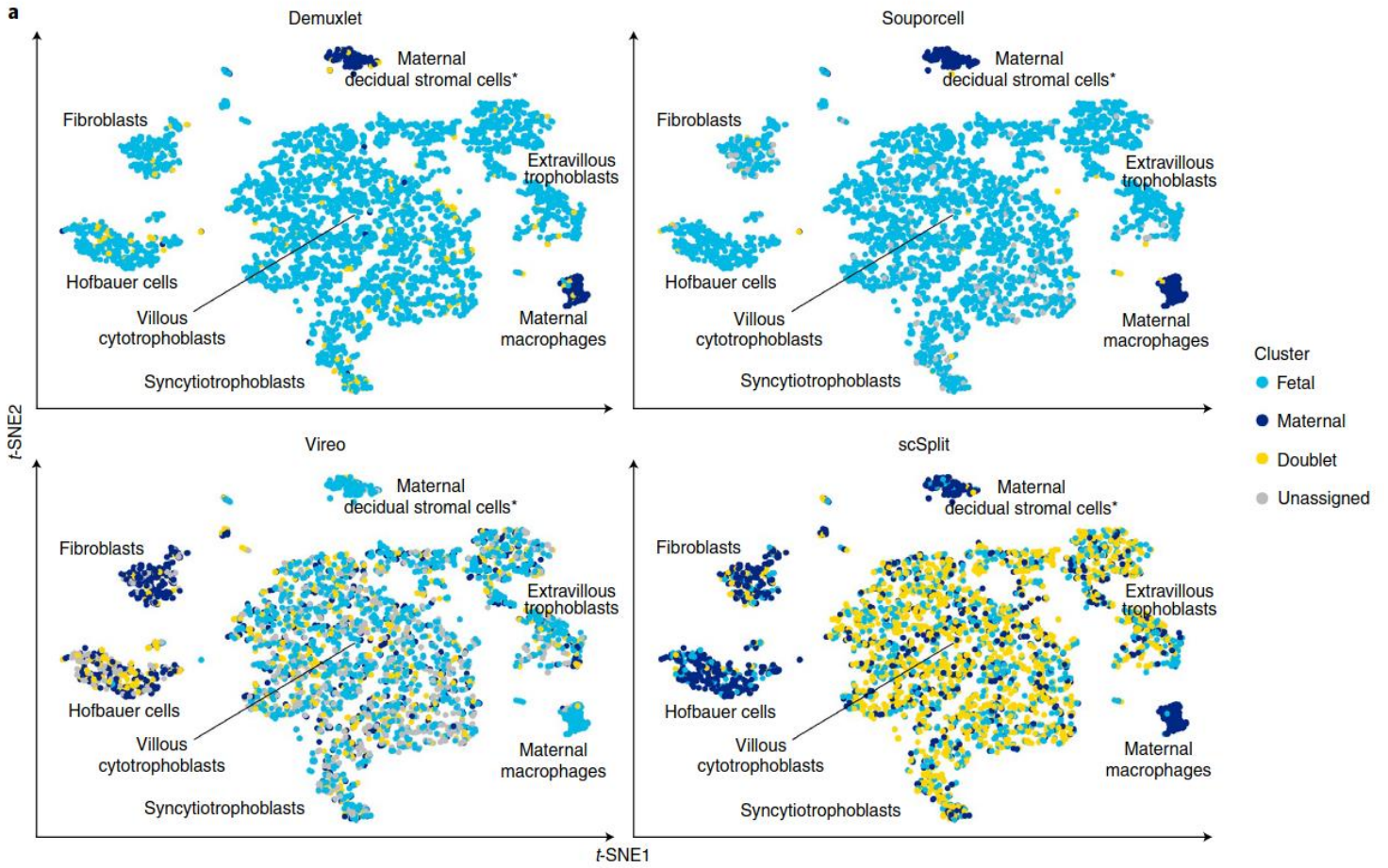
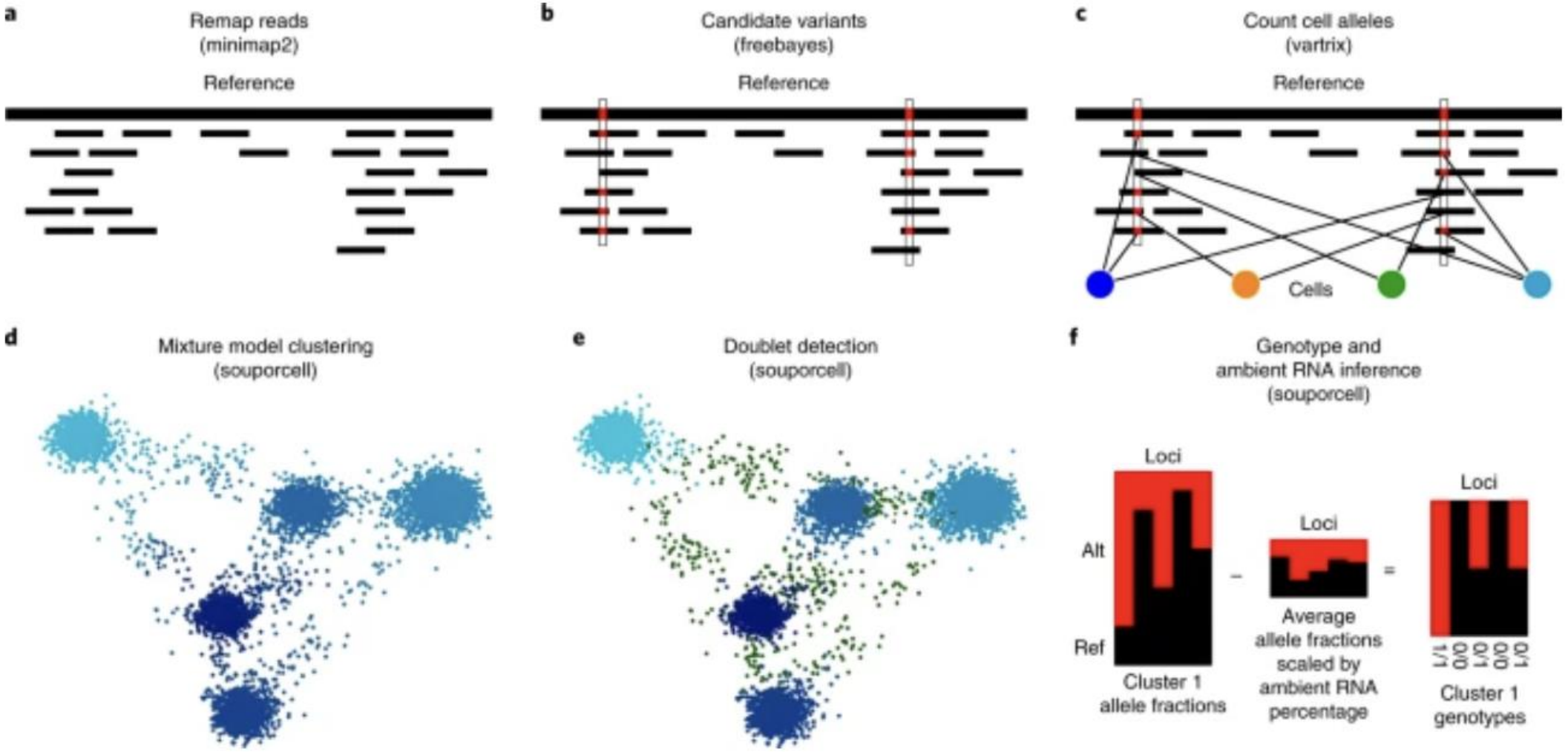
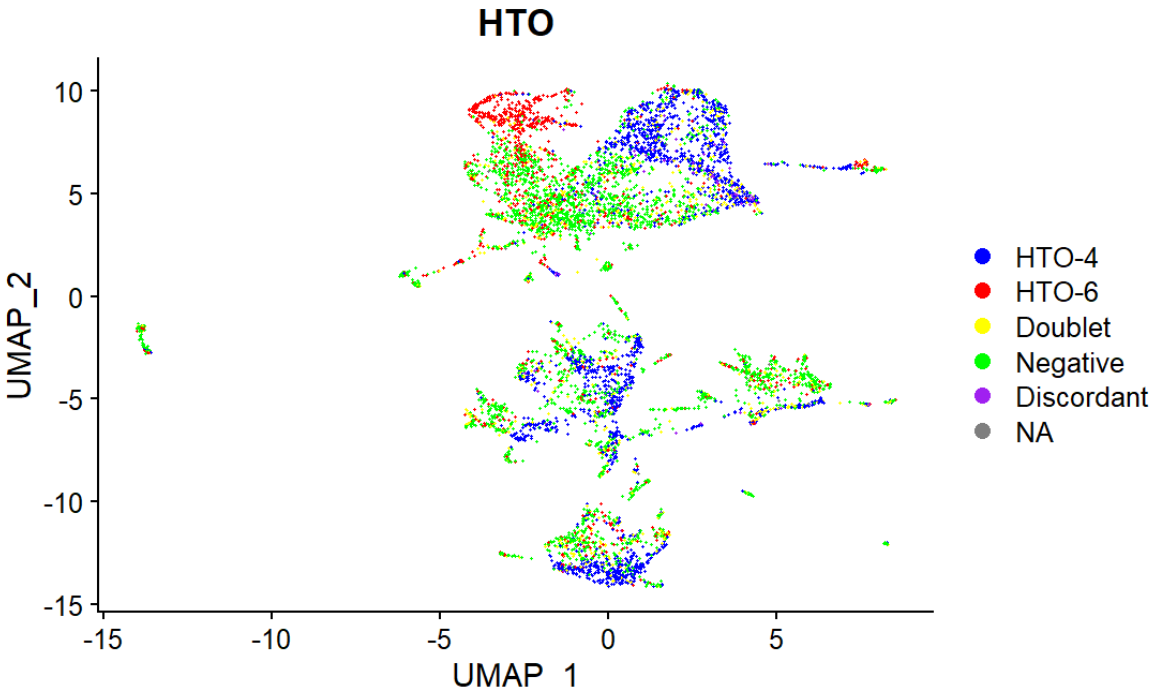
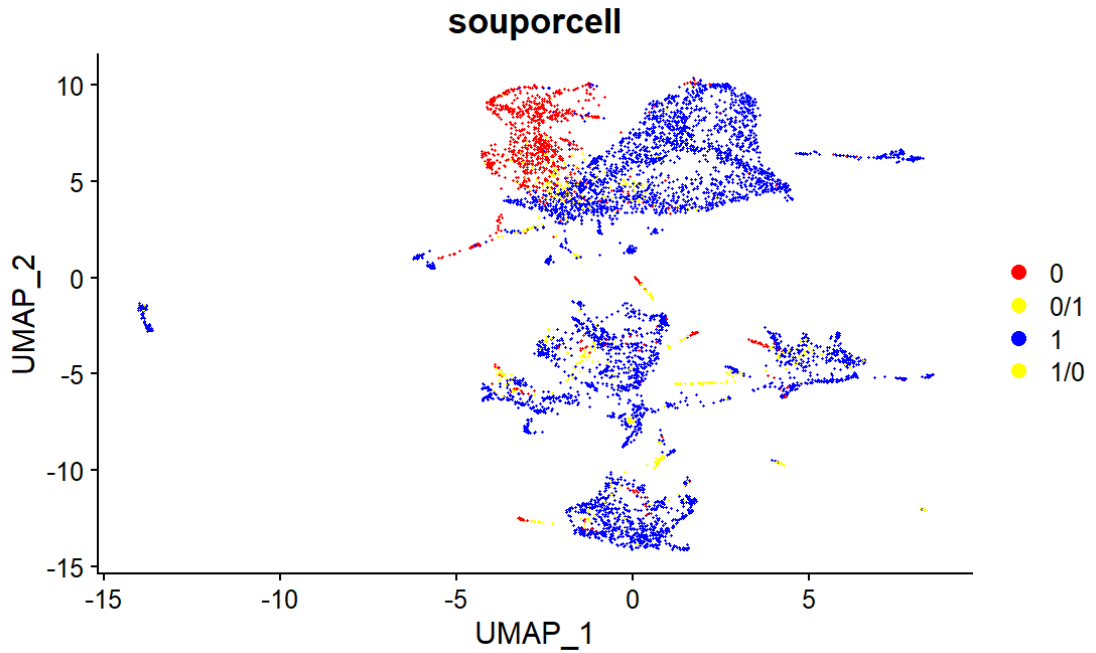


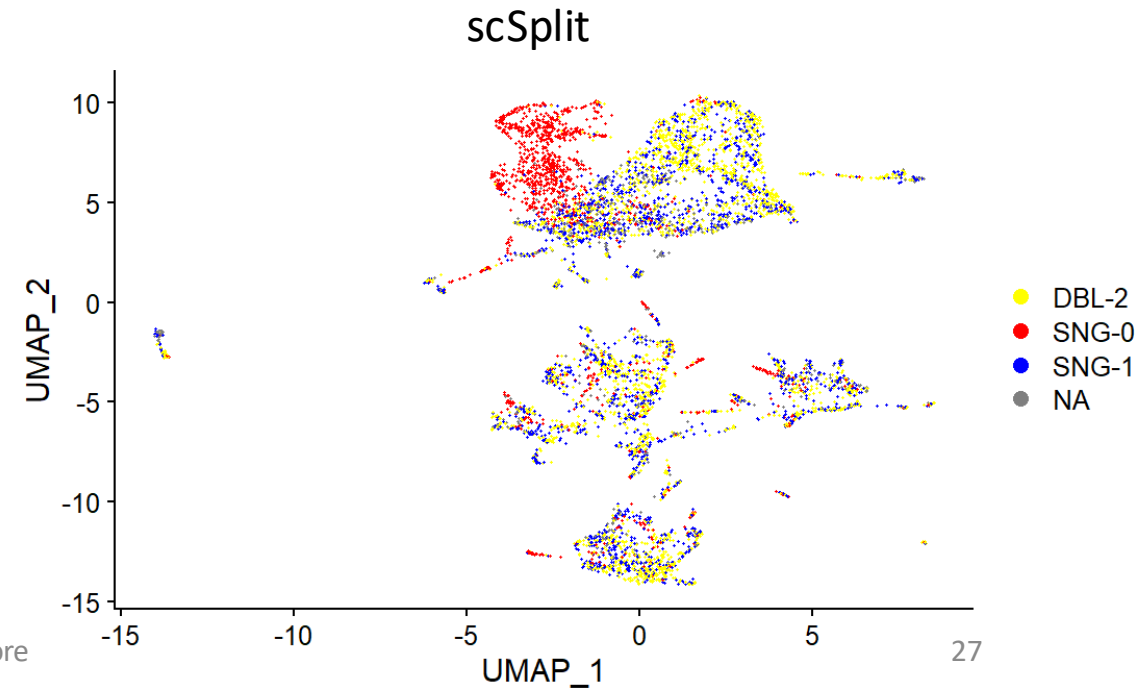
Fig. 1: Souporcell overview.



10X in one example

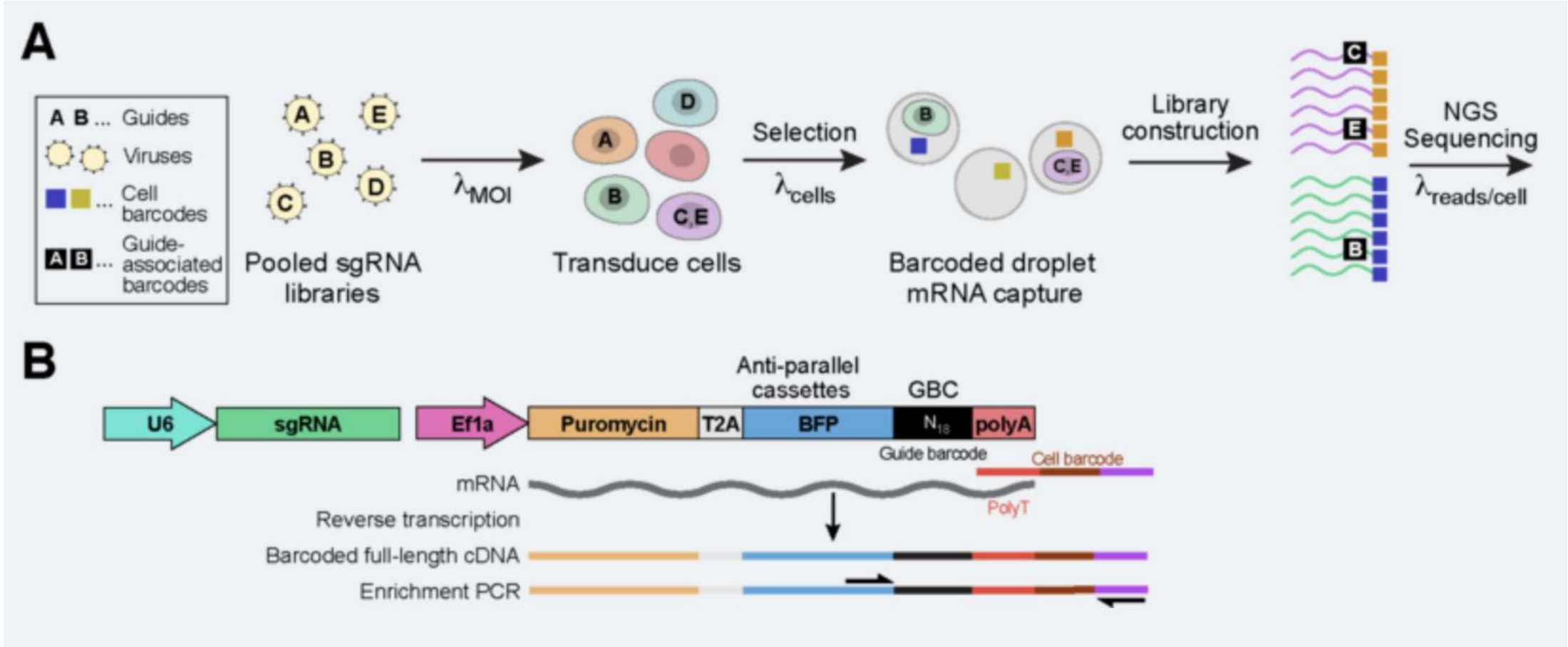






Perturb-Seq

CRISP-cas9
Do it on several samples
Pull them
sequence



https://singlecell.broadinstitute.org/single_cell/study/SCP24/perturb-seq

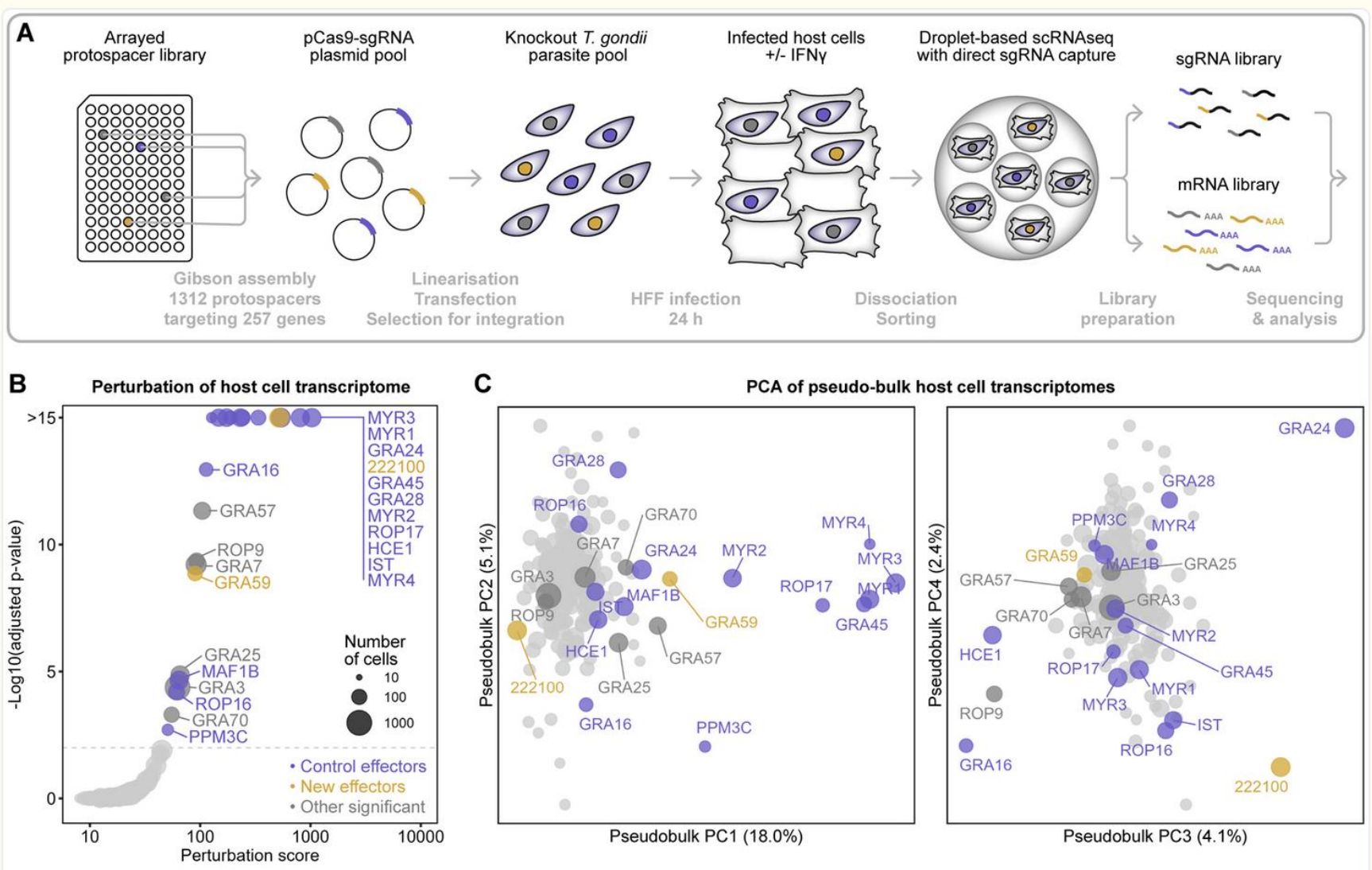
Cell Host Microbe. 2023 Oct 11;31(10):1748-1762.e8. doi: 10.1016/j.chom.2023.09.003.

High-throughput identification of Toxoplasma gondii effector proteins that target host cell transcription

Simon Butterworth [1], Kristina Kordova [1], Samba
Francesca Torelli [1], Eloise J Lockyer [1], Amelia E
Moritz Treeck [5]

Affiliations + expand

PMID: 37827122 PMCID: PMC12033024 DOI:



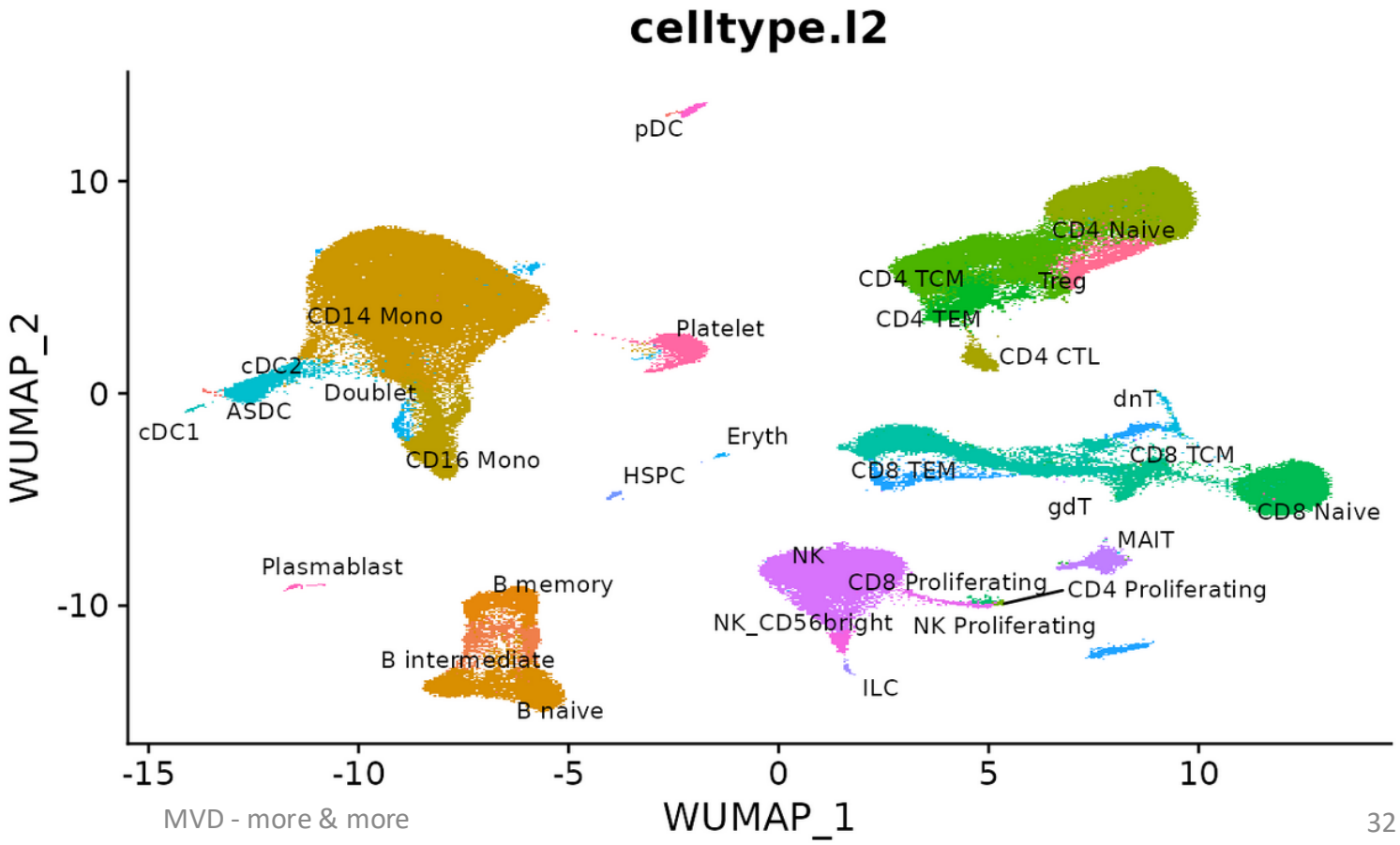
https://pubmed.ncbi.nlm.nih.gov/37827122/

- How to analyse
- What to consider?
- Why difficult?

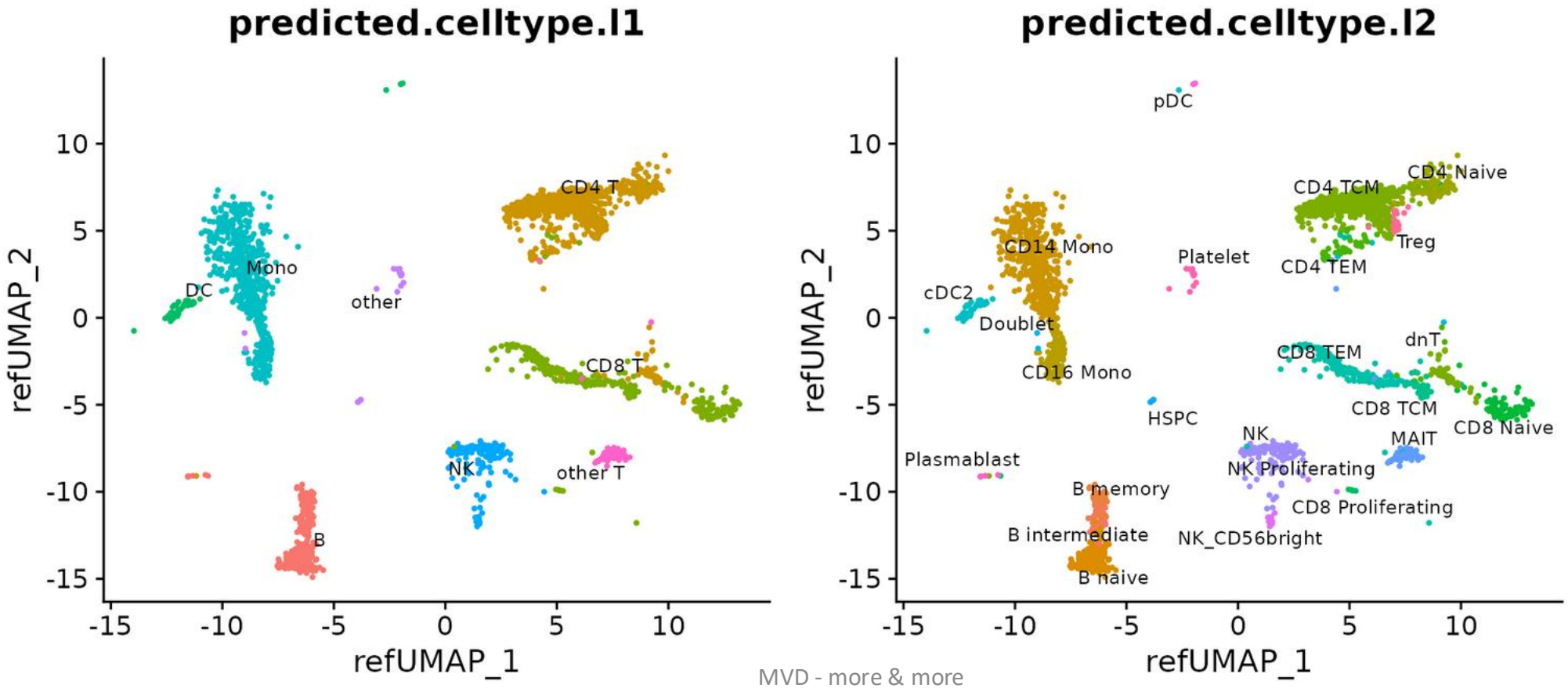
Automated reference annotation

- Annotation of cell population is difficult and time consuming
- Is it possible to transfer annotation from know datasets?

- You can transfer the annotation from a reference (singleR)
- Map your data onto a reference (Seurat)



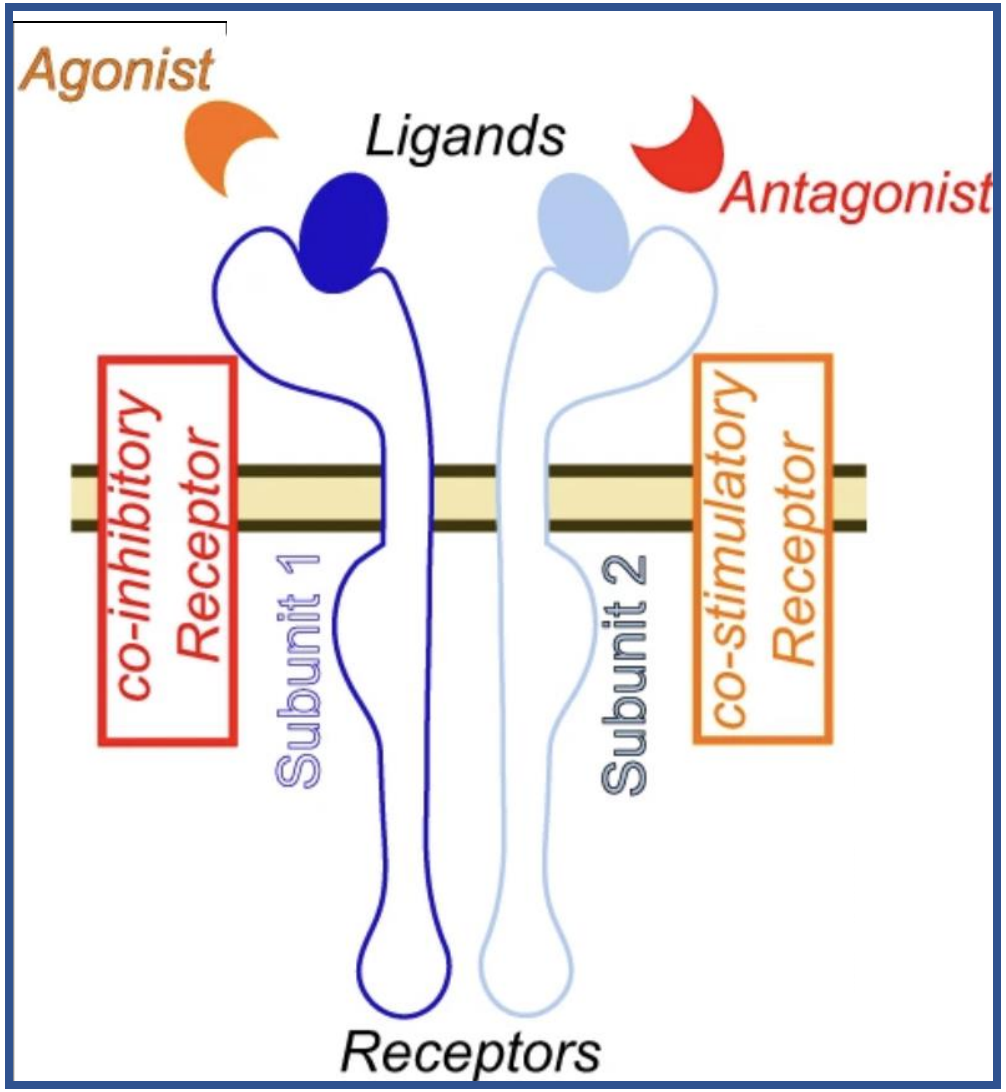
- https://satijalab.org/seurat/articles/multimodal_reference_mapping.html
- Might not need to do the cluster annotation?



- Mention Other methods like

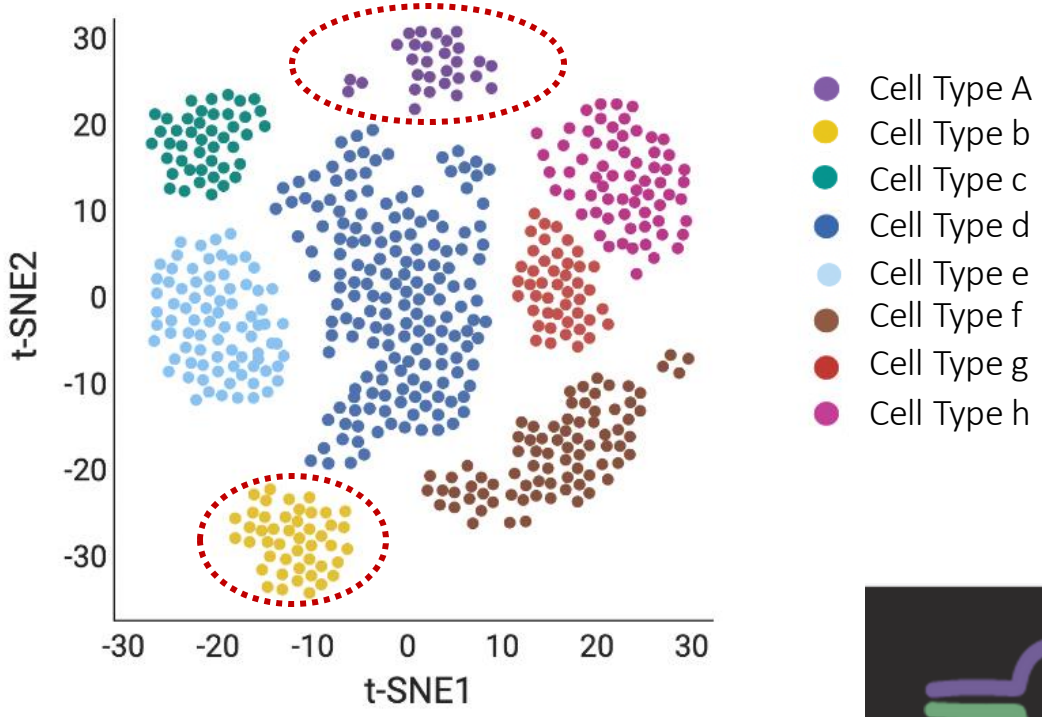
Cell-cell interaction

- scRNA-Seq allows us to interaction the dynamic of a cell population in terms of expression
- But can we deduce interaction **between** cells?



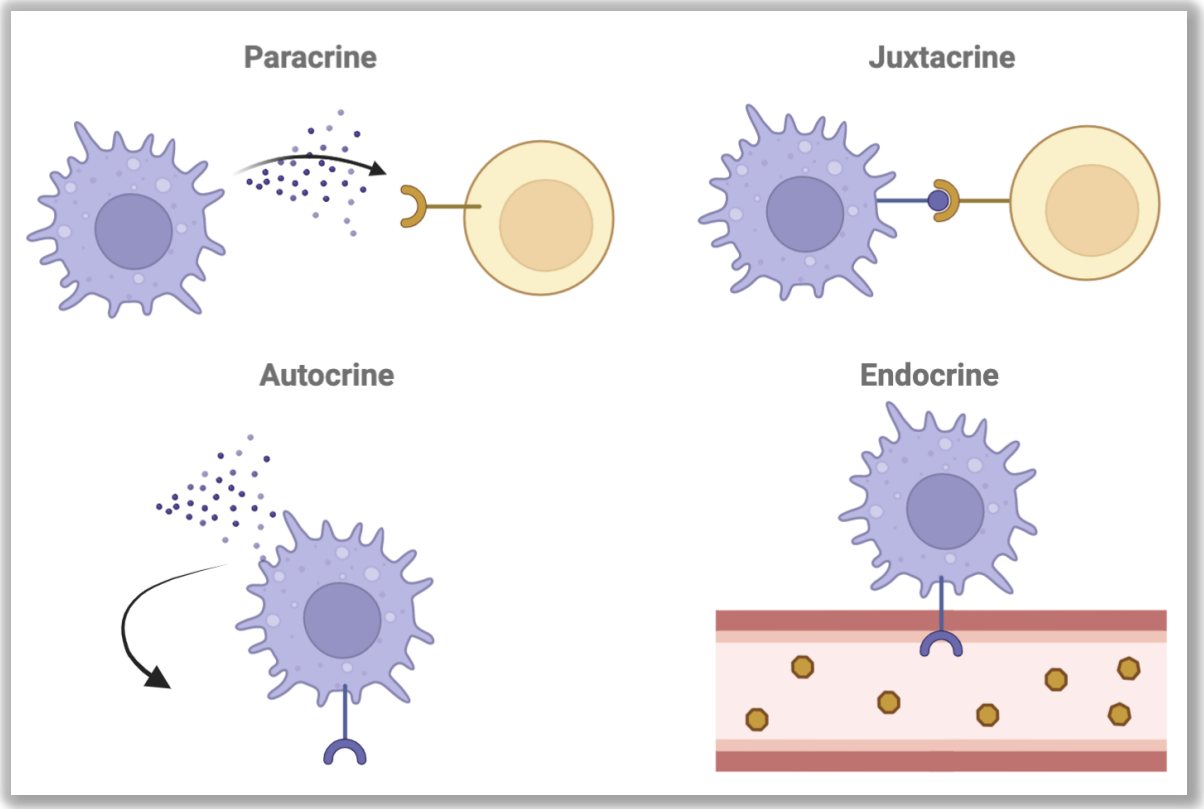
https://www.nature.com/articles/s41467-021-21246-9/figures/1

How do computational tools predict Cell-Cell Interaction (CCI)?

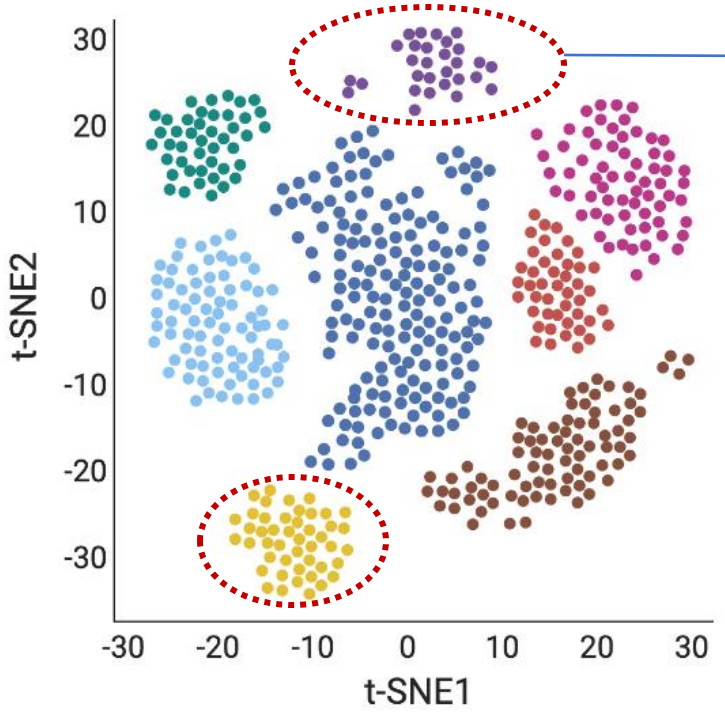


A database of know receptor-ligand interactions

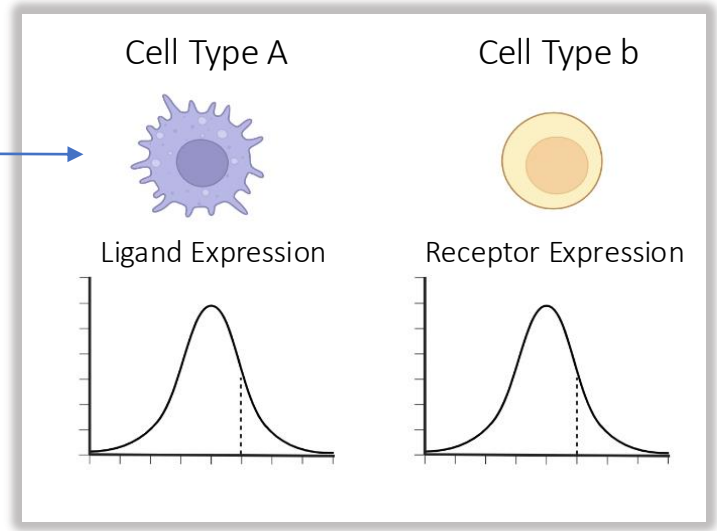
Types of cell-cell interactions

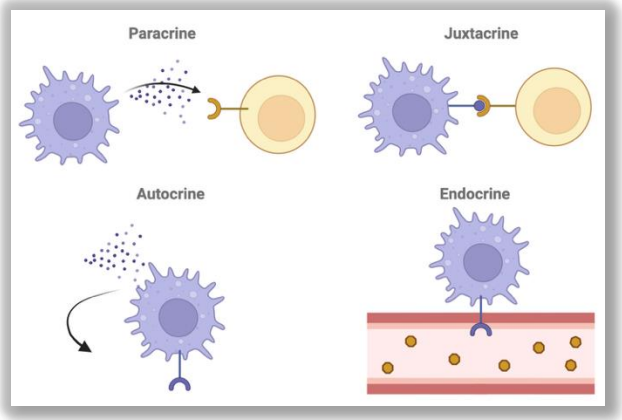


How do computational tools predict CCI?



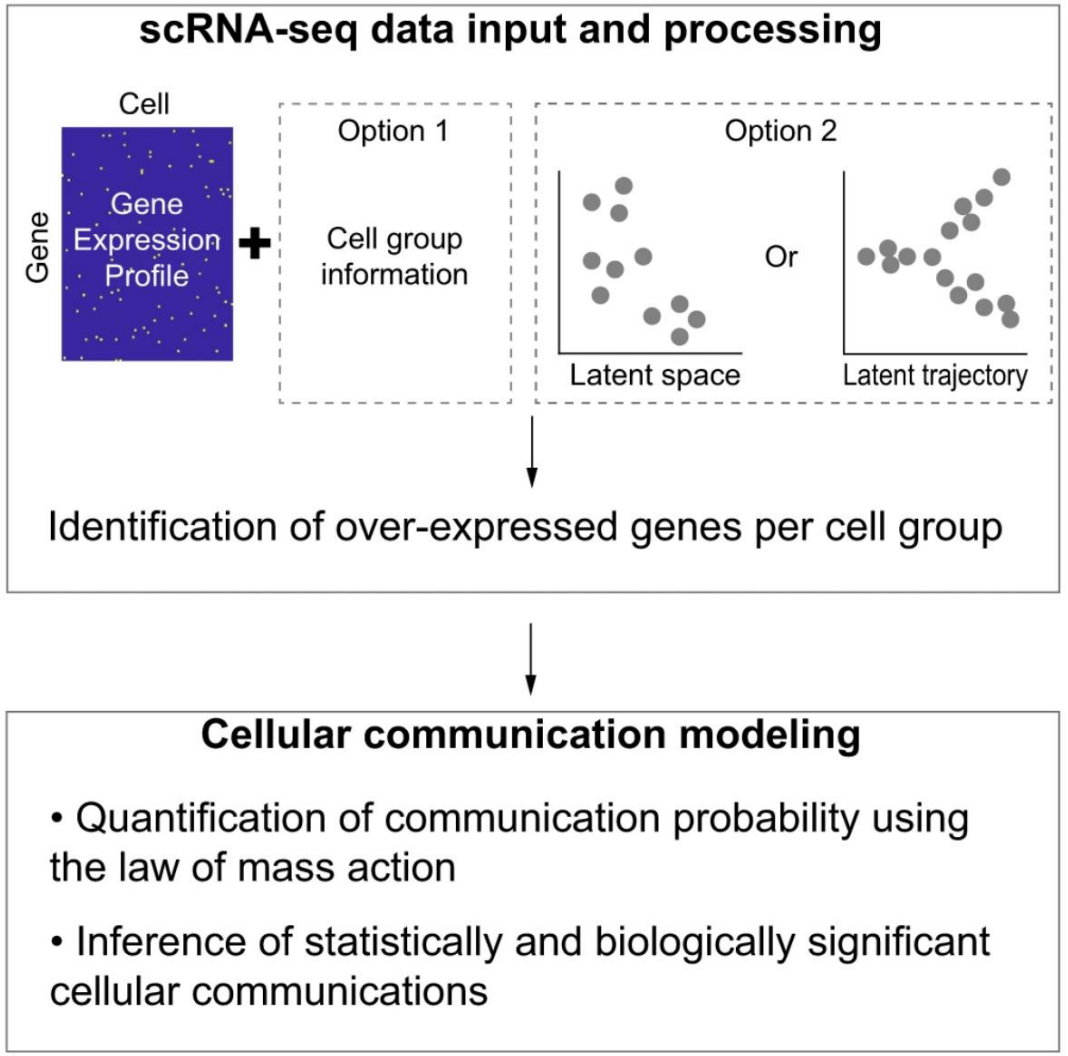
- Cell Type A
- Cell Type b
- Cell Type c
- Cell Type d
- Cell Type e
- Cell Type f
- Cell Type g
- Cell Type h



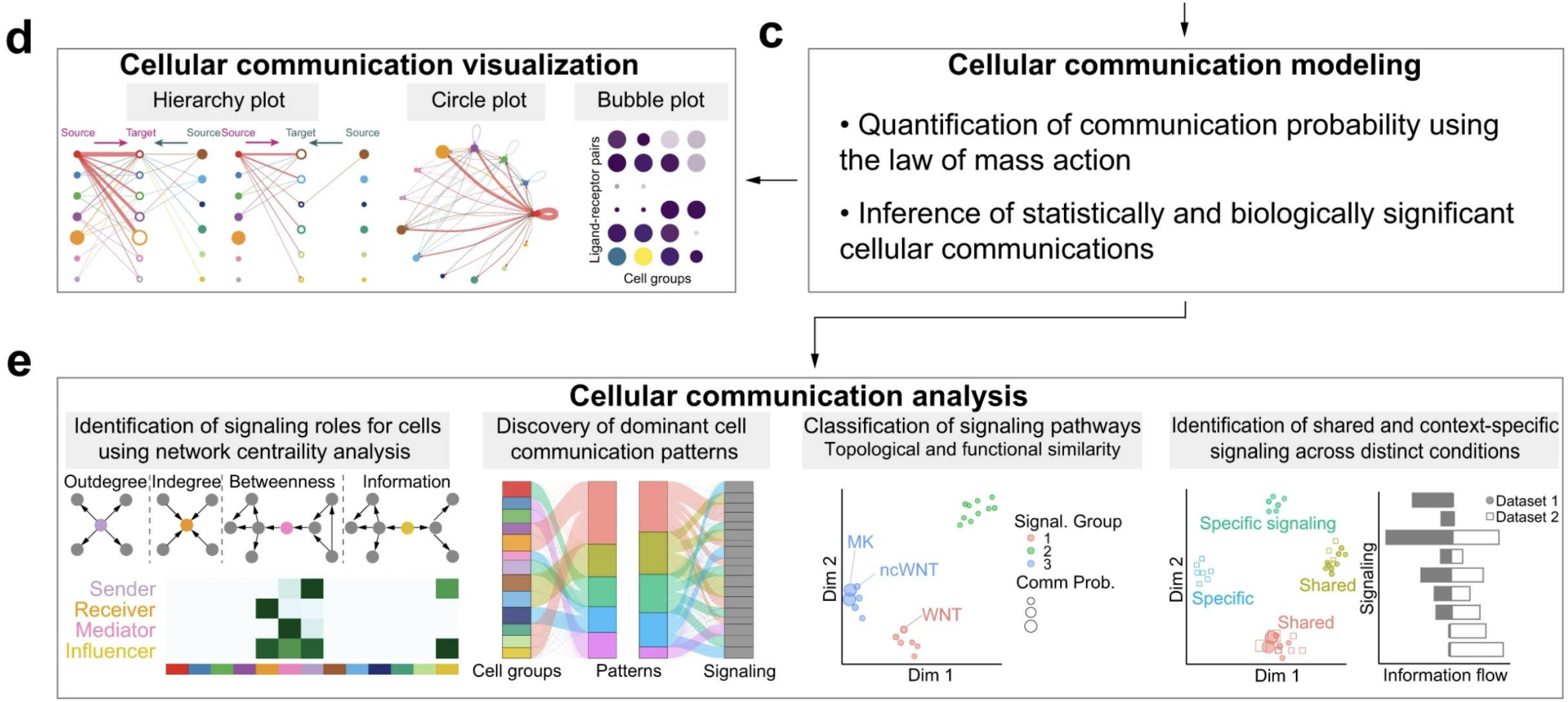


Cell-cell interaction databases

- We know current literature of ligand-receptors
- Several build databases of pairs (Cellchat, CellphoneDB)
- Use expressed genes to detect if pairs are expressed



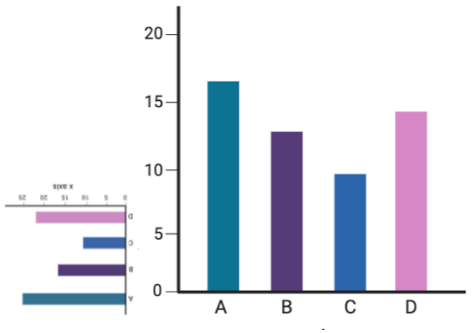
Cell-cell interaction graphs

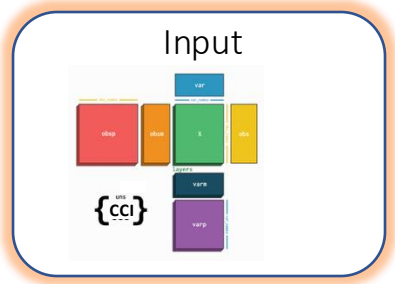


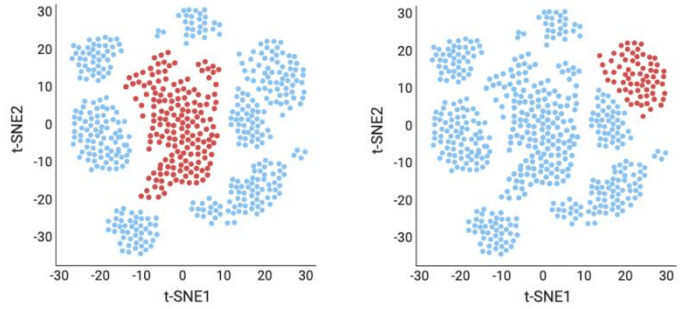
Cell-cell interaction

- A lot of graphs to look at
- Differential comparison most interesting – how does the cell-cell interaction changes, when one receptor is DE expressed between two conditions
- What are the limitations?
- What is the relation between expression and receptor?
- Just because the mRNA of two receptors is transcribed, does it might there is interaction?
- What would we need to know?

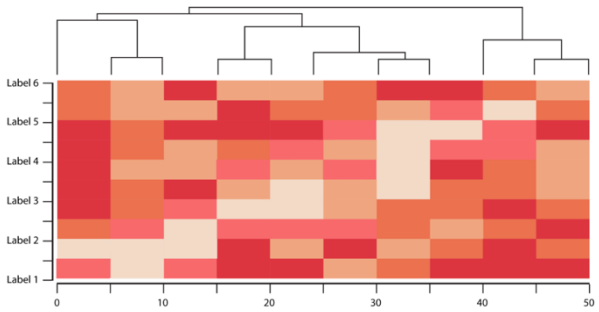
Cellxplore offers multiple visualisations to interrogate cell-cell interactions

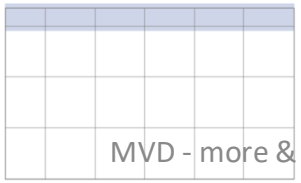




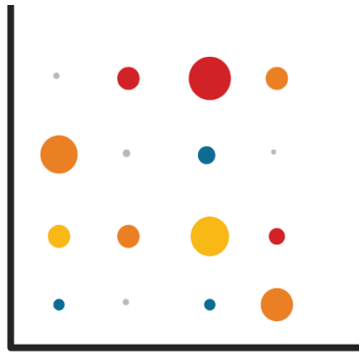


cellXplore



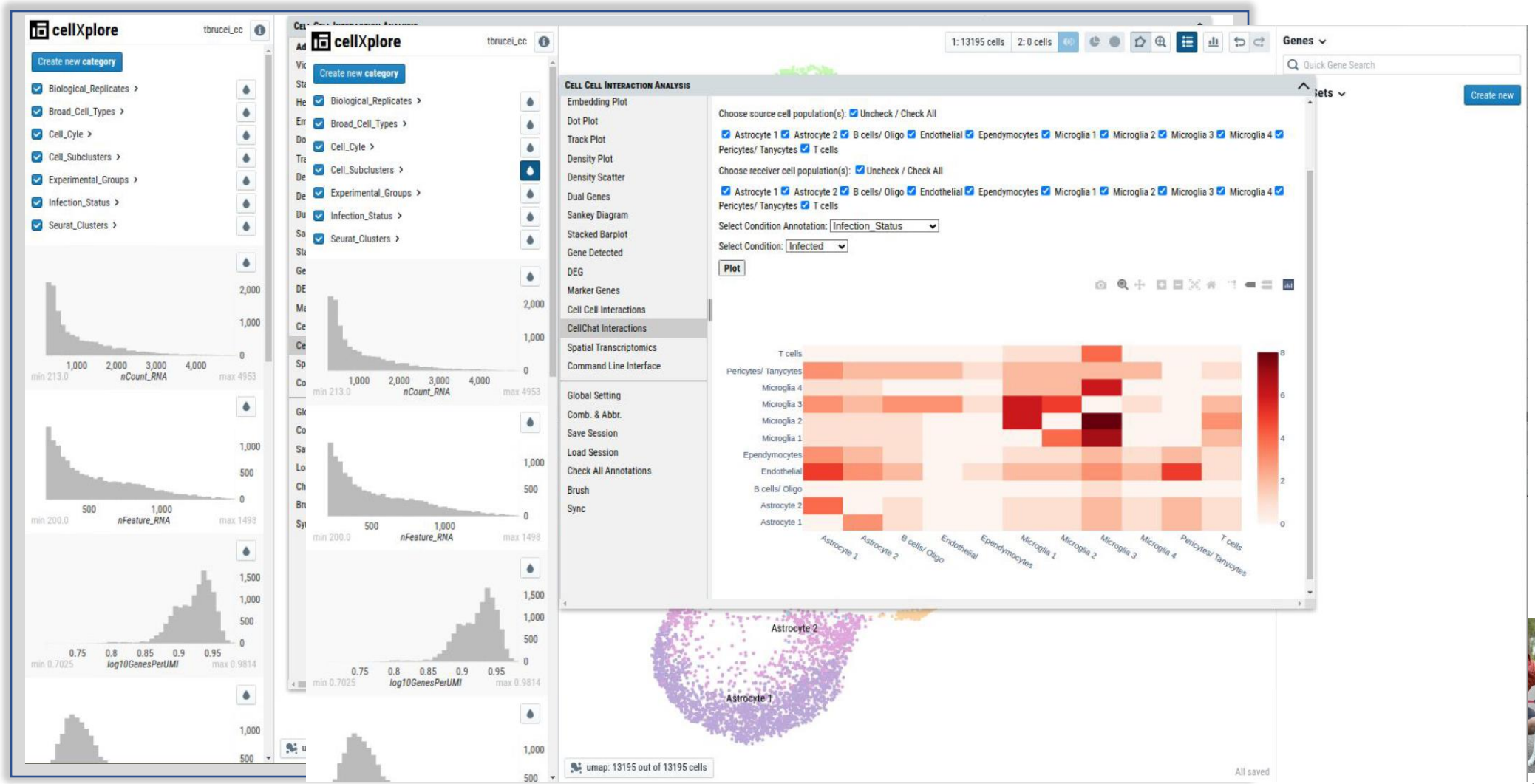


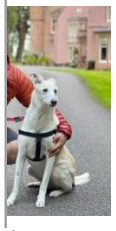
MVD - more & more



Olympia Hardy

Exploring cellular communications in the brain during *T. brucei* infection

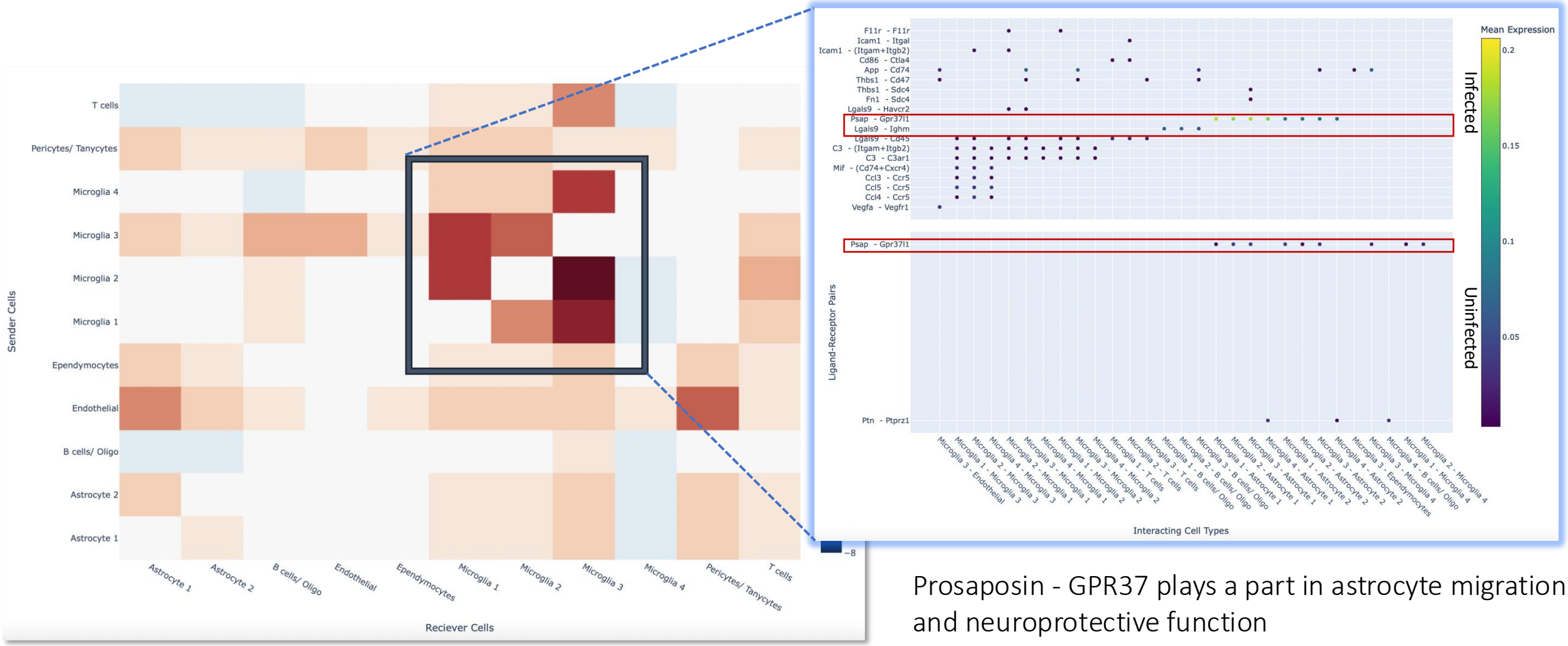




Dr. Juan Quintana

https://www.nature.com/articles/s41467-022-33542-z

Microglia subsets show increased cross-talk during *T. brucei* infection



Prosaposin - GPR37 plays a part in astrocyte migration and neuroprotective function

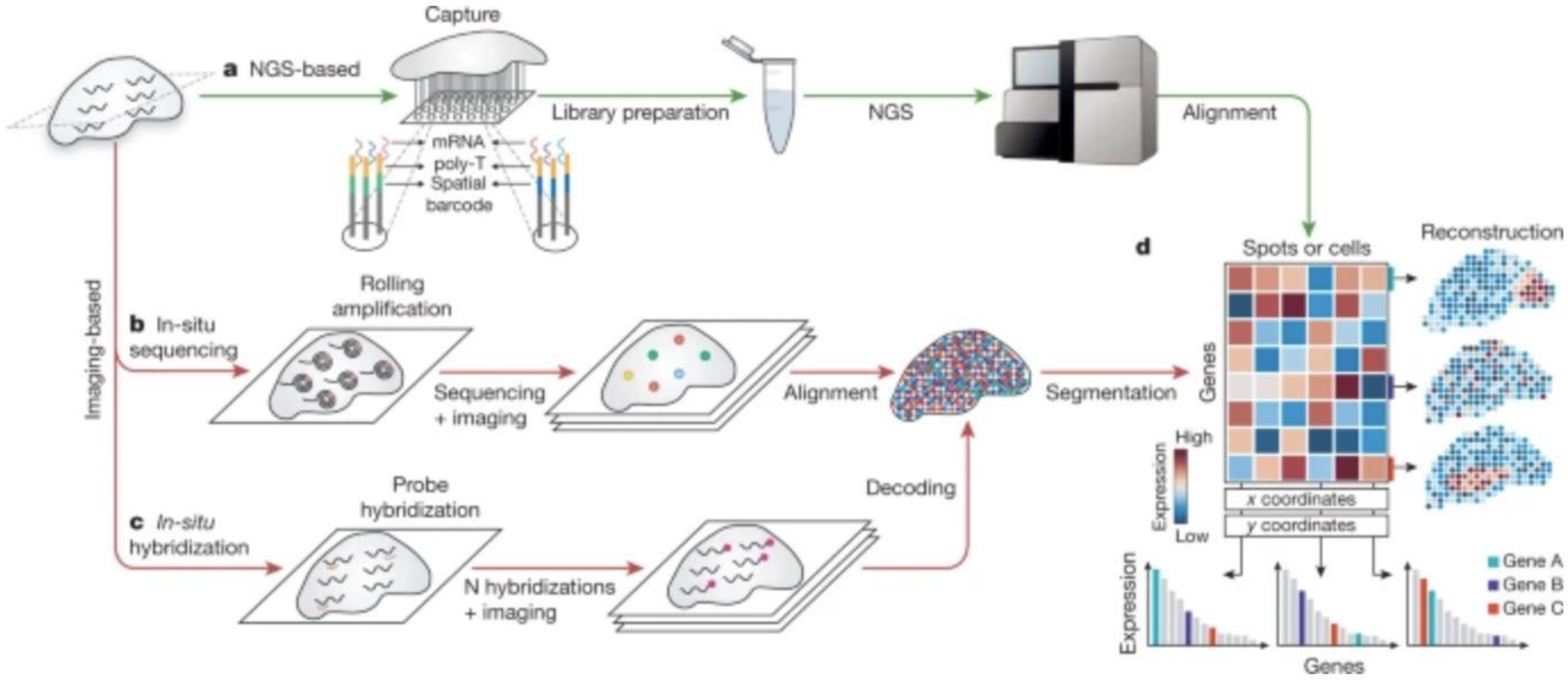
Lgals9-Ighm – Galectin-9 interaction regulates B cell signaling

Spatial transcriptomics

- cellular organization in tissues is intimately linked to biological function
- histopathology is often used as a conclusive diagnostic tool, precisely because many diseases are characterized by abnormal spatial organization within tissues
- Infectious and inflammatory processes can drastically change the cellular organization of tissues

Spatial transcriptomics

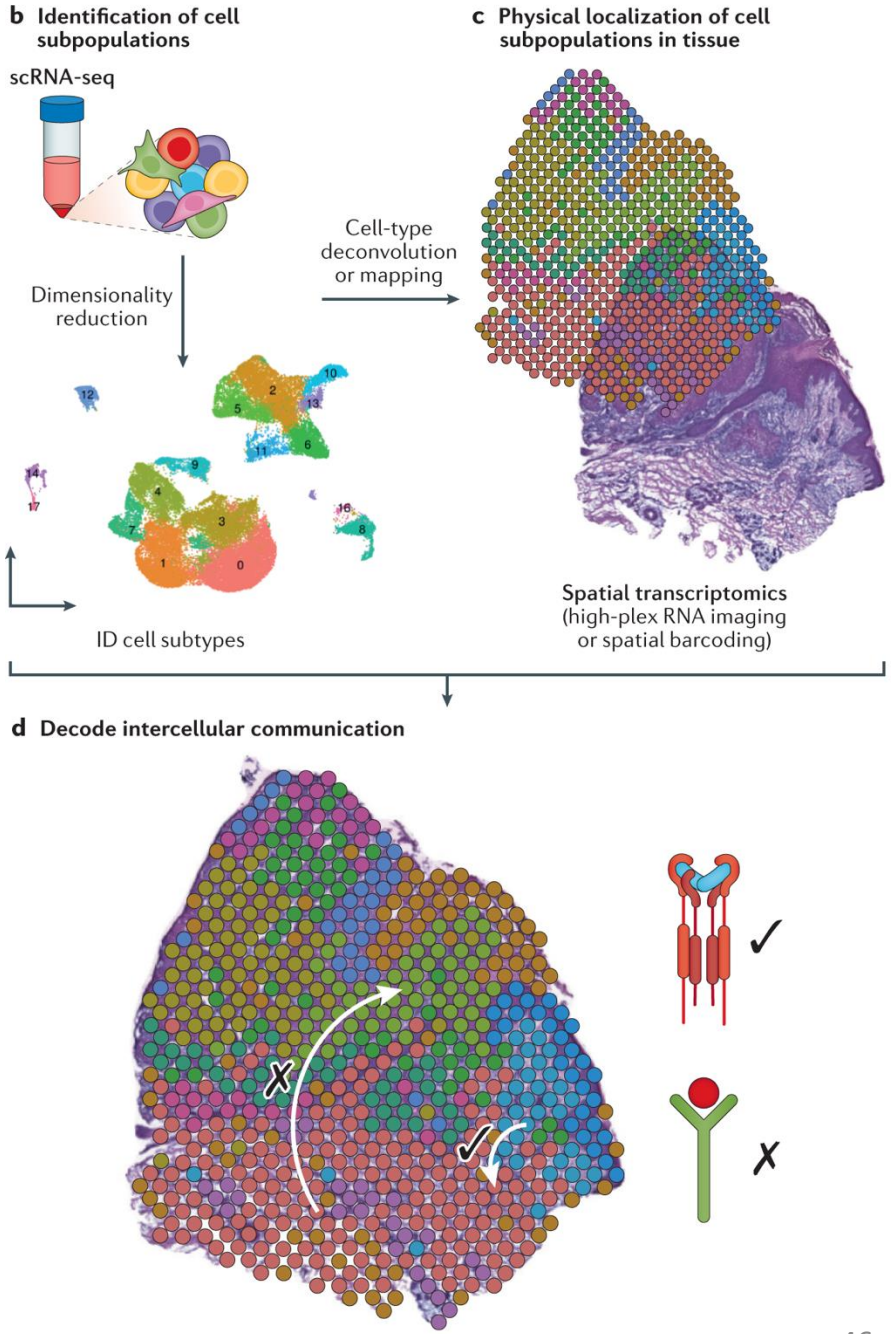
Fig. 1: The technologies of spatial transcriptomics provide a gene-expression matrix.



https://www.nature.com/articles/s41586-021-03634-9

Spatial transcriptomics + scRNA

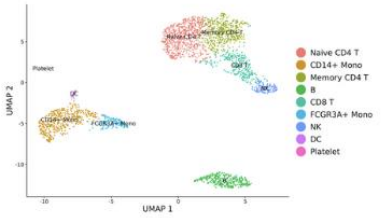
- methods exist
- Seurat has one ☺



https://www.nature.com/articles/s41576-021-00370-8

What else is there?

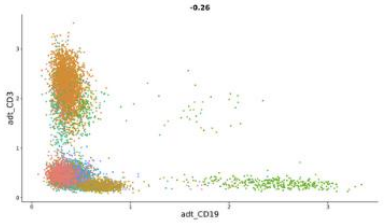
Guided tutorial — 2,700 PBMCs



A basic overview of Seurat that includes an introduction to common analytical workflows.

GO

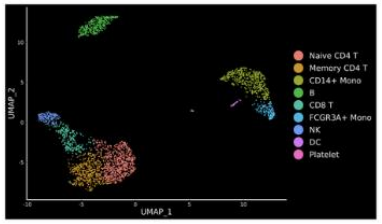
Multimodal analysis



An introduction to working with multi-modal datasets in Seurat.

GO

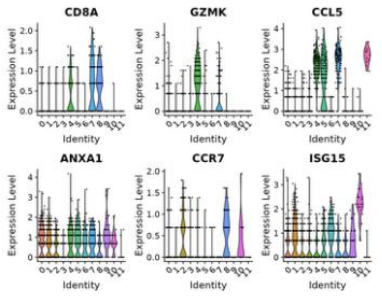
Visualization



An overview of the visualization capabilities within Seurat.

GO

SCTransform



Examples of how to perform normalization, feature selection, integration, and differential expression with an updated version of sctransform.

GO

Essential Commands Cheat Sheet

Standard `Seurat` workflow

`Seurat` Object data access

Pseudobulk analysis

Multi-Assay features

Subsetting and merging

Visualization in `Seurat`

Reference list of commonly used commands to store, access, explore, and analyze datasets.

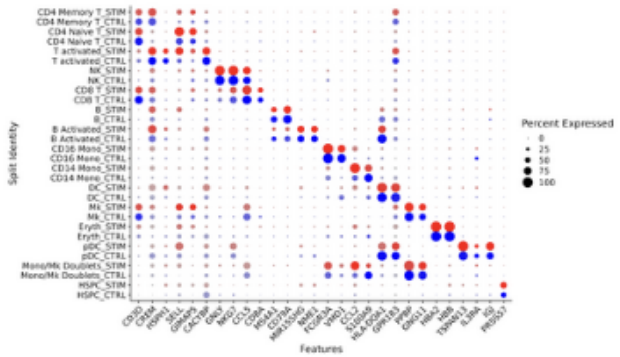
GO

https://satijalab.org/seurat/articles/get_started_v5_new

scRNA Data Integration

We have developed computational methods for integrated analysis of single-cell datasets generated across different conditions, technologies, or species. As an example, we provide a guided walk through for integrating and comparing PBMC datasets generated under different stimulation conditions. We provide additional vignettes demonstrating how to leverage an annotated scRNA-seq reference to map and label cells from a query, and to efficiently integrate large datasets.

Introduction to scRNA-seq integration



An introduction to integrating scRNA-seq datasets in order to identify and compare shared cell types across experiments.

GO

scRNA-seq Integration

Seurat v5

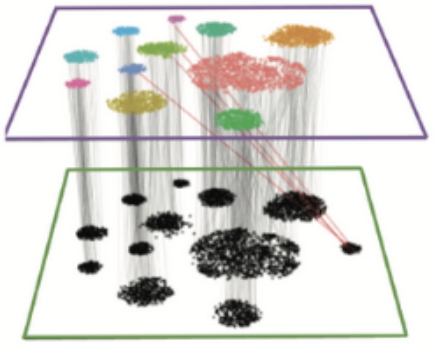
harmony

scvi-tools

Integrate scRNA-seq datasets using a variety of computational methods.

GO

Mapping and annotating query datasets



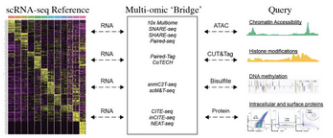
Learn how to map a query scRNA-seq dataset onto a reference in order to automate the annotation and visualization of query cells.

GO

Multi-assay data

Seurat also offers support for a suite of statistical methods for analyzing multimodal single-cell data. These include methods to integrate modalities that are simultaneously measured in the same cells, modalities that are measured in different cells, and techniques to analyze pooled CRISPR screens.

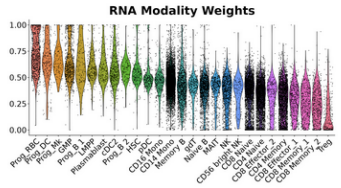
Cross-modality Bridge Integration



Map scATAC-seq onto an scRNA-seq reference using a multi-omic bridge dataset in Seurat v5.

GO

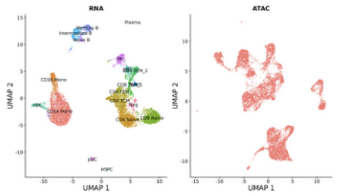
Weighted Nearest Neighbor Analysis



Analyze multimodal single-cell data with weighted nearest neighbor analysis in Seurat v4.

GO

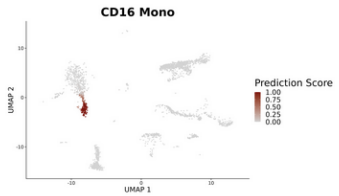
Integrating scRNA-seq and scATAC-seq data



Annotate, visualize, and interpret an scATAC-seq experiment using scRNA-seq data from the same biological system in Seurat v3.

GO

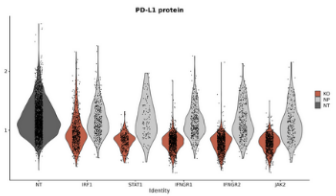
Reference Mapping for Multimodal Data



Analyze query data in the context of multimodal reference atlases.

GO

Mixscape



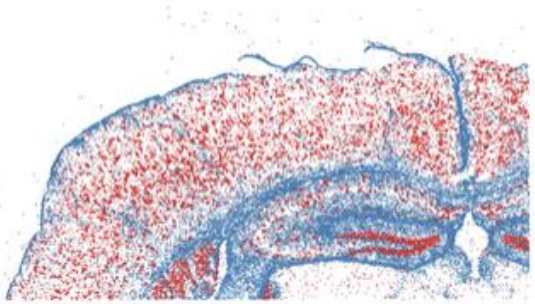
Explore new methods to analyze pooled single-celled perturbation screens.

GO

Spatial analysis

These vignettes will help introduce users to the analysis of spatial datasets in Seurat v5, including technologies that leverage sequencing-based readouts, as well as technologies that leverage in-situ imaging-based readouts. The vignettes introduce data from multiple platforms including 10x Visium, SLIDE-seq, Vizgen MERSCOPE, 10x Xenium, Nanostring CosMx, and Akoya CODEX.

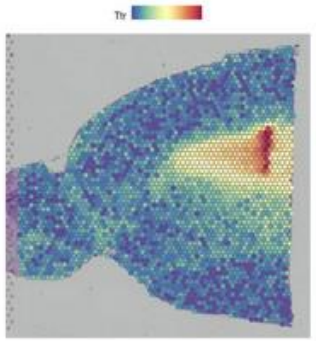
Analysis of spatial datasets (Imaging-based)



Learn to explore spatially-resolved data from multiplexed imaging technologies, including MERSCOPE, Xenium, CosMx SMI, and CODEX.

GO

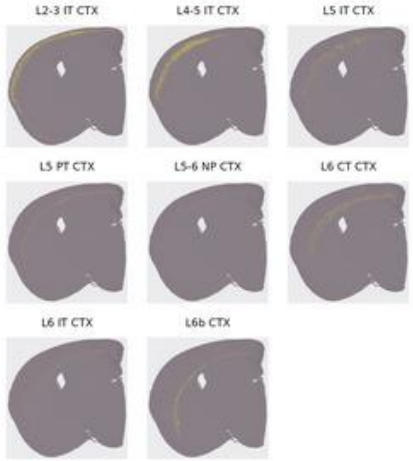
Analysis of spatial datasets (Sequencing-based)



Learn to explore spatially-resolved transcriptomic data with examples from 10x Visium and Slide-seq v2.

GO

Analysis of Visium HD spatial datasets



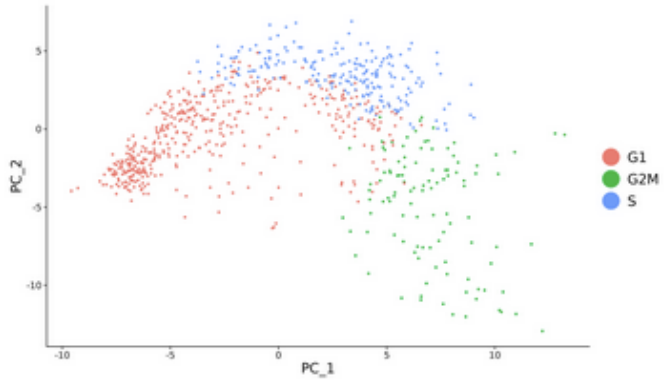
Learn to explore spatially-resolved transcriptomic data in high-definition from 10x Visium HD.

GO

Other

Here we provide a series of short vignettes to demonstrate a number of features that are commonly used in Seurat. We've focused the vignettes around questions that we frequently receive from users.

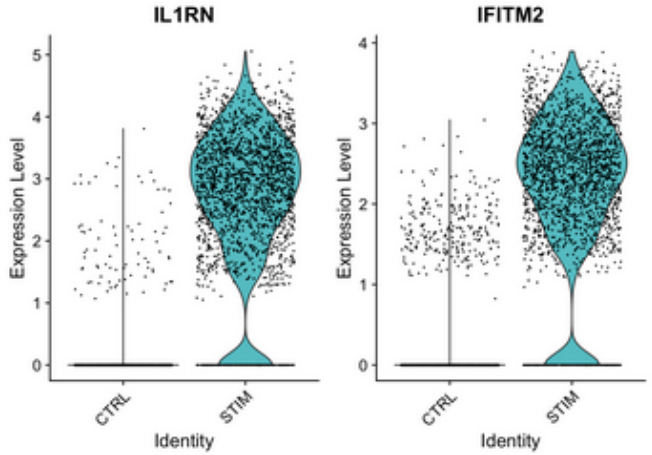
Cell Cycle Regression



Mitigate the effects of cell cycle heterogeneity by computing cell cycle phase scores based on marker genes.

GO

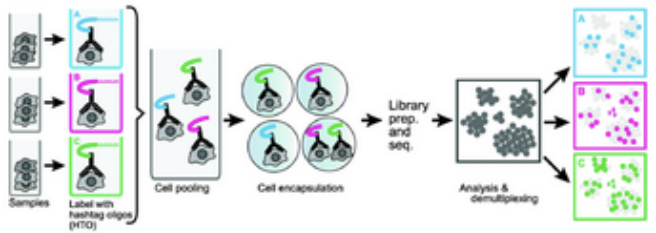
Differential Expression Testing



Perform differential expression (DE) testing in Seurat using a number of frameworks.

GO

Demultiplex Cell Hashing data



Learn how to work with data produced with Cell Hashing.

GO

Think about the opportunities

- Spatial transcriptomics will be the new hype
- Integrating different modalities (datatypes)

- Take home message: A lot of cool applications!

- Later in lecture looking into other methods

- Next, other “omics”

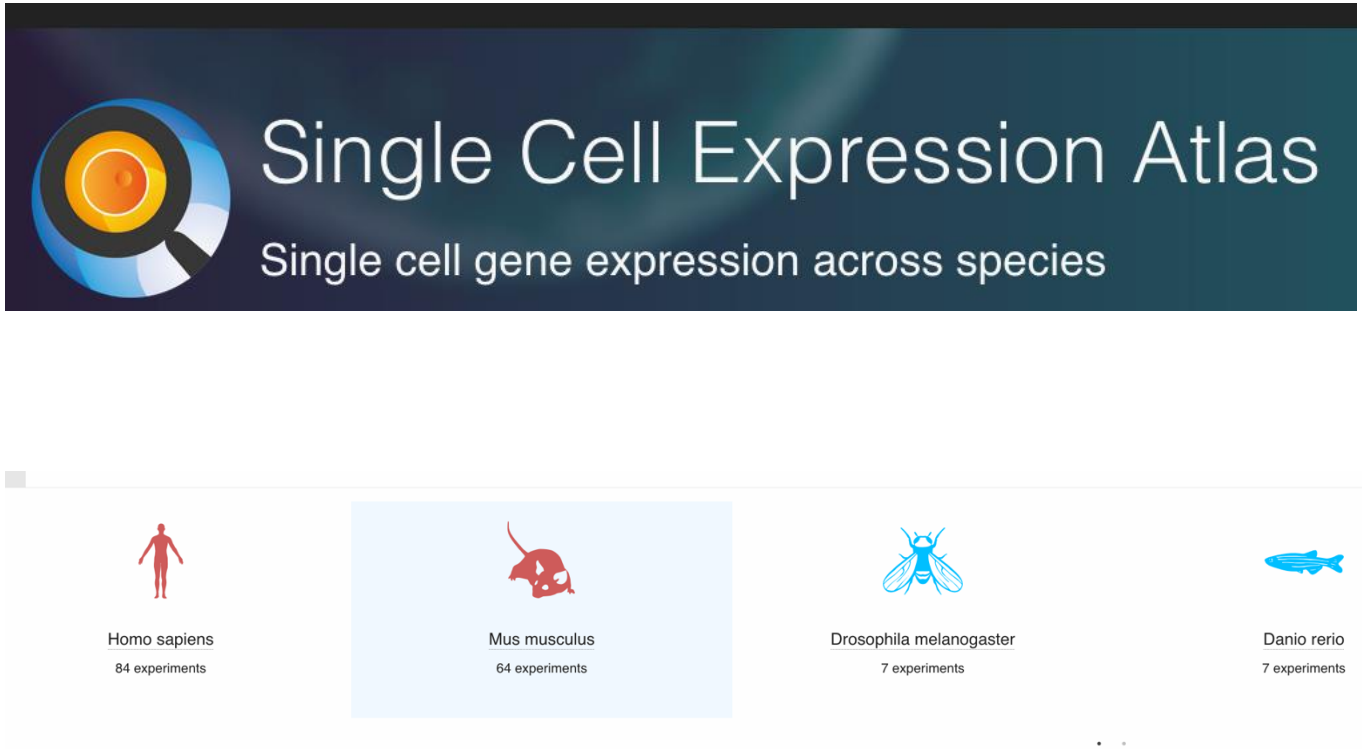
Resources

Many tools to visualise data!

HUMAN
CELL
ATLAS

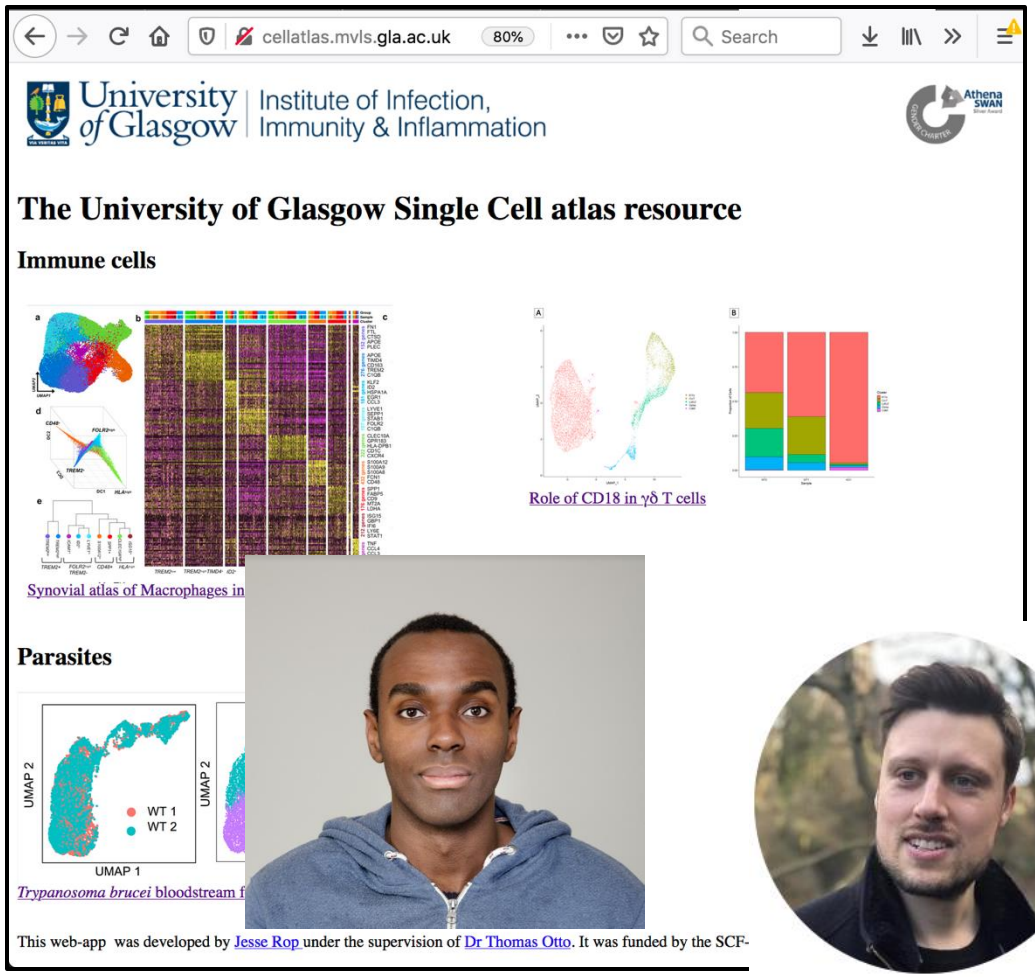
MISSION

To create comprehensive reference maps of all human cells—the fundamental units of life—as a basis for both understanding human health and diagnosing, monitoring, and treating disease.



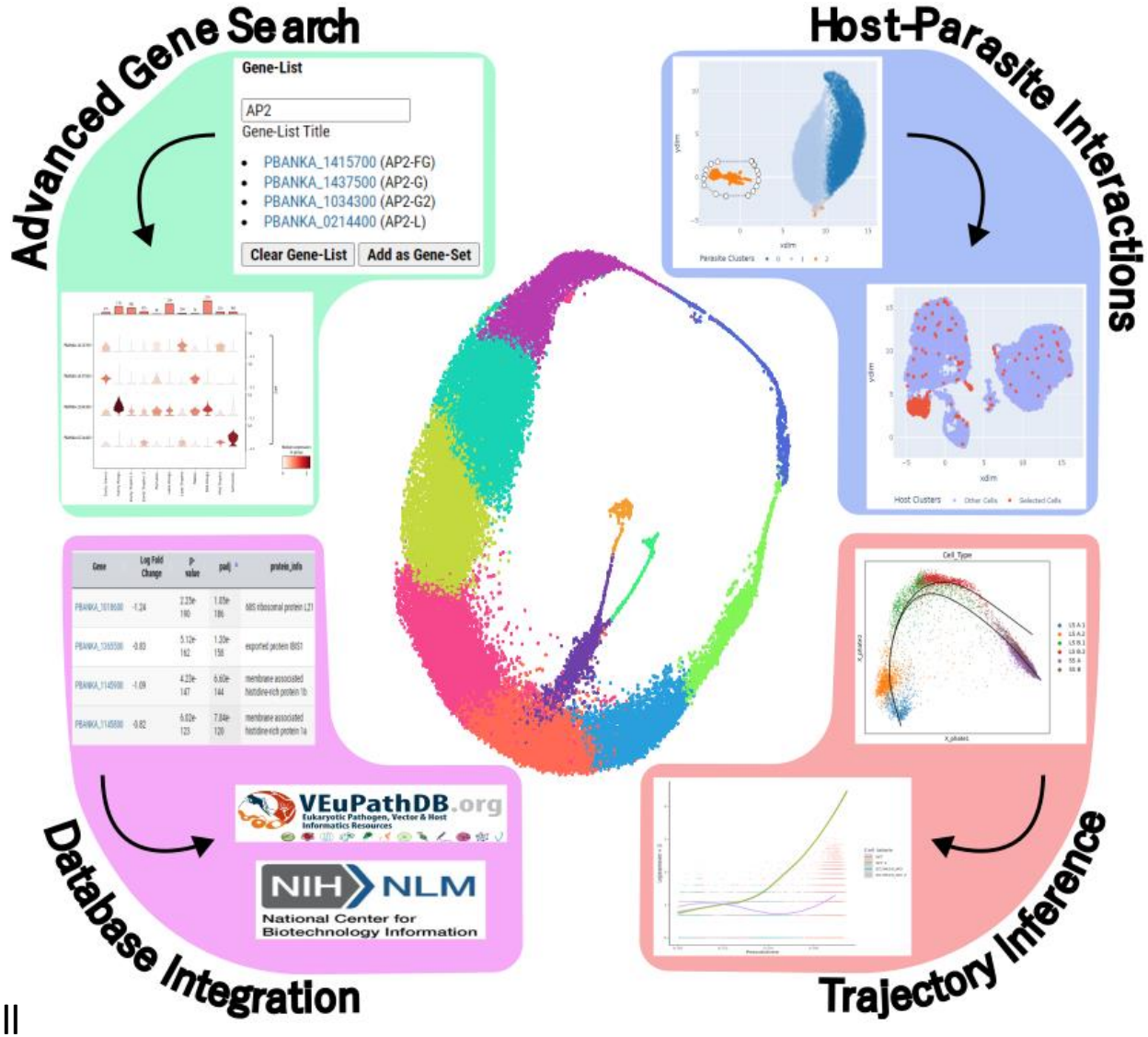
https://www.ebi.ac.uk/gxa/sc/home

Data accessibility - ParaCell



Edward Agboraw

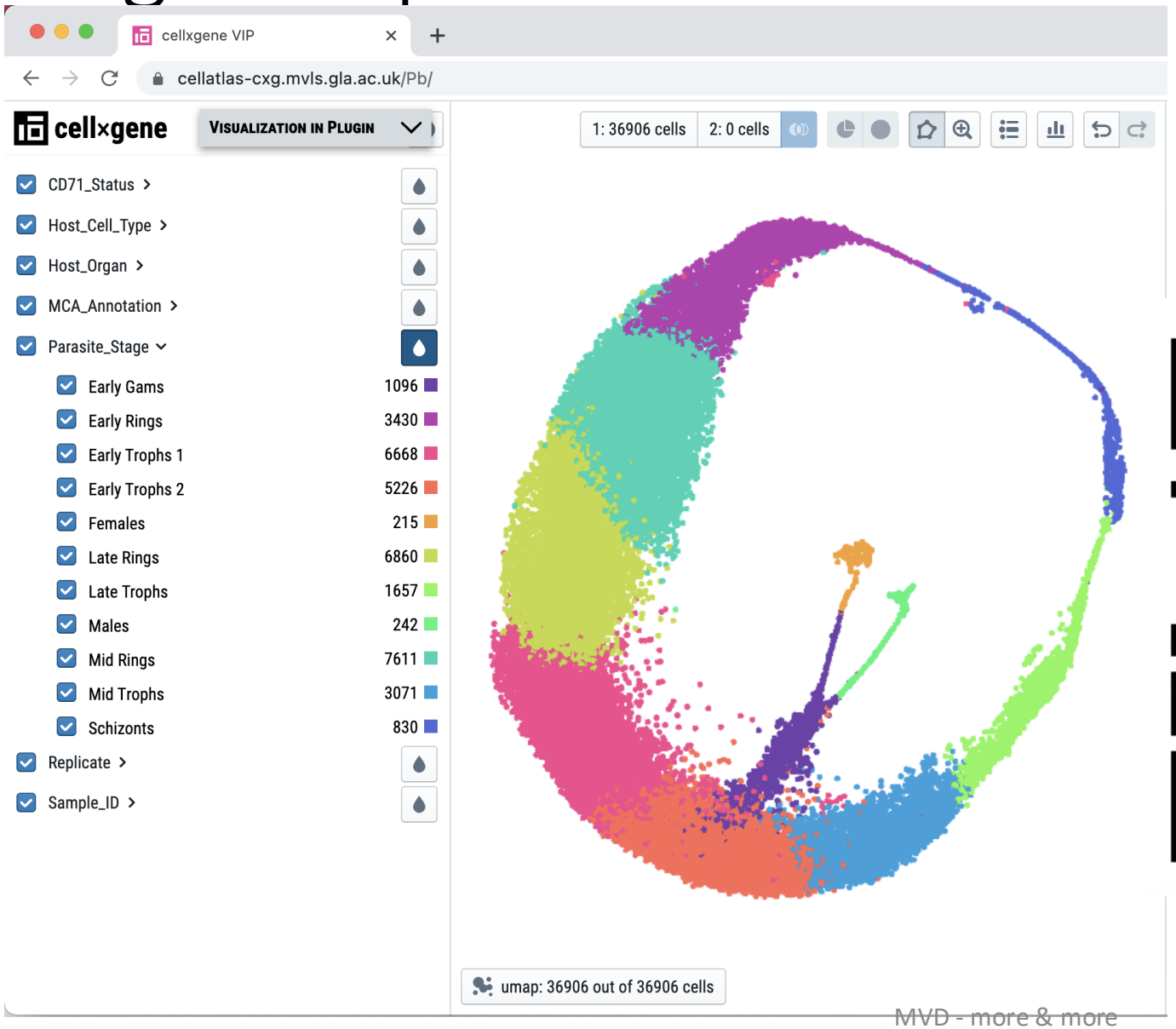
William Haese-Hill



http://cellatlas.mvls.gla.ac.uk/

Cellxgene - paraCell

https://cellatlas-cxg.mvls.gla.ac.uk/Pb/



Questions?