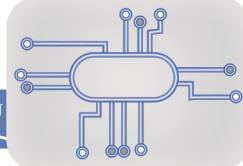


# Single-cell and spatial data analyses

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Sikander Hayat, PhD

Translational Data Science  
[@sikandhayat](https://twitter.com/sikandhayat)



10011011

Department for Renal and Hypertensive Diseases,  
Rheumatological and Immunological Diseases  
(Medical Clinic II)



# Overview

## **Module 1:**

What is Single-cell sequencing?

What can we do with Single-cell sequencing?

The next revolution – Spatial transcriptomics

## **Module 2:**

Sequencing basics

Cellranger – and preprocessing

## **Module 3:** (Hands-On)

QC

Batch effect correction

Clustering

Marker genes

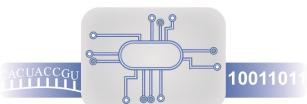
Cell-type annotation

Compositional Changes

## **Module 4:** (Tore Bleckwehl - Hands-On)

Spatial transcriptomics

Single-cell spatial transcriptomics



# Download data

From zenodo website:

<https://zenodo.org/record/6578047#.Yx8dtuxBxqt>

You should sub-sample the data to save computing time for different tasks in the workshop (code provided).

[https://github.com/hayatlab/singlecell\\_workshop](https://github.com/hayatlab/singlecell_workshop)

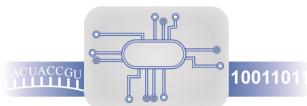
From our repo:

[https://drive.google.com/drive/folders/1y\\_-TyG4O-9ii2pk8Nw2ySwQm1eNQlg3S?usp=drive\\_link](https://drive.google.com/drive/folders/1y_-TyG4O-9ii2pk8Nw2ySwQm1eNQlg3S?usp=drive_link)



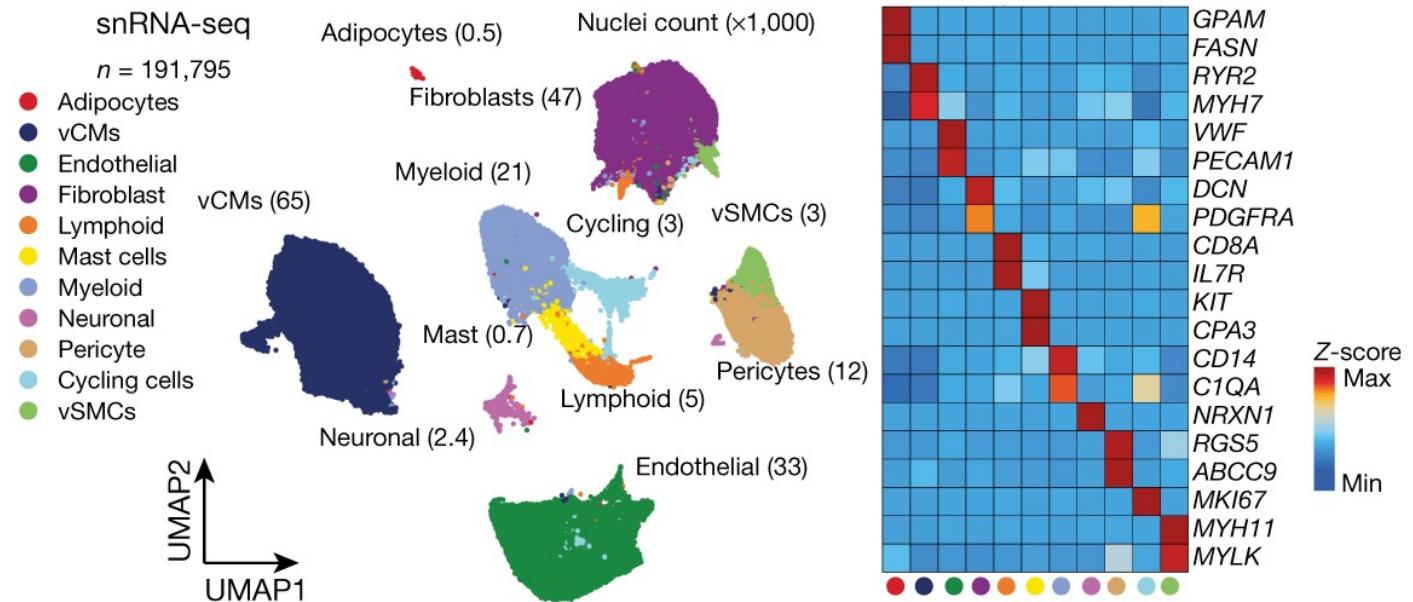
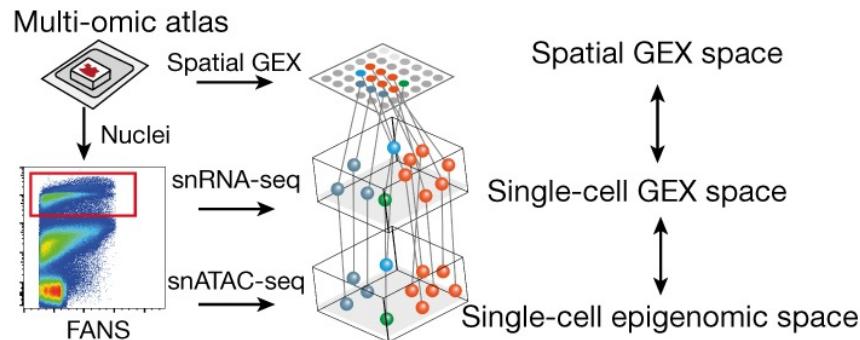
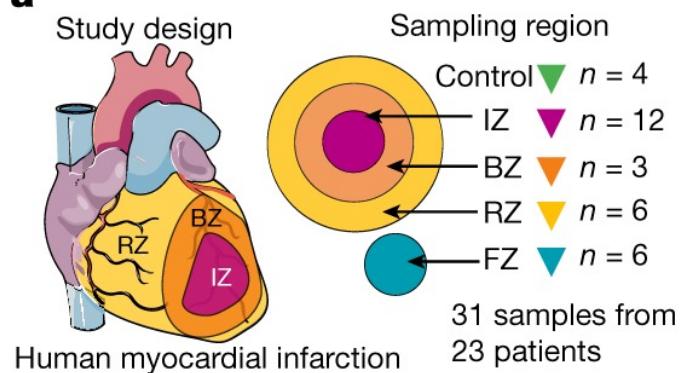
# Why study single-cells

- Understand heterogeneity of biological systems
- A given cell type can have different cell-states that might be reflective of disease phenotype
- Cell-type specific changes in gene expression patterns
- Changes in cellular composition
- Cellular communication
- Trajectories from one state to the other

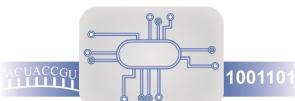
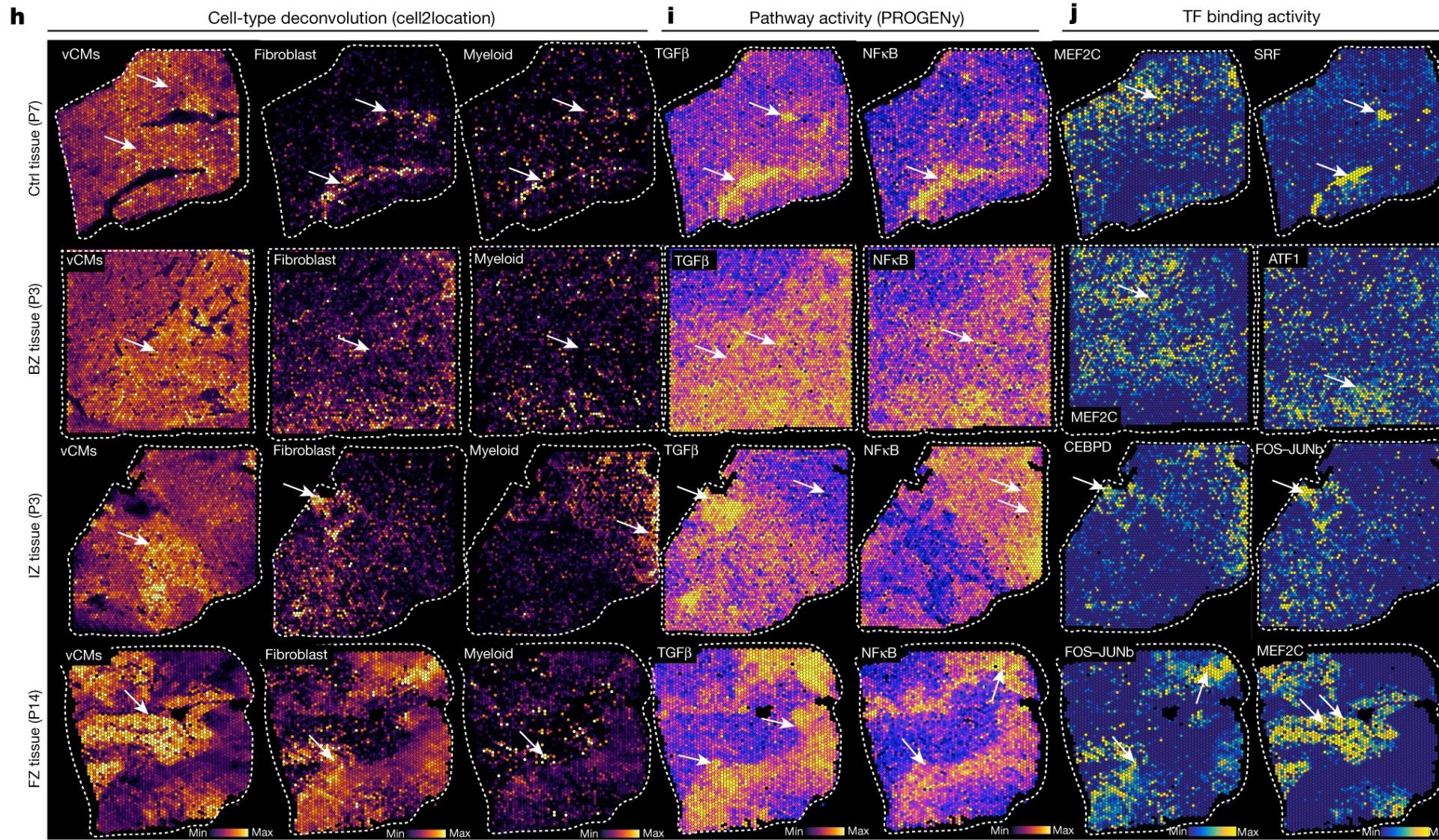


# Why study single-cells

a

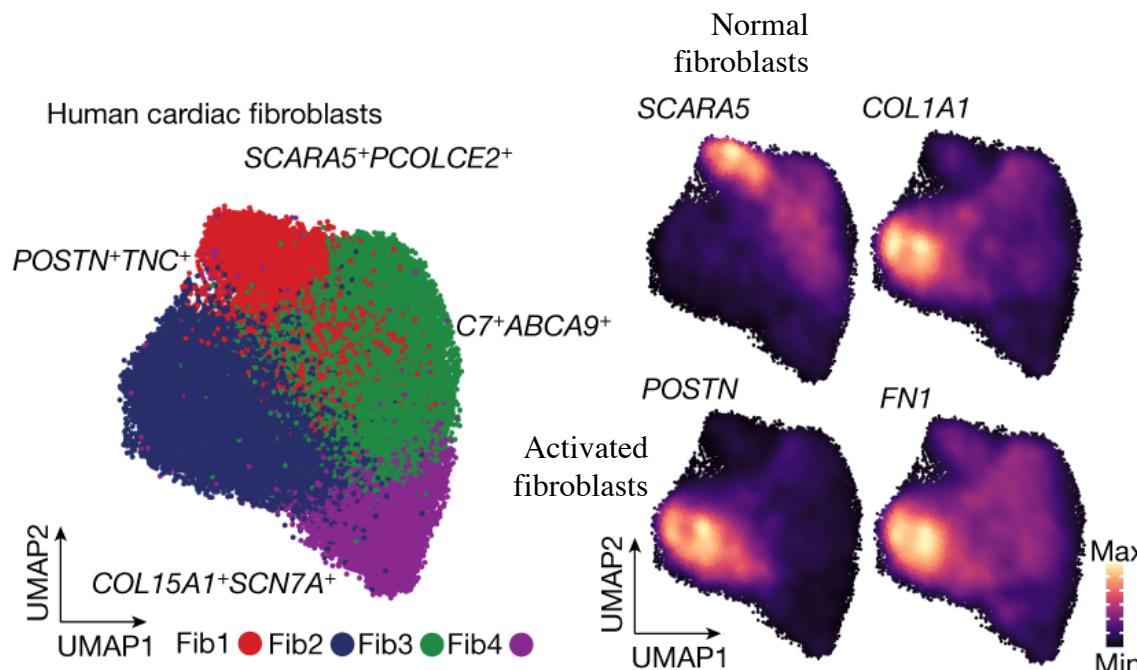


# Why study single-cells



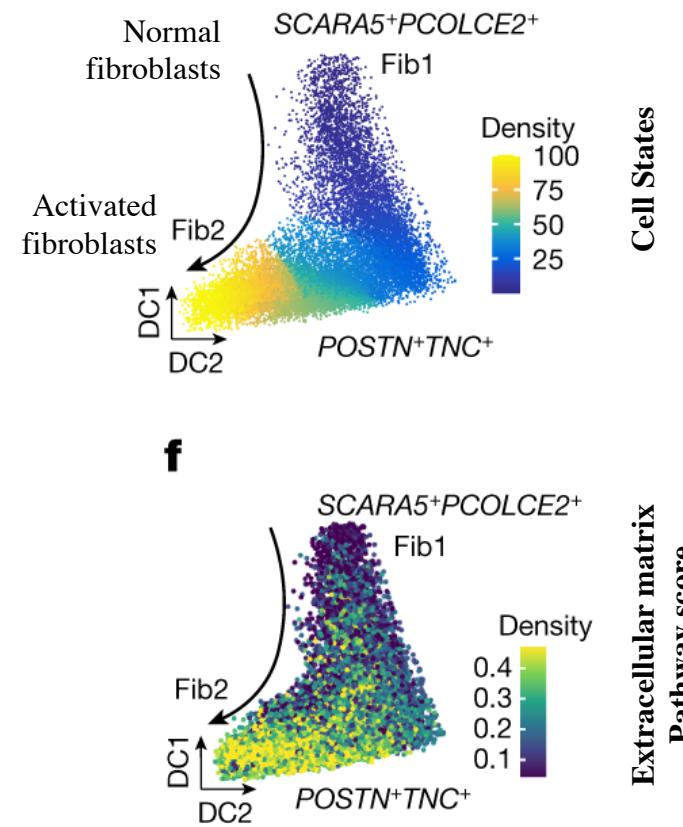
# Why study single-cells

## Discrete analyses

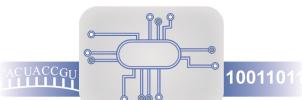


Sub clustering data to find cell-states

## Continuous analyses

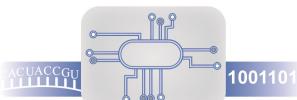
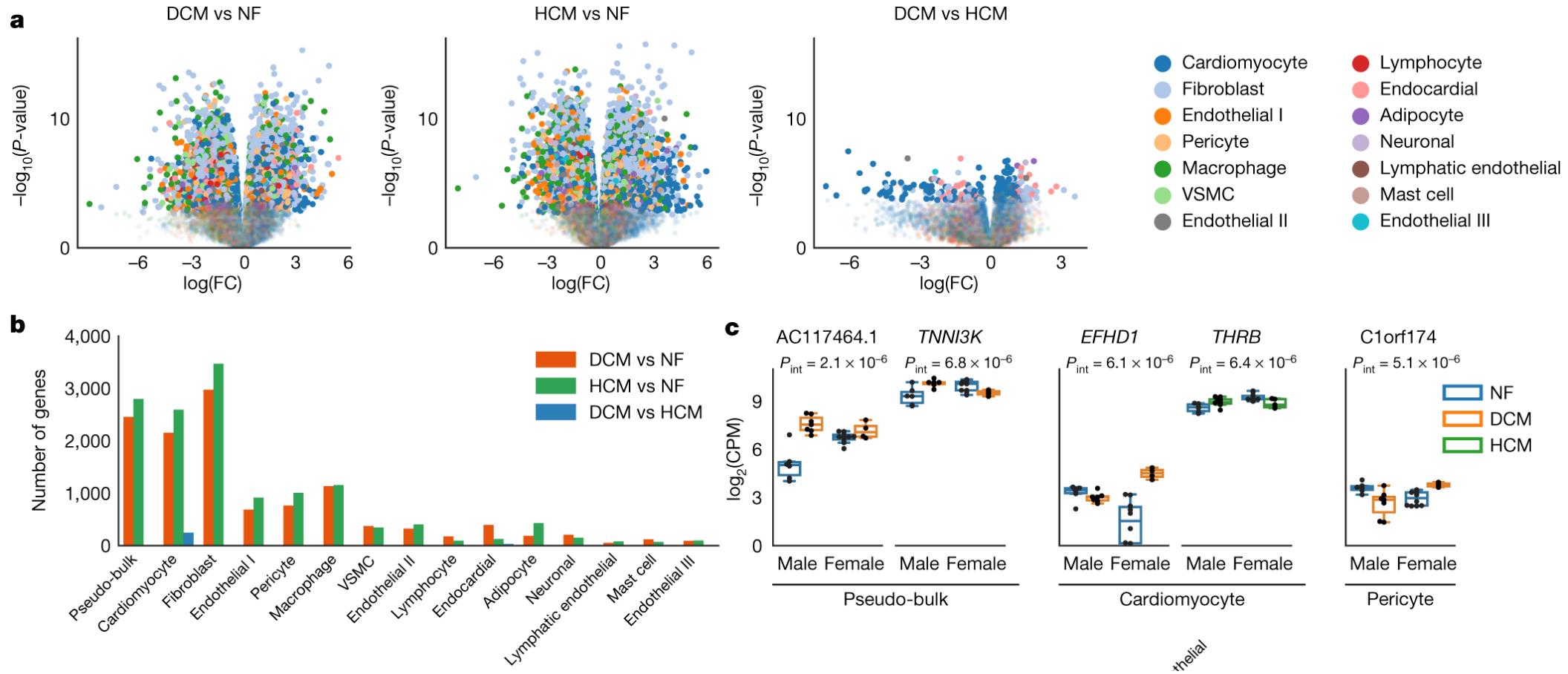


Trajectory analyses



# Why study single-cells

## Differentially Expressed genes

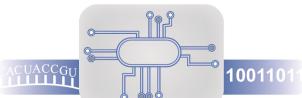
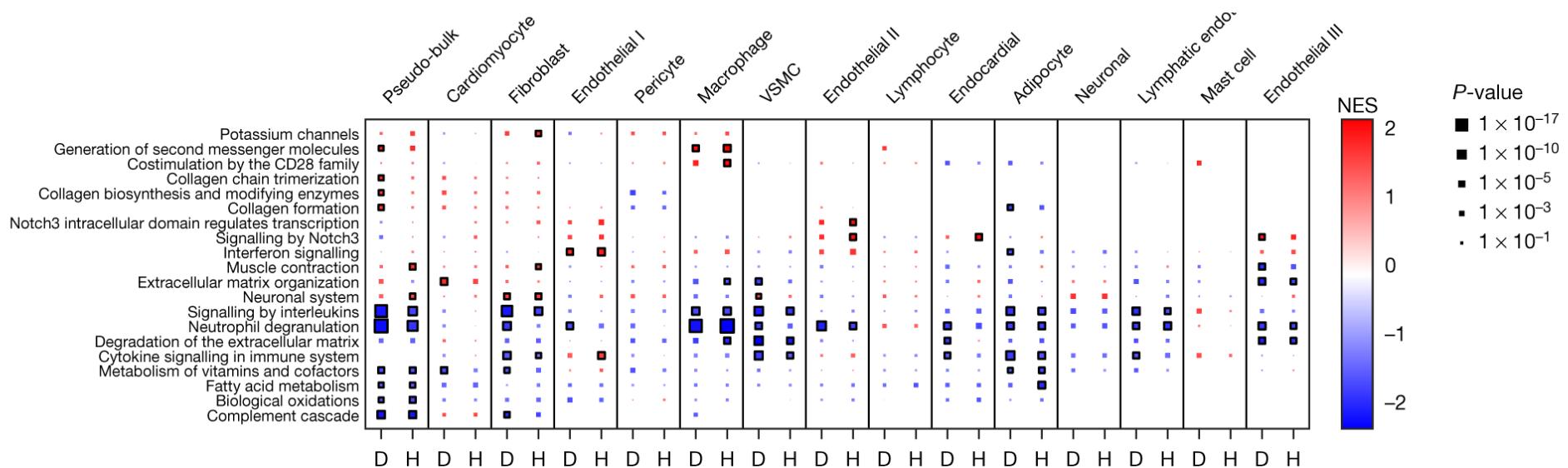


10011011

# Why study single-cells

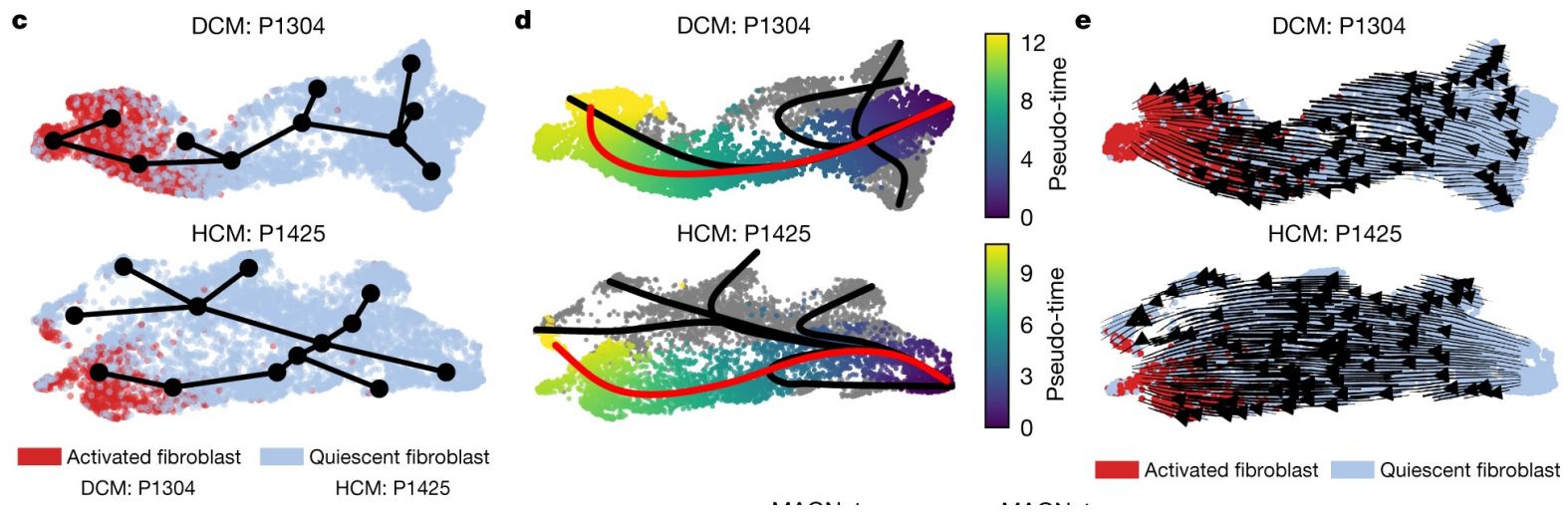
## Dysregulated Pathways

d



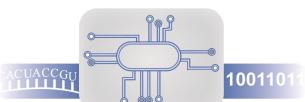
# Why study single-cells

Trajectory from normal to disease state

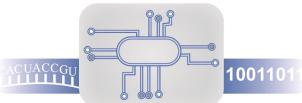
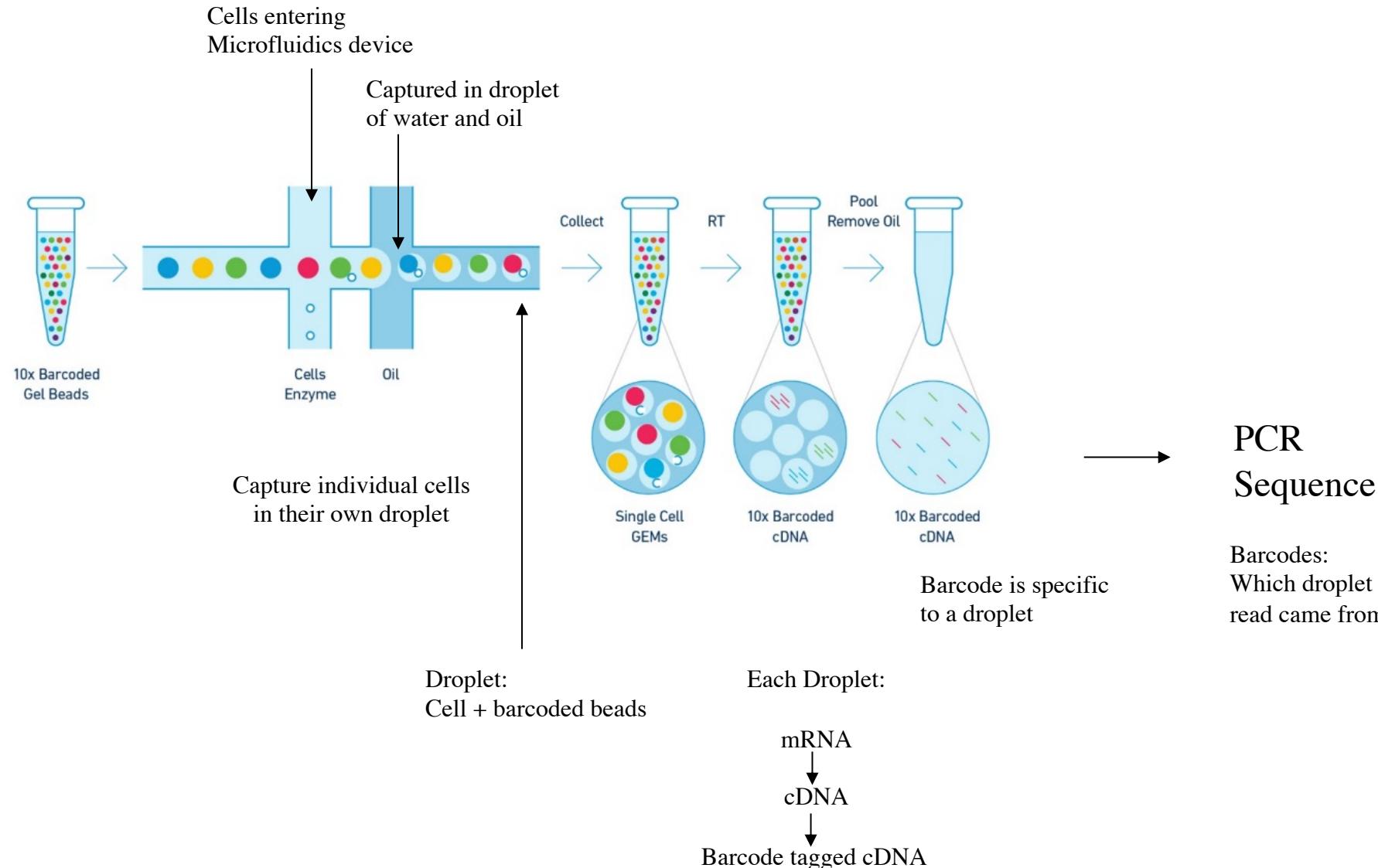


Changes from normal to activated fibroblast state

Genes changing along trajectory

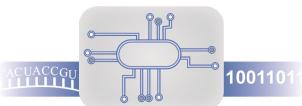
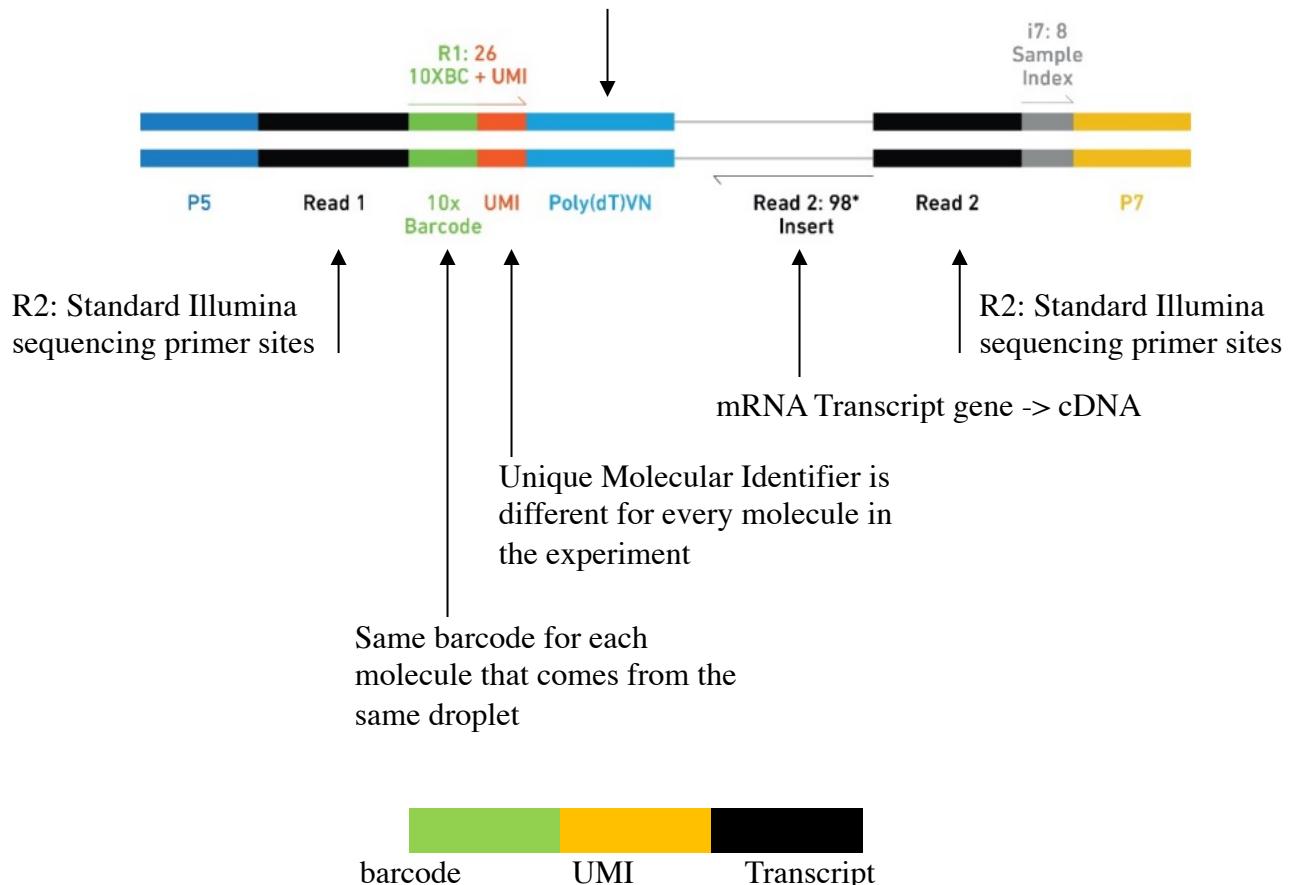


# 10x droplet bases single-cell sequencing

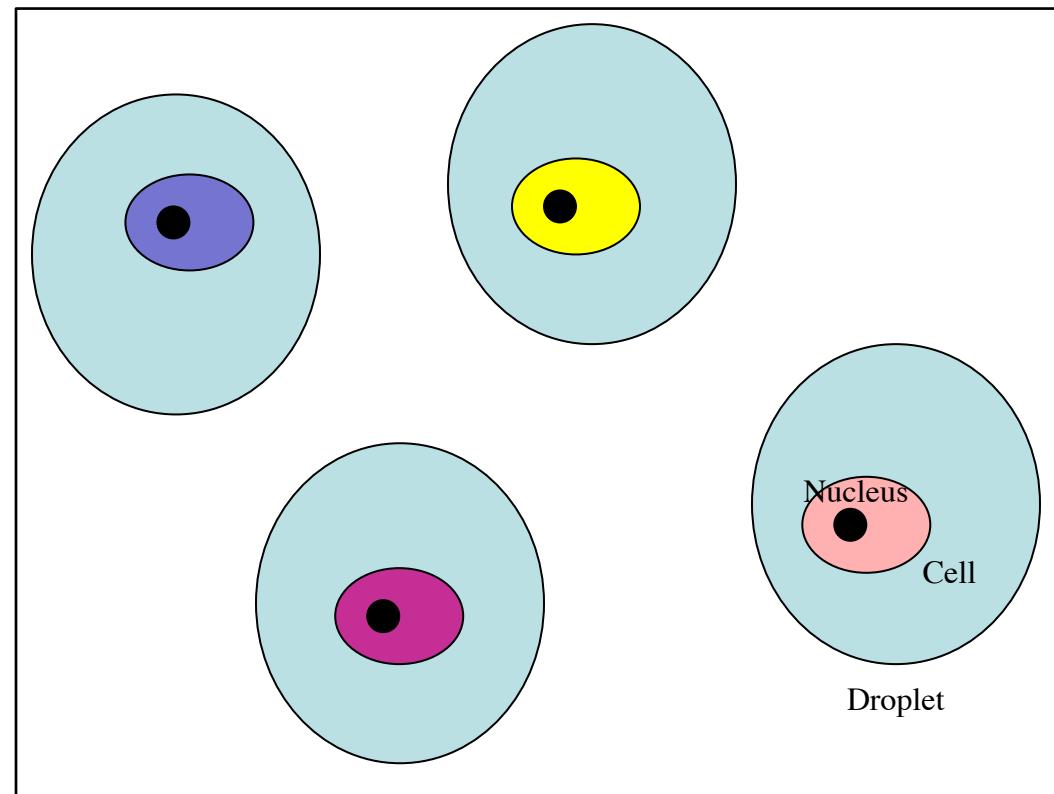


# Output of library preparation

Poly T hybridization:  
Acts as the bait for binding  
Polyadenylated mRNA from the  
lysed cell (mRNA) within a droplet



# From Library to count matrix

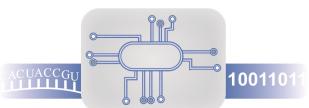
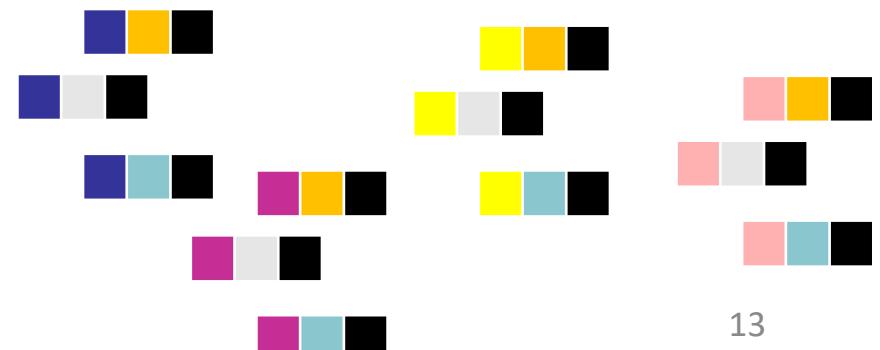
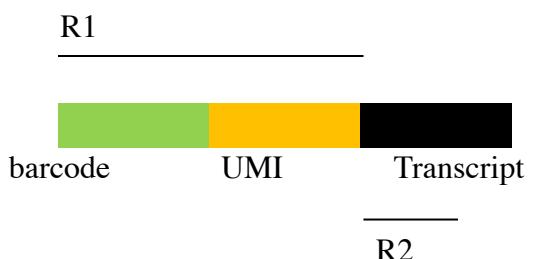
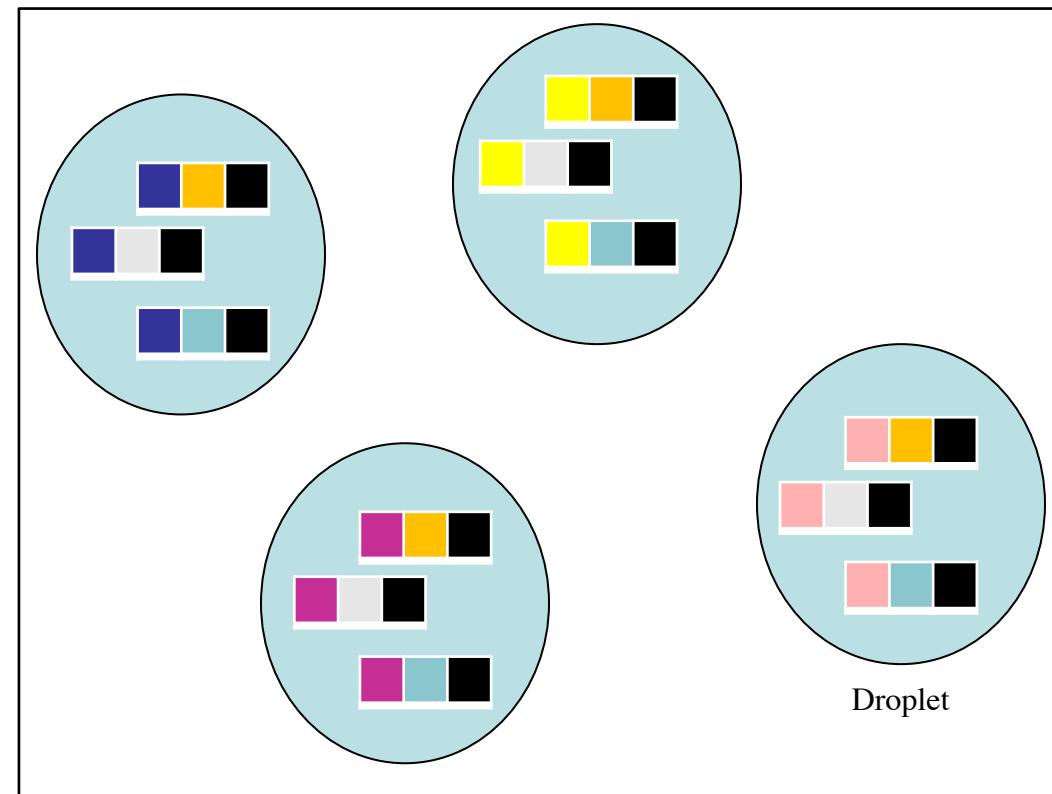


Lyse cells within each droplet

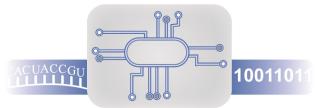
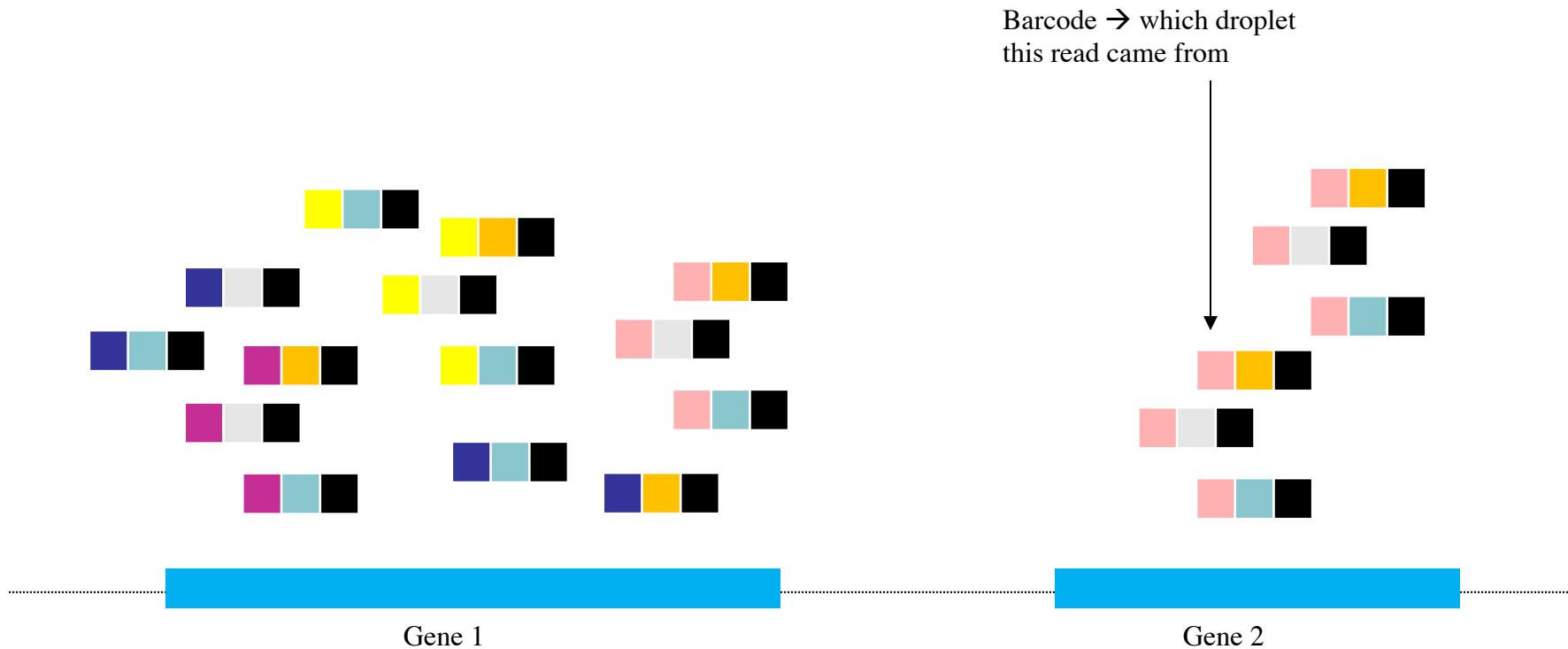
Reverse transcription  
Barcode+umi+PolyT

Break emulsion  
cDNA -> PCR  
amplification

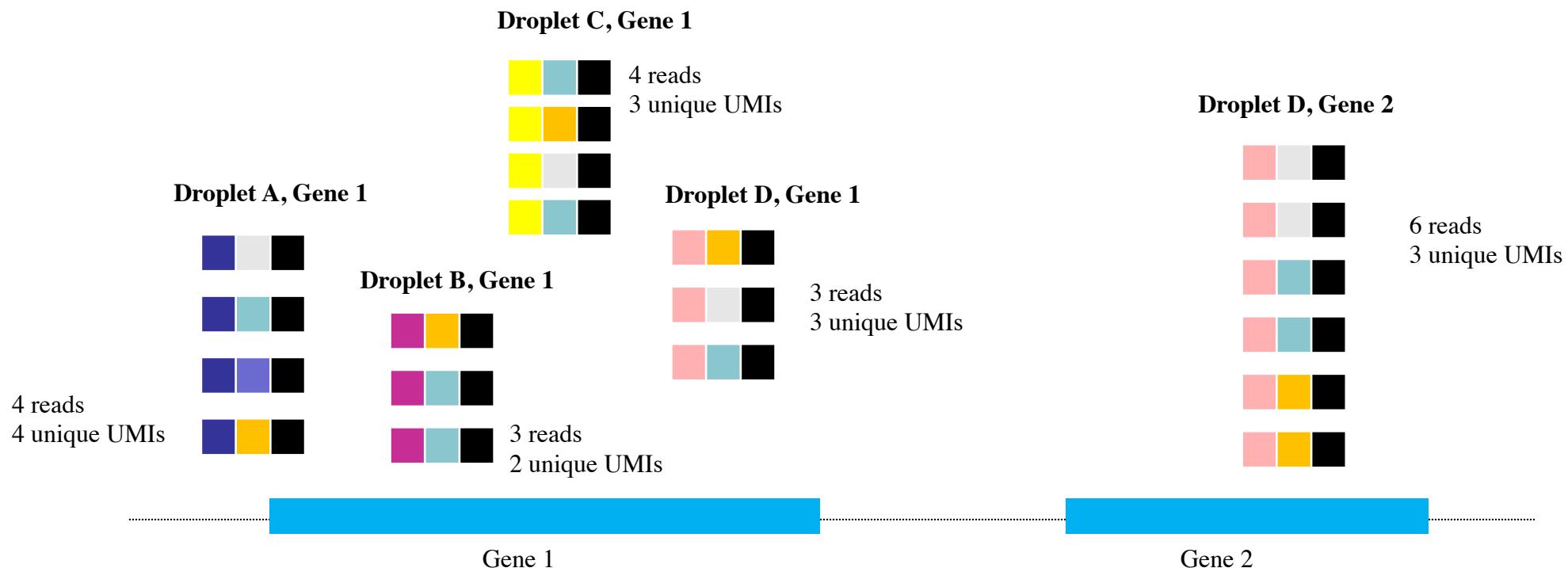
Sequencing



# From Library to count matrix – mapping



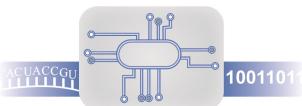
# From Library to count matrix – counting



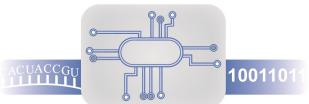
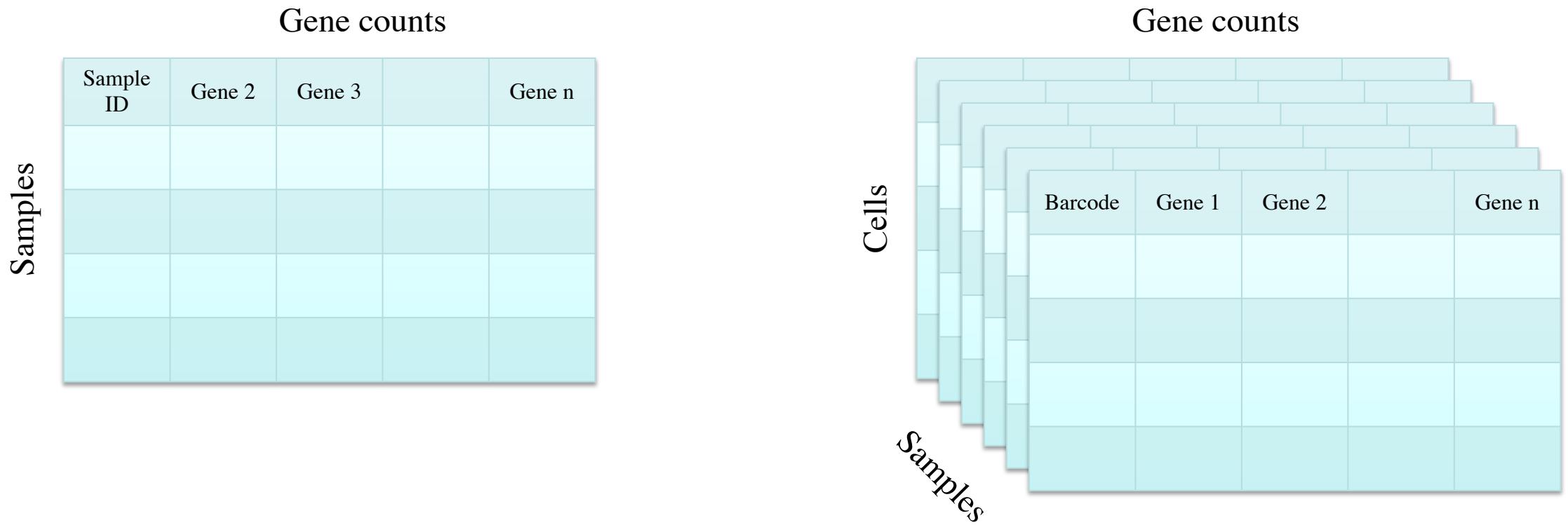
Barcode	Gene 1	Gene 2		Gene n
Droplet A	4	0		
Droplet B	2	0		
Droplet C	3	0		
Droplet D	3	3		

Count unique UMIs for each gene and droplet

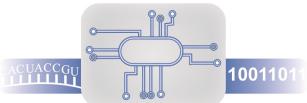
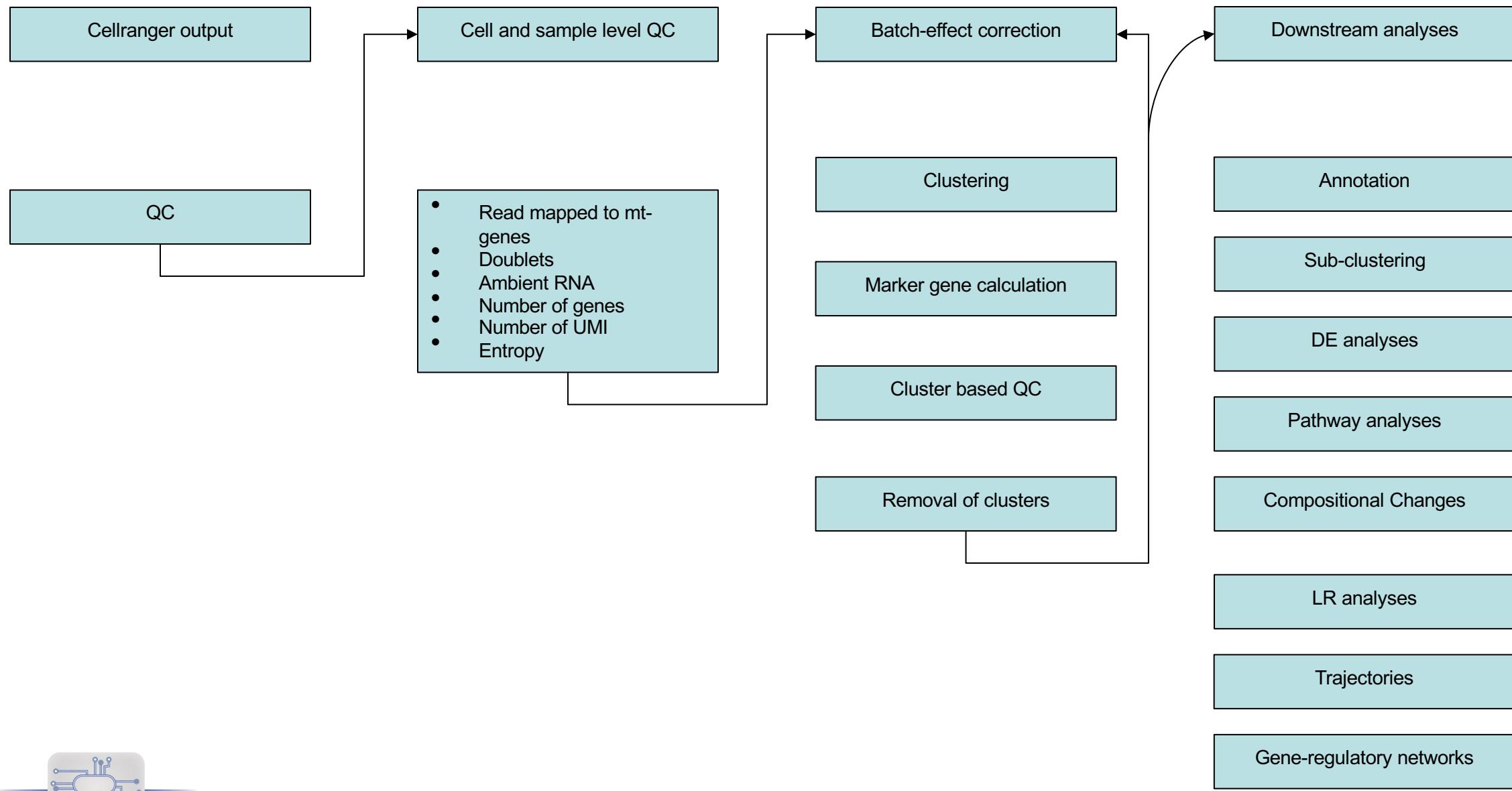
Count unique UMIs for each gene and droplet



# Bulk vs single-cell data matrix



# Data analyses workflow



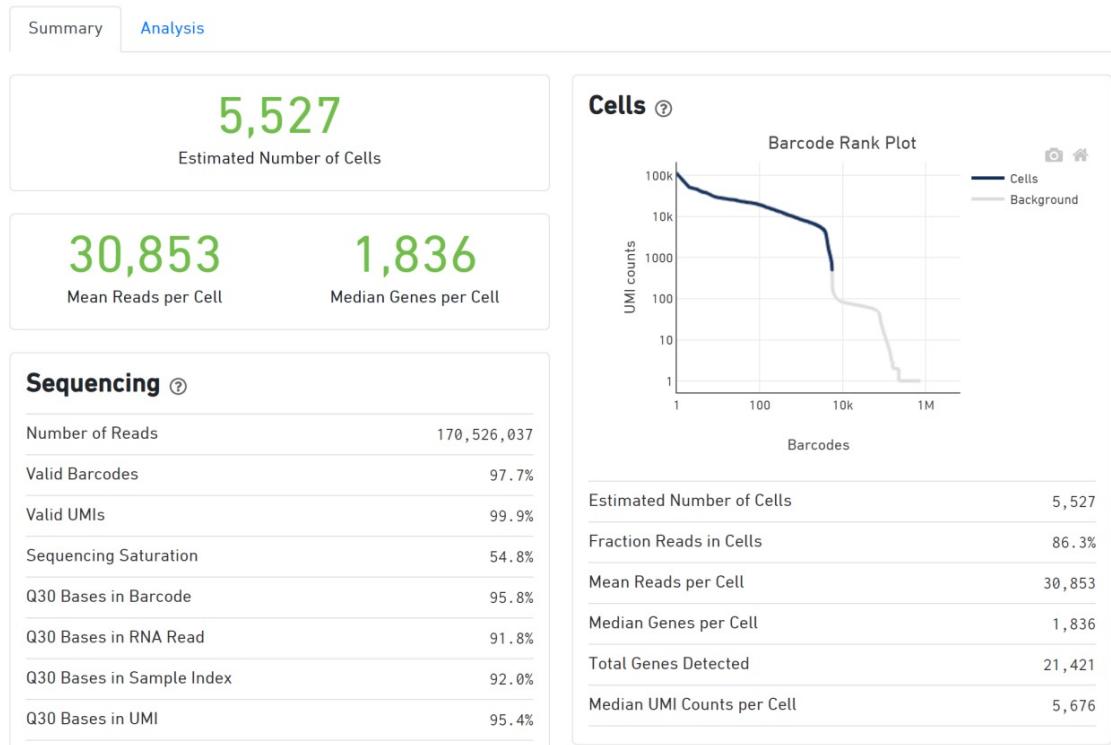
# Cell level QC (hands on)

For each droplet:

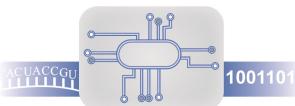
- Reads mapping to Mitochondrial genes
- Number of UMI
- Number of genes
- Doublet

Suggestions for cell QC:

- Outlier detection
- One strict cut-off
- QC per cell-type

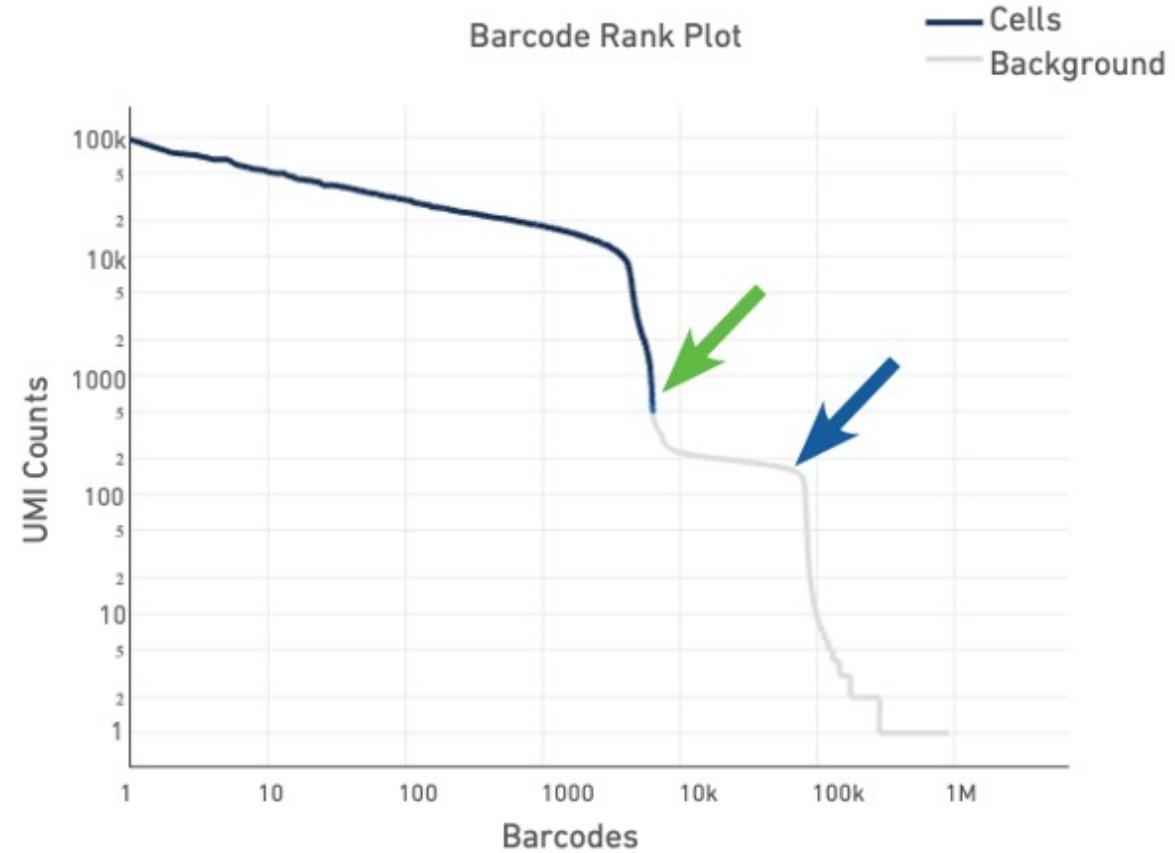
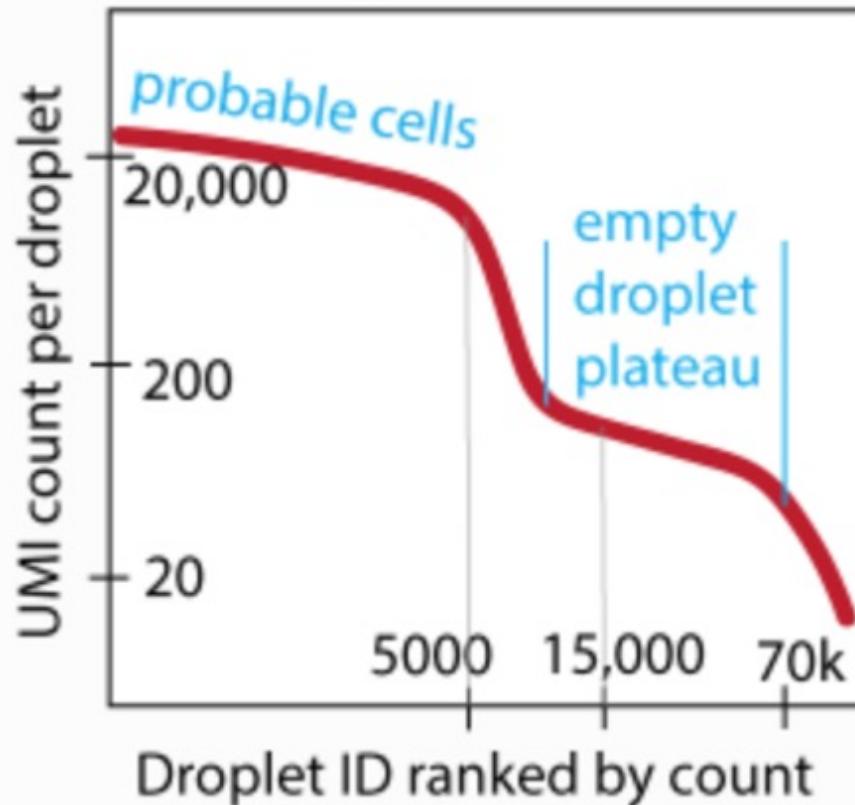


[https://cdn.10xgenomics.com/image/upload/v1660261286/support-documents/CG000329\\_TechnicalNote\\_InterpretingCellRangerWebSummaryFiles\\_RevA.pdf](https://cdn.10xgenomics.com/image/upload/v1660261286/support-documents/CG000329_TechnicalNote_InterpretingCellRangerWebSummaryFiles_RevA.pdf)

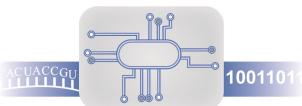


# Ambient RNA

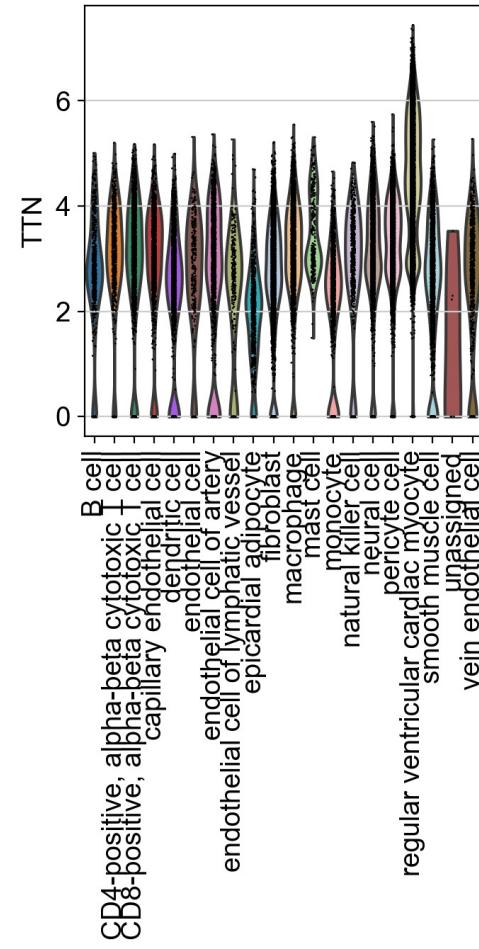
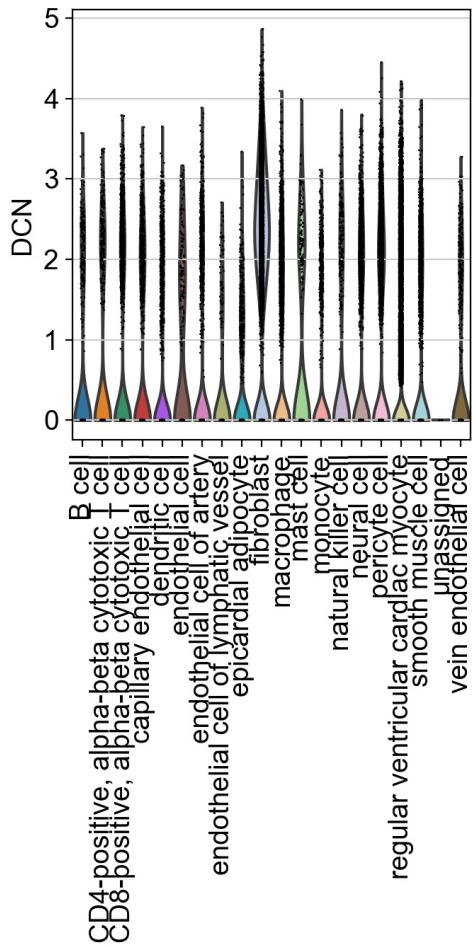
## Reading the UMI curve



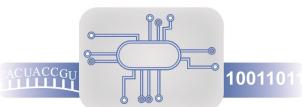
Cellbender



# Ambient RNA



Processed Results from MI Human Atlas



# Batch effect correction before integration

Batch-effect correction to just cluster the cells

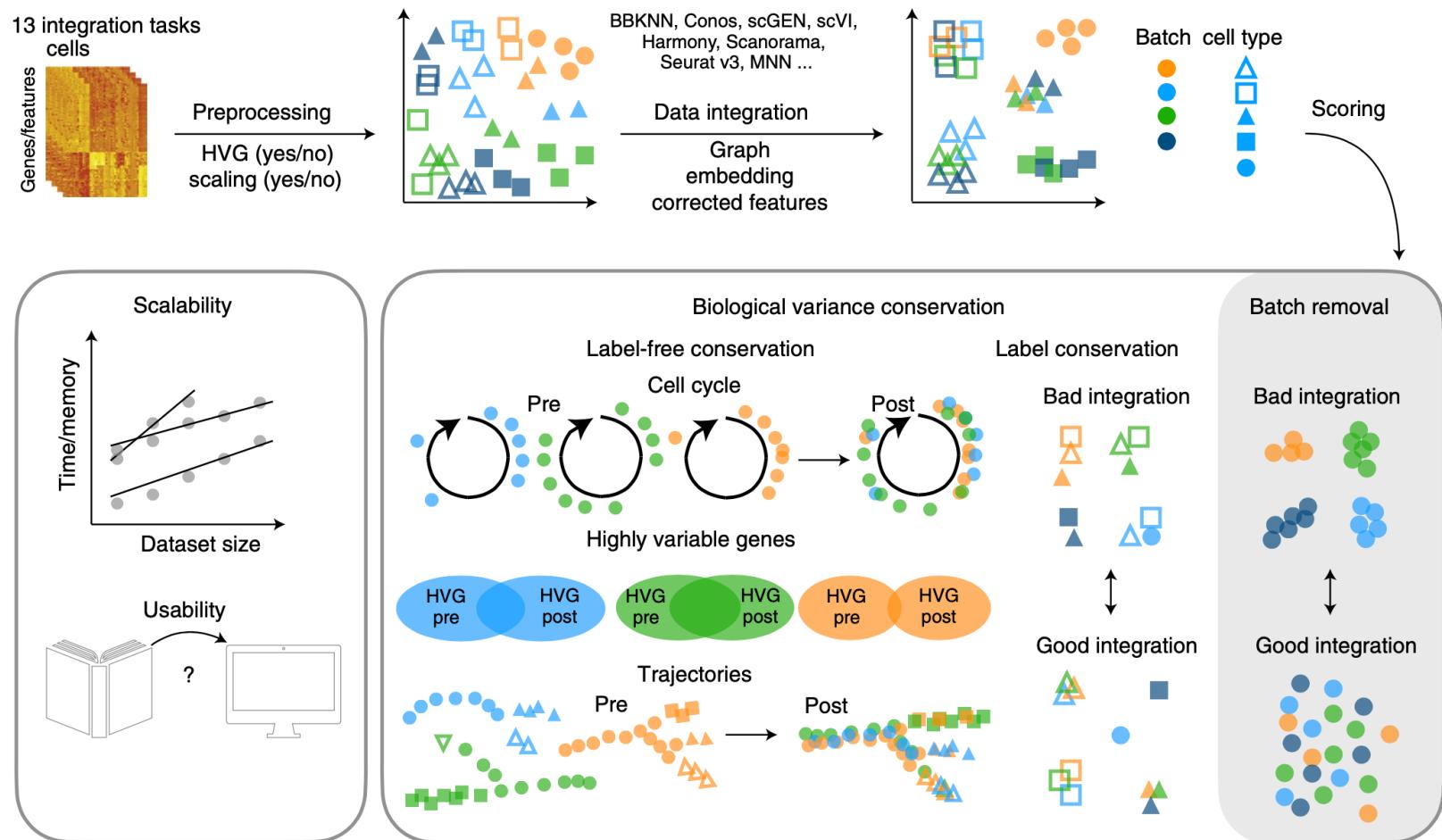
- DO not alter the underlying count data
- Model ‘batch’ variable separately

Correction that involves aligning the underlying counts

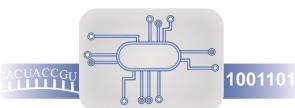
- Alter the count data

## Suggestions:

Harmony  
scVI  
MASI  
...  
...



Separate data by cell-type and not batch type



# Harmony – batch effect correction (hands on)

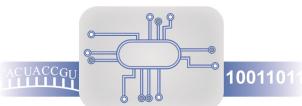
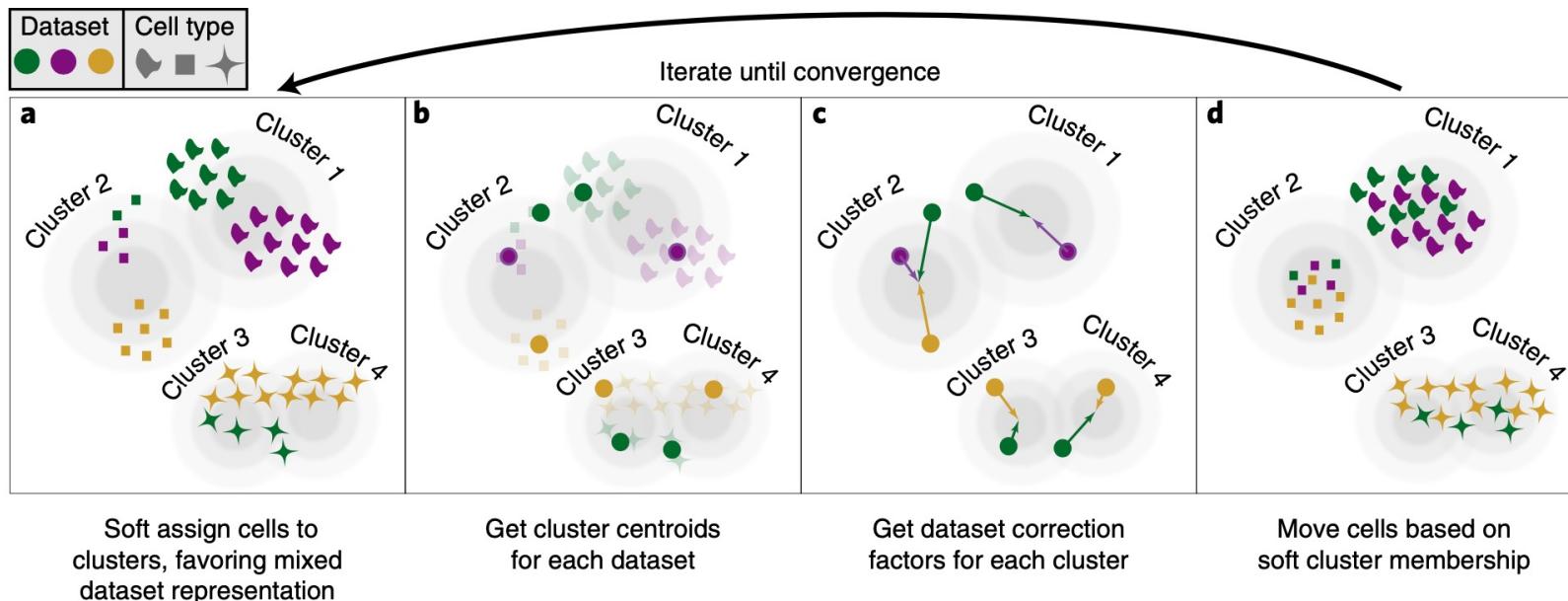
nature methods

ARTICLES

<https://doi.org/10.1038/s41592-019-0619-0>

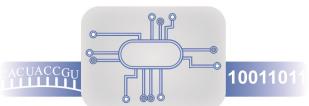
## Fast, sensitive and accurate integration of single-cell data with Harmony

Ilya Korsunsky<sup>1,2,3,4</sup>, Nghia Millard<sup>1,2,3,4</sup>, Jean Fan<sup>1,5</sup>, Kamil Slowikowski<sup>1,2,3,4</sup>,  
Fan Zhang<sup>1,2,3,4</sup>, Kevin Wei<sup>2</sup>, Yury Baglaenko<sup>1,2,3,4</sup>, Michael Brenner<sup>2</sup>, Po-ru Loh<sup>1,3,4</sup> and  
Soumya Raychaudhuri<sup>1,2,3,4,6\*</sup>

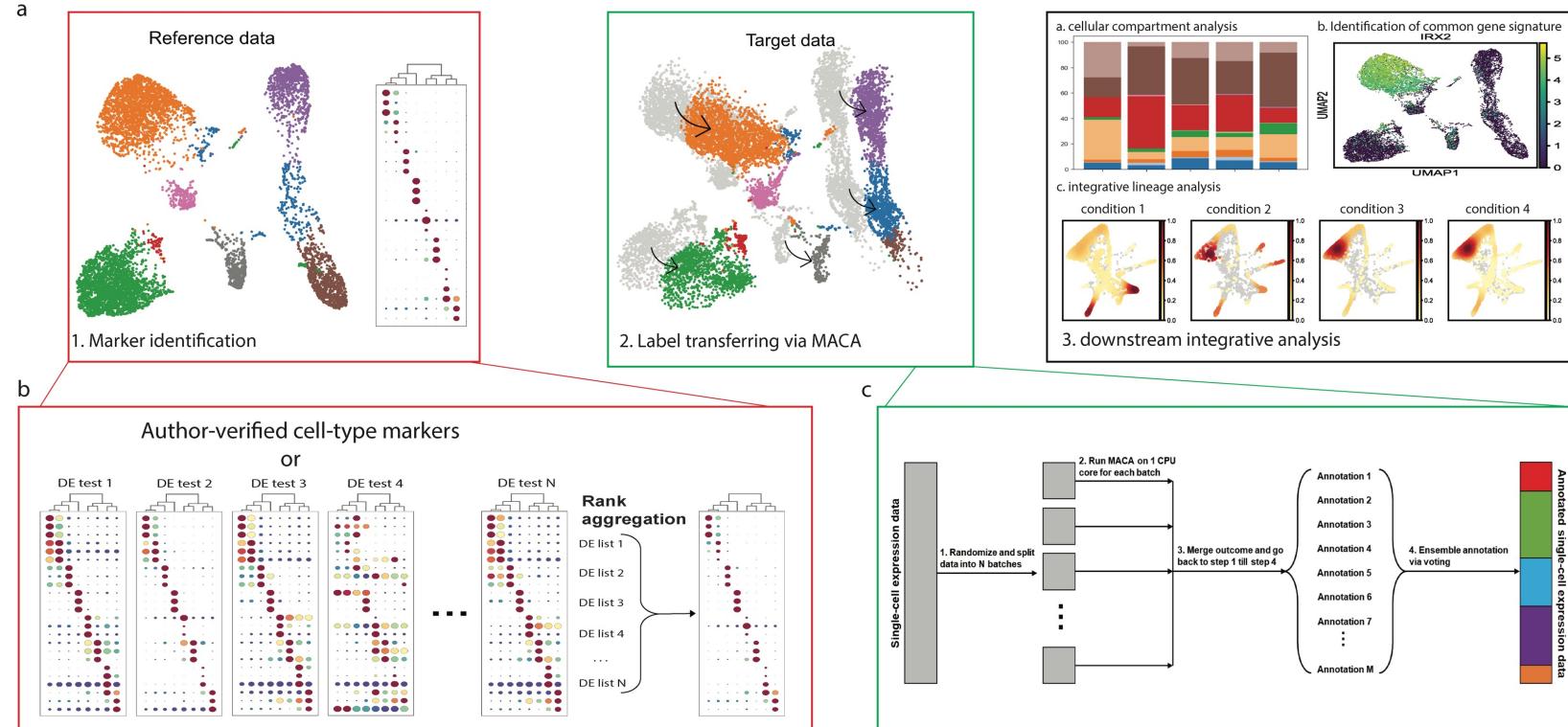


# Clustering (hands on)

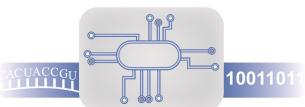
Graph based clustering Leiden or Louvain  
kNN  
Hierarchical clustering



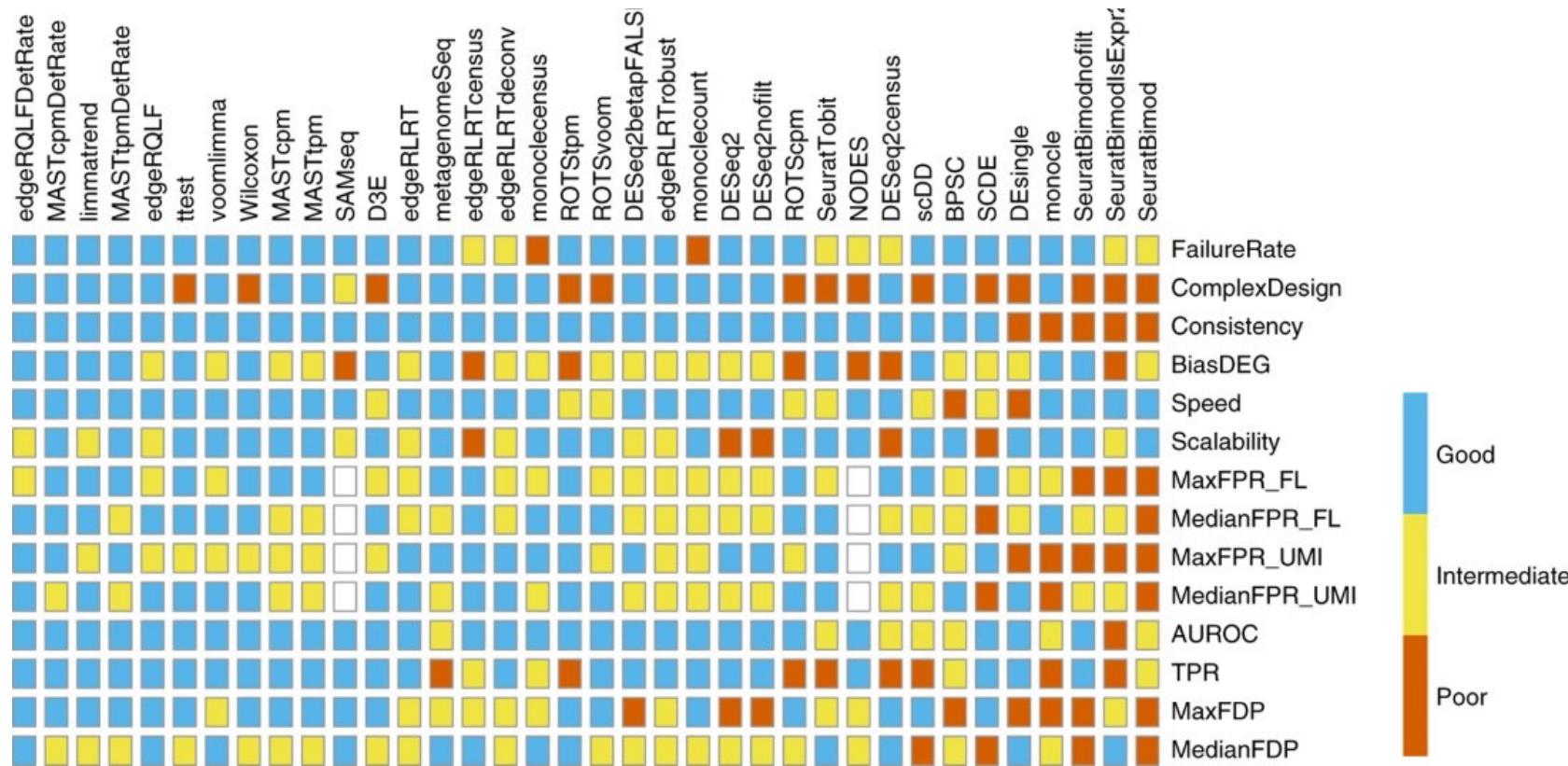
# Cell type annotation using MASI (hands on)



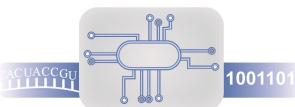
<https://www.nature.com/articles/s42003-023-04820-3>



# Differential expression analyses

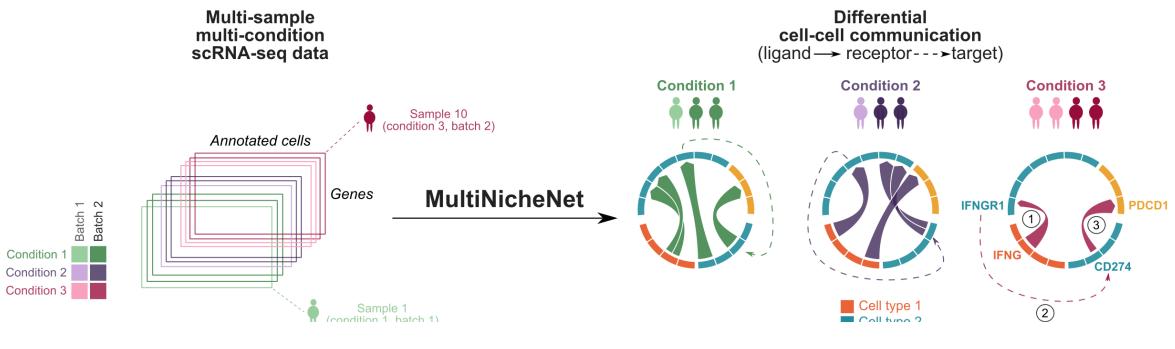


Soneson, C., Robinson, M. Bias, robustness and scalability in single-cell differential expression analysis. *Nat Methods* **15**, 255–261 (2018).

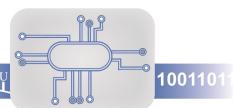


# Cell-cell communication

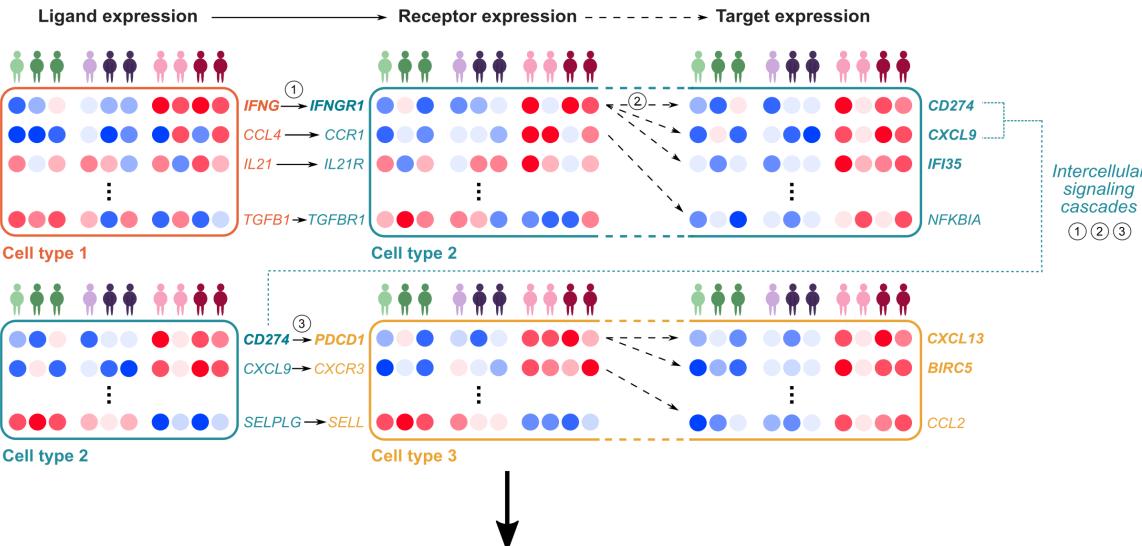
## a) Aim



Ranked ligand-receptor pairs	DE score ligand	DE score receptor	Cell-type specificity ligand	Cell-type specificity receptor	Downstream signaling activity	Fraction samples with ligand present	Fraction samples with receptor present	Aggregated prioritization score
<i>IFNG</i> → <i>IFNGR1</i>	█	█	█	█	█	█	█	█
<i>CD274</i> → <i>PDCD1</i>	█	█	█	█	█	█	█	█
<i>CXCL9</i> → <i>CXCR3</i>	█	█	█	█	█	█	█	█
<i>CCL4</i> → <i>CCR1</i>	█	█	█	█	█	█	█	█
<i>IL21</i> → <i>IL21R</i>	█	█	█	█	█	█	█	█
⋮								
<i>TGFB1</i> → <i>TGFB1</i>	█	█	█	█	█	█	█	█
<i>SELL</i> → <i>SELPLG</i>	█	█	█	█	█	█	█	█

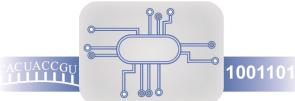
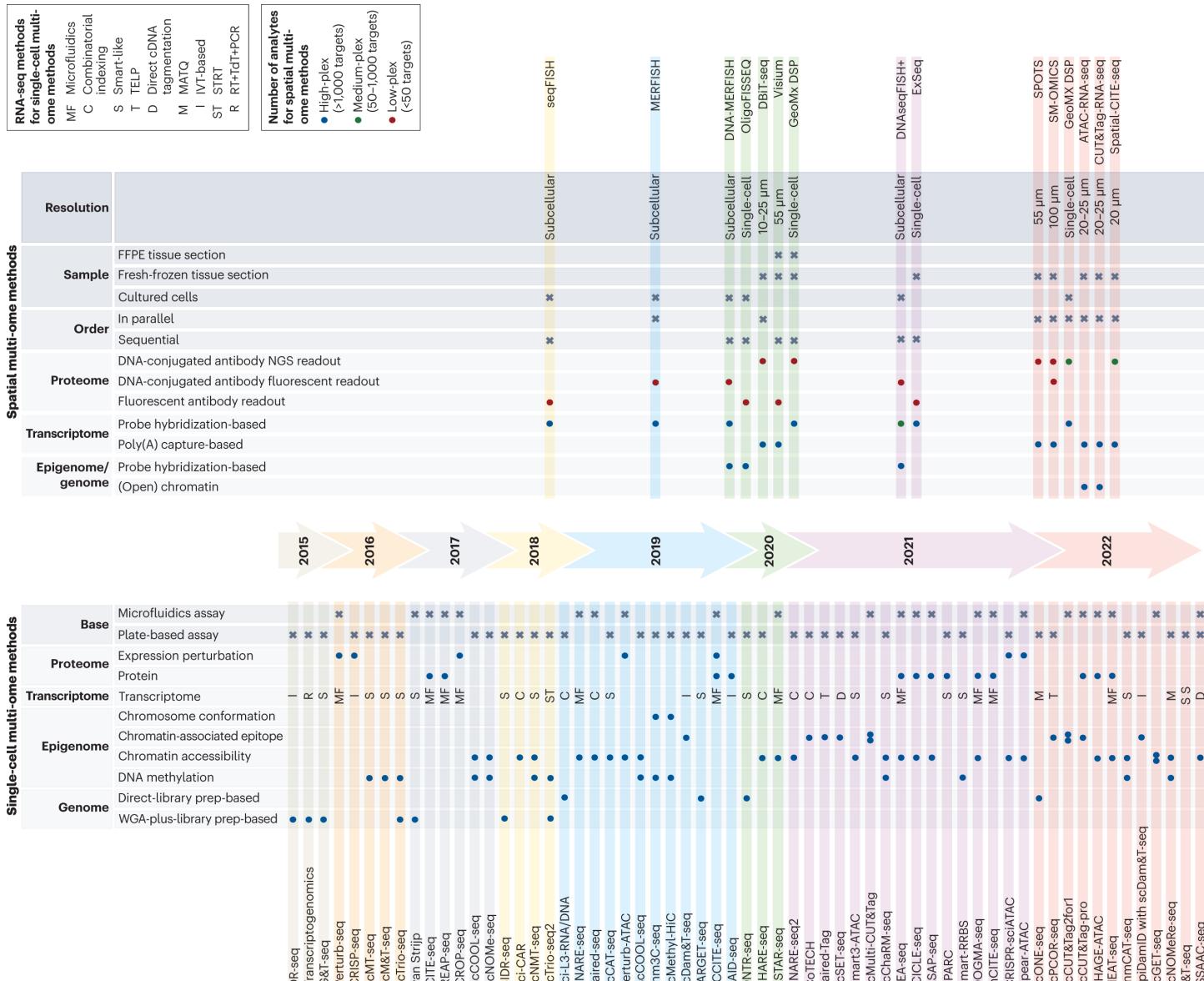


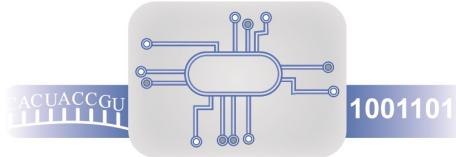
## b) Conceptual workflow



<https://github.com/saeyslab/multinichennetr>

# Single cell multi-omics methods





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[www.kramannlab.com](http://www.kramannlab.com)



Ministry of Culture and Science  
of the State of  
North Rhine-Westphalia

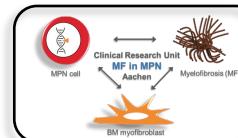


IZKF  
Institut für  
Klinische  
Forschung

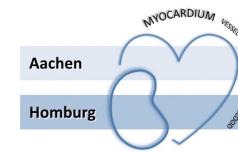
RWTHAACHEN  
UNIVERSITY



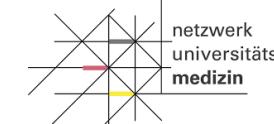
European  
Research  
Council



SFB-TRR219



CRU-344



"la Caixa" Foundation



Federal Ministry  
of Education  
and Research



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